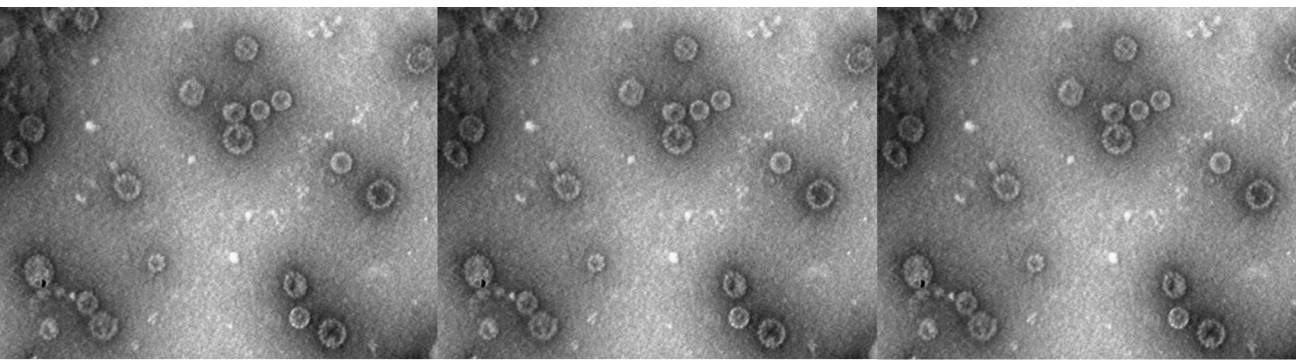
Infections and Cancers



Tata Memorial Centre

Infections and Cancers

Editors

Dr. Aruna Alahari Dhir MD
Professor & Head Dept. of Medicine

Dr. Sheela P. Sawant MD DNB
Associate Professor & Assistant Physician

Published by
Tata Memorial Centre
Mumbai

Tata Memorial Hospital
Dr. Ernest Borges Road, Parel
Mumbai 400 012. INDIA.
Tel.: +91-22-2417 7000
Fax: +91-22-2414 6937
Email: crs@tmc.gov.in
Website: http: //tmc.gov.in

Infections and Cancers

ISBN: 978-93-80251-13-5

Published by the Tata Memorial Hospital, Mumbai
Printed at the Sundaram Art Printing Press, Mumbai

List of Contributors

Amit Gulhane	Dept of Digestive Diseases and Clinical Nutrition
Amit Yelsangikar	Dept of Digestive Diseases and Clinical Nutrition
Anuprita Daddi	Dept of Medicine
Aruna Alahari Dhir	Dept of Medicine
Gauravi Mishra	Dept of Preventive oncology
Manju Sengar	Dept of Medical Oncology
Prachi Patil	Dept of Digestive Diseases and Clinical Nutrition
R K Shrivastava	Dept of Radiation Oncology
Reena Engineer	Dept of Radiation Oncology
Rohini Kelkar	Dept of Microbiology
Shubhada Chiplunkar	Chiplunkar Lab, ACTREC
Sagar Shah	Chiplunkar Lab, ACTREC
Sharmila Pimple	Dept of Preventive Oncology
Sheela Sawant	Dept of Medicine
Supriya Chopra	Dept of Radiation Oncology
Surendra Shastri	Dept. of Preventive Oncology
Tanuja Shet	Dept of Pathology

Contents

Preface

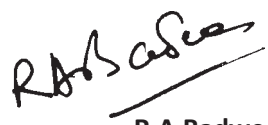
It is the 10th year of the "Evidence Based Management" meetings as Tata Memorial Hospital. This decade has seen a perceptible change in the academic lingo with off repeated use of terms "randomized evidence", "meta-analysis and "levels of evidence". Probably it has also induced uniform patterns to care across India reducing unnecessary long distance travel for patients to seek better patient care.

Infections and cancers is a relatively new topic where there may not be strong data from randomized trials at present but there is a need to do clinical research to answer various questions. Nearly 17.8% of the global cancer burden is attributable to infectious agents, with a higher percentage in developing countries. The principal infectious agents associated with cancer morbidity worldwide are Helicobacter pylori , human papilloma viruses , hepatitis B ,hepatitis C viruses and human immuno-deficiency viruses. A consensus is emerging that efforts to curb infection and infectious disease will also reduce the global burden of cancer. Vaccinations could be key to preventing some of these cancers that are associated with infection.

Malignancies are an important cause of morbidity and mortality in HIV infected patients. Cancer has been associated with AIDS even before the human immunodeficiency virus was identified. Many of the HIV related cancers are associated with oncogenic viruses. In the current era, HAART, better prophylaxis and treatment of opportunistic infections and advances in cancer therapy and supportive care, will translate into improved survival and quality of life for patients with HIV and Cancer. A multidisciplinary approach is required to develop therapeutic options.

The Section I of this book covers viruses, parasites, and bacteria that cause cancer. It familiarizes the busy physician with the important developments, infectious agents that are important causes of human pathology over and above the realm of traditional communicable diseases. Section II deals primarily with diagnosis and management of AIDS defining malignancies. Prevention and screening for non AIDS defining malignancies is discussed.

I hope that you find this book and the meeting useful for your day to day practice and also gives you opportunity do some clinical research which can have impact on the patient care in future.



R A Badwe
Director,
Tata Memorial Centre

Mumbai
February 2012

Section I
Infections and Cancers

Introduction

Infection, Inflammation and Cancer

Viral Causes of Cancer

Epstein-Barr Virus

Human Herpes Virus Type 8

Hepatitis B virus, Hepatitis C virus and Hepatocellular Carcinoma

Human Papilloma Viruses

- Oncogenic Human Papilloma Viruses
- HPV and Cervical Cancer
- Cervical Cancer Screening in India and HPV Vaccination
- HPV and Head & Neck Cancer

Retroviruses

Polyoma Viruses

Bacterial Causes of Cancer

Helicobacter Pylori

Parasitic Causes of Cancer

Schistosomiasis and Cancer

Infection, Inflammation and Cancer

Viral, bacterial and parasitic diseases have accompanied human beings since earliest times. Other than the direct toll inflicted by infectious diseases, numerous studies have established a relationship between microorganisms and chronic diseases such as atherosclerosis, neurologic disorders, cancer and obesity. Infection is one of the most important causes of cancer (Pisani et al., 1997). Among infection related neoplasms, cancer of the stomach, liver, cervix, bladder and colon are predominant and are associated with Helicobacter pylori, hepatitis B and C viruses, human papilloma viruses, infection with Schistosoma or Bacteroides species respectively.

A strong link between persistent infection, inflammation caused due to such infections and inflammation leading to cancers have been established (de Martel & Franceschi, 2009). Several types of inflammation differing by cause, mechanism, outcome and intensity can promote cancer development and progression. The inflammatory response triggered by infection precedes tumor development and is also a part of the normal host defense to eliminate the pathogen. Besides infection, chronic inflammation can result from exposure to environmental agents (Punturieri et al., 2009). Although inflammation acts as a host defense mechanism against infection or injury and is usually a self limiting process, inadequate resolution of inflammatory responses represents a major pathological basis for tumor development. About 25% of all cancer cases worldwide are linked to chronic inflammation caused by chronic infections, autoimmune diseases(eg.inflammatory bowel diseases) or inflammatory conditions of uncertain origin e.g. prostatitis (Porta et al., 2011). Colotta (Colotta et al., 2009) surmised that cancer related inflammation represents the seventh hallmark of cancer and suggested that this gets incorporated into the seminal contribution of Hanahan and Weinberg (Hanahan & Weinberg, 2000) that identified the six hallmarks of cancer. Inflammation also involves a well coordinated response of an innate and adaptive immune system following infection or injury.

Recently, however, rather than directly proposing link between infection and cancer, certain mechanisms related to inflammation are assumed to play an important role in causing infection mediated carcinogenesis. It has been proposed that infection in host causes chronic inflammation which in turn contributes to oncogenesis, provides growth and angiogenic factors, thus enhancing the proliferation and spread of tumor cells. A failure to regulate inflammation could result in chronic inflammatory disorders and also cancers (Rook & Dalgleish, 2011). Inflammation is found to persist in the tumors and is the base for their growth and spread. Inflammation enhances mutations (Colotta et al., 2009), growth, vascularization (K.J. O'Byrne, 2000) and metastasis. Infections are, however, considered the best understood activators of inflammation (Rakoff-Nahoum & Medzhitov, 2009; Terzic et al., 2010). Understanding the role of viruses, bacteria and parasites in causing chronic inflammation may facilitate development of therapeutic strategies for prevention and treatment of malignancies associated with these infections.

A) Viruses

Epstein Barr Virus (EBV)

Dennis Burkitt in the year 1958, first described a childhood B cell malignancy, which is now known as Burkitt's lymphoma and also suspected to have a viral etiology (Burkitt, 1962). In the year 1965, Anthony Epstein and Yvonne Barr identified a new type of herpesvirus and named it as human herpes virus-4 (HHV-4) or Epstein Barr Virus (EBV) (Epstein et al., 1965). Following primary infection, EBV persists within the memory B cells of the host in a latent non replicative state. EBV is found to be associated with human benign disease (infectious mononucleosis) and with many human malignancies viz. African Burkitt's lymphoma, gastric carcinoma, nasopharyngeal carcinoma, Hodgkin's lymphoma and B cell lymphoma in immunocompromised patients (Javier & Butel, 2008; Parkin, 2006). Of the 90% of the world population infected with EBV, majority of the patients develop benign disease at the time of primary infection. Genetic and the environmental factors e.g., infections such as malaria, nutrition, immune status and translocation of myc that is usually found in Burkitt's lymphoma cells may be responsible for progression towards malignancy (Lombardi et al., 1987; Martin & Gutkind, 2008).

Hepatitis B Virus (HBV)

Blumberg in the year 1965 identified a blood antigen that reacted with the antibodies from patients with hepatitis(Blumberg, 1977). This antigen was a surface protein of a DNA virus which was called Hepatitis B virus (HBV)(Hirschman et al., 1969). HBV is found to be a major risk factor in causing hepatocellular carcinoma (HCC).In most individuals, infection with this virus is asymptomatic or leads to acute hepatitis, after which the infection is cleared by the immune response(Guidotti et al., 1999). However, in some cases HBV infection fails to get cleared and leads to chronic infection resulting in progression towards HCC. Epidemiological studies have established a link between chronic HBV infection and liver cancer (Beasley &

Hwang, 1984). Persistent infection with HBV is associated with varying degree of chronic liver disease, progressing to cirrhosis and may culminate into HCC (Guidotti & Chisari, 2006). During this period of progression, sustained immune mediated tissue injury, hepatocellular regeneration and continuous inflammation are thought to result in random genetic and chromosomal damage, ultimately leading to HCC (Nakamoto et al., 1998). HBx the oncoprotein of HBV has been proposed to activate several molecular mechanisms involved in proliferation and inflammation such as AP-1and NF- êB transcription factors, activation of JAK1, proten kinase C, PI3K and MAPK (Tang et al., 2006). The development of vaccine in the late 1970s (Buynak et al., 1976), lead to a significant decrease in the rate of new HBV cases and subsequently in HBV associated HCC cases.

Hepatitis C Virus (HCV)

Houghton and colleagues identified a RNA virus of the flaviviridae family in 1989 and termed it as Hepatitis C virus (HCV). HCV is also found to cause HCC and chronic liver disease (Colombo et al., 1989). There are more than 270 million infected with HCV worldwide and nearly 20% of them tend to develop liver complications and cirrhosis and among these, 4-7% progress towards HCC (Thomas et al., 2000). HCV leads to cirrhosis and cancer by causing strong immunological reaction or by sustaining liver damage and inflammation. Unfortunately no HCV vaccine has been developed due to weak immune response mounted by HCV.

Human Papilloma Virus (HPV)

In 1842, Rigoni-Stern, the Italian physician noted that the cases of cervical cancer and genital warts in women were associated with sexual contacts (Gasparini & Panatto, 2009), however the contagious nature of genital warts was understood only in 1907, when Ciuffo observed the transmission of warts using cell free extracts. HPV is found to be associated with cervix, anus and oropharynx head and neck cancer. More than 130 HPV types have been identified and classified into low and high risk groups while 70% of the cervical cancers are related to high risk HPV types 16 and 18 (Beaudenon et al., 1986; zur Hausen, 2009). HPV is considered to be the most common sexually transmitted disease and infection with HPV is asymptomatic and cleared within 1 or 2 years. Nearly 500,000 new annual cases of human cancers worldwide are related to HPV (Parkin, 2006; zur Hausen, 2009). The recent development of HPV vaccines is expected to reduce the annual number of HPV related cervical cancer (zur Hausen, 2009) and also prevent the spread of oral cancers (Chaturvedi et al., 2008).

Human T-cell Lymphotropic Virus (HTLV-1)

The first human oncogenic retrovirus was identified by Robert Gallo in cultured human T cell lymphoma cells in 1980 and was named as Human T–cell lymphotropic virus-1 (HTLV-1) (Poiesz et al., 1980). HTLV-1 is responsible for most of adult T cell leukemia and around 10 to 20 million people worldwide are infected with HTLV-1, and the prognosis remains poor (Parkin, 2006). There is no effective vaccine or antiviral therapy available for treating HTLV-1 patients (Martin & Gutkind, 2008).Tax protein of HTLV-1 involves perturbation of several cell growth

regulatory pathways, epigenetic mechanisms and interference with the cellular DNA repair apparatus leading to genomic instability and transformation.

Table 1: Viruses associated with cancers.

Infectious Agent	Name	Associated Cancers
Viruses	Epstein-Barr virus (EBV)	Burkitt Lymphoma
		Hodgkin Lymphoma
		Non-Hodgkin Lymphoma
		T cell Lymphoma
		Nasopharyngeal carcinoma
	Hepatitis B virus (HBV)	Hepatocellular Carcinoma
	Hepatitis C virus (HCV)	Hepatocellular Carcinoma
		Non-Hodgkin Lymphoma
	Human papillomavirus types 16, 18, and others (HPV)	Anal cancer
		Cervical cancer
		Oral cancer
		Oropharyngeal cancer
		Penile cancer
		Vaginal cancer
		Vulvar cancer
	Human immunodeficiency virus 1 (HIV 1)	Anal cancer
		Cervical cancer
		Conjunctiva cancer
		Hodgkin lymphoma
		Kaposi sarcoma
		Non-Hodgkin lymphoma
	Human T-cell lymphotropic virus 1 (HTLV 1)	Adult T-cell leukemia/ lymphoma
	Kaposi sarcoma herpesvirus/ human herpesvirus 8 (KSHV/HHV 8)	Kaposi sarcoma
		Primary effusion lymphoma
	Merkel cell polyoma virus	Merkel cell carcinoma

Kaposi's Sarcoma-Associated Herpes Virus (KSHV)

Kaposi's sarcoma associated herpes virus, also known as Human herpes virus 8 (HHV-8) causes Kaposi's sarcoma which is an angioproliferative tumor that was first described by the Hungarian pathologist Moritz Kaposi in 1872. The incidence of this disease has changed during the last few decades because of the widespread appearance of lymphoadenopathic Kaposi sarcoma cases in Africa in transplant patients undergoing immunosuppressive therapy and because of its association with AIDS (Ganem, 2006). AIDS-KS was considered to be one of the severe conditions until the introduction of antiretroviral therapy (HAART) for the treatment of HIV infected individuals, which lowered the incidence of Kaposi sarcoma. However it still remains the most prevalent cancer among children in Africa and among minority groups and in developing countries. KSHV genes represents pirated versions of cellular genes including those encoding molecules involved in cell cycle control, apoptosis prevention, immune system regulation and inter-and intracellular communication, thus harbouring proangiogenic and oncogenic potential (Russo et al., 1996).

B) Bacteria

Earlier bacterial infections were usually believed to be acute, but it has been now been accepted that several bacteria cause chronic infections and diseases, including cancers (Lax & Thomas, 2002; Vogelmann & Amieva, 2007). Table 2 highlights various bacteria and parasites that cause chronic inflammation and show causal association with several malignancies.

Table 2: Bacteria and parasites associated with cancers.

Infectious Agent	Name	Associated Cancers
Bacteria	*Helicobacter pylori*	Gastric cancers
	Helicobacter hepaticus	Hepatocellular carcinoma
	Streptococcus bovis	Colon cancer
	Citrobacter rodentium	Colon cancer
	Chlamydiae pneumonie	Lung cancer
	Bartonella species	Vascular tumor formation
	Fusobacterium nucleatum	Colorectal cancer
	Helicobacter bilis	Gallbladder cancer
	Salmonella typhi	Gallbladder cancer
Parasites	Liver flukes	Cholangiocarcinoma
	Schistosomes	Bladder cancer

Robin Warren and Barry Marshall in 1984 isolated and demonstrated that certain curved bacilli were responsible for causing peptic ulcer disease (Marshall & Warren, 1984). Thereafter many researchers have tried to link this bacterium and gastric cancers. It was in the year 1994,

International Agency for Research on Cancer (IARC) and World Health Organization (WHO) classified Helicobacter pylori (*H.pylori*) as human carcinogen (group1). It has been estimated that *H.pylori* is the major cause of stomach infection that cause peptic ulcers (10-20%), distal gastric adenocarcinoma (1-2%) and gastric mucosal-associated lymphoid tissue (MALT) lymphoma (Dorer et al., 2009; Parsonnet, 1995; Porta et al., ; Porta et al., 2011). *H.pylori* infections accounts for about 5.5% of all cancers worldwide (Herrera & Parsonnet, 2009). *H.pylori* has developed the property of behaving as a pathogen and as commensal bacterium.

Risk factors identified in gallbladder cancer (GBC) are cholelithiasis, chronic infection of the gall bladder, obesity, reproductive factors ,diet , hepatobiliary anamolies and environmental exposure to specific chemicals. Recently, certain Helicobacter species (*H.bilis, H.canis* etc.) have also been found to be associated with GBC (Mishra et al., 2010). Similarly, although GBC is rare, the highest incidences are seen in populations where chronic infections like *Salmonella typhi* and *S. paratyphi* are prevalent (Randi et al., 2006).

Studies have linked colonic carcinogenesis to chronic inflammation generated by pathogenic bacteria. Although, the specific bacterial species is yet to be defined for colon cancer, the minor gut colonizer *Streptococcus bovis* has been implicated in some of the clinical studies (Greer & O'Keefe, 2011). Enterotoxin producing *Bacteriodes fragilis* organisms that produce inflammatory diarrhea in humans trigger colitis and strongly induced colonic tumors (Wu et al., 2009). It has been clearly established that the use of anti-inflammatory medications suppresses colon cancer risk (Baron & Sandler, 2000).

C) Parasites

Helminth infections are of great importance worldwide with millions of humans getting affected or are at risk of infection. IARC has considered three species of trematodes, *Schistosoma haematobium*, *Opisthorchis viverrini* and *Clonorchis sinensis* responsible for causing helminth induced human cancer. *S.haematobium* and *O.viverrini* were classified as group 1 carcinogens while *C. sinensis* as a group 2 carcinogen. Helminths have complicated life cycle and long asymptomatic latent periods due to which their role in cancer still remains uncertain (Herrera & Ostrosky-Wegman, 2001). Helminth infections are largely diffused among populations of developing countries and their association with cancer was mainly suggested by epidemiological studies (Porta et al., 2011). Cholangiocarcinoma (CCA) is usually less common then hepatocellular carcinoma (HCC), but in Thailand, where the prevalence of *O.viverrini* is the highest in the world, CCA is predominant type of liver cancer (Porta et al., 2011; Vatanasapt et al., 1990). Similarly in Korea, where there is *C.sinensis* endemism, the incidence of CCA is high (Kim et al., 2009). High prevalence of *S.haematobium*, in Egypt, has led to increase incidence of squamous cell carcinoma of bladder (Mostafa et al., 1999). These heminthic infections can be prevented by avoiding contaminated water (*S.haematobium*) and raw or improperly cooked tainted freshwater fish (*O.viverrini* and *C.sinensis*) (Fried et al., 2011). However, the role of other trematodes such as *Schistosoma japonicum*, *Fascicola hepatica* and many other is still under investigation (Vennervald & Polman, 2009).

Infection associated Inflammation and the roadway to cancer development

There are many mechanisms by which chronic bacterial infections lead to carcinogenesis. The release of various mediators of inflammation, cytokines and activation of several genes during chronic infections may lead to cancer development. Although inflammation acts as a host defense mechanism, inadequate resolution of inflammatory responses represents a major pathological basis of tumor development (Porta et al., 2011). There are two pathways that connect inflammation to cancer: a) the extrinsic pathway, triggered by factors that are able to induce persistent inflammatory response and b) the intrinsic pathway, induced by alteration in cancer associated genes (tumor suppressor genes or oncogenes).

Mutations seen in tumor suppressor genes such Von Hippel-Lindau tumor suppressor (VHL), transforming growth factor-b (TGF-β) and phosphatase and tensin homologue (PTEN), are some of the factors that cause an increase in the activation of transcription factors such as nuclear factor-κB (NF-κB), hypoxia inducible factor 1a (HIF-1α) and signal transducer and activator of transcription 3 (STAT3) that are involved in inflammation and vascularization (Mantovani et al., 2008). Many carcinomas secrete factors that up regulate fibronectin and recruit vascular endothelial growth factor receptor 1 (VEGFR-1), positive hematopoietic progenitors and inflammatory cells to the tumor site (Rook & Dalgleish, 2011). These cells induce upregulation of certain tumor promoting cytokines such as TNF-α, IL-6, IL-1β (Balkwill, 2009; Lin & Karin, 2007). The NF-κB family of transcription factors triggered by microbial infections and proinflammatory cytokines results in inflammation driven carcinogenesis (Karin & Greten, 2005).This in turn activates the IKK complex (Hacker & Karin, 2006) resulting in degradation of NF-κB inhibitors and thus enables NF-κB to enter the nucleus and mediate the transcription of target genes such as cyclin D1, CDK2 kinase, c-myc (cell cycle regulators), p21 etc. Some genes which are involved in cell cycle control are upregulated such as cyclin D1, c-myc while some involved in apoptosis such as p53, p21 are downregulated by NF-κB. NF-κB activation also leads to upregulation of certain proinflammatory cytokines like IL-6, IL-1α while it downregulates TNF, thus allowing tumor growth. Several infections, particularly those in which the pathogens are intracellular, are associated with the suppression of apoptosis- often through the modulation of the expression of Bcl-2 family proteins (Lax & Thomas, 2002). This creates a niche for the survival of pathogens defying the host immune response (Van Antwerp et al., 1996). During inflammation, the epithelial cells respond by producing reactive oxygen species (ROS) and nitric oxide (NO). These compounds increase mutations in genes responsible for controlling malignant transformations. ROS inhibit tyrosine phosphatases, causing overexpression of Mox1 (catalytic subunit of NADPH oxidases). On the other hand NO, inhibits the Fpg protein, a DNA repair enzyme (Wink & Laval, 1994), leading to failure of damage control. Chronic inflammation also leads to over expression of epidermal growth factor receptor family member ErbB2, which is involved in cell proliferation, differentiation and oncogenesis. All these events may orchestrate the mechanism of carcinogenesis (Figure 1).

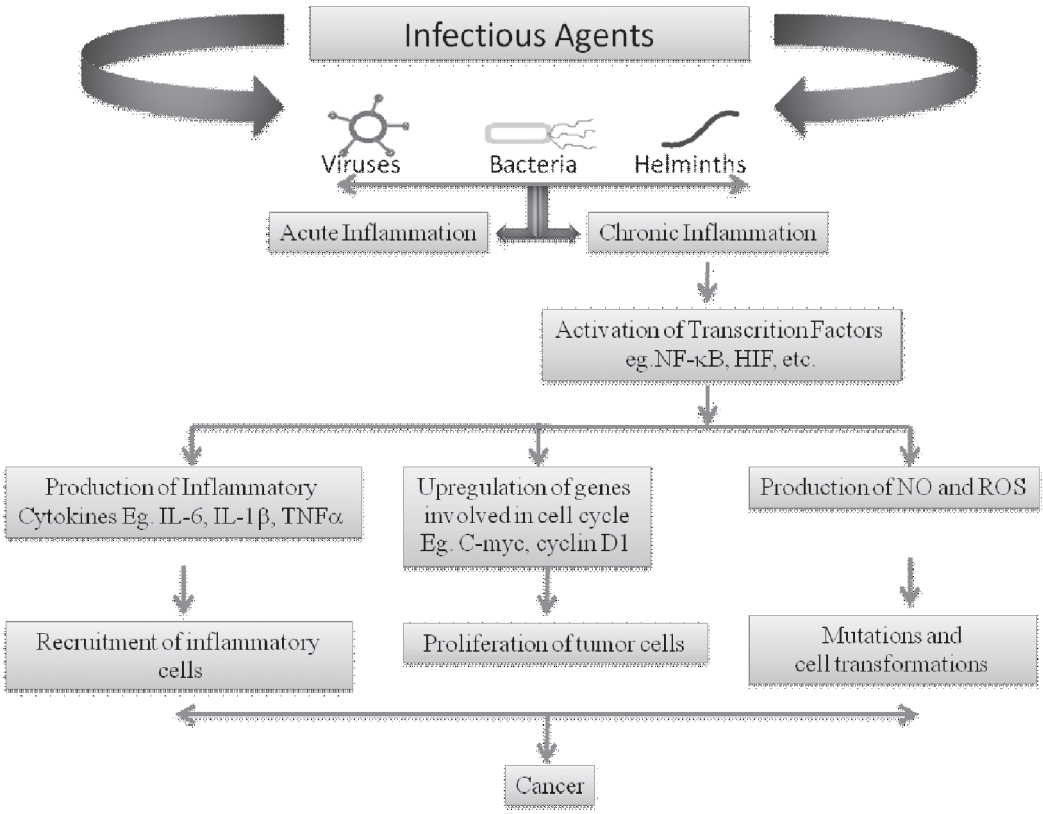


Figure 1: Roadway- From Infection to Cancer

Hypoxia-inducible factors (HIF), which are important mediators of the cellular oxygen-signaling pathway, promote tumorigenesis. During persistent HBV and HCV infections, activation of HIFs fails to result in eradication of these viruses. The consequence is the activation of pathways contributing to oncogenic transformations (Porta et al., 2011).

In case of *H.pylori* associated gastric cancers, the bacterium induces strong innate and adaptive immune responses (Robinson et al., 2007). Interaction of *H.pylori* with the gastric epithelial cells leads to the production of inflammatory cytokines and chemokines, which recruits leukocytes to the inflamed tissues and amplifies the immune response (Ernst & Gold, 2000). The innate immune responses against *H.pylori* generate mucosal damage, leading to increased exposure of leukocytes to bacteria and consequent amplification of the inflammatory response (Porta et al., 2011). However, *H.pylori* has the tendency to escape innate and adaptive immune responses. The bacterial proteins, CagA (Paziak-Domanska et al., 2000) and VacA (Gebert et al., 2003; Molinari et al., 1998) have the ability to inhibit T cell activation and subsequently trigger the recruitment of regulatory T cells (Tregs) in the gastric mucosa (Kandulski et al., 2008). These downregulate T cell functions during the course of infection and provide

optimal conditions for the prevalence of *H.pylori* infections. Helminths tend to induce the expression of an inflammatory program that is M2 (Arginase 1, Fizz 1, Ym 1, MGL2) and Th2 (IL-4, IL-5, IL-9, IL-10, IL-13) related, which persists for the duration of the infection (Hoffmann et al., 2002; Porta et al., 2011). Long lived helminths may affect host immune surveillance and thereby contribute to clonal expansion of malignant transformed cells (Pettit et al., 2000).

Conclusion

Cancer related inflammation is a key component in tumor progression and represents the seventh hall mark of cancer. Chronic inflammation in selected organs increases the risk of cancer. Inflammatory responses play decisive roles at different stages of tumor development i.e. initiation, promotion, progression and metastasis. Infections, autoimmune disorders and inflammatory conditions provide triggers of chronic inflammation that increases cancer risk. Clinical and epidemiological studies have suggested an association between infectious agents and chronic inflammatory disorders and cancer. A wealth of data suggests that induction of genetic instability by inflammatory mediators, epigenetic changes and activation of transcription factors orchestrate the inflammation mediated tumor progression. Some of these mediators may have both pro-tumorigenic and anti-tumorigenic functions. A complex interplay exists between malignant transformed cells, the surrounding stroma and the cells of the innate and adaptive immune system. Future challenging opportunities for diagnosis, prevention and /or therapy of chronic illnesses will require an integrated understanding of the developmental phases of inflammation induced immune dysfunctions. Such studies will provide important clues to understanding cancer development on the background of chronic inflammation.

References

Balkwill, F. (2009). Tumour necrosis factor and cancer. *Nat Rev Cancer*, 9, 361-71.

Balkwill, F. & Mantovani, A. (2001). Inflammation and cancer: back to Virchow? *Lancet*, 357, 539-45.

Baron, J.A. & Sandler, R.S. (2000). Nonsteroidal anti-inflammatory drugs and cancer prevention. *Annu Rev Med*, 51, 511-23.

Beasley, R.P. & Hwang, L.Y. (1984). Hepatocellular carcinoma and hepatitis B virus. *Semin Liver Dis*, 4, 113-21.

Beaudenon, S., Kremsdorf, D., Croissant, O., Jablonska, S., Wain-Hobson, S. & Orth, G. (1986). A novel type of human papillomavirus associated with genital neoplasias. *Nature*, 321, 246-9.

Blumberg, B.S. (1977). Australia antigen and the biology of hepatitis B. *Science*, 197, 17-25.

Burkitt, D. (1962). A children's cancer dependent on climatic factors. *Nature*, 194, 232-4.

Buynak, E.B., Roehm, R.R., Tytell, A.A., Bertland, A.U., 2nd, Lampson, G.P. & Hilleman, M.R. (1976). Vaccine against human hepatitis B. *Jama*, 235, 2832-4.

Chaturvedi, A.K., Engels, E.A., Anderson, W.F. & Gillison, M.L. (2008). Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. *J Clin Oncol*, 26, 612-9.

Colombo, M., Kuo, G., Choo, Q.L., Donato, M.F., Del Ninno, E., Tommasini, M.A., Dioguardi, N. & Houghton, M. (1989). Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *Lancet*, 2, 1006-8.

Colotta, F., Allavena, P., Sica, A., Garlanda, C. & Mantovani, A. (2009). Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis*, 30, 1073-81.

de Martel, C. & Franceschi, S. (2009). Infections and cancer: established associations and new hypotheses. *Crit Rev Oncol Hematol*, 70, 183-94.

Dorer, M.S., Talarico, S. & Salama, N.R. (2009). Helicobacter pylori's unconventional role in health and disease. *PLoS Pathog*, 5, e1000544.

Epstein, M.A., Henle, G., Achong, B.G. & Barr, Y.M. (1965). Morphological and Biological Studies on a Virus in Cultured Lymphoblasts from Burkitt's Lymphoma. *J Exp Med*, 121, 761-70.

Ernst, P.B. & Gold, B.D. (2000). The disease spectrum of Helicobacter pylori: the immunopathogenesis of gastroduodenal ulcer and gastric cancer. *Annu Rev Microbiol*, 54, 615-40.

Fried, B., Reddy, A. & Mayer, D. (2011). Helminths in human carcinogenesis. *Cancer Lett*, 305, 239-49.

Ganem, D. (2006). KSHV infection and the pathogenesis of Kaposi's sarcoma. *Annu Rev Pathol*, 1, 273-96.

Gasparini, R. & Panatto, D. (2009). Cervical cancer: from Hippocrates through Rigoni-Stern to zur Hausen. *Vaccine*, 27 Suppl 1, A4-5.

Gebert, B., Fischer, W., Weiss, E., Hoffmann, R. & Haas, R. (2003). Helicobacter pylori vacuolating cytotoxin inhibits T lymphocyte activation. *Science*, 301, 1099-102.

Greer, J.B. & O'Keefe, S.J. (2011). Microbial induction of immunity, inflammation, and cancer. *Front Physiol*, 1, 168.

Guidotti, L.G. & Chisari, F.V. (2006). Immunobiology and pathogenesis of viral hepatitis. *Annu Rev Pathol*, 1, 23-61.

Guidotti, L.G., Rochford, R., Chung, J., Shapiro, M., Purcell, R. & Chisari, F.V. (1999). Viral clearance without destruction of infected cells during acute HBV infection. *Science*, 284, 825-9.

Hacker, H. & Karin, M. (2006). Regulation and function of IKK and IKK-related kinases. *Sci STKE*, 2006, re13.

Hanahan, D. & Weinberg, R.A. (2000). The hallmarks of cancer. *Cell*, 100, 57-70.

Herrera, L.A. & Ostrosky-Wegman, P. (2001). Do helminths play a role in carcinogenesis? *Trends Parasitol*, 17, 172-5.

Herrera, V. & Parsonnet, J. (2009). Helicobacter pylori and gastric adenocarcinoma. *Clin Microbiol Infect*, 15, 971-6.

Hirschman, R.J., Shulman, N.R., Barker, L.F. & Smith, K.O. (1969). Virus-like particles in sera of patients with infectious and serum hepatitis. *Jama*, 208, 1667-70.

Hoffmann, K.F., Wynn, T.A. & Dunne, D.W. (2002). Cytokine-mediated host responses during schistosome infections; walking the fine line between immunological control and immunopathology. *Adv Parasitol*, 52, 265-307.

Javier, R.T. & Butel, J.S. (2008). The history of tumor virology. *Cancer Res*, 68, 7693-706.

K.J. O'Byrne, A.G.D., M.J. Browning, W.P. Steward, A.L. Harris. (2000). The relationship between angiogenesis and the immune response in carcinogenesis and the progression of malignant disease. *European Journal of Cancer*, 36, 151-169.

Kandulski, A., Wex, T., Kuester, D., Peitz, U., Gebert, I., Roessner, A. & Malfertheiner, P. (2008). Naturally occurring regulatory T cells (CD4+, CD25high, FOXP3+) in the antrum and cardia are associated with higher H. pylori colonization and increased gene expression of TGF-beta1. *Helicobacter*, 13, 295-303.

Karin, M. & Greten, F.R. (2005). NF-kappaB: linking inflammation and immunity to cancer development and progression. *Nat Rev Immunol*, 5, 749-59.

Kim, H.G., Han, J., Kim, M.H., Cho, K.H., Shin, I.H., Kim, G.H., Kim, J.S., et al (2009). Prevalence of clonorchiasis in patients with gastrointestinal disease: a Korean nationwide multicenter survey. *World J Gastroenterol*, 15, 86-94.

Lax, A.J. & Thomas, W. (2002). How bacteria could cause cancer: one step at a time. *Trends Microbiol*, 10, 293-9.

Lin, W.W. & Karin, M. (2007). A cytokine-mediated link between innate immunity, inflammation, and cancer. *J Clin Invest*, 117, 1175-83.

Lombardi, L., Newcomb, E.W. & Dalla-Favera, R. (1987). Pathogenesis of Burkitt lymphoma: expression of an activated c-myc oncogene causes the tumorigenic conversion of EBV-infected human B lymphoblasts. *Cell*, 49, 161-70.

Mantovani, A., Allavena, P., Sica, A. & Balkwill, F. (2008). Cancer-related inflammation. *Nature*, 454, 436-44.

Marshall, B.J. & Warren, J.R. (1984). Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet*, 1, 1311-5.

Martin, D. & Gutkind, J.S. (2008). Human tumor-associated viruses and new insights into the molecular mechanisms of cancer. *Oncogene*, 27 Suppl 2, S31-42.

Mishra, R.R., Tewari, M. & Shukla, H.S. (2010). Helicobacter species and pathogenesis of gallbladder cancer. *Hepatobiliary Pancreat Dis Int*, 9, 129-34.

Molinari, M., Salio, M., Galli, C., Norais, N., Rappuoli, R., Lanzavecchia, A. & Montecucco, C. (1998). Selective inhibition of Ii-dependent antigen presentation by Helicobacter pylori toxin VacA. *J Exp Med*, 187, 135-40.

Mostafa, M.H., Sheweita, S.A. & O'Connor, P.J. (1999). Relationship between schistosomiasis and bladder cancer. *Clin Microbiol Rev*, 12, 97-111.

Nakamoto, Y., Guidotti, L.G., Kuhlen, C.V., Fowler, P. & Chisari, F.V. (1998). Immune pathogenesis of hepatocellular carcinoma. *J Exp Med*, 188, 341-50.

Parkin, D.M. (2006). The global health burden of infection-associated cancers in the year 2002. *Int J Cancer*, 118, 3030-44.

Parsonnet, J. (1995). Bacterial infection as a cause of cancer. *Environ Health Perspect*, 103 Suppl 8, 263-8.

Paziak-Domanska, B., Chmiela, M., Jarosinska, A. & Rudnicka, W. (2000). Potential role of CagA in the inhibition of T cell reactivity in Helicobacter pylori infections. *Cell Immunol*, 202, 136-9.

Pettit, S.J., Seymour, K., O'Flaherty, E. & Kirby, J.A. (2000). Immune selection in neoplasia: towards a microevolutionary model of cancer development. *Br J Cancer*, 82, 1900-6.

Pisani, P., Parkin, D.M., Munoz, N. & Ferlay, J. (1997). Cancer and infection: estimates of the attributable fraction in 1990. *Cancer Epidemiol Biomarkers Prev*, 6, 387-400.

Poiesz, B.J., Ruscetti, F.W., Gazdar, A.F., Bunn, P.A., Minna, J.D. & Gallo, R.C. (1980). Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci U S A*, 77, 7415-9.

Porta, C., Riboldi, E. & Sica, A. Mechanisms linking pathogens-associated inflammation and cancer. *Cancer Lett*, 305, 250-62.

Porta, C., Riboldi, E. & Sica, A. (2011). Mechanisms linking pathogens-associated inflammation and cancer. *Cancer Lett*, 305, 250-62.

Punturieri, A., Szabo, E., Croxton, T.L., Shapiro, S.D. & Dubinett, S.M. (2009). Lung cancer and chronic obstructive pulmonary disease: needs and opportunities for integrated research. *J Natl Cancer Inst*, 101, 554-9.

Rakoff-Nahoum, S. & Medzhitov, R. (2009). Toll-like receptors and cancer. *Nat Rev Cancer*, 9, 57-63.

Randi, G., Franceschi, S. & La Vecchia, C. (2006). Gallbladder cancer worldwide: geographical distribution and risk factors. *Int J Cancer*, 118, 1591-602.

Robinson, K., Argent, R.H. & Atherton, J.C. (2007). The inflammatory and immune response to Helicobacter pylori infection. *Best Pract Res Clin Gastroenterol*, 21, 237-59.

Rook, G.A. & Dalgleish, A. (2011). Infection, immunoregulation, and cancer. *Immunol Rev*, 240, 141-59.

Russo, J.J., Bohenzky, R.A., Chien, M.C., Chen, J., Yan, M., Maddalena, D., Parry, J.P., Peruzzi, D., Edelman, I.S., Chang, Y. & Moore, P.S. (1996). Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus (HHV8). *Proc Natl Acad Sci U S A*, 93, 14862-7.

Tang, H., Oishi, N., Kaneko, S. & Murakami, S. (2006). Molecular functions and biological roles of hepatitis B virus x protein. *Cancer Sci*, 97, 977-83.

Terzic, J., Grivennikov, S., Karin, E. & Karin, M. (2010). Inflammation and colon cancer. *Gastroenterology*, 138, 2101-2114 e5.

Thomas, D.L., Astemborski, J., Rai, R.M., Anania, F.A., Schaeffer, M., Galai, N., Nolt, K., Nelson, K.E., Strathdee, S.A., Johnson, L., Laeyendecker, O., Boitnott, J., Wilson, L.E. & Vlahov, D. (2000). The natural history of hepatitis C virus infection: host, viral, and environmental factors. *Jama*, 284, 450-6.

Van Antwerp, D.J., Martin, S.J., Kafri, T., Green, D.R. & Verma, I.M. (1996). Suppression of TNF-alpha-induced apoptosis by NF-kappaB. *Science*, 274, 787-9.

Vatanasapt, V., Uttaravichien, T., Mairiang, E.O., Pairojkul, C., Chartbanchachai, W. & Haswell-Elkins, M. (1990). Cholangiocarcinoma in north-east Thailand. *Lancet*, 335, 116-7.

Vennervald, B.J. & Polman, K. (2009). Helminths and malignancy. *Parasite Immunol*, 31, 686-96.

Vogelmann, R. & Amieva, M.R. (2007). The role of bacterial pathogens in cancer. *Curr Opin Microbiol*, 10, 76-81.

Wink, D.A. & Laval, J. (1994). The Fpg protein, a DNA repair enzyme, is inhibited by the biomediator nitric oxide in vitro and in vivo. *Carcinogenesis*, 15, 2125-9.

Wu, S., Rhee, K.J., Albesiano, E., Rabizadeh, S., Wu, X., Yen, H.R., Huso, D.L., Brancati, F.L., Wick, E., McAllister, F., Housseau, F., Pardoll, D.M. & Sears, C.L. (2009). A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. *Nat Med*, 15, 1016-22.

zur Hausen, H. (2009). Papillomaviruses in the causation of human cancers - a brief historical account. *Virology*, 384, 260-5.

Epstein-Barr Virus

Epstein-Barr virus (EBV) is a human gamma herpes virus that is best known for being the causative agent of infectious mononucleosis in man. EBV can transform latently infected primary cells from healthy individuals into cancerous ones, thereby causing important human cancers such as B-cell neoplasms (e.g. Burkitt's lymphoma and Post-transplant lymphomas), certain forms of T-cell lymphoma, and some epithelial tumours (e.g. gastric carcinomas ,nasopharyngeal carcinomas).

EBV was first isolated from cases of Burkitt's lymphoma in 1964, the first form of cancer to be associated with a virus. EBV is named after Michael Anthony Epstein and Yvonne Barr, who discovered and documented the virus. EBV is a double stranded DNA enveloped virus . The EBV genome is a linear ds DNA molecule with 172 kbp .The viral genome does not normally integrate into the cellular DNA but forms circular episomes which reside in the nucleus. The genome is large enough to code for 100 - 200 proteins but only a few have been identified.

The primary infection with EBV is believed to start within the oropharyngeal epithelial cells with viruses subsequently passing to subepithelial B cells through direct contact. The invasion of the immune system by EBV infection of B cells stimulates a vigorous CD8+ T cell response. The development of a virus-specific adaptive immune response is largely responsible for the elimination, to a large extent of EBV infection by reducing the number of EBV infected B cells. This elimination is however incomplete. As a common virus infection, EBV appears to have evolved to exploit the process of B cell development to persist as a life-long asymptomatic infection. Persistent EBV infection remains as a latent infection in peripheral blood lymphocytes and as a lytic infection in the oral cavity, which results in the shedding of infectious viruses via oral secretions .

EBV infects resting B cells and turns them into continuously proliferating lymphoblastoid cell lines that express nine latency-associated viral proteins, including six nuclear antigens (Epstein-Barr nuclear antigen (EBNA)1, 2, 3A,3B, 3C and LP) and three membrane proteins (latent membrane protein (LMP) 1, 2A and 2B) . However, only some of these proteins are expressed in EBV-positive malignancies.

Protein/gene/antigen	Description
EBNA-1	EBNA-1 protein binds to a replication origin (oriP) within the viral genome and mediates replication and partitioning of the episome during division of the host cell. It is the only viral protein expressed during group I latency.
EBNA-2	EBNA-2 is the main viral transactivator.
EBNA-3	These genes also bind the host RBP-Jê protein.
LMP-1	LMP-1 is a six-span transmembrane protein that is also essential for EBV-mediated growth transformation.
LMP-2	LMP-2A/LMP-2B are transmembrane proteins that act to block tyrosine kinase signaling.
EBER	EBER-1/EBER-2 (EBV encoded RNA) are small nuclear RNAs, which bind to certain nucleoprotein particles, enabling binding to PKR (dsRNA dependent serin/threonin protein kinase) thus inhibiting its function. EBER-particles also induce the production of IL-10 which enhances growth and inhibits cytotoxic T-cells.
miRNAs	EBV microRNAs are encoded by two transcripts, one set in the BART gene and one set near the BHRF1 cluster. The three BHRF1 miRNAS are expressed during type III latency while the large cluster of BART miRNAs (up to 20 miRNAs) are expressed during type II latency. The functions of these miRNAs are currently unknown.

Thus once infected, a lifelong carrier state develops whereby a low grade infection is kept in check by the immune defences. Low grade virus replication and shedding can be demonstrated in the epithelial cells of the pharynx of all seropositive individuals. Furthermore a few EBV-immortalized B-cells can be demonstrated in the circulation which are continually cleared by immune surveillance mechanisms.

On infecting the B-lymphocyte by binding to the complement receptor, the linear genome circularizes and the virus subsequently persists within the cell as an episome. In primary infection, EBV replicates in oro-pharyngeal epithelial cells and establishes Latency III, II, and I infections in B-lymphocytes.The three types of latent infection (type I-III) are according to the patterns of viral latent genes expression (from studies of EBV gene expression in cultured Burkitt's lymphoma cell lines).EBER1&2 EBNA1 (Latency I) ,EBER1&2 LMP2A LMP2B EBNA1 LMP1 (Latency II),EBER1&2 LMP2A LMP2B EBNA1 LMP1 EBNA2,3,4,5,6 (Latency III) .It is also postulated that a program exists in which all viral protein expression is shut off (latency 0).

EBV latent infection of B-lymphocytes is necessary for virus persistence, subsequent replication in epithelial cells, and release of infectious virus into saliva. EBV Latency III and II infections of B-lymphocytes, Latency II infection of oral epithelial cells, and Latency II infection of NK or T-cell can result in malignancies, marked by uniform EBV genome presence and gene expression.

EBV oncogenesis

Gruhne *et al.* showed the specific roles of three EBV latency proteins. They demonstrated that EBNA-1 and EBNA-3C, and LMP-1 independently promote genomic instability, as detected by nonclonal chromosomal aberrations, DNA breaks and phosphorylation of histone H2AX. EBNA-1 promotes the generation of DNA damage by inducing reactive oxygen species (ROS), whereas DNA repair is inhibited in LMP-1-expressing cells through down regulation of the DNA damage-sensing kinase, ataxia telangiectasia mutated (ATM), reduction of phosphorylation of its downstream targets Chk2 and inactivation of the G2 checkpoint. EBNA-3C enhances the propagation of damaged DNA through inactivation of the mitotic spindle checkpoint and transcriptional down regulation of BubR1. Thus, multiple cellular functions involved in the maintenance of genome integrity seem to be independently targeted by EBV, pointing to the induction of genomic instability as a critical event in viral oncogenesis.

In several cancer cells types, the EBV genome is heavily methylated . Methylation of DNA is usually associated with inhibition of gene expression; partly mediated by the association of specific methyl- CpG -binding proteins with methylated DNA, leading to transcriptional silencing and chromatin remodelling; and also by the inhibition of DNA binding of some transcription factors through DNA methylation.

EBV and Burkitt's lymphoma

EBV is highly efficient at transforming primary B cells in culture into proliferating, latently

infected lymphoblastoid cell lines (LCLs). In LCLs, EBV expresses the full spectrum of latent genes.In EBV-positive BL an EBV-infected cell undergoes a germinal center reaction and acquires a MYC translocation. Although MYC activates p53, LMP2A provides survival signals, such as increasing levels of anti-apoptotic proteins which protect the cell from apoptosis, leading to an expansion of pretumor cells. Although the frequency of *p53* mutation in BL tumors is not dependent on EBV status, *p53* mutations are detected in a higher percentage of BL cell lines than in fresh biopsies. This likely reflects an association of p53 mutation with tumor progression. The expansion of cells mediated by MYC and LMP2A increases the probability of acquiring a mutation in p53 in an EBV-positive cell. Mutation in p53 lead to tumor progression. The p53 bypass function of LMP2A is no longer needed once a p53 mutation has occurred, consistent with the low levels of LMP2A detected in BL biopsies. Transcripts of the EBV latency protein LMP2A are present at low levels in BL biopsies. LMP2A increases the levels of pro-survival Bcl family members in B lymphocytes, shifting the balance of pro-apoptotic and anti-apoptotic factors to promote cell survival. This function of LMP2A allows for bypass of p53 inactivation

Table 1 - EBC associated cancers

Lymphoma	Distribution	% EBV positive	EBV latent gene expression
Burkitt's lymphoma Endemic Sporadic AIDS associated	 Central Africa Worldwide Worldwide	 95% 15-25% ~30%	EBNA1, EBERs, BARTs, miRNA (subsets express EBNA3s, or LMP1 and/or BZLF1, or BARF11
Polyclonal (PTLD, AIDS ELPS)[a]	Organ-transplant recipients	100%	Majority express EBNA1/2/3s/LP, LMP1/2A, EBERs, CSTs, (subsets express only EBNA1, EBERs, BARTs, down-regulate EBNA2, LMP1)
Hodgkin's disease[b] MC/LD NS	Worldwide 80-90% 30%		LMP1, LMP2a, EBNA1, EBERs, BARTs
Nasopharyngeal carcinoma	China and Southeast Asia	90%	EBNA1, EBERs, BARTs, miRNAs, in 50% LMP1, LMP2 (subsets express BARF1)
Gastric carcinoma (GC)	Worldwide	~10%	EBNA1, EBERs, BARTs, miRNAs, LMP1, LMP2

a PTLD, posttransplant lymphoproliferative disease; XLPS, X-linked lymphoproliferative syndrome.

b MC, mixed cellularity; LD, lymphocyte depleted; NS, nodular sclerosing.

in a MYC tumor model. It is proposed that LMP2A plays a role early in development of BL, where the survival signal allows for expansion of cells that contain a MYC translocation. The expanded cells increase the probability of acquiring a p53 mutation, which leads to tumor progression. After the p53 mutation, the tumor cells become less dependent on LMP2A and immune selection may explain the low levels of LMP2A present in tumor biopsies.

EBV and nasopharyngeal cancer (NPC)

EBV infects the epithelial cells of the posterior nasopharynx in Rosenmuller's fossa in Waldeyer's ring . There have been two models to explain infection of these cells by EBV. Although an EBV-compatible receptor on epithelial cells has not been found, a surface protein is antigenically related to the B cell. CD21 receptor has been described . Alternatively, it has been suggested that EBV may gain entry into nasopharyngeal cells through IgA-mediated endocytosis.

EBV undergoes latency II expression in undifferentiated nasopharyngeal carcinoma. The most common and outstanding genetic changes are the loss of chromosomal region 9p21 (p16, p15, and p14ARF) and 3p (RASSF1A),which occur early in the progression of this tumor. The highest deletion frequencies were found on chromosome 3p (95%) and 9p (85%) in the invasive tumors . Bearing the aberrant target genes p16 and RASSF1A, the abnormal genetic changes in chromosomes 3p and 9p appear to predispose nasopharyngeal cells to sustain latent EBV infection . Such genetic alterations detected in nasopharyngeal epithelium may even precede EBV infection. EBV infection in premalignant nasopharyngeal epithelium may drive the clonal expansion of genetically altered NP cells, transforming them into malignant cells. A unique feature of this unusual undifferentiated cancer is its universal association with the EBV that exists in a latent form exclusively in the cancer cells and not in the adjacent surrounding tissues .Higher EBV antibody titers, particularly of the IgA class, occur in NPC. Although nasopharyngeal carcinoma cells possess normal antigen processing and are effectively recognized by EBV-specific CTLs, these cells are not damaged. EBV-encoded viral IL-10 is increased in nasopharyngeal carcinoma and has been associated with increased production of IL-1α ?and IL-1ß by epithelial cells and by CD4+ T cells, which may, in turn, contribute to the growth of the tumor and to immune evasion. Over expression of bcl-2 may also play a role in oncogenesis by allowing the cell to bypass apoptosis.

EBV and Hodgkin's disease (HD)

In HD the malignant Hodgkin's and Reed-Sternberg (HRS) cells are EBV genome positive in up to 50% of cases. The transcriptional program of the virus in HRS cells is similar to that seen in NPC in several respects: (a) selective expression of EBNA1 mRNA from the BamHI F promoter; (b) downregulation of the BamHI C and W promoters and their associated EBNA mRNAs; (c) expression of LMP1 and, in most cases, LMP2A and 2B transcripts; and (d) expression of the "rightward-running" BamHI A transcripts once thought to be unique to NPC. This form of latency, consistently detected in EBV-positive HD irrespective of histological subtype, implies an active role for the virus in the pathogenesis of HD and also suggests that the tumor may remain sensitive to at least certain facets of the EBV-induced cytotoxic T cell response.

Epstein-Barr virus and gastric carcinoma

100% of carcinoma cells in EBV positive gastric carcinomas are EBV positive, this suggests that EBV plays an important role in the development of cancer. Of the six types of EBV determined nuclear antigens (EBNAs), only EBNA1 is expressed, and of the three latent membrane proteins (LMPs), LMP1 and LMP2B are not expressed, although LMP2A is expressed in some cases. In addition, the BARF0 gene from the BamHI-A region and the EBERs are always expressed. This EBV gene expression pattern is similar to that of Burkitt's lymphoma. In non-neoplastic gastric mucosa, scattered EBV positive cells are seen in dysplastic mucosa bordering the tumours by means of EBER ISH, but are absent in surrounding lymphocytes, other normal stromal cells, intestinal metaplasia, and normal gastric mucosa. These observations suggest

that EBV infection occurs in the dysplastic phase and that an apparent growth advantage is conferred by the EBV infection.

EBV and Posttransplant lymphoproliferative disorder (PTLD)

PTLD is a well recognized, although relatively uncommon, complication of both solid organ and allogeneic bone marrow transplantation. In most cases, PTLD is associated with Epstein-Barr virus (EBV) infection of B cells, either as a consequence of reactivation of the virus posttransplantation or from primary posttransplantation EBV infection acquired from the donor. While T-cell lymphoproliferative disorders not associated with EBV infection have also been documented after solid organ and bone marrow transplantation, the vast majority are B-cell proliferations.

Almost all lymphoproliferative disease tissue has demonstrated the presence of EBV DNA. Analysis indicates expression of 3 antigens in particular—EBNA-1, EBNA-2, and LMP-1. Two out of these 3 proteins usually are not expressed in other EBV-related malignancies and so are distinguishing features. Of note, the classic 8;14 or 8;22 translocations observed in Burkitt lymphoma are not observed in patients with PTLD.

The immunosuppression required to preserve graft function post transplantation results in impairment of T-cell immunity and allows for uncontrolled proliferation of EBV-infected B cells, resulting in monoclonal or polyclonal plasmacytic hyperplasia, B-cell hyperplasia, B-cell lymphoma, or immunoblastic lymphoma. Immune surveillance is impaired. As discussed above, this outgrowth usually is regulated by cytotoxic T cells and natural killer cells.In the initial stages, the proliferation is polyclonal. With mutation and selective growth, the lesion becomes oligoclonal and, later, monoclonal.

Laboratory Tests for EBV

Name	Purpose
In situ hybridization	Identify EBER transcripts or EBV DNA in specific cell types within histologic lesions. It is the gold standard for detecting and localizing latent EBV in tissue samples. EBER *in situ* hybridization can be accomplished on paraffin sections or on cytology preparations
EBV clonality assay by Southern blot analysis	Assess clonality of lesions with respect to EBV DNA structure; distinguish latent from replicative infection based on the episomal versus linear structure of the EBV genome
EBV DNA amplification	Detect viral DNA in patient tissues; disease specificity is lacking
EBV viral load	Quantitate EBV DNA in blood or body fluids to monitor disease status over time

Name	Purpose
Immunohistochemistry (LMP1, EBNA1, EBNA2, LMP2A, BZLF1)	Identify EBV protein expression in specific cell types within histologic lesions; distinguish latent from replicative infection based on expression profiles
Culture of EBV or of EBV-infected B lymphocytes	Detect and semiquantitatively measure infectious virions or latently-infected B lymphocytes; impractical for routine clinical use
Electron microscopy	Identify whole virions representing replicative viral infection; impractical for routine clinical use
Serology (VCA, EBNA, EA, heterophile antibodies)	Measure antibody response to viral proteins in serum samples; distinguish acute from remote infection; monitor disease status over time. Primary EBV infection is indicated if IgM antibody to the viral capsid antigen is present and antibody to EBV nuclear antigen, or EBNA, is absent. If antibodies to both the viral capsid antigen and EBNA are present, then past infection (from 4 to 6 months to years earlier) is indicated. In the presence of antibodies to EBNA, an elevation of antibodies to early antigen suggests reactivation

EBV vaccines

There is no vaccine to prevent EBV infection and no way for doctors to predict whether an EBV-infected person will develop virus-associated cancer.

Priorities for future research include determining which immune system responses to vaccination correlate with protection from infection or disease, identifying biological markers that would enable clinicians to predict development of EBV-related cancers; and establishing collaboration among government, academic and industry scientists to further improve an experimental EBV vaccine and to spur development of second-generation EBV vaccines

References

1. Rickinson A, Kieff E. Epstein-Barr virus. In: Knipe DM, Howley PM, editors. Fields virology, 4th ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2001. p. 2575–627.
2. Dalla-Favera R, Lombardi L, Pelicci PG, Lanfrancone L, Cesarman E, Neri A. Mechanism of activation and biological role of the c-*myc* oncogene in B-cell lymphomagenesis. Ann NY Acad Sci 1987;511:207–18.
3. Raab-Traub N. Epstein-Barr virus and nasopharyngeal carcinoma.In: Goedert JJ, editor. Infectious causes of cancer: targets for intervention.Totowa, NJ: Humana; 2000. p. 93–111.

4. Miller WE, Cheshire JL, Raab-Traub N. Interaction of tumor necrosis factor receptor-associated factor signaling proteins with the latent membrane protein 1 PXQXT motif is essential for induction of epidermal growth factor receptor expression. Mol Cell Biol 1998;18:2835–44.
5. Wakisaka N, Pagano JS. Epstein-Barr virus induces invasion and metastasis factors. Anticancer Res 2003;23:2133–8.
6. Wakisaka N, Yoshizaki T, Murono S, Furukawa M, Pagano JS. Epstein-Barr virus latent membrane protein-1 induces synthesis of hypoxia-inducible factor-l. Mol Cell Biol 2004;24:5223–34.
7. Zhang L, Pagano JS. Structure and function of interferon regulatory factor 7. Interferon and Cytokine Res 2002;22:95–101.
8. Miller CL, Lee JH, Kieff E, Longnecker R. An integral membrane protein (LMP2) blocks reactivation of Epstein-Barr virus from latency following surface immunoglobulin crosslinking. Proc Natl Acad Sci USA 1994;91:772–6.
9. Dykstra ML, Pierce SK. Epstein-Barr virus coopts lipid rafts to block the signaling and antigen transport functions of the BCR. Immunity 2001;14:57–67.
10. Scholle F, Raab-Traub N. Epstein Barr virus LMP2A transforms epithelial cells, inhibits cell differentiation, and activates Akt. J Virol 2000;74:10681–9.
11. Morrison JA, Raab-Traub N. Epstein-Barr virus latent membrane protein 2A activates beta-catenin signaling in epithelial cells. J Virol 2003;22:12276–84.
12. Shackelford J, Maier C, Pagano JS. Epstein-Barr virus activates catenin in type III latently infected B-lymphocyte lines: association with deubiquitinating enzymes. Proc Natl Acad Sci USA.
13. Deacon EM, Pallesen G, Niedobitek G, Crocker J,Brooks L, Rickinson AB, et al. Epstein-Barr virus and Hodgkin's disease: transcriptional analysis of virus latency in the malignant cells. J Exp Med 1993; 177:339-49.
14. Young LS, Dawson CW, Clark D, Rupani H, Busson P, Tursz T, Johnson A, Rickinson AB. Epstein-Barr virus gene expression in nasopharyngeal carcinoma.Gen Virol. 1988 May;69 (Pt 5):1051-65.
15. Shibata D, Tokunaga M, Uemura Y, *et al*. Association of Epstein-Barr virus with undifferentiated gastric carcinomas with intense lymphoid infiltration. Am J Pathol 1991;139:469–74.
16. Tokunaga M, Uemura Y, Tokudome T, *et al*. Epstein-Barr virus related gastric cancer in Japan: a molecular patho-epidemiological study. Acta Pathol Jpn 1993;43:574–81.
17. Zur Hausen A, Brink AATP, Craanen ME, *et al*. Unique transcription pattern of Epstein-Barr virus (EBV) in EBV-carrying gastric adenocarcinomas: expression of the transforming BARF1 gene. Cancer Res 2000;60:2745–8.

18. Rooney CM, Loftin SK, Holladay MS. Early identification of Epstein-Barr virus-associated post- transplantation lymphoproliferative disease. *Br J Haematol*. Jan 1995;89(1):98-103.

19. Bonnet, M., J. M. Guinebretiere, E. Kremmer, V. Grunewald, E. Benhamou, G. Contesso, and I. Joab. 1999. Detection of Epstein-Barr virus in invasive breast cancers. J. Natl. Cancer Inst. 91:1376–1381.

20. Margaret L. Gulley. Molecular Diagnosis of Epstein-Barr Virus-Related Diseases J Mol Diagn. 2001 February; 3(1): 1–10.

Selected Abstracts- Indian studies

Rao CR, Gutierrez MI, Bhatia K, Fend F, Franklin J .Association of Burkitt's lymphoma with the Epstein-Barr virus in two developing countries. Leuk Lymphoma. 2000 Oct;39(3-4):329-37.

Abstract

The clinical presentation of Burkitt's lymphoma (BL) and it's association with the Epstein-Barr virus (EBV) varies in different geographic areas, BL in developing countries being "intermediate" between the sporadic and endemic types, both in it's clinical presentation and it's association with EBV, which varies from 25-80%. In this study we have analysed the clinical features, EBV association, subtype and prevalence of the deleted variant of the Latent Membrane Protein-1 (LMP-1) of EBV in forty-two cases from two developing countries- India (n = 25) and Argentina (n = 17). In both countries the abdomen was the site most commonly involved while jaw involvement was rare. EBV was detected by in-situ hybridization using the EBER-1 RNA probe. 47% of cases from Argentina and 80% of cases from India were EBER positive. EBV typing using EBNA-3C primers showed a predominance of Type A in both countries (India-13/16 and Argentina-(7/8)). The 30bp deletion of the LMP-1 gene was detected in all evaluated cases from Argentina while the wild type of the gene was seen in all the evaluable Indian cases. Our study highlights the similarities and differences in the clinical presentation and EBV association of BL in two developing countries and also indicates that the subtype of EBV and prevalence of the LMP-1 deletion may reflect the predominant subtype in a particular population

Krishna SM, James S, Kattoor J, Balaram P.Serum EBV DNA as a biomarker in primary nasopharyngeal carcinoma of Indian origin. Jpn J Clin Oncol. 2004 Jun;34(6):307-11.

Abstract

BACKGROUND: Nasopharyngeal carcinoma (NPC) is a unique tumor due to its etiology and endemic distribution. Ethnic and regional factors are found to strongly influence the risk of disease; however, there have been no well-conducted studies on Indian patients. The present study assesses the relationship between Epstein-Barr Virus (EBV) and sporadic Indian NPC and the role of serum EBV DNA in NPC detection.

METHODS: Primers directed against non-polymorphic Epstein-Barr nuclear antigen-1 (EBNA-1) gene were used to detect the presence of EBV DNA from fresh tissue and serum in NPC, using PCR.

RESULTS: EBV DNA was detected in 69% of the biopsies and 58% of the serum of the NPC patients. With respect to histology, WHO Type III NPC, WHO Type II tumors and WHO I tumors showed 100%, 72.2% and 33% EBV positivity, respectively. EBV positivity was also observed in 23% (6/26) of benign samples. All biopsies of patients with positive serum samples were positive for EBV DNA.

CONCLUSION: EBV infection was found in sporadic NPC of South Indian origin, which confirms the etiological role of EBV in NPC. Detection of EBNA-1 in the serum and corresponding tissues of NPC patients suggests that the serum EBV DNA originates from NPC and also indicates the benefit of circulating viral DNA as an early marker in the diagnosis of NPC. Serum DNA-PCR methods can be extrapolated to follow-up studies involving tumor regression or to assess the response to various therapies.

Dinand V, Dawar R, Arya LS, Unni R, Mohanty B, Singh R.Hodgkin's lymphoma in Indian children: prevalence and significance of Epstein-Barr virus detection in Hodgkin's and Reed-Sternberg cells. Eur J Cancer. 2007 Jan; 43(1):161-8.

Abstract

AIM: This study was done to document the prevalence of Epstein-Barr virus (EBV) in Hodgkin's lymphoma (HL) in children of North India.

METHODS: 145 previously untreated children diagnosed with HL from 1991 to 2003 were included. Lymph node (LN) biopsies were studied and classified using World Health Organisation (WHO) classification. EBV detection was done by immunohistochemistry (IHC) and in situ hybridisation (ISH) in 145 cases and 25 age- and sex-matched controls. Patients were treated with chemotherapy alone.

RESULTS: EBV was detected by IHC in 131 (90.3%) cases and by ISH in 126 (93.3%) out of 135 cases, and in none of the controls examined. With IHC and ISH combined, EBV positivity was seen in 96.6% and was significantly associated with younger age (p=0.012) and lower socioeconomic level (p=0.007). EBV status had no implication on treatment response and survival.

CONCLUSION: EBV detection in 96.6% of childhood HL in a population with almost universal EBV seroconversion, and in none of the control lymph nodes, suggests a causative role of EBV in most cases of Indian childhood HL.

Kattoor J, Koriyama C, Akiba S, Itoh T, Ding S, Eizuru Y, Abraham EK et al. Epstein-Barr virus-associated gastric carcinoma in southern India: A comparison with a large-scale Japanese series. J Med Virol. 2002 Nov;68(3):384-9.

Abstract

Epidemiological and clinicopathological features of Epstein-Barr virus (EBV) associated gastric carcinoma was compared in India and Japan, two countries differing markedly in gastric cancer incidence. Using in situ hybridization assay, the presence of EBV-encoded small RNA

(EBER) was examined in 215, and 2,011 gastric cancer cases in Kerala, India, and Japan, respectively. Ten cases (5%), all males, in the Indian series were EBER-positive. This frequency was similar to that in the Japanese series (6.2%). As was the case with Japanese series, the EBV-associated gastric carcinoma in the Indian series was observed most frequently in the middle part of the stomach (1 in antrum, 4 in middle part, 2 in cardia, and 3 unknown), and, histologically, the diffuse type Lauren's classification (8 cases) was more common than the intestinal type (2 cases). Virus subtyping by PCR-RFLP revealed that all of the 10 EBV strains isolated from the EBER-positive Indian cases were subtype A, and wild-type F for Bam HI F region. In Bam HI I region, 8 cases were type C and the remaining 2 cases were type D. In either series, there was no significant difference in the frequency of tumors with p53 overexpression between EBER-positive and -negative cases. However, the proportion of cells with p53 overexpression in EBER-negative tumors was significantly higher than that in EBER-positive tumors regardless of histological type in both series. In conclusion, the frequency and major clinicopathological features of EBV-associated gastric carcinoma in south India were similar to those observed in Japanese series although gastric cancer incidence in these two countries differs markedly.

Human Herpes Virus Type 8 (Kaposi's sarcoma herpes virus)

In 1981, the emergence of Kaposi sarcoma (KS) among young gay men in New York, Los Angeles, and San Francisco heralded the beginning of the AIDS pandemic. In 1994 Chang, and Moore co discovered Human herpes virus 8 from lesions of KS patients by representational differential analysis . HHV-8 is now considered to be the etiological agent of all the clinico-epidemiological forms of KS (including AIDS KS, classic KS, endemic KS, and iatrogenic KS), primary effusion lymphoma(PEL), body cavity-based lymphoma, and multicentric Castleman's disease(MCD).

More than 95% of all KS lesions, regardless of type, contain KSHV viral DNA , 100% of PELs are KSHV-positive. Nearly 100% of AIDS-associated MCD is positive for the presence of KSHV. Approximately 50% of non-AIDS associated MCD contains KSHV viral DNA. This strong molecular epidemiological link associating KSHV with KS, PEL and MCD suggests that KSHV is necessary for the development of these malignancies. However, it is not entirely clear whether KSHV alone is sufficient for development of these neoplasms, since co-factors such as HIV co-infection and immunosuppression often play a contributory role in the induction of disease.

Ablashi et al studied the seroprevalence of HHV-8 has been studied in Malaysia, India, Sri Lanka, Thailand, Trinidad, Jamaica and the USA, in both healthy individuals and those infected with HIV. Seroprevalence was found to be low in these countries in both the healthy and the HIV-infected populations. This correlates with the fact that there are only few reported cases of AIDS-related Kaposi's sarcoma in India.

In endemic areas where HHV-8 seroprevalence is high during childhood and adolescence, viral transmission might occur through nonsexual contact. Several studies have demonstrated that saliva is the principal reservoir for HHV-8, whereas the viral load of HHV-8 is consistently

lower in peripheral blood, secretions from genital sites, and semen. In non-endemic countries, heterosexual transmission is probably not frequent. In contrast, sexual transmission is more common among men who have sex with men in non-endemic countries .

HHV8/KSHV is a gammaherpesvirus in the Rhadinovirus genus The genome is a linear, double-stranded DNA of about 165 to 170 kilobases in length. It may also exist in a circular episomal form during latency. Characteristic for HHV-8 is the high homology of several viral and cellular genes suggesting viral genes were pirated from host chromosomes during viral evolution.

HHV8 life cycle

KSHV has two major modes of replication. In the lytic phase, entry, uncoating, and nuclear import are followed by a coordinated sequence of viral transcription, DNA replication, and assembly, followed by the final release of nascent virions. KSHV can also undergo a "latent" life cycle where only a small subset of viral genes is expressed. In latent phase , after entry and translocation to the nucleus, the viral DNA circularizes, and multiple copies are maintained as episomes attached to the host chromosome via the viral latency associated nuclear antigen (LANA-1). Viral genomes are then replicated at roughly the same rate as the host chromosome, such that each daughter cell receives several copies of the viral genome at cell division.

HHV-8 is present in a latent form in KS spindle cells and lesional endothelial cells but yields a lytic infection in lymphocytes and monocytes infiltrating KS lesions. In addition, HHV-8 can infect circulating B cells, monocytes/macrophages, T cells, and KS-like spindle cell progenitors that are increased in number in the blood of patients with all forms of KS. It has been shown that the episomal viral DNA is tethered to metaphase chromosomes and copied in tandem with host cell DNA during cell division . Latent viral specific genes well demonstrated in infected KS SC are the latent nuclear antigen (LANA-1), viral cyclin (v-cyclin), v-FLIP and kaposin a small membrane protein. Lytic virus expression is most frequent in MCD, moderate in KS and relatively rare in PEL cells. Common viral genes found during lytic expression include K1 transmembrane protein, v-GPCR, v-IRF, v-IL-6 and v-MIP .

LANA-1 protein is considered important in the generation and maintenance of HHV-8 associated malignancies by its cell cycle regulation .It competes with E2F for binding of hypo phosphorylated pRb thus freeing E2F to activate gene transcription involved in cell cycle progression. LANA-1 interacts with p53, repressing its gene transcriptional activity and ability to induce apoptosis. This allows latent HHV-8 to promote cell cycle progression whilst inhibiting apoptosis . V*iral cyclin D(v cyclin)* overrides host cell-cycle growth arrest imposed by cyclin-dependent kinases and pRb .*vFLIP* protects cells latently infected with HHV-8 from apoptosis by preventing the activation of the Fas death receptor pathway and thereby blocking the killing of infected cells by cytotoxic T-cell surveillance. *Kaposin* causes transformation (Kaposin A) and cytokine and AU-rich mRNA stabilization by induction of p38 or MK2 signaling(Kaposin B).

VIP causes transformation; B cell activation; inhibition of apoptosis; downregulation of surface B cell receptor (BCR); and activation of PI3 K/Akt/mTOR kinases. K2(vIL-6) B cell proliferation; and autocrine/paracrine signaling. *vIRF-1* causes inhibition of type I interferon, p300, p53, and TGF-b; and transformation. vMIP-I has a role in angiogenesis; CCR5 and CCR8 binding; and chemoattraction of TH2 cells (immune modulation).

The two main immune evasion strategies used by KSHV are the establishment of latency and the expression of immunomodulatory genes. KSHV encodes a number of proteins that actively hinder the innate and adaptive antiviral responses. The HHV-8 infected cells escape immune response targeting by down regulation of surface MHC mediated by two transmembrane proteins, MIR1 and MIR2. Downregulation of MHC poses the risk of initiating a natural killer (NK) cell response by initiating apoptosis in cells lacking appropriate MHC I expression. HHV-8 can inhibit NK mediated killing through expression of the anti apoptotic v-FLICE-inhibitory proteins (v-FLIPs).

Kaposi's sarcoma(KS)

Kaposi's sarcoma (KS) is a malignant mesenchymal tumour involving blood and lymphatic vessels of multifactorial origin Viral oncogenesis by HHV8 and cytokine-induced growth together with some state of immunocompromise represent important conditions for this tumour to develop. The most frequent manifestation of the disease is skin lesions but mucous membranes, lymphatic system and viscera particularly the lung and gastrointestinal tract can be involved.

Initial histologic studies suggested that SC were of lymphatic endothelial cell (LEC) origin. Another possibility is that KS spindle cell originates from an uncommitted endothelial progenitor cell (EPC) defined as being CD34+ and VEGFR2+. In addition to plasma virus, KSHV has been detected in peripheral blood mononuclear cells (PBMC) such as B cells, T cells and monocytes of persons with KS . Thus, precursor SC may be primary targets or could be infected following contact with PBMC. SC do not behave like typical cancer cells . After initial KSHV infection, the development of KS depends upon the generation of a unique local microenvironment. This involves virus infection, proliferation and migration of infected endothelial cells, induction of a pro-inflammatory response and finally tumor formation. **C**urrent data suggest that the KS tumor microenvironment must play a critical role in maintaining the KSHV trans formed spindle cell in vivo.

Because of KSHV's tendency towards latent gene expression and its ability to induce tumors in infected individuals, a great deal of focus has been placed upon the latent gene products as likely mediators of tumorigenesis(LANA1 , vCyc, vFLIP, Kaposin A and miRNAs .Whille KSHV latent genes are essential for genome maintenance, lytic genes probably play an important role in driving tumorigenesis via direct as well as paracrine mechanisms (Vgpcr,K1,vIRF1). KSHV encodes many proteins that individually have the ability to immortalize/transform cells in vitro and invivo , but how they all work together to perform this function in the context of a normal

viral infection remains obscure. In addition to expressing its own potential oncogenes, KSHV induces the expression of a variety of cellular genes with transforming abilities(c Kit, CXCR7).

Many aspects of KS suggest that chronic inflammation associated with the lesion and/or viral infection plays a role in tumor pathogenesis. This is exemplified by the association of KS with the Koebner (or isomorphic) phenomenon(KP). KSHV encodes three chemokine ligands, vCCL-1 (K6),vCCL-2 (K4) and vCCL-3 (K4.1) that share homology with the cellular macrophage infammatory protein MIP.The pathogenesis of KS is still unclear and involves various mechanisms dependent on both viral and cellular activities related to inflammation and also angiogenesis promoted by endothelial growth factors (β-FGF, PDGF, VEGF) including HIV-Tat as well as cell proliferation and anti-apoptosis (vBCL2).

Most of the pro-inflammatory cytokines and angiogenic factors produced or induced by KSHV have likely evolved to create a highly proliferative environment that favors viral genome maintenance as well as the consistent, yet low level of reactivation from latency. Both lytic and latent genes are responsible, suggesting that both autocrine and paracrine mechanisms contribute to the microenvironment. In addition to virally induced factors, the persistent viral infection attracts a chronic leukocyte infiltrate, which in turn secretes cytokines, chemokines, enzymes, and growth factors that favor the growth of infected cells and contribute to KS progression.HIV is a co-factor for development of malignancy since the prevalence of KS in AIDS patients is unusually high. Immunosuppressive therapy is also a co-factor for the iatrogenic form of KS.

Primary effusion lymphoma(PEL)

PEL is a rare subset of AIDS non-Hodgkin's lymphoma predominantly growing in the pleural, pericardial, and peritoneal cavities as neoplastic effusions, usually without a contiguous tumor mass. The immunophenotypic and immunogenotypic characteristics of PEL suggest that this lymphoma represents the malignant counterpart of a B-cell that has reached a mature stage of development and is shifting toward terminal plasma cell differentiation. Recently, KSHV/HHV8 has been detectedby immunohistochemistry and/or polymerase chain reaction even in lymphoma cases presenting as tissue masses. These KSHV/HHV8-associated lymphomas,also called "solid PEL", have been reported prior the development of an effusion lymphoma and/or following resolution of PEL.

The exact mechanism by which HHV-8 promotes oncogenesis in PEL is an area of active investigation. HHV-8 genomes exist in PEL cells as mono- or oligoclonal episomes. Most infected cells express a latent pattern of gene expression, while a very small percentage expresses genes characteristic of the lytic phase . Even with the expression of latent genes, infected cells can undergo clonal expansion, eventually leading to neoplastic transformation through mechanisms of increased proliferation and impaired apoptosis. Three latent gene products that are thought to play significant roles are latency-associated nuclear antigen-1 (LANA-1), viral cyclin (v-Cyc),

and viral FLICE inhibitory protein (vFLIP). While the majority of cases of PEL show evidence of infection with EBV in addition to HHV-8, EBV plays an unclear role in PEL oncogenesis.

Detection of HHV8

The LANA-1 antigen is well detectable by imunohistochemistry also in routinely formalin fixed paraffin embedded biopsies. It is expressed by most SC in both early and late stage lesions of all different clinical KS forms (AKS,EKS, CKS and IKS) and therefore used as a diagnostic marker in suspected HHV-8 related lesions and also for serology of LANA-1 antibodies in patients by immunocytochemistry that gives a characteristic speckled nuclear staining on HHV-8 infected BCBL cells. Several studies have shown an increase in LANA-1 positive cells during progression of KS lesions allowing quantification and phenotyping of these cells in KS lesions.

Vaccines

There are no vaccines available for KSHV and research in this area has not been very active likely due to the lack of a suitable animal model and to the efficacy of HAART in dramatically lowering the incidence of KS.

References

1. Antman K, Chang Y. Kaposi's sarcoma. N Engl J Med 2000;342:1027–38.
2. Nador RG, Cesarman E, Chadburn A, Dawson DB, Ansari MQ, Sald J, et al. Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi's sarcoma-associated herpes virus.Blood 1996;88:645–56.
3. Ablashi DV, Chatlynne LG, Whitman Jr JE, Cesarman E. Spectrum of Kaposi's sarcoma-associated herpesvirus, or human herpesvirus 8, diseases. Clin Microbiol Rev 2002;15:439–64.
4. Boshoff C, Weiss RA. Epidemiology and pathogenesis of Kaposi's sarcoma-associated herpesvirus. Philos Trans R Soc Lond B Biol Sci 2001;356:517–34.
5. Moore PS, Chang Y. Kaposi's sarcoma-associated herpesvirus immunoevasion and tumorigenesis: two sides of the same coin? Annu Rev Microbiol 2003;57:609–39.
6. Kliche S, Kremmer E, Hammerschmidt W, Koszinowski U, Haas J. Persistent infection of Epstein-Barr virus-positive B lymphocytes by human herpesvirus 8. J Virol 1998;72:8143–9.
7. Flore O, Rafii S, Ely S, O'Leary JJ, Hyjek EM, Cesarman E. Transformation of primary human endothelial cells by Kaposi's sarcoma-associated herpesvirus. Nature 1998;394:588–92.
8. Damania B. Modulation of cell signaling pathways by Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8). Cell Biochem Biophys 2004;40:1–18.
9. Damania B. Gammaherpesviruses of non-human primates. The human herpesviruses: biology, therapy and immunoprophylaxis. Cambridge University; 2004; in press.

10. Wang L, Wakisaka N, Tomlinson CC, DeWire S, Krall S, Pagano JS, et al. The Kaposi's sarcoma-associated herpesvirus (KSHV/HHV8) K1 protein induces expression of angiogenic and invasion factors. Cancer Res 2004; 64.
11. Cesarman E, Mesri EA, Gershengorn MC. Viral G protein-coupled receptor and Kaposi's sarcoma: a model of paracrine neoplasia? J Exp Med 2000;191:417–22.
12. Nicholas J. Human herpesvirus-8-encoded signalling ligands and receptors. J Biomed Sci 2003;10(5):475–89.
13. Pagano JS, Blaser M, Buendia MA, *et al.* (December 2004). "Infectious agents and cancer: criteria for a causal relation". *Semin. Cancer Biol.* 14 (6): 453–71.
14. Ablashi D, Chatlynne L, Cooper H, Thomas D, Yadav M. Seroprevalence of HHV-8 in countries of south East Asia compared to USA, the Caribbean and Africa. Br J Cancer 1999;81:893-7

Selected Abstract – Indian study

Ablashi D, Chatlynne L, Cooper H, Thomas D, Yadav M, Norhanom AW et al Seroprevalence of human herpesvirus-8 (HHV-8) in countries of Southeast Asia compared to the USA, the Caribbean and Africa. Br J Cancer. 1999 Nov;81(5):893-7.

Abstract

Seroprevalence of HHV-8 has been studied in Malaysia, India, Sri Lanka, Thailand, Trinidad, Jamaica and the USA, in both healthy individuals and those infected with HIV. Seroprevalence was found to be low in these countries in both the healthy and the HIV-infected populations. This correlates with the fact that hardly any AIDS-related Kaposi's sarcoma has been reported in these countries. In contrast, the African countries of Ghana, Uganda and Zambia showed high seroprevalences in both healthy and HIV-infected populations. This suggests that human herpes virus-8 (HHV-8) may be either a recently introduced virus or one that has extremely low infectivity. Nasopharyngeal and oral carcinoma patients from Malaysia, Hong Kong and Sri Lanka who have very high EBV titres show that only 3/82 (3.7%) have antibody to HHV-8, demonstrating that there is little, if any, cross-reactivity between antibodies to these two gamma viruses

Hepatitis B Virus, Hepatitis C Virus and Hepatocellular Carcinoma

I. Introduction

Chronic viral hepatitis caused by the hepatitis B and C viruses is the commonest cause of chronic liver disease and liver cancer worldwide. We present a review of evidence of association and causation between HBV, HCV and HCC, and the effect of treatment of these infections on incidence of HCC.

II. Epidemiology

Hepatitis B virus (HBV) infection is seen in 2.4 % of the non-tribal population in our country [1]. Of the estimated 350 million HBV carriers worldwide, over 28 million are in India. It has been estimated that, of the 25 million infants born every year in India, over one million run the lifetime risk of developing chronic HBV infection [2]. Approximately 50% of HCC patients are infected with HBV worldwide. This increases to 75-80% in endemic countries in Asia and Africa. In India, HBV infection in patients with HCC varies between 36-74% (mean 47%) [3]. In most population studies there is a male preponderance with male: female ratio between 3:1 and 2:1.The incidence of HCC due to HBV related liver disease peaks in 7th decade suggesting that viral transmission in these individuals is probably horizontal. In most Asian countries there is a fall in incidence of HCC over the last 3 decades whereas in India there is a small increase in incidence in both males and females [3].

The estimated global prevalence of **Hepatitis C virus (HCV) infection** is 2.2%, corresponding to about 130 million HCV-positive people worldwide [4]. Studies conducted on voluntary blood donors estimate the prevalence of chronic HCV infection between 0.3-1.8%, while the prevalence could be as high as 50-80% in professional blood donors [5]. A community-based survey reported an overall rate of HCV infection of 0·9% in India [6].HCV infection is

responsible for 25-75% of HCC in Western Europe and 80-90% in Japan. In the United States, between 1993-1998, age adjusted incidence rates of HCV related HCC increased by 3 fold from 2.3 to 7 per 100,000. There was a significant trend towards younger age at presentation [7]. In India, 5-30% patients with HCC have HCV infection [3]. HCC develops at an annual rate of 0-3% after 30 years and at 1-4% per year in patients with HCV related cirrhosis.

Liver cancer is one of the leading causes of cancer related deaths worldwide. The incidence of Hepatocellular cancer (HCC) equals mortality in most parts of the world because patients with HCC frequently have underlying advanced chronic liver disease (CLD) and advanced HCC. An estimated 748,300 new liver cancer cases and 695,900 cancer deaths occurred worldwide in 2008 [8]. Chronic liver disease caused by infection with HBV and HCV constitutes over 75 % of the attributable risk for HCC [9]. Over the last three decades, the incidence of HCC is decreasing in high incidence countries (e.g. China, Taiwan, Korea, etc) following universal HBV vaccination and decrease in aflatoxin exposure, while the incidence of HCC is rising in most developed countries (USA, Europe, Australia etc), possibly secondary to increase in prevalence of HCV infection [10]. India has one of the lowest incidence rates of HCC in the world with an Age standardized rate (ASR) of 2.3/ 100,000 population in males and 1.1/100,000 population in females which translates into approximately 20,000 cases of HCC every year [11].

III. Risk of HCC in HBV infection

Many studies have shown the increased risk of developing HCC in HBV infection. In the western countries, HCC occurs most often in HBV carriers in the setting of cirrhosis, but in Africa and South-East Asia, where the HBV infection is acquired early in life and coincides with other oncogenic agents like aflatoxins, HCC may develop more frequently in a non-cirrhotic liver [12]. HCC develops at the rate of 2% per year in HBV cirrhotics and between 0.4-0.6% per year in chronic carriers without cirrhosis [12].

The relative risk (RR) of HCC in HBV infection ranges from 5-49 in case control studies and 7-98 in cohort studies [13]. In a meta-analysis of 32 case-control studies, the pooled odds ratio (OR) and 95% confidence interval (CI) for HBsAg positivity and anti-HCV/HCV RNA negativity were 15.6 (95% CI: 11.5-21.3); for HBsAg negativity and anti-HCV/HCV RNA positivity were 8.1 (95% CI: 5.0-13.0); and positivity for both HBsAg and anti-HCV/HCV RNA was 35.7 (95% CI: 26.2-48.5) [14].In an Indian case-control study, ORs and 95% CI of HCC were 48.02 (25.06-91.98) for any HBV marker, 38.98 (19.55-77.71) for HBsAg positivity, 12.34 (2.84-53.61) for HBsAg negative and antibody positive (either of anti-HBe or total anti-HBc), 5.45 (2.02-14.71) for anti-HCV positive and HCV RNA positive. An Indian study also indicated this increased risk amongst HBV infected cases compared to non infected controls with OR of 40 (95% CI 19.6-77.7) [15].

Factors associated with increased risk of HCC in HBV infected persons include demographic, lifestyle, virological, environmental and clinical factors [13]. Certain ethnic groups like Asians, Africans, and Yupik Eskimos with chronic HBV infection are at a higher risk of developing HCC.

HBV genotype, core promoter and X gene mutations and HBV replication status are other high risk features. Genotypes B and C are found to be associated with high levels of pre-core and core promoter mutations and are associated with an increased risk. So also, elevated Alfa-fetoprotein levels are associated with an increased risk for HCC. The risk factors are summarized in Table 1.

Table 1: Risk factors for HCC in patients with chronic HBV infection

Risk factor	Relative risk / Hazards Ratio (95% CI)	Reference
Older age	Age 40–49 yr: 4.39-5.4 Age 50–59 yr: 9.22-13.5 Age >60 yr: 9.63-17.7 Age in 1-y increment 1.09 (1.07-1.11)	Yang et al [16] Wang et al [17] Chen et al [18]
Male sex	2.1 (1.3-3.3)	Chen et al [18]
Diabetes mellitus	2.27 (1.10-4.66)	Chen et al [19]
Obesity and Diabetes mellitus	>100	Chen et al [19]
Cigarette smoking	8.9 (5.1–15.5) 1.55 (1.06–2.26) >25 years of smoking: 1.91 (1.25–2.91)	Yang et al [16] Wang et al [17]
Alcohol consumption	11.4 (5.0–26.3) 1.46 (0.97–2.21) >20 years duration of alcohol consumption: 1.56 (0.95–2.55) 1.6 (1.1-2.4)	Yang et al [16] Wang et al [17] Chen et al [18]
Abnormal LFT	AST > 30 IU/L or ALT > 35 IU/L: 3.15 (2.13–4.67) ALT >45: 4.1 (2.8-6.0)	Wang et al [17] Chen et al [18]
HBV DNA	>13.0 pg/ml: 6.0 (1.7–21.4) 10,000-99,999: 2.3 (1.1-4.9) 100,000-999,999: 6.6 (3.3-13.1) >1 million: 6.1 (2.9-12.7)	Yang et al [16] Chen et al [18]
HBV genotype	Genotype C vs. others: 5.11 (3.20-8.18) 5.97 (3.44-10.34)	Yu et al [23] Wu et al [24]
Co-infection with HCV	35.7 (26.2-48.5)	Shi et al [14]
Aflatoxin exposure	59.4 (16.6–212.0)	Yu et al [20]

The possible mechanisms of HBV-Induced HCC include direct factors like integration of HBV DNA into chromosomes of hepatocytes and the HBx protein which is a transcriptional activator which activates the Ras-Raf-MAPK pathway and interacts with p53, a tumor suppressor. Indirect mechanisms include inûammation and regeneration associated with chronic HBV infection and via cirrhosis associated with chronic HBV infection [21, 22].

- Level of evidence 2

IV. Risk of HCC in HCV infection

In patients with hepatitis C virus (HCV) infection the increased risk of HCC appears to coincide with the establishment of cirrhosis, when the yearly incidence varies between 3 and 8% [12.] HCV increases the risk for HCC probably by promoting ûbrosis and cirrhosis; virtually all HCV-related HCC cases occur among patients with cirrhosis [25]. Once cirrhosis develops, old age, male sex and severity of underlying cirrhosis are associated with a higher risk of HCC.

A meta-analysis of 32 case control studies showed an increased risk of HCC with an OR of 11.5 for anti-HCV/HCV RNA positivity [26]. The OR for anti-HCV was lower among studies using second- or third-generation anti-HCV or HCV RNA (OR: 8.2) with respect to studies with first-generation anti-HCV test (OR: 19.1). When combining data from the studies with second- or third-generation anti-HCV or HCV RNA, the OR for anti-HCV/HCV RNA positivity and HBsAg negativity was 17.3 (95% CI, 13.9-21.6), and the OR for both markers positivity was 165 (95% CI: 81.2-374). In a systematic review of 21 papers looking at prospective HCV infected cohorts in the general population, time to development of HCC was between 17-31 years. The mode of HCV acquisition was a strong predictor of development of cirrhosis and cancer [27].

Another systematic review of the literature included 21 published studies in which chronic HCV infection was defined by elevation of liver enzymes or persistent RNA and excluded studies in which cohorts were selected from patients with prevalent liver disease or in whom the onset of infection could not be estimated [28]. This review found a large variation in the incidence estimates of cirrhosis and HCC. The studies varied in sample size (17-1,680), duration of follow-up (8-45 yr), total person-years (157-34,098), and the mean age at onset of HCV (5-58 yr). The mean time to end stage liver disease (ESLD) was 4-23 yr and to HCC was 9-31 yr. There was a possible publication bias against studies with low incidence of ESLD and HCC. The pooled weighted incidence rates for ESLD and HCC based on infection mode were as follows: community-acquired HCV, 1.9 and 0 per 1,000 person-years; transfusion associated, 4.5 and 0.7; hemophilia patients, 7.9 and 1.0.

In HCV-infected patients, factors related to the host or environment or both appear to be more important than viral factors in determining the progression of HCV infection to cirrhosis and hence increased risk of HCC [25]. These factors include older age, male sex, co infection with HBV, alcohol intake, older age at the time of acquisition of infection, transfusion-related mode of acquisition of HCV infection and obesity with or without diabetes. Diabetes is a risk factor

for nonalcoholic fatty liver disease, which may progress to cirrhosis in up to 5% of cases. Obesity, which frequently accompanies diabetes, has been reported to be associated with an increased risk for hepatic steatosis and fibrosis in HCV-infected patients. The role of the duration and treatment of diabetes and diet in modifying this risk are still unclear. HIV co-infection has not shown any significant and consistent increased risk of HCC.

The risk factors are summarized in Table 2. There is insufficient evidence that HCV viral factors, such as viral genotype, viral load, or quasi-species determine the risk for progression to cirrhosis or HCC. All HCV genotypes have been described in HCV-related HCC. There are conûicting data as to whether genotype 1 is a risk factor for cirrhosis or HCC independent of older age [25].

Further well designed studies are required to probe these associations. Studies evaluating polymorphisms of metabolic enzymes such as CYP (cytochrome P450), NAT (N-acetyltransferase), GST (glutathione-S-transferase), and ALDH2 (aldehyde dehydrogenase), have largely been equivocal.

- Level of evidence 2

Table 2: Risk factors for HCC in patients with chronic HCV infection

Risk factor	Relative risk / Hazards Ratio (95% CI)	Reference
Older age	Age in 1-y increment: 1.05 Age > 60 yrs: 2.06 (1.00-4.23)	Degos et al [30] Chiba et al [31]
Male sex	2.13 4.20 (1.80-9.78)	Degos et al [30] Chiba et al [31]
Co-infection with HBV	165 (81.2-374)	Donato et al [26]
Diabetes mellitus	3.52 (1.29-9.24)	Chen et al [19]
Obesity	Body mass index e" 30 kg/m^2: 4.13 (1.38-12.4)	Chen et al [19]
Obesity and Diabetes	>100	Chen et al [19]
Alcohol consumption	0-40 g/day: 26.1 (12.6-54.0) 41-80 g/day: 62.6 (23.3-168) >80 g/day: 126 (42.8-373) Habitual heavy drinkers: 3.27 (1.46-7.30)	Tagger et al [29] Chiba et al [31]

V. Prevention of HCC

Va) Vaccination

Primary prevention of HCC is facilitated by the anti HBV vaccine. A vaccine against hepatitis B has been available since 1982. Hepatitis B vaccine is 95% effective in preventing HBV infection

and its chronic consequences like liver cirrhosis and HCC, and is the first vaccine against a major human cancer [32]. The duration of protection is at least 15 years. Routine vaccination of all infants against HBV infection was recommended by the World Health Organization (WHO) in 1992 and is now an integral part of national immunization schedules in 177 countries. In many countries where 8-15% of children used to become chronically infected with HBV, vaccination has dramatically reduced the rate of chronic infection to less than 1% among immunized children.

The results of universal vaccination have been seen in Taiwan, where, since the institution of universal hepatitis B vaccination in July 1984, the average annual incidence of HCC in children 6 to 14 years of age declined from 0.70 per 100,000 children between 1981-1986 to 0.57 between 1986-1990, and to 0.36 between 1990-1994 with a decrease in the corresponding rates of mortality from HCC [33]. In a subsequent study from Taiwan, vaccine failure and failure to receive hepatitis B immunoglobulin at birth were found to be the main problems preventing eradication of HCC [34].

Similar results have been seen in Malaysia where HBV vaccination was implemented in 1989 with a dramatic impact on hepatitis B virus (HBV) infection in school children. A cross-sectional sero-prevalence study of HBV infection in 190,077 school children aged 7-12 years from 1997-2003 showed a steady decline of HBV surface antigen (HBsAg) prevalence rate from 2.5% for children born in 1985 to 0.4% among school children born in 1996 [35].

In Singapore, universal HBV vaccination was introduced in 1987 and the incidence of acute hepatitis B declined from 10.4/100,000 in 1985 to 4.8/ 100,000 in 1996. There was a noticeable reduction in HBsAg prevalence in selected populations (school children, national servicemen and antenatal women). The ASR of primary liver cancer among males had also dropped from 27.8/100,000 per year during 1978-1982 to 19.0/100,000 per year during 1988-1992 [36].

Reduction in HCC rates is difficult to demonstrate at present since it may occur after a few decades. However in countries with high rates of HCC, the incidence in younger persons is expected to fall. In Alaska, a statewide hepatitis B virus immunization program was started in late 1982. In a study on childhood cancers in Alaska natives, although 16 children born before 1983 developed HCC, no child who was born in the 20 years since hepatitis B immunization was instituted among infants has received a diagnosis of HCC, a significant difference [37]. Data from a study from Thailand also showed similar results [38].

- Level of evidence 2

Vb) Treatment of HBV:

Antiviral therapy in pre-cirrhotic patients with chronic Hepatitis B infection can suppress the viral load, improve serum aminotransferase levels, and reduce hepatic necro-inflammation thereby slowing the progression of fibrosis and thus reducing the risk of progression to cirrhosis and HCC and ultimately improve survival.**Interferon (IFN) treatment:**

In patients with HBeAg positive chronic hepatitis, IFN treatment leads to e antigen seroconversion, normalization of liver enzymes, effective suppression of HBV DNA and rarely loss of HBsAg. In a case-control study, multivariate analysis showed that IFN treatment, HBeAg loss and genotype B were independently associated with reduced incidence of HCC in HBeAg positive chronic hepatitis [39]. Compared with untreated controls with persistent HBeAg, HBeAg seroconverters in untreated and IFN-treated group showed significantly lower incidence of cirrhosis and HCC.

A meta-analysis published in 2001 of 3 studies comparing treated patients versus untreated controls had shown that IFN does not seem to affect the rate of HCC in HBV-related compensated cirrhosis [40]. Another trial also showed that in patients with hepatic cirrhosis secondary to HBV infection, the risk of HCC did not seem to be modified by alpha-interferon treatment, even though a greater, but not significant risk (RR) of 4.9 (p=0.3) was calculated for untreated patients [41].

However, in contrast, studies from Asia have demonstrated a diminished incidence of HCC with Interferon treatment. One study on patients with HBV related cirrhosis showed that early intervention and prophylaxis with lymphoblastoid IFN-alpha can reduce the incidence of HCC in these high-risk persons wherein none of the treated group consisting of 518 cirrhotic patients, 82 male relatives of HCC patients and 20 post-resection cases developed HCC [42]. Another study from Japan and a small randomized controlled trial in Taiwanese men also showed that Interferon therapy for patients with HBV-related cirrhosis significantly decreased the HCC rate [43, 44].

A recent meta-analysis of 12 studies (n = 2742) enrolling patients treated by IFN vs. control showed that the risk of HCC after treatment was reduced by 34% (RR: 0.66, 95% CI: 0.48-0.89). Patients with early cirrhosis benefited more than those without cirrhosis [45]. However, among these 12 studies using IFN, only one was a randomized controlled trial (RCT), one was case–control study and 10 were cohort studies.

Thus, interferon treatment may reduce development of HCC in HBV infected patients. However the short follow up period, the heterogeneous population and the different types and doses of IFN and duration of treatment used in various studies are a problem in interpreting the results. Overall, the evidence shows a trend towards efficacy of treatment of HBV with IFN for prevention of HCC but is not convincing at present.

- Level of evidence 2

Treatment with oral nucleotide/side analogs (NA):

The other antivirals used in the treatment of chronic HBV infection are various nucleotide and nucleoside analogues. Lamivudine is a cytidine analogue, which inhibits HBV replication, improves liver enzymes and inflammatory scores and arrests progression to fibrosis [46]. It is well tolerated with an acceptable safety profile. However its prolonged use is associated with

the development of mutants which may adversely affect outcomes in the long run and the high rate of lamivudine resistance which could reactivate HBV DNA replication.

A retrospective study in 377 Japanese patients comparing patients treated with lamivudine to the same number of untreated HBV infected controls showed a beneficial effect of lamivudine on the development of HCC. HCC occurred in 1.1% of patients with an annual incidence of 0.4% (patient/year) in the treated group and in 13.3% of patients with an annual incidence of 2.5% (patient/year) in the untreated group (P< 0.001) [47].

A prospective multicenter double blinded RCT in 651 patients with chronic hepatitis B and cirrhosis or advanced fibrosis which used HCC as one of the endpoints showed that continuous treatment with lamivudine delays clinical progression in these patients by significantly reducing the incidence of hepatic decompensation and the risk of HCC [48]. The study was terminated after a median duration of treatment of 32.4 months because of a significant difference between the treated groups in the number of endpoints reached. HCC occurred in 3.9% in the lamivudine group and 7.4% in the placebo group (Hazard ratio, 0.49; P = 0.047). The emergence of YMDD mutations reduced the benefit of lamivudine but did not negate it, despite the occurrence of more end points due to decompensation among patients with YMDD mutations than among those without the mutations confirming the role of HBV DNA suppression.

In another large Italian retrospective multicenter study on patients with HBeAg-negative chronic hepatitis treated with Lamivudine, patients who had cirrhosis and who maintained virological response were less likely than those with viral breakthrough to develop HCC and disease worsening [49]. In patients with chronic HBV without cirrhosis, another case control study showed significantly higher rate of HCC in untreated patients than those treated with lamivudine without resistance (p = 0.03). Although appearance of YMDD mutants reduced the benefits from lamivudine therapy, the outcome of these patients was still better than untreated patients [50].

A meta- analysis of 5 studies (n = 2289) evaluating the impact of oral NA treatment showed a reduced risk of HCC by 78% in those treated with lamivudine compared to untreated individuals (RR: 0.22, 95% CI: 0.10-0.50) [45]. Patients who were HBeAg-positive and those without cirrhosis had a more significantly reduced risk. Among these 5 studies, there were two RCTs, one case–control study and two cohort studies. NA treatment demonstrated a more profound reduction in HCC risk of 78% compared to IFN which produced only a modest effect of 34%. The more effective reduction in HCC risk may be related to the more profound effects of viral suppression of oral anti-viral agents than IFN. Also there were a limited number of studies with significant heterogeneity in the studies included.

NA treatment has also been evaluated in the post-operative setting following resection of HCC. In a prospective cohort study of patients with HCC treated by liver resection, post-operative treatment with antiviral therapy significantly reduced the 5 year recurrence rates in

the non viremic group (54.7%) compared to the viremic group (72.9%). In multivariate analysis, sustained viraemia increased recurrence independently after surgery. To prevent long-term recurrences, antiviral therapy should be initiated in those with detectable serum HBV DNA [51]. A recent meta-analysis of 6 studies showed that postoperative antiviral therapy, interferon in particular, may serve as a favorable alternative to reduce recurrence and mortality in patients with HBV related HCC [52].

The advent of newer and more efficacious drugs like Adefovir, Entecavir, Telbivudine, Tenofovir, Emtricitabine etc. for the treatment of chronic HBV infection, may offer more opportunities in this setting. Ongoing studies using newer agents may consolidate the role of NA treatment of chronic HBV infection for prevention of HCC. Although antiviral drugs may play a role in reducing the risk of HCC by suppressing viral replication and thereby the progression of the underlying liver disease, the development of HCC in these patients is probably multifactorial and multimodal therapy needs to be explored in clinical trials.

- Level of evidence 1

Vc) Treatment of HCV:

In the absence of a vaccine, treatment with IFN is aimed at preventing chronic complications of HCV infection. The indicator of treatment efficacy is the absence of detectable HCV in serum after a period of 6 months from cessation of treatment, known as sustained virological response (SVR). SVR rate is inversely proportional to the risk of progression of liver disease. The role of interferon in the prevention of HCC in patients with hepatitis C remains controversial.

Only one small RCT from Japan compared IFN treatment with observation only for 90 patients with HCV-related cirrhosis [53]. IFN-alpha improved liver function in chronic active hepatitis C with cirrhosis, and its use was associated with a decreased incidence of HCC. After a follow up of 2-7 years, the rate of HCC was significantly less in those treated with IFN (4.4%) compared to untreated group (37.8%), RR for IFN treatment: 0.067 (0.009-0.530; p = 0.010). Subsequent studies from Europe could not reproduce the results of the Japanese trial which was criticized for the unusually high rates of HCC in the control group.

A meta-analysis was done of 3 RCTs and 15 nonrandomized controlled trials, including 4614 patients with HCV-related cirrhosis, comparing IFN to no treatment [54]. The incidence of HCC was less for treated as compared to untreated patients (overall Risk Difference -12.8%; 95% CI -8.3 to -17.2%, P < 0.0001). The preventive effect was more evident among sustained responders to IFN. The rate of HCC development was also lower in non-responders than in untreated patients. Inconsistency among the studies was a major problem. Consistent results were only observed when assessing data from European reports: in this subgroup only a weak effect was seen for HCV (overall RD -10%; 95% CI -5.9 to -14.2%; P < 0.0001). The authors concluded that the magnitude of the preventive effect of IFN on HCC development in patients

with HCV-related cirrhosis was low and the observed benefit could be due to spurious associations.

Another meta-analysis of 11 studies with 2178 patients evaluated the HCC incidence in IFN-treated and untreated patients with HCV-related cirrhosis [55]. HCC development was significantly more frequent in untreated (21.5%) than in IFN-treated patients (8.2%; OR: 3.0, 95% CI: 2.3-3.9). In the five studies reporting HCC incidence in patients with and without SVR to IFN, HCC was detected at a much higher rate in patients without (9%) than with a SVR (0.9%; OR: 3.7, 95% CI: 1.7-7.8). Moreover, HCC developed significantly more frequently in the untreated patients than in the non-SVR (OR: 2.7, 95% CI: 1.9-3.9). Interferon therapy significantly reduced the HCC risk in patients with HCV-related cirrhosis. HCC development was almost negligible among sustained responders, but a reduction in HCC incidence was also achieved in the non-sustained responders.

A recent systematic review of the literature to establish the outcome of compensated HCV cirrhosis included 13 papers involving 2386 patients. In compensated HCV cirrhosis, the estimated annual rate of HCC was 3.36%. When compared with studies of untreated patients, studies that included treated patients reported significantly lower mean annual percentage rates of HCC (2.52% vs. 4.79%, P = 0.02), but not decompensation (5.34% vs. 7.88%, P = 0.026) and death/transplantation (3.79% vs. 4.62%, P = 0.25).

Thus, IFN treatment for chronic HCV infection significantly decreases the risk of HCC. The maximum benefit is seen in those who have a SVR on treatment. However, a reduction in HCC incidence is also achieved in non-sustained responders.

- Level of evidence 1

VI. Summary

HBV and HCV are well established risk factors for HCC. Older age, male sex, alcohol consumption, cigarette smoking, elevated transaminases, elevated HBV DNA, HBV genotype C, co-infection with HCV and aflatoxin exposure are all associated with an increased risk of HCC in patients with chronic viral hepatitis B infection. Older age, male sex, co-infection with HBV, diabetes mellitus, obesity and alcohol consumption are associated with an increased risk of HCC in patients with chronic viral hepatitis C infection. Hepatitis B vaccination has dramatically reduced the rate of chronic HBV infection to less than 1% among immunized children and will result in decreased rates of HCC in areas with high immunization coverage. Clinical and epidemiological studies suggest that in chronic hepatitis B, IFN therapy reduces the risk of HCC development in HBeAg-positive and cirrhotic patients who achieve persistent suppression of viral replication, while in HBeAg-negative patients the beneûcial effect of IFN is not deûnitively conûrmed. However the short follow up period and the heterogeneity of the studies could lead to overestimation of the treatment effect. Oral NA treatment is also associated with a decreased risk of HCC and also may be associated with a decreased risk of post-operative

recurrence of HCC. IFN treatment for chronic HCV infection significantly decreases the risk of HCC. The maximum benefit is seen in those who have a SVR on treatment. However, a reduction in HCC incidence is also achieved in non-sustained responders. Further prospective randomized controlled trials with newer drugs and regimens are needed for a better quality of evidence.

References

1. Batham A, Narula D, Toteja T, Sreenivas V, Puliyel JM. Sytematic review and meta-analysis of prevalence of hepatitis B in India. Indian Pediatr. 2007 Sep;44(9):663-74.
2. Verma R, Khanna P, Prinja S, Rajput M, Chawla S, Bairwa M. Hepatitis B Vaccine in national immunization schedule: A preventive step in India. Hum Vaccin. 2011 Dec 1;7(12).
3. Dhir V, Mohandas KM. Epidemiology of digestive cancer in India-III. Liver. Indian J Gastroenterol 1998; 17: 100-3
4. Global Burden Of Hepatitis C Working Group. Global burden of disease (GBD) for hepatitis C. J Clin Pharmacol. 2004 Jan;44(1):20–9.
5. Mukhopadhya A. Hepatitis C in India. J. Biosci. 33(4), November 2008, 465–473
6. Chowdhury A, Santra A, Chaudhuri S, et al. Hepatitis C virus infection in the general population: a community-based study in West Bengal, India. Hepatology 2003; 37: 802–09.
7. El-Serag HB, Mason AC. Risk factors for the rising rates of primary liver cancer in the United States. Arch Intern Med. 2000;160:3227-3230.
8. Jemal A, Bray F, Center M, Ferlay J, Ward E, Forman D. Global Cancer Statistics. CA CANCER J CLIN 2011;000:000-000 (e-print)
9. Parkin DM, Pisani P, Mun˜oz N, Ferlay J. The Global Health Burden of Infection Associated Cancer, in Weiss RA, Beral V, Newton R (eds). Infections and Human Cancer. Cancer Surveys 1999; 33:5–33.
10. McGlynn KA, Tsao L, Hsing AW, Devesa SS, Fraumeni JF, Jr. International trends and patterns of primary liver cancer. Int J Cancer. 2001;94:290-296.
11. Ferlay J, Bray F, Pisani P et al. GLOBOCAN 2002. Cancer Incidence, Mortality and Prevalence Worldwide. IARC Cancer Base No.5, Version 2.0. Lyon, France :IARC Press; 2004
12. Bruix J, Sherman M, Llovet JM et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol 2001; 35:421–430.
13. Nguyen VT, Law MG, Dore GJ. Hepatitis B-related hepatocellular carcinoma: epidemiological characteristics and disease burden. J Viral Hepat. 2009;16(7):453-63.
14. Shi J, Zhu L, Liu S, Xie WF.A meta-analysis of case-control studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma in China. Br J Cancer. 2005 Feb 14;92(3):607-12.

15. Kumar M, Kumar R, Hissar SS et al. Risk factors analysis for hepatocellular carcinoma in patients with and without cirrhosis: a case-control study of 213 hepatocellular carcinoma patients from India. J Gastroenterol Hepatol 2007; 22(7): 1104–1111.
16. Yang H-I, Lu S-N, Liaw Y-F et al. Hepatitis B e antigen and the risk of hepatocellular carcinoma. N Engl J Med 2002;347(3): 168–174.
17. Wang LY, You SL, Lu SN et al. Risk of hepatocellular carcinoma and habits of alcohol drinking, betel quid chewing and cigarette smoking: a cohort of 2416 HBsAg-seropositive and 9421 HBsAg-seronegative male residents in Taiwan. Cancer Causes Control 2003; 14(3): 241–250.
18. Chen C-J, Yang H-I, Su J et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006; 295(1): 65–73
19. Chen C-L, Yang H-I, Yang W-S et al. Metabolic factors and risk of hepatocellular carcinoma by chronic hepatitis B/C infection: a follow-up study in Taiwan. Gastroenterology 2008; 135(1): 111–121.
20. Yu MC, Yuan JM. (2004) Environmental factors and risk for hepatocellular carcinoma. Gastroenterology 2004 Nov; 127(5 Suppl 1): S72–S78
21. Blum HE,Moradpour D, Blum HE,Moradpour D. Viral pathogenesis of hepatocellular carcinoma. J Gastroenterol Hepatol 2002;17(Suppl 3): S413-S420
22. Di Bisceglie AM. Hepatitis B and hepatocellular carcinoma. Hepatology. 2009 May;49(5 Suppl):S56-60
23. Yu M-W, Yeh S-H, Chen P-J et al. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. J Natl Cancer Inst 2005; 97(4): 265–272.
24. Wu C-F, Yu M-W, Lin C-L et al. Long-term tracking of hepatitis B viral load and the relationship with risk for hepatocellular carcinoma in men. Carcinogenesis 2008;29(1): 106–112.
25. El-Serag HB. Hepatocellular carcinoma and hepatitis C in the United States. Hepatology. 2002 Nov;36(5 Suppl 1):S74-83.
26. Donato F, Boffetta P, Puoti M. A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma. Int J Cancer 1998;75:347-354.
27. El-Serag HB, Goodgame B, Shaheen N. The risk of cirrhosis and Hepatocellular carcinoma (HCC) among HCV-infected patients: a systematic review. Gastroenterology 2002;123:S50.
28. Goodgame B, Shaheen NJ, Galanko J, El-Serag HB. The risk of end stage liver disease and hepatocellular carcinoma among persons infected with hepatitis C virus: publication bias? Am J Gastroenterol. 2003 Nov;98(11):2535-42.
29. Tagger A, Donato F, Ribero ML, Chiesa R, Portera G, Gelatti U, Albertini A, Fasola M, Boffetta P, Nardi G.Case-control study on hepatitis C virus (HCV) as a risk factor for hepatocellular carcinoma: the role of HCV genotypes and the synergism with hepatitis B virus and alcohol. Brescia HCC Study.Int J Cancer. 1999 May 31;81(5):695-9.

30. Degos F, Christidis C, Ganne-Carrie N, et al. Hepatitis C virus related cirrhosis: time to occurrence of hepatocellular carcinoma and death. Gut. 2000;47:131-136.
31. Chiba T, Matsuzaki Y, Abei M, et al. Multivariate analysis of risk factors for hepatocellular carcinoma in patients with hepatitis C virus-related liver cirrhosis. J Gastroenterol. 1996;31:552-558.
32. World Health Organization. Hepatitis B. WHO position paper. French and English versions (July 2004) . Accessed online: http://www.who.int/immunization/topics/hepatitis_b/en/ on 24th December 2011.
33. Chang MH, Chen CJ, Lai MS, et al. Universal Hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. N Engl J Med 1997;336:1855–1859.
34. Chang MH, Chen TH, Hsu HM, Wu TC, Kong MS, Liang DC,et al; Taiwan Childhood HCC Study Group. Prevention of hepatocellular carcinoma by universal vaccination against hepatitis B virus: the effect and problems. Clin Cancer Res. 2005 Nov 1;11(21):7953-7
35. Ng KP, Saw TL, Baki A, Rozainah K, Pang KW, Ramanathan M. Impact of the expanded program of immunization against hepatitis B infection in school children in Malaysia. Med. Microbiol. Immunol.2005; 194: 163–8.
36. Goh KT. Prevention and control of hepatitis B virus infection in Singapore. Ann. Acad. Med. Singapore1997; 26: 671–81.
37. Lanier AP, Holck P, Ehrsam Day G, Key C. Childhood cancer among Alaska Natives. Pediatrics 2003; 112: e396
38. Wichajarn K, Kosalaraksa P, Wiangnon S. Incidence of hepatocellular carcinoma in children in Khon Kaen before and after national hepatitis B vaccine program. Asian Pac J. Cancer Prev. 2008; 9: 507– 10.
39. Lin SM, Yu ML, Lee CM et al. Interferon therapy in HBeAg positive chronic hepatitis reduces progression to cirrhosis and hepatocellular carcinoma. J. Hepatol. 2007; 46: 45–52.
40. Camma C, Giunta M, Andreone P, Craxi A. Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach. J Hepatol 2001; 34: 593-602
41. Mazzella G, Accogli E, Sottili S, et al. Alpha interferon treatment may prevent hepatocellular carcinoma in HCV related liver cirrhosis. J Hepatol 1996; 24:141–147.
42. Oon CJ. Long-term survival following treatment of hepatocellular carcinoma in Singapore: evaluation of Wellferon in the prophylaxis of high-risk pre-cancerous conditions. Cancer Chemother Pharmacol 1992; 31(suppl):S137–142.
43. Ikeda K, Saitoh S, Suzuki Y, et al. Interferon decreases hepatocellular carcinogenesis in patients with cirrhosis caused by the hepatitis B virus: a pilot study. Cancer 1998; 82:827–835.
44. Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatology 1999; 29: 971-975

45. Sung JJ, Tsoi KK, Wong VW, Li KC, Chan HL.Meta-analysis: Treatment of hepatitis B infection reduces risk of hepatocellular carcinoma. Aliment Pharmacol Ther. 2008 Nov 1;28(9):1067-77.

46. Karayiannis P. Hepatitis B virus: old, new and future approaches to antiviral treatment. J Antimicrob Chemother 2003; 51:761-785

47. Matsumoto A, Tanaka E, Rokuhara A, Kiyosawa K, Kumada H, Omata M, et al. Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: A multicenter retrospective study of 2795 patients. Hepatol Res 2005; 32: 173-184

48. Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 2004; 351: 1521-1531

49. Di Marco V, Marzano A, Lampertico P, Andreone P, Santantonio T, Almasio PL, et al. Clinical outcome of HBeAg-negative chronic hepatitis B in relation to virological response to lamivudine. Hepatology 2004; 40:883-891

50. Yuen MF, Seto WK, Chow DH et al. Long-term lamivudine therapy reduces the risk of long-term complications of chronic hepatitis B infection even in patients without advanced disease. Antivir. Ther. 2007; 12: 1295–303.

51. Kim BK, Park JY, Kim do Y et al. Persistent hepatitis B viral replication affects recurrence of hepatocellular carcinoma after curative resection. Liver Int. 2008; 28: 393–401.

52. Miao RY, Zhao HT, Yang HY, Mao YL, Lu X, Zhao Y, et al. Postoperative adjuvant antiviral therapy for hepatitis B/C virus-related hepatocellular carcinoma: A meta-analysis. World J Gastroenterol 2010 June 21; 16(23): 2931-2942

53. Nishiguchi S, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima S, et al. Randomised trial of effects of interferon-alpha on incidence of Hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet 1995;346:1051-1055.

54. Cammà C, Giunta M, Andreone P, Craxì A. Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach. J Hepatol. 2001 Apr;34(4):593-602.

55. Papatheodoridis GV, Papadimitropoulos VC, Hadziyannis SJ.Effect of interferon therapy on the development of hepatocellular carcinoma in patients with hepatitis C virus-related cirrhosis: a meta-analysis. Aliment Pharmacol Ther. 2001 May;15(5):689-98.

56. Alazawi W, Cunningham M, Dearden J, Foster GR.Systematic review: outcome of compensated cirrhosis due to chronic hepatitis C infection. Aliment Pharmacol Ther. 2010 Aug;32(3):344-55.

Selected abstract-Indian study

Kumar M, Kumar R, Hissar SS, Saraswat MK, Sharma BC, Sakhuja P .Risk factors analysis for hepatocellular carcinoma in patients with and without cirrhosis: a case-control study of 213 hepatocellular carcinoma patients from IndiaJ Gastroenterol Hepatol. 2007 Jul;22(7):1104-11

Abstract

AIM: To assess the role of hepatitis B virus (HBV), hepatitis C virus (HCV) and alcohol intake as risk factors for hepatocellular carcinoma (HCC) in the presence or absence of cirrhosis in Indian population.

METHODS: A total of 213 patients with HCC and 254 control subjects not affected with hepatic diseases or neoplasm were recruited. Odds ratios (ORs) were estimated for each risk factor and synergism among various risk factors was also studied.

RESULTS: The ORs and 95% confidence intervals (CI) of HCC were 48.02 (25.06-91.98) for any HBV marker, 38.98 (19.55-77.71) for HBsAg positivity, 12.34 (2.84-53.61) for HBsAg negative and antibody positive (either of anti-HBe or total anti-HBc), 5.45 (2.02-14.71) for anti-HCV positive and HCV RNA positive, and 2.83 (1.51-5.28) for heavy alcohol use. No significant risk increase was evident for subjects who were anti-HCV positive and HCV RNA negative. Synergism between alcohol and HCV infection in causing HCC was found, but not between alcohol and HBV. Overall, conclusive evidence of the presence or absence of cirrhosis was reached in 189 (88.73%) HCC patients; cirrhosis was present in 137 (72.48%) of them. ORs with 95% CI of HCC in the presence and absence of cirrhosis, respectively, for HBV were as follows: (i) 48.90 (24.61-97.19) and 35.03 (15.59-78.66) for any HBV marker; (ii) 39.88 (19.41-81.97) and 24.40 (10.60-56.18) for HBsAg positivity; and (iii) 12.10 (2.67-54.88) and 19.60 (3.94-97.39) for HBsAg negativity and antibody positivity. Significantly increased risk was found among cirrhotic patients for anti-HCV positivity and HCV RNA positivity [OR = 7.53 (2.73-20.78)] and for heavy alcohol use [OR = 3.32 (1.70-6.47)]; however, in the absence of cirrhosis, no significant risk increase was evident for subjects who were anti-HCV positive and HCV RNA positive [OR = 0.97 (0.11-8.54)], or who had history of heavy alcohol use [OR = 1.58 (0.55-4.53)].

CONCLUSIONS: Infection with HBV and HCV are the major risk factors for the development of HCC in Indian patients. Presence of HBV antibodies even in the absence of HBsAg conferred increased risk for HCC in the presence or absence of cirrhosis. Anti-HCV positivity in the absence of HCV RNA conferred no increased risk. HCV RNA positivity and heavy alcohol use significantly increased the risk of HCC among cirrhotic patients, but not non-cirrhotic patients.

Oncogenic Human Papilloma Viruses

Worldwide in 2002, an estimated 561,200 new cancer cases (5.2% of all new cancers) were attributable to HPV, making HPV one of the most important infectious cause of cancer. Papillomaviruses were first identified, cloned and sequenced from cervical tumor specimens and were subsequently established as important causative agents for development of cervical cancer, the discovery which was honored by conferring Nobel Prize of Physiology and Medicine for the year 2008 to its inventor Harald zur Hausen.

Human papilloma viruses (HPVs) are small, non enveloped, double-stranded DNA viruses. More than 150 types of HPV are acknowledged to exist . HPVs establish productive infections only in keratinocytes of the skin or mucous membranes. 23-30 types of HPVs infect almost exclusively the skin of the lower genital tract(genital HPV). Infection with HPV typically leads to benign epithelial proliferations; however, a growing number of viral subsets have been associated with epithelial cancers.

The genital HPV types can be divided into two broad groups (low-risk and high-risk HPVs) depending upon their association with cancers of the lower genital tract. Low-risk HPV types (6, 11, 42, 43, 44, 54, 61, 70, 72, and 81) are virtually never found in cancers. High-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) have been identified in cancers of the cervix, vagina, vulva, anus, and penis. They are also called oncogenic HPVs. Types 16 and 18 are generally acknowledged to cause about 70% of cervical cancer cases. Together with type 31, they are the prime risk factors for cervical cancer . Studies show a link between HPV infection and penile and anal cancer ,HPV-positive oropharyngeal cancer (OSCC), a form of head and neck cancer and squamous cell carcinoma of the skin . HPV infection is also reported in lung cancer and in retinoblastoma, but the frequency has been low in India. Though controversial, presence of HPV DNA sequences has also been reported in breast cancer.

Genital HPV infection is the most common viral sexually transmitted infection (STI) and affects roughly 80 percent of all sexually active people The risk of exposure to HPV is estimated to be approximately 15-25% per partner. More than 60% of men without HIV and 90 % of men with HIV who have sex with men are infected with HPV in their anal canals. The peak prevalence is between ages 18 and 30. Early age of first intercourse, multiple sexual partners, unprotected sex and sex with uncircumcised men, have been found to increase the risk of contracting HPV infection Immune suppression is a risk factor for all people exposed to HPV.

95% of HPV infected patients acquired the disease through sexual contact.It is primarily transmitted through genital skin-to-genital skin sexual contact and penetrative intercourse is not required. Oral and anal HPV infections occur and oral and anal intercourse may not be required. HPV can be transmitted nonsexually from direct contact with caretaker contaminated with genital HPV .Vertical transmission can occur through the bloodstream prior to birth, or at the time of birth, as the infant passes through the infected birth canal .

HPV genome

The HPV genome consists of double stranded DNA (dsDNA) approximately 8kbp long contained within a capsid . Only one coding strand of the dsDNA acts as a template for transcription. The HPV genome is divided into three regions: an Early region containing genes encoding non-structural proteins (E), a Late region containing genes encoding capsid proteins (L), and an Upstream Regulatory Region (URR) (or Long Control Region), which contains a DNA replication origin, transcription regulatory sequences and one or more promoters which control expression of the viral oncoproteins E6 and E7.HPV genome encode eight major proteins, six located in the early region and two in the late region.

E1 and E2 are DNA binding proteins that regulate transcription and replication of the viral genome . E4 is thought to be involved in activating the productive phase of the HPV life cycle. E5 protein binds with platelet derived growth factor â receptor ,promoting a sustained mitotic signal. E6 has a close relationship with the cellular protein E6-AP (E6-associated protein). E6-AP is involved in the ubiquitin ligase pathway, a system that acts to degrade proteins. E6-AP binds ubiquitin to the p53 protein, thereby flagging it for proteosomal degradation which results in prevention of apoptosis. E7 has numerous interactions with cellular proteins involved in cell growth regulation ,such as cyclin dependent kinases (CDK) and CDK inhibitors, but it interacts particularly with retinoblastoma suppressor protein (Rb)by binding to G0/G1 specific hypophosphorylated form of Rb disrupting the pRb/E2F complex and bypassing cell cycle arrest. L1 and L2 comprise the virus capsid required for virus transmission, spread and survival in the environment.

HPV life cycle

HPV is strictly epitheliotropic and infects basal epithelial cells of stratified squamous epithelium either of the skin or mucous membranes, particularly of the anogenital tract and

oropharynx. HPV is dependent on host cell for replication, transcription and translation. The viral functions are tightly linked to cellular differentiation .The HPV genome encodes only eight open reading frames hence the virus must recruit host cell functions to maintain and replicate itself. Since the differentiating epithelial cell normally does not divide and frequently loses its nucleus after leaving the basal layer, HPV proteins have evolved to maintain their host cells in a cycling state. As a result, infected epithelia contain a much higher proportion of nucleated and dividing cells in all layers.

The virus infects the basal layer of the epithelium via minor abrasions in the skin. It enters the cell, uncoats and delivers its DNA to the cell's nucleus to be expressed as autonomous replicating episomal or extrachromosomal elements. Using host cell machinery , viral DNA is replicated and then segregated into progeny cells. E6 and E7, the major transforming genes, are expressed, causing an increase in cell division. The net result of both viral products, E6 and E7, is dysregulation of the cell cycle, allowing cells with genomic defects to enter the S-phase (DNA replication phase) The cells divide and differentiate, carrying the HPV DNA with them.

The infected basal cells divide and their progeny take HPV DNA with them. During the early phases of infection the copy number of the viral genome is between 50 and 100, and the viral genome exists as extrachromosomal plasmid (episome) that replicates as the host cell chromosomes replicate.

As the infected cells differentiate, the E1, 2, 4 and 5 genes are expressed. As the cells approach terminal differentiation the late genes, L1 and L2, are activated - they encode the major and minor viral capsid proteins respectively.Thousands of virus particles are produced per cell. As these cells approach the surface of the skin they are sloughed off, and the virus particles are released to infect other cells and spread to other hosts. HPV infections have not been shown to be cytolytic; rather, viral particles are released as a result of degeneration of desquamating cells

Low copy numbers of the mucosotrophic HPV DNA plasmids are maintained in the basal and parabasal cells that divide, whereas the productive phase takes place only in postmitotic, differentiated cell strata and progeny virus shed within the sloughing superficial cells. Thus, it is paramount that, in either latent or active infections, HPV DNA must partition into the two daughters of dividing cells for viral persistence.High-risk HPVs can frequently persist in an infected host cell at a low copy number for decades, often without causing clinically overt lesions.

Natural history of HPV infection

In most cases, HPV infection is cleared by the immune system. The average episode of HPV infection lasts four to 20 months. Less than half of women who develop HPV infection with a high-risk type will have persistence of the same high-risk HPV type 12 months later. Persistent infection has been defined as detection of the same high-risk HPV genotype two or

more times within a given interval of time; however, the duration of time that defines "persistence" is not yet agreed upon. There are currently no data on the natural history of high-risk HPV infection in men. Persistent infections with high-risk HPV types can cause precancerous lesions and cancer.

HPV Oncogenesis

One of the key events of HPV-induced carcinogenesis is the integration of the HPV genome into a host chromosome. HPV genome integration often occurs near common fragile sites of the human genome , but there are no apparent hot spots for integration and no evidence for insertional mutagenesis . Integration follows a more specific pattern with respect to the HPV genome.

Expression of the viral E6 and E7 genes is consistently maintained, whereas other portions of the viral DNA are deleted or their expression is disturbed .Loss of expression of the HPV E2 transcriptional repressor is significant, as it may result in deregulated HPV E6 and E7 expression. There is also evidence for increased HPV-16 E6/E7 mRNA stability after integration , and specific alterations of host cellular gene expression have been detected upon HPV genome integration . Cells that express E6/E7 from integrated HPV sequences have a selective growth advantage over cells with episomal HPV genomes . The concept that loss of E2 repressor function may be critical for malignant progression is supported by experiments showing that reexpression of E2 in cervical cancer cell lines causes growth suppression . These experiments clearly demonstrate that continued E6/E7 expression in cervical cancers is necessary for the maintenance of the transformed phenotype . Expression of high-risk HPV E6 and E7 genes in primary human keratinocytes effectively facilitates their immortalization .Integration of the viral genome into a host cell chromosome also leads to loss of E5 expression.

The rate of spontaneous mutagenesis in normal human cells is exceedingly low, but the expression of high-risk HPV E6/E7 proteins dramatically augments genomic instability . Therefore, expression of the high-risk HPV E6/E7 genes not only is necessary for the induction of premalignant alterations but also directly contributes to malignant progression by subverting genomic stability

Several types of cancer are associated with HPV .Almost all cervical cancers are caused by HPV. 50% vulval cancer, 65%vaginal cancers, 35% penile cancer and 95% anal cancers are linked to HPV. About 60% oropharyngeal cancers are linked to HPV .

Immune-mediated regression

Most HPV lesions eventually resolve due to a host immune response to the virus. Because an individual's immune system can usually suppress (and perhaps even clear) HPV most individuals are not at great risk of getting these cancers. Whether an immune-mediated regression clears that HPV type from the body completely, or just suppresses it to the point where it is not likely to be contagious nor cause HPV-induced disease in the future is not known.

However, the end-result is essentially the same since neither “cleared” nor “permanently suppressed” HPV would be likely to cause cancer in the short to medium term.

Long-term persistence of HPV is not very common. When it happens, the complex interplay of HPV, host immunity, various co-factors, and perhaps, spontaneous mutations in the host cell may eventually result in the development of pre-cancers and cancer of the cervix, vagina, vulva, anus, or penis. Failure of the immune response may contribute to the development of premalignant and malignant lesions, as is suggested by an increased incidence of HPV-associated lesions in immunocompromised hosts .

Co factors

The genetic predisposition of patients with epidermodysplasia verruciformis to skin cancer caused by HPV raises the possibility of another more subtle susceptibility to cervical cancer. There is evidence for a genetically determined risk for cervical cancer . Cigarette smoking is associated with accumulation of a tobacco carcinogen in cervical mucus. Sunlight as well as a genetic defect are cofactors for skin carcinomas caused by HPV5 and 8 in patients with EV. Anal cancer associated with HPV is much more frequent in HIV-infected persons with immune deficiency, especially males. Chronic immunosuppression increases the risk of high grade cervical dysplasia and progression to cancer. There are no reports that HPV can secondarily alter tumor cell behavior such as invasiveness or aggressivity of tumor cell growth.

References

1. Fehrmann F, Laimins LA. Human papillomaviruses: targeting differentiating epithelial cells for malignant transformation. Oncogene 2003;22:5201–7.
2. Zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical Application. Nature 2002;2:342–50.
3. Parkin DM. “The global health burden of infection-associated cancers in the year 2002”. *Int. J. Cancer 2006* 118 (12): 3030–44
4. Boshart M, Gissmann L, Ikenberg H, Kleinheinz A, Scheurlen W, zur Hausen H. A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. *Embo J* 1984; *3* : 1151-7.
5. Schiffman M, Castle PE . “Human papillomavirus: epidemiology and public health”. Arch Pathol Lab Med 2003,127 (8): 930–4.
6. Walboomers JM, Jacobs MV, Manos MM (1999). “Human papillomavirus is a necessary cause of invasive cervical cancer worldwide”. J. Pathol. 189 (1): 12–9
7. D’Souza G, Kreimer AR, Viscidi R (2007). “Case-control study of human papillomavirus and oropharyngeal cancer”. N. Engl. J. Med. 356 (19): 1944–56
8. Karagas MR, Waterboer T, Li Z, Nelson HH, Michael KM, Bavinck JNB, Perry AE, Spencer SK, Daling J, Green AC, Pawlita M (2010). “Genus â human papillomaviruses and incidence

of basal cell and squamous cell carcinomas of skin: population based case-control study". *BMJ* 341: 2986

9. IARC. *Human papillomaviruses*. Lyon: IARC Press; 2007.
10. de Villiers EM, Sandstrom RE, zur Hausen H, Buck CE. Presence of papillomavirus sequences in condylomatous lesions of the mamillae and in invasive carcinoma of the breast. *Breast Cancer Res* 2005; *7* : R1-11.
11. Koutsky L. Epidemiology of genital human papillomavirus infection. Am J Med. 1997;102:3-8.
12. Winer RL, Lee S-K, Hughes JP, Adam DE, Kiviat NB, Koutsky LA. Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students.Am J Epidemiol.2003;157:218-26.
13. Centers for Disease Control and Prevention. Human Papillomavirus: HPV Information for Clinicians. November 2006.
14. Hernandez BY, Wilkens LR, Zhu X, Thompson P, McDuffie K, Shvetsov YB. Transmission of human papillomavirus in heterosexual couples. Emerg Infect Dis.2008; 14:888-92.
15. Burchell AN, Winer RL, de Sanjose S, Franco EL. Epidemiology and transmission dynamics of genital HPV infection. Vaccine. 2006;24(Suppl 3):52-62.
16. Stoppler MC, Straight SW, Tsao G, Schlegel R, McCance DJ. The E5 gene of HPV-16 enhances keratinocyte immortalization by fulllength DNA. Virology 1996; 223: 251-4.
17. Maufort JP, Shai A, Pitot HC, Lambert PF. A role for HPV16 E5 incervical carcinogenesis. Cancer Res 2010; 70: 2924-31.
18. Bosch FX, Schwarz E, Boukamp P, Fusenig NE, Bartsch D, zur Hausen H. Suppression *in vivo* of human papillomavirus type 18 E6-E7 gene expression in nontumorigenic HeLa X fibroblast hybrid cells. J Virol 1990; 64: 4743-54.
19. von Knebel Doeberitz M, Oltersdorf T, Schwarz E, Gissmann L. Correlation of modified human papilloma virus early gene expression with altered growth properties in C4-1 cervical carcinoma cells. Cancer Res 1988; 48: 3780-6. Ganguly, N.; Parihar, S. P. (2009). "Human papillomavirus E6 and E7 oncoproteins as risk factors for tumorigenesis". *Journal of biosciences* 34 (1): 113–123.
20. Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, *et al*. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a metaanalysis update. *Int J Cancer* 2007; *121* : 621-32.
21. Bosch FX. Epidemiology of human papillomavirus infections: new options for cervical cancer prevention. *Salud Publica Mex* 2003; *45* (Suppl 3) : S326-39.
22. Pagano JS, Blaser M, Buendia MA, *et al.* (December 2004). "Infectious agents and cancer: criteria for a causal relation". *Semin. Cancer Biol.* **14** (6): 453–71.

HPV and Cervical Cancer

Worldwide, cervical cancer is the second most common cancer in women and the most common cancer in women in developing countries. In developed countries, cervical cancer accounts for 1.7% of all cancers while in developing countries this figure is 7% . In 2008, the World Health Organisation estimated that there were 529,828 newly diagnosed cases of cervical cancer and this disease was associated with 275128 deaths annually ,80% of these deaths occur in developing countries . The higher prevalence of cervical cancer in developing countries may be largely attributable to the limited access women in these countries have to screening programs combined with high-risk characteristics such as poor nutrition and high parity.Early epidemiological studies of cervical neoplasia suggested a causal relation with sexual activity and HPVs emerged as prime suspects as sexually transmitted carcinogens. It is now well established that HPV infection is the primary cause of virtually all cervical cancers and is a necessary cause for the disease.

India has a population of 366.58 millions women ages 15 years and older who are at risk of developing cervical cancer. In India cervical cancer ranks as the 1st most frequentcancer among women in India, and the 1st most frequent cancer among women between 15 and 44years of age. About 7.9% of women in the general population are estimated to harbour cervical HPVinfection at a given time and 82.5% of invasive cervical cancers are attributed to HPVs 16 or 18.

Epidemiological strength of association of HPV with cervical neoplasia

A landmark study has shown that HPV DNA can be found in 99.7% of cervical cancer specimens. Data from large case-control studies using sensitive and specific polymerase chain reaction (PCR) based assays strongly suggest that HPV infections cause most CIN lesions. Munoz and Bosch have reviewed the available epidemiological data on the association of HPV and cervical cancer and found that the data fulfill the Bradford Hill criteria of causality

The strength of association between HPV and cervical cancer is considered one of the strongest for a human cancer. Recent studies have shown that HPV (all types combined) is present in >90% of cervical cancers. The presence of HPV in cervical cancer is consistent among a large number of studies, regardless of the HPV testing system used. There are no published studies with negative observations that challenge the association of HPV and cervical cancer. HPV type is also important in the development of specific cancers. HPV is present in the tumour cells. Viral oncogene expression (E6 and E7) occurs in tumour material, but not in stromal cells. HPV infections precede pre-cancerous cervical lesions and cervical cancer by years to decades. HPV is a powerful carcinogen that immortalizes human keratinocytes *in vitro*. There are no animal models in which a sexually transmitted HPV produces cervical cancer. HPV is present in cervical cancer, where it expresses the oncogenic proteins E6 and E7 that inactivate the host regulatory proteins p53 and RB, respectively. Epidemiological studies support a role for HPV in cervical cancer. The association does not conflict with what is known about the natural history of cervical cancer development. *In vitro* and *in vivo* evidence supports a causal role for HPV in the development of cervical cancer

Worldwide, the HPV types causing cervical cancer varies from one country to another, however, over 70%, in any given country, are caused by only 2 types, HPV16 and HPV 18. In a pooled analyses of data from 11 case–control studies of women with histologically confirmed squamous cell cervical cancer conducted in 9 different countries, HPV DNA could be detected in 90.7% of the cases by one detection method and in 96.6% by another; the proportion of control women testing positive were, respectively, 13.4% and 15.6%. This study tested either cytology smears or biopsied tissue and found that the most common types, in order of frequency, were HPV-16, -18, -45, -31, -33, -52, -58 and -35 .

The current etiologic model for the development of invasive cervical cancer highlights infection with high-risk HPV types as a necessary, but not sufficient, cause of cervical cancer. This model also incorporates the role of co-factors, such as smoking, nutritional deficiencies, oral contraceptive (OC) use, and parity status in the development of cancer. Persistent infection with high-risk types of HPV is necessary for the progression of high-grade lesions to invasive cancer. Smoking is clearly associated with neoplastic progression. Some, but not all, studies have demonstrated a longer persistence of infection among ever smokers than non-smokers.

Recent studies have suggested that condom use reduces the risk of high-grade cervical lesions and increases HPV clearance. Women with human immunodeficiency virus (HIV) have prolonged HPV persistence, even if their CD4 counts are normal . Persistence of infection with high-risk HPV leads to abnormal clonal progression in the cervical epithelium and eventually may lead to invasive cervical carcinoma. There is insufficient evidence to recommend that women who have infection with a high-risk HPV type should discontinue OC use. Some studies have suggested that OC use and the presence of other STIs may act as co-factors in neoplastic progression, persistence of HPV infection, or both.

Natural history of cervical cancer

Cervical cancer begins with the development of pre-cancerous, benign lesions in the cervicular area.According to WHO classification, the first stage of development is mild dysplasia, which can then progress to becoming moderate dysplasia, severe dysplasia, and then carcinoma in situ (CIS) or invasive cervical cancer.

Mild dysplasia usually regresses on its own without treatment, and doesn't progress to moderate or severe dysplasia. A small percentage of women with mild dysplasia, however, will progress to more severe forms, although this can take as long as 10 years. Women with moderate to severe dysplasia are at high risk of developing invasive cancer, although the progression from severe pre-cancerous lesions to cancer may take several years as well .

There are two other systems of classification. According to the Cervical Intraepithelial Neoplasia (CIN) system, mild to moderate dysplasia are classified as CIN1, intermediate dysplasia as CIN2,and severe dysplasia and carcinoma in situ are together classified as CIN3. The Bethesda system simplifies it further, by classifying CIN1 as Low Grade Squamous Intraepithelial Lesion (LSIL),and both CIN2 and CIN3 as High Grade Intraepithelial Lesion (HSIL).

Once invasive cancer develops, it is further classified into various stages, as per the InternationalFederation of Gynaecology and Obstetrics (FIGO).

Prevention

Cervical cancer is a disease that can be prevented through both primary prevention and early detection using screening techniques. Currently two HPV vaccines are commercially available Gardasil (Quadrivalent - HPV 16,18,6 and 11) and Cervarix (Bivalent – HPV 16 and 18).Several screening modalities are now available for early detection of cervical cancer and its precursor lesions.

References

1. Koutsky L. Epidemiology of genital human papillomavirus infection. *Am J Med.* 1997; 102:3-8.
2. Walboomers JMM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189:12–9.
3. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55(2):74–108.
4. Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. J Clin Pathol 2002;55(4):244–65.
5. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348(6):518–27.

6. Human papilloma viruses and related cancers, India. WHO/ICO Information Centre on HPV and Cervical Cancer (HPV Information Centre) 2010. (www.who.int/hpvcentre)
7. Parkin DM. The global health burden of infection-associated cancers in the year 2002. Int J Cancer 2006;118:3030–44.
8. Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. *Vaccine.* 2006;24(Suppl 1):S4-15.
9. Burchell AN, Winer RL, de Sanjose S, Franco EL. Epidemiology and transmission dynamics of genital HPV infection. *Vaccine.* 2006;24(Suppl 3):52-62.
10. Giuliano AR, Sedjo RL, Roe, DJ, Harris R, Baldwin S, Papenfuss MR, et al. Clearance of oncogenic human papillomavirus (HPV) infection: effect of smoking (United States). *Cancer Causes Control.* 2002;13:839-46.
11. Hogewoning CJ, Bleeker MC, van den Brule AJ, Voorhorst FJ, Snijders PJ, Berkhof J, et al. Condom use promotes regression of cervical intraepithelial neoplasia and clearance of human papillomavirus: a randomized clinical trial. *Int J Cancer.* 2003;107:811-6.
12. Moscicki AB, Ellenberg JH, Farhat S, Xu J. Persistence of human papillomavirus infection in HIV-infected and -uninfected adolescent girls: risk factors and differences, by phylogenetic type. *J Infect Dis.* 2004;190:37-45.
13. Hernandez BY, Wilkens LR, Zhu X, Thompson P, McDuffie K, Shvetsov YB. Transmission of human papillomavirus in heterosexual couples. *Emerg Infect Dis.* 2008; 14:888-92.
14. Alliance for Cervical Cancer Prevention. “Natural History of Cervical Cancer: Even Infrequent screening of Older Women Saves Lives”, Cervical Cancer Prevention: Fact Sheet, April 2003.
15. Pagano JS, Blaser M, Buendia MA, *et al.* (December 2004). “Infectious agents and cancer: criteria for a causal relation”. *Semin. Cancer Biol.* **14** (6): 453–71.

Selected Abstracts-Indian studies

Singh A, Datta P, Jain SK, Bhatla N, Dutta Gupta S, Dey B, Singh N.Human papilloma virus genotyping, variants and viral load in tumors, squamous intraepithelial lesions, and controls in a north Indian population subset. Int J Gynecol Cancer. 2009 Dec;19(9):1642-8.

Abstract

A study of human papilloma virus (HPV) types and variants is important for developing preventive protocols and appropriate intervention targets. The presence of HPV types, their variants, and viral load in a population subset from North India was studied. Polymerase chain reaction (PCR) and line blots were used for HPV genotyping; HPV 16 and 18 viral loads were measured using real-time PCR. Variant analysis was done by sequencing of the PCR-amplified E6/E7 regions of HPV 16 and the long control region and E6/E7 regions of HPV 18. The 93.6%, 78.6%, and 10% of tumors, squamous intraepithelial lesions (SILs), and controls were HPV-positive, respectively. The most commonly observed type was HPV 16. Human papilloma virus 73 which is uncommonly observed was seen in 2 tumors. Multiple infections were more

common in controls and SILs than tumors. The majority (86.4%) of the HPV 16-positive and all of the HPV 18-positive samples belonged to the European variant class. Five novel nonsynonymous changes were seen in the HPV 16-positive and 2 in HPV 18-positive samples. There was a significant increase in viral loads from controls through SILs to tumors, but no significant differences in viral loads were observed between different stages of cancer. In tumors, a significant increase in HPV 16 viral loads was seen with increasing age. The study shows a similar HPV type and variant distribution to European studies, with some differences in type distribution. Viral load does not appear to be good marker for stage wise progression and intralesional variability may affect its use as a differentiating parameter between high-grade squamous intraepithelial lesion and low-grade squamous intraepithelial lesions.

Cervical Cancer Screening in India and HPV Vaccination

Cervical Cancer Burden in India

Cervical cancer is a major global public health problem affecting the socioeconomically deprived populations. It is the third most common cancer in women with an estimated 530,000 new cases in 2008. More than 85% of the global burden occurs in developing countries, where it accounts for 13% of all female cancers.*(1)*

In India, cervical cancer is a significant problem in terms of incidence, mortality and morbidity. Every year cervical cancer is diagnosed in about 134420 women (ASIR 27 per 100,000 women) in India and is responsible for more than 72,000 deaths (ASRs 15.2 per 100,000 women) annually. India recorded an estimated 141768 new cases and 77096 deaths due to Cervical Cancer in 2010, contributing to over 25% of the disease burden and more than 26% of the deaths due to cervical cancer worldwide. Cervical cancer ranks as the first most frequent cancer among women in India, and the first most frequent cancer among women between 15 and 44 years of age. About 7.9% of women in the general population are estimated to harbour cervical Human Papilloma Virus (HPV) infection at a given time., and 82.5% of invasive cervical cancers are attributed to HPVs 16 or 18.*(1)*

India has a population of 366.58 million women with ages 15 years and older who are at risk of developing cervical cancer. One out of every five women in the world suffering from this disease belongs to India. More than three-fourths of these patients are diagnosed at advanced stages leading to poor prospects of long term survival and cure *(1)*

There is a wide variation in the incidence of cervical cancer across the country. The age-adjusted incidence is highest in Chennai (ASR 28.0), and lowest in Thiruvanathapuram, the capital of Kerela (National Cancer Registry Programme and IARC GLOBOCAN 2008). (National

Cancer Registry Programme and World Health Organisation). According to IARC estimates incidence of cervical cancer will increase from 134420 in 2008 to 203757 by 2025 (45% increase) for the age group of 0-64 years. Currently the age standardized cervical cancer mortality rate for India is 15.2 (Rates per 100,000 women per year) which is almost double than that of the world average of 7.8. The mortality from cervical cancer is also expected to witness a 79% increase from 72825 deaths in 2008 to 115171 deaths by 2025 (National Cancer Registry Programme2009,WHO2004).

The prevalence and burden of cervical cancer is much higher among women of low SES, as well as among rural women in India. The primary reason given for this is lack of access to screening and health services, and lack of awareness of the risk factors of cervical cancer.*(2,3)*

Cervix cancer screening in India

India is one of the few countries to have a National Cancer Control Programme since 1975–76. However National Cancer Control Program has somehow lacked the required thrust for community based strategies in prevention and control of cervical cancer. Cervical cancer screening coverage in India is highly sub optimal with 2.6% for all women aged 18-69 yrs screened every 3yrs, 4.9% for Urban women aged 18-69 yrs screened every 3yrs and 2.3% for rural women aged 18-69 yrs screened every 3yrs) (WHO/ICO HPV Information Centre) verses study estimates of 63 percent of women in developed countries receiving cervical cancer screening, with an upper range of 80 to 90 percent.*(4)* There has been excessive reliance on treatment-oriented approaches, neglecting prevention strategies. Also the late stage at presentation does not seem to have changed much over the past 30 years. The District Cancer Control Programme in selected districts was initiated but did not result in sustainable and productive activity.*(5)* This may be due to the low priority given to non communicable diseases and also absence of adequate health infrastructure and trained manpower.

Organized cancer prevention and control activities for the entire country do not exist. Current cancer control and screening activities are provided as opportunistic interventions in a sporadic manner, mostly at tertiary or secondary care facilities, mostly in urban settings. Diagnostic infrastructure in the country are also limited especially in the rural areas as most of pathology/cytology and treatment facilities remain concentrated in urban areas of the country. Appropriate and effective linkages between primary care and secondary or tertiary care facilities for diagnosis and treatment are lacking or are non-existent at places.

Cervical cancer is a disease that can be prevented through both primary prevention and early detection using screening techniques. Several screening modalities are now available for early detection of cervical cancer and its precursor lesions.

Cytology screening **:**

Even though the efficacy of cytology screening has never been proven through randomized trials, in developed countries, cytology-based services utilizing the Pap smear have been the

basis of cervical cancer screening and detection programs for many years. In fact, the Papanicolaou (Pap) smear is one of a unique group of tests that have been widely adopted into standard (western) clinical practice without first being subjected to rigorous, prospective blinded studies to examine their effectiveness. The data on the efficacy and effectiveness of cytology based screening come from observational studies of screening in defined populations. Studies using cohort, case-control of geographical correlation (before/after analysis) designs indicate substantial effects in reducing the cervical cancer incidence and mortality rates. *(6,7,8).*

The decreases in invasive cervical cancer incidence and mortality since the introduction of the Pap smear in the above regions have been so dramatic that it is one of the few interventions to receive an "A" recommendation from the U.S. Preventive Services Task Force even though there are no randomized trials demonstrating its effectiveness.*(9)*

It is the organized programmes that have shown the greatest effect, while using fewer resources than the unorganized programmes. Evidence of the effectiveness of this approach has led to the adoption of cervical cytology screening in all developed and some developing nations. However, no significant reduction in incidence and mortality from cervical cancer has been observed in developing countries where cytology screening programmes exist. *(10)*

Several large meta-analyses have indicated that both the sensitivity and specificity of cervical cytology are lower than previously thought. There is general agreement that high quality cytology is a highly specific screening test, with estimates of the order of 98-99%. Several recent meta-analyses have reported quite low Pap smear sensitivities—in the range of 50 percent but as low as 20 percent.*(11,12)* Authors of these studies note that decision makers should consider these findings highlighting low Pap test sensitivity when establishing health policies.

In the context of the successes of the cytology programs in reducing the burden of cervical cancers in selected developed countries and regions, it is important to recognize the limitations of cytology based programmes in developing country settings.

Cytology is a subjective test requiring highly trained manpower and in programmes without quality control/quality assurance it is virtually impossible to achieve and maintain the clinical performance of cytology.(13,14).

Cytology-based screening programs are logistically burdensome and hence can be costly. The many steps are required to be completed from both a clinical and programmatic perspective such as getting the women to participate in screening program, taking an adequate smear, transport of samples to the nearest secondary or tertiary care facility for further processing and analyzing the specimen. The report thus generated needs a system in place where by not only the women is informed of the results, but in case of an abnormal result can get the women back for follow up investigations. If any of these steps suffers setbacks due to technical, logistical or financial constraints the entire screening program collapses and, with it, offsets the potential public health benefit the program can offer. Thus despite the low cost of consumables and

because of the reasons cited above, high-quality cytology is expensive in absolute terms and may not necessarily be the most cost-effective option for screening.

Conventional cervical cytology is highly specific but false negative rates had always been an area of greatest concern in cytology based programmes. To address this issue and in the efforts to improve Pap smear performance, the focus had been on reducing the number of false negative smears. Several new technologies are being explored in an effort to improve the accuracy of Pap smears, liquid based cytology (LBC) being one of them.

Liquid-based cytology has logistical and operational advantages (interpretation at higher speed, lower rate of unsatisfactory smears and possibility of ancillary molecular testing using remnant fluid), but is more expensive and is neither more sensitive nor more specific than conventional cytology with respect to detection of histologically confirmed high-grade CIN *(15)*

Automation-assisted devices like computerized rescreening, algorithm-based rescreening is aimed at enhancing the performance of manual microscopic screening by excluding some of the normal slides from manual screening. In addition it is possible to identify the most abnormal areas of the slide or suspicious cells under the microscope and collect images into a gallery for review on computer screens. The USPSTF(2003) found poor evidence to determine whether new technologies, such as liquid-based cytology, computerized rescreening, and algorithm based screening, are more effective than conventional Pap smear screening in reducing incidence of or mortality from invasive cervical cancer. It is suggested that automation-assisted screening would not improve the outcome of an optimal cervical cytology service*.(9)*

HPV Screening

HPV causality for cervical cancer is now firmly established and is considered a necessary cause of the disease. In most women, the infection becomes undetectable relatively quickly. Women persistently infected with certain carcinogenic types are at increased risk of developing severe dysplasia and cervical cancer. Worldwide interest has grown in the potential for HPV testing in cervical cancer prevention programmes, both as an adjunct to cytological screening approaches and in primary screening. HPV 16 and 18 account for 70% of cervical cancer cases.

Results from various studies highlight that the accuracy of Hybrid Capture®2 (HC2) (Qiagen Gaithersburg, Inc. MD, USA (previously Digene Corp.)) for detection of high-grade lesions, the sensitivity ranged from 80-90% and specificity from 57-89%. For low-grade lesions, there was a drop in both sensitivity (64%) and specificity (65%). The pooled sensitivity for HPV (hybrid capture II) has been stated to be 66.5% (range 45.7-80.9%) with a specificity of 93.8%. *(16).* Several studies have shown that HPV negativity alone or in combination with negative cytology signiûes a longer disease free interval against CIN2+ than being negative for cytology alone. *(17,18)*

A single negative HPV DNA test thus provides a substantial 5–10 year reassurance against CIN3*(19)* and even cancer as shown in the trial in India*,(20)* thereby permitting cost-effective interval extensions between screenings for those women who are HPV DNA negative.

All reviews of the HPV-based screening have shown an improved sensitivity (by 30-35%) and a reduced specificity (by 8-12%) compared to conventional cytology.*(21,22)*

From the meta-analyses summarised above, it is abundantly clear that HPV DNA testing is substantially more sensitive than cytology at detecting high-grade CIN. However, HPV testing is somewhat less speciûc than cytology due primarily to the detection of transient infections that have not produced cytologic changes.*(14)*

In a cluster randomized, controlled trial in rural India, one of the largest randomised controlled trial of the three screening methods for cervical cancer in a low-resource setting, carried out from 2000 to the present, researchers evaluated the effects of a single round of HPV screening on the rates of advanced cervical cancer and cervical cancer deaths. More than 130,000 women (age range, 30-59 years) were assigned to 4 groups: HPV testing, cytologic testing, visual cervical inspection with acetic acid (VIA), and control group or no screening (the current standard of care in this area of India). Women screened for either HPV or abnormal cells had fewer cervical cancers in the following 8 years of follow-up compared with unscreened women. Rates of advanced cervical cancer and cancer-related deaths also were substantially lower in the HPV-screened group than in the cytologic-screened and VIA-screened groups. The significant reduction in advanced cancers and cervical cancer deaths following a single HPV testing is due to the possibility that HPV screening detected more pre cancer lesions with a higher potential of becoming cancer than those detected by visual screening or Pap smear. *(20)* Other studies also have shown that HPV screening is much more sensitive than cytologic testing for the detection of precancerous conditions. *(23,24)*

Detection of high –risk HPV DNA is considered to be potentially useful in four clinical applications: (1) as a primary screening test, solely or in combination with a Pap smear to detect cervical cancer precursors; (2) as a triage test to triage women with equivocal Pap smear who would need further diagnostic investigations. (3) in the continuing management of women referred for colposcopy for whom no lesion could be visualised; and (4) as a follow-up test for women treated for high-grade intraepithelial lesion with local ablative or excisional therapy to more rapidly and accurately identify women who have or have not been cured by their treatment.*(14)*

Self sampling for HPV DNA

In an attempt to improve population coverage of screening in settings where acceptance in the socio cultural context as well as accessibility towards screening procedures limits acceptance, several studies have evaluated the diagnostic accuracy of self-collected vaginal specimens using swabs, tampons, or brushes for HPV and found an overall relative sensitivity

of 74% and specificity of 84% for the self-taken sample, though not as good as a clinician taken sample. The studies showed good acceptance among women and the sensitivity compares favourably to cytology though less specific ,thus could be a valuable screening method for women who refuse to attend clinic-based screening.*(25-28).*

The parallel development of fast, accurate and affordable HPV tests, suitable for use in developing countries, makes HPV testing a feasible screening approach in low-resource settings and should go hand in hand with further developments in affordable and effective vaccines to prevent infection by the two major types of HPV responsible for cervical cancer development. "Although HPV testing will avoid the variation and subjectivity in test interpretation and minimize efforts required in quality assurance, high participation for screening and treatment of precancers and cancers are critical to successful screening programmes leading to reduce disease burden in all settings.

However, the applicability of these success stories in Low Middle Income Countries is questionable; cytology based screening is resource intensive in terms of infrastructure, equipment and manpower, HPV screening still not a feasible approach due its cost .

This has led to a search for alternative screening methods that can be more cost-effective for application in low-resources settings. Visual inspection-based screening tests, such as naked eye visual inspection or 'downstaging', visual inspection with 3-5% acetic acid (VIA), VIA with magnification (VIAM), and visual inspection post application of Lugol's iodine (VILI), are a set of alternative screening mechanisms which have been studied for their effectiveness in LMIC settings, including in India

Visual inspection Tests

Visual inspection of the cervix has re-emerged as a screening tool for LMICs, despite its limited specificity, since it is economical and provides immediate results. The test is cost effective and even paramedical including non medical health care providers at primary care facilities can be easily trained to perform visual inspection in a relatively short period of time for primary screening requiring minimal health care infrastructure with results of the procedure available immediately for initiating treatment at the same visit. The test characteristics of VIA have been evaluated in several cross-sectional studies in LMICs. These studies together have involved more than 150,000 women and have shown promising results that support its use as an alternative to conventional cytology. The sensitivity of VIA to detect high-grade precursor lesions and invasive cervical cancer has varied from 49-96% and the specificity from 49-98%. *(29-33)*

Pooled estimates of sensitivity vary from 62-80% and specificity from 77-84% for VIA (Visual Inspection with Acetic Acid) to detect high-grade CIN, after adjusting for the effects of verification bias. VIA showed a sensitivity of 79% (95% CI 73–85%) and 83% (95% CI 77–89%), and a specificity of 85% (95% CI 81–89%) and 84% (95% CI 80–88%) for the outcomes CIN2+ or CIN3+, respectively. VIAM (Visual inspection with Acetic Acid plus Magnification) showed similar

results as VIA. VILI (Visual Inspection with Lugo's Iodine) was on an average 10% more sensitive and equally specific. The specificity values of VILI varied over a similar range as VIA, between 73.0 and 91.6%. The overall pooled sensitivity for VILI (91.2%; CI 87.8–94.6%) was statistically significantly higher than for VIA. On the other hand, the pooled specificity of VILI (84.5%; CI 81.3–87.8%) was not significantly different from that of VIA. *(34,35)*

VIA screening followed by treatment reduces the rate of cervical cancer compared with no screening was demonstrated in the only large cluster randomized trial in Southern India to address the use of this test in a low resource setting. At seven-year follow-up, there were 167 cervical cancer cases and 83 cervical cancer deaths in the intervention group, compared with 158 cases and 92 deaths in the control group (incidence hazard ratio: 0.75; 95% CI 0.55–0.95; and mortality hazard ratio: 0.65; 95% CI: 0.47–0.89) . The trial thus showed that a single round of screening with VIA reduces cervical cancer incidence and mortality by 25% and 35% respectively. The authors concluded that good training of providers and sustained quality assurance are vital for VIA screening to succeed in preventing cervical cancer in routine settings.*(36)*

Another cluster randomized, controlled trial of cervix cancer screening in Mumbai, India, is showing a 24% reduction in cervical cancer mortality after three rounds of VIA screening.*(37)*

Coexisting vaginal or cervical infection with Trichomonas vaginalis, Chlamydia trachomatis, or Neisseria gonorrhoeae did not alter the sensitivity or specificity of VIA for CIN2+ in a cross-sectional study of 2754 women in South Africa.*(38)*

Cost effectiveness studies based on data from India, Kenya, Peru, South Africa, and Thailand indicate that the most cost-effective strategies for cervical screening are those approaches requiring the fewest visits, leading to improved follow-up testing and treatment. The analyses reports that screening women once in their lifetime, at the age of 35 years, with a one- or two-visit screening strategy involving VIA or HPV testing reduced the lifetime risk of cancer by approximately 25–36%, and costs less than 500 dollars per year of life saved.*(39,40)*

HPV Vaccine

Currently two HPV vaccines are commercially available Gardasil (Quadrivalent - HPV 16,18,6 and 11) and Cervarix (Bivalent – HPV 16 and 18). The results of Phase III clinical trials are now available and these two vaccines are now licensed in some 120 countries. Many HICs (High Income Countries) have introduced HPV vaccines into routine vaccination programs. The majority of the critical Phase III results for both vaccines are available for women in the 15-26 age range.*(41,42)* Several supplementary studies have been completed or are under way, including immune-bridging studies in 9-15 year-old girls and boys and efficacy studies in 26-45 year-old women, as well as studies in adult men and special populations (immune-suppressed and other).

These two vaccines have so far shown high efficacy with reference to the predefined end point lesions (HPV 16 or 18-related CIN 2 or more, CIN 2+), adequate safety and tolerability, high immunogenicity, long-term protection (currently 7-8 years) and strong indications of its ability to induce immune memory. Some degree of cross-protection against CIN 2+ related to HPV types 31 for both vaccines and HPV 33 and 45 for the bivalent vaccine has been documented.

None of the vaccines has shown therapeutic activity. Very high coverage rates (65-70%) are seen in Australia (with the quadrivalent vaccine up to 26 years) and in UK (with the bivalent vaccine in girls aged 12-13 years and the catch-up population of up to 18 years).*(43)* Results on the reduction of CIN 1 + and CIN 2 + in these populations are expected to be seen by the end of 2011.

The limitations of current vaccines i.e. the lack of therapeutic effect and the limited impact of cross-protection effect requires the continuation of screening programs in the vaccinated women. The cost of the vaccine is very high and is currently unaffordable in LMICs.*(44-48)*

Conclusion :

The health infrastructure and organizational aspects for population based screening programmes in India is currently lacking. There are various limitations in undertaking population based cervical cancer screening programmes in India. Barriers to implementing programs for cervical cancer prevention include competing health care demands, economic, social, health policy and programmatic issues. The resources and infrastructure too vary widely in different parts of the country and within states.

Prevention of cervical cancer through screening has been identified as one of the main goals under the National Cancer Control Program of the Government of India (NCCP) and would be implemented through Districts Cancer Control Program (DCCP) as and when feasible. In the context of adopting any feasible cost effective evidence based technology the program needs health care organizational restructuring to meet the requirements of a population based screening program which includes.

1. Strengthening the primary health care system coupled with cancer awareness
2. Training of relevant health care personnel.
3. Deciding on the appropriate age for screening to ensure that the appropriate target group for the programme is screened.
4. Regular supply of logistics.
5. Establishment of linkages to a reliable laboratory at secondary or tertiary care facility.
6. Establishing information systems for timely communication of test results with follow up for diagnostic workup and treatment where necessary.

7. Adopting strategies to minimize multiple visits eg. 'see-and-treat' approach in the same sitting to improve compliance for diagnosis and treatment.
8. Establishing functional and effective referral systems for diagnosis and treatment.
9. Minimizing reporting delays and subsequent loss of follow up:

The magnitude of the problem of cervical cancer, and the potential for prevention, makes it imperative to identify a feasible strategy in the Indian settings.

Evidence and experience generated from district level programmes will help in substantially refining the approaches to develop a nationwide cervical cancer screening programme in the near future.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C and Parkin DM. GLOBOCAN 2008, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet]. Lyon, France: International Agency for Research on Cancer; 2010. Available from: ttp://globocan.iarc.frGlobocan 2008).
2. Vallikad E, "Cervical Cancer: The Indian Perspective", International Journal of Gynecology Obstetrics, 95(1) (November 2006): S215-S233.
3. Kurkure AP, and Yeole BB, "Social inequalities in cancer with special reference to South Asian countries", Asian Pacific Journal of Cancer Prevention, 7(1) (Jan-March 2006): 36-40.)
4. Gakidou E, Nordhagen S, Obermeyer Z. Coverage of cervical cancer screening in 57 countries: low average levels and large inequalities. PLoS Med 2008; 5:e132
5. Krishnan Nair, Cherian Varghese, R. Swaminathan .Cancer: Current scenario, intervention strategies and projections for 2015; NCMH Background Papers· Burden of Disease in India.
6. *Evaluation of Cervical Cytology*. Summary, Evidence Report/Technology Assessment: Number 5, January 1999. Agency for Health Care Policy and Research, Rockville, MD. http://www.ahrq.gov/clinic/epcsums/cervsumm.htm
7. IARC handbooks of cancer prevention, Cervix cancer screening vol. 10, International Agency for Research on Cancer Press, Lyon, France (2005).
8. Henry C. Kitchener, Philip E. Castle and J. Thomas Cox, Chapter 7: Achievements and limitations of cervical cytology screening, Vaccine Volume 24, Supplement 3, 21 August 2006, Pages S63-S70)
9. U.S. Preventive Services Task Force. *Guide to Clinical Preventive Services*, 2nd ed. Washington, DC: Office of Disease Prevention and Health Promotion; 2003. http://www.ahrq.gov/clinic/epcsums/cervsumm.htm
10. Sankaranarayanan R, Budhuk A, Rajkumar R. Effective screening programs for cervical cancer in low- and middle-income developing countries. Bull World Health Organization 2001;79:954–62.

11. Nanda K, McCrory D, Myers E, et al. Accuracy of the Papanicolaou test in screening for and followup of cervical cytologic abnormalities: a systematic review. Annals of Internal Medicine 16;132(10): 810–819 (May 2000).
12. Fahey M, Irwig L, Macaskill P. Meta-analysis of Pap test accuracy. American Journal of Epidemiology 141:680–689 (1995)
13. Goldie SJ, Gaffikin L, Goldhaber-Fiebert JD, Gordillo-Tobar A, Levin C, Mahe C, et al. Cost-effectiveness of cervical-cancer screening in five developing countries. N Engl J Med 2005;353(20):2158–68.
14. Jack Cuzick, Marc Arbyn, Rengaswamy Sankaranarayanan, Vivien Tsu, Guglielmo Ronco etal. ICO Monograph Series on HPV and Cervical Cancer: Overview of Human Papillomavirus-Based and Other Novel Options for Cervical Cancer Screening in Developed and Developing Countries ;Vaccine Volume 26, Supplement 10, 19 August 2008, K29-K41.
15. Arbyn M, Bergeron C, Klinkhamer P, Martin-Hirsch P, Siebers AG, Bulten J. Liquid compared with conventional cervical cytology: a systematic review and metaanalysis. Obstet Gynecol 2008;111(1):167–77.
16. Cuzick J, Sasieni P, Davies P, Adams J, Normand C, Frater A, et al . A systematic review of the role of human papilloma virus (HPV) testing within a cervical screening program: Summary and conclusions. Br J Cancer 2000;83:561-5.
17. Sherman ME, Lorincz AT, Scott DR, Wacholder S, Castle PE, Glass AG, et al. Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. J Natl Cancer Inst 2003;95(1):46–52.
18. Clavel C, Cucherousset J, Lorenzato M, Caudroy S, Nou JM, Nazeyrollas P, etal. Negative human papillomavirus testing in normal smears selects a population at low risk for developing high-grade cervical lesions. Br J Cancer 2004;90(9):1803–8.
19. Dillner J, Rebolj M, Birembaut P, Petry KU, Szarewski A, etal. Long term predictive values of cytology and human papillomavirus testing in cervical cancer screening: joint European cohort study. BMJ. 2008 Oct 13;337:a1754. doi: 10.1136/bmj.a1754.
20. Sankaranarayanan R, Nene BM, Shastri SS, Jayant K, Muwonge R, Budukh AM, Hingmire S, Malvi SG, Thorat R, Kothari A, Chinoy R, Kelkar R, et al. HPV screening for cervical cancer in rural India. N Engl J Med 2009;360:1385-94.
21. Cuzick J, Arbyn M, Sankaranarayanan R, et al. Overview of human papillomavirus-based and other novel options for cervical cancer screening in developed and developing countries. Vaccine 2008;26(Suppl 10):K29-41.
22. Arbyn M, Sasieni P, Meijer CJ, et al. Clinical applications of HPV testing: a summary of meta-analyses. Vaccine 2006;24(3):S378-89.
23. Bulkmans NW, Berkhof J, Rozendaal L, van Kemenade FJ, Boeke AJ, Bulk S, et al. Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial. Lancet 2007;370:1764-72.

24. Mayrand MH, Duarte-Franco E, Rodrigues I, Walter SD, Hanley J, Ferenczy A, et al. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. N Engl J Med 2007;357:1579-88.

25. Ogilvie GS, Patrick DM, Schulzer M, Sellors JW, Petric M, Chambers K, et al. Diagnostic accuracy of self collected vaginal specimens for human papillomavirus compared to clinician collected human papillomavirus specimens: a meta-analysis. Sex Transm Infect 2005;81(3):207–12.

26. Nobbenhuis MA, Helmerhorst TJ, van den Brule AJ, Rozendaal L, Jaspars LH, Voorhorst FJ, et al. Primary screening for high risk HPV by home obtained cervicovaginal lavage is an alternative screening tool for unscreenedwomen. J Clin Pathol 2002;55(6):435–9.

27. Brink AA,Meijer CJ, WiegerinckMA, Nieboer TE, Kruitwagen RF, van Kemenade F, et al. High concordance of results of testing for human papillomavirus in cervicovaginal samples collected by two methods, with comparison of a novel self-sampling device to a conventional endocervical brush. J Clin Microbiol 2006;44(7):2518–23.

28. Szarewski A, Cadman L, Mallett S, Austin J, Londesborough P, Waller J, et al. Human papillomavirus testing by self-sampling: assessment of accuracy in an unsupervised clinical setting. J Med Screen 2007;14(1):34–42

29. Sankaranarayanan R, Basu P, Wesley RS, Mah_e C, Keita N, et al. Accuracy of visual screening for cervical neoplasia: results from an IARC multicentre study in India and Africa. Int J Cancer 2004;110:907–13.

30. Sankaranarayanan R, Wesley R, Thara S, Dhakad N, Chandralekha B et al . Test characteristics of visual inspection with 4% acetic acid (VIA) and Lugol's iodine (VILI) in cervical cancer screening in Kerala, India. Int J Cancer. 2003;106:404–8.

31. Sankaranarayanan R, Shastri SS, Basu P, Mahe C, Mandal R, AminG, et al. The role of low-level magnification in visual inspection with acetic acid for the early detection of cervical neoplasia. Cancer Detect Prev 2004;28:345–51.

32. R. Sankaranarayanan, L. Gaffikin, M. Jacob, J. Sellors and S. Robles, A critical assessment of screening methods for cervical neoplasia. Int J Gynaecol Obstet, 89 Suppl. 2 (2005), pp. S4–S12.

33. Marc Arbyn, Rengaswamy Sankaranarayanan, Richard Muwonge, Namory Keita, Amadou Dolo, Int. Journal Of 2008 Jul 1;123(1):153-60

34. C. Mahe and L. Gaffikin, Screening test accuracy studies: how valid are our conclusions? Application to visual inspection methods for cervical screening. Cancer Causes Control, 16 6 (2005), pp. 657–666.

35. Catherine Sauvaget , Jean-Marie Fayette a, Richard Muwonge,Ramani Wesley, Rengaswamy Sankaranarayanan Accuracy of visual inspection with acetic acid for cervical cancer screening International Journal of Gynecology and Obstetrics 113 (2011) 14–24.

36. Sankaranarayanan R, Esmy PO, Rajkumar R, Muwonge R, Swaminathan R, Shanthakumari S, Fayette JM, Cherian J. Effect of visual screening on cervical cancer incidence and mortality in Tamil Nadu India: a cluster-randomised trial. Lancet 2007;370:398–406.
37. Unpublished personal communication dated October 1, 2011, from Dr. S. S. Shastri, PI – Mumbai trial for Breast and Cervical Cancer Screening, NIH RO1 awarded study.
38. Denny L, Kuhn L, Pollack A, Wright TC Jr. Direct visual inspection for cervical cancer screening: an analysis of factors influencing test performance. Cancer 2002; 94:1699.
39. Goldie S, Gaffikin L, Goldhaber-Fiebert J, Gordillo-Tobar A, Levin C, Mahe C, Wright T: Cost effectiveness of cervical screening in five developing countries. N Engl J Med 2005; 353: 2158–2168.
40. Legood R, Gray AM,Mahe C,Wolstenholme J, Jayant K,Nene BM, et al. Screening for cervical cancer in India: How much will it cost? A trial based analysis of the cost per case detected. Int J Cancer 2005;117:981–7.
41. Bosch FX, Castellsague X, de Sanjose S. HPV and cervical cancer: screening or vaccination? Br J Cancer 2008;98:15-21.
42. Bosch FX, de Sanjose S, Castellsague X. Evaluating the potential benefits of universal worldwide human papillomavirus vaccination. Therapy 2008;5:305-12.
43. Fairley CK, Hocking JS, Gurrin LC, et al. Rapid decline in presentations of genital warts after the implementation of a national quadrivalent human papillomavirus vaccination programme for young women. Sex Transm Infect 2009;85:499-502.
44. Markowitz LE, Dunne EF, Saraiya M, et al. Quadrivalent human papillomavirus vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2007;56(RR-2):1-24.
45. European Center for Disease Prevention and Control (ECDC). Guidance for the introduction of HPV vaccines European countries. Available from: http://ecdc. europa.eu/pdf/ HPV_report pdf. September 2008.
46. World Health Organization. Global Advisory Committee on Vaccine Safety, 17 — 18 December 2008. Wkly Epidemiol Rec 2009;84:37-40 Available from: http:// www.who.int/ vaccine_safety/en/
47. Schiller JT, Castellsague X, Villa LL, Hildesheim A. An update of prophylactic human papillomavirus L1 virus-like particle vaccine clinical trial results. Vaccine 2008;26(Suppl 10):K53-61.
48. Centers for Disease Control and Prevention. Reports of health concerns following HPV vaccination. Available from: http://www.cdc.gov/vaccinesafety/ vaers/gardasil.htm.

Selected Abstracts – Indian studies

Sankaranarayanan R, Nene BM, Shastri SS, Jayant K, Muwonge R, Budukh AM, et al .HPV screening for cervical cancer in rural India. N Engl J Med. 2009 Apr 2;360(14):1385-94.

Abstract

BACKGROUND: In October 1999, we began to measure the effect of a single round of screening by testing for human papillomavirus (HPV), cytologic testing, or visual inspection of the cervix with acetic acid (VIA) on the incidence of cervical cancer and the associated rates of death in the Osmanabad district in India.

METHODS: In this cluster-randomized trial, 52 clusters of villages, with a total of 131,746 healthy women between the ages of 30 and 59 years, were randomly assigned to four groups of 13 clusters each. The groups were randomly assigned to undergo screening by HPV testing (34,126 women), cytologic testing (32,058), or VIA (34,074) or to receive standard care (31,488, control group). Women who had positive results on screening underwent colposcopy and directed biopsies, and those with cervical precancerous lesions or cancer received appropriate treatment.

RESULTS: In the HPV-testing group, cervical cancer was diagnosed in 127 subjects (of whom 39 had stage II or higher), as compared with 118 subjects (of whom 82 had advanced disease) in the control group (hazard ratio for the detection of advanced cancer in the HPV-testing group, 0.47; 95% confidence interval [CI], 0.32 to 0.69). There were 34 deaths from cancer in the HPV-testing group, as compared with 64 in the control group (hazard ratio, 0.52; 95% CI, 0.33 to 0.83). No significant reductions in the numbers of advanced cancers or deaths were observed in the cytologic-testing group or in the VIA group, as compared with the control group. Mild adverse events were reported in 0.1% of screened women.

CONCLUSIONS: In a low-resource setting, a single round of HPV testing was associated with a significant reduction in the numbers of advanced cervical cancers and deaths from cervical cancer.

Gravitt PE, Paul P, Katki HA, Vendantham H, Ramakrishna G, Sudula M et al .Effectiveness of VIA, Pap, and HPV DNA testing in a cervical cancer screening program in a peri-urban community in Andhra Pradesh, India. PLoS One. 2010 Oct 28;5(10):e13711.

Abstract

BACKGROUND: While many studies have compared the efficacy of Pap cytology, visual inspection with acetic acid (VIA) and human papillomavirus (HPV) DNA assays for the detection cervical intraepithelial neoplasia and cancer, few have evaluated the program effectiveness.

METHODS AND FINDINGS: A population-based sample of 5603 women from Medchal Mandal in Andhra Pradesh, India were invited to participate in a study comparing Pap cytology, VIA, and HPV DNA screening for the detection of CIN3+. Participation in primary screening and all subsequent follow-up visits was rigorously tracked. A 20% random sample of all women screened, in addition to all women with a positive screening test result underwent colposcopy with directed biopsy for final diagnosis. Sensitivity, specificity, positive and negative predictive values were adjusted for verification bias. HPV testing had a higher sensitivity (100%) and specificity (90.6%) compared to Pap cytology (sensitivity = 78.2%; specificity = 86.0%) and VIA

(sensitivity = 31.6%; specificity = 87.5%). Since 58% of the sample refused involvement and another 28% refused colposcopy or biopsy, we estimated that potentially 87.6% of the total underlying cases of CIN3 and cancer may have been missed due to program failures.

CONCLUSIONS: We conclude that despite our use of available resources, infrastructure, and guidelines for cervical cancer screening implementation in resource limited areas, community participation and non-compliance remain the major obstacles to successful reduction in cervical cancer mortality in this Indian population. HPV DNA testing was both more sensitive and specific than Pap cytology and VIA. The use of a less invasive and more user-friendly primary screening strategy (such as self-collected swabs for HPV DNA testing) may be required to achieve the coverage necessary for effective reduction in cervical cancer mortality

Bhatla N, Suri V, Basu P, Shastri S, Datta SK, Bi D et al .Immunogenicity and safety of human papillomavirus-16/18 AS04-adjuvanted cervical cancer vaccine in healthy Indian women. J Obstet Gynaecol Res. 2010 Feb;36(1):123-32.

Abstract

AIM: India has the highest number of annual incident cases and mortality rates for cervical cancer worldwide. This study was conducted to assess the immunogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted cervical cancer vaccine in healthy Indian women aged 18-35 years old.

METHODS: This double-blind, randomized (1:1), controlled and multicenter trial with two parallel groups, the Vaccine and Placebo groups, included 354 subjects in four centers across India. Subjects were given GlaxoSmithKline's HPV-16/18 AS04-adjuvanted cervical cancer vaccine or aluminum hydroxide placebo according to a 0, 1 and 6 month schedule and followed up until month 7. Serum samples were drawn at pre-vaccination and at month 7. Safety data were collected throughout the study.

RESULTS: A total of 330 subjects completed the study. One month post-Dose 3, all initially seronegative subjects in the Vaccine group had seroconverted for HPV-16 and HPV-18 antibodies with anti-HPV-16 and anti-HPV-18 geometric mean titer levels of 10226.5 EL.U/ml (95% confidence interval: 8847.1-11821.0) and 3953.0 EL.U/ml (95% confidence interval: 3421.8-4566.8), respectively. Initially seropositive subjects also showed an increase to similar geometric mean titer levels. Six serious adverse events (two in the Vaccine group and four in the Placebo group), all unrelated to vaccination, were reported. Commonly reported solicited local (injection-site pain) and general (fatigue, headache and fever) symptoms were similar in both groups. Compliance to the three-dose vaccination course was >97%.

CONCLUSIONS: The AS04-adjuvanted HPV-16/18 cervical cancer vaccine was highly immunogenic and generally well-tolerated making it a potential tool for prevention and control of cervical cancer in India.

HPV and Head and Neck Cancer

Head and neck cancer includes cancers of the oral cavity, oropharynx, hypopharynx, larynx, sinonasal tract, and nasopharynx and is commonly of the squamous cell carcinoma type (HNSCC). HNSCC is the sixth most common type of cancer in the world reported annually, and of these, approximately 10% (or more for some geographic locations) are OSCC(oropharyngeal squamous cell carcinoma) . Globally, the incidence and localization of HNSCC varies widely. It is the most common form of cancer in India.

Demographics

Men are generally more often affected than women. Smoking, alcohol consumption, and betel chewing are traditional risk factors for HNSCC and OSCC However, during the past decade several reports have documented HPV in OSCC. The first clue to this was suggested in 1983 by a Finnish team led by Stina Syrjänen, who noted that 40% of the cancers in their study contained histological and morphological similarities with HPV-associated lesions.

Several studies indicate that oral HPV infection is likely to be sexually acquired. Risk factors among men include young age at first intercourse, number of sexual partners and a history of genital warts. Risk is elevated among women with a high number of sexual partners. Specific sexual behaviors have been more strongly associated with risk of an HPV-positive tumor, including a history of performing oral sex and oral-anal contact.

Clinical evidence also suggest that HPV-associated OSCC could be biologically distinct from classical OSCC. Tobacco-associated OSCC are more frequent in men, while men and women are at equal risk of HPV-associated OSCC. In addition, patients with HPV-associated OSCC are often nonsmokers and nondrinkers and on average 5 years younger than their tobacco-use-associated counterparts. Some reports have indicated that the HPV subtypes associated with OSCC are similar but not identical to HPVs found in cervical carcinoma. Chen *et al*. showed that

HPV-associated HNSCC harbor HPV-16 subtypes with characteristic changes in their promoter/enhancer region that render them particularly active in oral keratinocytes.

Pathogenesis

The dominance of HPV 16 in HPV+ HNSCC (85%-95%) is even greater than that seen in cervical carcinoma (approximately 50-60%) of total worldwide cases. This is likely to reflect a difference in life cycles of the different HPV subtypes in different mucosal locations, with an associated difference in mucosal immune responses.It is still unknown how persistent infection gives rise to intraepithelial lesions, and, in turn, to invasive HNC. It is known that oncogenic types of HPV may produce non productive-infection and persist in the cells in low number episomic molecules. It is well known that HPVs exert their oncogenic role after DNA integration, gene expression of E5, E6 and E7 loci and p53/pRb host proteins suppression, leading to increased cell proliferation and contributing to carcinogenesis .Although integration of HPV DNA into genomic DNA is a common event in cervical carcinoma and intraepithelial lesions, however, 15-30% of cervical cancer, containing HPV only in the episomal form, shows a plasmidic expression of oncogenic protein E5, E6, E7. The situation in head and neck cancers is not clear, but heterogeneity and the existence of multiple pathways in carcinogenesis is highly likely.

A recent study of lymph-node metastases in HNSCC reported that p16 protein overexpression is a surrogate marker for oropharyngeal origin and HPV-association. A role for HPV in oropharyngeal tumors is further substantiated by distinct molecular genetic alterations in HPV-positive versus HPV negative tumors. As for many cancers, inactivation of the p53 and pRb pathways is a common event in the molecular progression of HNSCC. However, inactivation occurs by different mechanisms in HPV positive and negative tumors. In HPV-positive HNSCC, genetic alterations are reflective of viral oncogene function. For instance, HPV-positive tumors tend to have wild-type p53, because p53 is functionally inactivated by viral E6 oncoprotein. By contrast, HPV-negative tumors have specific p53 mutations demonstrated to be induced by carcinogens in tobacco smoke. As another example, pRb function is inactivated by viral E7 protein in the HPV-positive tumor, but in HPV-negative tumors, the pRb pathway is altered by other mechanisms. More complex differences in regions of chromosomal loss and gain have been demonstrated in HPV-positive versus -negative tumors through techniques such as comparative genomic hybridization and microsatellite analysis.

Clinical implications of HPV positive Head and neck cancers

There is sufficient evidence to conclude that a diagnosis of HPV-positive HNSCC has significant prognostic implications; these patients have at least half the risk of death from HNSCC when compared with the HPV-negative patient. The HPV etiology of these tumors may have future clinical implications for the diagnosis, therapy, screening, and prevention of HNSCC.

HPV related oropharyngeal carcinoma is a distinct disease entity. Patients often present with a small primary tumour and large neck nodes. This may lead to delayed diagnosis.

Accumulating evidence suggests that HPVpositive status is an important prognostic factor associated with a favourable outcome in head and neck cancers The favorable outcome of HPV induced oropharyngeal cancers might be attributable to the absence of field cancerization or enhanced radiation sensitivity.

HPV detection may have future implications for the diagnosis, prognosis, therapeutics, and prevention of HNSCC. For diagnostic purposes, HPV detection in cervical lymph nodes of patients presenting with an occult primary may be used to establish with high specificity, the location of the primary within the oropharynx.

With regard to prognosis, patients with HPV-positive tumors have improved prognosis when compared with patients with HPVnegative tumors in the majority of studies. Studies to date have suggested that HPV positive patients may have as much as 60% to 80% reduction in risk of dying from their cancer when compared with the HPV-negative patient Negative studies may be explained by inadequate sample size, follow-up time, and residual confounding by other prognostically significant variables. The reason for the improved survival is unclear; however, improved radiation responsiveness, immune surveillance to viral antigens, and the absence of field cancerization in these patients who tend to be nonsmokers, have been postulated. The term 'field cancerization' is used to describe the presence of carcinogen induced early genetic changes in the epithelium from which multiple independent lesions arise, leading to the development of multifocal tumors.

In addition, E6-related degradation of p53 in HPV-positive cancers may be functionally inequivalent to HPV-negative p53 mutations, and therefore, HPV-positive tumors may have an intact apoptotic response to radiation and chemotherapy. Possible therapeutic implications of an HPV-positive diagnosis are an active area of investigation. This includes selection of patients for organ preservation therapy, which may be more successful in patients with HPV-HNSCC.**I** The improved prognosis and treatment responses to chemotherapy and radiotherapy by HPV positive tumours may suggest that HPV status detection is required to better plan and individualize patient treatment regimes.

How to make a diagnosis of HPV-HNSCC

The diagnosis of HPV-HNSCC should be considered in all squamous cell carcinomas that arise from the lingual and palatine tonsils. Suspicion should be high for cancers in nonsmokers and nondrinkers, patients with basaloid or poorly differentiated histology, the young patient, the immunosuppressed patient and the patient with Fanconi anemia. HPV-DNA detection *per se* in an OSCC does not prove causal association. Only HPV DNA that is transcriptionally active is biologically and clinically relevant.

Molecular detection of HPV

Paradoxically, the low number of HPV DNA copies in integration or episomic status may be underestimated by standard immunopathological studies. When performing the molecular

detection of HPV DNA, it is essential that the diagnostic procedures employed are highly sensitive, specific and reliable and it should kept in mind that the efficiency of HPV detection may be affected by several methodological variables.

In situ hybridization and in situ oncogenic protein staining techniques have increased sensitivity and specificity of HN diagnostic practices. These techniques have allowed not only detection and identification of low risk/high risk HPV in cytological smears or histopathological immune-sections but, in addition, also the definition of the topographical level of infection, and/or viral integration status (basal layer-integration/upper layers - lytic - episomic phases). Furthermore, these techniques have provided to calculate the copy number per cell (one-two nuclear spot for integration/diffuse nuclear signals for episomic

In routine IHC the expression of viral HPV proteins E5, E6, E7 as surrogate markers of HPV infection, and the resulting down-regulation of critical tumor suppressor (p53, pRB, p-16) in histopathologically analyzed HNC can be demonstrated. In any case, we must remember that p16 immunohistochemical positivity is unable to discriminate between HPV integrated vs HPV not integrated OSCCs. IHC and ISH are considered methods with a low sensitivity because of the limited availability of antibodies against specific types of HPV (IHC) or the low applicability in clinical routine for the long and hard technical word required (ISH).

An highly sensitive broad-spectrum detection of human papillomaviruses should be performed for HPV detection on paraffin-embedded sections collected during diagnostic procedures. Today methods with higher sensitivity (PCR) than the classical imunohistochemical or ISH techniques are able to identify HPV, by detection with type-specific primers or consensus primers.The high sensitive and specific SPF10 HPV DNA test, determined by direct sequencing of PCR fragments and genotyping assay, as good screening test, can be performed for HPV detection on paraffin-embedded sections collected during diagnostic procedures as good screening test .PGMY/GP nested PCR system is able to perform consistently at a high level of sensitivity, namely 0.1-1 copy per cell

However the possibility of overestimation or underestimation of HPV positivity due to technical limitations, the absence of standardization in collection, storing and analyses of tissue samples, the impact in the clinical practice, the cost and the commercial availability are important aspects to consider, in order to establish the exact role of HPV in oral and oropharyngeal lesions and its real tumoral frequency in an anatomical region where HPV has a yet high prevalence.

In clinical setting, nowadays we have to perform a sensible, specific and accurate HPV test. In case of false negative results, patients are potentially deprived of important curative tools i.e. radio and chemotherapy. Similarly, false positive patients are potentially deprived of another curative tool: the surgery. Only standardized technical procedures could assist clinicians to provide effective diagnostic test, innovative treatment and more efficient screening systems for OSCC patients.

Prevention

Clinical trials to evaluate the efficacy of the quadrivalent HPV vaccine in protecting against oral HPV infection are currently in development. Current generation HPV 16 and 18 L1 VLP vaccines hold potential promise for the prevention of a greater majority of HPV positive oral cancers than for cervical cancer. This is due to the narrow HPV type distribution for oral cancers. Worldwide, HPV 16 consistently accounts for 86% to 95% of HPV-DNA positive head and neck cancers and the remainder of these cancers are positive for HPV-DNA from phylogenetically-related members of the A9 clade.

The increasing incidence of HPV-associated oral cancer (oropharyngeal cancer) underscores the potential importance of cancer prevention via HPV prophylactic vaccination of both women and men. Currently, vaccines targeted against oncogenic HPV infection have been indicated for use in women only. Vaccinating males against oncogenic HPV infection may be a particularly important approach for the prevention of oral cancer, given the incidence is higher in men. Clinical trials to evaluate the potential for vaccines to prevent oral HPV 16 infection are in the developmental stages. Therapeutic HPV vaccination strategies are also being developed.

References

1. Peter KC Goon, Margaret A Stanley, Jörg Ebmeyer, et al.HPV & head and neck cancer: a descriptive update: *Head & Neck Oncology* 2009.
2. H Mehanna,1 V Paleri, C M L West,C Nutting Head and neck cancer—Part 1: Epidemiology, presentation, and prevention BMJ *2010;341:c4684*
3. Pannone et al .The role of human papillomavirus in the pathogenesis of head & neck squamous cell carcinoma: an overview;. Infectious Agents and Cancer 2011, 6:4
4. Torbjörn Ramqvist and Tina Dalianis ,Oropharyngeal Cancer Epidemic and Human Papillomavirus Emerging Infectious Diseases 2010: 16(11)
5. Maura L. Gillison,Human papillomavirus-related Diseases: Oropharynx Cancers and Potential Implications for Adolescent HPV Vaccination J Adolesc Health. 2008 October ; 43(4 Suppl): S52–S60

Selected Abstract- Indian Studies

Barwad A, Sood S, Gupta N, Rajwanshi A, Panda N, Srinivasan R.Human papilloma virus associated head and neck cancer: A PCR based study. Diagn Cytopathol. 2011 Apr 6.

Abstract

Head and neck cancers (HNC), 90% of which are squamous cell carcinomas (SCC), rank sixth among all malignancies worldwide and comprise 40-50% of the total number of malignancies in India. In addition to alcohol and tobacco usage, which is the major source of oral carcinogens, viruses such as human papilloma virus (HPV) may also contribute to

development of the malignancy. The aim of this study was to identify the prevalence of HPV in head and neck cancers using material from metastatic site. A total of 111 cases of neck nodal metastases were included in this study. The primary was identified as oral cavity, oropharynx and nasopharynx. In a subset, the primary remained "unknown." Polymerase chain reaction was carried out to detect HPV DNA on the fine needle aspirates. HPV was detected in 32.4% cases. Maximum positivity was observed in metastases from primary in the oral cavity (47.1%) with tongue (55%), followed by oropharynx (25%) and nasopharynx (5%) cases. In the unknown primary group, HPV was detected in 52.9% cases. Study defines the association of HPV with HNC in population of northern India. There was varied association of HPV depending on site of primary tumor arising in mucosal surfaces of head and neck region.

Koppikar P, deVilliers EM, Mulherkar R.Identification of human papillomaviruses in tumors of the oral cavity in an Indian community. Int J Cancer. 2005 Mar 1;113(6):946-50.

Abstract

Oral cancers and other squamous cell cancers of the head and neck are common cancers in India, primarily due to tobacco chewing/smoking and alcohol consumption. Recent reports indicate involvement of human papillomavirus (HPV), HPV 16, in a subset of squamous cell carcinoma of head and neck (SCCHN) cases. To investigate the types of HPVs present in 83 oral cancers and 19 other head and neck tumors, degenerate primers directed to consensus regions in the HPV L1 open reading frame (ORF) were employed to amplify genomic DNA from tumor and when available, the adjacent normal mucosa. PCR-amplified products were cloned and sequenced. Similar studies were done on exfoliated buccal cells of 102 individuals visiting a dental hospital for dental complaints. HPV was detected in 32 out of 102 patients (31%), in either the tumor or the adjacent normal mucosa, while 5% (5/102) of the comparative group were found to be HPV-positive. Sequence analysis revealed a number of cutaneous HPVs, predominantly HPV types of the genus Beta-Papillomavirus, in the oral cavity. Multiple HPV infections were also commonly observed in patients (14/102; 14%). HPV 16 and 18 were each detected in 6 patients (6/102; 6%). Neither high-risk HPVs nor multiple infections were observed in the mouthwash samples of the comparative group. We report that the oral cavity harbors a variety of different HPVs. These viruses, in conjunction with the carcinogens present in tobacco could contribute to carcinogenesis

Retroviruses and Cancer

Retroviruses comprise a large and diverse family of enveloped RNA viruses. Viruses are etiologically linked to approximately 20% of all malignancies worldwide. Retroviruses account for approximately 8%-10% of the total. Human retroviruses cause malignancy via direct effects (Human T lymphotropic virus type 1 ,HTLV1) as well as through interactions with other oncogenic herpesviruses and other viruses(Human immunodeficiency virus,HIV).

HTLV1

Human T lymphotropic virus type 1 (HTLV1) was the first human retrovirus to be discovered and is endemic in certain areas (especially SW Japan, the Caribbean and parts of Africa and South America) where up to 10% or more of the population may be infected. In most cases the infection is harmless. However, as many as 1 in 20 infected individuals eventually develop a type of adult T cell leukaemia (ATL) in which every tumour cell carries a clonally integrated HTLV1 provirus. HTLV1 is considered the sole causal agent for ATL whether it occurs in endemic areas or in sporadic cases.

HTLV-1 is primarily found in CD4+ T cells, but other cell types in the peripheral blood of infected individuals have been found to contain HTLV-1, including CD8+ T cells, dendritic cells, and B cells. Transmission of HTLV-I is believed to occur from mother to child via breastfeeding; by sexual contact; and through exposure to contaminated blood, either through blood transfusion or sharing of contaminated needles.

After infection Reverse Transcriptase (RT) in the virion uses genomic RNA as template, and synthesizes proviral DNA that is then integrated into the host cell genome by virally encoded integrase. Replication of virus is directed from these integrated viral genomes. Typical retroviral genes are encoded by the genome (gag, pro, pol, env and IN), but in addition there are six functional proteins encoded within the px region of the genome that are unique. HTLV1 proviral

DNA integrates in a common chromosomal site in all ATL cells in a given patient, producing a state of clonal integration . However, the integration site is not unique but differs in different cases of ATL, and it does not produce insertional mutagenesis.

HTLV1 does not contain an oncogene derived from a cellular protooncogene. Rather the *trans*-acting factor Tax encoded within the px region is essential for cellular transformation and induces and interacts with specific sets of cellular genes. Tax binds to factors that regulate these genes, which are important for disease pathogenesis by the virus. Tax activates the IL-2 receptor and several cytokines involved in T-cell growth as well as other genes, in part by destabilizing IκB and activating NFκB. Tax also dysregulates cellular gene expression through CREB/CRE. Tax can induce Bcl-XL and resistance to apoptosis. Tax interferes with the DNA polymerase α component of DNA repair mechanisms and inactivates p16 INK4A, an inhibitor of cyclin-dependent kinases 4–6 . Finally by causing mislocation of hsMAD1 and hsMAD2, Tax can produce loss of mitotic checkpoint. The viral genome persists in cells as a DNA copy or proviral genome in CD4+ T cells. HTLV1 infection also leads to chromosomal instability caused by Tax. The virus itself, in contrast to HIV, is genetically stable because the HTLV1 proviral genomes are replicated in their cellular context by cellular polymerase á, not by reverse transcriptase, which is error-prone, and is used for replication of virus.

HTLV1 induces a rather weak growth transformation of T cells in the laboratory but, in the body, is probably never sufficiently strong to induce T cell leukaemia on its own. However, a virally infected cell in which growth controls have even partly broken down, is more susceptible to further genetic accidents. During persistent infection a gradual build-up of HTLV1-positive T cells which have accumulated additional genetic changes may occur. Eventually this can lead to selection and outgrowth of a fully malignant, HTLV1-positive clone . At this stage malignant cell growth can occur in the absence of tax gene expression.

After a long latent period, adult T-cell leukemia/lymphoma (ATL) occurs in 1 per 1000 carriers per year, resulting in 2500-3000 cases per year worldwide and over half of the adult lymphoid malignancies in endemic areas. Certain HLA alleles increase the risk of ATL.

HIV

Human immunodeficiency virus (HIV)has been indirectly associated with carcinogenesis. It induces a chronic state of immunosuppression reducing immunosurveillance for neoplastic cells and increasing the risk of reactivation of latent viruses as well as the risk of acquiring new infections and its transactivating regulatory protein Tat enhances direct and indirect cytokine and immunological dysregulation to cause diverse cancers. Kaposi's sarcoma (KS) is a very rare tumor except after HIV-1 infection, when its incidence is greatly amplified reaching seventy thousand-fold in HIV-infected homosexual men. Human herpesvirus 8 (HHV-8), which is also known as Kaposi's sarcoma-associated virus (KSHV), is a necessary but not sufficient etiological factor in KS. The dramatic decline of KS since the introduction of highly active antiretroviral

therapy (HAART) could be due to suppression of HIV-1 tat. B-cell non-Hodgkin's lymphoma occurs as their first acquired immunodeficiency syndrome-defining diagnosis in 3%-4% of HIV-infected patients. Hodgkin's lymphoma is also associated with HIV infection but at a lower risk. Human papillomaviruses are linked to invasive cervical cancer and anogenital cancers among HIV-infected patients.

References

1. Proietti, F.A., et al., Global epidemiology of HTLV-I infection and associated diseases. Oncogene, 2005. 24(39): 6058-68.
2. Yoshida, M., Discovery of HTLV-1, the first human retrovirus, its unique regulatory mechanisms, and insights into pathogenesis. Oncogene, 2005. 24(39): 5931-5937.
3. Grassmann, R., M. ABoud, and K. Jeang, Molecular mechanisms of cellular transformation by HTLV-1 Tax. Oncogene, 2005. 24(39): 5976-5985.
4. Gallo R. Human retroviruses after 20 years: a perspective from the past and prospects for their future control. Immunol Rev 2002;185:236–65
5. Beral V and Newton R :Overview of epidemiology of immunodeficiency associated cancers .Monogr Natl Cancer Inst 23:1-6,1998
6. Newton R,Beral Vand Weiss R.Human immunodeficiency virus infection and cancer .Cancer Surv33:237-262,1999.
7. Pagano JS, Blaser M, Buendia MA, et al.Infectious agents and cancer: criteria for a causal relation. Semin. Cancer Biol.2004; 14 (6): 453–71.

Selected Abstracts -Indian Study

Chaudhari CN, Shah T, Misra RN. Prevalence of human T cell leukaemia virus amongst blood donors. Armed Forces Med J India 2009;65:38-40

Abstract

Background: Human T cell leukaemia virus (HTLV) I/II are retroviruses implicated in transfusion transmitted infection. Present study was undertaken to assess seroprevalence of HTLV in voluntary blood donors along with pattern of blood utilisation.

Methods: A total of 258 healthy blood donors who were free from infectious markers in transfusion as per current transfusion guidelines were enrolled. They were screened for HTLV-I/II antibodies by commercially available enzyme immuno assay (EIA) and their blood utilisation data was analysed.

Result: Five (1.9%) donors were found seropositive for HTLV-I/II of which 1.2 % were first time and 0.9% were repeat donors. Blood utilisation data revealed 20.9% and 38.8% units were utilised within 5 and 6-14 days of collection respectively. 45.9% recipients were transfused with single blood unit. 42.9% recipients were immunosuppressed due to underlying disease.

Conclusion: The high prevalence of HTLV in blood donors, coupled with single unit transfusion, use of fresh blood, non availability of acellular blood products and immunosuppression in recipients can lead to significant transfusion transmitted HTLV infection. We suggest judicious use of blood products and screening of blood donors in prevention of transfusion transmitted HTLV-I/II.

Polyoma Viruses – Merkel Cell Virus MCV

Prior to MCV, there were four known human polyomaviruses called JC virus (JCV), BK virus (BKV), KI virus (KIV) and WU virus (WUV). A fifth virus, SV40, is a monkey polyomavirus that may have infected some humans through contamination in the late 1950s-early 1960s. Although the other human polyomaviruses can cause cancer in animals, MCV is the only polyomavirus that has been shown to cause cancer in humans. In 2008 Feng, Moore, Chang discovered the human polyomavirus, the Merkel cell polyomavirus(MCPyV).

Normal Merkel cells are widely distributed in the epidermis near the end of nerve axons and may function as mechanoreceptors or chemoreceptors. Merkel cell carcinoma (MCC; formerly called trabecular carcinoma) was first described by Toker in 1972. It is a rare, aggressive carcinoma of cutaneous neuroendocrine cells

Epidemiology

Epidemiology of MCPyV infection is similar to the other known human polyomaviruses . Exposure to MCPyV as measured by serum antibodies to viral capsid proteins appears to be widely prevalent among healthy subjects . In one study, the prevalence of MCPyV seropositivity was 0% in infants, 43% among children aged 2–5 years old and increased to 80% among adults older than 50 years .A similar trend of increasing seroprevalence with age was seen in another study, suggesting that primary exposure to MCPyV occurs during childhood. Consistent with the serologic data, MCPyV DNA was detected in cutaneous swabs from clinically healthy subjects with a prevalence of 40%–100% in three independent studies.Iit appears that the virus is being shed chronically from clinically normal skin in the form of assembled virions. Besides the skin, viral DNA has been detected in lower frequencies among respiratory secretions, oral and anogenital mucosa ,and in the digestive tract . The exact mode of transmission remains to be elucidated and could involve cutaneous, fecal-oral, mucosal, or respiratory routes. Importantly, although widely prevalent, active MCPyV infection appears to be asymptomatic and with the

exception of Merkel cell carcinoma (MCC), this virus has not yet been convincingly associated with any other human disease.

Role of MCPyV in Pathogenesis of MCC

Cancer-associated viruses may contribute to carcinogenesis directly via expression of viral oncogenes that promote cell transformation or indirectly via chronic infection and inflammation, which may predispose host cells to acquire carcinogenic mutations. In humans, MCPyV is the first polyomavirus with demonstrated integration into genomic DNA. Several significant observations suggest that MCPyV contributes to the pathogenesis of MCC:

1) It is present in a substantial proportion of MCC tumors .
2) Monoclonality of MCPyV integration in MCC tumor cells suggests viral integration is an early event in tumorigenesis.
3) T-antigen transcripts and oncoproteins are expressed in most MCC tumors
4) The MCPyV LTantigen expressed in MCC tumors is truncated due to mutations that preserve critical cell-cycle progression functions, but eliminate cell-lethal virus-replication activities
5) Persistent expression of these MCPyV proteins is required for continued growth of MCC cell lines in vitro.

These findings strongly suggest that MCPyV plays a key role in MCC carcinogenesis rather than merely being a passenger virus that secondarily infects tumor cells.The genome of polyomavirus contains early and late regions. The former encodes nonstructural proteins, ST and LT, that are responsible for viral replication; and the latter encodes viral proteins (VPs) that constitute viral particles. LT has been shown not only to initiate viral replication by binding to DNA and recruiting cellular replication factors but also to interact with several key cellular proteins to drive cells into the S phase and is thus thought to play a central role in cellular transformation.

The MCPyV LT-antigen appears to retain the major conserved features of other polyomavirus LT-antigens, including the DnaJ motif (binds to heat-shock proteins) and the LxCxEmotif (inactivates retinoblastoma family proteins), and the origin-binding and helicase/ ATPase domains (promote viral replication) .These various domains allow the polyomaviruses to use host cell machinery for viral genome replication, but can also target tumor suppressor proteins resulting in cellular transformation . The LT-antigen transcripts are commonly expressed in MCC tumors .

The mechanisms by which MCPyV may contribute to MCC carcinogenesis continue to be elucidated. MCPyV Tantigen appears to be essential for cell survival among tumors infected with the virus. In MCPyV-infected MCC cell lines and xenograft models, the expression of T-antigen appears to be essential for sustained proliferation; knockdown of this viral protein leads to growth arrest and/or cell death while restoration of T-antigen expression rescues cell

growth. Furthermore, interaction with the retinoblastoma (Rb) tumor suppressor protein appears to be critical to the observed growth-promoting effects of LT-antigen .

Immunohistochemistry (IHC) data from human MCC tumors shows strong positive association between tumor Rb expression and MCPyV LT-antigen expression, with LT-antigen–positive MCC tumors also expressing Rb and 87% of LT-antigen– negative tumors being Rb-negative as well. Similar to the well-characterized interactions between SV40 LT-antigen and the Rb family of proteins (Rb, p107, p130), the MCPyV LT-antigen is likely to sequester hypophosphorylated Rb that usually binds to E2F transcription factors. This sequestration of Rb allows E2F-mediated transcription that leads to the entry of the cell into S-phase. The integrity of the DnaJ and the LxCxE motifs is required for this mechanism in SV40, and the retention of these domains (with intact Rb-binding ability) in the truncated MCPyV LT-antigen is consistent with this mechanism being relevant to MCC pathogenesis.

The other putative mechanism by which polyomaviruses contribute to transformation is interference with the p53 tumor suppressor pathway. The usual functions of p53 are not conducive to viral replication as p53 transactivates genes that lead to cell cycle arrest, which could deprive the virus of essential replication factors. Additionally, active p53 could lead to cellular apoptosis in response to the presence of viral or cellular oncoproteins. In order to complete their normal infectious cycles, the polyomaviruses have developed the ability to block p53 function through several mechanisms. The bipartite domain of the SV40 LTantigen can bind directly to the specific DNA-binding domain of p53, hence interfering with p53-dependent gene transcription (this binding has also been shown to increase the half-life and steady-state levels of p53 in cells.

As the MCPyV LT-antigen seems to be prematurely truncated in the MCC tumor cells lacking the helicase domain and the supposed p53-binding sites, the significance of the p53 pathway in pathogenesis of MCPyV-associated MCC is unclear. However, even if the truncated T-antigen does not bind to p53, MCPyV may play a role in suppressing p53 function in MCC tumors via other mechanisms. For example, there is evidence that the binding of T-antigen to p53 in SV40 may not be sufficient to block p53 function and that other indirect mechanism (involving small T-antigen and/or the J-binding and Rbbinding domains of the LT-antigen) are also important in functional suppression of p53. Consistent with MCPyV somehow disabling p53 function in MCC tumors, inactivating mutations in TP53 gene and/or over expression of p53 have been seen only in a small subset ofMCC tumors. Moreover, recent studies have indicated an inverse relationship between p53 expression and MCPyV viral abundance in MCC tumors as well as p53 overexpression potentially being associated with poor outcome.

Merkel cell carcinoma

Merkel cell carcinoma (MCC) is a rare, aggressive neuroendocrine skin cancer that occurs more frequently in immunosuppressed in-dividuals, such as those infected with HIV and/or diagnosed with AIDS. Many individuals who are diagnosed with MCC have a history of other

sun exposure–associated skin cancers, and MCC may also share etiologic factors with other malignancies. For example, increased joint risks of MCC and multiple myeloma, chronic lymphocytic leukemia (4, non-Hodgkin lymphoma, and malignant mela-noma have been reported. MCCis rare, with an estimated annual incidence of three occurrences per million people in the United States. The mortality rate within 2 years of MCC diagnosis is 28%,

Merkel cells are mechanoreceptors (fine touch) within basal epidermis.There are three histologic patterns (all with similar prognosis):Intermediate type, most common type ,Small cell type and Trabecular type. On immunohistochemistry, 87% of MCC vs 4.6% of SCLC are CK20 positive. Peri-nuclear dot pattern of cytokeratin is pathognomonic. The CM2B4 antibody recognizes the Merkel polyomavirus Large T antigen. This antibody is highly specific for MCC but it has a low sensitivity (~60% of MCC tumors are positive).

MCC is seldom suspected at presentation. The clinician's index of suspicion of MCC is increased with a lesion that is red but asymptomatic and that is expanding rapidly in a patient who is immune deficient, is old, or has UV-damaged skin. Immunocompromise and immunosuppression seems to play a role, because MCC has a higher incidence in transplant patients and those affected by human immunodeficiency virus (HIV). Engels et al. found the relative risk of MCC in HIV positive patients to be 13.4 as compared with the general population There is also a reported 100-fold increase in the incidence of MCC in patients with psoriasis treated with PUVA. Prognosis is poor with distant metastasis.

References

1. Merkel Cell Carcinoma: Recent Progress and Current Priorities on Etiology, Pathogenesis, and Clinical Management *The Rockville Merkel Cell Carcinoma GroupJ Clin Oncol 27:4021-4026.* NCCN Clinical Practice Guidelines in Oncology, Merkel cell carcinoma version 1, 2012.
2. Bhatia S, Afanasiev O, Nghiem P .Immunobiology of Merkel cell carcinoma: implications for immunotherapy of a polyomavirus-associated cancer.Curr Oncol Rep. 2011 Dec;13(6):488-97.
3. Merkel cell polyomavirus infection and Merkel cell carcinoma in HIV-positive individuals. Wieland U, Kreuter A. Curr Opin Oncol. 2011 Sep;23(5):488-93.
4. Kuwamoto S Recent advances i.n the biology of Merkel cell carcinoma. Hum Pathol. 2011 Aug;42(8):1063-77.
5. Jeanette Kaae, Anne V. Hansen, Robert J. Biggar, Heather A. Boyd, Patrick S. Moore, Jan Wohlfahrt, Mads Melbye,Merkel Cell Carcinoma: Incidence, Mortality, and Risk of Other Cancers .JNCI J Natl Cancer Inst (2010) 102 (11): 793-801.
6. Engels EA, Frisch M, Goedert JJ, et al: Merkel cell carcinoma and HIV infection. Lancet 2002;359:497-498.
7. Feng H, Shuda M, Chang Y, et al: Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science 2008;319:1096-1100.

Selected Abstract-Indian study

Medhi S, Purandare NC, Dua SG, Gujral S.Bilateral renal metastases in a case of Merkel cell carcinoma. J Cancer Res Ther. 2010 Jul-Sep;6(3):353-5.

Abstract

Merkel cell carcinoma is a primary neuroendocrine carcinoma of the skin. It is a highly aggressive tumor which commonly metastasizes to lymph nodes, liver, lung and bone. The diagnosis is based on histology and immunohistochemistry. Renal metastasis, with sparing of other common sites of hematogenous spread (lung and liver), is a unique feature of this case.

Helicobacter Pylori and Gastric Cancer

I. Helicobacter pylori: Epidemiology

Helicobacter pylori, a Gram-negative bacterial pathogen that selectively colonizes the gastric epithelium was isolated by culture from gastric biopsy specimens by Robin Warren and Barry Marshall in 1983 [1]. They were awarded the Nobel Prize in Physiology or Medicine in 2005 for their discovery that *H. pylori* cause most peptic ulcers. *H. pylori* has been implicated in the causation of gastritis, peptic ulcers, mucosa-associated lymphoid tissue (MALT) - lymphomas and even, gastric cancer.

Almost 50% of the world's population is thought to be colonized with *H. pylori*. The prevalence varies amongst different ages, socioeconomic strata and geographical regions. Globally, different strains of *H. pylori* appear to be associated with differences in virulence, and the resulting interplay with host factors and environmental factors leads to subsequent differences in the expression of disease [2]. The transmission of *H. pylori* is feco-oral. The prevalence is higher in developing countries most probably due to lower socio-economic status, poor sanitation, overcrowding and lack of safe drinking water. Here infection is more prevalent in children. In contrast the prevalence is lower in the developed countries where it increases with age which is probably a cohort effect of an earlier generation exposed to poor sanitation. *H. pylori* infection is common in India. The frequency of *H. pylori* infection increases with age. Exposure occurs in childhood and approximately 80% of adults have been infected at some time [3]. The infection persists throughout life, unless treated. Sero-epidemiological studies show a prevalence of 22%-57% in children under the age of five, increasing to 80%-90% by the age of 20, and remaining constant thereafter [3-6]. There is no gender-related difference in the prevalence.

II. Gastric cancer: Epidemiology

Gastric cancer (GC) is the 4th commonest cancer in the world and accounts for 8% of the total cancer cases and 10% of total cancer-related deaths worldwide with over 70% of new cases and deaths occurring in developing countries [7]. GC rates are about twice as high in males as in females. The highest incidence rates are in Eastern Asia, Eastern Europe, and South America and the lowest rates are in North America and most parts of Africa [7]. The percentage of non-cardia cancers is higher in females than in males and higher in the developing world and Japan. Regional variations are probably related to differences in diet and in the prevalence of Helicobacter pylori infection [8]. India has an Age standardized rate (ASR) of 4.7/ 100,000 population for males and 2.9/100,000 population for females [9].

Cancers in the fundus and pylorus are commoner in high incidence areas, and these have been declining in the last few decades. The reasons for declining rates include: increased use and availability of refrigeration, decreased reliance on salted and preserved foods, reductions in chronic H. pylori infection in most parts of the world and reduction in smoking. Also, in countries like Japan, aggressive screening programs have contributed to the decreasing mortality.

Adenocarcinoma of the cardia which is related to gastro-esophageal reflux disease and lifestyle factors like obesity has been rising in North America and Europe in the last 2 decades.

III. H. pylori and Gastric cancer causation- The evidence

Approximately half of the world's population has colonization of the stomach with *H. pylori*. The organism survives in the acidic environment of the stomach due to its remarkably high urease activity; which converts urea in gastric juice to alkaline ammonia and carbon dioxide. In most persons, *H. pylori* colonization does not cause any symptoms although a majority of colonized individuals develop histological signs of chronic gastritis [10]. Infection with *H. pylori* is a cofactor for duodenal or gastric ulcers (reported to develop in 1 to 10% of infected patients), gastric cancer (in 0.1 to 3%), and gastric mucosa associated lymphoid tissue (MALT) lymphoma (in <0.01%) [11]. The risk of these disease outcomes in infected patients varies widely among populations.

Pathogenesis

H. pylori induced chronic gastritis involves interplay of several virulence factors [12]. The *cag* pathogenicity island (*cag* PAI) is a 40-kb DNA insertion element which contains 27 to 31 genes flanked by 31-bp direct repeats and encodes one of the most intensely investigated *H.pylori* proteins, CagA [13,14] . CagA is the most important single virulence determinant that has been investigated extensively for mechanistic and functional evidence to its being cytotoxic and carcinogenic [15,16]. An independent *H. pylori* locus linked with increased disease risk is *vacA*, which encodes the toxin VacA which is a cytotoxin that induces intracellular vacuolation of cultured cells. Host genes and the environment also play a role in imparting susceptibility (or

otherwise) towards more serious outcomes of the colonization [10]. Adherence of *H. pylori* to the gastric epithelium facilitates initial colonization, persistence of infection, and delivery of virulence factors to host epithelial cells [14]. Adhesins and Outer membrane proteins (OMPs), whose expression has been associated with gastroduodenal diseases may heighten the risk for developing GC [17].

Critical host responses that influence the progression to *H. pylori*-induced carcinogenesis include gastric inflammation and a reduction in acid secretion. Polymorphisms that change the production of pro- and anti-inflammatory cytokines and which have an impact on the COX expression will also have a bearing on the risk of GC [14]. Other factors that may lead to increased risk of GC are: oxidative damage caused by *H. pylori*, dysregulated host immune response and environmental factors like increased salt intake, decreased dietary antioxidant intake and cigarette smoking.

H. pylori was classified as a human carcinogen in 1994 [18]. It is presently considered to be the most common etiologic agent of infection-related cancers, which represent 5.5% of the global cancer burden [8]. The best evidence regarding the risk of GC with *H. pylori* comes from prospective studies. *H. pylori* infection and the circulating antibody response can be lost with development of cancer; thus retrospective studies are subject to bias resulting from classification of cases as *H. pylori* negative when they were infected in the past and this may lead to significant underestimation of the prevalence of infection [19].

Gastric carcinogenesis involves various stages like superficial gastritis, chronic inflammation, atrophic gastritis, intestinal metaplasia, dysplasia and finally gastric cancer. Apart from *H. pylori*, salt, bacterial overgrowth and N-nitroso carcinogens have also been implicated. The risk of cancer is highest among patients in whom the infection induces inflammation of both the antral and fundic mucosa and causes mucosal atrophy and intestinal metaplasia.

Clinical studies

In a prospective study of 1526 Japanese patients who had duodenal ulcers, gastric ulcers, gastric hyperplasia, or non-ulcer dyspepsia at the time of enrollment; and who were followed up for a mean 7.8 years (range, 1.0 to 10.6), GC developed in 36 (2.9 %) of the infected and none of the uninfected patients [20]. Among the patients with *H. pylori* infection, those with severe gastric atrophy, corpus-predominant gastritis, and intestinal metaplasia were at significantly higher risk for GC. Another prospective study evaluated 49 subjects negative for *H. pylori* and 58 positive subjects for a mean follow-up of 11.5 years (range 10-13 years) [21]. Development of atrophic gastritis and intestinal metaplasia occurred in 2 (4%) uninfected and 16 (28%) infected subjects. Regression of atrophy was noted in 4 (7%) infected subjects. Development of atrophic gastritis and intestinal metaplasia was significantly associated with *H. pylori* infection ($p = 0.0014$; OR 9.0, 95% CI 1.9-41.3). The proportion of atrophic gastritis in the study population showed an annual increase of 1.15% (0.5-1.8%).

In a meta-analyses of 12 studies with 1228 GC cases, the association with *H. pylori* was restricted to non-cardia cancers (Odds Ration [OR] 3.0; 95% Confidence Intervals [CI] 2.3-3.8) and was stronger when blood samples for *H. pylori* serology were collected 10+ years before cancer diagnosis (OR 5.9; 95% CI 3.4-10.3) [19]. *H. pylori* infection was not associated with an altered overall risk of cardia cancer (OR 1.0; 95% CI 0.7-1.4). The meta-analysis also supported the idea that when *H. pylori* status is assessed close to cancer diagnosis, the magnitude of the non-cardia association may be underestimated. Another meta-analysis of 19 cohort or case-control studies with 2491 patients and 3959 controls showed a significant difference in OR between patients with early and advanced GC (6.35 vs. 2.13; P = 0.01) and patients with cardiac and non-cardiac gastric cancer (1.23 vs. 3.08; P = 0.003) [22]. *H.* pylori-infected younger patients have a higher relative risk for gastric cancer than older patients with odds ratios decreasing from 9.29 at age d" 29 years to 1.05 at age e" 70 years. *H.* pylori infection was equally associated with the intestinal or diffuse type of GC.

A systematic review of 10 'nested' case-control comparisons in prospective studies included 800 gastric cancers, and combined analysis of them yielded a risk ratio of 2.5 (95% CI: 1.9-3.4; 2P < 0.00001) for GC in people sero-positive for *H. pylori* antibodies [23]. Another meta-analysis of 42 observational epidemiological studies (8 cohort and 34 case-control studies), showed a pooled OR for *H. pylori* in relation to GC of 2.04 (95% CI: 1.69-2.45) [24]. There was no relationship with female gender (OR 0.76, 95% CI: 0.64-0.89) or stage of cancer (advanced %) (OR 1.12, 95% CI: 0.88-1.43). The quality of the studies varied considerably, with the majority of excellent studies producing positive results and the very poor to moderate studies producing mixed results.

Indian studies

A retrospective small study done on 50 cases and 30 controls in Kashmir, India did not find a significant association between *H. pylori* and GC [25.] In another small study from Varanasi, 68% of the 50 cases of GC were found to be positive for *H. pylori* infection as compared to 74% of healthy controls [26]. The prevalence rate of *H. pylori* infection was non-significantly lower in the GC group than in the control population though statistically not significant. Another small study from Lucknow investigated 20 patients each with GC and non ulcer dyspepsia (NUD) [27]. Patients with GC more often had anti-*H. pylori* IgG (16⁄20 vs. 8⁄20; p=0.02) and a trend towards higher apoptotic index (AI) (48.6 [19.2 to 71.7] vs. 41.4 [11.7 to 63.6]; p=0.06) than NUD. AI was higher in GC and NUD infected with *H. pylori* than in those without infection. AI was also higher in GC than in NUD with *H. pylori* infection. Exaggerated apoptosis may play a role in *H. pylori*-mediated gastric diseases including carcinogenesis. A study from Allahabad showed that the prevalence of *H. pylori* in controls was slightly higher than the patients with GC (80% Vs 78%) [28]. Ulcerated type of gross appearance had maximum prevalence of *H. pylori* (88%). Prevalence of *H. pylori* was more in diffuse type of gastric cancer than intestinal type (86% Vs 68%). A significant association between *H. pylori* and grades of gastritis was noted (P < 0.01) in controls as well as in patient group but it failed to show a significant association with tumour

grades, intestinal metaplasia, site of the tumour and age of the patients. *H pylori* infection was not found to be an independent risk factor for carcinogenesis of GC in a hospital based case-control study from Mizoram [29]. However, when *H. pylori* infection interacted with consumption of fermented pork fat or smoked dried meat, it showed a significant association.

Most of the studies conducted in India fail to confirm the association between *H. pylori* infection and GC. This could be due to small sample size of most of the studies, lack of adjustment for other confounding variables and retrospective nature of most studies, which may underestimate the *H. pylori* prevalence in GC subjects. We need large, adequately powered studies to evaluate the association of *H. pylori* with GC in India.

Prospective studies mainly done in the west suggest that GC is 2- 3 times as common in those chronically infected by *H.* pylori. **(Level 2 evidence)**

IV. H. pylori and Gastric Lymphomas

A nested case-control study involving two large cohorts (230,593 participants) showed that patients with gastric non-Hodgkin's lymphoma were significantly more likely than matched controls to have evidence of previous *H.* pylori infection (matched OR, 6.3; 95 % CI, 2.0 to 19.9) [30]. No association was found between non-gastric non-Hodgkin's lymphoma and previous *H.* pylori infection (matched odds ratio, 1.2; 95 percent confidence interval, 0.5 to 3.0). A causative role for the organism is plausible, but remains unproved. **(Level 2 evidence)**

V. H. pylori treatment

Since a majority of patients with *H. pylori* infection do not have any related clinical disease, routine testing is not considered appropriate [31]. *H. pylori* eradication is recommended in duodenal or gastric ulcer (**Level of evidence - 1a** ; Grade of recommendation – A), MALToma (**Level of evidence – 1c** ; Grade of recommendation – A), Atrophic gastritis (**Level of evidence - 2a** ; Grade of recommendation – B), After gastric cancer resection (**Level of evidence – 3b** ; Grade of recommendation – B), in subjects who are first degree relatives of patients with GC (**Level of evidence – 3b** ; Grade of recommendation – B) and, if the patient so desires (**Level of evidence - 5** ; Grade of recommendation – A) [31].

A proton pump inhibitor (PPI) with 2 antibiotics (clarithromycin-amoxicillin or metronidazole) is the recommended first choice treatment in populations with less than 15–20% clarithromycin resistance. In populations with less than 40% metronidazole resistance use of metronidazole is preferable to amoxycillin. Quadruple treatments are alternative first choice treatments. Bismuth-containing quadruple treatments remain the best second choice treatment, if available [31]. The recommended duration of treatment is 7-14 days. Choice and duration of treatment depends on prevailing antibiotic resistance rates and patterns. An alternative initial regimen is 10-day sequential therapy, involving a PPI plus amoxicillin for 5 days followed by a PPI clarithromycin and tinidazole for 5 more days [32]. Treatment should achieve an eradication rate of e" 80%.

(Level 1 evidence)

Studies from India have shown high prevalence rates of antibiotic resistance (Metronidazole: 77.9-85%, Clarithromycin: 44.7%, Amoxycillin: 32.8%, Tetracycline: 7.5%) [33, 34]. *H. pylori* eradication rates vary in Indian studies from 31-96% [35-37]. Widespread antibiotic resistance and high rate of reinfection may be responsible for the low eradication rates seen with standard fixed-dose *H. pylori* treatment kits in India.

VI. H. pylori eradication and prevention of Gastric cancer – evidence

In a randomized trial, *H. pylori* eradication was associated with a reduced risk of histological progression of Intestinal Metaplasia (IM) which is considered to be a pre-neoplastic lesion in Gastric carcinogenesis. Conversely, persistent *H.* pylori infection (OR 2.13 (95% CI 1.41-3.24)), was associated with IM progression [38]. Other randomized prospective studies have demonstrated that *H. pylori* eradication gave more favorable gastric histopathologies over 1 year than no treatment and also significantly reduced the severity and activity of chronic gastritis and led to a marked resolution of IM in the antrum [39, 40]. On the other hand, continuous *H. pylori* infection leads to progressive aggravation of atrophy and IM. *H. pylori* eradication prevents development, or leads to regression or decreased progression of pre-neoplastic changes (atrophic gastritis and IM) of the gastric mucosa which indicates its effect on early stages of gastric carcinogenesis. It is still unclear whether prophylactic *H. pylori* eradication will reduce the risk of GC. Results from randomized trials are eagerly awaited, but availability of strong conclusive results may take many years.

Cost-effectiveness analysis and economic models have shown that screening and treatment for *H.* pylori infection is potentially cost-effective in the prevention of GC, particularly in high-risk populations [41, 42]. Large cancer prevention trials using this strategy will be needed to give conclusive evidence regarding the same.

H. pylori infection strongly enhanced gastric carcinogenesis initiated with a chemical carcinogen in a study on Mongolian Gerbils [43]. Following eradication at an early period this enhancing effect was effectively reduced. Eradication at an early stage of inflammation might be effective in preventing *H. pylori* related gastric carcinogenesis. A prospective, randomized, placebo-controlled, population-based primary prevention study involved 1630 healthy carriers of *H. pylori* infection in a high-risk region of China [44]. Subjects were randomized to receive *H. pylori* eradication treatment or placebo. The incidence of GC development at the population level was similar between participants receiving *H. pylori* eradication treatment and those receiving placebo during a period of 7.5 years. (7 vs. 11, P =.33). In the subgroup of *H. pylori* carriers without precancerous lesions, eradication of *H. pylori* significantly decreased the development of GC (0 vs. 6; P =.02). A multi-centre, open-label, randomized controlled trial on 544 patients investigated the prophylactic effect of *H. pylori* eradication on the development of metachronous GC after endoscopic resection for early GC [45]. The odds ratio for metachronous GC was 0.353 (95% CI 0.161-0.775; p=0.009) in the eradication group. Prophylactic eradication of *H. pylori* after endoscopic resection of early GC prevented the development of metachronous GC.

Although a number of studies have showed that atrophic gastritis and IM are strongly associated with *H. pylori*, and decrease with eradication, evidence that *H. pylori* eradication may reduce the risk of GC is based on non-randomized controlled studies in animal and humans. The optimal time to eradicate *H. pylori* is probably before pre-neoplastic lesions develop. There is no conclusive evidence showing a decreased risk of GC with *H. pylori* eradication. Larger trials and additional cost-effectiveness studies, are needed before decisions about large-scale *H. pylori* eradication can be made. **(Level 1 evidence)**

Eradication of *H. pylori* infection causes regression of most localized gastric MALT lymphomas [46] and is the recommended treatment. **(Level 2 evidence)**

VII. H. pylori and Gastric cancer: The Asian enigma

H. pylori infection is common in developing Asian countries like India, Bangladesh, Pakistan, and Thailand with sero-prevalence rates of 55-92%. Here, the infection is acquired at an earlier age as compared to developed Asian countries like Japan and China who have lower sero-prevalence rates of 55% and 44% respectively [47]. The ASRs for GC for India, Pakistan and Bangladesh are much lower (Males: 4.7, 8 and 5.9 per 100,000 population respectively; Females: 2.9, 4.5 and 4.4 per 100,000 population respectively) in contrast to those in China and Japan (Males: 41.3 and 46.8 per 100,000 population respectively; Females: 18.5 and 18.2 per 100,000 population respectively) [9]. Similar scenario is seen in Africa where countries like Ethiopia and Nigeria have sero-prevalence rates of more than 90% [2]. The ASRs for GC for Ethiopia and Nigeria are also very low (Males: 4.4 and 3.2 per 100,000 population respectively; Females: 2.8 and 1.4 per 100,000 population respectively) [9]. Thus we see that despite established etiological role of *H. pylori*, the situation is somewhat enigmatic in Asian and some African countries because in countries with higher frequency of infection, there is lower rate of GC [47]. Studies from India have also failed to show a convincing association between *H. pylori* infection and GC. The host's genetic makeup and dietary and environmental factors might explain this enigma.

VIII. Conclusion

Approximately half of the world's population has colonization of the stomach with *H. pylori*. In most people, *H. pylori* colonization does not cause any symptoms although a majority of colonized individuals develop histological signs of chronic gastritis. Infection with *H. pylori* is a cofactor for duodenal or gastric ulcers, gastric cancer and gastric MALT lymphoma. The risk of these disease outcomes in infected patients varies widely among populations. Prospective studies mainly done in the west suggest that Gastric cancer is 2- 3 times as common in those chronically infected by *H.* pylori. *H pylori* eradication is recommended in duodenal or gastric ulcer, MALToma, Atrophic gastritis, after gastric cancer resection and in subjects who are first degree relatives of patients with gastric cancer. A PPI combined with antibiotics given for 7-14 days is the treatment of choice which should ideally yield eradication rates of more than 80%. Although a number of studies have showed that atrophic gastritis and intestinal

metaplasia are strongly associated with *H. pylori*, and decrease with eradication, there is no conclusive evidence showing a decreased risk of GC with *H. pylori* eradication. However, eradication of *H. pylori* infection causes regression of most localized gastric MALT lymphomas and is the recommended treatment. Adequately powered trials and additional cost-effectiveness studies are needed before decisions about large-scale *H. pylori* eradication to prevent GC can be made.

References

1. Warren JR, Marshall BJ. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983; 1:1273-5.
2. Hunt RH, Xiao SD, Megraud F, Leon-Barua R, Bazzoli F, van der Merwe S, et al; World Gastroenterology Organization. Helicobacter pylori in developing countries. World Gastroenterology Organisation Global Guideline. J Gastrointestin Liver Dis. 2011 Sep;20(3):299-304.
3. Ramakrishna BS. Helicobacter pylori infection in India: the case against eradication. Indian J Gastroenterol. 2006 Jan-Feb; 25(1):25-8.
4. Graham DY, Adam E, Reddy GT, Agarwal JP, Agarwal R, Evans DJ Jr, et al. Seroepidemiology of Helicobacter pylori infection in India. Comparison of developing and developed countries. Dig Dis Sci. 1991 Aug;36(8):1084-8.
5. Gill HH, Majmudar P, Shankaran K, Desai HG. Age-related prevalence of Helicobacter pylori antibodies in Indian subjects. Indian J Gastroenterol. 1994 Jul;13(3):92-4.
6. Kang G, Rajan DP, Patra S, Chacko A, Mathan MM. Use of serology, the urease test & histology in diagnosis of Helicobacterpylori infection in symptomatic & asymptomatic Indians. Indian J Med Res. 1999 Sep; 110:86-90.
7. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011 Mar-Apr;61(2):69-90.
8. Parkin DM. The global health burden of infection-associated cancers in the year 2002. Int J Cancer. 2006; 118:3030-3044.
9. GLOBOCAN 2008. Accessed at: http://globocan.iarc.fr/factsheets/cancers/stomach.asp on 4th January 2012.
10. Ahmed N, Tenguria S, Nandanwar N. Helicobacter pylori—a seasoned pathogen by any other name. Gut Pathog. 2009 Dec 23; 1:24.
11. McColl KE. Clinical practice. Helicobacter pylori infection. N Engl J Med. 2010 Apr 29;362(17):1597-604
12. Suerbaum S, Michetti P. Helicobacter pylori infection. N Engl J Med. 2002 Oct 10; 347(15):1175-86.

13. Akopyants, N. S., S. W. Clifton, D. Kersulyte, J. E. Crabtree, B. E. Youree, C. A. Reece, N. O. Bukanov, E. S. Drazek, B. A. Roe, and D. E. Berg. 1998. Analyses of the cag pathogenicity island of Helicobacter pylori. Mol. Microbiol. 28:37–53.
14. Wroblewski LE, Peek RM Jr, Wilson KT. Helicobacter pylori and gastric cancer: factors that modulate disease risk. Clin Microbiol Rev. 2010 Oct; 23(4):713-39.
15. Ohnishi N, Yuasa H, Tanaka S, Sawa H, Miura M, Matsui A, Higashi H, Musashi M, Iwabuchi K, Suzuki M, Yamada G, Azuma T, Hatakeyama M: Transgenic expression of Helicobacter pylori CagA induces gastrointestinal and hematopoietic neoplasms in mouse. Proc Natl Acad Sci USA 2008, 105:1003-1008.
16. Suzuki M, Mimuro H, Kiga K, Fukumatsu M, Ishijima N, Morikawa H, Nagai S, Koyasu S, Gilman RH, Kersulyte D, Berg DE, Sasakawa C: Helicobacter pylori CagA phosphorylation-independent function in epithelial proliferation and inflammation. Cell Host Microbe 2009, 5:23-34.
17. Dossumbekova, A., C. Prinz, M. Gerhard, L. Brenner, S. Backert, J. G. Kusters, R. M. Schmid, and R. Rad. 2006. Helicobacter pylori outer membrane proteins and gastric inflammation. Gut 55:1360–1361.
18. Infection with Helicobacter pylori. In: IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. IARC monographs on the evaluation of carcinogenic risks to humans. Vol. 61. Schistosomes, liver flukes and Helicobacter pylori. Lyon, France: International Agency for Research on Cancer, 1994:177-240.
19. Helicobacter and Cancer Collaborative Group. Gastric cancer and Helicobacter pylori: a combined analysis of 12 case-control studies nested within prospective cohorts. Gut 2001; 49:347–53.
20. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. N Engl J Med. 2001 Sep 13; 345(11):784-9.
21. Kuipers EJ, Uyterlinde AM, Peña AS, Roosendaal R, Pals G, Nelis GF, Festen HP, Meuwissen SG. Long-term sequelae of Helicobacter pylori gastritis. Lancet. 1995 Jun 17; 345(8964):1525-8.
22. Huang J-Q, Sridhar S, Chen Y, Hunt RH. Meta-analysis of the relationship between Helicobacter pylori seropositivity and gastric cancer. Gastroenterology 1998; 114:1169–79.
23. Danesh J. Helicobacter pylori infection and gastric cancer: systematic review of the epidemiological studies. Aliment Pharmacol Ther 1999; 13:851–6.
24. Eslick GD, Lim LL, Byles JE, Xia HH, Talley NJ. Association of Helicobacter pylori infection with gastric carcinoma: a meta-analysis. Am J Gastroenterol 1999; 94:2373–9.
25. Malik GM, Mubarik M, Kadla SA and Durrani HA. Gastric Cancer Profile in Kashmiri Population with Special Dietary Habits. Diagn Ther Endosc. 2000;6(2):83-6

26. Khanna AK, Seth P, Nath G, Dixit VK, Kumar M. Correlation of Helicobacter pylori and gastric carcinoma. J Postgrad Med 2002;48:27

27. Tiwari S, Ghoshal U, Ghoshal UC, Dhingra S, Pandey R, Singh M, Ayyagari A, Naik S. Helicobacter pylori-induced apoptosis in pathogenesis of gastric carcinoma. Indian J Gastroenterol. 2005 Sep-Oct; 24(5):193-6.

28. Misra V, Misra SP, Singh MK, Singh PA, Dwivedi M. Prevalence of H. pylori in patients with gastric cancer. Indian J Pathol Microbiol 2007; 50:702-7.

29. Phukan RK, Narain K, Zomawia E, Hazarika NC, Mahanta J. Dietary habits and stomach cancer in Mizoram, India. J Gastroenterol. 2006 May; 41(5):418-24.

30. Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, Orentreich N, Vogelman JH, Friedman GD. Helicobacter pylori infection and gastric lymphoma. N Engl J Med 1994; 330:1267–71.

31. Malfertheiner P, Megraud F, O'Morain C, et al. Current concepts in the management of Helicobacter pylori infection: the Maastricht III consensus report. Gut 2007; 56:772-81.

32. Jafri NS, Hornung CA, Howden CW. Meta-analysis: sequential therapy appears superior to standard therapy for Helicobacter pylori infection in patients naive to treatment. Ann Intern Med 2008; 148:923- 31. [Erratum, Ann Intern Med 2008; 149: 439.]

33. Thyagarajan SP, Ray P, Das BK, Ayyagari A, Khan AA, Dharmalingam S, et al. Geographical difference in antimicrobial resistance pattern of Helicobacter pylori clinical isolates from Indian patients: multicentric study. J Gastroenterol Hepatol 2003; 18:1373-8.

34. Datta S, Chattopadhyay S, Patra R, De R, Ramamurthy T, Hembram J, et al. Most Helicobacter pylori strains of Kolkata in India are resistant to metronidazole but susceptible to other drugs commonly used for eradication and ulcer therapy. Aliment Pharmacol Ther 2005; 22:51-7.

35. Bhasin DK, Sharma BC, Ray P, Pathak CM, Singh K. Comparison of seven and fourteen days of lansoprazole, clarithromycin, and amoxicillin therapy for eradication of Helicobacter pylori: a report from India. Helicobacter 2000; 5:84-7.

36. Bapat MR, Abraham P, Bhandarkar PV, Phadke AY, Joshi AS. Acquisition of Helicobacter pylori infection and reinfection after its eradication are uncommon in Indian adults. Indian J Gastroenterol 2000; 19:172-4.

37. Bhatia V, Ahuja V, Das B, Bal C, Sharma MP. Use of imidazole-based eradication regimens for Helicobacter pylori should be abandoned in North India regardless of in vitro antibiotic sensitivity. J Gastroenterol Hepatol 2004; 19:619-25.

38. Leung WK, Lin SR, Ching JY, et al. Factors predicting progression of gastric intestinal metaplasia: results of a randomized trial on Helicobacter pylori eradication. Gut 2004; 53:1244-9.

39. Ley C, Mohar A, Guarner J, et al. Helicobacter pylori eradication and gastric preneoplastic conditions: a randomized, double-blind, placebo-controlled trial. Cancer Epidemiol Biomarkers Prev 2004; 13:4–10.

40. Zhou L, Sung JJ, Lin S, et al. A five-year follow-up study on the pathological changes of gastric mucosa after H. pylori eradication. Chinese Med J 2003; 116:11–14.

41. Parsonnet J, Harris RA, Hack HM, Owens DK. Modelling cost-effectiveness of Helicobacter pylori screening to prevent gastric cancer: a mandate for clinical trials. Lancet 1996;348:150–4.

42. Mason J, Axon AT, Forman D, Duffett S, Drummond M, CrocombeW, Feltbower R, Mason S, Brown J, Moayyedi P. Leeds HELP Study Group. The cost-effectiveness of population Helicobacter pylori screening and treatment: a Markov model using economic data from a randomized controlled trial. Aliment Pharmacol Ther 2002; 16:559–68.

43. Nozaki K, Shimizu N, Ikehara Y, et al. Effect of early eradication on Helicobacter pylori-related gastric carcinogenesis in Mongolian gerbils. Cancer Sci 2003; 94:235–9.

44. Wong BC, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, Lai KC, Hu WH, Yuen ST, Leung SY, Fong DY, Ho J, et al. Helicobacter pylori eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. JAMA 2004;291: 187–94.

45. Fukase K, Kato M, Kikuchi S, et al. Effect of eradication of Helicobacter pylori on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomized controlled trial. Lancet. 2008; 372:392-7.

46. Fischbach W, Goebeler-Kolve ME, Dragosics B, Greiner A, Stolte M. Long term outcome of patients with gastric marginal zone B cell lymphoma of mucosa associated lymphoid tissue (MALT) following exclusive Helicobacter pylori eradication therapy: experience from a large prospective series. Gut 2004; 53: 34-7.

47. Kartar Singh, Uday C Ghoshal. Causal role of Helicobacter pylori infection in gastric cancer: An Asian enigma. World J Gastroenterol 2006 March 7; 12(9):1346-1351.

Selected Abstracts –Indian Studies

Dikshit RP, Mathur G, Mhatre S, Yeole BB.Epidemiological review of gastric cancer in India. Indian J Med Paediatr Oncol. 2011 Jan;32(1):3-11.

Abstract

Stomach cancer is the one of the leading cause of cancer in southern region of India. Its incidence is decreasing worldwide yet on global scale stomach cancer remains one of the most common causes of cancer death. Etiology of gastric cancer includes Helicobacter pylori infection, diet and lifestyle, tobacco, alcohol and genetic susceptibility. In this review, we tried to find the contribution of Indian scientist in understanding the descriptive and observational epidemiology of stomach cancer. PubMed was used as a search platform using key words such as “stomach cancer, treatment, clinical characteristics, stomach cancer outcome, epidemiology,

etiological factor and their corresponding Mesh terms were used in combination with Boolean operators OR, AND". Most of the reported studies on gastric cancer from India are case report or case series and few are case-control studies. Indian studies on this topic are limited and have observed H. pylori infection, salted tea, pickled food, rice intake, spicy food, soda (additive of food), tobacco and alcohol as risk factors for gastric cancer. More research is required to understand the etiology, develop suitable screening test, to demarcate high-risk population and to develop and evaluate the effect of primary prevention programs

Misra V, Misra SP, Singh MK, Singh PA, Dwivedi M.Prevalence of H. pylori in patients with gastric cancer. Indian J Pathol Microbiol. 2007 Oct;50(4):702-7.

Abstract

The present study was taken with an aim to assess the prevalence of H. pylori in patients with gastric carcinoma and correlate it with gross appearance and histological type. Endoscopic biopsies from 54 patients with gastric carcinoma and 50 age and sex matched controls were taken after thorough upper gastrointestinal examination. Gross appearance of the tumour was noted and two biopsies each from the site of malignancy and from normal appearing areas were taken. Sections were stained with Haematoxylin & Eosin and Loeffler's methylene blue for histopathological details and presence of H. pylori. Prevalence of H. pylori in controls was slightly higher than the patients group (80% Vs 78%). Ulcerated type of gross appearance had maximum prevalence of H. pylori (88%). Prevalence of H. pylori was more in diffuse type of gastric cancer than intestinal type (86% Vs 68%). A significant association between H. pylori and grades of gastritis was noted ($P < 0.01$) in controls as well as in patient group but it failed to show a significant association with tumour grades, intestinal metaplasia, site of the tumour and age of the patients. So, it can be inferred that prevalence of H. pylori infection is not directly associated with pathogenesis of gastric cancer but it may act as a co-carcinogen by damaging the mucosa and thereby making it more susceptible to effects of carcinogen.

Schistosomiasis and Cancer

Introduction

Schistosomiasis or bilharziasis is a parasitic infection caused by Schistosomes which are blood trematodes that reside in the venous system. Schistosomiasis is an ancient disease and Schistosome ova have been detected in Egyptian mummies of 1250 BC.

In 1951, Bilharzfirst described the presence of the worm in a vein and therefore the disease is also called bilharziasis. There are 4 species known to cause human disease *Schistosoma haematobium*, *Schistosoma mansoni*, *Schistosoma japonicum* and Schistosoma intercalatum.

Schistosomiasis

Schistosomiasis is a common parasitic infection. More than 200 million people are infected globally across 76 countries.The infection is transmitted to man via an intermediate host which is the snail. These snails which proliferate in canals and water reservoirs are of different species. Schistosomiasis is thus a disease affecting agricultural communities and the life cycle is maintained through the unsanitary habits of man.

Transmission and Life Cycle

The ova from an infected person are liberated through the urine or faeces into water reservoirs like lakes, canals and rivers. They contain a fully formed larva called the miracidium. This is the free living form which seeks an intermediate host the snail. Only certain species of snails support the parasite.Miracidia penetrate the snail and through a second larval stage develop into cercaria which leave the snail and have a free living existence in the water. Humans who enter such water reservoirs get infected with the cercaria (larva) which can penetrate through intact skin.

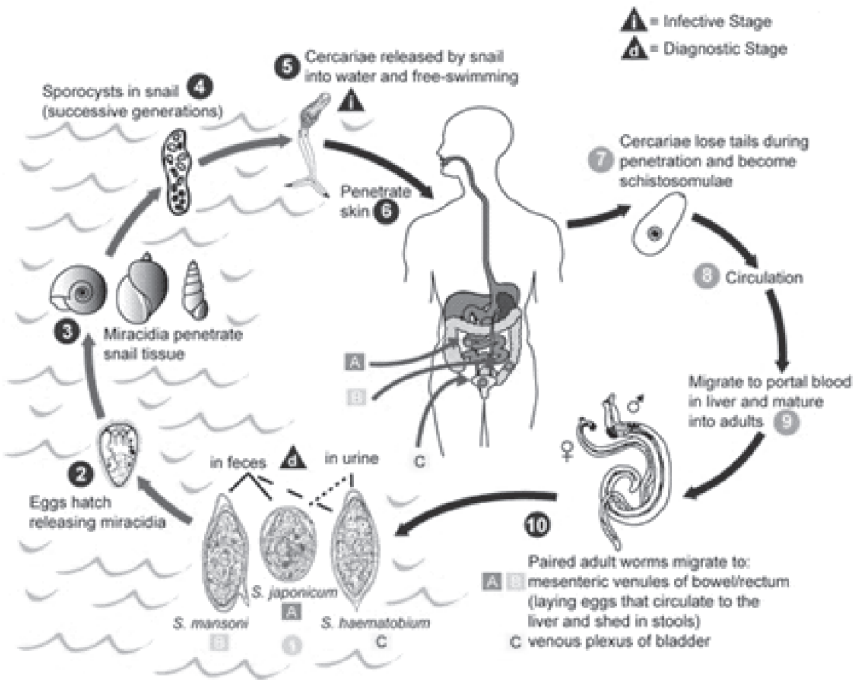


Figure 1: Life Cycle of Schistosomes

In the definitive host the cercaria loses its tail to form schistosomulae. These pass through the tissues to reach the liver where they develop into adult worms. Schistosomes have separate sexes and the female releases ova following copulation.

Clinical Features of Infection

Infection does not always result in clinical disease. Many infections are asymptomatic. Symptomatic patients may have diarrhea, weakness hepato-splenomegaly and later carcinoma of the intestine, liver, uterus and bladder.

Pathology

The adult worms do not cause any tissue damage. The deposited ova are propelled by muscular contractions through the vessel wall into the muscle. This is a slow process and damages the wall of the bladder or intestine. When the ovum passes into the lumen there is associated bleeding. Many ova remain in the muscles and initiate a granulomatous reaction around the ovum. Ova may be carried to the liver, lungs and other organs through the blood stream. The inflammatory reaction results in fibrosis and cirrhosis of the liver or corpulmonale in the lungs.

Intensity of infection and morbidity correlates directly with the worm burden that is the number of worms present in the tissues.

Laboratory diagnosis

Demonstration of ova is the mainstay for diagnosis. Ova are found in urine or in the faeces. Serological tests and molecular tests including PCR are available.

Epidemiology

Schistosomiasis is widespread in the developing countries. Indiscriminate defecation and urination into water reservoirs are of utmost importance for the spread of infection. Schistosomiasis has been endemic in Egypt since the time of the ancient Pharaohs and is currently reported from 75 countries, infecting more than 200 million people with around 600 million at risk of infection. The disease infects the agricultural communities.

Schistosomiasis in India

A small endemic focus of urinary Schistosomiasis was reported by Gadgil and Shah in 1951. The infection is believed to be imported by travelers from endemic areas. They found 250 persons infected with *S haematobium* residing in a village called Gimvi near Ratnagiri, 10 miles from the Konkan coast. The infected population included children.

Extensive studies by Gadgil and Shah have identified a different species of the intermediate host the snail in this region. They have grown and infected this snail under experimental conditions identified as Ferrissiatenuis. This snail was present in large numbers in a stream that passed through the village and was used by the villagers for washing clothes and agricultural activities. Studies of the bladder mucosa in a few of these cases revealed inflammatory changes and calcification of the bladder wall. The primary symptom in all cases was hematuria. Ova of *Schistosoma haematobium* were demonstrated in urine samples of infected cases. Infected children showed a high worm burden from the number of ova. One of the cases was used for experimental infection of the snails grown in an artificially created environment in glass aquaria to establish the life cycle of Schistosomiasis in this endemic focus. The species was further identified as *Schistosoma haematobium* with the intermediate host as Ferrissiatenuis.

Schistosoma haematobium

The adult worm resides in the veins of the vesical plexus and cause symptoms related to the urinary tract. Chronic infections show a wide range of lesions from mucosal ulceration and fibrosis to granulomas, polyps and predisposition to carcinoma of the bladder.Association with prostate cancer is also shown. Diagnosis is by detecting the characteristicova in the urine with a delicate terminal spine.

Schistosoma mansoni

Infections are widespread in Africa, Brazil and the Carribbeanislands. The adult worm resides in the inferior mesenteric veins causing symptoms related to the gastrointestinal tract in the form of abdominal pain and diarrhea.

The intestine shows ulceration, polyps,fibrosis and can lead to portal fibrosis.

Ova are found in the feces and have a prominent lateral spine. In tissue sections these ova are acid fast in contrast to *Schistosoma haematobium* which are non-acid fast. There is no definitive evidence to link this species with Hepato-cellular carcinoma.

Schistosoma japonicum

Infections occur in South East Asia namely Japan, China, Philippines, Thailand, Cambodia, Vietnam and Malaysia. The adult worm resides in the superior mesenteric veins and is known to cause symptoms related to the gastrointestinal tract. The ova released affect the liver resulting in fibrosis. Involvement of the lungs and the central nervous system is known.

Ova are found in the feces and have a small projection tubercle on one side. There is a strong indication that *Schistosoma japonicum* is a causative agent in the development of liver cancer in Japan and Colorectal cancer in China.

Schistosomiasis and bladder cancer

The association between bladder cancer and schistosomiasis was first suspected in 1911 by Ferguson who attributed this process to prolonged irritation caused by the ova of *Schistosoma haematobium* as "cancer of the urinary bladder was the irritation cancer of Egypt." In 1950 many scientists favored the carcinogenic action of this disease. Most of the studies showing this association are from Egypt. This association is also related to the endemicity of the parasite. The association between *Schistosoma haematobium* infection and induction of bladder cancer is strong. The Cairo Cancer Institute data from 1970 to 1974 shows that 27.6% of bladder cancers were associated with Schistosomisasis. There are several such reports that support the association between Schistosomiasis and bladder cancer from countries where Schistosomiasis is known to be endemic.

The major histological cell type of bladder cancer associated with Schistosomiasis is squamous cell carcinoma (SCC). In areas endemic for Schistosomiasis a higher proportion of SCC of the bladder are seen in comparison with bladder cancers from schistosoma free countries. This SCC constitutes 54-81% of bladder cancers in endemic areas of Schistosomiasis compared with 3-10% SCC in the Western Countries.

Squamous cell carcinomas are also associated with high parasite burden and transitional cell carcinomas in areas with lower degrees of infection. The peak incidence of bladder cancer associated with schistosomiasis is between 40-49 years in comparison with schistosoma free countries where the peak is between 65 to 75 years. There is also a male preponderance in the endemic regions of bladder cancer linked with greater exposure of males to infected water during agricultural activities.

Evidence is available in the form of correlation from the demonstration of eggs in histological sections. In Egyptian cases of bladder cancer it is common to find ova of

schistosomiasis. Various studies have shown that 63.5 to 88.7% of specimens from bladder cancers were positive for ova of Schistosomiasis. Further bladder carcinoma was seen at an earlier age in patients with schistosome eggs (46.7 years) than those of egg negative cases (53.2years). The predominant histological type was squamous cell carcinoma (75.9%).

Infection with S. haematobium is associated with a five- fold risk of bladder cancer.

Mechanism of Carcinogenesis

A number of host and environmental factors have been evaluated:

1. *Chronic inflammation:* Chronic inflammation induced by Schistosomiasis and irritation to the bladder mucosa is associated with increased risk of cancer induction.

   Inflammatory cells can enhance the carcinogenic potential of bladder carcinogens.
2. *Bacterial infection:* Several studies have been described to determine a relationship between urinary Schistosomiasis and bacterial infection. It seems possible that infection with nitrate reducing bacteria increases the production of N- nitrosamines. This increases the risk of formation of carcinogenic alkylating agents and thereby the risk of bladder cancer.
3. *Genetic changes:* Activation or inactivation of oncogenes and tumour suppressor genes due to mutations is known to play an important role in tumour progression. The p53 gene is the most intensively studied tumour suppressor gene. Mutation of this gene is the most fragment genetic change found in a variety of malignancies. This association has been described in an Egyptian study on bladder cancers where 86% had p 53 mutations. Changes in cell cycle control are also known to be associated with the development of cancer. However the role of the proteins that cause this change and association with human cancer is unclear. It is evident that several genetic changes occur during the development of bladder cancer and some of these may be used to identify Schistosomiasis associated bladder cancer.
4. *Diet:* Carcinogens associated with foodstuffs have been implicated as causative agents. The presence of N-nitroso compounds in traditional foods may play a significant role in the induction of Schistosomiasis associated bladder cancer.

Schistosomiasis and Carcinogens

Most carcinogens are chemically inert and need to be activated before the initiation of biological consequences. Polycyclic aromatic hydrocarbons are known to cause cancer. These are activated by the cytochrome P-450 system predominantly in the liver and to a lesser extent in the bladder. N-nitrosamines and Aromatic amines are important carcinogens associated with Schistosomiasis and bladder cancer.

Various hypotheses have been proposed to explain the process of carcinogenesis in the bladder by Schistosomiasis.

The N-Nitrosamines are an important class ofcChemical carcinogens in the development of bladder cancer associated with schistosomiasis. Aromatic amines are potent environmental carcinogens and occupational exposure to aromatic amines is a known important cause of bladder cancer.

In conclusion there is a compelling body of evidence in support of the association between Schistosomiasis of the urinary tract and bladder cancer. The mechanisms involved are not completely understood. Chronic irritation and inflammation caused by Schistosomiasis could facilitate a premalignant transformation and along with the other host and environmental factors complete this conversion from premalignant to malignant state.

Preventive strategies for bladder cancer include elimination of the parasite through improved hygienic standards, better sanitation and living conditions, identification of early infections and appropriate management.

Schistosoma japonicum

Schistosomiasis caused by this species has been associated with liver and colorectal cancer. However, the evidence supporting this is weaker and presently infection with *Schistosoma japonicum* is considered as a possible carcinogen. Studies from China and Japan favour the role of infection with *Schistosoma japonicum* as a risk factor in the development of hepatocellular carcinoma. Additional risk factors include infections with Hepatitis B, Hepatitis C and alcohol abuse. A study from China has shown a strong association with colorectal cancer.

Schistosoma mansoni

The association between infection with this species and hepatocellular carcinoma seems to be an indirect one. Patients with this form of Schistosomiaisis have a higher risk of exposure to Hepatitis B virus and Hepatitis C virus during blood transfusion via contaminated blood, needles and syringes.

Intestinal Schistosomiasis causes immunosuppression and these patients take a long time to clear infections with HBV or HCV. This is associated with a higher risk of developing complications including hepatocellular carcinoma.

A review of the literature shows that there is a scarcity of data from India . Schistosomiasis is considered next to malaria among the parasitic diseases of man. There are several unexplored curiosities with this infection. The snail intermediate host described in Gimvi, near Ratnagari by Gadgil and Shah has not been described elsewhere. There have been isolated reports of Schistosomiasis of the urinary tract associated with bladder cancer, hepatic infections from Punjab and Andhra Pradesh. The Gimvi focus is now reported to be a dead focus. However, there is no follow up data on the occurrence of bladder cancer in this region.

This highlights the need for surveillance on the prevalence of schistosomiasis in India and histopathological and molecular studies on the squamous cell cancers of the bladder to

evaluate the role of schistosomiasis in bladder cancers. Similar data on hepatocellular cancers and association with schistosomiasis is necessary.

References

1. Harkness A H. 1922. Bilharzia haematobia in India. The British Medical Journal 475-476.
2. Fairley N H. 1951. Schistosomiasis and some of its problems. Transactions of the Royal Society of Tropical Medicine and hygiene 45: 279-306.
3. Gadgil R K, Shah S N. 1952. Human Schistosomiasis in India Discovery of an Endemic Focus in the Bombay State. IJMR 6: 760-763.
4. Shah S N, Gadgil R K. 1955. Human Schistosomiasis in India part I, The Study of Snails. IJMR 43: 689-694.
5. Gadgil R K, Shah S N. 1955. Human Schistosomiasis in India part II, Infection of Snails with SchistosomaHematobium. IJMR 43: 695-701.
6. Shah S N, Gadgil R K. 1955. Human Schistosomiasis in India part III, Note on the Clinical Survey of the Endemic Focus. IJMR 43: 703-706.
7. Gadgil R K, Shah S N. 1956. Human Schistosomiasis in India part IV, Establishing the Life Cycle in the Laboratory. IJMR 44: 577-580.
8. Editorial. 1956. Human Schistosomiasis in India. Journal of the Indian Medical Association 26:430-431.
9. Gadgil R K. 1963. Human Schistosomiasis in India. IJMR 51:244-251.
10. EL-Bolkainy M N, Mokhtar N M, Ghoneim M A, Hussein M H. 1981. The impact of Schistosomiasis on the Pathology of Bladder Carcinoma. American Cancer Society 48:2643-2648.
11. Koroltchouk V, Stanley K, Stjernsward J, Kott K. 1987. Bladder Cancer: approaches to prevention and control. World Health Organization 65(4): 513-520.
12. Kelkar S S, Kelkar R S. 1993. A Textbook of Parasitology. Published by Popular Prakashan.
13. Mostafa M H, Sheweita S A, O'Connor P J. 1999. Relationship between Schistosomiasis and Bladder Cancer. CMR 12(1):97-111.
14. Amonkar P, Murali G, Krishnamurthy S. 2001. Schistosoma Induced Squamous Cell Carcinoma of the Bladder. IJPM 44(3):363-364.
15. Khurana K, Dubey M L. 2005. Association of Parasitic Infections and Cancers. IJMM 23(2):74-79.
16. Bacelar A. 2007. Association between prostate cancer and schistosomiasis in young patients: A Case Report and Literature Review. The Brazillian Journal of Infectious Diseases 11(5): 520-522.
17. Agarwal M C, Rao V G. 2011. Indian Schistosomes: A Need for Further Investigations. JPR 1-4.

Selected Abstract Indian study

Amonkar P, Murali G, Krishnamurthy S .Schistosoma induced squamous cell carcinoma of the bladder. Indian J Pathol Microbiol. 2001 Jul;44(3):363-4.

Abstract

Schistosomiasis or Bilharziasis caused by S. hematobium is endemic in Africa, Egypt, southern tips of Europe and Japan. Though not unknown in India, it is a much less common occurrence. Schistosomiasis of the bladder is known to be a causative factor for bladder carcinoma; which is usually of the squamous type. These cancers are usually of a higher grade and the average initial stage is higher than those for transitional cell carcinomas. We present a case of schistosoma induced squamous carcinoma of the bladder as this is not a common association in India

Section II
HIV and Cancers

Introduction

HIV Related Lymphoma

- Pathology of AIDS Related Lymphomas
- Management of HIV Related Lymphomas

HIV and Cervical Cancer

HIV Associated Kaposi's Sarcoma

Non AIDS Defining Cancers

HIV and Cancer

Cancer has been associated with AIDS before the human immunodeficiency virus was even identified . The association between viruses, the immune system, and cancer has been better explored and defined since the discovery of HIV. Individuals with HIV infection and cancer are faced with two complex life-threatening diseases.

The AIDS defining cancers are Kaposi's sarcoma (KS), non Hodgkin's Lymphoma (NHL) and cervical carcinoma. All AIDS-defining cancers are caused by or strongly associated with viruses . NHL is associated with Epstein-Barr virus,KS with Kaposi's sarcoma-associated herpesvirus (or human herpes virus 8) and invasive cervical cancer, is associated with human papilloma virus.

Since the advent of highly active antiretroviral therapy (HAART) for HIV infection, and optimal treatment of opportunistic infections, the clinical outcomes for persons living with AIDS have improved substantially. This set of patients in whom severe immunosuppression is not a factor , certain non –AIDS defining cancers like Hodgkin's ,anal canal cancer, lung ,testicular and others are being seen in increased frequencies as compared to the general population. In the current era, HAART as well as better prophylaxis and treatment of opportunistic infections and advances in cancer therapy as well as supportive care, would translate into improved survival and quality of life for patients with HIV and Cancer.

Selected Abstract Indian study

Dhir AA, Sawant S, Dikshit RP, Parikh P, Srivastava S, Badwe R et al Spectrum of HIV/AIDS related cancers in India. Cancer Causes Control. 2008 Mar;19(2):147-53.

Abstract

OBJECTIVE: To study the cancer pattern among HIV positive cancer cases.

METHOD: The study group included patients registered in the HIV Cancer clinic at the Tata Memorial Hospital (TMH), Mumbai, which is the largest tertiary referral cancer center in India. We used the gender and age-specific proportions of each cancer site of the year 2002 that was recorded in the Hospital Cancer Registry to estimate an expected number of various cancer sites among HIV positive cancer patients during the period 2001-2005. The observed number of site-specific cancer cases was divided by the expected number to obtain proportional incidence ratio (PIR).

RESULTS: No case of Kaposi's sarcoma was observed. Increased proportion of non-Hodgkin's lymphoma (NHL) was observed (PIR in males = 17.1, 95%CI 13.33-21.84, females = 10.3, 95%CI 6.10-17.41). In males, PIR was increased for anal cancer (PIR = 10.3, 95%CI 4.30-24.83), Hodgkin's disease, testicular cancer, colon cancer, and few head and neck cancer sites. Among females, the PIRs for cervical cancer (PIR = 4.1, 95%CI 2.90-5.75), vaginal cancer (PIR = 7.7, 95%CI 2.48-23.85), and anal cancer (PIR = 6.5, 95%CI 0.91-45.88) were increased.

CONCLUSIONS: The absence of Kaposi's sarcoma and increased PIRs for certain non-AIDS defining cancers among HIV infected cancer cases indicates a different spectrum of HIV associated malignancies in this region. The raised PIR for cervical cancer emphasizes the urgent need for screening programs for cervical cancer among HIV infected individuals in India.

Pathology of AIDS Related Lymphomas

Introduction

In the highly active antiretroviral therapy (HAART) era, AIDS related Non-Hodgkin's lymphomas (ARL) and their treatment still represent an open issue, because HAART may not be sufficient to prevent the development of Non Hodgkin's Lymphoma (NHL) [1].

As we embark well into the fourth decade of the HIV pandemic, AIDS-related NHL remains a major cause of morbidity and mortality globally. While HAART has been successful in preventing some AIDS defining illnesses like Kaposi sarcoma probably, an increased duration of therapy is required to prevent NHL thus the rates are not yet down and may be increasing in some continents[2]. The improved CD4 counts have however resulted in a shift in the morphology from lesser Burkitt's lymphoma (BL) to larger cell lymphomas[3].

A rising Indian epidemic

Though the estimated prevalence of HIV infection in India is low, the actual numbers are next to African countries due to the dense population. Likewise a study reported from our institute reported that 30% of HIV positive patients registered at the Tata Memorial Hospital with cancer had Non Hodgkin's lymphoma making it the highest prevalent cancer in HIV patients[4]. In an autopsy study in HIV positive patients from Mumbai the incidence of Non Hodgkin's lymphomas was 3%[5]. In a study from our institute in 2002, 35 ARL cases included 7 cases of Hodgkin disease, 4 cases of plasmacytoma, 3 Burkitt lymphoma, 4 diffuse large B-cell lymphoma (DLBL), 10 immunoblastic DLBL (IBL), 4 high-grade B-cell lymphoma (unspecified) and the rest were other subtypes[6]. EBV-association was noted in all cases of HD, 2 of 3 BL, and 3 of 10 immunoblastic lymphoma[6]. Since that study from 2000 to 2011 we have accessioned 265 AIDS associated lymphoma (publication in process, part of an ICMR funded study) hinting at the rising nature of incidence with lesser patients dying from infective causes. Of these after

thorough evaluation 39% were DLBL (including IBL), 45% were plasmablastic lymphomas (PBL), 10% were Burkitt lymphomas, 4 % were unclassifiable, 1% was plasmacytoma and 1% had other tumor types. EBER- ISH was positive in 30% DLBL and in 83% PBL cases. The unclassifiable categories were reduced chiefly due to availability of extended immunohistochemistry panel for diagnosis.

General features of ARL

While Non Hodgkin's lymphomas have been labeled as AIDS defining illness way back in 1985 in HIV patients, the Hodgkin's lymphoma do not form "AIDS defining illness" even though they occur with increased frequency in HIV patients. Non Hodgkin lymphoma (NHL) is 60 times more common in AIDS patients than in the general population[7]. Essentially ARL are characterized by high incidence of extranodal presentation, B cell derivation, aggressive behavior and increased association with Epstein Barr Virus(EBV) or Human Herpes or Kaposi sarcoma Virus -8(HHV8/KSV). Lymphomas in such settings more commonly affect patients with hemophilia or clotting disorders or homosexuals and IV drug abusers having HIV infection than those who have acquired HIV by heterosexual contact[7,8]. Though most studies are retrospective, 4-10% of HIV patients are affected by lymphomas. The Multicentric AIDS Cohort Study (MACS) actually found an increase in the incidence of NHL at a rate of 21% per year while the incidence of Kaposi's sarcoma fell during the same period and is likely to reflect the reduction in other causes of AIDS more than a true rise in incidence of NHL[9]. Eighty percent of NHLs in AIDS patients are systemic NHLs while primary central nervous lymphomas (PCNSL) form 20%[10, 11]. Of the systemic lymphomas 40% are diffuse large B cell lymphoma (DLBL) and 30% each are formed by immunoblastic and Burkitt's lymphoma. A drop in incidence of PCNSL after HAART therapy has been observed but the spectrum of ARL varies in different populations. A Japanese study showed that EBV-positive lymphomas decreased from 88% in the pre-HAART era to 58% in the HAART era, but the actual incidence did not differ significantly between HAART users (73%) and nonusers (74%)[12].

Factors causing ARL in HIV patients:-

1. Chronic B cell stimulation and proliferation – It is now clear that HIV virus is not directly involved in transformation of B cells[11]. Dendritic histiocytes in germinal center of lymphoid follicles serve as sanctuary for HIV virus proliferation leading to chronic B cell stimulation and these stimulated B cells are prone to mutational changes. HIV stimulates monocytes and macrophages to produce IL6 a B cell differentiation factor that functions as an autocrine growth factor.
2. Immunosuppression predisposes B cells to a genetic mutation. Many studies have documented that nodes that exhibit florid follicular hyperplasia contain clonal B cell population that are immortalized but not transformed. Lack of adequate immune function leads to defective T cell function, lack of removal of hyper stimulated clonal B cells that are genetically unstable and prone to develop translocations especially in bcl6/ cmyc .

3. Susceptibility to infection by transforming viruses – EBER/HHV8 infection in HIV patients builds an oncogenic environment. EBV is capable of immortalizing B cells but may be insufficient by itself for tumor development. Likewise KSHV is known to activate cyclin D1 and some cell cycle proteins that can lead to the development of lymphomas indirectly[11].

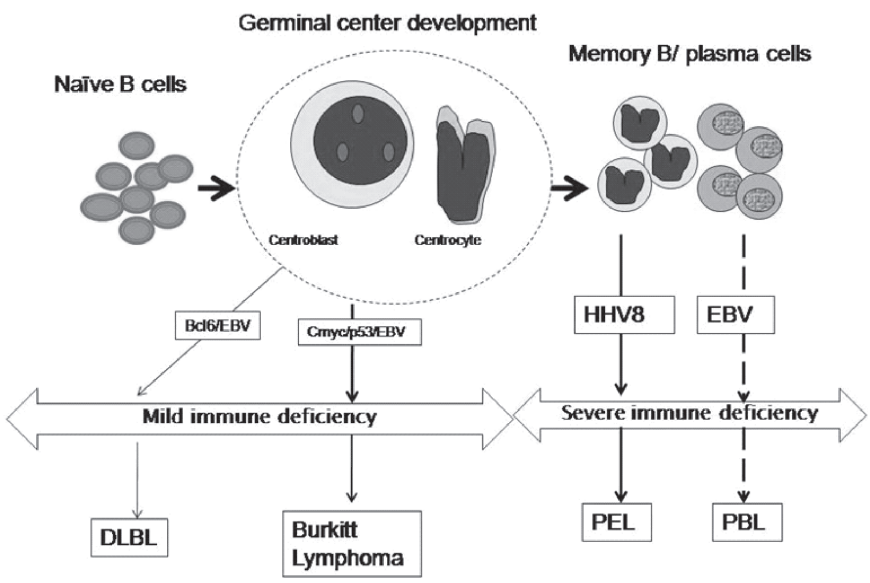
   Three factors that determine what type of lymphoma occurs in a HIV infected person and are also responsible for the heterogeneity of ARL[13] viz:-

a) The degree of immune deficiency in the host – the more preserved an immune status of a patient the more differentiated subtypes of lymphomas develop. Thus Hodgkin's lymphoma and diffuse large B cell lymphoma are common in patients on HAART while plasmablastic lymphomas occur in patients with severe immune deficiency.

b) Coexistent viral infection and molecular pathway affected in different ethnic groups: While HHV8 infection gives rise to PEL in a severely immunosuppressed patients, in populations where HHV8 seroprevalence is low like in India, plasmablastic lymphomas occur more commonly.

c) Cell of origin involved in the development- Depending on the stage at which the B cell development is affected the resultant tumor varies.

At present, 4 major molecular pathways in ARL have been identified. The first pathway involves AIDS-BL and is characterized by mild immunodeficiency in the host and multiple genetic lesions of the tumor, including activation of c-myc, disruption of p53, and less frequently, infection by EBV. Two distinct pathways involve AIDS DLBL/Burkitt lymphoma (BL) and IBL. Expression of LMP1 yields immunoblastic subtypes while BCL-6 rearrangement lead to BL/DLBL. Finally, the fourth pathway associated with HHV8 and AIDS-PEL. A summary of these pathways involved in ARL is given in Figure 1.



Histological Classification of AIDS related lymphomas

AIDS related B cell lymphomas express immunophenotype similar to those expressed by lymphomas of comparable morphology occurring in immunocompetent individuals. The present WHO classification 2008 does not acknowledge the category of immunoblastic B cell lymphomas separately, but the fact remains that a chunk of B cell lymphomas in HIV positive patients cannot be classified into clear cut categories. A summary of ARL is listed in Table 1.

Table 1: Histological Classification of AIDS associated lymphomas:-

Lymphomas also occurring in immunocompetent patients
A) Burkitt lymphoma
B) Diffuse large B-cell lymphoma
- Centroblastic
- Immunoblastic
D) Peripheral T-cell lymphoma (rare)
E) Classic Hodgkin lymphoma
Lymphomas occurring more specifically in patients who are HIV patients
- Primary effusion lymphoma
- Plasmablastic lymphoma of the oral cavity
Lymphomas occurring in other immunodeficiency states
- Polymorphic B-cell lymphoma

Primary CNS lymphoma (PCNSL) in HIV patients:- As opposed to non AIDS-related PCSNL in Norway, the incidence of AIDS-related PCNSL was shown to be falling[14]. On MRI and CT scan PCNSL closely mimics toxoplasmosis and though diffusion or perfusion MRI, magnetic resonance spectroscopy, positron emission tomography, EBV PCR in CSF might help to select patients for biopsy none of these tests are completely specific. PCNSL are uncommon in HIV patients in India and one study quoted 1/56 PCNSL being HIV positive[15]. The PCNSL in HIV positive patients is often associated with Epstein-Barr virus (EBV), whereas EBV is rare in immunocompetent PCNSL patients. Pathologically PCNSL in immunocompetent and immunodeficient patients is similar and show a centroblastic or large B cell morphology with the characteristic angiocentric pattern. Most PCNSLs (96%) are positive for MUM-1 (a marker of germinal center/ early post–germinal center B cells), indicating a late germinal center/early post–germinal center stage of differentiation[16].

Diffuse large B cell lymphoma / DLBL in AIDS:- The heterogeneity of DLBL in non HIV patients is maintained even in the AIDS patients. Amongst the unique morphology the immunoblastic variant is the hallmark of AIDS related DLBL. DLBLs containing > 90% immunoblasts and usually exhibiting plasmacytoid features are classified as the immunoblastic

(IBL) variants and 90% of these harbors an EBV infection. It is still unclear if these morphologic variants should continued to be recognized in a pathology report as the present WHO 2008 classification does not classify these separately.

Immunohistochemical markers that can place DLBCL into prognostically relevant categories have been identified, sometimes based on the data gleaned from the gene expression profiling research[17]. In the AIDS malignancy consortium study the relative proportion of GCDLBCLs was higher than in immunocompetent patients and the frequency of EBV in DLBCLs is not increased in AIDS patients. However in a study at our institute (unpublished observations) we observed that in a third of ARL neither germinal center nor MUM1 expression was noted[17]. Similarly in a study that compared AIDS DLBL with non AIDS DLBL it was observed that while non AIDS DLBL clustered into the germinal centre and activated B cell groups easily, AIDS DLBL did not cluster similarly and showed profiles with overlapping features[18]. A study of 81 DLBCLs from patients with AIDS in AIDS malignancy consortium compared the immunophenotype with survival data, Epstein-Barr virus (EBV) positivity, and CD4 counts and found none of these factors except Ki-67 affected survival, where a higher proliferation index was associated with better survival[17]. However another study documented poor OAS (overall survival) and DFS (disease free survival) in patients with post–germinal center (GC) differentiation (BCL-6 and CD10 negative but MUM1/IRF4 and/or CD138+) when compared with GC (CD10 and bcl6 positive) differentiation[19].

Burkitt lymphoma:- Burkitt lymphoma (BL) is a germinal center (GC) tumor with high proliferation index that needs to be treated with intensive regimens and this often becomes a problem in HIV positive patients with immunosuppression and low CD4 counts. While the survival of patients with DLBL has improved in the HAART era, survival of similarly treated patients with HIV-BL remains poor[20]. While BL in immunocompetent patients has extranodal presentation, in HIV patients BL is often nodal in distribution. HIV positive patients with harboring a BL are often young, do not usually carry a diagnosis of AIDS and have fairly preserved CD 4 counts[21]. The reason may be that without a cognate CD4 lymphocyte cell help, B cells are shunted into programmed cell death and therefore, the rate of generating c-*myc/*Ig translocation positive B cells would decrease, reducing the incidence of BL in patients with low CD4 lymphocyte counts[22].

Many AIDS associated DLBL show high proliferative index and often it is not possible to differentiate BL from DLBL. Some reports describe atypical immunophenotypic features in HIV-BL which include absence of CD20 and surface immunoglobulin expression which mimics plasmablastic lymphoma[23].

Polymorphic B cell Lymphomas:- Nador et al described 10 cases of lymphomas in AIDS patients which morphologically resemble polymorphic lymphoproliferations occurring in post transplant patients[24].Similar to post transplant lymphoproliferative disorders /PTLPD these were characterized by a polymorphous lymphoid cell population exhibiting variable plasmacytic differentiation, cytologic atypia, epithelioid histiocytes, numbers of atypical immunoblasts,

and focal coagulative necrosis. None of the patients had a history of severe opportunistic infections or Kaposi's sarcoma or carried a known diagnosis of AIDS at the time of presentation and had low CD4 counts. Unfortunately, due to lack of clinical information, definite prognostic implications could not be elucidated.

Plasmablastic lymphoma (PBL):- Delecluse et al. were the first to describe 16 cases of a highly aggressive B-cell lymphoma with plasmacytic differentiation in HIV positive patients which commonly affected extranodal sites like oral cavity, gastrointestinal tract, and lymph nodes[25].

While the clinical spectrum of PBL is well elucidated the pathology diagnostic scene has become complex. The vast majority of PBL cases are seen in patients with chronic HIV infection and low CD4 cell counts and HAART is of value not only to treat but also to prevent PBL in HIV patients. Given these factors it is not surprising that this unique lymphoma occurs commonly in Indian patients. Most cases of PBL present with either stage I or stage IV disease. EBV was detected in 74%. Complete response to CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) was obtained in 66% of treated cases. The refractory/relapsed disease rate was 54%. Death occurred in 53% of patients, with a median overall survival of 15 months. Longer survival times in patients with PBL are being reported in the literature and have been attributed to response to HAART, the patient's inherent immunological status, improved supportive care (i.e., antibiotic prophylaxis and growth factors), and improved delivery of chemotherapy[26].

PL are CD20 negative or weak positive, LCA +/CD138+/CD38+/MUM1+/PAX5 negative post germinal center immunophenotype. The histological spectrum of DLBCL with plasmablastic features has however widened to include subtypes, such as PBL of oral mucosa type, PBL with plasmacytic differentiation, classic primary effusion lymphoma (PEL), extracavitary/solid PEL or HHV8-associated DLBCL, and ALK-positive DLBCL. This has lead to many questions around the diagnostic criteria for PBL. Essentially PBL have a blastic NHL like or myeloma like histology. Some tumors with frank DLBL histology may show immunophenotype of PBL and this poses problem of labeling this as DLBL/PBL. Myeloma like PBL and myeloma share similar antigen profiles however the clinical presentation, high MIB1 in PL and the HIV status are useful in the distinction[27].

More recently the MYC rearrangement which is a characteristic of Burkitt's lymphoma (BL) and some DLBCL with very aggressive clinical behavior have been identified as the most common recurrent structural chromosomal alteration in PBL[28]. *MYC* rearrangements were reported in 20 (49%) of the 41 PBL in a recent study with I*GH* gene as the partner in 15 cases. These rearrangements were more frequent in monomorphic PBL than in PBL with plasmacytic differentiation but this difference was not statistically significant[28]. With the detection of these MYC rearrangements the border between BL and PBL has become more blurred in HIV positive patients.

Primary effusion lymphomas (PEL):- Cesarman et al in 1995 identified Kaposi sarcoma associated human herpes virus (KHSV) DNA in a unique group of AIDS associated lymphomas that primarily affected body cavities and presented with effusions[29]. Subsequently the same team coined the term PEL or primary effusion lymphomas for these tumors. PEL form 4% of all ARL but have been reported in patients with other forms of immunodeficiency, in non immunocompromised elderly patients and in geographic areas with high KHSV prevalence[30.] This subtype is not common in Indian patients just as Kaposi sarcoma as the seroprevalence of HHV8 in India is low. In a multicontinent study the seroprevalence of HHV8 in healthy individuals was 4 % while it was 2.4% in patients with HIV-1 infection[31].

Microscopically PEL has morphologic features that bridge between Immunoblastic lymphoma and anaplastic large cell lymphoma[30]. Their cytoplasm is very much like plasmablastic lymphomas but the PEL cells have enormous size and prominent nucleoli. PEL also share their most of their immunophenotype with plasmablastic lymphomas except their association with KSHV and clonal Ig rearrangements and somatic hypermutations. Hence PEL are LCA positive (95%), show absence of B and T antigens, express activation marker CD30, EMA and plasma cell markers CD138, CD38 and MUM1 with the expression of the KHSV latent protein LNA1. Co infection with EBV is recorded in PEL. While diagnostically more defined than PBL, the clinical spectrum of PEL is expanding and it is now recorded at nodal and extranodal locations like oral cavity (solid PEL) preceding or following resolution of PEL in AIDS patients[32]. Sometimes these solid PEL's are not associated with PEL and such patients have longer survival. Most of PEL carry a dismal prognosis but cases with complete remission after only HAART administration have been reported.

Large B cell lymphoma arising in multicentric Castlemans disease (MCD) in HIV patients:- HHV8 is specifically associated with a variant of MCD with lambda light chain restricted KHSV positive plasmablasts in the mantle zone of B cell follicles[33]. These plasmablasts in MCD are large with moderate amphophilic cytoplasm and large vesicular nucleus with one or two prominent nucleoli and showed CD20+ /CD30-/LNA 1+ /CD138+ and l light chain restriction. Though these large cells are called plasmablasts they show clear cut B cell differentiation, resemble immunoblasts, show light chain restriction, express IgM and are strongly CD20 positive. Small collections of these plasmablasts may be seen in usual MCD but only when they destroy the node and show clonality they are labeled as large B cell lymphomas.

Peripheral T cell lymphoma[34]**:-** Data on HIV-associated PTCL are largely limited to case reports and small case series. As with poor prognosis types of ARL, PTCL is associated with low CD4 counts and poor survival. While PTCL-NOS and ALK negative ALCL morphology is common the response rate is similar to immunocompetent patients with PTCL, and ranges from 50 to 70%. While diagnosing a PTCL in untreated HIV patients with poor immune status pathologist should remember that clonal CD8 positive reactive proliferations that resolve after HAART therapy are known.

Hodgkin's Lymphoma(HL) in HIV patients[35-37] **:-** HL represents the most common type of non–AIDS-defining tumor that occurs in the HIV-infected population. After a 12-year follow-up, the Multicenter AIDS Cohort Study (MACS) found the relative risk for HL in HIV positive individuals versus the US general population to be 7.0. HIV-HL patients have widespread disease at presentation, high incidence of systemic ''B'' symptoms with frequent involvement of extranodal sites like bone marrow, liver, and spleen and noncontiguous spread of tumor. HL occurring in HIV-infected individuals exhibits pathologic features different from those of HL in the general population including a higher incidence of unfavorable histologic subtypes like mixed cellularity (40%–100%) and lymphocyte depleted (20%), and a relatively low prevalence of the nodular sclerosis and lymphocyte predominant subtypes[35]. HL in the HIV setting displays a distinct cellular background, including the presence of a fibrohistiocytoid stromal cell proliferation in the involved lymph nodes. Another morphologic characteristic is the abundance of Reed Sternberg cells, mimicking ALCL. A large majority of HIV-related Hodgkin's lymphoma (80%–100%) are linked pathogenically to EBV as opposed to HIV negative HL[37].

Differential Diagnosis within ARL spectrum

A certain degree of diagnostic confusion occurs in the classification of NHL in HIV patients. The flow chart of these diagnostic issues in CD20 positive and CD20 negative subgroups of ARL is given in Table 2 and Table 3. Essentially appropriate segregation depends on use of extended immunohistochemistry panel, clinical profile and molecular genetics. A summary of

Table 2: Differential diagnosis in CD20 positive AIDS Related Lymphomas

Diffuse Large B cell germinal center Vs Burkitt		**Diffuse large B cell(Immunoblastic) Vs Plasmablastic**	
Large to intermediate size cells	Intermediate size cells	Nuclei have single prominent nucleolus	Nuclei in blastic have prominent nucleolus
Pleomorphic cells	Monomorphic	Cell cytoplasm pale eosinophilic	Cell cytoplasm plasmacytoid and amphophilic
EBV latency varies	EBV latency I pattern EBER+, occasionally EBNA1+, LMP1-/ EBNA2-	EBER in 30% tumors Variable latency	EBER in 80% tumors-latency I
Ki67 variable but less likely 100%	Ki67 – 100%	CD 20 variable	CD 20 always focal, never strong positive
Molecular genetics : complex rearrangements including MYC may be seen	Molecular genetics : MYC rearrangements without other rearrangements	IgH gene rearrangements seen	IgH gene rearrangements absent

Table 3: Differential diagnosis of CD20 negative AIDS related Non Hodgkins Lymphomas

Features	Plasmablastic lymphoma	Primary effusion lymphoma	Immunoblastic DLBL
Morphology	Plasmacytoid cells	Immunoblastic or anaplastic cells	Immunoblastic cells
Immunohistochemistry	LCA + w, CD20(1-2%) CD138/ MUM1(90%) EMA(42.5%)*	LCA + w, CD20(13%), CD138/MUM1 (40%) EMA(70-0%)	LCA+/- , CD20(+/-) , CD138/MUM1 +, EMA negative
EBER - ISH	80% positive	Variably positive	Variably positive
EBV-LMP1	Negative	Negative	Positive
HHV8	Absent	Always present	Absent

Table 4: Immunophenotypic differences in ARL

	PBL	PEL	DLBL	Immunoblastic	Burkitt lymphoma
LCA	+/weak(w)	+/weak	+++	+++	+++
CD20	w(1-2%)	+/w(13%)	+++	w/+++	+++
CD79a	W	+/w(25%)	+++	+++	+++
CD138	+++	+(40%)	Negative	-/+++	Negative
CD38	+++	++	Negative	Not known	Negative
MUM1	++	++	++	+	Negative
Bcl6	Negative	Negative	+++	Negative	+++
PAX5	Negative	Negative	+++	Negative/+	++
EBER (ISH)	++++	++	+	+++	+++
HHV8 (LNA1)	Negative	++++	+	+	Negative

immunohistochemistry panel required to differentiate common subtypes of ARL is given in Table 4.

Conclusion

ARL represent a heterogeneous yet unique spectrum on Non Hodgkins lymphoma and Hodgkin's lymphoma. The geographic distribution of subtypes and behavior is largely dependent on seroprevalence of EBV/HHV8, prior CD 4 counts and HAART therapy. Therapy related guidelines should target each of these facets to reduce incidence of ARL in HIV positive patients.

References

1) Carbone A. AIDS-related non-Hodgkin's lymphomas: from pathology and molecular pathogenesis to treatment. Hum Pathol. 2002;33(4):392-04.
2) Stebbing J, Gazzard B, Mandalia S, Teague A, Waterston A, Marvin V, et al. Antiretroviral treatment regimens and immune parameters in the prevention of systemic AIDS-related non-Hodgkin's lymphoma. J Clin Oncol. 2004; 22:2177-83.
3) Levine AM, Seneviratne L, Espina BM, Wohl AR, Tulpule A, Nathwani BN, Gill PS. Evolving characteristics of AIDS-related lymphoma. Blood. 2000;96(13):4084-90.
4) Dhir AA, Sawant S, Dikshit RP, Parikh P, Srivastava S, Badwe R, et al. Spectrum of HIV/AIDS related cancers in India. Cancer Causes Control. 2008;19(2):147-53.
5) Lanjewar DN. The spectrum of clinical and pathological manifestations of AIDS in a consecutive series of 236 autopsied cases in mumbai, India. Patholog Res Int. 2011;20:547-18.
6) Agarwal B, Ramanathan U, Lokeshwar N, Nair R, Gopal R, Bhatia K, Naresh KN. Lymphoid neoplasms in HIV-positive individuals in India. J Acquir Immune Defic Syndr. 2002;29(2):181-3.
7) Beral V, Peterman T, Berkelman R, Jaffe H. AIDS-associated non-Hodgkin lymphoma. Lancet. 1991;338(8771):884-5.
8) Knowles DM.Immunodeficiency-associated lymphoproliferative disorders. Mod Pathol. 1999;12(2):200-17.
9) Powles T, Matthews G, Bower M.AIDS related systemic non-Hodgkin's lymphoma. Sex Transm Infect. 2000;76(5):335-41.
10) Knowles DM. Molecular pathology of acquired immunodeficiency syndrome-related non-Hodgkin's lymphoma. Semin Diagn Pathol. 1997;14(1):67-82.
11) Knowles DM. Etiology and pathogenesis of AIDS-related non-Hodgkin's lymphoma. Hematol Oncol Clin North Am. 2003;17(3):785-820.
12) Hishima T, Oyaizu N, Fujii T, et al: Decrease in Epstein-Barr virus-positive AIDS-related lymphoma in the era of highly active antiretroviral therapy. Microbes Infect 2006;8:1301-07.
13) Carbone A. Emerging pathways in the development of AIDS-related lymphomas. Lancet Oncol. 2003 ;4(1):22-9.
14) Haldorsen IS, Kråkenes J, Goplen AK, Dunlop O, Mella O, Espeland A. AIDS-related primary central nervous system lymphoma: a Norwegian national survey 1989-2003. BMC Cancer. 2008; 8:225-32.
15) Paul T, Challa S, Tandon A, Panigrahi M, Purohit A. Primary central nervous system lymphomas: Indian experience, and review of literature. Indian J Cancer. 2008; 45:112-8.

16) Bhagavathi S, Wilson JD. Primary central nervous system lymphoma. Arch Pathol Lab Med. 2008;132:1830-4.

17) Chadburn A, Chiu A, Lee JY, Chen X, Hyjek E, Banham AH, Noy A, et al. Immunophenotypic analysis of AIDS-related diffuse large B-cell lymphoma and clinical implications in patients from AIDS Malignancies Consortium clinical trials 010 and 034. J Clin Oncol. 2009;27:5039-48.

18) Gormley RP, Madan R, Dulau AE, Xu D, Tamas EF, Bhattacharyya PK et al. Germinal center and activated b-cell profiles separate Burkitt lymphoma and diffuse large B-cell lymphoma in AIDS and non-AIDS cases Am J Clin Pathol. 2005;124:790-8

19) Hoffmann C, Tiemann M, Schrader C, Janssen D, Wolf E, Vierbuchen M, et al. AIDS-related B-cell lymphoma (ARL): correlation of prognosis with differentiation profiles assessed by immunophenotyping. Blood. 2005;106(5):1762-9.

20) Lim ST, Karim R, Nathwani BN, Tulpule A, Espina B, Levine AM. AIDS-related Burkitt's lymphoma versus diffuse large-cell lymphoma in the pre-highly active antiretroviral therapy (HAART) and HAART eras: significant differences in survival with standard chemotherapy. J Clin Oncol. 2005;23:4430-8.

21) Davi F, Delecluse HJ, Guiet P, Gabarre J, Fayon A, Gentilhomme O, Felman P, Bayle C, Berger F, Audouin J, Bryon PA, Diebold J, Raphaël M. Burkitt-like lymphomas in AIDS patients: characterization within a series of 103 human immunodeficiency virus-associated non-Hodgkin's lymphomas. Burkitt's Lymphoma Study Group. J Clin Oncol. 1998 ;16:3788-95.

22) Guech-Ongey M, Simard EP, Anderson WF, Engels EA, Bhatia K, Devesa SS, Mbulaiteye SM. AIDS-related Burkitt lymphoma in the United States: what do age and CD4 lymphocyte patterns tell us about etiology and/or biology? Blood. 2010;116: 5600-5604.

23) Kelemen K, Braziel RM, Gatter K, Bakke TC, Olson S, Fan G. Immunophenotypic variations of Burkitt lymphoma. Am J Clin Pathol. 2010;134:127-38.

24) Nador RG, Chadburn A, Gundappa G, Cesarman E, Said JW, Knowles DM.Human immunodeficiency virus (HIV)-associated polymorphic lymphoproliferative disorders. Am J Surg Pathol. 2003;27:293-302.

25) Delecluse HJ, Anagnostopoulos I, Dallenbach F, Hummel M, Marafioti T, Schneider U, Plasmablastic lymphomas of the oral cavity: a new entity associated with the human immunodeficiency virus infection. Blood. 1997;89:1413-20.

26) Castillo J, Pantanowitz L, Dezube BJ. HIV-associated plasmablastic lymphoma: lessons learned from 112 published cases. Am J Hematol. 2008;83(10):804-9.

27) Vega F, Chang CC, Medeiros LJ, Udden MM, Cho-Vega JH, Lau CC, et al. Plasmablastic lymphomas and plasmablastic plasma cell myelomas have nearly identical immunophenotypic profiles. Mod Pathol. 2005;18:806-15.

28) Valera A, Balagué O, Colomo L, Martínez A, Delabie J, Taddesse-Heath L, Jaffe ES, Campo E. IG/MYC rearrangements are the main cytogenetic alteration in plasmablastic lymphomas. Am J Surg Pathol. 2010 Nov;34(11):1686-94.

29) Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM.Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. N Engl J Med. 1995;332:1186-91.

30) Ablashi D, Chatlynne L, Cooper H, Thomas D, Yadav M, Norhanom AW, et al Seroprevalence of human herpesvirus-8 (HHV-8) in countries of Southeast Asia compared to the USA, the Caribbean and Africa. Br J Cancer. 1999;81:893-7.

31) Carbone A, Gloghini A. KSHV/HHV8-associated lymphomas.Br J Haematol. 2008;140:13-24.

32) Chadburn A, Hyjek E, Mathew S, Cesarman E, Said J, Knowles DM. KSHV-positive solid lymphomas represent an extra-cavitary variant of primary effusion lymphoma. Am J Surg Pathol. 2004;28:1401-16.

33) Dupin N, Diss TL, Kellam P, Tulliez M, Du MQ, Sicard D, Weiss RA, Isaacson PG, Boshoff C. HHV-8 is associated with a plasmablastic variant of Castleman's disease that is linked to HHV-8-positive plasmablastic lymphoma. Blood. 2000;95:1406-12.

34) Castillo JJ, Beltran BE, Bibas M, Bower M, Collins JA, Cwynarski K, et al Prognostic factors in patients with HIV-associated peripheral T-cell lymphoma: a multicenter study. Am. J. Hematol. 2011; 86:256–61.

35) Tirelli U, Errante D, Dolcetti R, Gloghini A, Serraino D, Vaccher E, et al. Hodgkin's disease and human immmunodeficiency virus infection: clinicopathologic and virologic features of 114 patients from the Italian Cooperative Group on AIDS and Tumors. J Clin Oncol 1995;13: 1758– 67.

36) Dolcetti R, Boiocchi M, Gloghini A, Carbone A. Pathogenetic and histogenetic features of HIV-associated Hodgkin's disease. Eur J Cancer. 2001;37:1276-87.

37) Herndier BG, Sanchez HC, Chang KL, Chen YY, Weiss LM. High prevalence of Epstein-Barr virus in the Reed-Sternberg cells of HIV-associated Hodgkin's disease. Am J Pathol 1993;142: 1073-9.

Management of HIV Related Lymphomas

Human acquired immunodeficiency virus (HIV) infection increases the risk of developing non-Hodgkin lymphoma (NHL) by 165-folds as compared to non-infected individuals. Approximately 4% of individuals with acquired immunodeficiency syndrome (AIDS) have NHL either at the time of diagnosis or develop during the course of illness. This has lead to incorporation of NHL as one of the AIDS defining illnesses since 1985. Introduction of combination antiretroviral therapy (ART) has changed the biology as well as decline in incidence of NHL over the years. However the significant reduction in opportunistic infections and kaposi's sarcoma has made lymphomas as the most common AIDS defining illness.

Diffuse large B cell lymphomas, Burkitt's lymphoma, immunoblastic lymphomas and primary CNS lymphomas are the commonly reported subtypes in these patients. Other rare but unique subtypes include plasmablastic and primary effusion lymphomas.

Non-Hodgkin lymphomas in HIV/AIDS patients have important differences as compared to immunocompetent patients with respect to clinical features- advanced stage, higher frequency of B symptoms, extranodal involvement and central nervous system (CNS) involvement at presentation.

Evaluation and investigations at diagnosis

History: B symptoms, prior AIDS defining illness, features suggestive of ongoing opportunistic infections, CNS symptoms and other comorbidities

Examination: Performance status, documentation of sites and size of lymphadenopathy, organomegaly, extranodal sites.

Investigations for diagnosis:

Excisional / incisional / core needle biopsy from most representative and accessible nodes or extranodal site. This should be subjected for histopathological diagnosis, immunohistochemistry and other optional special tests (EBER, HHV-8).

Imaging – CECT of thorax , abdomen and pelvis and Brain. PET-CT is optional

Bone marrow biopsy and imprint

CSF cytology

Serum LDH

Complete blood count

Renal and live function tests

CD4 counts

Preferably Viral load

Treatment of HIV related NHL

Before deriving any conclusions from studies published on management of HIV related NHL two important conclusions and observations from the Cochrane reviews 2009 should be kept in mind

1. All reported studies in HIV related NHL are from developed countries whereas almost 95% of HIV infected individuals live in developing world and have no representation in these studies
2. There is no evidence that the systemic interventions for untreated patients with AIDS-related NHL provide superior clinical effectiveness for improving overall survival, disease free survival, and tumour response rate; however, this conclusion is based on four randomized controlled trials with limited sample size and variable quality. More adequately powered randomized controlled trials that have low risk of bias are necessary to determine the real benefit or harm of interventions to treat this population.(**Level of evidence IA**)

In addition, one important fact to be considered is very low rates of ART usage in countries like ours and thus our HIV related lymphoma biology and outcomes mimic that of pre- ART era.

Treatment of HIV-related NHL has been a difficult task due to tumour regrowth, poor response rates and high rates of infections secondary to chemotherapy. Risk stratified approach based on performance status, CD4 counts and stage has lead to use of modified or low dose chemotherapy (CHOP, mBACOD). With this approach median survival has been 3-6 months. Patients who received full dose chemotherapy died of treatment related complications whereas in those with low dose chemotherapy, lymphoma progression was the cause of death. **(Level of evidence II B)**

Use of ART along with chemotherapy (CHOP, ACBVP) lead to better response rates (70-75%) and median survival (upto 27 months) **(Level of evidence II B)**

In order to reduce the tumour regrowth by overcoming tumour cell resistance infusional chemotherapies have been tested. Preclinical and clinical evidence suggest that tumor cells are relatively less resistant to prolonged low concentration exposure to the natural product-derived agents like etoposide, doxorubicin, vincristine, compared with brief high concentration exposure. Dose adjusted EPOCH was tested in 39 patients in a single center phase II trial. Continuous 96 hours intravenous infusion of etoposide, doxorubicin y, vincristine were given along with cyclophosphamide prednisolone. This study reported 74% complete response rates and 70% overall survival after a median follow up of 62 months. A multicentre phase II trial of infusional CDE plus concurrent ART resulted in complete response rate of 45% and 2-year survival of 44% ,complete response rates of 50%, with a 2-year survival of 61% and median survival of 26 months, have been reported by others using this regimen.**(Level of evidence IIID)**.These schedules are effective as compared to bolus therapy where median survival ranges from 9- 12 months. Administration of prolonged infusion requires additional resources, including hospitalization, central venous catheters, infusion pumps, growth factors with their antecedent cost and complications and makes them less feasible for application particularly in developing countries.

The addition of rituximab to the CHOP regimen has resulted in remarkable prolongation

in disease-free and overall survival among patients with HIV-negative diffuse large Bcell lymphoma (DLBCL),and early results among patients with HIV-associated lymphoma appeared also to show benefit. However, a randomized phase 3 study of CHOP with or without rituximab, performed by the AIDS Malignancy Consortium (AMC), found only a trend toward greater efficacy in patients on the rituximab arm, with a statistically significant increase in death due to infection. However when patients with CD4 count less than 50/mL were excluded from the analysis, no significant difference in infectious death was seen. In addition, rituximab has been associated with various viral infections, as well as progressive multifocal leukoencephalopathy and hepatitis B reactivation, and could theoretically be a concern in patients with HIV. However subsequent phase II studies have shown improved results with addition of rituximab to chemotherapy (CHOP and abbreviated course of DA-EPOCH). Therefore one can conclude that though rituximab has a potential to increase the risk of infection in patients with HIV related lymphoma in presence of severe immunodeficiency, it gives a clear benefit to patients with HIV-related CD20 positive systemic NHL.**(Level of evidence IIID)**

Use of concomitant anti-retroviral therapy

There has been a lot of controversy on whether to continue ART during chemotherapy or not. The rationale behind interrupting ART is to try to avoid adverse drug reactions that could reduce the chance of cure. There is probability of drug interactions, prolongation of

myelosuppression (Zidovudine) and neutropenia (Protease inhibitors) which can jeopardize the outcomes of lymphoma treatment and increase the toxicity. In addition, HAART may not prevent chemotherapy-induced lymphocyte depletion. The period of interruption of ART during chemotherapy would be small to have a significant HIV dependent CD4+ cell loss. Other potential reasons to consider omission of HAART for patients receiving chemotherapy included compliance issues in the setting of polypharmacy, as well as problems with chemotherapy-induced nausea, vomiting and mucositis which may reduce ability to comply with treatment.

However, a large majority of our patients have severe immunosuppression and are not on ART at the time of diagnosis, thus it would be optimal to continue ART during chemotherapy to enhance the immunological recovery which can improve lymphoma response rates. Care should be taken to avoid zidovudine and protease inhibitors in ART combinations. **(Level of Evidence VD)**

Use of central nervous system prophylaxis

The frequency of CNS involvement at diagnosis and relapse is as high as 20%. This suggests the need for adequate CNS prophylaxis. The data from published studies does not provide one single best method and frequency of its use. It varies from single to multiple courses of intrathecal methotrexate, triple intrathecal therapy (methotrexate, cytosar, hydrocortisone), cranial radiotherapy and high-dose methotrexate. **(Level of Evidence VD)**

Treatment of special subtypes

Burkitt's Lymphoma: In the era of ART, most of the studies recommend treatment with similar regimens as immunocompetent patients with Burkitt's lymphoma along with use of rituximab. Combination of DA-EPOCH with rituximab has been used in HIV-related patients.

Plasmablastic Lymphoma: The reported literature shows that median survival of these patients is 15 months with CHOP and CHOP like chemotherapy regimens. It occurs in patients with CD4 counts <200/mL. These lymphomas are CD20 negative and thus there is no role for rituximab. Given the rarity of the histopathologic subtypes it is difficult to make any recommendation on treatment of this entity.

Primary effusion lymphoma: This is a rare subtype seen exclusively in patients with HIV and occurs in patients with severe immunodeficiency. The reported cases have been treated with CHOP with median survival of 6 months.

Primary CNS lymphoma: Most of these patients have CD4 counts **<**50/mL and thus have limited tolerance to intensive chemotherapy as used in their immunocompetent counterparts. However with wider application of ART, similar treatment regimens incorporating high-dose methotrexate, cytarabine and radiation are being used.

Treatment of Hodgkin Lymphoma (HL)

Similar to NHLs, patient with HIV related HL also present with advanced stage disease, extranodal sites and B- symptoms. Lymphocyte predominant and mixed cellularity is histology is commonly seen in these patienst. Data on treatment of these patients is limited to phase II studies. Use of ABVD chemotherapy along with use ART and growth factor have shown complete response rates of 87% and 5-year overall and event-free survival probabilities were 76% and 71%,respectively. Use of more intensive regimens like Stanford V and escalated BEACOPP has been shown to be feasible but with high toxicity. Given the wider experience with ABVD in immunocompetent individuals it would be a preferable regimen to use in view of lack of randomized and large studies. **(Level of evidence IVD)**

Suggested Readings

1. Carbone A, Gloghini A, Serraino D, Spina M. HIV-associated Hodgkin lymphoma.Curr Opin HIV AIDS. 2009 ;4(1):3-10. Review
2. Levine AM. Management of AIDS-related lymphoma. Curr Opin Oncol. 2008;20(5):522-8. Review.
3. Martí-Carvajal AJ, Cardona AF, Rodríguez ML. Interventions for treating AIDS-associated Hodgkin s lymphoma in treatment-naive adults. Cochrane DatabaseSyst Rev. 2007,18;(2):CD006149. Review.

Selected Abstracts- Indian Studies

Gujral S, Shet TM, Kane SV. Morphological spectrum of AIDS-related plasmablastic lymphomas. Indian J Pathol Microbiol. 2008 Jan-Mar;51(1):121-4.

Abstract

We have had a recent spurt in cases of AIDS-related lymphoma (ARL) at our centre. Most of these cases are aggressive mature B cell lymphomas, mainly plasmablastic lymphoma (PBL) and diffuse large B-cell lymphoma (DLBCL). Most of the PBL are extranodal in location and are mucosa-based. We reviewed the morphological features of 34 cases of PBL. Diagnosis was based on morphology, immunohistochemistry, proliferation index, HIV positive status and its preference to extranodal sites (mostly mucosa based). We classified PBL into three morphological subtypes (immunoblastic - 25, Burkitt's - 7, plasmacytic - 2). Tumor cells expressed as leucocyte common antigen (LCA) in 60%, CD138 in 100%, EMA in 45% and light chain restriction in 86% cases. CD20 was negative in all cases. Pathologists need to be aware of PBL and its various morphological subtypes as the identification of this entity from its close differentials carries major therapeutic implications.

Sharma A, Bajpai J, Raina V, Mohanti BK et al.HIV-associated non-Hodgkin's lymphoma: experience from a regional cancer center. Indian J Cancer. 2010 Jan-Mar;47(1):35-9.

AIMS: To analyze clinical features and survival in HIV-associated non-Hodgkin lymphoma (NHL) cases registered at Dr BRA Institute Rotary Cancer Hospital of AIIMS, New

Delhi.MATERIALS AND METHODS: We have retrospectively reviewed records of NHL patients registered, from January 2003 to July 2007 to analyze HIV-associated NHL.

RESULTS: Seven cases of HIV-associated NHL cases were identified. Age range was 14-56 years. Five were males. Baseline performance status (ECOG-PS) was >I in 6. Mean LDH was 409 U/L. Mean hemoglobin was 10.5 g% and mean CD4 count was 243/mm3 (range 18-454). Three cases had nodal lymphoma and four had extra nodal lymphoma. No primary CNS (PCNSL) lymphoma was seen. All patients were of advanced stages and of intermediate to high-risk group based on international prognostic index (IPI). Six cases had high-grade NHL. None had CNS involvement. Five had B symptoms. HIV infection was diagnosed as part of NHL work-up in five patients. All patients received HAART. All were planned for chemotherapy with CNS prophylaxis. Protocols used were CVP, CHOP, R-CHOP or MCP-842. One patient received IFRT. RESPONSE: One patient achieved complete response (CR) and continues to be disease free, with 4.5 years of follow-up. Three cases achieved partial response (PR) and 2 had progressive disease (PD). Currently, three patients are on follow-up.

CONCLUSIONS: These NHL are of higher grade and advanced stage. Response and tolerance to chemotherapy is poor. Appropriate supportive care and CNS prophylaxis might improve outcome. We need to improve epidemiological data collection system in this part of world. With HAART, the goal of therapy is durable CR rather than palliation

HIV and Cervical Cancer

Introduction

Cervical cancer is one of the most common cancers amongst Indian women. Population Based Cancer Registry (PBCR) reported 90,708 new cases of cervical cancer in 2007(1). Though urban PBCR reports decline in incidence of cervical cancer in the last two decades, an increase may be anticipated in view of increasing incidence of Human Immunodeficiency Virus (HIV) infection amongst Indian women and increasing survival as a result of Highly Active Antiretroviral Treatment (HAART). Decrease in local and systemic immunity due to HIV predisposes women to rapid progression of Human Papilloma Virus (HPV) induced cervical lesions such that there may be increased incidence of premalignant and malignant cervical lesions in women with HIV(2). A recent report from UNAIDS/WHO has estimated that upto 1.2 million women may be infected with HIV in India (3) and a significant proportion of these women may present with dysplastic, squamous intraepithelial lesions (SIL) or invasive cervical lesions during their lifetime(4). The present chapter reviews the etiopathogenesis, screening, diagnosis, treatment and outcome of preinvasive and invasive cervical cancer in patients with HIV infection.

Etiopathogenesis and Epidemiology

High risk oncogenic HPV subtypes have been implicated in the pathogenesis of cervical cancer in immunocompetent patients (5). United States national health and nutritional examination survey reported that 16-42% of women in reproductive age group may harbour HPV infection (6) . While upto 90% of those infected have spontaneous resolution of the HPV within 2 years, the persistence of high risk oncogenic HPV in a small proportion may lead to premalignant and malignant transformation within 10-15 years of infection. While HPV 16 and 18 are commonest subtypes in general population, non 16-18 HPV subtypes are common in those infected with HIV. The common HPV subtypes in HIV infected women include HPV 35,

39,43,44,51,54,59,66 (7-9). Though multiplicity of infections is common, the overall prevalence of HPV is modulated by CD4 counts. HPV prevalence of 70, 56 and 43% is reported in those with CD4 count of 200, 200-500 and more than 500 cells/ìl respectively (10).

Apart from multiplicity of infection, alteration of natural history is known in those coinfected with HIV and HPV such that dysplastic and preinvasive lesions may develop within 2-3 years of HPV infection. This rapid transformation is attributed to modification of cell cycle protein expression (11) and decrease in local cellular immunity to HPV (2, 12)

Screening of cervical cytological abnormalities in women with HIV

Cervical dysplasia has been reported in 11-60% of women with HIV (4, 13, 14). Immunosuppression characterized by reduction in CD4 counts is correlated with early onset of dysplasias and SIL(15). The annual incidence of SIL is estimated to be five times higher in seropositive women (8.3/100 vs 1.8/100) with a three fold risk of developing invasive cervical cancer(15). The US Centre of Disease Control and Prevention (CDC) guidelines recommend initial screening with two Papinocolau (PAP) smears obtained six months apart followed by annual screening if results of both tests are normal(16). Though early reports have raised concerns regarding accuracy of cervical cytological analysis in women with HIV (17, 18), prospective studies have not reported any difference in sensitivity and specificity of PAP smear. However, false negativity is reported to be higher in patients with CD4 counts< 500 cells/ ìl (19). Based on initial reports of high false negativity some authors have recommended semiannual or annual colposcopies for all patients (16, 20, 21). Prospective studies demonstrate that the existent policy of biannual PAP screening ensures detection of 95% of cervical abnormalities within one year. (19) This questions the need for colposcopy for all comers (including those with low CD4 counts). Furthermore, cost efficacy analyses report that annual PAP after two negative semiannual smears provides quality adjusted life expectancy that is no different than that of colposcopy (22). Hence adherence to the US CDC guidelines for screening is recommended(16) in immunocompetent patients.

Management of dysplasias and preinvasive carcinoma in HIV positive patients

Atypical Squamous Cells of unknown Significance (ASCUS)

HIV infected women with PAP report of atypical squamous cells of uncertain significance (ASCUS) are at a higher risk (2.4 times) of presenting with SIL. In immunocompetent women a report of ASCUS necessitates one of the following options 1) Repeat cytological examination 2) HPV DNA testing or 3) Colposcopy. By contrast wherein high grade SIL cannot be ruled out colposcopy is the only recommended option. Only few studies have compared the incidence of SIL after PAP test reporting ASCUS in HIV positive women. Despite limited evidence, HIV epidemiology Research Group recommends referral for colposcopy for HIV positive women regardless of immune status or CD4 count(23).

Squamous Intraepithelial Lesions (SIL)

- *Low Grade Squamous Intraepithelial Lesions (LSIL)*

In immunocompetent patients, 60-70% of histologically suspected cases of LSIL revert back to normal(24). Regression is however rarely observed in patients coinfected with HIV(25). High rate of persistence and recurrence of SIL and poor compliance to follow up has been reported in patients coinfected with HIV. Hence, wait and see policy adopted for LSIL in immunocompetent women may not be appropriate in HIV positive women. Though use of HAART is associated with increase in regression rates, the published literature demonstrates only modest benefit and policy of observation cannot be recommended in women receiving HAART(26).

- *High Grade Squamous Intraepithelial Lesions (H-SIL)*

Large Loop Excision of Transformation zone (LLETZ) or conisation is the procedure of choice in women presenting with HSIL. LLETZ or conisation is associated with high success rate (83-100%) in HIV untested women(27, 28). However, therapeutic outcomes of LLETZ are less predictable in women coinfected with HIV. HIV infected women are twice as likely to have a positive margin after LLETZ (29, 30) and are at a high risk of recurrence. This has been attributed to extent of lesion, multicentricity, and presence of endocervical extension which is often missed during colposcopy. In a prospective study Foulot et al demonstrated that therapeutic decision (LLETZ or electrosurgical cone biopsy) made after careful colposcopy and endocervical curettage is associated with success rates of upwards of 70% in HIV infected women. However, higher rates of failure and recurrence may be noted in women with low CD4 counts. Two large prospective studies (Women Intergency and HIV epidemiology research group) reported higher rates of treatment failure in women with CD4 counts<200/ìl and persisting oncogenic HPV after treatment (31).

Recent years have witnessed increase in use of HAART and hence lower incidence of various opportunistic infections in retropositive women. Use of HAART has been associated with only modest increase in rates of HPV clearance and SIL resolution (26, 32). However, the use of HAART is often limited to women in low CD4 counts. Only few publications address the role of HAART in immunocompetent patients (CD4>200/ ìl) with carcinoma cervix(32). The exact role of HAART on HSIL resolution in seropositive immunocompetent women (CD4>200/ ìl) merits further investigation.

In summary the existing evidence cautions against observation in LSIL and supports the use of colposcopy for evaluation of HSIL. Wherever the proximal extent of the lesion cannot be identified endocervical curettage should be performed. LLETZ may be appropriate only for select women with small lesions. For others conisation is recommended.

Invasive cervical cancer in HIV positive women

The standard approaches to the management of invasive cervical cancer in HIV negative women are applicable to immunocompetent seropositive women as well. The stage wise therapeutic management is as follows:

Early Stage Cervical Cancer (Stage IA-IIA)

For Stage IA, simple hysterectomy or brachytherapy are equally effective approaches. Brachytherapy alone to a dose of 60 Gy to point A in a single low dose rate application provides equivalent control. Wertheim's hysterectomy or concomitant chemoradiotherapy provide equivalent results in stage IB-IIA. Radical radiotherapy should consist of 40 Gy/ 20Fraction / 4 weeks with external beam radiotherapy (EBRT) with midline block applied at 20 Gy along with two intracavitary brachytherapy applications to the low dose rate (LDR) equivalent of 25-30 Gy to point 'A' given during and after conclusion of EBRT. Concomitant chemotherapy should contain weekly cisplatin (30-40 mg/m^2).

Locally Advanced Cervical Cancer (IIB-IVA)

Combined chemoradiotherapy is the treatment of choice for stage IIB-IIIB. EBRT should consist of 45-50 Gy in 22-25 fractions over 5 weeks with external beam therapy and one intracavitary treatment to the LDR equivalent of 25-30 Gy to point 'A' given before or after the end of external beam therapy. As indicated above concomitant chemotherapy should contain weekly cisplatin (30-40 mg/m^2). Though patients with focal adjacent organ infiltration can be treated radically, the intent of treatment changes in patients with frank infiltration of adjacent organs. Various palliative radiotherapy fractionation schedules have been recommended for those with advanced disease. Single fractions of 8-10 Gy delivered every month will often relieve pain and bleeding and can be repeated to a total of three fractions (30 Gy in 3 fractions)(33, 34). Weekly fractions of 3 Gy once per week for 4 fractions, followed by a 3-week gap and then another course of 3 Gy per week for 4 fractions (24 Gy in 8 fractions in 9 weeks) have been recommended in IAEA TECDOC 2001 (35). Radiation Therapy Oncology Group (RTOG) recommends 3.7-Gy twice a day for 2 days (14.8 Gy) and then a repeat courseg after 2-4 weeks for a maximum of 44.4 Gy. The late toxicity of RTOG group is significantly lower (7%-actuarial) than other highly abbreviated schedules(36), therefore for patients who may survive 6 months or longer, protracted fractionation as that recommended by RTOG is preferable.

Special Considerations in Seropositive women

Patients with Intact Immune System

While adherence to standard therapeutic recommendations is advised in women with intact immune system (CD4>200µl) and invasive cervical cancer, enhanced toxicity may be anticipated. Only few studies have systematically reported treatment related morbidity in this cohort of patients. High incidence of acute grade III-IV gastrointestinal (34%) and genitourinary (19.5%) toxicity has been observed in patients receiving radiotherapy alone (37). Authors of

the aforesaid report recorded almost seven times higher incidence of all acute toxicities. This high rate of toxicity was associated with high incidence of treatment interruptions (48.8%) and low compliance to treatment. The high incidence of toxicity recorded by Gichangi et al has not been observed by other authors who have reported 3-12% incidence of grade III-IV toxicity (38). In a retrospective analysis of 42 patients treated at our centre we observed Grade III–IV acute gastrointestinal and skin toxicity in 14 and 27% of patients respectively, leading to treatment delays and poor compliance to treatment (38). Though there is heterogeneity in the reported incidence of acute grade III-IV toxicity, the reported toxicity rates necessitate attention towards radiation planning techniques and supportive care during treatment. Pelvic radiotherapy+/- chemotherapy could lead to further immune impairment hence modulation of antiretroviral schedule in consultation with infectious disease specialist is recommended.

Patients with Immune Impairment (CD4: 50 to 200µl)

In patients with moderate immune impairment while all attempts should be made to adhere to standard recommendations, the incidence of treatment induced toxicity may be higher leading to frequent treatment interruptions. Use of antiretrovirals and prophylaxis against opportunistic infections may prevent gastrointestinal and genitourinary mucosal infections however needs systematic evaluation. The prognosis of severely immnuocompromised patients (CD4<50/ìl) may be guided by the severity of HIV infection rather than malignancy hence radical chemoradiotherapy may not be appropriate. Palliative hypofractionated radiotherapy may provide good palliation in such patients.

Treatment Response in seropositive women with cervical cancer

Poor response to radiotherapy is observed in retropositive patients with almost six fold incidence of pelvic failure (37). Maiman et al reported that immunocompetent seropositive patients with cervical cancer died because of cancer rather than HIV infection related complications at 10 months after treatment completion (39). Though inherent radioresistance (due to alteration of cell cycle kinetics) is proposed to be the cause of treatment failure, presence of pretreatment anemia and tumor hypoxia, frequent treatment interruptions and poor compliance may be a contributing factor. Only a few studies have evaluated patterns of local and systemic failure on women coinfected with HIV. Prospective documentation of sites of failure may help treatment regimen modulation.

Conclusions

Seropositive women with preinvasive and invasive carcinoma of cervix are at an increased risk of persistent and recurrent disease after standard therapeutic management. Therapeutic outcomes in invasive cancer can be improved by treatment intensification and toxicity reduction with the use of highly conformal image guided radiotherapy. The safety, tolerability and outcomes following concurrent chemoradiotherapy also needs prospective evaluation. To this end, IAEA initiated a multicentric phase III study that randomized HIV positive patients to radical

radiotherapy or concurrent chemoradiotherapy (weekly cisplatin 30 mg/m^2). This trial is closed to accrual and results are awaited. In immunocompetent seropositive women the overall outcome depends on response to treatment following diagnosis of invasive cancer and presence of HIV should not preclude radical treatment. Systematic treatment intensification within clinical trials and multidisciplinary care (includes oncologist, infectious disease specialist, psychologist, medical social worker) needs to be evolved for improving outcomes.

References

1. Nandakumar A, Ramnath T, Chaturvedi M. The magnitude of cancer cervix in India. Indian J Med Res 2009;130:219-221.
2. Hessol NA, Seaberg EC, Preston-Martin S, et al. Cancer risk among participants in the women's interagency HIV study. J Acquir Immune Defic Syndr 2004;36:978-985.
3. Rodrigo C, Rajapakse S. Current Status of HIV/AIDS in South Asia. J Glob Infect Dis 2009;1:93-101.
4. Sirivongrangson P, Bollen LJ, Chaovavanich A, et al. Screening HIV-infected women for cervical cancer in Thailand: findings from a demonstration project. Sex Transm Dis 2007;34:104-107.
5. Anton HNH, Frank S, Wendy D, et al. Transition of high-grade cervical intraepithelial neoplasia to micro-invasive carcinoma is characterized by integration of HPV 16⁄18 and numerical chromosome abnormalities. The Journal of Pathology 2004;202:23-33.
6. Markowitz LE, Sternberg M, Dunne EF, et al. Seroprevalence of human papillomavirus types 6, 11, 16, and 18 in the United States: National Health and Nutrition Examination Survey 2003-2004. J Infect Dis 2009;200:1059-1067.
7. Cappiello G, Garbuglia AR, Salvi R, et al. HIV infection increases the risk of squamous intra-epithelial lesions in women with HPV infection: An analysis of HPV genotypes. International Journal of Cancer 1997;72:982-986.
8. Baay MFD, Kjetland EF, Ndhlovu PD, et al. Human papillomavirus in a rural community in Zimbabwe: The impact of HIV co-infection on HPV genotype distribution. Journal of Medical Virology 2004;73:481-485.
9. Peedicayil A, Thiyagarajan K, Gnanamony M, et al. Prevalence and risk factors for human papillomavirus and cervical intraepithelial neoplasia among HIV-positive women at a tertiary level hospital in India. J Low Genit Tract Dis 2009;13:159-164.
10. Goldie SJ, Freedberg KA, Weinstein MC, et al. Cost effectiveness of human papillomavirus testing to augment cervical cancer screening in women infected with the human immunodeficiency virus. The American Journal of Medicine 2001;111:140-149.
11. Nicol AF, Pires AR, de Souza SR, et al. Cell-cycle and suppressor proteins expression in uterine cervix in HIV/HPV co-infection: comparative study by tissue micro-array (TMA). BMC Cancer 2008;8:289.

12. Sun X-W, Kuhn L, Ellerbrock TV, et al. Human Papillomavirus Infection in Women Infected with the Human Immunodeficiency Virus. N Engl J Med 1997;337:1343-1349.
13. Massad LS, Riester KA, Anastos KM, et al. Prevalence and predictors of squamous cell abnormalities in Papanicolaou smears from women infected with HIV-1. Women's Interagency HIV Study Group. J Acquir Immune Defic Syndr 1999;21:33-41.
14. Fruchter RG, Maiman M, Sillman FH, et al. Characteristics of cervical intraepithelial neoplasia in women infected with the human immunodeficiency virus. Am J Obstet Gynecol 1994;171:531-537.
15. Ellerbrock TV, Chiasson MA, Bush TJ, et al. Incidence of Cervical Squamous Intraepithelial Lesions in HIV-Infected Women. JAMA 2000;283:1031-1037.
16. USPHS/IDSA guidelines for the prevention of opportunistic infections in persons infected with human immunodeficiency virus. USPHS/IDSA Prevention of Opportunistic Infections Working Group. MMWR Morb Mortal Wkly Rep 1997;46:1-46.
17. Maiman M, Tarricone N, Vieira J, et al. Colposcopic evaluation of human immunodeficiency virus-seropositive women. Obstet Gynecol 1991;78:84-88.
18. Fink MJ, Fruchter RG, Maiman M, et al. The Adequacy of Cytology and Colposcopy in Diagnosing Cervical Neoplasia in HIV-Seropositive Women. Gynecologic Oncology 1994;55:133-137.
19. Anderson JR, Paramsothy P, Heilig C, et al. Accuracy of Papanicolaou test among HIV-infected women. Clin Infect Dis 2006;42:562-568.
20. Northfelt DW. Cervical and anal neoplasia and HPV infection in persons with HIV infection. Oncology (Williston Park) 1994;8:33-37; discussion 38-40.
21. Hankins CA LJ, Handley MA. Cervicovaginal screening in women with HIV infection: a need for increased vigilance? Can Med Assoc J 1994;150:681-686.
22. Goldie SJMDMPH, Weinstein MCP, Kuntz KMS, et al. The Costs, Clinical Benefits, and Cost-Effectiveness of Screening for Cervical Cancer in HIV-Infected Women. Annals of Internal Medicine 1999;130:97-107.
23. Duerr A, Paramsothy P, Jamieson DeniseÂ J, et al. Effect of HIV Infection on Atypical Squamous Cells of Undetermined Significance. Clinical Infectious Diseases 2006;42:855-861.
24. Jordan J, Martin-Hirsch P, Arbyn M, et al. European guidelines for clinical management of abnormal cervical cytology, Part 2. Cytopathology 2009;20:5-16.
25. De Vuyst Hab, Lillo Fc, Broutet Nd, et al. HIV, human papillomavirus, and cervical neoplasia and cancer in the era of highly active antiretroviral therapy. European Journal of Cancer Prevention 2008;17:545-554.
26. Ahdieh-Grant L, Li R, Levine AM, et al. Highly Active Antiretroviral Therapy and Cervical Squamous Intraepithelial Lesions in Human Immunodeficiency Virus-Positive Women. J. Natl. Cancer Inst. 2004;96:1070-1076.

27. Lopes A, Morgan P, Murdoch J, et al. The Case for Conservative Management of "Incomplete Excision" of CIN after Laser Conization. Gynecologic Oncology 1993;49:247-249.
28. Mohamed-Noor K, Quinn MA, Tan J. Outcomes after Cervical Cold Knife Conization with Complete and Incomplete Excision of Abnormal Epithelium: A Review of 699 Cases. Gynecologic Oncology 1997;67:34-38.
29. Gilles C, Manigart Y, Konopnicki D, et al. Management and outcome of cervical intraepithelial neoplasia lesions: a study of matched cases according to HIV status. Gynecol Oncol 2005;96:112-118.
30. Boardman LA, Peipert JF, Hogan JW, et al. Positive cone biopsy specimen margins in women infected with the human immunodeficiency virus. Am J Obstet Gynecol 1999;181:1395-1399.
31. Massad LSMD, Fazzari MJMS, Anastos KMD, et al. Outcomes After Treatment of Cervical Intraepithelial Neoplasia Among Women With HIV. [Article]. Journal of Lower Genital Tract Disease 2007;11:90-97.
32. Sirera G, Videla S, Lopez-Blazquez R, et al. Highly active antiretroviral therapy and incidence of cervical squamous intraepithelial lesions among HIV-infected women with normal cytology and CD4 counts above 350 cells/mm3. J. Antimicrob. Chemother. 2008;61:191-194.
33. Halle JS, Rosenman JG, Varia MA, et al. 1000 cGy single dose palliation for advanced carcinoma of the cervix or endometrium. International Journal of Radiation Oncology*Biology*Physics 1986;12:1947-1950.
34. Mishra S, Laskar S, Muckaden MA, et al. Monthly palliative pelvic radiotherapy in advanced carcinoma of uterine cervix. Vol 1; 2005.
35. IAEA. The role of radiotherapy in the management of cancer patients infected by Human Immunodeficiency Virus (HIV). IAEA-TECDOC-1224 2001:32-36.
36. Spanos WJ, Jr., Clery M, Perez CA, et al. Late effect of multiple daily fraction palliation schedule for advanced pelvic malignancies (RTOG 8502). Int J Radiat Oncol Biol Phys 1994;29:961-967.
37. Gichangi P, Bwayo J, Estambale B, et al. HIV impact on acute morbidity and pelvic tumor control following radiotherapy for cervical cancer. Gynecologic Oncology 2006;100:405-411.
38. Shrivastava SK, Engineer R, Rajadhyaksha S, et al. HIV infection and invasive cervical cancers, treatment with radiation therapy: toxicity and outcome. Radiother Oncol 2005;74:31-35.
39. Maiman M. Management of Cervical Neoplasia in Human Immunodeficiency Virus-Infected Women. J Natl Cancer Inst Monogr 1998;1998:43-49.

Selected Abstracts – Indian studies

Joshi SN, Gopalkrishna V, Kumar BK, Dutta S, Nyaynirgune P, Thakar M.Cervical squamous intra-epithelial changes and human papillomavirus infection in women infected with human immunodeficiency virus in Pune, India. J Med Virol. 2005 Aug;76 (4):470-5.

Abstract

In view of the dual burden of HIV infection and cervical cancers in India, this study was undertaken to estimate the prevalence of Pap smear abnormalities and human papillomavirus infection among HIV-infected women. Consecutive HIV-infected women attending voluntary counseling testing clinics were enrolled. Written informed consent, demographic information, Pap smears, cervical swabs for HPV typing and a blood sample for CD4+ cell count were collected. Treatment for opportunistic and sexually transmitted infections and reproductive tract infections was provided. Women with Pap smear abnormality were referred for further intervention. Between January 2003 and May 2004, 287 HIV-infected women were enrolled. Pap smear abnormalities were seen in 6.3% women and were more common among women aged 30 and above (P=0.042) and those who had suffered from opportunistic infections (P=0.004). In multivariate analysis, Pap smear abnormalities were associated independently with opportunistic infections (P=0.02, AOR 3.8, 95% CI 1.2—11.5). Of the 100 random cervical specimens screened for HPV 16 and 18 genotypes, 33% (95 CI 23.9—43.1) were positive for HPV 16⁄18. Of the 122 patients who returned for a follow-up visit, 5 patients (4.1%) who did not have Pap smear abnormality at baseline, had developed Pap smear abnormality. The incidence of Pap smear abnormalities was 5.5 per 100 person year of follow-up. In order to prevent thousands of deaths due to cervical cancer in India, there is a need for strengthening the Pap smear screening program and HPV vaccine development.

Shrivastava SK, Engineer R, Rajadhyaksha S, Dinshaw KA.HIV infection and invasive cervical cancers, treatment with radiation therapy: toxicity and outcome. Radiother Oncol. 2005 Jan; 74 (1):31-5.

Abstract

BACKGROUND AND PURPOSE: To determine the effect of radiotherapy in HIV seropositive cervical cancer patients, tumour response and toxicity and compliance of patients to the treatment.

PATIENTS AND METHODS: This study is a retrospective review of 42 HIV seropositive patients diagnosed with carcinoma cervix, between 1997 and 2003 at the Tata Memorial Hospital. The age and symptoms of presentation, clinical stage, response, compliance and tolerance to radiotherapy were studied.

RESULTS: Mean age at presentation was 41 years. All patients presented with the symptoms of cervical disease. Of these patients 31(74%) patients had 'Karnofsky Performance Scale' (KPS) more than 80%. Twenty-one (50%) of the patients were of Stage IIIb-IVa. Thirty-

two (76%) were started on radiotherapy with radical intent. Compliance to radiotherapy was poor with 24% patients discontinuing after few fractions of radiotherapy. Seven (17%) patients were given palliative radiotherapy. Twenty-two patients completed prescribed radical radiotherapy and 50% of these achieved complete response. Grade III-IV acute gastrointestinal toxicity was seen in 14% of the patients, and grade III acute skin toxicity was seen in 27% of patients, leading to treatment delays. There was good relief of symptoms in patients treated with palliative intent.

CONCLUSIONS: Radiotherapy is effective in this set of patients. Palliative fractionation schedules are effective for patients with poor performance status and locally advanced cancers in relieving the symptoms related to carcinoma cervix. An emphasis should be given to the increased acute mucosal and skin toxicity and to improving compliance and clinical outcome of these patients

HIV Associated Kaposi's Sarcoma

Kaposi's sarcoma(KS) was first described in 1872 by the Hungarian dermatologist ,Moritz Kaposi. From that time until the present AIDS epidemic, KS remained a rare tumour. KS is rare in India.

In HIV infected patients , KS is an AIDS-defining illness. In patients with severe and untreated immunodeficiency the course of the disease is aggressive with lethal outcomes and an average survival time following diagnosis less than one year. Almost 95% of all epidemics of KS in United States have been diagnosed in homosexual or bisexual men.Since the introduction of Higly Active Anti Retroviral Therapy(HAART) in 1996, the incidence of KS in HIV infected patients has decreased sharply almost by 90%.

The seroprevalence of HHV 8 is low in India. In India, only 11 cases of KS exist in the published literature.

Clinical Manifestations

Clinical diagnosis of cutaneous form of KS is based on identification of a red or violaceous skin or mucosal papule. Cutaneous lesions are concentrated on the face ,trunk and lower extremities. Lesions progress from flat to nodular to ulcerated lesins. Most common site of extracutaneous KS is lymph nodes followed by gastointestinal tract (GI) and lungs. Pulmonary KS adversely affects prognosis. KS is one of the most common causes of pleural effusions in AIDS. No organ is spared.

Diagnosis

Diagnosis of KS in the skin and mucous membranes can usually be made based on the following clinical features;

1. Purple macules or nodules

2. Distribution along skin tension lines
3. Green-yellow discoloration around the tumours corresponding haemmorrhage
4. Tumor associated edema –hallmark of advanced KS
5. Dissemination of lesions,possibly with mucocutaneous involvement.

Biopsy of the mucocutaneous lesion is the **gold standard** .

FNA cytology specimens are also adequate for diagnosis.

RTPCR (reverse transcriptase in situ polymerase chain reaction) for HHV-8 is useful in differential diagnosis between KS and other atypical vascular proliferative lesions.

Important features of KS on routine histology include:

1. Epidermis is usually intact.
2. Slit like spaces formed by new,thin walled and partly aberrant blood vessels running alongside normal dermal vessels and adnexal structures.
3. Extravasated erythrocytes around the new vessels.
4. Haemosiderin deposits.
5. Lymphocytic inflammatory infiltrate.
6. An infiltrate of oval or spindle –shaped cells (spindle cell KS)

Staging

Following investigations are useful for staging KS:

1. Complete cutaneous inspection of the patient (including oral and mucosal membranes)
2. Lymph node ultrasound
3. Abdominal ultrasound
4. Upper GI scopy (for mucocutaneous tumours)
5. Lower GI scopy
6. Chest radiography
7 Determination of CD4 cells and HIV viral load.

Staging of HIV –associated KS

(AIDS Clinical Trial Group/ACTG, Krown et al.1997)

Early Stage good risk (0)

If **all** following criteria are met

1. Tumor (T): 0
   KS limited to skin and/or lymph nodes;minimal oral disease (non- nodular KS confined to hard palate)

Late Stage poor risk(1)

If **one** of the following applies

1. Tumor (T): 1
   Pulmonary or gastrointestinal KS; Extensive oral KS;tumor associated oedemaor ulceration.

2. Immune system (I): 0
   CD4 T cells > 200/ul
3. Systemic illness (S): 0
   No history of OI or thrush,no B symptoms of HIV infection

2. Immune system (I): 1
   CD4 T cells < 200/ul
3. Systemic illness (S) : 1
   History of opportunistic infections, thrush,malignant lymphoma or HIV neurological disease,B symptoms of HIV infection.

KS is a multicentric disease and the standard TNM classification is not applicable.

Prognosis

Survival in HIV –related KS is influenced more by the depth of immunosuppression and the control of the underlying HIV disease ,than by tumor burden. Immune function is the single most important predictor of survival. Only in patients with CD4 lymphocyte count greater than 200 cells/ul, tumor burden contributed to additional predictive value.

Treatment

All therapies for KS have been markedly influenced by HAART (highly active antiretroviral therapy), which has decreased the incidence and severity of this disease.

Antiretroviral therapy

HAART treatment has changed the goal in Kaposi sarcoma treatment from short-term palliation to long-term remission and control.HAART must be started in all cases once diagnosed KS . Response to HAART ranges from 20-80% based on stage of disease and the amount of pretreatment. The effects of HAART on KS are multifactorial and include inhibition of HIV replication, diminished production of the HIV-1 transactivating protein Tat, amelioration of the immune response against KSHV and perhaps some direct antiangiogenic activity of protease inhibitors.

Effective combination antiretroviral therapy usually is comprised of a combination of either a protease inhibitor(PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI) with 2 nucleoside reverse transcriptase inhibitors (NRTI). Some evidence suggests a direct antitumor effect on angioproliferative Kaposi sarcoma–type lesions. Yet presently, no level 1 evidence supports this clinically and no difference is apparent between PI-based and NNRTI-based antiretroviral regimens in terms of response of Kaposi sarcoma.

Patients with poor-risk Kaposi sarcoma rarely respond to HAART alone. Kaposi sarcoma immune reactivation inflammatory syndrome associated with HAART initiation consistent with a reactivation of the immune system and associated inflammatory response may occur. In such cases, chemotherapy may be necessary to ameliorate and reverse the disease progression.

HAART may be tried as the sole modality used in nonvisceral disease. For visceral disease, chemotherapy may be added. For locally symptomatic disease, radiation therapy may be introduced.

Local therapy

Local therapy is best suited for individuals who require palliation of locally advanced symptomatic disease (eg, radiation) or for individuals who have cosmetically unacceptable lesions. This therapy is also well suited for individuals with significant comorbidities and disease refractory to systemic modalities. It can provide better cosmesis, control bulky lesions that cause bleeding, pain or, edema, and treat extensive skin disease. Local therapy fails to halt the development of new Kaposi sarcoma lesions.

1. Radiation therapy: Radiation therapy is the most widely used and effective local therapy. This can palliate bleeding, pain, or unsightly lesions. This may be given in the form of low-voltage (100 kv) photons or electron-beam radiotherapy. Responses occur in 80-90% of patients. A higher cumulative dose (40 Gy) results in better local control than lower doses (8 Gy or 20 Gy). Electron beam therapy is reserved for treatment of superficial lesions. This is usually giving once weekly in 4 Gy fractions. Recurrence may be common in adjacent, untreated areas, leading some authors to recommend extended- field radiotherapy to affect a higher cure rate. Patients with HIV are more prone to develop radiation-induced mucositis as well and hyperpigmentation, desquamation, and ulceration of treated lesions.

   In patients with widespread skin involvement, extended-field electron beam radiation therapy (EBRT) has been effective in controlling the disease. This approach appears to give better long-term control than piecemeal radiation of individual lesions. This type of therapy is also given in 4-Gy fractions weekly for 6-8 weeks.
2. Surgical excision: Surgical excision may be of benefit for patients with small superficial lesions. The major problem is local recurrence. The presence of clear surgical margins does not mean that Kaposi sarcoma has been permanently controlled at a given anatomical site. Local recurrence is very common.
3. Intralesional therapy: Intralesional therapy with vinca alkaloids with low-dose vincristine or vinblastine as well as bleomycin has been used in a limited fashion primarily for the classic form of Kaposi sarcoma where localized skin disease predominates. Responses occur in 60-90% of patients with little in the way of systemic side effects with duration of 4-6 months. Dosing is done at about one-tenth the systemic dose of drug with 3- to 4-week intervals between treatment. Side effects include changes in pigmentation, swelling, blistering, ulceration, and pain on injection as well as localized but usually transient neuropathic symptoms. Because the disease recurs in other areas, its use is relatively limited.
4. Cryotherapy: May be useful for small facial lesions less than 1 cm in dimensions. It induces response in more than 85% of cases. It has the advantage of short duration, minimal

discomfort, and ability to be used repeatedly and in combination with other forms of treatment. It has limited penetration and is not ideal for large, deep lesions.

5. Laser and surgical therapies can be used locally. Laser photocoagulation can shrink smaller lesions and be used to palliate bleeding and pain in larger lesions. Similar to cryotherapy, it has limited application to deep, bulky lesions. Surgery is usually limited to patients with a visceral crisis such as obstruction or bleeding or for very deep, localized, painful lesions.
6. Topical retinoids

   IL-6 is a cytokine implicated in the pathogenesis of Kaposi sarcoma. In vitro, retinoic acid down-regulates IL-6 receptor expression. A 0.1% (alitretinoin [Panretin]) gel is available commercially and may be applied topically 2-4 times daily. This agent is generally well tolerated but may cause local erythema and irritation. It induces responses in one third to one half of the patients at a time interval of 2-14 weeks after initiation of therapy. Common side effects of local inflammation and depigmentation.

Systemic therapy

Administration of systemic cytotoxic chemotherapy is warranted in patients with more advanced or rapidly progressive disease. It is indicated in

1. Widespread skin involvement such as more than 20 lesions
2. Extensive KS of the oral cavity,
3. Tumour-associated oedema or ulceration,
4. Symptomatic visceral involvement
5. Immune reconstitution inflammatory syndrome-induced KS flare

The decision to initiate systemic chemotherapy is usually based on a number of parameters including the prognostic index, response to HAART alone, patient performance status and end organ function, including hepatic and bone marrow reserve. No randomized prospective clinical trials compare one adjuvant therapy to the other for classic Kaposi sarcoma Liposomal anthracyclines and taxanes have become established as the backbone of current standard systemic cytotoxic therapy against KS.

1. Liposomal anthracyclines:

The trials of liposomal anthracyclines for HIV-associated KS were undertaken in the pre-HAART era but clinicians continue to regard them as the gold-standard first-line chemotherapy for KS. Both liposome-encapsulated daunorubicin (DaunoXome 40 mg/m2 every 2 weeks) and the pegylated liposomal doxorubicin, which is known variously as Caelyx, Doxil or PLD (20 mg/m2 every 3 weeks), have been shown to have good antitumour activity. Since the widespread introduction of HAART, the duration of responses to treatment for KS have increased and no further randomized trials have comparedliposomal anthracyclines with nonencapsulated chemotherapy regimens.

2. Taxanes:

Paclitaxel is given at a dose of 100mg/m2 every two weeks with premedication of dexamethasone 20 mg intravenously given just prior to the chemotherapy.This agent has response rates from 60-70% in phase 2 reports and is effective even in anthracycline resistant disease. Paclitaxel is less preferred as first line therapy than anthracyclines because of the need for a three hour infusion and increased side effects.

3. Systemic interferon –alpha Systemic IFNX may be considered for patients who have attained appropriate immune reconstitution with HAART therapy but have residual cutaneous KS, with responses detected in 20-40% of patients.

Novel agents used to treat KS include angiogeneisis inhibitors , tyrosine kinase inhibitors, and matrix metalloproteinase inhibitors. Thalidomide, which has signifcant antian-giogenic activity partly through inhibition of basic fbroblastgrowth factor-induced angiogenesis, has been shown to provide a partial response in treating KS. Bevacizumab, a monoclonal antibody against VEGF, may provide similar potential activity in the treatment of KS. Imatinib mesylate, a PDGF-R andc-Kit inhibitor, has been found to result in marked clinical and histologic regression of KS. Several trials have shown that COL-3, a matrix metalloproteinase inhibitor, is benefcial in the treatment ofAIDS-related KS]. Ra-pamycin (sirolimus), an inhibitor of the PI3K/Akt/mTor pathway, has been very effective in treating posttransplant as wellas classic KS and are currently being investigated for HIV-related KS.

Summary

Early-stage KS (T0 stage)

- HAART (level of evidence III B).
- Consider local radiotherapy or liposomal anthracycline for rapidly progressing or cosmetically disfiguring disease (level of evidence III B).

Advanced-stage KS (T1 stage)

- HAART and liposomal anthracycline (either DaunoXome 40 mg/m2 every 14 days or Caelyx 20 mg/m2 every 21 days) (level of evidence Ib A).
- Anthracycline-refractory KS-HAART and paclitaxel (100 mg/m2 every 14 days) (level of evidence III B).

References

1. Krown SE, Testa MA, Huang J. AIDS-related Kaposi's sarcoma: prospective validation of the AIDS Clinical Trials Group staging classification. AIDS Clinical Trials Group Oncology Committee. J Clin Oncol 1997;15: 3085–309
2. Nasti G, Talamini R, Antinori A et al. AIDS-related Kaposi's sarcoma: evaluation of potential new prognostic factors and assessment of the AIDS Clinical Trial Group Staging System in the HAART Era – the Italian Cooperative Group on AIDS and Tumors and the Italian Cohort of Patients Naý¨ve from Antiretrovirals. J Clin Oncol 2003; 21: 2876–2882.

3. Stebbing J, Sanitt A, Nelson M, Powles T, Gazzard B, Bower M. A prognostic index for AIDS-associated Kaposi's sarcoma in the era of highly active antiretroviral therapy. Lancet 2006; 367: 1495–1502
4. Kigula-Mugambe JB, Kavuma A. Epidemic and endemic Kaposi's sarcoma: a comparison of outcomes and survival after radiotherapy. Radiother Oncol 2005; 76: 59–62
5. Bodsworth NJ, Bloch M, Bower M, Donnell D, Yocum R. Phase III vehicle-controlled, multi-centered study of topical alitretinoin gel 0.1% in cutaneous AIDSrelated Kaposi's sarcoma. Am J Clin Dermatol 2001; 2: 77–87.
6. Ledergerber B, Telenti A, Egger M. Risk of HIV-related Kaposi's sarcoma and non-Hodgkin's lymphoma with potent antiretroviral therapy: prospective cohort study. Swiss HIV Cohort Study. BMJ 1999; 319: 23–24.
7. Northfelt DW, Dezube BJ, Thommes JA et al. Pegylated- liposomal doxorubicin versus doxorubicin, bleomycin, and vincristine in the treatment of AIDSrelated Kaposi's sarcoma: results of a randomized phase III clinical trial. J Clin Oncol 1998; 16: 2445–2451
8. Lichterfeld M, Qurishi N, Hoffmann C et al. Treatment liposomal doxorubicin and HAART simultaneously induces effective tumor remission and CD4 T cell recovery. Infection 2005; 33: 140–147.
9. Gill PS, Tulpule A, Espina BM et al. Paclitaxel is safe and effective in the treatment of advanced AIDS-related Kaposi's sarcoma. J Clin Oncol 1999; 17: 1876–1883.
10. Dhillon T, Stebbing J, Bower M. Paclitaxel for AIDSassociated Kaposi's sarcoma. Expert Rev Anticancer Ther 2005; 5: 215–219.
11. Kreuter A, Rasokat H, Klouche M et al. Liposomal pegylated doxorubicin versus low-dose recombinant interferon alfa-2a in the treatment of advanced classic Kaposi's sarcoma; retrospective analysis of three German centers. Cancer Invest 2005; 23: 653–659.
12. Dedicoat M, Newton R. Treatment of Kaposi's sarcoma in resource-poor settings. Trop Doct 2005; 35: 60.
13. Pagano JS, Blaser M, Buendia MA, *et al.* (December 2004). "Infectious agents and cancer: criteria for a causal relation". *Semin. Cancer Biol.* **14** (6): 453–71.

Selected Abstracts Indian Studies

Dongre A, Montaldo C. Kaposi's sarcoma in an HIV-positive person successfully treated with paclitaxel. Indian J Dermatol Venereol Leprol. 2009 May-Jun;75(3):290-2.

Abstract

Epidemic Kaposi's sarcoma is one of the malignant neoplasms, which can develop in HIV-infected patients. Although the prevalence of HIV infection is reported to be high in Asian countries, Kaposi's sarcoma is rarely reported. We report a case of Kaposi's sarcoma involving the skin and oral mucosa along with extensive bilateral lymphedema of lower extremities, treated successfully with paclitaxel and antiretrovirals.

Kumarasamy N, Venkatesh KK, Devaleenol B, Poongulali S, Ahilasamy N. Regression of Kaposi's sarcoma lesions following highly active antiretroviral therapy in an HIV-infected patient. Int J STD AIDS. 2008 Nov;19(11):786-8.

Abstract

This case report documents that highly active antiretroviral therapy (HAART) can lead to the regression of Kaposi's sarcoma (KS) lesions in the auditory canal of an HIV-infected male from Chennai, India. In resource-limited settings where administering anti-KS chemotherapeutic agents may not be feasible, HAART alone can be an option in HIV-infected individuals with KS

Non-AIDS Defining Cancers

Cancer has been associated with AIDS from the beginning of the epidemic when the first few patients presented with Kaposi's sarcoma. Kaposi's sarcoma, Non-Hodgkin's lymphoma and cervical cancer are considered as AIDS defining malignancies (ADMs). All these cancers are associated with viruses HHV-8, EBV and HPV respectively. Immune suppression is associated with Kaposi's and NHL. In developed countries, the availability of highly active antiretroviral therapy (HAART) beginning in 1996 has led to improvements in immunity and declining AIDS-related morbidity and mortality . HAART substantially reduces risk of KS and EBV-related NHL. Yet, the incidence of malignancies not known to be associated with immunosuppression [non-AIDS–defining malignancies (NADMs)] has been found to be significantly more common than in the general population.

The incidence of non–AIDS-defining malignancies has increased by greater than 3-fold over the past 10 years and has now surpassed that of AIDS-defining malignancies in HIV infected patients. Some of this increase is associated with the longer survival and aging of patients in the potent antiretroviral therapy era, but some also appears to be associated with direct effects of HIV that increase susceptibility to such cancers. The increased incidence of non-AIDS-defining malignancies is an important factor contributing to mortality in HIV-infected people now that survival can be prolonged through the use of HAART.

Contributing factors to NADMs: In addition to increased life expectancy and reduction of other causes of death in HIV-infected persons, contributors to the increased prevalence of NADMs are,

(1) greater prevalence of co infection with viruses that have etiologic roles in cancer, including **human papilloma virus** (anal, penile, and possibly head and neck cancers,conjunctival cancer), **Epstein-Barr virus** (Hodgkin's disease, pediatric leiomyosarcoma) and **hepatitis B and C viruses** (hepatomas).

(2) behaviours and environmental toxins, including tobacco and alcohol use

(3) Effects of HIV infection, including potential direct effects of the virus and the consequences of long-term immunosuppression. Potential direct effects of HIV include HIV Tat protein transactivation of cellular genes or proto-oncogenes.

NADMs tend to occur at a younger age in HIV-infected persons compared to those not infected with HIV. These cancers are usually aggressive tumours with atypical histopathlogy and present with metastatic disease. These factors contribute to rapid progression and poor response to treatment.

Hodgkin lymphoma

Hodgkin lymphoma (HL) is among the most common non-AIDS-defining malignancies. Large series and reviews of the literature indicate that there is a 3- to 18-fold increased risk of HL in HIV infected individuals.

Unusual features of HL in patients with HIV infection include the following:

- Unfavorable histology is particularly common in HIV-positive patients, with mixed cellularity and lymphocyte depletion in distinction to a predominance of nodular sclerosis subtype seen in young adults in the general population.
- Nearly all patients with HIV-associated HL are EBV positive, with a 75 to 100 percent rate of EBV coinfection.

HL and immune suppression: HL tends to develop early in HIV infection. The relationship between HL and CD4 cell count is unclear, with some studies indicating that HL is associated with advanced HIV-related immunosuppression .In immunocompromised persons, Hodgkin Reed- Sternberg cells are almost always EBV positive suggesting that the increased risk of Hodgkin lymphoma in HIV-infected persons is due to loss of control of EBV infection. For Hodgkin lymphoma, an additional possibility is that some cases arise as part of an immune reconstitution syndrome. This hypothesis is consistent with the nonlinear relationship between CD4 count and Hodgkin lymphoma risk and with rising Hodgkin lymphoma incidence during the HAART era. Specifically, the increase in CD4 count associated with HAART use among extremely immune deficient individuals may shift them to a level of immunosuppression (i.e., a CD4 count 200-250 cells/mm3,) that puts them at greatest risk for Hodgkin lymphoma. In that setting, Hodgkin lymphoma may develop because the malignant Hodgkin Reed-Sternberg cell may already be present, and partial restoration of immunity allows recruitment of surrounding immune cells and manifestation of the tumor.

Clinically, HL in HIV-positive patients often presents with more aggressive disease. Systemic B symptoms are frequent, and widely disseminated extra nodal disease may be seen in 75 to 90 percent of patients.

Guidelines recommend that all patients should be treated with HAART and that a standard chemotherapy regimen such as ABVD be used as the initial treatment of advanced disease.

HPV infection

Human papillomavirus (HPV) infection is responsible for a significant subset of cancers of the anus, penis, oropharynx, vagina, and vulva

Anal cancers

Anal cancer is caused by persistent infection with oncogenic subtypes of HPV. Anal cancer incidence is especially high in HIV-infected men-who-have-sex-with men ,due to sexual transmission of HPV through anal intercourse. In this group, anal HPV infection is almost universal, chronic, and frequently characterized by the presence of multiple HPV subtypes. Anal cancer incidence is also elevated among other HIV-infected men and women, which could partly reflect acquisition of HPV through anal sex, but also probably reflects indirect transmission of HPV related to other sexual acts. The role of HIV-related immunosuppression in promoting development of anal cancer has been somewhat difficult to establish .A number of epidemiologic studies have found either a stable or increasing occurrence of anal cancer since the advent of HAART.

HIV-infected men have poorer treatment outcomes and shorter survival times than the general population. Tumor size is an important prognostic factor; tumors 2 cm in diameter or less have a cure rate of 80%. Tumors 5 cm in diameter or larger are cured in less than 50% of cases .In the general population, the majority of tumors are controlled locally with combined chemotherapy and radiation, and 5-year survival rates range from 65% to 85%.Despite the potential drawbacks of combined chemotherapy and radiation, relatively healthy HIV-positive patients with anal squamous cell carcinoma should be treated with combined modality therapy.

Lung Cancer

Lung cancer is one of the most common non-AIDS-defining malignancies among HIV-infected patients. Recent studies suggest that lung cancer risk is 3 to 4 times higher in HIV-infected patients than in uninfected persons after adjusting for other factors such as smoking intensity and duration.

Tobacco smoking has been implicated for the excess of lung cancers seen in the HIV infected population. Although patients with HIV smoke more cigarettes than the HIV-negative population it has been previously shown that this alone cannot account for the excess of lung cancers seen in HIV. The pre-HAART studies assumed the whole HIV-positive population smoked, and still found an excess risk of lung cancer. Moreover, adenocarcinoma, which is the histologic subtype least strongly associated with smoking cigarettes, is most commonly seen in this population. Additionally, there is no data to suggest that the incidence of smoking has gone up

since the introduction of HAART, which would be necessary if this was the cause of the increased incidence of the cancer.

Lung cancer is diagnosed when locally advanced or metastatic (stage III-IV) in 75-90% of cases. Adenocarcinoma is the most frequent histological type. The prognosis is worse in HIV infected patients than in the general lung cancer population. Efficacy and toxicity data for chemotherapy and radiation therapy are few and imprecise. Surgery remains the treatment of choice for localized disease in patients with adequate pulmonary function and general good health, regardless of immune status. Prospective clinical trials are needed to define the optimal detection and treatment strategies for lung cancer in HIV infected patients

Conjunctival cancer

Conjunctival squamous cell carcinoma (SCC) appears to be several-fold more common among HIV-infected individuals compared to those who are HIV-negative. In HIV-infected patients, the spectrum of conjunctival involvement includes intraepithelial dysplasia, carcinoma in situ, and invasive SCC, most commonly originating in the limbus (transition zone) of the eye. In sub-Saharan Africa, the HIV epidemic has been associated with a dramatic increase in the incidence of conjunctival neoplasia. The association of conjunctival SCC with HIV infection in other geographic regions is less well established, although one epidemiologic study has reported an increased incidence in North America as well .Risk factors for SCC of the conjunctiva include age greater than 50 years, high solar ultraviolet radiation exposure, geography (sub-Saharan Africa), and HPV infection The relatively lower incidence in America and Europe has been attributed, at least in part, to the lower solar ultraviolet exposure associated with higher latitudes.

Conjunctival SCC in HIV-positive patients occurs much earlier in life than in their HIV-negative counterparts and may be particularly aggressive .Presentation ranges from eye irritation or erythema to a plaque or nodular lesion. SCC of the conjunctiva has a high propensity for local invasion into the orbit, and occasionally distant metastases occur.Aggressive histologic variants may be diagnosed, such as spindle cell carcinoma. Treatment is mainly surgical .Other potential therapies include photodynamic therapy or topical treatment with either mitomycin-C or interferon .

Prevention, screening and early detection

Cancer screening is now an important component of health maintenance in HIV clinical practice. The decision to screen an HIV-infected patient for cancer should include an assessment of individualized risk for the particular cancer, life expectancy, and the harms and benefits associated with the screening test and its potential outcome. Potential opportunities for prevention, screening, and early detection for selected non-AIDS-defining malignancies

Tata Memorial Centre,
Dr. Ernest Borges Road, Parel, Mumbai - 400 012. INDIA
Website : www.tatamemorialcentre.com

Malignancy	Prevention	Screening	Early detection
Lung cancer	Smoking cessation	-	Chest radiography and computed tomography
Hodgkin lymphoma	-	-	-
Anal cancer	Reduced sexual exposures, HPV vaccination	Anal Pap smears HPV DNA testing	Anal Pap smears
Liver cancer	Reduced sexual exposures Alcohol cessation HBV vaccination HCV and HBV treatment		AFP and ultrasound
Non-melanoma skin cancer	Reduced sun exposure	Skin examination	Skin examination

Screening for breast and colorectal cancer should follow standard, age-appropriate screening recommendations that apply to the general population. Screening HIV-infected men for prostate cancer, as with the general population, lacks a clear benefit.

Counselling by clinicians will play a role in prevention programs. The cornerstone of prevention for lung cancer is tobacco cessation, and given the role of tobacco in other illnesses, programs to encourage HIV-infected individuals to quit smoking should be a priority. Encouraging HIV-infected people to limit the number of their sexual partners may reduce their risk of acquiring new HBV and HPV infections, but condom use is of unproven benefit (e.g., for anal HPV). For individuals with liver disease, abstinence from alcohol may help prevent progression to liver cancer. Finally, encouraging HIV-infected persons to minimize unnecessary sun exposure is prudent to reduce skin cancer risk.

Vaccination strategies may also help prevent cancers. HBV vaccination prevents HBV infection and is associated with reduced occurrence of liver cancer. HBV vaccine is less immunogenic among HIV-infected individuals than healthy persons but should still be offered .A vaccine against HPV subtypes 16 and 18 is available to prevent cervical infection .This vaccine could conceivably prevent anal infections due to these oncogenic subtypes (although this is unproven), and thus might prevent anal cancer .To be effective, HBV and HPV vaccines would need to be administered before acquisition of these infections, which often occurs before acquisition of HIV. Thus, it remains unclear whether vaccination of persons who are already HIV-infected could prevent a sizeable number of liver or anal cancers. Among individuals with

chronic liver disease, treatment of HCV and HBV infection may prevent progression to liver cancer.

References

1. Roger J. Bedimo, Kathleen A. McGinnis, MS, Melinda Dunlap, Maria C. Rodriguez-Barradas, etal .Incidence of Non-AIDS–Defining Malignancies in HIV-Infected Versus Noninfected Patients in the HAART Era: Impact of Immunosuppression. (J Acquir Immune Defic Syndr 2009; 52:203–208
2. E.A. Engels. Non-AIDS-defining malignancies in HIV-infected persons: etiologic puzzles epidemiologic perils, prevention opportunities *AIDS*. 2009 May 15; 23(8): 875–885
3. Del Mistro A, Chieco BL. HPV-related neoplasias in HIV-infected individuals. Eur J Cancer.2001; 37:1227–1235.
4. Palefsky JM, Holly EA, Efirdc JT, et al. Anal intraepithelial neoplasia in the highly active antiretroviral therapy era among HIV-positive men who have sex with men. AIDS. 2005
5. Hessol NA, Pipkin S, Schwarcz S, Cress RD, Bacchetti P, Scheer S. The impact of highly active antiretroviral therapy on non-AIDS-defining cancers among adults with AIDS. Am J Epidemiol.2007; 165:1143–1153.
6. Goldie SJ, Kuntz KM, Weinstein MC, Freedberg KA, Welton ML, Palefsky JM. The clinicaleffectiveness and cost-effectiveness of screening for anal squamous intraepithelial lesions in homosexual and bisexual HIV-positive men. JAMA. 1999; 19(281):1822–1829.
7. Chin-Hong PV, Berry JM, Cheng SC, et al. Comparison of patient- and clinician-collected anal cytology samples to screen for human papillomavirus-associated anal intraepithelial neoplasia in men who have sex with men. Ann Intern Med. 2008; 149:300–306.
8. Katz KA, Clarke CA, Bernstein KT, Katz MH, Klausner JD. Is there a proven link between anal cancer screening and reduced morbidity or mortality? Ann Intern Med. 2009; 150:283–284.
9. Biggar RJ, Jaffe ES, Goedert JJ, Chaturvedi A, Pfeiffer R, Engels EA. Hodgkin lymphoma and immunodeficiency in persons with HIV/AIDS. Blood. 2006; 108:3786–3791.
10. Clifford GM, Rickenbach M, Lise M, et al. Hodgkin lymphoma in the Swiss HIV Cohort Study.Blood. 2009; 113:5737–5742.
11. Engels EA, Brock MV, Chen J, Hooker CM, Gillison M, Moore RD. Elevated incidence of lung cancer among HIV-infected individuals. J Clin Oncol. 2006; 20(24):1383–1388.
12. Epidemiology and Clinical Characteristics of Non-AIDS-Defining Malignancies Elizabeth Y. Chiao;Molecular basis of therapy for AIDS defining cancers Dirk P Dittmer, Susan E Krown p17-40.

Selected Abstracts -Indian Studies

Dhir AA, Sawant SP.Malignancies in HIV: the Indian scenario. Curr Opin Oncol. 2008 Sep;20(5):517-21.

Abstract

PURPOSE OF REVIEW: India has the second largest number of HIV/AIDS patients in the world; however, studies done in the area of HIV-related malignancies are few. With the availability of highly active antiretroviral therapy and treatment and prevention of opportunistic infections, an increase in life expectancy of HIV-infected individuals and an increase in HIV-related malignancies is expected. The purpose of this review is to put forth the Indian scenario of HIV-related malignancies.

RECENT FINDINGS: About 2.5 million Indians have HIV/AIDS. Non-Hodgkin's lymphoma and cervical cancer were found to occur in a higher proportion among the HIV-infected individuals in India as compared with non-HIV-infected individuals. The incidence of AIDS-related primary central nervous system lymphoma is low in India. Kaposi's sarcoma is rare in India. Amongst the non-AIDS defining cancers anal cancer, testicular cancer, Hodgkin's disease, colon cancer and certain head and neck cancer sites in men and vaginal cancers among women were found to occur more frequently.

SUMMARY: With the availability of highly active antiretroviral therapy an increased mortality and morbidity due to neoplastic diseases is expected in the future. As India is a large country and geographically and culturally diverse, large-scale studies need to be done linking the regional cancer centres with the AIDS centres across the country to evaluate the exact burden of HIV-related malignancies.