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Editorial

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Immuno-Genomics and Score: Stepping Stone for Immune Design

Immunogenomics is referred as identification of prognostic and predictive neoantigens for immunotherapeutic interventions with recent high-throughput sequencing technologies and bioinformatics data analysis. In cancer cells, neoantigens are generated by non-synonymous somatic gene mutations, presented on the surface of cells, remained bound to human leukocyte antigen (HLA) molecules, and in response to that T cell immunity might be provoked. Therefore, immunogenic neoantigens identification are of utmost importance for improvement of efficacy of cancer immunotherapy.

Identification of Neo-antigen

Neoantigens may have diverse sources, viral and mutated proteins. In cancers with virus associated etiology such as Merkel cell carcinoma, adult T cell Leukemia and HPV associated cancers, viral associated antigens have been considered as tumor specific antigens. On the other hand, mutated protein generate as a result of single nucleotide variants (SNV) resulting in non-synonymous substitutions, frame-shift derived mutations due to insertion or deletion of nucleotides, chromosomal translocation from break-point mutations and post-translational modifications such as phosphorylation and deamidation. Identification of neoantigens can be carried out using high-throughput genomics techniques viz whole-exome sequencing, whole-genome sequencing and more recent technique HLA peptidomics.[1] After introduction of immune checkpoint modulating antibodies such as anti-CTLA4 and anti-PD1 and its association with mutation burden response, neoantigens have become potential biomarkers for immunotherapies.

Immune Check Point Molecules

Immune Check Point molecules includes stimulatory molecules and inhibitory molecules, regulate immune activation and maintain immune homeostasis. The neo-antigens can be potentially recognized by specific T cells and mount T cell specific immune response. Inhibitory check point molecules like Cytotoxic T-lymphocyte associated antigen-4 (CTLA-4), programmed death-1 (PD-1), PD-1 ligand-1 (PD-L1), and lymphocyte activation gene 3 (LAG-3), suppress T-cell mediated immune response.CTLA-4 may be expressed on tumor cells, tumor infiltrating T regulatory cells or exhausted conventional T cells. The prognostic value of CTLA-4 expression of tumor cells has been described by few studies and have shown association with decreased survival in nasopharyngeal cancer and increased survival in non-small cell lung cancer. The ligand PD-L1 for PD-1 receptor is commonly over expressed on tumor cells or on non-transformed cells in the tumor microenvironment. PD-L1 expressed on the tumor cells binds to PD-1 receptors on the activated T cells, which leads to the inhibition of the cytotoxic T cells in the tumor microenvironment. Further PD-1 and PD-L1 expression when correlated with patient's survival, dissimilar findings were noted in several studies. The higher expression of PD-1 and PD-L1 expression associated with decreased survival in melanoma, renal cell cancer, esophageal cancer, gastric cancer and ovarian cancer, and improved survival in angiosarcoma.[2] The immune checkpoint modulating antibodies such as anti-CTLA4 and anti-PD1 suppress host immune response against cancer cells and then allow activation of host immune system resulting in eradication of cancer cells.Anti-CTLA-4 and anti-PD-1 antibodies are shown effective in immunogenic tumors prior to treatment having T cell infiltration and high mutation rates. A meta-analysis have shown a benefit of immune checkpoint inhibitors only in a subset of patients; no or minimum clinical benefit in majority of patients and severe immune-related adverse reactions in some patients. This study highlighted identification of predictive biomarker(s) that can be used to select patients who are more likely to expect clinical benefit with minimal risk of autoimmune adverse events, contributing to reduction of unnecessary medical costs.

Tumor Infiltrating Lymphocytes (TIL)

To understand relationship between immune system and tumor, evaluation of TIL is an important component because it reflects host antitumor response.[3] TIL are evaluated by immuno-

histochemical staining of CD3 and CD8 in two core regions of the tumor and the invasive margin defined as immunoscore. An international consortium of 14 centres in 13 countries includes our institute GCRI the only centre of India, which was led by society of immunotherapy of cancer (SITC) recommended to evaluate immunoscore in routine pathological diagnosis because it is valid and cost-effective.[4] High immunoscore predicts better overall survival and response to neoadjuvant and adjuvant therapy. Immunoscore has recently been established as a new cancer prognosticator for survival in spinal chordoma, colon, lung, bladder and liver cancer.[4,5,6,7] Patients with high immunoscore exhibited significantly higher PDL-1 expression of tumor cells and immune cells than low immunoscore, however, survival did not differ significantly with respect to PDL-1 expression in high and low immunoscore groups in colorectal cancer.[8] In another study on gastric cancer, a significant association of tumor-PD-L1 (+) and immune cells-PD-L1 (+) with a high immunoscore was observed. Further, PD-L1 expression of tumor and immune cells alone was not significantly correlated with the overall survival of patients. But in combination PD-L1 (+)/immunoscore Low group showed the poorer overall survival and the PD-L1 (+)/immunoscoreHigh group showed the better overall survival.[9] In addition to immunoscore, recently immunophenoscore which is insilico constructed transcriptome sequencing based score having clinically robust 32 gene panel has been proposed as a possible predictor of immune check point inhibitors for immunogenic tumors.[10]

In nutshell, identification of prognostic and predictive neoantigens by immunogenomics are expected to generate new insights for development of neoantigen-formulated vaccines. Further, immunoscore and pan-cancer immunophenoscore evaluation prior to treatment help to overcome therapeutic resistance to immunotherapy.

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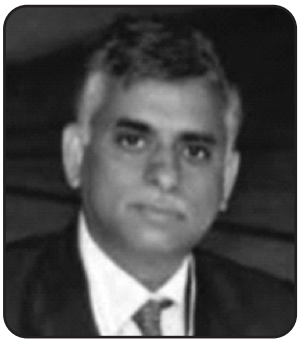
Dr. T. B. Patel Oration Award 2018



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Nuclear Medicine Cancer Theranostics: Exploring the New Horizons

The term 'Theranostics' encapsulates the integration of diagnostics and therapeutics in the individualized management of disease. Implicit in the Theranostic paradigm is the assumption that diagnostic test results can precisely determine whether an individual is likely to benefit from a specific treatment. This assumption underpins the recent focus on companion diagnostics as an integral part of drug development.

An excellent example of the concept of theranostics in oncology is the requirement that, to be selected for Trastuzumab therapy (Herceptin; Genentech), a candidate must have a tumor on which the presence of human epidermal growth factor receptor 2 (HER2) has been demonstrated. However, this requirement is limited by the potential sampling bias intrinsic in tissue biopsy. Molecular imaging of HER2 expression using ^{89}Zr-radiolabeled Trastuzumab provides an alternative vision of how the selection of candidates for expensive and sometimes toxic therapies might look in the future. Molecular imaging with ^{89}Zr-trastuzumab can be used to detect heterogeneity of HER2 expression. Because it can image the whole body, it has the potential to improve selection of patients for Trastuzumab and antibody–drug conjugate therapy. It also opens the way for therapeutic application of radionuclide therapy.

Nuclear medicine is ideally placed to play a central role in Theranostics by allowing visualization of molecular targets and thus enabling so-called in vivo Immune-Histochemistry, by which noninvasive biomarkers can be provided to select targeted drugs labeled with therapeutic Radionuclides and monitor the response to them. The staging and treatment of thyroid cancer with the diagnostic use of ^{123}I or ^{124}I complementing the therapeutic efficacy of ^{131}I has paved the way for theranostics in therapeutic nuclear medicine. Successful treatment of metastatic thyroid cancer was achieved even before the molecular basis of radioiodine uptake through the sodium-iodide symporter was characterized, speaking to the power of this paradigm. The use of radiolabeled meta-iodo-benzyl-guanidine in diagnosis and treatment of metastatic Neuroblastoma, Paraganglioma, and Pheochromocytoma or of radiolabeled somatostatin analogs in Neuroendocrine tumors (NETs) has extended the paradigm to other cancers. Despite the impressive results achieved using these agents, they have generally been developed in academic centers and used on a compassionate basis. This has led to limited resources for establishing the evidence base that usually accompanies registration and approval of cancer therapies. Beyond the expected clinical benefits of personalized medicine, theranostics could also have a significant positive economic effect.

Nuclear medicine is ideally placed to play a central role in Theranostics by allowing visualization of molecular targets and thus enabling so-called in vivo immunohistochemistry, by which noninvasive biomarkers can be provided to select targeted drugs labeled with therapeutic radionuclides and monitor the response to them. Limited resources for establishing the evidence that usually accompanies registration and approval of cancer therapies, in particular there has been a lack of randomized controlled trial data comparing radionuclide therapies with other forms of therapy and virtually none testing the integrated theranostic approach. In order to take benefit of the unique qualities of theranostics with radionuclides therapies and save costs in developing countries with huge patient load we need to develop theranostics tools locally and identify agents that can modulate target expression or increase radiation-induced cellular damage (radio sensitizing agents), and encourages the combination of Cytostatic treatments between radioactive sessions while identifying reliable and accurate biomarkers of therapeutic response.

Shri Madanmohan Ramanlal GCRI Luminary Oration Award - 2018

Dr. Shreedevi B. Patel
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More than Two Decades of Career at GCRI and GCS: My Strength as a Radiologist

I feel blessed by Shri Krishnabhagvan for being selected to deliver Shri Madanmohan Ramanlal GCRI luminary oration award. Shri Madanmohan Ramanlal ignited the flame of service to mankind and gave the impetus to one and all to surge ahead in building up an institution which has been a helping hand to the society in difficult time of fighting one of the still dreaded medical conditions like "The Cancer". His excellence in business was extended to social service to build up a institute of excellence till date.

As the process of striving for excellence continued, the siblings of GCRI who have sacrificed the prime of their life and made exceptional contribution are awarded this prestigious Shri Madanmohan Ramanlal oration. I really feel honoured to be awarded this prestigious status.

If Shreedevi Patel is one side of the coin, the other side is Dr. Babu N. Patel, my husband. I would like to clarify that this prestigious oration equally belongs to my better half as he has been my mentor in my professional and academic carrier till date. Without his support I cannot imagine what I would have been...

My contribution to GCRI commenced in 1985 as assistant professor of radiology and incharge of CT scan department. The vision of late Dr. T. B. Patel the then director of GCRI gave me the opportunity to start my carrier in radiology department. Working under "SAHEB" the one and only Dr. Narendra Patel a great visionary in radiology and excellent teacher not only in academics but his foresight in keeping the radiology department at the forefront in western India taught me to keep the department updated.

The radiology department must be best equipped and the most efficient department has been the motto. With this zeal my contribution did not limit my skills just to one modality or specialty of radiology. In the initial days high frequency X ray unit with image intensifier system was a unique feature and IITV guided cine radiographic procedures like cerebral, aortic and peripheral angiography procedures were unique feature at GCRI only. Procedures like lymphography were also done when USG and CT scan was not commonly available investigation tool. Mammography for breast cancer and screening was a rarity in the state of Gujarat. The first ever modality available in market would be imbibed at GCRI. Whether it be spiral CT, MRI, DSA, color Doppler or even OPG. I have seen a sea change and the metamorphosis of radiology from simple x ray to most recent MDCT and MRI or in other words when RADIOLOGY branch not very hot or preferred specialty to the most sought after branch of present time.

My broad vision in learning, experimenting and implementing new modalities came from my exposure for studying in London for DMRD in 1977 at Conjoint Board London and working for a short period as clinical assistant in Mount Vernon Hospital Northwood England in 1974 and in 1975 as a locum registrar in radiology for one year at Watford general hospital England. Prior to this I graduated in 1969 as MBBS, DMRD in 1972 and MD in radiology in 1973 from Gujarat University.

I had a spell of ill health and social responsibilities which kept me away from my professional carrier.

My conviction, perseverance and support from my husband Dr. Babu Patel followed by immense encouragement, trust and support from SAHEB/ Dr. N.L.Patel, Dr. D.D. Patel, Dr. Pankaj M. Shah set my stronger innings in professional carrier. I certainly feel blessed to work under such dynamic leadership.

Why Gujarat Cancer and Research Institute was the only preferred institute? As all of us are aware that patients with cancer need a multidisciplinary aggressive approach. Isolated piecemeal management by only surgery or chemotherapy is less than half the work done. A concomitant treatment with all possible modalities including rehabilitation under one roof is what cancer care is all about. Very few institutions in the country were established with such broad vision.

The visionaries who established this institute had the guts to attack the incurable disease like cancer received whole hearted support from philanthropic people, donors, doctors and society to shape the vision into reality. GCRI became the largest cancer care center in western India and the government of India recognized it as a "Regional Cancer Center". It was not just the western India but patients as far as from Bihar, UP and MP came to receive state of art treatment at most affordable rate.

Radiology is always the backbone of any institute and plays a pivotal role in the development of patient care. This role is even more vital in cancer care.

The work culture at our institute was simple. No patient as far as possible should leave the radiology department without a possible conclusive radiological diagnosis. Utilize all possible radiological modalities to narrow down the differential diagnosis and if need be, do a tissue sampling to conclude the pathology whenever possible. The entire department is synchronized and tuned to achieve this goal.

For a reasonable number of years CT scan, MRI and even ultrasonography like modalities at GCRI was the only source for investigation available in the new civil hospital campus including Saurashtra and Kutch area. This gifted us a wide spectrum of pathologies from head to toe and our department became the final destination for diagnosis whether it be stroke, head injury or complex pathological conditions of the human body.

Radiology department being at the forefront of getting all the latest modalities, I had the opportunity in analyzing the specifications, technological qualities, cost, durability, service for the betterment of patient care and institute.

This experience of mine gave me the opportunity to be special advisor not only for Gujarat but to Jammu and Kashmir government for purchase of new CT scan units for medical colleges.

Academic activities to uplift the medical expertise have always been my passion. I have conducted various CME's like paediatric onco imaging, breast imaging, regular training sessions for post graduate students. Numerous case presentations in national and state conferences helped me to continuously upgrade my skills. Some of my exclusive achievements include as author of "Atlas of computed tomography – the eye and orbit" published in 2006 by GCRI and GCS at Ahmedabad. The atlas was released by the present prime minister and the then chief minister of Gujarat honorable Shri Narendra Modiji. Audiovisual presentation on pediatric GI radiology in national conference on pediatrics at Ahmedabad. I have attended and participated in more than a dozen international and national conferences and courses including the RSNA at Chicago in 1984 and NICER course held at New Delhi. I have contributed more than fifty odd publications and presentations at various conferences and in journals. My academic development was sharpened by opportunities of being trained for short period on CT scan at NYU under Dr. Norman Chase in 1984 in USA. In 1994 for a period of four months under ODA phase II project visited Royal Hallamshire Hospital Sheffield for special training on mammography, breast screening project, MRI and CT scan imaging in oncology. I had also been deputed in 1998 to visit Singapore for spiral CT & MRI training by Hitachi Company Ltd. and Sydney, Australia for training in "Radiosurgery" at Prince of Wales hospital in 1998.

If I look upon my journey as a MBBS graduate in 1969, a post graduate in Radiology in 1973, my qualifications in UK, work experience and academic exposure in western world and at home, my contribution at GCRI for about 22 years and as professor in Radiology at Waghodia, SBK Shah Medical Institute & Research centre, a crusade of around 50 years has passed, more than 2/3rd of my present age.

I experience a great sense of satisfaction as I have been a stepping stone in development of cancer care by my radiological skills. In this process I was able to be a guide to numerous post graduate students. Juniors and colleagues could interact with me and gain experience and excel in their professional carrier.

I know that there is no thank you or sorry in a family but from the bottom of my heart I value each and every one who has been with me, seniors, teachers, colleagues, students and staff with special mention to CT Scan department residents and doctor colleagues, technologists, nursing staff including class IV employees for making me worthy of standing before you all for this prestigious oration.

At this juncture, of **कल , आज औऱ कल** (yesterday, today and tomorrow) of my professional carrier and my contribution to GCRI, I sincerely believe that someone from present generation will carry the torch of enlightening and GCRI will remain and cross the pinnacles of success in cancer care.

Alteration of Vitamin D and Vitamin D Signaling Pathway in Breast Cancer: A Preliminary Study from Western India

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Summary

Recent evidences suggest protective mechanism of vitamin D against breast cancer by autocrine/paracrine manner and may modestly reduce risk of breast cancer. It also plays an important role in apoptosis, cell cycle regulation and metastasis. Therefore, the present study aimed to study vitamin D and its derivatives in breast cancer development.

Total 88 subjects including 51 breast cancer patients and 37 healthy individuals were enrolled for the study. Serum 25(OH) D levels were measured by HPLC. The transcript levels of CYP27B1, VDR and CYP24A1 were investigated in malignant tissues and adjacent normal tissues by RT-PCR. Statistical analysis was carried out using SPSS software. 'p' value <0.05 was consider as a statistical significant.

In the study, serum 25(OH) D was lower in breast cancer patients as compared to the controls. Based on serum 25 (OH) D levels, odds ratio analysis showed increased risk of breast cancer from mild to moderate to severe vitamin D deficiency. RT-PCR analysis showed that mRNA expression of CYP27B1 was lower whereas CYP24A1 and VDR were higher in malignant tissues as compared to adjacent normal tissues. ROC curve analysis for VDR suggested significant difference between malignant tissues and adjacent normal tissues. Multivariate analysis revealed that CYP24A1 was significantly associated with various clinicopathological parameters like menopausal status, stage, molecular subtypes, ER and HER2 receptors.

Best of our knowledge, this is the first Indian study in relation with vitamin D signaling pathway and breast cancer. It suggests that lower levels of 25(OH) D may be associated with breast cancer risk. Altered expression of VDR suggests its role in breast carcinogenesis. The data warrant in depth analysis with large number of sample to stamp influential role of vitamin D and signaling molecules in breast carcinogenesis.

Keywords: Breast cancer, CYP24A1, CYP27B1, 25 Hydroxyvitamin D [25(OH) D], VDR, Vitamin D signaling pathway

Introduction

Breast cancer ranks first among all other cancer with an incidence rate of 25.2% worldwide in case of female. Collectively, India accounts for almost one third of the global breast cancer burden which is 27.0%.[1] At The Gujarat Cancer and Research Institute (GCRI) which is the regional cancer center for western part of India, breast cancer emerged as major female health hazard. According to the population based registry of GCRI, out of 40% of female cancer cases registered, Various etiolological factors are associated with breast cancer including genetic factors, lifestyle, and diet.[2] Recently, it has been suggested that there is protective mechanism of vitamin D against breast cancer by autocrine/paracrine manner and various studies suggested that it may modestly reduced risk of breast cancer.[3,4] In autocrine/paracrine mechanism breast epithelium produces 1α 25(OH) 2D3 from the circulatory 25(OH) D with the help of anabolic enzyme 1α-OHase encoded by CYP27B1 gene. 1α 25(OH)2D3 is the biologically active metabolite and relatively small, lipophilic molecule that can easily penetrates by simple cell diffusion in the cell membrane and binds to the vitamin D receptor (VDR), thereby causing its dimerization with the retinoid X receptor (RXR) and its translocation to the nucleus. The ligand-bound 1α 25(OH)2D3-VDR–RXR complex binds to vitamin D response elements (VDREs) in multiple regulatory regions located in the promoters of target genes and this causes the recruitment of co-activators or co-repressors, which leads to positive or negative transcriptional regulation of gene expression. These target genes are involved in diverse molecular pathways, thereby resulting in a wide range of 1α 25(OH) 2D3 mediated anticancer actions in an autocrine/paracrine manner. Degradation of unneeded 1α 25(OH) 2D3 is accomplished by the catabolic enzyme 24 Hydroxylase (24-OHase) encoded by CYP24A1 gene for regulation of 1α 25(OH) 2D3 synthesis. In addition, 1α-OHase (CYP27B1) and 24-OHase (CYP24A1) also plays an important role in the vitamin D metabolic cascade.[5] Thus, alterations in vitamin D receptor and its associated anabolic enzyme CYP27B1 as well as catabolic enzyme CYP24A1 are important for maintenance of circulatory 25-hydroxyvitamin D levels, thus mRNA expression of CYP27B1, VDR and CYP24A1 as well as circulatory 25(OH) D plays crucial role in development of breast

cancer. However there is dearth of data from India, regarding circulating 25(OH) D levels and signaling molecules in breast cancer. Therefore, the aim of the present study was to evaluate role of circulating 25(OH) D levels and its associated genes involved in vitamin D signaling in breast cancer.

Materials and Methodology

Subjects

The study was approved by Institutional Review Board (IRB) and Institutional ethics committee of GCRI. Total 51 female breast cancer patients and 37 female controls were enrolled. Due consent was taken from all the subjects prior to enrollment in the study. Histopathologically confirmed breast cancer patients prior to any anti-cancer treatment were selected for present study. Moreover, any other illness as well as breast cancer patients supplemented with vitamin D or multivitamins were excluded from the present study. Premenopausal, postmenopausal and perimenopausal groups were divided according to subject questionnaires.

Blood and tissue collection and processing

Blood samples were collected into plain vials. Serum was separated and stored at -80^{0}C until analyzed. Malignant and adjacent normal tissues were collected after surgical resection. Adjacent normal tissues were selected from the remaining tumor free margins (at least 1-2 cm away) as defined by the pathologist and it was epithelial containing tissues (Fat tissues were avoided). The tissues were washed with ice-cold phosphate buffer saline (pH-7.4) and immediately stored at -80^{0}C in RNA stabilizing reagent. If sample contain small amount of fat tissues lipid was removed using lipid removal kit.

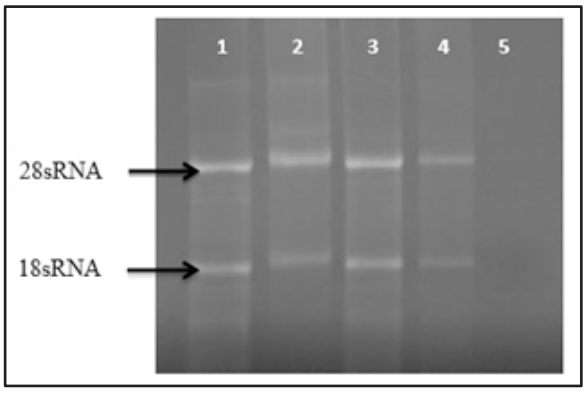

Figure 1: Separation of 18s RNA and 28s RNA on agarose gel electrophoresis. Lanes 1 and 3 shows intact bands of RNA from malignant tissues and lanes 2 and 4 shows intact bands of RNA from adjacent normal tissues. While lane 5 depicts negative control (NC)

Methodology

Circulatory 25(OH) D Levels by high performance liquid chromatography (HPLC)

Serum 25(OH) D levels were carried out by HPLC using recipe circulatory 25-hydroxy vitamin D2/D3 kit from Germany. Calibrator was used as a standard (component of kit), It is lyophilized pooled calf serum containing 25(OH) D3 and 25 (OH) D2 concentrations. Moreover, the mean values of calibrator are traceable to NIST-SRM972a (National Institute of Standard and Technology-Standard Reference Material 927a) (vitamin D metabolites in frozen human serum). According to manufacturer's instruction 25(OH) D levels were categorized into four type; severe deficiency (< 5µg/l), moderate deficiency (5-10µg/l), mild deficiency (10-20µg/l) and sufficiency (20-70µg/l).

Transcript Levels of CYP27B1, VDR and CYP24A1 by reverse transcriptase polymerase chain reaction (RT-PCR)

RNA isolation was done by RNAeasy mini kit from Qaigen, USA according to manufacturer's instructions. RNA integrity was carried out to check quality of RNA on 1% agarose gel electrophoresis. Figure 1 depicts 18s ribosomal RNA and 28s ribosomal RNA after separation of isolated RNA samples from malignant and adjacent normal tissues. RT-PCR was carried out using one-step RT-PCR kit (Qiagen, USA) for mRNA expression. 500ng of RNA was used for mRNA expression of CYP27B1, VDR and CYP24A1. PCR products were run on 1.5% agarose gel electrophoresis and bands were visualized under gel documentation system (Alpha Inotech Inc. USA) and quantified by integrated density values

Table 1: Primers used for mRNA expression of CYP27B1, VDR, CYP24A1 and 28sRNA

No	Parameter	Primer sequences	Reference
1.	CYP27B1	5'-GCTACACGAGCTGCAGGTGCAGGG -3'	Segersten et al 2005[6]
		5'-AGCGGGGCCAGGAGACTGCGGAGC -3'	
2.	VDR	5'-TGCCTGACCCTGGAGACTTTGACC -3'	
		5'-CATCATGCCGATGTCCACACAGCG -3'	
3.	CYP24A1	5'-GGCTTCTCCAGAAGAATGTAGGGGATGAAG -3'	
		5'-TGAGGCTCTTGTGCAGCTCGACTGGAG -3'	
4.	28 sRNA (HKG)	5'-GTTCACCCACTAATAGGGAACGTG-3'	
		5'-CATCATGCCGATGTCCACACAGCG -3'	

(IDV). 28s RNA was used as a house keeping gene (HKG). The primers were selected according to segersten et al.[6] The list of primers used for CYP27B1, VDR and CYP24A1 as well as 28 sRNA (HKG) for mRNA expressions is listed in Table 1, In which, gene bank number for CYP27B1 was AB006987, for CYP24A1 was NM_000782, for VDR was NM_000376.

Statistical analysis

Statistical analysis was carried out using SPSS statistical software (version 15.0; SPSS Inc., Chicago, IL, USA). Student's independent't' test was performed to assess the level of significance for circulatory 25(OH) D. Student's paired 't' test was used to compare the mRNA expression of CYP27B1, VDR and CYP24A1 between adjacent normal and malignant tissues of the breast cancer patients. Multivariate analysis was performed to correlate the markers like CYP27B1, VDR and CYP24A1 with various clinicopathological parameters. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for the risk estimation of breast cancer development in relation to 25(OH) D levels. Receiver's operating characteristic (ROC) curve was constructed to evaluate discriminatory efficacy of the circulatory 25(OH) D levels, CYP27B1, VDR and CYP24A1. The ideal cutoff was determined from multiple points on the ROC curve that resembled the mean value in the control group. Power analysis was performed for the study and it was greater than 80%. Moreover, effect size was also performed using z statistic test. The values were expressed as the mean ± Standard error of mean (SEM). 'P' value ≤ 0.05 was considered as statistically significant.

Results

Socio-demographical details of the subjects are depicted in Table 2. Age and menopause matched cases and controls were included in the present study. The age range of breast cancer patients was between 31-70 years, whereas the age range of controls was between 25-68 years. Most of the subjects were postmenopausal (49.1%) followed by premenopausal (43.1%) and perimenopausal (7.8%). Moreover most of the subjects were without family history of cancer.

Clinicopathological details of the breast cancer patients

As per Table 3, Pathological tumor, Node and Metastasis (pTNM) staging of breast cancer patients were determined as per American Joint Committee on Cancer (AJCC). Breast cancer patients also

Table 2: Details of breast cancer patients and controls

Characteristics of breast cancer patients		Characteristics of controls	
No. of breast cancer patients	**51 (100%)**	**No. of healthy individuals**	**37(100%)**
Age (Years)			
Mean	47	Mean	45
Median	46	Median	46
Range	31-70	Range	25-68
Menopausal status			
Premenopausal	22(43.1%)	Premenopausal	15(40.5%)
Perimenopausal	4 (7.8%)	Perimenopausal	6(16.2%)
Postmenopausal	25 (49.1%)	Postmenopausal	16(43.3%)
Familial history			
Yes	2 (3.9%)	Yes	1(2.7%)
No	49 (96.1%)	No	36(97.3%)

Table 3: Clinicopathological details of the breast cancer patients

Characteristics	N (%)
Diagnosis	
IDC	49(96.1%)
Others	2(3.9%)
Stage	
Early	23(45.1%)
Advance	20(39.2%)
Undefined	8(15.7%)
Lymph Node (LN) involvement	
LN positive	20(39.2%)
LN negative	12(23.5%)
Undefined	19(37.3%)
Estrogen receptor	
Positive	29(56.9%)
Negative	19(37.2%)
Undefined	3(5.9%)
Progesterone receptor	
Positive	25(49.0%)
Negative	23(45.1%)
Undefined	3(5.9%)
HER2	
Positive	26(50.9%)
Negative	16(31.4%)
Undefined	9(17.7%)
Molecular subtypes	
Luminal A	10(19.6%)
Luminal B	20(39.2%)
TNBC	9(17.6%)
HER2 enriched	3(6.0%)
Undefined	9(17.6%)

categorized according to hormone receptor status i.e. ER, PR and HER2 as well as molecular subtypes. Most of the breast cancer patients showed invasive ducal type of cancer (96.1%). 39.2% breast cancer patients had advanced breast cancer and 45.1% breast cancer patients had early stage of the disease. 39.2% cases were with lymph node (LN) involvement. Majority of patients were with luminal B subtype i.e. 39.2% followed by luminal A (19.6%), triple negative breast cancer (17.6%) and HER2 enriched (6.0%).

Comparison of circulatory 25(OH) D levels between breast cancer patients and controls

Figure 2 depicts mean levels of serum 25(OH) D in breast cancer patients and controls. It revealed that mean levels of 25(OH) D were 21.68µg/l or ng/ml and 19.86µg/l or ng/ml in controls and breast cancer patients respectively. Thus, serum 25(OH) D levels were lower in breast cancer patients as compared to the controls. However, the 25(OH) D levels were not statistically significant (p=0.57).

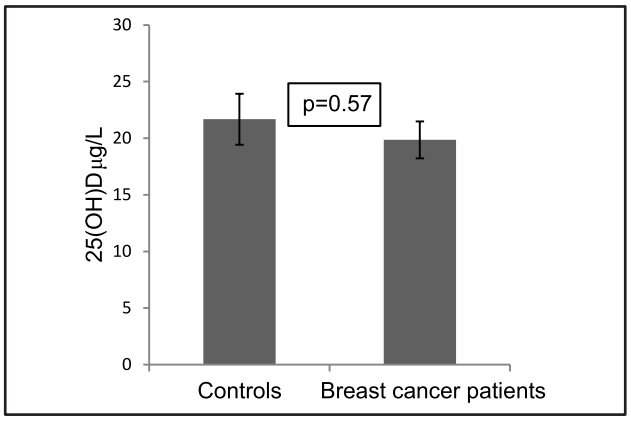

Figure 2: Mean levels of serum 25(OH) D in breast cancer patients and controls

Prevalence of 25(OH) D deficiency and sufficiency among subjects according to category

According to Figure 3, the prevalence of severe25 (OH)Ddeficiency was almost five fold higher in breast cancer patients as compared to controls (11.8% in breast cancer and 2.7 % among controls). Moreover, the prevalence of moderate 25(OH)D deficiency was also higher in breast cancer patients (17.6%) as compared to controls (13.6%).

Risk assessment of breast cancer by 25(OH) D levels

Table 4 shows association between 25(OH) D levels and breast cancer risk. Increased trend for odds ratio was observed from mild 25(OH) D deficiency (OR=0.67, 95% CI=0.25 to 1.79, p=0.43) to moderate 25(OH) D deficiency (OR= 1.30, 95% CI=0.36 to 4.65, p= 0.67) to severe 25(OH) D deficiency (OR=4.36, 95% CI= 0.47 to 39.89, p= 0.19). Further, odds ratio analysis for 25(OH)D levels in menopausal status showed that, the odds ratio was increased in post-menopausal breast cancer patients (OR=1.16, 95% CI=0.32 to 4.15, p=0.81) compared to pre (OR=0.96, 95% CI=0.25 to 3.66, p=0.95) and peri menopausal breast cancer patients (OR=0.33, 95% CI=0.02 to 5.32, p=0.43). However, there was no statistical significant difference was observed (Table 5). Sufficient Vitamin D levels were considered as a referent group for risk assessment.

Table 4: Odds ratio analysis for serum 25(OH) D levels and breast cancer

Circulatory 25(OH) D levels	Odds ratio	95% CI	p value
Total 25(OH) D deficiency	1.00	0.42 to 2.36	p= 0.99
Severe 25(OH) D deficiency	4.36	0.47 to 39.89	p= 0.19
Moderate 25(OH) D deficiency	1.30	0.36 to 4.65	p= 0.67
Mild 25(OH) D deficiency	0.67	0.25 to 1.79	p=0.43

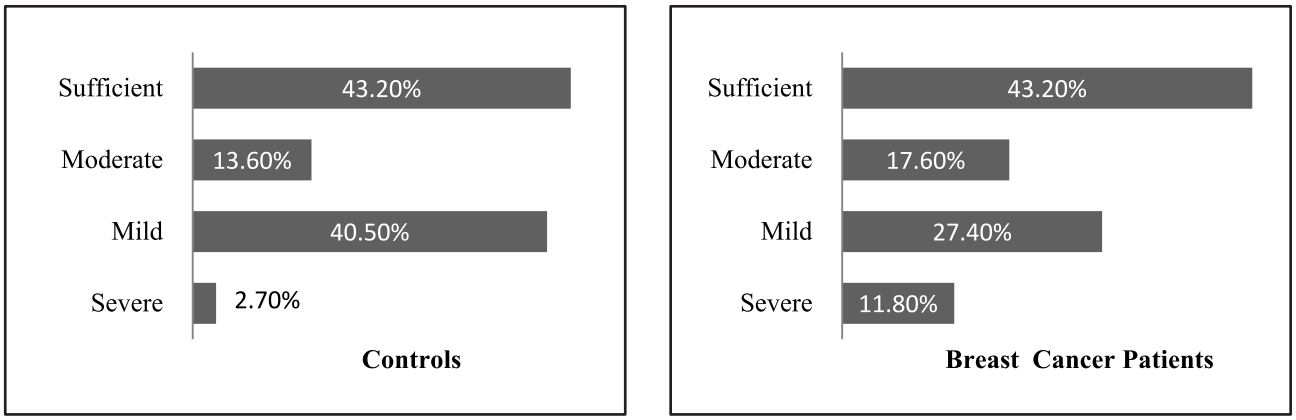

Figure 3: Prevalence of severe, moderate, mild 25(OH) D deficiency and 25(OH) D sufficiency among controls and breast cancer patients

Receiver operating characteristic (ROC) curve analysis for circulatory 25(OH) D levels

ROC curve plotted using SPSS version 15 and it is a meaningful statistical approach as it considers both sensitivity and specificity of the parameters. ROC curve was constructed for serum 25 (OH) D levels to evaluate their discriminatory efficiency between controls and breast cancer patients. Figure 4 shows ROC curve analysis for serum 25(OH)D levels, in which there was no significant difference have been found between breast cancer patients and controls (p=0.66).

Table 5: Odds ratio analysis for serum 25(OH) D levels and menopausal status of breast cancer patients

Menopausal status	Odds ratio	95% CI	p value
Premenopausal	0.96	0.25 to 3.66	p= 0.95
Perimenopausal	0.33	0.02 to 5.32	p= 0.43
Postmenopausal	1.16	0.32 to 4.15	p= 0.81

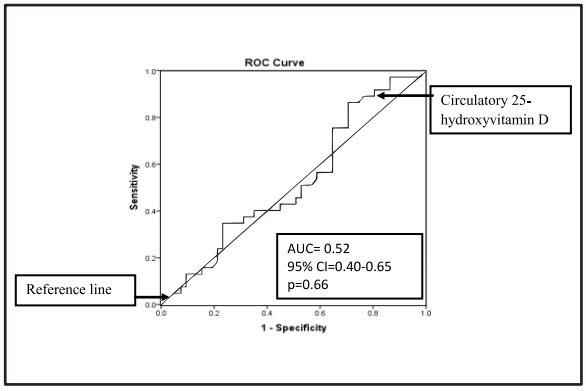

Figure 4: ROC curve analysis for 25(OH) D

Transcript levels of CYP27B1, VDR and CYP24A1 in malignant and adjacent normal tissues

Figures 5-7 show representative pattern of CYP27B1, VDR and CYP24A1, respectively. Mean integrated density value (IDV) of CYP27B1 (0.20 adjacent normal and 0.13 for malignant tissues), VDR (0.44 adjacent normal and 1.01 for malignant tissues) andCYP24A1 (0.44 adjacent normal and 0.64 for malignant tissues) in malignant tissues and adjacent normal tissues, in which the mean IDV values of CYP27B1 was lower in malignant tissues as compared to adjacent normal tissues (p=0.26). However, the difference was not statistical. Mean IDV of VDR and CYP24A1 was higher in malignant tissues as compared to adjacent normal tissues (p=0.04 and p=0.18).

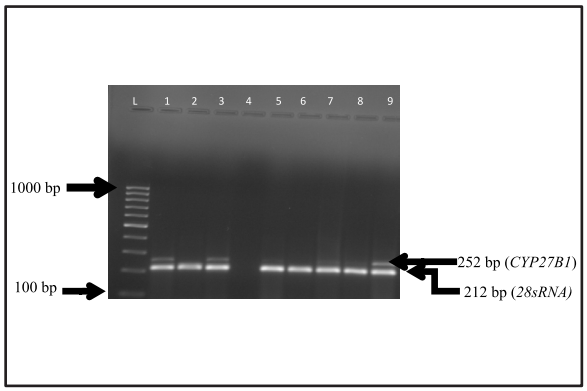

Figure 5: Representative pattern of CYP27B1 expression in malignant and adjacent normal tissues. Lanes 2, 6 and 8 represent the amplicon pairs of CYP27B1 and 28sRNA from malignant tissues, whereas lanes 1, 3, 5, 7 and 9 represents the amplicon pairs of CYP27B1 (252bp) and 28sRNA (212bp) from adjacent normal tissues. Lane L represents DNA Ladder (100-1000 bp) and Lane 4 represents negative control

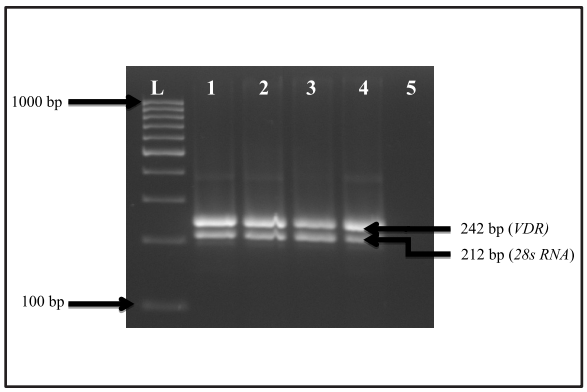

Figure 6: Representative pattern of VDR expression in malignant and adjacent normal tissues. Lane L shows 100bp ladder, lane 1 and lane 3 shows m RNA expression of VDR gene (242bp) as well as housekeeping gene 28s RNA (212bp) from malignant breast tissues, lane 2 and lane 4 shows m RNA expression of VDR gene as well as housekeeping gene 28s RNA from adjacent normal breast tissues, lane 5 shows negative control

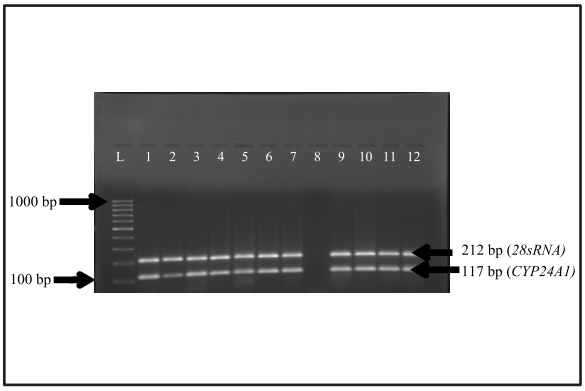

Figure 7: Representative pattern of CYP24A1 expression in malignant and adjacent normal tissues. Lanes 1, 3, 5, 7, 9 and 11 represents the amplicon pairs of CYP24A1 (117bp) and 28sRNA (212bp) from malignant tissues, whereas lanes 2, 4, 6, 10 and 12 represents the amplicon pairs of CYP24A1 and 28sRNA from adjacent normal tissues. Lane L represents DNA Ladder (100-1000 bp) and Lane 8 represents negative control

ROC curve analysis for CYP27B1, VDR and CYP24A1 in malignant and adjacent normal tissues

Table 6 depicts area under curve (AUC) and 95% Confidence interval (CI) for CYP27B1, VDR and CYP24A1. Among all transcript levels, VDR could significantly discriminate between malignant tissues and adjacent normal tissues (p=0.02) (Figure 8).

Table 6: Area under curve (AUC) for CYP27B1, VDR and CYP24A1

Parameter	Area	95% CI	p value
CYP27B1	0.43	0.11-0.75	p=0.67
VDR	0.82	0.62-1.00	p=0.02
CYP24A1	0.64	0.37-0.91	p=0.29

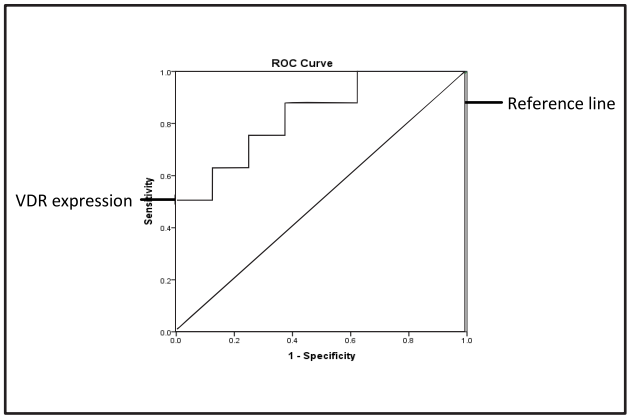

Figure 8: Reservoirs operating curve for VDRexpression between adjacent normal and malignant tissues

Multivariate analysis for CYP27B1, VDR and CYP24A1 transcripts levels with various clinicopathological parameters

The mRNA expression of CYP27B1, VDR and CYP24A1 were compared with menopausal status, age, molecular subtypes and various hormone receptor status (ER, PR, HER2). According to Table 7, F is factor and it is determined by degrees of freedom. Moreover, the test of main effect and the interaction effect are calculated by dividing the calculated variances by the variance (clinicopathological parameters) within the groups. However, for levels of significance we had considered p values. According to Table 7, CYP24A1 was associated with various clinicopathological parameter like menopausal status, stage, molecular subtype, ER and HER2 receptors (p=0.07, p=0.06, p=0.04, p=0.05 and p=0.03 respectively).

Table 7: Comparison of CYP27B1, VDR and CYP24A1with clinico-pathological parameters using multivariate analysis

Parameter	CYP27B1	VDR	CYP24A1
Menopausal status	F=0.43 p=0.62	F=0.74 p=0.54	F=66.76 p=0.07
Stage	F=1.82 p=0.40	F=2.02 p=0.39	F=99.21 p=0.06
Age	F=0.80 p=0.53	F=0.59 p=0.58	F=2.08 p=0.38
Molecular subtypes	F=0.03 p=0.88	F=1.19 p=0.47	F=193.32 p=0.04
Her2 Receptor	F=0.58 p=0.58	F=1.43 p=0.44	F=115.57 p=0.05
Progesterone Receptor	F=0.002 p=0.97	F=1.14 p=0.47	F=22.81 p=0.13
Estrogen Receptor	F=0.62 p=0.57	F=0.95 p=0.50	F=367.57 p=0.03

Discussion

Vitamin D, specifically in autocrine/ paracrine manner showed tremendous capacity to modulate important cancer features like regulation of proliferation and differentiation, angiogenesis, invasion, metastasis and enhance apoptosis suggesting its importance in cancer.[3,5,7, 21] In Indian population, there are very few studies which showed a relation between vitamin D levels and cancer.[8] Although there are no study documented regarding vitamin D and breast cancer especially molecular basis of vitamin D or genes in involved vitamin D signaling and breast cancer. Hence, the present study was concentrated to elucidate role of circulatory 25-hydroxyvitamin D, CYP27B1, VDR and CYP24A1 in breast cancer. To the best of our knowledge, this is first Indian study that showed vitamin D levels and its signaling molecules in breast cancer.

Various methods are available for estimation of 25-hydroxyvitamin D such as ELISA, RIA, LC-MS etc. However, determination of circulatory 25-hydroxyvitamin D by HPLC with UV detection can be considered the gold standard method.[9] In the present study, mean levels of circulatory 25-hydroxyvitamin D was lower in breast cancer patients as compared to the controls. Furthermore, odds ratio was increased in severe 25-hydroxyvitamin D deficiency followed by moderate and mild deficiency as compared to sufficient vitamin D levels, indicating that the risk of breast cancer increases when move from mild to

moderate to severe vitamin D deficiency. A study conducted by su x et al have shown that an inverse association was observed between proliferative benign breast disorders and amounts of vitamin D consumption.[10] In accordance with our results, two studies from India showed lower vitamin D levels in pediatric cancer and ovarian cancer patients compared controls.[8,11] Similarly, In Iran and other Middle East countries, the prevalence of vitamin D deficiency has been observed in approximately 30- 80% of breast cancer patients.[12] In another study by Rossi et al showed that circulatory 25-hydroxyvitamin D levels were significantly lower in patients than controls.[13] Shamsi et al and other studieshas also observed protective effect of vitamin D against breast cancer.[14-17] Moreover, in our study, we have also found that breast cancer risk was increased in post menopausal breast cancer patients as compared to pre and peri menopausal women. Contradictory, a significant association was demonstrated only in premenopausal and perimenopausal cases by Chlebowski.[18] Moreover, Bener and El Ayoubi found a high frequency of vitamin D deficiency in 635 postmenopausal breast cancer patients as compared to the pre and peri menopausal breast cancer patients.[19] Various studies have shown that responses to steroid hormones are modulated by crucial "pre-receptor" mechanisms involving tissue-specific activation or inactivation via locally expressed steroidogenic enzymes.[20] The enzymes 1α hydroxylase encoded by the gene CYP27B1 and 24-hydroxylase encoded by the gene CYP24A1 are important in vitamin D signaling pathway. CYP27B1 is responsible for the synthesis of the biologically active form of vitamin D (1, 25-dihydroxyvitamin D), whereas CYP24A1 mediates the catabolism of vitamin D.[21] In our study we found decreased mRNA expression of CYP27B1 in malignant breast tissues as compared to the adjacent normal tissues. Similarly various studies have demonstrated that CYP27B1 mRNA expression in breast tumors was decreased in comparison with normal mammary tissue.[22] It is speculated that tumors secrete endocrine/paracrine factors, which influence CYP27B1 expression, however other studies suggested that down regulation of CYP27B1 is caused by hypermethylation of its promoter.[23]

Present study demonstrated an increase CYP24A1 mRNA expression in breast cancer tissues as compared to adjacent normal tissues. Similarly, Segersten et al also showed that CYP24A1 mRNA expression was overall 2-fold higher in breast carcinoma as compared to normal tissues.[6] According to, Chen et al high CYP24A1 transcript levels seem to be a common feature of several solid tumors.[24] Thus, from the data we can presume that lower expression of CYP27B1 and higher expression of CYP24A1 resultant into alteration in optimal 25(OH) D levels.

In present study, mRNA expression of VDR was increased in malignant tissues as compared to the adjacent normal tissues. Moreover, ROC curve analysis revealed that VDR mRNA expression could significantly distinguish between malignant and adjacent normal tissues. VDR is expressed in the mammary gland and vitamin D has been shown to display anti-carcinogenic properties, this hormone has emerged as a promising targeted therapy. But in order to keep the homeostasis of the organism the amount of circulating vitamin D has to be tightly regulated. Some studies have demonstrated that the VDR protein is expressed in samples from normal breast tissues and also in breast cancer biopsy specimens.[6,25]

In our study, multivariate analysis results indicated that CYP24A1 expression was associated with menopausal status, stage, molecular subtype, ER and HER2 receptors. According to Albertson et al elevated tumor CYP24A1 expression is associated with a poorer prognosis of breast tumors and analysis of the data sets from the cancer genome atlas confirms that a subset of human breast cancers (10–13%) exhibit alterations in the CYP24 gene, with the most frequent changes being amplifications and up regulation at the mRNA level.[26] De Lyra et al has showed there were no differences in the expression of the CYP27B1, VDR and CYP24A1 mRNA in breast cancer and non-neoplastic mammary tissue.[27] Elevated as well as decreased CYP24A1 or CYP27B1 expressions are reported in different cancer cell lines.[28] Moreover, studies on human cancer biopsies agree with the hypothesis that the expression of VDR and CYP27B1 increases initially when a tumor develops, but while the tumor becomes more malignant and starts to dedifferentiate, the expression of VDR and CYP27B1 decreases while the expression of CYP24A1 strongly increases in human tissues of breast cancer and colorectal cancer.[29] This suggests that during early tumorigenesis the synthesis and signaling of 1,25(OH)2D3 are upregulated as a physiological defense system against epithelial tumor progression. When tumors dedifferentiate, VDR and

CYP27B1 levels drop while CYP24A1 expression increases, implicating that local 1,25(OH)2D3 concentrations decrease since less 1,25(OH)2D3 is synthesized while more is metabolized. The sequential acquisition of mutations that occur during tumor progression and metastasis could possibly negatively influence the expression of 1,25(OH)2D3-metabolizing enzymes.[30] Collectively these three genes i.e. CYP27B1, VDR and CYP24A1 are inter-correlated and play important role in vitamin D signaling pathway and ultimately play crucial role in development of breast cancer. The limitation of the study is small sample size though it is preliminary study. However, it is useful for public awareness and women health specially in Indian population. Moreover, the strength of the study is correlation of Vitamin D deficiency with various clinico pathological parameters such as correlation of vitamin deficiency with molecular subtypes of breast cancer.

Conclusion

Our findings suggest that low serum levels of 25(OH) D may be associated with an increased risk of breast cancer. Apart from that circulatory 25-hydroxy vitamin D deficiency is also associated with increased risk of breast cancer particularly, in postmenopausal women. Increased expression of VDR and CYP24A1 in malignant tissues suggests its role in breast cancer pathogenesis. The decreased expression of CYP27B1 in malignant tissues may be important in their predisposition to the development of breast cancer. Hence, vitamin D and its derivatives can evidently influence tumorigenesis and /or facilitate tumor progression.

Conflict of Interest: None

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Rituximab-Induced Late Onset Neutropenia: Case Report and Review of Literature

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Summary

Single agent rituximab has the potential to cause delayed and late-onset neutropenia that may vary in severity. In this report, we present a case of patient who was treated for mantle cell lymphoma with rituximab that lead to severe late-onset isolated neutropenia, which resulted in delay and subsequent omission of further rituximab cycles. It is important to be aware of this uncommon adverse event, which can occur long after cessation of rituximab therapy.

Keywords: Rituximab, Late onset neutropenia, Absolute neutrophil count.

Introduction

Drug induced neutropenia is a potentially serious and life-threatening adverse event that may occur secondary to variety of agents. Cytotoxic chemotherapy can cause a predictable, reversible and dose-related decrease in neutrophil count. Isolated neutropenia secondary to other medications tends to be an idiosyncratic reaction either as an immune-mediated reaction or because of direct myeloid cell line damage.Rituximab is an IgG1 chimeric human/mouse, anti-CD20 monoclonal antibody indicated for the treatment of variety of B-cell lymphocytic malignancies, including chronic lymphocytic leukemia, follicular lymphoma, mantle cell lymphoma (MCL) and diffuse large B-cell lymphoma.[1] Mechanism of actions of rituximab may occur by antibody-dependent cellular cyto-toxicity (ADCC), complement-dependent cytotoxicity and direct signaling (apoptosis).[2] Delayed and late-onset serious side effects associated with rituximab may include reactivation of hepatitis B (20-55%), interstitial pneumonitis (5.4%) and progressive multifocal leukoencephalopathy (1-2%).[1] When rituximab was added onto chemotherapy regimens, it was found to be safe and tolerable without adding significant hematological toxicities. Post-marketing studies and case reports have shown that rituximab has the potential to cause delayed and late-onset neutropenia (LON) that may vary in severity.[3-5] We report case of a patient who was treated for MCL with rituximab based chemo-immunotherapy followed by single agentrituximab maintenance every two monthly that led to severe LON before fifth cycle, which resulted in delay and subsequent omission of further maintenance rituximab cycles.

Case Report

A 51-year-old male patient presented to our hospital on 22/09/2017 with history of multiple bilateral cervical lymphadenopathy,since 2 months. His ECOG performance status was one. On detailed history and physical examination multiple, firm, discrete lymphadenopathy was found in bilateral cervical region, rest of the physical examination was normal. Excisional biopsy of cervical node was undertaken, which on histopathological examination showed intermediate to high grade non-Hodgkin lymphoma. On immunohistochemistry examination CD5, CD20 and Cyclin D1 were positive and CD23 was negative, hence a diagnosis of MCL was made. On further staging work up, which included contrast enhanced computerized tomography (CECT) scan of neck, thorax, abdomen and pelvis; MCL stage III, according to Lugano staging system was found.[6] Bone marrow aspiration and trephine bone biopsy was normal. Complete blood count (CBC) and serum biochemistry were normal. Following which patient was administered one cycle of cytoreductive chemotherapy consisting of cyclophosphamide, vincristine and prednisolone; followed by three cycles of rituximab, cyclophosphamide, adriamycin, vincristine and prednisolone (R-CHOP) every 21 days, after checking CBC before each dose of chemotherapy cycle. Patient tolerated all chemotherapy cycles very well, with no delay in any scheduled cycle. Interim CECT scan was done after three cycles, which showed more than partial response, according to response evaluation criteria in solid tumor (RECIST) version 1.1. In view of good response, further three cycles of R-CHOP were administered to the patient. CT scan evaluation after six cycles of R-CHOP showed complete response (according to RECIST v1.1). Subsequently patient was started on maintenance therapy with single agent rituximab every two monthly. Patient tolerated four cycles of single agent rituximab maintenance very well. CBC examination done just before scheduled fifth cycle showed, hemoglobin of 13.9 gm/dl, total leucocyte count (TLC) of 2800/cumm, with absolute neutrophil count (ANC) of 448/cumm, which was confirmed manually, and platelet count of 2.45

Table 1: Serial Complete blood count post fourth cycle of single agent rituximab

Date	Total Leucocyte Count(cells/cumm)	Absolute Neutrophil Count(cells/cumm)	Hemoglobin (gm/dl)	Platelet Count(cells/cumm)
03/08/2018	2800	448	13.9	2,45,000
04/08/2018	2300	500	13.5	2,04,000
06/08/2018	2600	640	13.3	1,70,000
09/08/2018	2400	1200	12.9	2,08,000
13/08/2018	4000	1600	13.4	2,19,000

lakh/cumm. Grade IV neutropenia according to common terminology criteria for adverse events (CTCAE) v5.0 was found. Patient was asymptomatic, with no history of any fever episode, cough, rash, malaise, anorexia or nausea/vomiting. Hence infectious causes or viral fever were not suspected or tested. Vitamin B12 deficiency is usually associated with severe anaemia, yellow skin and variable amount of neurological abnormality. Our patient only had isolated neutropenia with normal haemoglobin and platelet count. And also,the temporal course of neutropenia was correlating with previous rituximab administration. Diagnosis of rituximab-induced LON was made. In view of low ANC, rituximab maintenance was deferred, and serial CBC examinations were done biweekly (Table1). ANC gradually recovered to normal value (>1500) over a period of ten days. Granulocyte colony stimulating factors (G-CSFs) administration for ANC recovery was not required. In view of risk of recurrent, severe and prolonged neutropenia, rituximab re-challenge was not attempted. Patient was explained regarding the nature of adverse event and the option of high dose chemotherapy with autologous hematopoietic stem cell transplantation was discussed. Patient opted for no further treatment.

Review of Literature

Pathophysiology

Neutropenia is defined as having an ANC of less than 1500 cells/cumm and is a common adverse event associated with many cytotoxic chemotherapy agents.[7] In patients receiving cancer treatment regimens containing rituximab with cytotoxic chemotherapy (e.g. anthracycline, alkylating agents), the nadir (lowest value) of the patients neutrophil count is expected to occur 10-14 days following administration of each cycle of treatment. Neutrophil recovery will usually occur in three to four weeks following chemotherapy. Single agent rituximab has been reported to cause neutropenia, but with a delayed and often unpredictable onset. Single agent rituximab associated LON has been defined in the literature as developing at least three to four weeks following the end of rituximab administration despite a complete recovery of ANC following chemotherapy.[8] Rituximab-induced LON may be prolonged and result in very unpredictable recovery time.

The mechanism by which rituximab may induce neutropenia has yet to be fully elucidated, variety of theories exist. Direct toxicity is very unlikely. Several studies suggest that LON could be related to an excess of T-large cell lymphoma(T-LGL) in the bone marrow and peripheral blood which express and secrete large amounts of Fas and Fas ligand leading to apoptosis of mature neutrophils, or to a production of autoantibodies binding to the neutrophil surface during recovery of a new immune repertoire.[9] On the other hand, recent studies suggest that LON is not related to circulating factors but to perturbations of stromal derived factor 1 and B cell activating factors, cytokine, affecting granulopoiesis homeostasis during B cell recovery.[10] This is reinforced in the same study showing the hypo cellularity of the bone marrow at time of LON and absence of anti-neutrophil antibodies in the serum or T-LGL in peripheral blood.[10] The intricate balance of lymphopoiesis and granulopoiesis governed by a complex cytokine balance in the bone marrow environment may be hampered by rituximab, resulting in B-cell lymphopoiesis over granulopoiesis within common developmental niches. A recent study correlated specific polymorphism in the immunoglobulin G Fc receptor FCgammaRIIIa 158 V/F with increased rates of LON. Polymorphism in FCGR3A, a low-affinity receptor capable of binding to the Fc portion of complexed IgG, have been implicated in this process.[11] The presence of this polymorphism may facilitate neutropenia by mediating ADCC on malignant and non-malignant B cells, thus increasing the degree of B-cell depletion.[11]

Incidence and risk factors

The reported incidence of rituximab-induce LON varies within the literature. This adverse drug reaction (ADR) may occur in 8% to 27% of cancer patients treated with single agent rituximab.[12] Single agent rituximab-induced LON occurs a median of 38

to 175 days following the last rituximab dose, with a median duration of 5 to 77 days.[13] Despite the proposed high incidence of this ADR, many of the episodes are self-limiting and without any apparent clinical significance. Multiple studies have evaluated the risk factors for developing rituximab-induced LON. Patients with advanced stages of malignancy and those more than 60 years of age are at greater risk.4 Previous treatment with purine analogs or methotrexate and prior autologous peripheral blood stem cell transplantation may also be risk factors for developing rituximab-induced LON.

Management

Most cases of rituximab-induced LON, are, grade 1–2 which are self-limiting and resolve without any complications. However,in grade 3 or 4 neutropenia, there is a potential for prolonged andserious life-threatening infectious complications.[14,15] The delayed onset, unpredictable occurrence and neutrophil recovery associated with single agent rituximab-induced LON can create a clinical challenge for practitioners. Infectious complications, such as neutropenic fever, that may occur because of severe and prolonged neutropenia secondary to rituximab treatment should be managed with antimicrobial therapy. Antimicrobials should be selected and modified based on guideline recommendations.[7] Granulocyte -colony stimulating factors (G-CSFs) can also be used in patients with neutropenic fever with additional risk factors for severe complications, such as those with an ANC of less than 100 cells/cumm and/or with pneumonia, hypotension, multi-organ failure, or invasive fungal infections.[16] G-CSFs are especially useful in managing patients treated with rituximab because they address the unpredictable nature of neutrophil recovery and possible prolonged neutropenic duration. No specific recommendations regarding the optimal ANC target, frequency, and duration of administration of filgrastim products have been proposed to manage this adverse event. The drug is typically administered once daily until neutrophil recovery when it is utilized for neutropenia prophylaxis in patients with non-myeloid malignancies receiving myelosuppressive chemotherapy.[17] Although rituximab-induced LON has the potential to be a long-lasting complication, neutrophil recovery with the use of a filgrastimproduct can occur in as few as four days.7 To keep a patient's ANC greater than 1,000 cells/cumm, maintenance strategies using the drug once or twice weekly may be employed for several months for patients with prolonged neutropenia despite initial neutrophil recovery.[3] Given the unclear nature and mechanism of rituximab-induced LON, it is not fully known and understood if re-treatment with rituximab is a viable and safe option for patients. It has been previously reported that re-challenging a patient with rituximab following an episode of severe LON can lead to recurrent episodes.[13] With the possibility of recurrence and the unclear risks and implications of retreatment, the decision to administer further doses of rituximab should be made on a case-by-case basis. Future research is needed in this area.

Conclusion

Single agent rituximab can cause delayed and LON that may last for an unpredictable amount of time. Although most cases appear to be self-limiting and resolve without issue, rituximab-induced LON may result in serious life-threatening complications requiring immediate medical intervention. Diligent patient follow-up is needed to monitor for this adverse event, which may occur long after therapy cessation and therapeutic intervention may be necessary in severe cases that may result in neutropenic fever. This adverse event can pose challenge for clinicians and requires close patient follow-up with CBC monitoring during rituximab administration as well as after therapy has ended.

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Rare Case Report of Cerebral Ganglioneuroblastoma

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Summary

Ganglioneuroblastomas (GNBs) are rare embryonic neoplasm in the spectrum of neuroblastic tumours and 80% of cases occur in the first decade. We describe a 14-year-old boy with acute onset headaches and limb weakness. On imaging he was diagnosed of having intra-cranial mass. Patient underwent partial excision of tumor, followed by chemo-radiotherapy. This mass was pathologically confirmed as a primary intracranial ganglioneuroblastoma, a rare finding in the pediatric population. For cerebral ganglioneuroblastoma, the preferred regimen would seem to be neurosurgical removal, followed by chemoradiotherapy including temozolomide.

Keywords: Brain, Ganglioneuroblastoma, Neuroblastoma

Introduction

Ganglioneuroblastoma (GNB) represents a subgroup of neuroblastoma tumors with prominent, mature ganglion cell differentiation, usually located in the adrenal gland, posterior mediastinum, or retro peritoneum. Neuroblastomas are neural crest tumors composed of undifferentiated neuroblasts with stroma poorly represented. In the presence of ganglion cells and stroma-rich areas, they are designated as ganglioneuroblastoma.[1-3] Neuroblastomas are classified among the group of primitive neuroectodermal tumors (PNET), which also include medulloepithelioma and ependymoblastoma (containing ependymoblastic rosettes).[1] Some author also include medulloblastoma within the group of PNET's. More than 90% of all ganglioneuroblastomas are seen in children younger than 5 years old and it is rare that they appear in adults. These neoplasm arise wherever sympathetic tissue exists and may be seen in the neck, posterior mediastinum, adrenal gland, retro peritoneum, and pelvis.[4,5] Central nervous system neuroblastomas and GNB are uncommon. Signs and symptoms of cerebral neuroblastic tumors are related to the site of origin, and include seizures, disturbances of consciousness, increased intracranial pressure, and motor deficit.

Case Report

We report a case of 14-year-old child presented with gradually increasing headache since two months, nausea and vomiting since 15 days and left upper limb and lower limb weakness since five days. The previous history was otherwise negative. Physical examination showed a left upper limb weakness (power 2/5) and left lower limb weakness (power 3/5). MRI Brain showed 25 x 24 x 24 mm lesion, at left cerebellopontine angle with mass effect which is T1 hypointense, T2 hyperintense, homogenously enhancing with restricted diffusion with lepto meningeal enhancement. The patient underwent subtotal excision of space occupying lesion. Post operative MRI Brain with whole spine screening was suggestive of progression of disease in form of, altered signal intensity solid cystic lesion in left posterior cerebral region (77x15 mm), left temporal lobe region (47x33 mm) with post contrast enhancement and diffuse meningeal enhancement, suggestive of meningeal metastasis. CSF cytology was positive for malignant cells. Further work-up including CT scan of thorax and abdomen and bone scan showed no other tumor locations elsewhere. After surgery, the treatment was followed by chemoradiotherapy (40 Gy in 20 fractions with concomitant temozolomide (75 mg/m^2/day), post chemo radiotherapy MRI Brain with whole spine screening was suggestive of stable disease. It was followed by 6, monthly cycles of temozolomide (200 mg/m^2/day for 5 days, every 4 weeks). At present patient is receiving last cycle of adjuvant chemotherapy.

Histology

The histology of tumor was GNB. (Figure 1).The brain parenchyma was infiltrated by focally highly cellular proliferation of cells with round and hyperchromatic nuclei and a relatively small rim of cytoplasm, showing positive staining for

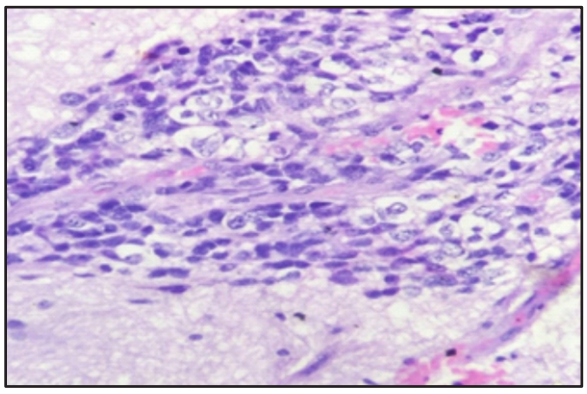

Figure 1: Histopathology of H & E stained slide of cerebral GNB

synaptophysin, NSE, CD-56. INI1 retained. In between these cells we observed several larger cells with neuronal features, often with irregular nuclei, sometimes showing a double nucleus. MAP2 was positive in ganglion cell. Ki-67 positivity was 25%.

Discussion

GNB is defined by the International Neuroblastoma Pathology Committee (INPC 1999)[3] and classified as a subgroup of neuroblastoma.[6] GNB is a mixed tumor including mature ganglion cells and malignant neuroblastoma simultaneously.[2,3] Degree of GNB differentiation is between high malignant neuroblastoma and benign ganglioneuroma.[7] However, it is difficult to draw a clear demarcation line based on morphology or gene expression differences.

The most common sites of origin of GNB are the adrenal medulla, extra-adrenal retroperitoneum, and posterior mediastinum. Less common sites are the neck and pelvis.[5] although rare, the GNB may also occur at the central nervous system, mainly involving the cerebral hemispheres. The location of intracranial GNB, including frontal, temporal, parietal, occipital, parietal-occipital, pineal, cerebellar, cerebellopontine region and ventricle, determine its clinical symptoms, such as seizures, visual impairment, hemianesthesia, unilateral sensory, motor disturbance, headache, and transient global amnesia.MRI often showed features of low-grade gliomas, including a space-occupying lesion with a well-defined margin. Hyper-signal on DWI with a low ADC value was detected, which prompted high tumor malignancy. Furthermore, Magnetic Resonance Spectroscopy analysis showed an increase of Cho/NAA.

GNB is composed of neuroblastoma cells, ganglion cells with different degrees of differentiation, nerve sheath, and glial fibers.[8, 9] the common characteristic of pathological findings is the highly infiltrated and proliferated cells with dense chromatin. Ganglion-like large cells usually present with double nucleus.[10] Immunohistochemical staining for S100, neurofilaments, chromogranin, NSE, CD34, and synaptophysin was positive in ganglion cells and nerve sheath cells.[11–16] S100, synaptophysin, neurofilaments were positive in neuroblastoma

Table 1: Cases of ganglioneuroblastoma reported in the literature

First author	Location	Metastasis	Surgery (complete/partial resection)	Radiotherapy	Chemotherapy	Survival
Raina et al. [22]	Spinal cord	None	Complete	-	Adriamycin, cyclphosphamide, vincristine, etoposide, ifosphamide, cisplatinum	>24 m
Sibilla et al. [23*]	Spinal cord	Local	-	-	-	>3 m
Tripathy et al. [24]	Spinal cord	None	Complete	-	-	>6 m
Feigin and Cohen [7]	Brain	Metaststic disease		-	-	0.5 m
Nakajima et al. (1982)	Brain		Complete	RT	Chemoth, unknown	>39m
Tanaka et al. (19980)[25]	Brain	None	Complete	50 Gy	-	>15 m
Nishihara et al. (2008)[26]	Brain	None	Complete	**	-	-
Sabatino et al. [27]	Brain	None	Complete	60 Gy local	Temozolomide	>18 m
M.H. Schipper et al.[29]	Brain	None	Complete	60 Gy local	Temozolomide	>14 m
M.H. Schipper et al.[29]	Brain	None	Partial	60 Gy local	Temozolomide	>12 m

(*) – Not received any form of therapy
(**)- Radiotherapy details not available

cells.[17,18] In our case, histopathology showed the positive staining of synaptophysin , NSE , CD-56. INI1 retained. MAP2 - positive in ganglion cell. Ki-67 was 25%.

GNB is further divided into 2 subtypes (undifferentiated and poorly differentiated types) [24] under electron microscope. The undifferentiated type consists of small round-to-oval cells with hyper chromatic nuclei.[19] the poorly differentiated type is composed of large round-to-oval spindle-shaped cells with pale staining nuclei.[19]

Complete resection is the optimal treatment for intrarcranial GNB. Partial resection or subtotal resection should be performed if the tumor extends into the cavernous sinus. Moreover favorable outcome will be obtained after fractionated radiotherapy and chemotherapy. It was reported that the longest asymptomatic period of the patients with intracranial GNB is 60 months following the above treatment.[20] although the rare occurrence of ganglioneuroblastoma makes prospective trials virtually impossible; patients treated with combinations therapy including chemoradiation using temozolomide have shown the longest survival.[21]

For literature review, pubmed and google scholar database was chosen. On literature review ten adult cases of ganglioneuroblastoma have been reported with in the central nervous system, of whom three in the spinal cord and seven in the brain. Details of these patients are described above in table. Out of these ten patients, two patients did not receive any form of therapy. Survival is improved with trimodality therapy inform of surgery followed by chemo-radiotherapy. Chemotherapy agents used include: adriamycin, cyclophophamide, vincristine, etoposide, ifosphamide, cisplatin, temozolamide. Our patient underwent subtotal excision, and chemoradiotherapy (40 Gy) with temozolomide, according to the Stupp schedule.[21] At present patient is receiving last cycle of adjuvant chemotherapy.

Table 1 shows cases of ganglioneuroblastoma reported in the literature. Recently immunotherapy as treatment for neuroblastomas was reported as being successful.[28] For ganglioneuroblastoma it is unknown, whether immunotherapy may be beneficial.

Conclusion

Cerebral GNB is rare presentation of GNB. For cerebral GNB, the preferred regimen would now seem to be neurosurgical removal, followed by chemoradiotherapy including temozolomide followed by adjuvant chemotherapy with temozolamide.

Abbreviation

GNB: Ganglio Neuro Blastoma, MRI: Magnetic Resonance Imaging, PNET: Primitive Neuro Ectodermal Tumor.

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Classical Hodgkin's Lymphoma with Secondary Haemophagocytic Lymphohistiocytosis - A Rare Case Report

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Summary

Hemophagocytic lymphohistiocytosis (HLH) is a syndrome characterized by immune activation and subsequent widespread organ damage. Patients affected by HLH commonly develop fever, cytopenias, liver damage, neurologic manifestations, and hypercytokinemia. In this case, we describe a 20 years oldfemale who presented with HLH and was subsequently diagnosed with Hodgkin lymphoma. This case highlights the importance of considering a cancer diagnosis in the differential diagnosis of patients presenting with HLH.

Keywords: Hemophagocytic lymphohistiocytosis, Hodgkin's lymphoma, malignancy

Introduction

Hemophagocytic Lymphohistiocytosis (HLH) is a life threatening disorder causing multisystem organ failure. It is characterized by an excessive and uncontrolled immune response due to cytokine dysregulation and lymphohistiocytic proliferation.[1, 2] HLH is a secondary reaction to infection, medication, autoimmune or neoplastic diseases. Haematological malignancies are a well-known HLH aetiology, but the combination of Hodgkin's lymphoma and HLH is rarely reported at the time of diagnosis.[3] we report a case of Hodgkin's lymphoma revealed by HLH as an initial manifestation illustrating diagnostic difficulties and interest of rapid treatment.

It should be noted that the diagnostic criteria for HLH (Table 1) were devised for use in clinical trials and are therefore unlikely to capture every case of HLH. Because of the high mortality of HLH in the absence of appropriate treatment, we do not always require these diagnostic criteria to be met in order to initiate treatment. Specifically, treatment should not be delayed while awaiting the results of genetic or specialized immunologic testing.

Adults are more likely to have a secondary form of HLH than children, and adults with secondary HLH are more likely to have an underlying malignancy as the cause.

Table 1: Revised Diagnostic Guidelines for HLH4

The diagnosis HLH can be established if one of either 1 or 2 below is fulfilled
1. A molecular diagnosis consistent with HLH *
2. Diagnostic criteria for HLH fulfilled (five out of the eight criteria below)
·Fever
·Splenomegaly
·Cytopenias (affecting >=2 of 3 lineages in the peripheral blood)
·Haemoglobin< 9 gm/dl (in infants <weeks: haemoglobin <10gm/dl)
·Platelets <100000 per cu mm
·Neutrophils<1000 per cu mm
·Hypertriglyceridemia and/or hypofibrinogenemia: Fasting triglycerides >265 mg/dl;
·Fibrinogen <150 mg/dl
·Hemophagocytosis in bone marrow or spleen or lymph nodes. No evidence of malignancy
·Low or absent NK-cell activity (according to local laboratory reference)
·S. Ferritin 500 microgm/L
·S. Soluble CD25 (i.e., soluble IL-2 receptor) >= 2,400 U/ml

*Homozygosity or compound heterozygosity for verified HLH-associated mutations (eg, PRF1, UNC13D, STX11, STXBP2, Rab27A, SH2D1A, BIRC4, LYST, ITK, SLC7A7, XMEN, HPS) or gene defects of other immune regulatory genes (identified by whole exome sequencing [WES]).

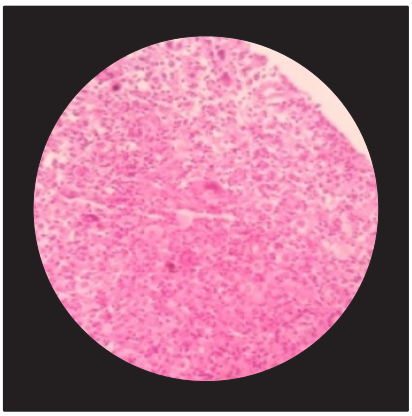

Figure 1: lymph node biopsy showing Reed Sternberg cells

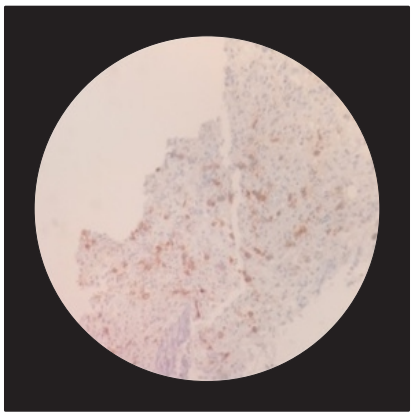

Figure 2: CD 15 positivity on immunohistochemistry

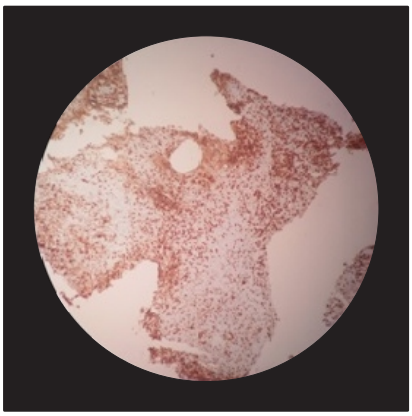

Figure 3: CD 30 positivity on histochemistry

Case Report

A 20 years old female presented to GCRI with 3 months history of persistent fever, generalized weakness, abdominal pain. On clinical examination hepatosplenomegaly with neck, axillary and inguinal nodes were palpable. Complete blood picture showed anaemia and thrombocytopenia with haemoglobin of 6.6 mg/dl, total leukocyte count of 4700 mg/dl and platelet count of 8000 per cu mm. Kidney function tests were normal. Total bilirubin was 2mg/dl, Serum glutamic oxaloacetic transaminase was 140 Units/L and serum glutamic pyruvate transaminase was 35 Units/L.S. Ferritin was 16000ng/ml and Fibrinogen was 139mg/dl. Urine routine microscopy was suggestive of +1 proteinuria. CECT of neck, thorax and abdomen suggested cervical, mediastinal, abdominal lymphadenopathy with splenic infiltration.

After confirming 5 out of 8 criteria for clinical diagnosis of HLH, and lymph node and bone marrow biopsy report being awaited, patient was started on iv dexamethasone, following which patient improved, that anasarca subsided, jaundice decreased

Bone marrow aspirate examination revealed normocellular marrow.Trephine biopsy suggestive of normocellular marrow with grade 3 fibrosis and large Reed Sternberg cells immunoreactive for CD15 and CD30.Sections of lymphnode biopsy shows Reed Sternberg cells immune reactive for CD15, CD30 and PAX5 favouring classical Hodgkin's lymphoma (Figure 1,2,3).

After confirmation of classical Hodgkin lymphoma with Immunohistochemistry on lymphnode biopsy as well as bone marrow biopsy, patient was started with ABVD chemotherapy (Adriamycin 25mg/m^2, Bleomycin 10 IU/m^2, Vinblastin 6mg/m^2, Dacarbazine 375mg/m^2). After completion of 2 cycles of ABVD, there was complete recovery of cytopenia, and resolution of systemic symptoms.

Discussion

HLH is a hyper inflammatory syndrome mediated uncontrolled activation of immune cells(macrophages, lymphocytes and histiocytes) and elevated cytokines such as Tumour necrosis factor alpha,interleukin-6,interferon gamma and macrophage inflammatory protein one alpha. Familial form is autosomal recessive presenting during childhood and diagnosed by identification of mutations in HLH associated genes PRF1, UNC13D, STX11, STXBP2, Rab27A, SH2D1A or BIRC.[4,5] These mutations affect exocytosis of cytotoxic granules in natural killer cells leading to a hyper inflammatory state. Acquired HLH can present at any age. The association between HLH and hematologic malignancies including Hodgkin's lymphoma is well described, with 1% of these patients developing HLH.[6]

In a multicentre retrospective case series of 68 patients with HLH[7], Schramm et.al found most common underlying disorder was malignancy(49%) followed by infection, auto immune and idiopathic HLH. Among malignancies, B lymphoid were most common followed by myeloid, T lymphoid and solid. Among B lymphoid neoplasms Hodgkin's lymphoma (6%) was the most common. Amongst infections EBV (9%) and CMV (9%) have the highest incidence.

A study in Sweden by Machaczka et al[6] studied 8 patients with haematological malignancy HLH. Out of them only 1 patient survived. Two patients treated with immunosuppressive therapy (steroids, IVIG) died shortly after HLH diagnosis. Six patients were treated with modified HLH-94 protocol, out of which 2 did not respond. Four patients who initially responded died within an average of 2.4 months. They concluded that HLH associated with lymphoma had poor outcome.

Consistent with other studies.[8, 9] patients with lymphoma associated HLH had a worse prognosis than those without it (median survival 2.8 months versus 10.7 months).

Among patients who are acutely ill or deteriorating, and no secondary causes identified, HLH specific therapy based on the HLH-2004 protocol or enrollment in a clinical trial is suggested. More than half of patients treated with the HLH-2004 regimen achieve five-year survival.

Therapy based on the HLH-2004 protocol consists of eight weeks of induction therapy with etoposide (VP-16) and dexamethasone with cyclosporine with intrathecal therapy for those with CNS involvement. For the intrathecal therapy, hydrocortisone and intrathecal methotrexate are given. Induction therapy is followed by continuation therapy with same agents.[4]

Conclusion

There should be strong suspicion of HLH in patients presenting with cytopenias, splenomegaly and recurrent fever as systemic symptoms. Apart from infection, malignancy should be strongly suspected as secondary cause in any patient with HLH.

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Synchronous Occurrence of Acute Myeloid Leukemia and Carcinoma of Upper Alveolus : A Rare Coexistence

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Summary

Synchronous occurrence of two malignancies of different histologies is a rare clinical scenario. Synchronous diagnosis of hematological and solid malignancy poses a challenge in treatment planning. Here, we report a case of 37 year old female patient with upper alveolar growth and pancytopenia. Bone marrow examination for pancytopenia suggested acute myeloid leukemia (AML M4). The upper alveolar growth was clinically suspected to be chloroma after marrow diagnosis of AML; however the histopathological diagnosis from the same was squamous cell carcinoma.

Keywords : Synchronous malignancies, acute myeloid leukemia, carcinoma upper alveolus

Introduction

Multiple primary cancers are not unusual phenomena in current oncology practice. These can be commonly metachronous (separated by duration of more than 6 months between diagnosis of two malignancies) or less commonly synchronous (within 6 months of diagnosis of first primary).[1] There are many cancer predisposition syndromes in which sequential occurrence of different types of primary malignancies have been reported.[1] However occurrence of synchronous malignancies of different histological origin is very rare. Immunosuppression is a well recognised cause of second malignancies, especially squamous cell carcinoma (SCC) of skin and other regions in patients with hematological malignancies undergoing chemotherapy. However SCC has been reported late in the course of the disease or many years after completion of treatment. Here in we report, a case of acute myeloid leukemia (AML) and carcinoma of the upper alveolus diagnosed at the same time in a patient as synchronous malignancies which is a rare scenario. The synchronous occurrence of the respective malignancies has not been reported in literature before.

Case Report

A 37-year old female patient presented to us with 2 months history of swelling over buccal mucosa in left upper alveolar region which was gradually increasing in size with proliferative growth over mucosa and intermittent mild bleeding from the same. Patient had consulted dental surgeon at local clinic and was treated with oral antibiotics and anti-inflammatory agents with no response. She was referred to us with complete blood counts suggestive of pancytopenia with haemoglobin (Hb) 10.8g/dl, total leucocyte count (TLC) of 1510/cmm , differential counts of 64% lymphocytes and 32% neutrophils with no abnormal cells on peripheral smear and platelet count (PC) of 84000/cmm. There was no positive family history of cancer. There was no history of blood or blood component transfusion. On physical examination she had mild pallor, normal vital parameters with no lymphadenopathy or hepatosplenomegaly, the systemic examination was normal. On oral examination she had a ulcero-proliferative growth over left upper alveolar buccal mucosa with irregular margins bleeding on pressure application (Figure 1).She was investigated with bone marrow aspiration and trephine bone biopsy on outpatient basis initially which was suspicious for acute leukemia with 15% blasts population. So she was adviced admission and work up for suspected acute leukemia. The investigations showed Hb 9.0g/dl, TLC 3700/cmm, PC 52000/cmm, manual differential counts showed 40% blast cells with normal renal and liver function tests. A repeat bone marrow examination was suggestive of acute myeloid leukemia (AML-M4) with 31% blast population (Figure2).Immunophenotyping of bone marrow aspiration showed positive expression for CD13, CD33, CD117, CD15, CD 11b, CD 11c, along with CD34 and HLA-DR consistent with AML. The conventional cytogenetic examination showed complex karyotype with multiple chromosomal rearrangements suggestive of poor risk group AML. The upper alveolar buccal mucosal lesion was clinically suspected as possible leukemic infiltration (chloroma) in view of AML-M4 being known to be associated with tissue infiltration including gums. However, with high index of suspicion the lesion was biopsied, the histopathology report of which showed 'moderately differentiated squamous cell carcinoma' (Figure3).The CECT scan of Paranasal sinuses and neck showed a locally eroding soft tissue density lesion of size 99x28x16 mm involving mucosa of upper alveolar buccal mucosa. The lesion was extending into left upper buccal space and left upper gingivobuccal sulcus, consistent with malignant neoplastic lesion (Figure4).The final diagnosis was AML M4 with SCC of left upper alveolus. With two synchronous different histological malignancies, the consideration of treatment was discussed and prioritized in terms of urgency, pace of disease and patient's medical condition and performance status. With falling haemoglobin and platelet counts, patient was started on induction treatment for AML with standard 7+3 protocol of daunorubicin (60mg/m^2 for 3 days) and cytarabine

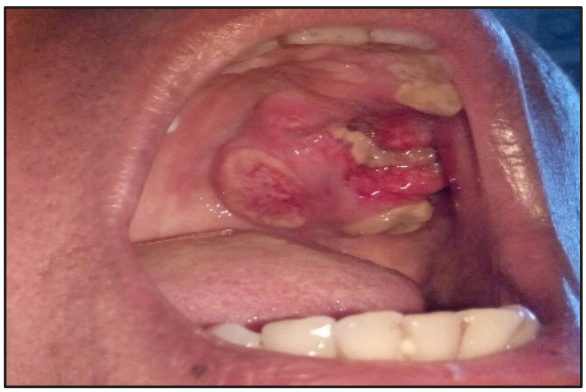

Figure 1 : Upper alveolar growth at presentation

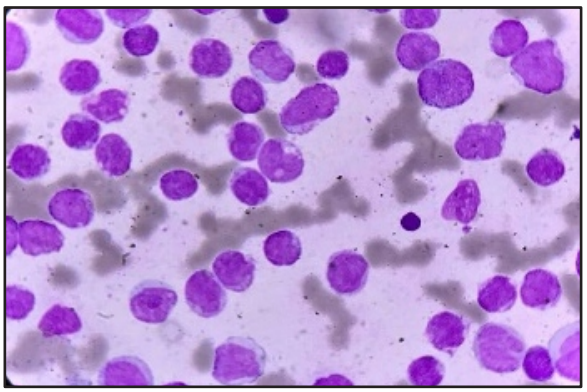

Figure 2: BM aspiration at diagnosis (AML M4)

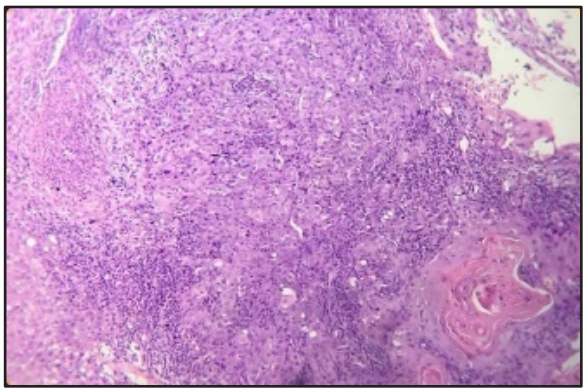

Figure 3: Upper alveolar growth biopsy
(Squamous cell carcinoma showing keratin formation)

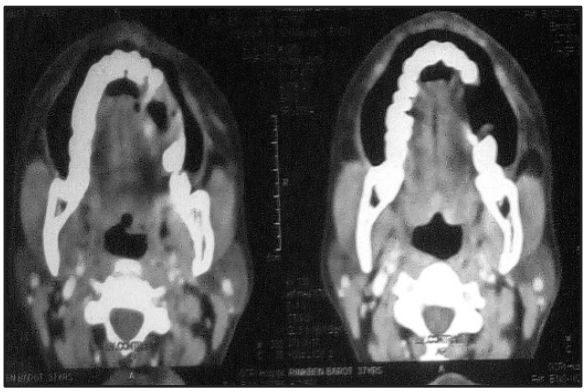

Figure 4: CECT picture of upper alveolar growth at diagnosis

(200mg/m^2 infusion for 7 days). Post chemotherapy course was complicated with neutropenic sepsis and pneumonia managed with broad spectrum IV antibiotics, blood component transfusion support. Post induction bone marrow was under morphological remission but with persistent thrombocytopenia (CRp). Surprisingly the upper alveolar lesion also decreased in size significantly. Patient was asked to follow up weekly with CBC monitoring for starting consolidation therapy, however she could not be given consolidation therapy in face of persistent thrombocytopenia, a repeat bone marrow aspiration done after 2 weeks for worsening thrombocytopenia and deteriorating performance status showed relapsed leukemic activity with 40% blast cells. In view of being a poor risk AML category with complex karyotype further plan of treatment was re-induction chemotherapy followed by high dose chemotherapy and hematopoetic stem cell transplant, however due to financial contraints and non-availability of HLA matched donor she did not remain a candidate for transplant and hence was started on palliative oral metronomic therapy with 6 MP + etoposide + prednisolone and supportive care with transfusions. The upper alveolar lesion was not planned for any local treatment in view of rapidly deteriorating performance status of the patient with very low blood counts, patient was not fit for any interventional procedure.With aggressive nature of disease, the patient expired within 2 months on supportive care.

Discussion

Synchronous diagnoses of AML and carcinoma of upper alveolus have not been reported till date.There have been sporadic case reports of synchronous occurance of AML with various malignancies like Gastrointestinal stromal tumors (GIST)[2], renal cell carcinoma (RCC)[3], gastric carcinoma[4], adenocarcinoma of large bowel[5] have been reported. The Warren and Gates criteria for diagnosis of multiple primary malignancies are as follows.[6]

1. Each of the tumors must be malignant, each confirmed by histology
2. Each must be geographically separate and distinct. The lesions should be separated by normal mucosa
3. Probability of one being the metastasis of the other must be excluded.

Billroth first reported multiple primary tumors of different histology, in different organs, at different time interval in same individual in 1860.[7] Perilongo et al reported a case of a child manifesting five different tumour types simultaneously.[8] These multiple primary cancers are known to occur with greater frequency in certain familial cancer predisposition syndromes like Li-Fraumeni syndrome.[9] However our patient had no familial history of malignancy in first or second degree relatives. Synchronous malignancies are thought to arise in certain populations following exposure to carcinogens, such as tobacco smoke, accounting for as

much as 17% of myeloid leukemias.[13] In our patient there was nohistory of any tobacco use or exposure, neither there was any history of radiation exposure. An early mutation occurring during embryonal development may predispose the individual for double malignancies involving the different tissue types.[10] This supports the stem cell theory of cancer origin that stem cells of cancer maintain the capacity to differentiate, migrate and develop into a new malignant tumour with completely new traits.[1] Some people with second malignancy have a known genetic susceptibility, such as point mutation of the p53 tumour suppressor gene and allele loss of Rb gene, neurofibromatosis and immunodeficiency.[12] The tumour genetic abnormality observed in our patient was a complex karyotype. Further mutational analysis could not be performed due to limited resources. Whole genome analysis of such cases may help in detecting the underlying mutation which may otherwise be missed by conventional cytogenetics. Of interest are rare 'Syndromes of Telomere shortening' like Dyskeratosiscongenita which has been reported to involve skin manifestations along with multi systemic involvement of bone marrow failure diseases and leukemia with squamous cell carcinomas and interstitial lung diseases.[13] However there was no obvious skin lesions or stigmata of previous lesions in this patient. This represents a particularly unusual and difficult oncologic scenario involving two significant hematological and solid tumor malignancies for which prioritizing the chronology and focus of treatment must be considered. The therapeutic dilemma raised by such cases is the simultaneous management of two cancers which may have quite different treatment strategies. Some suggest that treatment should be directed towards the malignancy that is more advanced and aggressive at presentation.[14] However ideal treatment option would be to use combination of treatment modalities likely to be effective against both. We could not find the literature on the management of patients with synchronous squamous cell carcinoma of upper alveolus and AML. In general, outcome in these cases with synchronous malignancies is likely to be poor and new novel treatment options need to evolve. This also illustrates the need for active involvement of multidisciplinary team for effective treatment strategies.

The synchronous presentation of AML and squamous cell carcinoma of upper alveolus is somewhat surprising. Although rare, we conclude that any questionable lesion should be assessed with biopsy in a case of leukemia. Early diagnosis by regular and thorough physical examination provides the best chance of successful outcome.

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Isolated Rib Metastasis as the First Manifestation of Hepatocellular Carcinoma in a Chronic Alcoholic Patient a Rare Case Report and a Literature Review

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Summary

Hepatocellular carcinoma (HCC) is the most common primary tumor of the liver and is the fifth most common cancer in the world. Extrahepatic spread of HCC during presentation is aseen in around 5 to 15% of patients. Skeletal metastasis of HCC less common and is a rare primary form of presentation. We report a case of HCC presenting with isolated right 4th rib metastasis, in a 60-year-old man presented with painless right chest wall swelling, loss of weight and decreased appetite. Histological Examination from chest wall swelling showed metastatic carcinoma, which on positive for IHC markers ; Heppar, AE1,CD138 and negative for vimentin.The histopathology was suggestive of metastatic HCC or carcinoma with hepatoid differentiation.Tc99m MDP whole body bone scan revealed increase radiotracer concentration in anterior aspect of right 4th rib. CECT thorax-abdomen showed lytic lesion involving right 4th rib with diffuse alteration in liver parenchyma. Portal venous doppler reveals hepatomegaly with multiple hypoechoic lesion and regenerating nodule of cirrhotic liver with portal vein thrombus. HCC with isolated rib metastasis as a primary presentation is rare. Present case and review of literature reinforces the view that HCC should be considered in differential diagnosis in patients presenting with bone metastasis.

Keywords: Hepatocelluar carcinoma, HCC, Ribs, Skeletal metastasis

Introduction

Primary cancer of the liver represents the fifth most common malignancy worldwide and is the second most common cause of death from cancer.[1-3] Three leading sites of metastasis in advance hepatocellular carcinoma are: lung (44%), portal vein (35%), and portal lymph node (27%).[3-6] Bone is an uncommon site of metastasis in hepatocellular carcinoma and is a site that is overlooked during investigation of patient.The incidence of bone metastasis is 3-20% in HCC with spine and pelvic bone being most common, and the prognosis is usually good in presence of isolated bone metastasis.[6-7] We present a case of metastatic HCC who presented with a painless right 4th rib swelling.

Case Report

A 60-year-old man whose was smoker and alcoholic (20 unit/day alcohol for 30 years and 75 pack year smoking) presented to our centre with right chest wall swelling with decreased weight and appetite. On clinical examination patient had swelling over anterior aspect of 4 th rib. The laboratory studies showed: Hb - 10.90 g/dL; WBC - 8400/cmm; serum creatinine - 0.79 mg/dL; AST - 393.20 u/L; ALT - 50 U/l; ALP - 194.20 IU/l; total protein - 7.09 g/dL; albumin - 2.93 g/dl; total bilirubin - 5.60 mg/dl (DBIL - 4.40, IBIL - 1.20); SAFP - >1000 IU/ml; SPSA - 1.3 ng/ml.

CT thorax and abdomen showed lytic lesion with soft tissue swelling involving right 4th rib with intrapleural extension (Figure 1, 2). USG Guided biopsy was performed from 4 th rib and histopathology showed features of metastatic carcinomas. IHC markers were suggestive of Metastatic HCC with hepatoid differentiation and were positive for hepatic marker Heppar, AE1, CD138, negative for vimentin. Whole body Tc99m MDP scintigraphy showed increased radiotracer concentration in anterior aspect of right 4th rib. (Figure 3) Patient underwent UGI endoscopy showed small oesophageal varices with mild PHG.

CT Abdomen showed liver parenchyma with diffused alteration with few nodular lesions in right upper lobe with mild perihepatic fluid collection suggestive of liver parenchymal disease. USG abdomen and pelvis showed liver is enlarged in size measured 17.9 cm and shows grossly altered echotexture, surface nodularity and irregularity with hypertrophied right lobe and atrophied left lobe. Liver had multiple hypoechoic areas with regenerative nodule;metastasis, with no dilation of IHBR. Portal vein measured 14 mm at porta on Doppler with echogenic material extending into right and left branches suggestive of thrombus. Visualised portal vein at confluence showed hepatopedal flow with reduced biphasic variation and velocity.

As per the investigation patients diagnois of Child Pugh 'C' disease and stage IV disease was reached. As per multidisplinary tumor discussion the patient was treated with best supportive care due to metastatic lesion. Patient was treated with Tablet Tamoxifen 40mg and Injection Zoledronic acid 4mg/100ml. Due to progressive disease patient died of disease after one month of treatment.

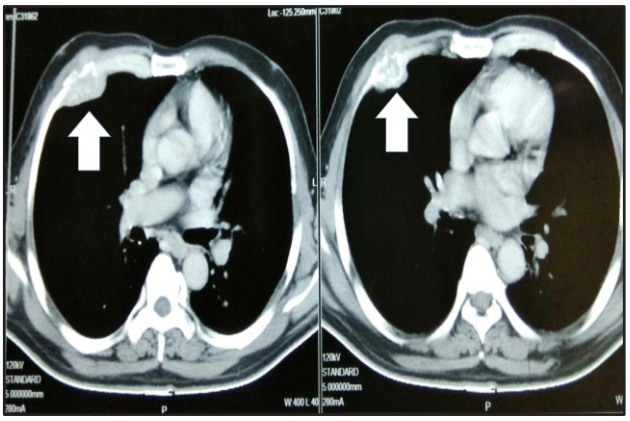

Figure 1: 4.3*3.55 cm osteolytic lesion in anterior aspect of fourth rib with surrounding soft tissue swelling with intrapleural extension; malignant bony lesion with surrounding soft tissue component.(White arrow over fourth rib lesion)

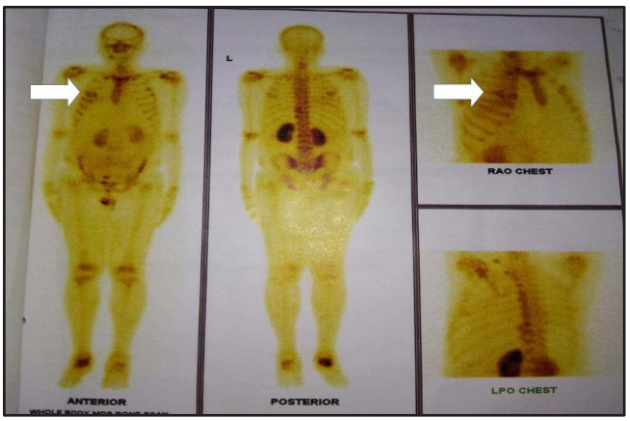

Figure 3: Whole body Tc99m MDP scintigraphy showed increased radiotracer concentration in right 4th rib anteriorly (White arrow over 4th rib)

Discussion

Hepatocellular carcinoma (HCC) is the fifth most common cancers worldwide. HCC metastasizes to the lung, portal vein, portal lymph nodes,rarely to bone and soft tissues.[1-7] Metastatic spread to bone is seen in 3-20% of HCC patients and the most common site of skeletal involvement in descending order are the vertebrae, pelvis, rib, skull, humerus and sternum.[3-7] As per review of literature about the initial clinical presentation of unsuspected HCC associated with bone metastasis is uncommon. The review of literature showed isolated reports of vertebrae involvment. Most reported cases of sacral/ lumbosacral metastasis were accompanied by multiple metastatic elsewhere in the body or previously known HCC. Isolated Rib metastasis from HCC as a primary presentation is a rare condition. In current study we have reported a patient who developed isolated rib metastasis from a hepatocellular carcinoma as a primary presentation.The treatment of extrahepatic metastasis is mainly depends on the clinical stage, Child Pugh

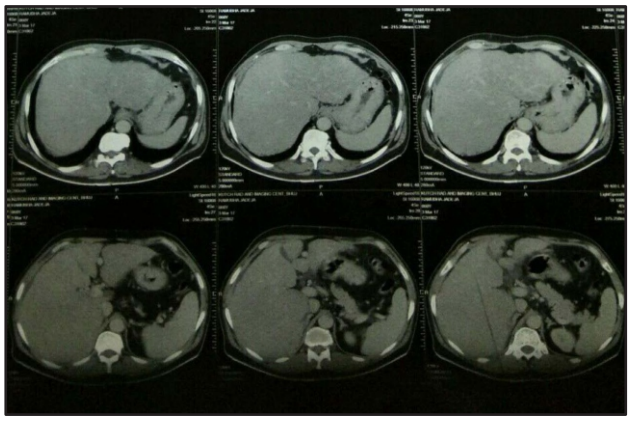

Figure 2: Moderate hepatomegaly with diffuse alteration of liver parenchymal density with few nodular lesion in right lobe

score and metastasis loci in order to prolong the survival of patients. Sugihara et al reported that for HCC patients with bone metastasis,combined treatment with radiation, zoledronate, and surgery, may possibly improve their quality of life resulting in a long clinical course. Metastatic HCC can be treated with surgery, followed by radio therapy, target therapy or other conservative treatments.The hypothesis for metastasis to the bone occurs via portal vein to vertebral vein plexuses (owing to either portal thrombus and/or portal hypertension which allows bypass through plexus), explaining the more frequent craniospinal and pelvic bone metastasis. HCC should be considered in the differential diagnosis in patient presenting with bone metastasis. Attili et al reported two cases of HCC with bone metastasis, first patient was 56 year old female with HCC having child Pugh 'A' disease with rib, clavicle, humerus, right femur and left tibia metastasis.[7] This patient was treated with chemotherapy along with zoledronic acid.[7] And second patient was 64 year old male HCC with child Pugh'C' disease with vertebral and iliac bone metastasis did not received any therapy and died of progressive disease.[7]

Conclusion

HCC with isolated rib metastasis as a primary presentation is rare. Present case and review of literature reinforces the view that HCC should be considered in differential diagnosis in patients presenting with bone metastasis.

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Presentations at the Clinical Meetings

(July 2018 to December 2018)

Sr. No.	Date	Speaker/Department	Title
1	12.07.2018	Goswami Parijat Microbiology	Real-Time Automated PCR for early Diagnosis and Monitoring of Cytomegalovirus Infection after Bone Marrow Transplantation
		Dadhania Jaykumar Medical Oncology	Precision Oncology: Who,How,What,When and When Not?
2	08.08.2018	Shivachhand Akshay Medical Oncology	Condensed Versus Standard Schedule of High-Dose Cytarabine Consolidation Therapy with Pegfilgrastim Growth Factor Support in Acute Myeloid Leukemia.
		Patel Dhara Blood Bank/Pathology	Blood Group Phenotype Frequencies in Blood Donors from a Tertiary Care Hospital in North India
3	05.09.2018	Shah Franky Stem Cell Biology Lab	Screening of over 1000 Indian Patient with Breast and/or Ovarian Cancer with Multi Gain Panel: Prevelence of BRACA 1/2 and Non-BRACA Mutations
		Arasu Nirmal Radiotherapy	Proton Beams in Cancer Treatments: Clinical Outcomes and Dosemitric Comparisions with Photon Therapy
4	10.10.2018	Trivedi Trupti Clinical Carcinogenesis Lab	The Role of IDH Mutations in Acute Myeloid Leukemia
		Guled Suvarna Gynec oncology	Letrozole may be a Valuable Maintenance Treatment in High-Grade Serous Ovarin Cancer Patients
5	05.11.2018	Kalita Nuri Library and Information Services	Is Copyright Infringement a form of Plagiarism?
		Singh Ashok Surgical Oncology	Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy for Colorectal Cancer
6	06.12.2018	Patel Suchita Pathology	Sentinel Lymph Nodes for Breast Carcinoma: An Update Current Practice
		Sadhwani Manish Surgical Oncology	Sentinel Lymph Nodes Dissection with & without Axillary Dissection in Women with Invasive Breast Cancer and Sentinel Node Metastasis

Panel Discussion at the Clinical Meetings

(July 2018 to December 2018)

Sr. No.	Date	Moderator/ Department	Panelist/ Department	Title
1	23.08.2018	Patel Prabhudas Cancer Biology	Trivedi Priti Onco-Pathology	Liquid Biopsy in Oncology: Hopes and Hypes
			Parikh Sonia Medical Oncology	
			Shah Franky Stem Cell Biology Lab	
2	19.09.2018	Sanghvi Priti Palliative Medicine	Joshi Geeta Ex-Professor, Palliative Medicine	Safe use of Essential Narcotic Drugs in Chronic Pain
			Bhatia M. T. Anesthesiology	
			Shah Bhavna Anesthesiology	
3	22.11.2018	Trivedi Priti Onco-Pathology	Gami Amisha Onco-Pathology	Recent AJCC Guidelines for oral Cancer Staging
			Tripathi Umank Surgical Oncology, (Head & Neck)	
4	20.12.2018	Panchal Harsha Medical Oncology	Sharma Mohit Surgical Oncology, (Breast & Thorax)	Current Trends in Breast Cancers
			Yadav Rajan RadioTherapy	
			Vora Hemangini Cancer Biology	

Case Presentations for Morbidity, Mortality at Clinical Meetings

(July 2018 to December 2018)

Sr. No.	Date	Presenter/Department	Case Discussion
1	28.07.2018	Dr Twinkal Patel Anesthesiology	Morbidity and Mortality Data presentation of Surgical and Medical Departments
2	25.08.2018	Dr Dushyant Vaidya Anesthesiology	Morbidity and Mortality Data presentation of Surgical and Medical Departments
3	22.09.2018	Dr Jinesha Chauhan Anesthesiology	Morbidity and Mortality Data presentation of Surgical and Medical Departments
4	27.10.2018	Dr Jinesha Chauhan Anesthesiology	Morbidity and Mortality Data presentation of Surgical and Medical Departments
5	24.11.2018	Dr Dushyant Vaidya Anesthesiology	Morbidity and Mortality Data presentation of Surgical and Medical Departments
6	22.12.2018	Dr Dushyant Vaidya Anesthesiology	Morbidity and Mortality Data presentation of Surgical and Medical Departments

About the Journal and Instructions to Author

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The Editorial Procss

A manuscript will be reviewed for possible publication with the understanding that it is being submitted to Gujarat Cancer Society Research Journal at that point in time and has not been published anywhere, simultaneously submitted, or already accepted for publication elsewhere. The journal expects that authors would authorize one of them to correspond with the journal for all matters related to the manuscript. On submission, editors review all submitted manuscripts initially for suitability for formal review. Manuscripts with insufficient originality, serious scientific or technical flaws, or lack of a significant message are rejected before proceeding for formal peer-review. Manuscripts that are unlikely to be of interest to the Gujarat Cancer Society Research Journal readers are also liable to be rejected at this stage itself.

Manuscripts that are found suitable for publication in Gujarat Cancer Society Research Journal are sent to expert reviewer/s. The journal follows a double-blind review process, therein the reviewer/s and authors are unaware of each other's identity. Every manuscript is also assigned to a member of the editorial team, who based on the comments from the reviewer/s takes a final decision on the manuscript. The comments and suggestions (acceptance/ rejection/ amendments in manuscript) received from reviewer/s are conveyed to the corresponding author. If required, the author is requested to provide a point by point response to reviewers' comments in a separate sheet and submit a revised version of the manuscript with the changes underlined in red. This process is repeated till reviewers and editors are satisfied with the manuscript.

Manuscripts accepted for publication are copy edited for grammar, punctuation, print style, and format. Page proofs are sent to the corresponding author. The corresponding author is expected to return the corrected proofs within two days. It may not be possible to incorporate corrections received after that period.

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The following documents are required for each submission: (Font: Times New Roman)

- Title Page (Font size: 12)
- Title of manuscript (Font size: 16)
- Summary and Keywords (Font size: 9)
- Text (Introduction, Aims and Objectives, Materials and Methods, Results and Analysis, Discussion with Conclusions; Font size: 12).
- Tables (separate page, Number Arabic numerals (e.g. 1,2,3) as it comes in results) (Font size: 12)
- Figures and Illustration (separate page, JPEG format, Number Arabic numerals (e.g. 1, 2,3) as in results, if photographs of persons are used, the subjects or patients must not be identifiable).
- Legends to Figures and Illustration: Present the legends for illustrations separate page using double-spacing, with Arabic numerals corresponding to the Illustrations. (Font size: 12)
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Avoid abbreviations in the title and abstract. All unusual abbreviations should be fully explained at their first occurrence in the text. All measurements should be expressed in SI units. Drug names Generic drug names should be used.

Abbreviations of units should conform to those shown below:

Decilitre	dl	Kilogram	kg
Milligram	mg	Hours	h
Micrometer	mm	Minutes	min
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Percent	%		

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The title page should include

1. Type of manuscript (article/case report)
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3. The name by which each contributor is known (Last name, First name and initials of middle name), with institutional affiliation;
4. The name of the department(s) and institution(s) to which the work should be attributed;
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8. 3-8 keywords

Language and grammar

- Uniformly American English
- Abbreviations spelt out in full for the first time

- Numerals from 1 to 10 spelt out
- Numerals at the beginning of the sentence spelt out

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Text: This should consist of **Introduction (including Aims and Objectives), Materials and Methods, Results, Discussion with Conclusions. Cite every Reference, Figures and Tables mentioned in the text in Arabic numerals (e.g. 1,2,3).**

Introduction/Aims and Objective: State the purpose of the article. Summarize the rationale for the study or observation. Give only strictly pertinent information and references, and do not review the subject extensively. Do not include data or conclusions from the work being reported.

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Identify precisely all drugs and chemicals used, including their generic names, their manufacturer's name, city and country in parenthesis, doses, and routes of administration.

Results: Present your results in a logical sequence in the text, Tables, and Illustrations. Do not repeat in the text all the data in the Tables or Illustrations. Emphasize or summaries only important observations. Specify the statistical methods used to analyze the data. Restrict Tables and Illustrations to those needed to explain the argument of the paper and to assess its support. Where possible, use Graphs as an alternative to Tables with many entries. Do not duplicate data in Graphs and Tables.

Discussion: Emphasize the new and important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material given in the Introduction or the Results section. Include in the Discussion section the implications of the findings and their limitations, including the implications for future research. Relate the observations to other relevant studies.

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Present the legends for illustrations using double-spacing, with Arabic numerals corresponding to the Illustrations.

Acknowledgements: State contributions that need to be acknowledged.

References
A list of all the references cited in the text should be given at the end of the manuscript and should be numbered consecutively in the order in which they are first mentioned in the text. Identify references in the text by Arabic numerals in superscript. Omit month and issue number. List all authors, but if the number is six or more, list first three followed by et al. The references should be cited according to the Vancouver agreement. Authors must check and ensure the accuracy of all references cited. Abbreviations of titles of medical periodicals should conform to the latest edition of Index Medicus. Some examples are shown below:

Standard Journal
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Online journal article
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Chapter in a book
Weinstein L, Swartz MN. Pathogenic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: Saunders, 1974: 457-472

Online book or website
Garrow A, Weinhouse GL. Anoxic brain injury: assessment and prognosis. In: Up To Date Cardiovascular Medicine [online] Available at: www.UpToDateInc.com/card.
Accessed February 22, 2000.

In press
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Stem Cell Transplantation (BMT) Services at GCRI

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Introduction

The Bone Marrow transplant (BMT) services is aptly called stem cell transplant services as there are various sources of hematopoietic stem cells- peripheral blood, bone marrow and umbilical cord. This specialized unit is an integral part of the Department of Medical and Pediatric Oncology. We perform both autologous and allogenic transplants in both adult and pediatric patients. All forms of allogenic transplants, such as matched related, matched unrelated, haplo-identical (half-matched) and cord blood transplants have been performed. India's first successful umbilical cord transplant was performed in 2007 at our centre. We cater to potential transplant candidates from many states such as Gujarat, Rajasthan, Uttar Pradesh, Bihar, Maharashtra and Madhya Pradesh. The success rate and mortality are comparable to any good transplant centre in the country.

Foundation/ Formation

The BMT unit was established in August 1999 under the guidance of Dr. Pankaj Shah, Director and HOD of Medical Department at that time, Dr. Kirti M. Patel and Dr. Shilin N. Shukla. It is a 4-bedded unit which is equipped with HEPA (High-efficiency particulate air) filters. There are two recovery rooms for stepping down of patients. Till date we have performed nearly 400 stem cell transplants. Haplo-identical stem cell transplants were started in the year 2015 for both benign and malignant conditions.

Our Team

We have a dedicated team of transplant physicians and nurses who are well-trained in the field of stem cell transplantation. Our unit Incharge is Dr. Sandip A Shah and in addition, we have Dr. Akanksha Garg. Our BMT officers are Dr. Kamlesh Shah and Dr. Kinnari A Patel who have been associated with the unit for more than a decade. BMT services is associated with the Medical & Paediatric Oncology Department and has full support from the faculty: Dr. Asha Anand, Dr. Harsha Panchal, Dr. Apurva Patel, Dr. Sonia Parikh, Dr. Nahush Tahiliani, Dr. Nitin Joshi and Dr. Chinmay Doctor. We have constant support of our DM Medical Oncology residents who are posted in the BMT unit. We have 14 specialized BMT nurses and committed class 4 workers who take immense care of all our transplant patients within the unit.

Clinical Services

1) Stem Cell Transplant Services

- Allogenic stem cell transplants are performed for leukemias (Acute lymphoblastic and acute myeloid leukemia), bone marrow failure syndromes (Aplastic anemia, Fanconi anemia), immunodeficiency disorders (Leucocyte adhesion defect, thalassemia, and myelodysplastic syndrome.
- Autologous stem cell transplants have been performed for multiple myeloma, lymphoma (both non Hodgkin and Hodgkin), acute promyelocytic leukemia and neuroblastoma.

Type of Transplant	Number
Autologous	195
Matched Related	156
Haplo-Identical	17
Cord Blood	17
Matched Unrelated	7
Total	392

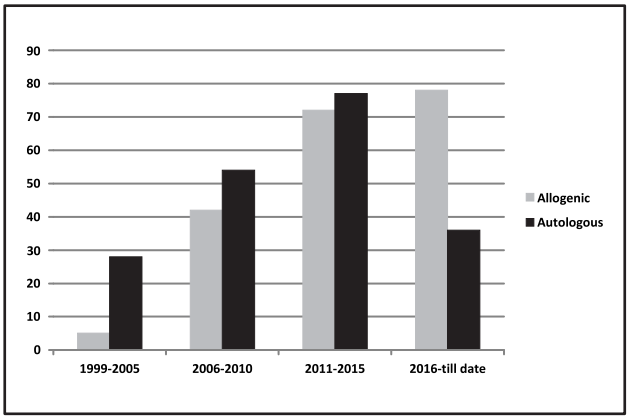

Figure 1: Number of stem cell transplants done over the years

- Matched related transplants- 6/6 HLA –matched donors are selected.
- Matched unrelated transplants- fully matched unrelated voluntary donors from transplant registries are selected.
- Haplo-identical transplants (half-matched) - have been performed for both benign and malignant disorders.
- Cord blood transplants have been performed for thalassemia and leukemia.

2) **Transplant Follow up OPD Services:** for post transplant patients runs from Monday to Saturday in the BMT unit.

3) **Hematology OPD:** Runs under the BMT team on Wednesday and Saturday where new and follow up cases are seen. We receive nearly 40-50 patients/OPD day of benign hematology, including referrals from civil hospital for expert opinion. In addition we also see few cases of hematological malignancies. Patients requiring admission are managed as well.

Success of our transplant program is not possible without the constant support of other departments.

- Stem cell harvesting and cryopreservation is being done at the Department of Transfusion Medicine under the guidance of Dr. Rima Kusumgar and Dr. Dhara Patel. They also help us with timely transfusion support services for the patients including single donor platelet apheresis.
- CD34 counting is done by Dr. Hemangini Vora, Head of the Immunohistochemistry and Flow Cytometry Lab.
- HLA typing which is essential for any transplant is being done in-house at subsidized cost by Dr. Nandita Ghosh, Head of Tumor Biology Lab. They perform 6-antigen HLA (low resolution) typing.
- Surveillance cultures of the BMT rooms and viral screening such as CMV-RT PCR are regularly performed by the Microbiology department under Dr. Parijat Goswami.
- Central venous access is required for our patients. Peripherally inserted central catheters, hickmann catheters and dialysis catheters (for stem cell harvesting) are placed by the help of Department of anaesthesia.
- Consultations with other departments such as pediatric surgery, pathology, radiology, dental OPD etc, help us manage the patients better.

Research Activities

- Many research articles have been published in esteemed Pubmed indexed journals. Data presentation at various imminent transplant conferences is done regularly which helps gain exposure and recognition.

Training Programs and Academic Activities

- DM Medical Oncology residents are posted in BMT unit on rotation where they gain transplant experience. Classes for DM students are held on important topics of stem cell transplantation every 15 days.
- The nursing staff is also actively involved in attending BMT programs for nurses held annually at centers like TMH, Mumbai, CMC Vellore, etc.
- Faculty is active in attending national and international conferences to improve clinical protocols and practices.

Future Prospects and Scope

- The new BMT unit being constructed will be a 6-bedded HEPA filter equipped unit with 5 additional recovery (step down) rooms.
- Good scope for post MD/DNB medicine /pediatricians/post DM Medical oncology/Pediatric oncology/Hematology doctors wanting to pursue training in BMT as a wide variety of transplants is being done.
- Plan to organize a national conference in hematology and BMT training program for nurses.

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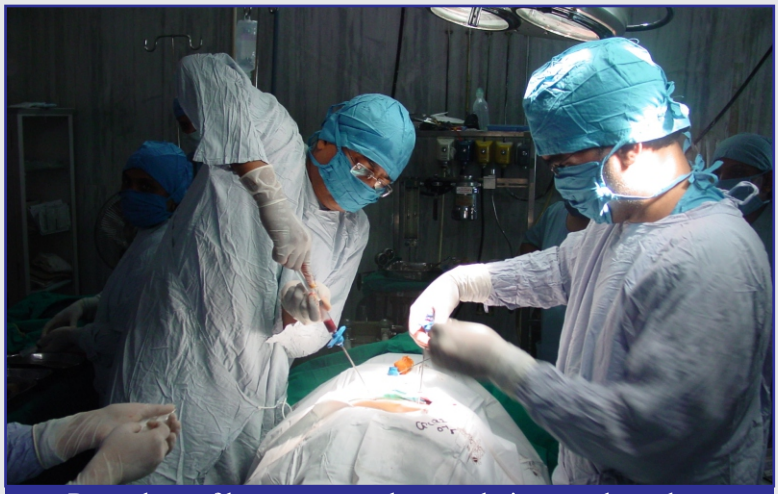
Bone Marrow Transplant Department Services at GCRI



Procedure of bone marrow harvest being conducted on healthy donor

Post transplant recipient patients in regular follow up OPD services

Haematopoeitic stem cell transplant patient admitted in the unit