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Editorial

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Anaesthesiologist- A Perioperative Physician? A Word of Caution …

Anaesthesiology has undergone revolutionary changes over the past century. Discovery of newer drugs, monitoring equipments and evidence based assessment of the patients have resulted in improved outcomes. Advances in surgical techniques too owe much to advances in Anaesthesiology like induced hypotension and good muscle relaxation. Apart from operation theatre duties, anaesthetists have ventured out long back and are involved in multiple outside operation room duties like PACU (Post Anaesthesia care unit), critical care and trauma unit, endoscopies, anaesthesia for radiological procedures, radiotherapy, obstetrics anaesthesia, ambulatory and day stay anaesthesia, pain management and clinical research.[1]

Due to the technical efficacy of anaesthesiologist in clinically invasive work like intubation and intravenous access and the basic knowledge of physiology, pharmacology and medicine; they are increasingly being recognised as "perioperative physician ".[2]

The concept of perioperative physician is newer; launched in 1990's. In western world a new group of physicians called "hospitalists" has emerged and established a role as perioperative physician.[3] The term "hospitalists" was coined by Wachter R M and Goldman L in 1996.[4]

What then is a perioperative physician? Preoperative medicine? Perioperative care implies assessing and optimizing the condition of the patient, choosing appropriate anaesthetic technique and intraoperative monitoring and supervising postoperative care from recovery unit until discharge.[1]

Clearly this broader horizon for patient care challenges the traditional role of anesthetic practice and creates a number of clinical, academic, financial and legal dilemmas which the speciality as a whole has to face in coming times and be prepared to workout logistic solutions for the same.

A physician is a professional who practices medicine, who is concerned with promoting, maintaining or restoring human health through the study, diagnosis and treatment of disease, injury and other physical and mental impairments. Thus meaning of physician conveys a sense of expertise in treatment by drugs or medication, rather than by the procedure of surgery.[5]

Modern day medicine too has undergone tremendous changes with discovery of newer antibiotics for infection control, newer cardiac drugs, antipsychotics, cancer chemotherapeutic agents etc to name a few, leading to vast spectrum of drug interactions and a wider effect on multiple systems of human body.

Taking this into consideration, a word of caution seems appropriate. Should we as anaesthesiologist take up the integrated responsibility of total patient care? This thought triggers a number of questions in our mind, which are left unanswered. Is our curriculum effective to make us competent enough to deal with various aspects of medicine? Are we not compromising patient safety and quality of anaesthesia under this increased clinical burden of perioperative care?

Anaesthesiology was the first medical speciality to champion patient safety as a specific focus. Changes in practice have decreased mortality and catastrophic morbidity caused by anaesthesia administration. Thus APSF (Anaesthesia Patient Safety Foundation) was launched in 1985 with the vision "that no patient shall be harmed by anaesthesia". It seems as a model for the pioneering collaboration and commitment of the entire constellation of anaesthesia related profession to the common goal of patient safety. The success of the anaesthesia patient safety movement was recognised significantly when an article (June 21, 2005) in the Wall Street Journal highlighted dramatic decrease in professional liability insurance premium paid by anaesthesiologists.[6] Thus this "culture of safety" has developed in anaesthesia and should be proposed for adoption of more system based approach.

There are still a number of aspects for which medicine man's help is sought by anaesthesiologist preoperatively and post-operatively. Preoperatively for cardiovascular assessment (for eg angioplasty? stress test?), respiratory assessment and cognitive and CNS assessment. Recognition of anesthetic team is no doubt established for periopertive pain

management but there is lack of fluid requirement understanding in postoperative period.[7] Thus, a change in curriculum needs to be done if we have to act as perioperative physician. Still better would be an idea of introducing "perioperative fellowship" for anaesthesiologist who would want to work as perioperative physician or hospitalist, just as there are fellowship programmes for critical care.[7,8] This programme should be recognised by official bodies (Medical Council of India, Diplomate of National Board or Central Government).

It will be necessary to define the care, knowledge, skills and experience expected of perioperative physician. Integrated cross speciality training programmes will be required to deliver this training and define appropriate qualification.[9]

"Anaesthesia is speciality that facilitates care but seldom cures", in contrast perioperative medicine deals with healing and cure, as surgeons increasingly focus on new and more specialized technical procedures, other specialists have to take more responsibility for the wider care of patient population with complex medical needs. Modern times with Consumer Protection Act, greater awareness and knowledge of patient and insurance claim should caution anaesthesiologist and encourage us to develop" collaborative culture"[9] so that anaesthesiologists can focus on their own standardisation, technology, pharmacy and clinical research. The anaesthesiologists should apply "innovative thinking".

As Albert Einstein said "The significant problems we face cannot be solved at the same level of thinking we were at, when we created them".[10]

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"Flowers always make people better, happier, and more helpful; they are sunshine, food and medicine for the soul."

Luther Burbank

Isochromosome der(17)(q10)t(15;17) with an Additional Copy of *RARa-PML* Fusion Gene in Two Young APML Patients

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Summary

Acute promyelocytic leukemia (APML) is associated with the t(15;17)(q22;q21) translocation. The two chimeric genes, *PML-RARa* and *RARa-PML* are reported to play a role in leukemogenesis. Isochromosome of the long arm of the derivative chromosome 17, originating from the translocation t(15;17) [ider(17)(q10)t(15;17) or ider(17q)] in APML, is a rare chromosomal aberration which has been associated with a poor prognosis. In the present study, we report two APML patients with ider(17q) with poor prognosis. Conventional cytogenetics and FISH (Fluorescence in situ hybridization) analysis with different FISH probes helped to confirm ider(17q). Studies on *RARa-PML* dosage, effect and the influence of ider(17)(q10)t(15;17) on clinical features such as prognosis, survival, and treatment response of APML cases have been documented earlier. The present study showed poor prognosis with ider(17)(q10) t(15;17) which is in accordance with the literature.

Keywords: APML, Chromosome, i(17)(q), ider(17)(q)

Introduction

Acute promyelocytic leukemia (APML) according to the World Health Organization (WHO) classification, and the M3 subtype according to the French American British (FAB) classification, is a well-defined subtype of acute myeloid leukemia (AML). It is a distinct molecularly defined subtype of AML characterized by the specific t(15; 17)(q22;q21) in 95% of cases. As a result of the t(15;17), a retinoic acid (RA) receptor (*RARa*) gene on 17q21 fuses with a transcription factor (promyelocytic leukemia, or *PML*) gene on 15q22 giving rise to the formation of two functional fusion genes, *PML-RARa* on the derivative chromosome 15 and *RARa-PML* on the derivative chromosome 17. Current treatment strategies with All Trans Retinoic Acid (ATRA) in combination with Anthracycline-based chemo-therapy, has transformed APML into the most curable type of AML. Although, approximately 70–80% of the patients with newly diagnosed APML carrying *PML-RARa* achieve long-term remission and are probably cured, some patients still show a poor outcome.[1,2] Lou Y et al (2013), have reported the incidence of additional chromosomal abnormalities (ACAs) was 27% (46/172) in APML cases with t(15;17). Trisomy 8 was the most recurrent abnormality, accounting for 30% (14/46) of patients with ACAs, followed by +21 (7%, 3/46) and -7/7q (7%, 3/46)[3]. In another study, out of 271 of patients, nine cases (14.1%) were found to have additional balanced translocation aberrations; most of them are new and non-recurrent. A large study by Amare et al (2011) documented 14% incidence of deletion/ complex variants of *PML-RARa*.[4]

Chromosomal rearrangements in addition to t(15;17) have been reported in 25-40% of APML patients, with a large predominance of trisomy 8. Other abnormalities are far less frequent, and they usually involve chromosomes 17, 9, and -7 particularly as ider(17)(q10), del(9q), and del(7q) abnormalities. The significance of additional chromosomal abnormalities is uncertain. Even though the number of published cases is small, ider(17)(q10) in APML patients might be related to a poor prognosis.[5] According to Cervera et al (2010) about 1% of the reported secondary cytogenetic abnormalities in APML patients are ider(17)(q10) t(15;17)(q22;q12), an infrequent type of additional recurrent chromosomal abnormality.[6]

In the present study, we report two new young APML patients with ider(17)(q10) in addition to t(15;17). ider(17q) was characterized and confirmed with the help of different FISH probes. Results revealed that an extra *RARa-PML* fusion gene and ider(17q) were associated with poor prognosis in both the patients.

Materials and Methods

The study was approved by the Institutional Review Board.

Case 1

A 20 year old female with complaints of red and black patches on all over body, fever, vomiting and headache since one week was registered at The Gujarat Cancer and Research Institute, Ahmedabad, India. The laboratory studies were as follows: hemoglobin 5.3 g/dL, white blood cell count 4500/cmm, platelet count 13000/cmm and blast 74%. Bone marrow examination revealed hyper cellular marrow with presence of large cells with high nuclear to chromatin (N:C) ratio, prominent nucleoli and moderate amount of finely granular cytoplasm. Some

of the cells showed grooved or reniform nuclei and findings were suggestive of APML. The patient expired within one week before administration of any anticancer treatment.

Case 2

A 17 year old male, with complaints of bleeding gums and weakness since 10 days registered at The Gujarat Cancer and Research Institute Ahmedabad, India. The laboratory test results were as follows: hemoglobin 10 g/dL, white blood cell count 1300/cmm, platelet count 55000/cmm and blast 45%. Bone marrow examination revealed hyper cellular marrow. Normal marrow components were almost replaced by large atypical cells (>90%). Cells with moderate N:C ratio, coarse chromatin, and hyper granular cytoplasm. Few cells showed bilobed nuclei and Auer rods. Occasional blasts showed multiple Auer rods. M:E (Myeloid to Erythroid) ratio was increased. Megakaryocytes were markedly decreased and findings were suggestive of APML. Patient was treated with ATRA and Daunorubicin for 3 months. Patient achieved complete haematological remission. However, patient expired after 2 months.

Conventional Cytogenetic Study: Short-term culture of bone marrow cells, harvesting and Giemsa banding were performed using Giemsa and Trypsin according to standard procedures following karyotyping according to International Standards for Chromosomal Nomenclatures 2009 guidelines.[7,8]

FISH Probes and Detection Systems: FISH for Locus Specific Identifier (LSI) probes for *PML-RARa* dual color dual fusion (DCDF) and dual color single fusion (DCSF) gene rearrangement, (Abbott Molecular-Vysis, Des Plaines, IL) were performed according to manufacturer's protocol. FISH probe, DCDF helped to study the formation of ider(17q). Using a dual-color, dual-fusion *PML-RARa* translocation DNA probe which hybridizes both to *PML-RARa* and *RARa-PML* fusion genes, the typical fusion pattern as well as the variant fusion pattern in both interphase and metaphase cells were observed. The variant fusion pattern with one fusion genes for *PML-RARa* and two for *RARa-PML* corresponded to clone with ider(17)(q10).

Results

Conventional Cytogenetics and FISH

Case 1

Conventional cytogenetic analysis from bone marrow sample showed 46,XX,der(15)t(15;17)(q22;q21),ider(17)(q10)t(15;17)(q22;q21)[15] in all metaphases. The analysis with *PML-RARa* DCSF probe showed 1R2G1F signal pattern. Whereas, FISH with *PML-RARa* DCDF probe showed 1R1G3F signal pattern. Two fusion signals were present on both the arms of der(17) indicating ider(17)(q10) with duplication of *RARa-PML* fusion and third fusion signal was present on der(15).

Case 2

Conventional cytogenetic analysis from bone marrow showed 46,XY, der(15)t(15;17)(q22;q21),ider(17)(q10)t(15;17)(q22;q21)[15] in all metaphases. Results with *PML-RARa* DCSF probe showed 1R2G1F signal pattern whereas, FISH with *PML-RARa* DCDF probe 1R1G3F signal pattern. Two yellow fusion signals were present on both the arms of der(17) indicating ider(17)(q10) with duplication of *RARa-PML* fusion and third yellow signal was present on der(15). Conventional karyotype result and FISH results are mentioned in Table 1. Representative partial karyotype and FISH results described in Figure 1.

Discussion

APML is a distinct subtype of AML and constitutes about 5-8% of all cases of AML. APML can be diagnosed when there is a t(15;17) or a *PML-RARa* rearrangement, even if peripheral blood or bone marrow studies show less than 20% promyelocytes.[9] As recently reported by Manola et al (2011) and Kim et al (2010), the ider(17)(q10)t(15;17), an isochromosomal abnormality occurs on the long arm of ider(17)(q10) t(15;17) after reciprocal translocation of t(15;17).[2,10] It is a relatively rare type of an additional recurrent cytogenetic ab-normality that has been reported in 63 APML patients worldwide. According to these studies, the influence of ider(17)(q10)t(15;17) on the prognosis of adult APML patients is less significant than its effect on

Table 1: Conventional cytogenetic data and FISH results using different LSI *PML-RARa* FISH probes

Patient No	Age/Sex	Bone marrow Morphology report At Diagnosis	Conventional cytogenetic results	FISH signal pattern - DCSF probe	FISH signal pattern - DCDF probe	Survival status of the patient
1	20/F	APML	46,XX,der(15)t(15;17)(q22;q21),ider(17)(q10)t(15;17)(q22;q22)[15]	OGGF	OGFFF	Expired within a week
2	17/M	APML	46,XY,der(15)t(15;17)(q22;q21),ider(17)(q10)t(15;17)(q22;q22)[15]	OGGF	OGFFF	Expired after 5 months

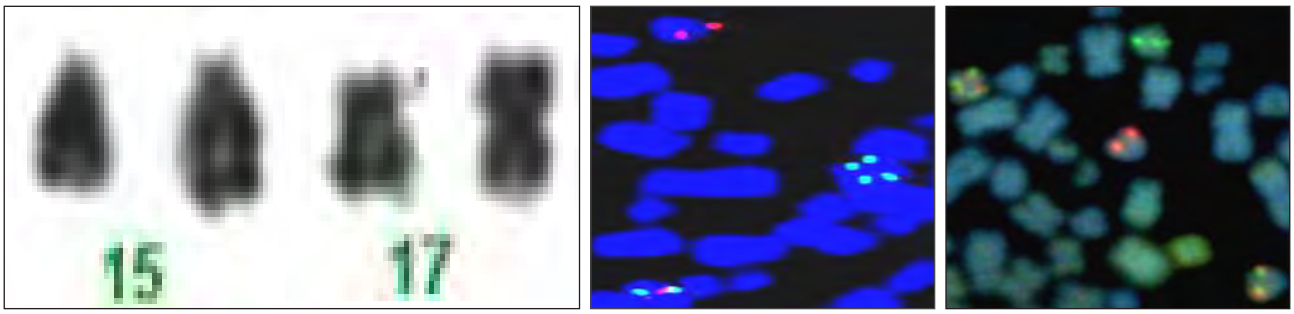

Figure 1: (a) Representative GTG banded partial karyotype showing ider(17)(q10)t(15;17), **(b)** Representative FISH results with DCSF showing i(17)(q) – two green signals on both arms of der(17), one yellow fusion of *PML-RARa* on der(15) and one orange signal on normal chromosome 15, **(c)** Representative FISH results with DCDF showing ider(17)(q) – two yellow fusion signals of extra *RARa-PML* fusion on both arms of der(17), one yellow fusion of *PML-RARa* on der(15) and one green signal on normal chromosome 17 and one orange signal on normal chromosome 15

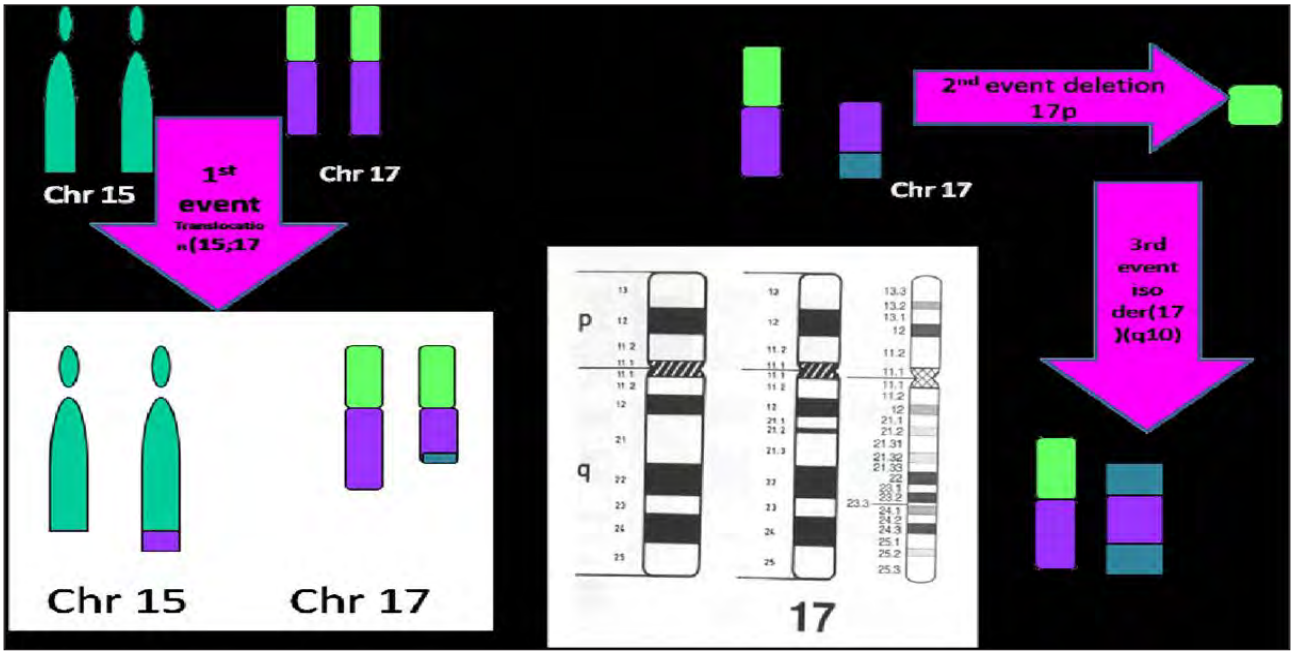

Figure 2: Three step mechanism of ider(17)(q10) formation

children.[11] Here, in the present investigations we have documented two more cases with ider(17)(q10) in young APML patients.

Isochromosome 17q is a structural abnormality that results from loss of the short arm and duplication of the long arm of chromosome 17, resulting in a single copy of 17p and three copies of 17q. Isochromosome 17q is the most common isochromosome in hematologic malignancies. It has been reported in lymphomas and in acute and chronic myeloid and lymphoid leukemias. Isochromosome 17q is also a common karyotype abnormality in solid tumors, most notably in medulloblastoma.[12] Isochromosome 17q is the most frequent genetic abnormality observed during disease progression of chronic myeloid leukemia (CML).[13]

ider(17q) is rather rare than i(17q). The mechanism behind formation of ider(17)(q10) is described in Figure 2. The formation of ider(17)(q10) is a three-step mechanism; the first step is formation of *PML-RARa* and *RARa-PML* fusion. The second step is deletion of p arm of chromosome 17 and the third step is duplication of *RARa-PML* fusion and formation of isochromosome ider(17)(q10) t(15;17). This indicates high genomic instability which may have correlation with poor prognosis. Currently, the prognostic significance of ider(17q) and the two copies of *RARa-PML* as a consequence of ider(17q) in APML patients is unknown. Furthermore, evidences also suggest that *RARa-PML* may potentiate the leukemogenesis of *PML-RARa* via mechanisms that are not yet understood.[3]

FISH with different types of probe revealed the formation of double ider(17)(q10), involving the *PML-RARa* fusion gene and providing a very important hint for clinicians. The clinical significance of ider(17)(q10) in APML includes a tendency toward short survival, which suggests that ider(17)(q10) might be an additional independent poor prognostic factor.[12] Xu et al (2001) found that the presence of the

additional or complex chromosome abnormalities was related to a poor prognosis in both newly diagnosed and relapsed patients.[14] Lee et al (2005) reported that an APML patient with ider(17)(q10) developed a therapy-related leukemia (AML-M5) at 1 year after complete remission with all-trans retinoic acid treatment. Over expression of *RARa-PML* fusion protein in ider(17)(q10) in t(15;17)(q22;q21) patients might be playing a crucial role in progression of the disease. Loss of copy of a *TP53* tumor suppressor gene on chromosome 17p is an important mechanism associated with tumorigenesis. Some investigators have insisted that, because of reduction of the total p53 levels, the integration of genetic repair and apoptosis may be interfered with and that this can contribute to disease progression.[15]

In conclusion, the data presented in this study showed adverse influence of the extra *RARa-PML* gene on prognosis of APML patients. Our study also illustrates the importance of a DCDF FISH probes and combination of molecular and conventional cytogenetics to decipher complex karyotypic abnormalities in leukemia. Present observations also underlined the importance of metaphase FISH to avoid erroneous interpretation of interphase FISH only as emphasized by our results. It also strengthens the fact that exact interpretation of any atypical interphase FISH pattern is dependent on FISH metaphase studies. From a diagnosis perspective minimal residual disease detection using such multiple abnormal fusion signals through the *PML-RARa* FISH analysis in APML patients associated with ider(17)(q10) t(15;17) would be considered to be a useful follow-up marker in clinics. Additional studies would contribute towards better understanding of the influence of ider(17)(q10) t(15;17) on the prognosis, survival, and treatment response of APML cases.

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Cancer Atlas in Gujarat, India

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Summary

The overall aim of this study wasto obtain an overview of patterns of cancer in different parts of Gujarat statefor three years period from January 2008 to December 2010.Since over 85-90% of cancers (as per the data of National Cancer Registry Programme (NCRP) have a microscopic diagnosis, the cases were obtained from pathology department of medical colleges and major hospitals (both government and private).The rationale for only microscopically confirmed cancer cases was that setting up cancer registries through the state would involve enormous cost in establishing and maintaining the same. Therefore, microscopically confirmed cancers registered across the Gujarat state during the period 2008 to 2010 were included. Demographic profile such as age, gender,address and diagnostic information (most valid microscopic diagnosis) was collected from various sources from all over Gujarat.All the newly diagnosed cancer cases during the year 2008, 2009 and 2010 were collected, entered and classified as per the International Classification of Diseases – Oncology (ICD-O) III edition. Out of50589 cases,29670 (58.65%) were males and 20919 (41.35%) were females. Male: Female sex ratio was 1.42:1. Maximum cancer cases, 25823 (51.04%), were observed in Central Gujarat. Most of the cancers were in the age group of 35-64 years. Most common cancer in male wasmouth (10.6%) and in females was breast (25.2%).The study described various patterns of cancers across various districts of Gujarat state which would provide important leads in targeting cancer control measures.

Keywords: Microscopically confirmed cancer, Age, Gender, Incident cases

Introduction

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. Cancer is caused by both external factors (tobacco, chemicals, radiation, and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism).According to recent World Health Organization (WHO) projections, cancer would replace ischemic heart disease as the overall leading cause of death worldwide in 2010.[1]According to estimates from the International Agency for Research on Cancer (IARC), there were 12.7 million new cancer cases in 2008 worldwide, of which 5.6 million occurred in economically developed countries and 7.1 million in economically developing countries. The corresponding estimates for total cancer deaths in 2008 were 7.6 million (about 21,000 cancer deaths a day), 2.8 million in economically developed countries and 4.8 million in economically developing countries.[2] By 2030, the global burden is expected to grow to 21.4 million new cancer cases and 13.2 million cancer deaths simply due to the growth and aging of the population, as well as reductions in childhood mortality and deaths from infectious diseases in developing countries.[3]

Looking at Gujarat state, for the new cancer cases, in Ahmedabad two cancer registries under the network of National Cancer Registry Programme (NCRP) have provided an idea of the magnitude and patterns of cancer in urban and rural areas since 2007 and 2004 respectively. However, extensive areas remain essentially uncovered and therefore the picture of cancer in other areas of Gujarat state remains largely unknown. Setting up of new registries throughout the state would involve enormous and probably prohibitive cost in establishing and maintaining the same. The data of the NCRP has shown 80-85% microscopically confirmed cancer cases as the basis of diagnosis. The basic and critical principle therefore, was that the department of pathology (in Medical Colleges and Hospitals) as well as private pathology laboratories constituted the nodal point for obtaining data on cancer.[4] On similar line, the Gujarat Cancer & Research Institute (GCRI) had an opportunity to work on Gujarat Cancer Atlas Project to know the cancer burden in the state of Gujarat.

Material and Methods

The study was based on cross-sectional study design. Duration for the study was three years 2008 to 2010 which covered entire population of Gujarat State. All microscopically confirmed cancer cases of Gujarat state were included in the study so there was no scope for sample selection. Whole of Gujarat state was considered under study area and was divided into four parts namely North Gujarat, South Gujarat, Central Gujarat and Katchchh & Saurashtra regions. Banaskantha, Mahesana, Patan and Sabarkantha districts were included on North Gujarat. In South Gujarat, Bharuch, Dang, Narmada, Navsari, Surat, Tapi and Valsad were included. Central Zone comprised of Ahmedabad, Anand, Dahod, Gandhinagar, Kheda, Panchmahal and Vadodara. Katchchh & Saurashtra Region comprised of Amreli, Bhavnagar, Jamnagar, Junagadh, Katchchh, Porbandar, Rajkot and Surendranagar.NCRP core proforma was used as a study tool for cancer data collection.

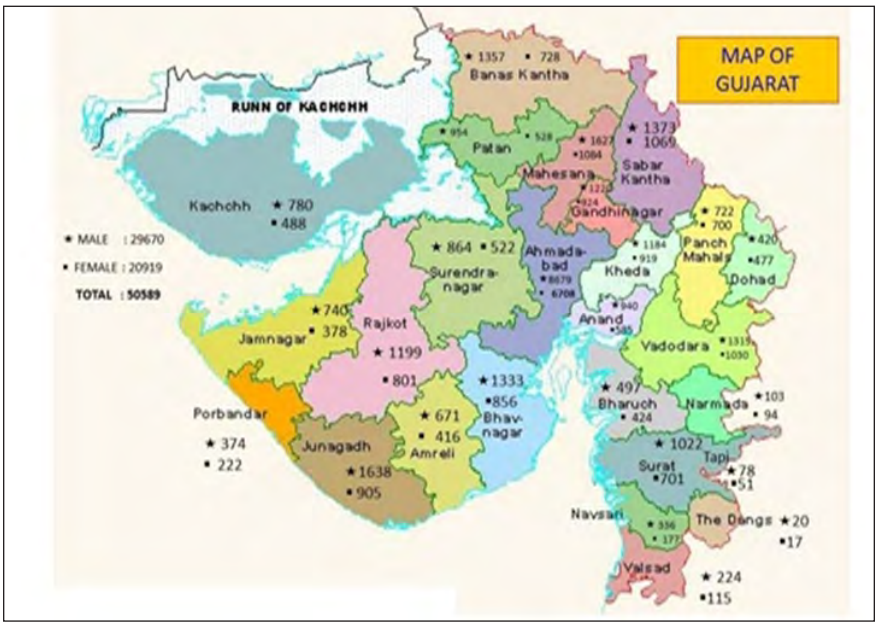

Figure1: Geographical distribution of cancer cases across Gujarat State year 2008-2010

Table 1: Geographical distribution of cancer cases in different regions of Gujarat state : year 2008-2010

District	Male		Female		Total	
	#	%	#	%	#	%
North Gujarat (4 Districts)						
Banaskantha	1627	4.57	728	3.48	2085	4.12
Mahesana	1357	5.48	1084	5.18	2711	5.36
Patan	954	3.22	528	2.52	1482	2.93
Sabarkantha	1373	4.63	1069	5.11	2442	4.83
Total	5311	17.90	3409	16.30	8720	17.24
South Gujarat (7 Districts)						
Bharuch	497	1.68	424	2.03	921	1.82
Dang	20	0.07	17	0.08	37	0.07
Narmada	103	0.35	94	0.45	197	0.39
Navsari	336	1.13	177	0.85	513	1.01
Surat	1022	3.44	701	3.35	1723	3.41
Tapi	78	0.26	51	0.24	129	0.25
Valsad	224	0.75	115	0.55	339	0.67
Total	2280	7.68	1579	7.55	3859	7.63
Central Gujarat (7 Districts)						
Ahmedabad	8679	29.25	6708	32.07	15387	30.42
Anand	940	3.17	585	2.80	1525	3.01
Dahod	420	1.42	477	2.28	897	1.77
Gandhinagar	1220	4.11	924	4.42	2144	4.24
Kheda	1184	3.99	919	4.39	2103	4.16
Panchmahal	722	2.43	700	3.35	1422	2.81
Vadodara	1315	4.43	1030	4.92	2345	4.64
Total	14480	48.80	11343	54.22	25823	51.04
Katchchh & Saurashtra Regions Gujarat (8 Districts)						
Amreli	671	2.26	416	1.99	1087	2.15
Bhavnagar	1333	4.49	856	4.09	2189	4.33
Jamnagar	740	2.49	378	1.81	1118	2.21
Junagadh	1638	5.52	905	4.33	2543	5.03
Kachchh	780	2.63	488	2.33	1268	2.51
Porbandar	374	1.26	222	1.06	596	1.18
Rajkot	1199	4.04	801	3.83	2000	3.95
Surendranagar	864	2.91	522	2.50	1386	2.74
Total	7599	25.61	4588	21.93	12187	24.09
Total Cancer Cases	**29670**	**58.65**	**20919**	**41.35**	**50589**	**100.00**

The first step towards collation is identification and recording of a malignant neoplasm. GCRI is a Regional Cancer Centre, where respected cancer cases are referred for diagnosis and diagnosed cases are referred for treatment. It is one of the best comprehensive cancer care in India. The method of obtaining this varies in different settings. At this institute, the identifying information is completed for all patients who attend this centre for the first time, regardless of whether a microscopic report of malignancy exists or not. A provisional diagnosis is made in the core proforma wherever a record/report of diagnosis of malignancy is available. The diagnostic portion is subsequently completed after reviewing the records/reports of the pathology department.The identifying information is made at the time of initial registration at O.P.D.

Medical College Hospitals and other General Hospitals (Government and Private): Usually, cancers constitute less than 10% of all diseases in a general hospital setting. Therefore, unlike that in the cancer centre, contact with the patient/relative/close friend is taken-up only after a diagnosis of malignancy is made by the department of pathology. However, centres use different approaches for histo-pathology, haematology and cytology. For the latter two methods of diagnosis, normally, patients personally visit the laboratory for giving blood or bone marrow samples or present themselves for smears to be taken. The chances of the pathologist looking up the patient and the patient's records for details of suspected cancer if any are high. The identifying information in the core proforma is completed for such patients wherein a malignancy is diagnosed or suspected. Whenever a histopathology diagnosis of malignancy is made, the concerned patients are followed back to the in-patient wards and where the patient is not admitted or has been discharged follow up is done through the concerned physician.

Pathology Laboratories: Histopathology specimens are often received at the pathology laboratory and the report collected by family members or friends of the patient. In these circumstances, identifying the report with a diagnosis of malignancy and contacting the patient's representative for the required identifying information, by the concerned pathologist with the help of his secretarial staff posed little difficulty. However, occasionally in some pathology laboratories, specimens are sent through courier or messengers, by the surgeons practicing in rural areas to the laboratory in the urban area. In such instances the collaborating pathologist has developed a rapport with the oncologists in the area and the required information is gathered.[4] Reported malignant neoplasms were classified and coded as per WHO Manual.[5] International Classification of Diseases for Oncology (ICD-O-III) had been used for coding of microscopically verified reports of pathology.[6]

Results

A total of 50,589 cases of cancers were reported during the study period from all over Gujarat. Cancer patients from various districts of Gujarat state registered during three years(2008-2009-2010)are shown in map of Gujarat state(Figure1). Out of them 31,208 (61.69%) cases were registered at GCRI, 13636(26.95%) were from sources of Ahmedabad district (other than GCRI) and 5745 (11.36%) cases were from sources of other districts.

Out of 50,589 cases, 8720 (17.24%) cases were from North Gujarat. South Gujarat registered 3859 (7.63%) cases were registered. More than half, 25,823 (51.04%), cases were from Central Gujarat while Kachchh and Saurashtra regions had 12187 (24.09%) registered cancer cases (Table1).

In North Gujarat, cancer of lung is more common in males and breast cancer in females. In South Gujarat, cancer of mouth in males and cancer of cervix in females were the leading cancers. Central Gujarat shows mouth cancers in males and cervical cancers in females as leading sites of cancers. In, Saurashtra region (including Kachchh), lung cancer is more common in males while breast cancer is more common in females.

Mouth was the most common site in males (10.55%) followed by lung (7.97%), tongue (6.81%), oesophagus (4.93%) and base of tongue (4.59%). Breast was the most common sites in females (25.2%) followed by cervix (16.3%), ovary (4.53%), oesophagus (3.36%) and tongue (3.25%) (Figure 2). Mouth, lung and tongue were observed to be in leading sites of male in every zone of Gujarat (Figure 3), while in females, the leading sites are breast, cervix and ovary (Figure 4).

The bulk of the cancer cases were from the age group 35-64 years among both sexes (65.44%). The proportion of paediatric age group (0-14 years) and geriatric age group (65+ years) was 3.67% and 22.36% respectively of total males while 2.79% and 16.85% respectively of total females. The male: female ratio in total cancer cases was 1.42: 1 (Table 2).

The mean age of cancer patients was 50.77 (SD=16.64) years among males and 49.03 (SD=15.16) years among females. The median age at diagnosis was 53 years in males and 50 years in females. The highest number of cases (12.5%) was observed in the age group 55-59 years in males and 45-49 years in females (14.25%) (Figure 5).

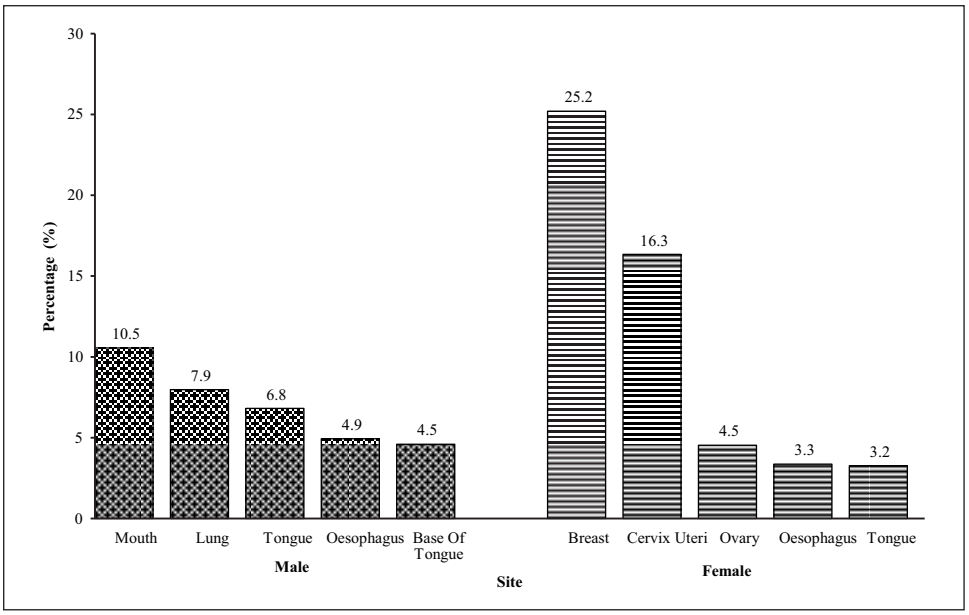

Figure 2: Gender wise distribution of five leading sites of cancer in Gujarat state year 2008-2010 (in percentage)

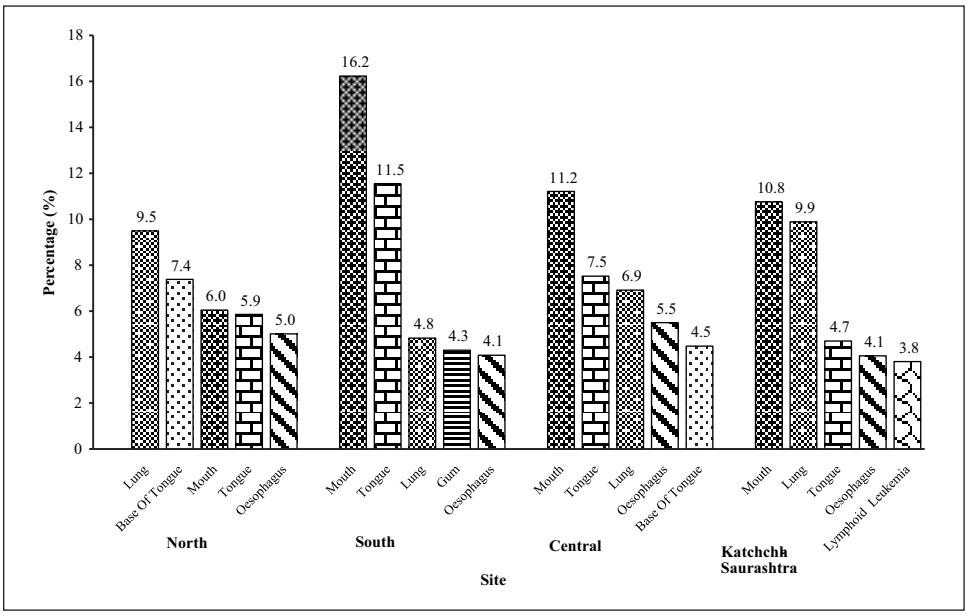

Figure 3: Five leading sites in males zone wise distribution in Gujarat state year 2008-2010 (in percentage)

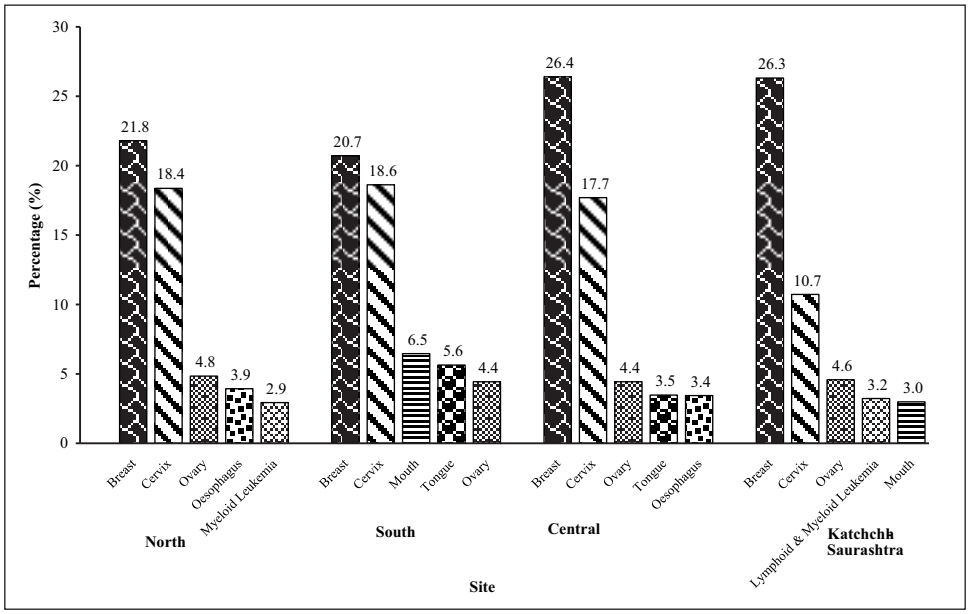

Figure 4: Five leading sites in females - zone wise distribution in Gujarat state year 2008-2010 (in percentage)

Table 2: Broad age group wise distribution of cancer cases Gujarat : year 2008-2010

Age Group (in years)	Gender					
	Male		Female		Total	
	#	%	#	%	#	%
00 – 14	147	3.67	584	2.79	1674	3.31
15 – 34	6634	10.86	2154	10.30	5375	10.62
35 – 64	18578	62.62	14526	69.44	33104	65.44
65 +	3221	22.36	3524	16.85	10158	20.08
Age unknown	1090	0.50	131	0.63	278	0.55
Total	**29670**	**100**	**20919**	**100**	**50589**	**100**

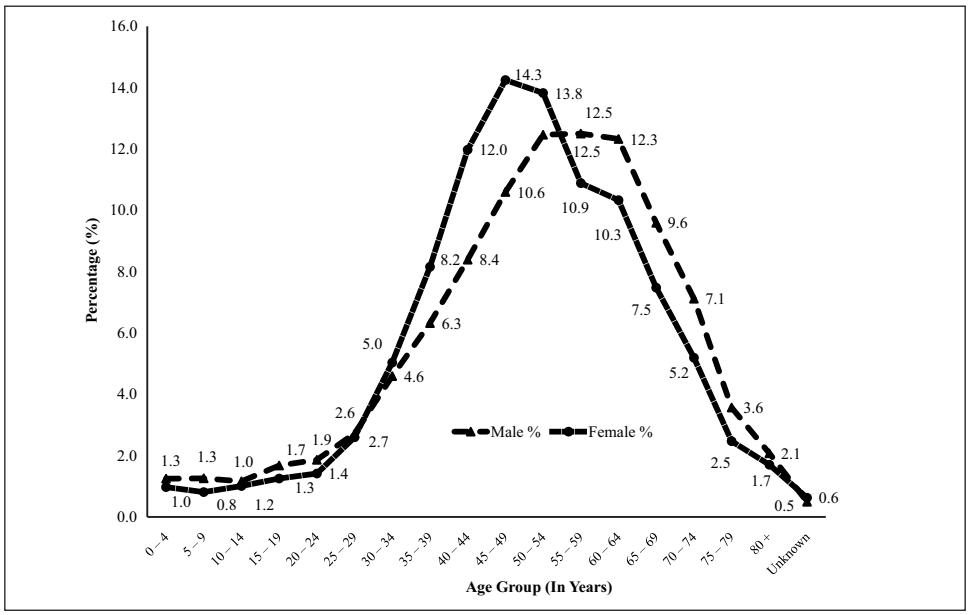

Figure 5: Distribution of cancer cases by five year age group Gujarat state: year 2008-2010 (in percentage)

Discussion

During the years 2008-2009-2010, there were 50589 (males: 29670; females: 20919) cancer patients recorded across the Gujarat state. The median age at diagnosis was 53 years in males and 50 years in females. Children (0-14 years) constituted 3.31% and nearly two thirds (65.44%) of all cancers were in the truncated age group (35-64 years). Male: female ratio was 1.42:1.

Head and neck cancers constituted 42.76% (12688 cases) of total male cancers and 14.22% (2975 cases) of total female cancers.

The proportion of tobacco related cancers relative to all cancers was 55.22% in males and 17.95% in females.

Cancer of mouth (C06) (10.55%) was the leading site among males followed by cancer of bronchus and lung (C34) (7.97%). Among females cancer of breast (C50) (25.20%) was the leading site followed by cancer of cervix (C53) (16.34%).

Proportion of oral cancers (ICD -10 code C00 -C06 - lip, base of tongue, other parts of tongue, gum, floor of mouth, palate, other parts of mouth) to total cancers in males and females were 27.64% and 8.53%, respect ively.

Proportion of pharyngeal cancers (ICD -10 code C09-C14-tonsil, oropharynx, nasopharynx, pyriform fossa, hypopharynx, pharynx) to total cancers in males and females were 9.84% and 2.94%, respectively.

Digestive system cancers (ICD -10 code C15 -C26 - esophagus, stomach, small intestine, colon, rectum, anal canal, liver, gall bladder, biliary duct, pancreas, other digestive organs etc) constituted 13.41% and 10.58% of total cancers in males and females, respectively.

Proportion of respiratory system cancers (ICD -10 codes C30-C38-nasal cavity, accessory sinuses, larynx, trachea, bronchus and lung, thymus, heart, mediastinum and pleura) to total cancers in males and females were 12.82% and 3.35%, respectively.

In children (0 -14 years), lymphoid leukemia (C91) (35.41% in boys and 32.70% in girls) was the predominant cancer in both sexes. In the 15-34 year age group, other parts of mouth (C06) were the predominant cancer in males (16.67%) and cancer of breast (C50) in females (17.22%). In the 35+ age group also, the leading cancer sites were other parts of mouth (C06) in males (10.22%) and breast (C50) in females (26.95%).

The burden of cancer is increasing worldwide despite advances in diagnosis and treatment. Epidemiological studies have shown that many cancers may be preventable. It is widely held that 80–90% of human cancers may be attributable to environmental and lifestyle factors such as tobacco, alcohol and dietary habits.[7]

Knowing the patterns of cancer across the districts of Gujarat would provide important leads in undertaking etiological research, in targeting cancer control measures and in examining clinical outcomes. Orientation of surgeons, gynecologists and pathologists in Cancer Epidemiology and Cancer registration would strengthen the proper diagnosis, systematic recording and reporting of cancer morbidity and mortality.

Acknowledgment: The authors express their sincere appreciation to the Health and Family Welfare Department Government of Gujarat for their valuable support in this study. We are also thankful to all the government and private hospitals, All CHCs, All PHCs, Cancer Specialists, private practitioners and various diagnostic laboratories across the Gujarat state for their valuable support for providing cancer information.

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"Medicine to produce health must examine disease; and music, to create harmony must investigate discord."

Plutarch

Nasopharyngeal Carcinoma in Pediatric Population: A Retrospective Analysis

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Summary

Nasopharyngeal carcinoma in pediatric patients is a rare malignancy. Majority of the patients present with locally advanced disease for which neoadjuvant chemotherapy followed by chemoradiation is the preferred regimen. A retrospective study was performed of 10 previously untreated pediatric nasopharyngeal carcinoma patients up to 14 years of age diagnosed and treated between 2010-2012. The histological diagnosis was made in all cases according to the World Health Organisation (WHO) classification. Overall response rate was seen in 87.5% of patients (7 out of 8 patients). Complete response seen in 3 out of 8 patients (37.5%). Partial response was seen in 4 out of 8 patients (50%). Out of 8 cases 7 developed mucositis (87.5); 4 (50%) had vomiting; 2 had febrile neutropenia (25%). Out of 8 patients 1 progressed (12.5%). Neoadjuvant chemotherapy followed by chemoradiation is a curative option in locally advanced nondistant metastatic nasopharyngeal carcinoma.

Keywords: Pediatric, Nasopharyngeal carcinoma, Treatment

Introduction

Nasopharyngeal carcinoma is an infrequent cancer of the childhood and it is one of the most confusing, commonly misdiagnosed, and poorly understood diseases with rhabdomyosarcoma and lymphoma being the most frequent differential diagnosis. An age-adjusted incidence is less than 1 per 100,000 people. The rates are twice as high in males as in females.[1] The incidence of nasopharyngeal carcinoma is estimated to be less than 1% of all pediatric cancers and endemic form type III which is undifferentiated histology virtually constitutes of all the cases of nasopharyngeal carcinoma in childhood.[2,3] This undifferentiated endemic form is usually associated with environmental and the genetic factors. The environmental factors that have shown to have casual relation with nasopharyngeal carcinoma are consumption of salted cured fish and meat and infection with EBV.[4] Nasopharyngeal carcinoma has a remarkable racial and geographical distribution, primarily affecting individuals from southern China and South East Asia[5]. In spite of the high incidence of cancer of the oral cavity and other parts of pharynx, nasopharyngeal carcinoma is uncommon in the Indian subcontinent except in the Northeastern part of the country.[6] Early stage nasopharyngeal carcinoma that is T1 can be effectively controlled with exclusive radiotherapy, but in patients with locally advance disease stages ranging from T2b N0 to T4 N3, definitive scientific evidence supports the use of concurrent platinum-based chemotherapy with standard external beam radiotherapy.[7] As nasopharyngeal carcinoma in childhood in a rare malignancy much of the understanding of the disease is based on the concepts and observations achieved in adult patients regarding the biology and course of the disease. To review clinical profile and outcome of treatment in pediatric patients of nasopharyngeal carcinoma seen at our institute from 2010 to 2012 in terms of demographic profile, treatment outcome and complications in a retrospective manner.

Methods

This review is based on a retrospective analysis of 10 patients less than or equal to 14 years of age who presented to our institute in between 2010 to 2012 and were subsequently diagnosed as a case of nasopharyngeal carcinoma. Patients with nasopharyngeal mass that were histopathologically proved to be undifferentiated, metastatic, squamous and anaplastic nasopharyngeal carcinoma are reviewed here whereas patients with reports of lymphoma, rhabdomyosarcoma, sarcoma, germ cell tumour, craniopharyngioma, neuroendocrine tumour, thyroid carcinoma and angiofibroma are not included. Patient's case records were reviewed in details in terms of history, physical examination and subsequently investigations of CBC, blood chemistry, chest X-ray, CT/MRI paranasal sinuses and metastatic work-up. Patients were staged according to the classification on the American Joint Committee on Cancer Staging (AJCC).[8] While on therapy, patient's complaints, examination, investigations and symptomatic therapy at every visit were analysed. Patient's response to therapy was documented by the RESICT criteria of target lesions (Table 1).[9] Patients who did not visit the institute for more than one month of the scheduled date are termed as lost to follow-up (LFU).

Toxicities related to chemotherapy and radiotherapy were graded according to Abridged Common Toxicity Criteria.[10]

Table 1: Evaluation of Target Lesions (Response evaluation criteria in solid tumors (RECIST) version1.1[9]

Response	Definition
Complete Response (CR)	Disappearance of all target lesions
Partial Response (PR)	At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started
Progressive Disease (PD)	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions

Results

Total number of pediatric malignancy cases registered at our institute from 2010 to 2012 is 2413 and out of these only 10 patients was diagnosed with nasopharyngeal carcinoma.

In this retrospective review of 10 naso-pharyngeal carcinoma patients (Table 2); 8 were males and 2 were females. The median age at diagnosis was 13 years. Most common sysptoms were neck swelling (unilateral or bilateral) and headache. Out of 10 reviewed cases the most common histology was undifferentiated carcinoma (70%). Eighty percent (80%) of the patients had stage IV A disease.

All the patients were given neoadjuvant chemotherapy (NACT): Nine had recieved cisplatin + 5-flurouracil while one had received taxol+ cisplatin+5-fluorouracil. Response was evaluated subjectively and according to RECIST criteria in 8 patients after NACT before starting curative chemoradiation (CT-RT). One patient was LFU after one cycle of chemotherapy and another patient after 2 cycles of chemotherapy and therefore were not included in further analysis.

Eight patients were evaluated after definitive CT-RT (Table 3). Three patients out of 8 patients had CR. Out of these, 2 patients are on surveillance presently while one patient is LFU. Four patients out of 8 patients were documented with PR. Of these only one patients is under surveillance; 3 patients are LFU of which one patient was documented with progression in form of spine metastasis before LFU. One patient did not achieve response and progressed in form of spine metastasis and presently is LFU.

The mean overall survival (OS) in the review in 10.2 months (range: 5 -16 months). The mean disease free survival (DFS) is 12.3 months (range: 9 -16 months). Out of 10 patients; 2 LFU patients are not reviewed for toxicity. Mucositis was the most common toxicity with 4 patients having grade III, 2 patients having grade II and 1 patient having grade I. The second most common toxicity was vomiting. One had grade I vomiting and 3 patients had grade II. Two patients had grade III febrile neutropenia. One patient developed grade I rash and 1 patient had grade IV. Grade II sensory neuropathy developed in 1 patient. A single patient developed both grade II anaemia and thrombocytopenia simultaneously (Table 4).

Table 2: Patient Characteristics

Characteristics	N (%)
Sex	
Male	8 (80%)
Female	2 (20%)
Median age (years)	13
Symptoms	
Bilateral neck swelling	6 (60%)
Unilateral neck swelling	3 (30%)
Headache	3 (30%)
Vomiting	1 (10%)
Neck pain	1 (10%)
Epistaxis	1 (10%)
Ear ache	1 (10%)
Nasal swelling	1 (10%)
Histology	
Undifferentiated	7 (70%)
Metastatic	2 (20%)
Anaplastic	1 (10%)
Stage	
III	2 (20%)
IV A	8 (80%)

Table 3: Status at 6 months (N=8)

Response	N%
Patients in CR at 6 months	3 (37.5%)
Patients in PR at 6 months	4 (50%)
Patients with progression	1 (12.5%)

Table 4: Toxicity profile

Toxicity	N%	
	Any grade	Grade III or more
Mucositis	7 (87.5%)	4 (50%)
Vomiting	4 (50%)	-
Febrile neutropenia	2 (25%)	2 (25%)
Neutropenia	2 (25%)	-
Anaemia	1 (12.5%)	-
Thrombocytopenia	1 (12.5%)	-
Fever without neutropenia	1 (12.5%)	-
Rash	2 (25%)	1 (12.5%)
Nausea	1 (12.5%)	-
Neuropathy	1 (12.5%)	-

Discussion

Nasopharyngeal cancinoma is extremely rare in childhood and accounts for less than 1% of all pediatric cancers.[11] Laskar et al[12] also reported that nasopharyngeal carcinoma accounts for 1.5% of all pediatric malignancies seen at the Tata Memorial Hospital annually. In this retrospective analysis the incidence of nasopharyngeal carcinoma is 0.4% over three year period from 2010 to 2012 with preponderance of male patients, type III histology and stage III or IV A disease. The median age of diagnosis reported by Laskar et al (in review of 81 pediatric patients)[12] and Ozyar et al (review of 165 patients)[13] was 14 years and in this analysis it is 13 years of age.

The median duration of symptoms was 2 months in this analysis versus 5 months as mentioned by Pappo et al[11] with neck swelling being the most common complaint. Ninety-one patients had clinically palpable nodes at presentation as reported by Laskar et al[12] which is similar to this analysis (90%). Since nasopharyngeal carcinoma is a very chemosensitive neoplasm; Children's Oncology Group recommends NACT before chemoradiation may be beneficial as the dose of radiation may be lowered if patient has good response to NACT, thereby averting severe toxicities related to higher doses of radiotherapy[14]. All patients at our institute received NACT followed by curative chemoradiation.

The response rate reported in study by Ayan et al[3] is 79% and alive number of patients reported is 22 out of 40 (55%). Though the 5 year OS and DFS reported by Ozyar et al[13] is 77.4% and 68.8%, respectively and; 45% and 54% after median follow-up of 50 months by Laskar et al, in this retrospective analysis the overall response rate is 87.5% (7 out of 8 patients) with 3 out of 10 enrolled patients (30%) alive and under surveillance presently. As the duration of median follow-up is still short in this review as compared to two above mentioned data so there is need for continued surveillance of patients. In this review 7 patients are LFU and therefore the present status of these patients is not known. Progression is documented in 12.5% (i.e. 1 out of 8 patients) of patients in this analysis which is less than that reported by Ayan et al[3] (43%). The less number of patients in this analysis along with unknown status of six LFU patients may have led to this difference in result.

Most common toxicity found in this retrospective review is mucositis (87.5%, of any grade) and acute toxicity of febrile neutropenia (25%) which is same as in Laskar et al[12] results (81% and 21%), respectively. Being highly chemosensitive, the combined modality of induction chemotherapy and chemoradiation for treating patients of naso-pharyngeal carcinoma is a curative treatment in pediatric patients. There is need for educating and encouraging parents or guardians for completing treatment protocol even if symptoms have resolved and for regular follow-up after completing treatment for surveillance.

Conclusion

This review suggests that pediatric patients though presenting with locally advanced naso-pharyngeal carcinoma can be treated with the curative intent employing neoadjuvant chemotherapy followed by chemoradiation.

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Solution to Crossword Puzzle- IV

O[1]	S	T[6]	E	O[10]	S	A	R	C	O	M	A[19]
N		E		I							R
D[2]	Y	S	P	L[11]	A[3]	S[15]	I	A	N		S
A		T		A		O					E
N[4]	C[9]	I	S	P[12]	L	A	T	I	N		N
S		S[7]		A		P[16]					I
E		I		R[13]		I					C[20]
T[5]	R	A	S	T	U	Z	U	M[18]	A[21]	B	L
R		D		O		Z		I			O
O		H[8]	C	G[14]		A[17]	C	N	E		U
N				O		L		E			D
	C[22]	H	O	D		K			B[23]	R	

Congratulation to the winner:

Dr. Chandrima Ray
Fellow, Gynaec Oncology

Answers:

ACROSS:

1. OSTEOSARCOMA
2. DYSPLASIA
3. ASIAN
4. NCI
5. TRASTUZUMAB
8. HCG
9. CISPLATIN
17. ACNE
21. ABL
22. CHOD
23. BR

VERTICAL:

1. ONDANSETRON
6. TESTIS
7. SIADH
10. OIL
11. LAPA
12. PART
13. RTOG
14. GOD
15. SOAP
16. PIZZA
17. ALK
18. MINE
19. ARSENIC
20. CLOUD

Relevance of Serum Interleukin-1α and Interleukin-1β in Thyroid Diseases

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Summary

It has been hypothesized that cytokines which play major role as inflammatory mediators might serve as triggers of chronic inflammation and increase the risk of developing thyroid cancer. Studies have shown that Interleukin-1 (IL-1) family of cytokines are primarily associated with inflammation. Thus, the aim of present study was to estimate serum levels of Interleukin-1α (IL-1α) and Interleukin-1β (IL-1β) from total 69 patients with different thyroid diseases: Goitre (N=21), Autoimmune diseases (N=16) and Carcinomas (N=32) and 19 healthy individuals by Enzyme Immunoassay (EIA). Results indicated that serum IL-1α was predominantly higher only in patients with goitre, while serum IL-1β was significantly elevated in patients with goitre, autoimmune diseases and thyroid carcinomas as compared to that of healthy individuals. Moreover, in thyroid carcinoma patients, inverse correlations of serum IL-1α levels with tumor size, lymphatic permeation and differentiation status were significant and serum IL-1β values were positively correlated with the lymphnode metastasis and the differentiation status of the tumors. Thus, both IL-1α and IL-1β seem to have a role in pathogenesis of thyroid diseases and monitoring their serum levels can be distinctively helpful in differentiating patients with thyroid disease from healthy subjects. Also the differential strategy of IL-1α and IL-1β in malignant cells or in the tumor's microenvironment can open new avenues for using IL-1 in cancer therapy.

Keywords: Interleukin-1, IL-1α, IL-1β, Goitre, Thyroid diseases, Thyroid carcinogenesis

Introduction

Thyroid diseases are arguably among the commonest endocrine disorders worldwide. In India too, there is a significant burden of thyroid diseases. According to a projection from various studies on thyroid disease, it has been estimated that about 42 million people in India suffer from thyroid diseases.[1] But, as their symptoms often appear gradually, they are commonly misdiagnosed. The three most common thyroid problems are the underactive thyroid, the overactive thyroid, and thyroid nodules. Based on these problems, the disorders of the thyroid gland include: Goitre, Autoimmune thyroid diseases and Thyroid carcinoma.

Thyroid cancer is the most common endocrine malignancy, and majority of thyroid carcinomas originate from follicular epithelial cells. The incidence of thyroid carcinomas derived from follicular cells varies worldwide depending on dietary iodine intake, but in most countries it has increased during the past few decades.[2] Accumulating evidences indicate that follicular cell derived thyroid cancer constitutes a biological continuum progressing from the highly curable well differentiated thyroid cancer to the universally fatal anaplastic thyroid cancer.[3] Also, literature reports association between the thyroid cancer and a history of several benign and autoimmune diseases.[4] Hence, to aid diagnosis, for differentiating benign from malignant thyroid tumors and, in the last group, to distinguish tumors with indolent and aggressive behavior, it is important to decipher the molecular mechanisms underlying thyroid tumorigenesis.

Several epidemiologic studies support the fact that chronic inflammatory diseases are frequently associated with increased risk of cancers [5, 6, 7] and it is estimated that underlying infections and inflammatory responses are linked to 15–20% of all deaths from cancer worldwide.[5] As various clinical and pathological evidences support the concept of step wise progression of thyroid cancer (progression of cancer from benign/autoimmune diseases), we hypothesized that cytokines which play major role as inflammatory mediators, might serve as triggers of chronic inflammation and increase the risk of developing thyroid cancer.

Cytokines are small cell signalling protein molecules which encompass a large and diverse family. They consist of immunomodulating agents such as interleukins and inteferons. Virtually all nucleated cells, especially endow/epithelial cells and macrophages are potent producers of cytokines[8] and thus understanding cytokine immunobiology is central to the development of rational therapies for destructive inflammatory diseases.

Moreover, studies have shown that the predominant effect of cytokines on the hypothalamic-pituitary-thyroid axis is inhibitory and the cytokines may play a role during physiological as well as pathophysiological conditions contributing to thyroid diseases.[9] Cytokines can also modulate both growth and function of thyroid follicular cells. In addition to these effects, exogenous administration of cytokines has been associated with impairment of thyroid

function ranging from the appearance of autoantibodies alone to the development of frank thyroid dysfunction.[10]

IL-1 family of cytokines is primarily associated with acute and chronic inflammation and has important roles in endocrinology and in the regulation of responses associated with inflammatory stress.[8] IL-1 serves as the prototypic "alarm" cytokine in healthy persons, affecting nearly every tissue and organ in the body. The induction of IL-1 by a virus, bacterium or toxin leads to the expression of many effector proteins, e.g. cytokines/chemokines, nitric oxide synthetase and matrix metalloproteinases (MMPs)[11] through signalling pathways. Some of the cytokine pathways induce immunological mechanisms, and others produce haematological changes. IL-1 is produced by a variety of cells that are part of the innate immune system. There is increasing evidence that constant activation of the innate immune system occurs in several chronic inflammatory processes. This persistent activation promotes constitutional changes, metabolic abnormalities and destruction and remodelling of tissues in persons with chronic, uncontrolled disease.[12]

IL-1α and IL-1β are the two main proinflammatory cytokines of the IL-1 family. IL-1α and IL-1β are implicated in inflammation-associated carcinogenesis. These two proinflammatory cytokines clearly differ in their cellular compartmentalization and function. IL-1β is solely active in its secreted form, whereas IL-1α is largely active as a membrane-bound cytokine and only to a lesser extent as a secreted molecule.[13] IL-1α has been demonstrated to induce loss of the thyroid epithelial barrier, measured as transepithelial resistance while, IL-1β is an important regulator of thyroid cell function. It has been repeatedly shown that IL-1β inhibited differentiated thyroid functions in vitro. Previous findings demonstrated that IL-1β induced inhibition of human thyroid cell adenylate cyclise (cAMP) and thyroglobulin release and at the same time increased IL-6 release. Inhibition of the production or function of IL-1 could be of potential interest in the management of immunoinflammatory disorders.[14] The origin of IL-1 could be from infiltrating monocytes/macrophages, endothelial cells as well as from the thyrocytes themselves. Thus, IL-1 activated thyrocyte may participate directly in the immunological process by reacting to and producing immunoinflammatory cytokines.[15]

Hence, the aim of this study was to explore the occurrence of inflammatory cytokines- IL-1α and IL-1β in sera of patients with various thyroid diseases (Goiter, Autoimmune thyroid disorders and Thyroid carcinoma) and to correlate the results with clinico-pathological parameters in thyroid cancer patients.

Materials and Methods

Total 88 individuals were included in the study, out of which 69 were patients with thyroid disorders: (Goiter: N=21, Autoimmune thyroid diseases: N=16, and thyroid carcinoma: N=32) and 19 were age matched disease free healthy individuals (Table 1). The mean age of healthy individuals included in the study was 30.57 years (range: 18-56 years) while, that of patients with Goiter was 34.23 years (range: 18-58 years), Autoimmune thyroid disease was 42.81 years (range: 26-61 years) and thyroid carcinoma was 43.96 years (range: 18-78 years). The patients were grouped into younger (<45 years) and older age groups ($\geqslant$45 years) according to the American Joint Committee on Cancer (AJCC) TNM staging system (Table 1).

Pretherapeutic fasting blood samples were collected in vaccutainers with gel for serum separation after taking written consent of the subjects. Moreover, none of the patients were diagnosed for any autoimmune disease previously, nor any of them were taking immunosuppressive or immunomodulant drugs. Serum was separated after centrifugation and was stored at -80˚C until analysis. IL-1α and IL-1β were determined from the serum samples using commercially available enzyme immunoassay (EIA) kits following the manufacturer's instructions. The detailed clinical and histopathological characteristics of all patients were noted from the case files maintained at Gujarat Cancer & Research Institute (Table 2).

Statistical analysis

The results were presented as mean standard error of mean (M±SE). The differences in serum IL-1α and IL-1β levels between healthy individuals and patients with thyroid diseases were assessed by performing Mann-Whitney U-test. The discriminating efficacy of IL-1α and IL-1β between healthy individuals and patients with thyroid diseases were also determined by constructing Receiver's Operating Characteristic (ROC) curves. p values <0.05 were considered statistically significant. Also, in thyroid cancer patients, Mann-Whitney U-test was performed to analyze the association between IL-1α and IL-1β levels and clinicopathological parameters and independent relationship between serum IL-1α and IL-1β levels was described by Spearman's correlation.

Table 1: Characterization of patients with thyroid diseases and healthy individuals

Subjects	N (%)	Gender		Age	
		Male	Female	<45 years	≥45 years
Healthy individuals	19	6	13	15	4
Total Patients	69	16	53	40	29
Goiter	21	3	18	14	7
Autoimmune diseases	16	4	12	9	7
Graves' disease	12	4	8	7	5
Hashimoto's disorder	4	-	4	2	2
Thyroid Carcinoma	32	9	23	17	15
Papillary Carcinoma	18	3	15	12	6
Follicular Carcinoma	7	2	5	2	5
Medullary Carcinoma	4	2	2	2	2
Anaplastic Carcinoma	3	2	1	1	2

Table 2: Clinicopathological parameters of thyroid cancer patients

Parameters	N (%)	Parameters	N (%)
Age		**Multifocality**	
<45 years	17 (53.10)	Present	14 (43.70)
⩾45 years	15 (46.90)	Absent	18 (56.30)
Gender		**Bilaterality**	
Male	09 (28.10)	Unilateral	23 (71.90)
Female	23 (71.90)	Bilateral	09 (28.10)
Tumor size		**Haemorrhagic area**	
T1+T2	16 (50.00)	Present	07 (21.90)
T3+T4	16 (50.00)	Absent	25 (78.10)
Lymph node metastasis		**Necrosis**	
Present	18 (56.30)	Present	03 (9.40)
Absent	14 (43.70)	Absent	29 (90.60)
Distant metastasis		**Calcification**	
Present	21 (65.60)	Present	19 (59.40)
Absent	11 (34.30)	Absent	13 (40.60)
Stage		**Sclerosis**	
Early stage (Stage I & II)	15 (46.90)	Present	04 (12.50)
Advanced stage (Stage III & IV)	17 (53.10)	Absent	28 (87.50)
Lymphatic permeation		**Extrathyroidal extension**	
Present	04 (12.50)	Present	13 (40.60)
Absent	28 (87.50)	Absent	19 (59.40)
Vascular permeation		**Fibrosis**	
Present	08 (25.00)	Present	08 (25.00)
Absent	24 (75.00)	Absent	24 (75.00)
Capsular Invasion		**Inflammation**	
Present	13 (40.60)	Present	14 (43.70)
Absent	19 (59.40)	Absent	18 (56.30)
Encapsulation		**Differentiation**	
Well encapsulated	27 (84.40)	Well	22 (68.75)
Not encapsulated	05 (15.60)	Moderate/ Poor	10 (31.25)

Results

Serum IL-1α levels were predominantly higher in patients with goitre, while the levels of IL-1β were significantly elevated in thyroid disorders (goitre, autoimmune thyroid disorders, and thyroid carcinoma) as compared to healthy individuals. IL-1α and IL-1β levels in thyroid carcinoma patients with different histopathological subgroups have also been compared to that of healthy individuals. Statistically significant higher levels of IL-1α were found in patients with follicular carcinoma while, noteworthy higher IL-1β levels were observed in papillary, medullar and anapaestic carcinoma patients as compared to healthy individuals (Tables 3 and 4).

ROC curve (Figure 1(a)) indicates that IL-1β exhibited a good discriminatory efficacy between healthy individuals and total patients with thyroid diseases (IL-1β: AUC-0.800). Moreover, the ROC curves for both the cytokines between healthy individuals and individual groups of patients, that is, goitre, autoimmune disease, and thyroid cancer, revealed that both IL-1α as well as IL-1β showed good sensitivity and specificity to discriminate between healthy individuals and patients having goitre (IL-1α: AUC-0.774, IL-β: AUC-0.690) (Figure 1(b)); while, only IL-1β could significantly differentiate between healthy individuals and patients with thyroid autoimmunity and carcinoma (Autoimmune thyroid disorders- IL-1β: AUC-0.875 and thyroid cancer- IL-1β: AUC-0.835) (Figures 1(c)–1(d)).

The incidence of patients with thyroid diseases having higher levels of IL-1α and IL-1β than those of healthy individuals has been shown in Figures 2. The levels of IL-1α > 5.00 pg/ml (maximum level of IL-1α in healthy individuals) were found in 71.4% of patients with goitre, 37.5% patients having autoimmune diseases and in 56.3% thyroid carcinoma patients. While levels of IL-1β >3.56 pg/ml (maximum level in healthy individuals) were observed in 61.9% patients with goitre, 81.3% patients with autoimmune diseases and in 78.1% thyroid carcinoma patients. Amongst the autoimmune thyroid disorder patients, incidence of higher IL-1β values was observed in both patients with Graves' disease and Hashimoto's disorder as compared to that of IL-1α values. Moreover, ≥ 50% patients had increased levels of both IL-1α and IL-1β with papillary, follicular and medulary carcinoma, while < 5.0 pg/ml of IL-1β and > 3.56 pg/ml of IL-1α levels were observed in all three (100%) anaplastic carcinoma patients.

Association of the serum IL-1α and IL-1β levels with different clinicopathological parameters have been studied by Mann-Whitney U test. It revealed that elevated serum IL-1α levels were significantly associated with tumor size, lymphatic permeation and differentiation status while, IL-1β levels exhibited predominant association with lymph node metastasis and differentiation status of thyroid carcinoma patients. In fact, serum IL-1α levels were significantly increased in patients having smaller tumor size, absence of lymphatic permeation and in patients with well differentiated tumor. Thyroid carcinoma patients with lymph node metastasis and moderate to poorly differentiated tumors had significantly elevated levels of IL-1β as compared to those with absence of lymph node metastasis and well differentiated tumors (Figure 3)

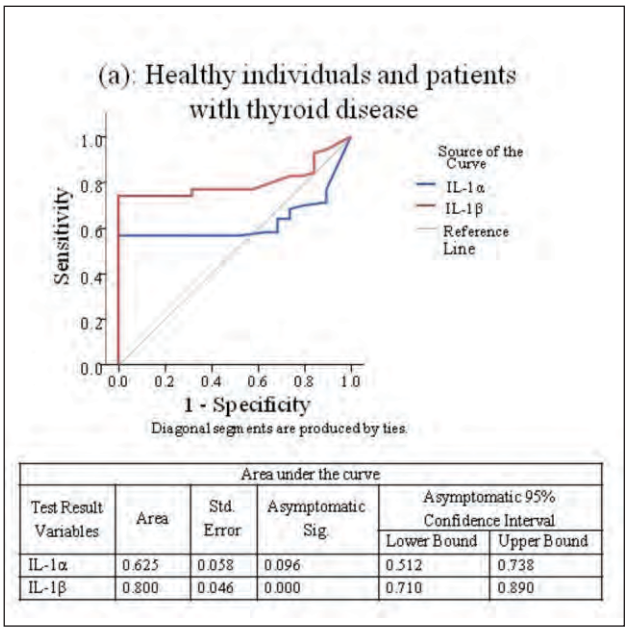
Moreover, Spearman rank's correlation analysis revealed significant inverse relationships of IL-1α levels with tumor size (r = -0.526, p = .002), lymphatic permeation (r = -0.385, p = .029) and differentiation status (r = -0.403, p = .022) of tumor while, correlation of IL-1β with lymphnode metastasis (r = 0.379, p = .032) and differentiation status of tumors (r = 0.629, p <0.001) was positively significant (Table 5).

Table 3: Significance of IL-1α levels in patients with thyroid diseases as compared to healthy individuals

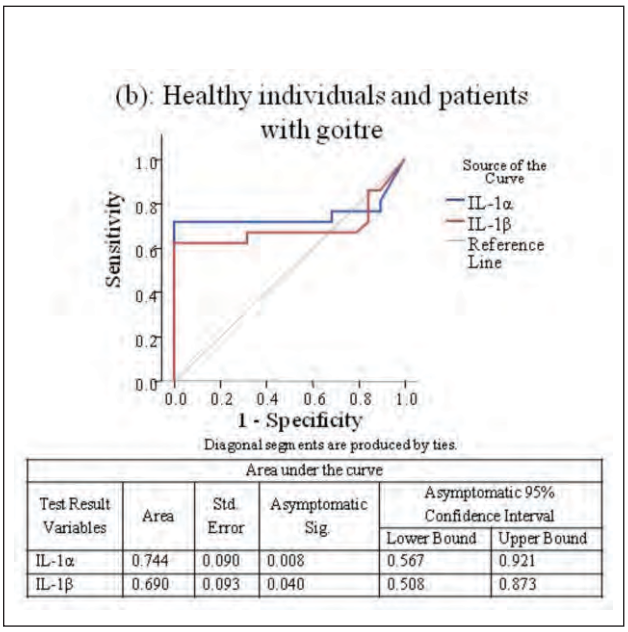
Subjects	Mean ± S.E (pg/ml)	Median	p value
Healthy Individuals	3.53 ± 0.39	4.10	
Total patients	30.48 ± 9.31	10.85	0.095
Goitre	69.11 ± 28.26	17.98	0.008
Autoimmune disorders	9.33 ± 3.98	3.05	0.683
Graves' disease	6.26 ± 2.27	3.05	0.541
Hashimoto disorder	18.54 ± 14.94	5.76	0.907
Thyroid Carcinoma	15.70 ± 4.68	7.45	0.121
Papillary carcinoma	14.85 ± 3.46	11.18	0.066
Follicular carcinoma	27.23 ± 19.55	11.53	0.048
Medullary carcinoma	10.12 ± 5.45	10.12	0.785
Anaplastic carcinoma	1.35 ± 0.78	1.35	0.069

Table 4: Significance of IL-1β levels in patients with thyroid diseases as compared to healthy individuals

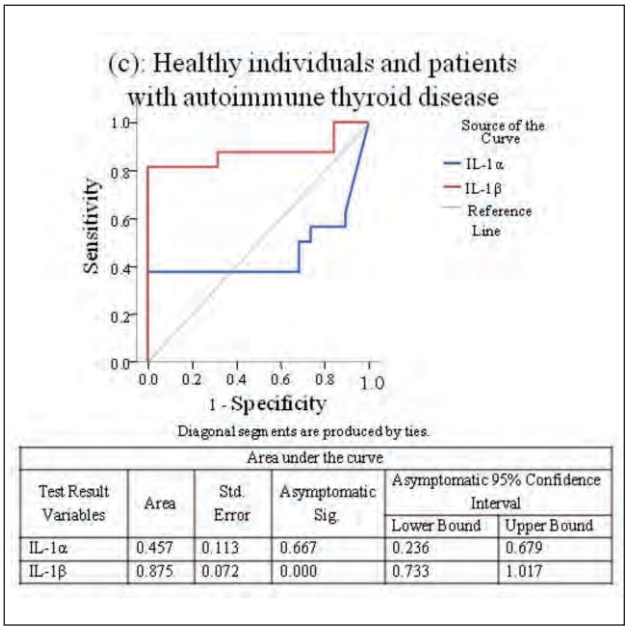
Subjects	Mean ± S.E (pg/ml)	Median	p value
Healthy Individuals	2.60 ± 0.23	2.80	
Total patients	9.27 ± 1.52	5.89	<0.001
Goitre	4.70 ± 0.88	4.50	0.039
Autoimmune disorders	8.09 ± 1.29	7.62	<0.001
Graves' disease	7.68 ± 1.56	6.06	0.002
Hashimoto disorder	9.33 ± 2.48	8.31	<0.001
Thyroid Carcinoma	12.87 ± 3.05	7.79	<0.001
Papillary carcinoma	8.32 ± 1.52	6.93	<0.001
Follicular carcinoma	4.65 ± 0.96	4.50	0.152
Medullary carcinoma	15.36 ± 4.29	12.82	<0.001
Anaplastic carcinoma	55.99 ± 17.46	53.87	0.001



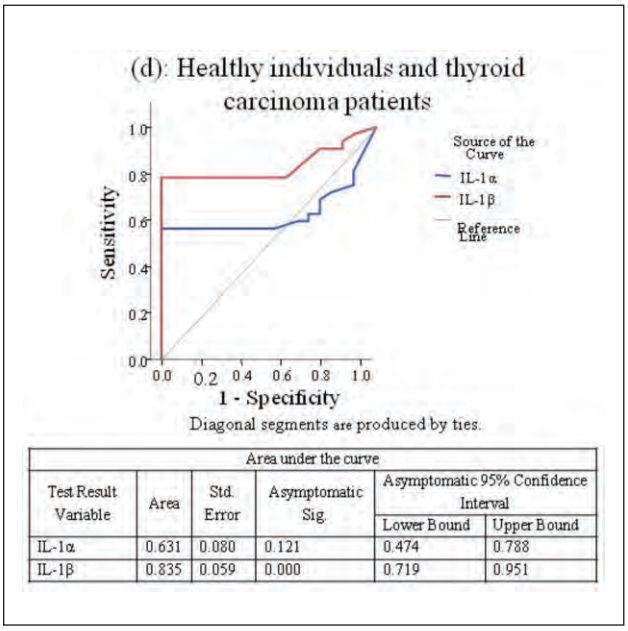
Area under the curve					
Test Result Variables	Area	Std. Error	Asymptomatic Sig.	Asymptomatic 95% Confidence Interval	
				Lower Bound	Upper Bound
IL-1α	0.625	0.058	0.096	0.512	0.738
IL-1β	0.800	0.046	0.000	0.710	0.890



Area under the curve					
Test Result Variables	Area	Std. Error	Asymptomatic Sig.	Asymptomatic 95% Confidence Interval	
				Lower Bound	Upper Bound
IL-1α	0.744	0.090	0.008	0.567	0.921
IL-1β	0.690	0.093	0.040	0.508	0.873



Area under the curve					
Test Result Variables	Area	Std. Error	Asymptomatic Sig.	Asymptomatic 95% Confidence Interval	
				Lower Bound	Upper Bound
IL-1α	0.457	0.113	0.667	0.236	0.679
IL-1β	0.875	0.072	0.000	0.733	1.017



Area under the curve					
Test Result Variable	Area	Std. Error	Asymptomatic Sig.	Asymptomatic 95% Confidence Interval	
				Lower Bound	Upper Bound
IL-1α	0.631	0.080	0.121	0.474	0.788
IL-1β	0.835	0.059	0.000	0.719	0.951

Figure 1: ROC curves for IL-1α and IL-1β

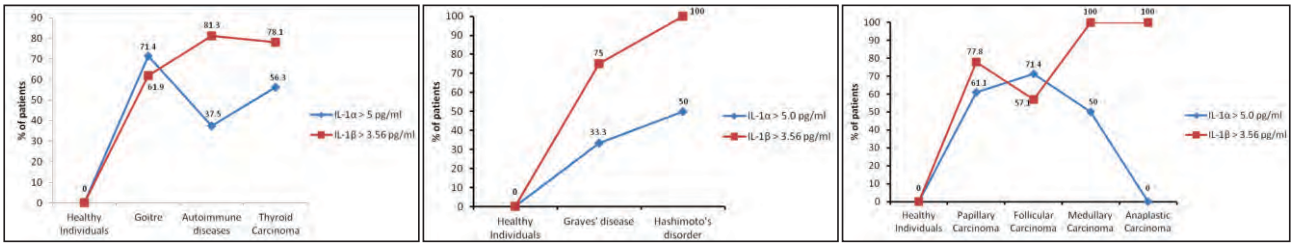


Figure 2: Incidence of patients with thyroid diseases having IL-1 α and IL-1β values higher than that of the healthy individuals

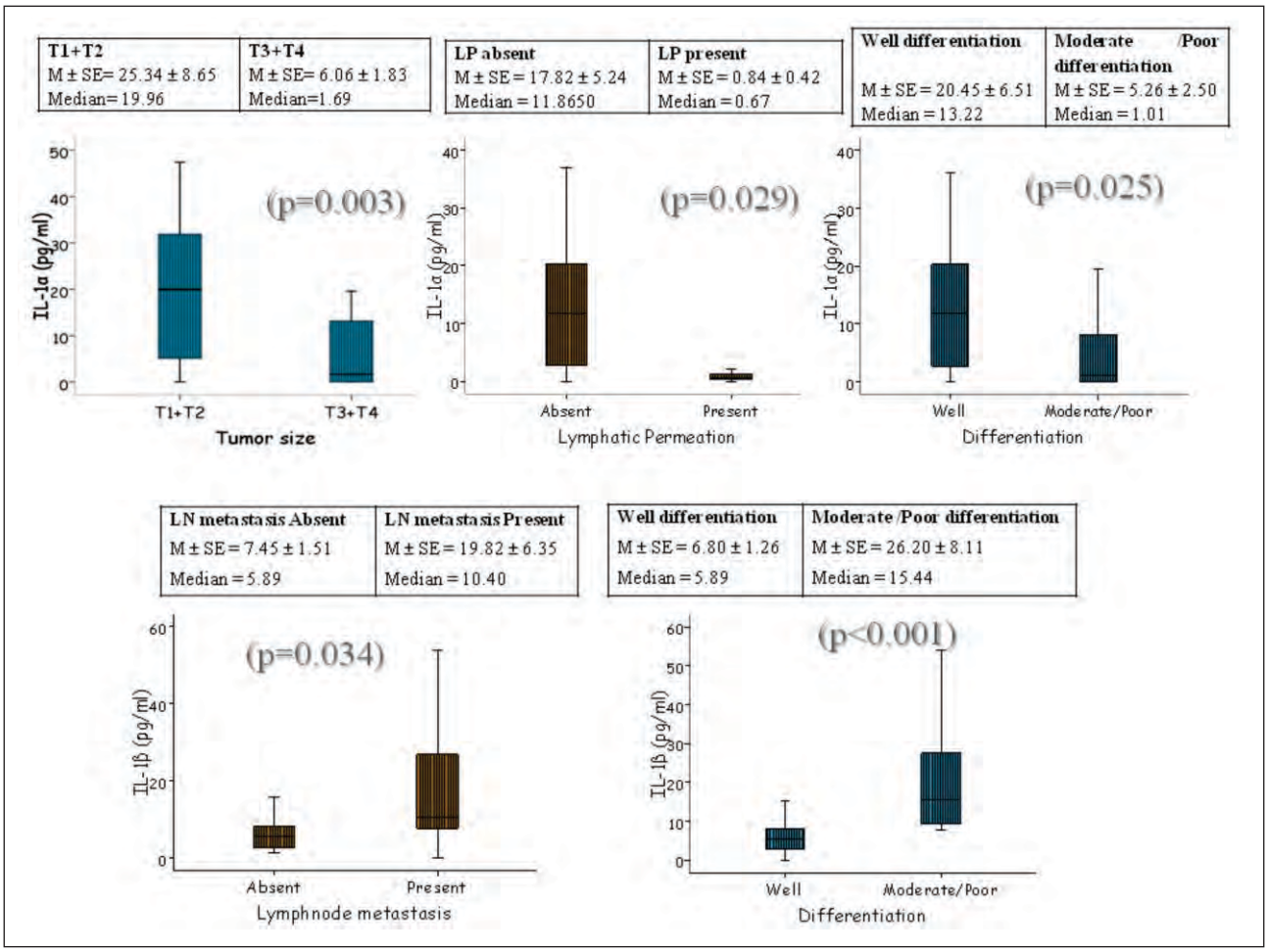


Figure 3: Association of IL-1α and IL-1β with clinicopathological parameters in thyroid carcinoma patients

Table 5: Correlation of IL-1α and IL-1β with clinicopathological parameters in thyroid carcinoma patients

Clinicopathological parameters	IL-1α	IL-1β
Tumor size	p= 0.002 r= -0.526	
Lymph node metastasis		p= 0.032 r= 0.379
Lymphatic permeation	p= 0.029 r= -0.385	
Differentiation	p= 0.022 r= -0.403	p< 0.001 r= 0.629

Discussion

Our results indicate elevated levels of IL-1α and IL-1β in patients having Goitre and the ROC curve showed that both the cytokines could efficiently discriminate between patients with goitre and the healthy subjects. It has also been clearly revealed that only IL-1β and not IL-1α levels were distinguishably higher in autoimmune thyroid disorders and thyroid carcinoma patients as compared to that of healthy individuals. It has been well implicated in a study by Ajjan et al that cytokines influence activation, growth, and differentiation of several target cells, play a crucial role both in autoimmune and neoplastic thyroid diseases, and interfere with thyroid hormone synthesis.[16] In accordance with our study, Krysiak R and Okopien B have shown that the activated monocytes from Hashimoto's thyroiditis patients produced larger amounts of IL-1β as compared to that from healthy subjects.[17] In fact, IL-1 and other proinflammatory cytokines like TNFα and IFNγ have been repeatedly detected in thyroid tissues of Graves' disease and multinodular goitre patients.[18,19,20]

In contrast to our observations, Phenekos et al have shown that patients with toxic nodular goitre, Hashimotos thyroiditis and Graves' disease had lower IL-1β serum levels compared to controls.[21] Moreover, Salvi et al and Siddiqi et al did not show rise of IL -1β in Graves' disease patients and thyrotoxicosis respectively.[22, 23] Reed and Davies had observed that amongst the Th1 cytokines, IL-1α was released in greatest amounts in Hashimoto's thyroiditis which is again not in accordance with our results.[24] One of the studies also revealed that serum IL-1β concentrations allowed the discrimination between atrophic thyroiditis and papillary thyroid cancer groups.[25]

Previous studies[26,27] addressing IL-1-induced alterations in thyroid cell growth, morphology, and apoptosis showed variable results but were primarily generated with IL-1β. A significant difference was found in the serum levels of IL-1α between the groups of controls and patients with thyroid eye disease while, serum IL-1β was higher in patients with thyroid eye disease in comparison with controls but the difference was statistically not significant.[28] It has already been implicated that IL-1α/IFN-γ effects may involve interferences between cytokine- and TSH-activated pathways that would lead to the inactivation of the latter through impaired cAMP production.[29]

IL-1 has been reported to be crucially involved in cell survival, proliferation, and angiogenesis in cancer cells.[30] IL-1 secretion seems to be associated with a more aggressive form of breast cancer.[31,32] Its production by breast cancer cells has been shown to increase RANKL expression and thus stimulate osteoclasts.[33,34] Moreover, several evidences support a role for IL-1 and other cytokines, including TNFα, IL-6, IL-8, and IL-18, in the pathogenesis of the insulin resistance and non-alcoholic fatty liver disease.[35,36,37] Elevated levels of IL-1 have also been observed in ovarian cancer specimens, and may play a role in tumor cell growth by up regulating expression of IL-6.[38] Increased susceptibility to colon carcinogenesis was also associated to increased permeability and local production of IL-1.[39,40] Inflamed colon mucosa from patients with inflammatory bowel disease and in human colon cancer cells stimulated with IL-1β, aberrantly induced IL-31 which further activated extracellular signal-regulated kinase (ERK)- and STAT mediated signalling in these colon cancer cells and increased the secretion of IL-8, thereby promoting cell proliferation and migration.[41] On the other hand, abnormal level (a higher than normal value) of IL-1β was detected in only 1/30 (3.3%) colorectal carcinoma cases by Huanrun et al.[42] Some investigators have described the presence of IL-1 in the synovial fluid of RA patients.[43,44] As in other organs, IL-1 has been implicated in the pathogenesis of immune, inflammatory, and fibrotic kidney diseases.[45] The elevated production of IL-1α by epithelial cells derived from human benign prostate hyperplasia has been implicated in increased proliferation of these cells.[46] In an unexpected twist, IL-1α was also shown recently to play a pivotal role in the pathogenesis of liver cancer.[47] Also, the role of IL-1 in the growth of pituitary adenomas has been suggested.[48]

IL-1, is one of the polypeptide messenger of inflammation that drives tumor angiogenesis. TNF-α and IL-1, present in host stromal cells surrounding breast, prostate, bladder and colorectal cancer, stimulate tumor growth. Kimura et al implicated that IL-1β, and to a minor extent IL-1α, were required for in vivo angiogenesis and invasiveness of tumors in vivo.[49,50]

Thus, the IL-1 levels are increased in many tumors, in which IL-1β promotes tumor growth and IL-1α induces antitumor immunity.[51] However, several other studies reported the tumor-promoting role of IL-1α.[13] IL-1α, expressed in both normal tissue and several tumor cells, is a regulatory cytokine that can induce the activation of transcription factors, including NF-kB and AP-1, and promotes the expression of genes involved in cell survival, proliferation, and angiogenesis.[30]

IL-1β role in cancer associated inflammation is controversial. Low concentration of IL-1β may induce a local inflammatory response leading to activation of protective immune response whereas, high concentration of IL-1β results in inflammation-associated cancer damage.[51] The importance of IL-1β in tumour spread was demonstrated by the observation that metastasis associated with melanoma, mammary and prostate cancer models were inhibited in IL-1β deficient mice.[52] In a study by Zhang GJ and Adachi I, only 4/46 Japanese patients with metastatic breast cancer had detectable IL-1β concentrations and no correlations were found between these levels and clinicopathological parameters.[53] Brailo et al[54] observed that serum IL-1β concentrations were below the level detection in all three groups- oral cancer, leukoplakia and healthy control group which is partially in concordance with results of Wong et al[55] who reported undetectable serum IL-1β concentrations in more than 50% of healthy individuals. Joblonska et al[56] reported significantly higher concentrations of IL-1β in oral cancer patients compared to healthy controls while Hathaway et al[57] and Hoffmann et al[58] found no significant differences between the two groups. A low concentration of IL-1β has been shown to induce local inflammatory responses followed by activation of protective immune response, while a high concentration of IL-1β leads to inflammation-associated tissue damage and tumor invasiveness.[51]

Moreover, in the present study, IL-1α inversely correlated with tumor size, lymphatic permeation and differential status, which indicates that IL-1α levels were elevated in patients having well differentiated tumors with small tumor size and absence of lymphatic permeation. So, IL-1α is not associated with more aggressive behaviour of thyroid cancer, in fact it can be well detected in goitre and at the early inset of thyroid carcinogenesis.

Contrarily, IL-1β was found to be significantly positively correlating to the lymphnode metastasis and the differential status of the tumor, indicative of higher levels of IL-1β in moderate/poorly differentiated tumors having lymphnode metastasis.

Thus, IL-1β showed appreciable specificity and sensitivity to discriminate between healthy individuals and patients having different thyroid diseases.

Conclusion

This study concludes that measuring IL-1α levels in the serum of patients with goitre could help to differentiate them from healthy individuals and IL-1β might prove useful as serum biomarker and may have role in thyroid cancer pathogenesis. Thus, differential strategy of IL-1α and IL-1β in malignant cells or in the tumor's microenvironment can open new avenues for using IL-1 in cancer therapy.

In summary, IL-1 family member processing and secretion mechanisms are unusual and complex compared with other cytokines. However, further studies including more number of patients are essential to define them fully and to understand their particular role in the initiation of immune responses following cellular stress during various inflammatory, infectious or autoimmune diseases and carcinogenesis.

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"A vigorous five-mile walk will do more good for an unhappy but otherwise healthy adult than all the medicine and psychology in the world."

Paul Dudley White

Cytoplasmic Her-2/neu Internal Domain Expression a Truncated form Confirmed by Double Staining Immunohistochemistry Identifies an Aggressive Breast Cancer Phenotype

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Summary

Her-2/neu over expression is found in women with breast cancers and recent studies indicated prognostic significance of Her-2/neu over expression may be due to associated expression of p95Her-2/neu, a truncated form of Her-2/neu lacking the extracellular domain. The aim of the study was to detect truncated form of Her-2/neu (p95Her-2/neu) by double staining immunohistochemistry method on formalin fixed paraffin embedded tumor tissues of breast cancer patients and compare its expression with clinicopathologic parameters and disease outcome. In this study, 90 patients with breast cancer were enrolled. Double staining immunohistochemistry method was performed to confirm cytoplasmic staining as truncated form of Her-2/neu, using antibodies against Her-2/neu internal domain (CB11), Cytokeratin (polyclonal, and AE1/AE3) and Her-2/neu external domain (SP3) in three combinations. Over expression of membranous Her-2/neu internal domain, cytoplasmic Her-2/neu internal domain and Her-2/neu external domain was found in 70%, 30% and 33% of patients with breast cancer, respectively. A significant positive correlation of membranous Her-2/neu internal domain expression was observed with cytoplasmic Her-2/neu internal domain expression (p=0.001) and Her-2/neu external domain expression (p=0.001). Further, univariate survival analysis indicated that patients with cytoplasmic Her-2/neu internal domain positivity significantly associated with reduced disease free survival (DFS) and overall survival (OS) as compared to cytoplasmic Her-2/neu internal domain negativity. Multivariate survival analysis also indicated patients with cytoplasmic Her-2/neu internal domain positivity significantly associated with reduced DFS as compared to respective counterpart. Cytoplasmic Her-2/neu (truncated Her-2/neu) identifies an aggressive phenotype of breast cancer. Double staining immunohistochemistry technique may provide a unique tool for evaluation of truncated Her-2/neu in breast cancer.

Keywords: Breast cancer, Membranous Her-2/neu internal domain, Cytoplasmic Her-2/neu internal domain, Her-2/Neu external domain, Double staining immunohistochemistry

Introduction

Her-2/neu, 185 kDa oncoprotein is one of the four family members of transmembrane tyrosine kinase receptors HER1 to HER4, that share a similar structure composed of an extracellular ligand-binding domain, a short hydrophobic transmembrane region, and a cytoplasmic tyrosine kinase domain. Her-2/neu emerged as an important cellular target for development of many new cancer therapies over the last few years and gained considerable interest, in its role as prognosticator and predictor of response to therapy.[1-4]

Ross et al have summarized results of 107 studies with 39,730 patients and observed differences in the study conclusions may be attributed to differences in number of patients, patient population including those receiving systemic adjuvant therapy, length of follow up, and most importantly Her-2/neu full length status determination and interpretation techniques.[5] Of various methods for determining Her-2/neu protein expression immunohistochemistry has been the predominant method utilized.

Recent reports indicated prognostic significance of Her-2/neu over expression may be due to associated expression of p95Her-2/neu, a truncated form of Her-2/neu lacking the extracellular domain.[6] The p95Her-2/neu was originally identified as amino-terminally truncated fragment(s) of Her-2/neu presumed to be remnant of metalloprotease mediated proteolytic cleavage of the Her-2/neu external domain (ECD).[7] A dimerization motif in the cytoplasmic domain of p185Her-2/neu may be responsible for constitutive activation following the removal of ectodomain.[8] Aminoterminally truncated fragments of Her-2/neu have been identified in human breast tumors that show similar but distinct migration patterns on Western blots with antibodies against Her-2/neu.[9,10] Deletion of Her-2/neu ECD increases the tyrosine kinase activity and transforming efficiency of the resulting truncated protein.[11,12] Truncated Her-2/neu has also been studied in cell lines and experimental models and treatment efficacy have been evaluated by two study groups.[13,14]

In humans, truncated Her-2/neu has been evaluated in tissues and peripheral blood. ECD of Her-2/neu is cleaved from cell surface by matrix metalloproteases and released into blood can be detected by ELISA.[15,16] In tissues western blot analysis has been described for detection of truncated Her-2/neu but requires a large amount of fresh frozen tissue.[7] On paraffin sections few methods have been described, one is conventional immuno-histochemistry determines amount of p95Her-2/neu based on a difference between amount of Her-2/neu

ICD and Her-2/neu ECD.[17] Second an immuno-flourescent based assay [13] and third an image analysis, by which p95Her-2/neu was evaluated using digitized ratio.[18] Fourth most recent method is VeraTag p95 assay which uses a novel antibody that can specifically detect and quantitate p95Her-2/neu on formalin fixed tumors .[14]

The idea behind present study for detection of p95Her-2/neu was based on our previous study findings where a splice variant of Her-2/neu mRNA was noted in breast tumors which suggested protein produced by spliced mRNA may be truncated. Interestingly by immunohistochemistry, cytoplasmic staining of Her-2/neu along with membranous staining was observed in breast tumors using CB11 antibody against Her-2/neu internal domain. This cytoplasmic staining as truncated form was confirmed by double staining immunohistochemistry method using antibodies against Her-2/neu internal domain and Her-2/neu external domain along with Cytokeratin. Further, expression of cytoplasmic Her-2/neu, membranous Her-2/neu internal domain and external domain were correlated with clinicopathological parameters, disease outcome and Her-2/neu allelic expression.

Patients and Methods

Patients

In this study, 90 untreated breast cancer patients (stage I N=9, stage II N=55, stage III N=24, stage IV N=02) diagnosed and treated at Gujarat Cancer & Research Institute, a regional cancer centre of Western India, were enrolled with exclusion of triple negative cases associated with worst disease outcome. Patients with Luminal A subtypes (Her-2/neu negative subtypes) were included for comparison between Her-2/neu positive subtypes (Luminal B and Her-2 positive subtypes). Detailed clinical history was recorded from case files maintained at Medical Record Department. UICC TNM classification was followed for disease staging. Primary treatment offered was surgery followed by adjuvant treatment [Cyclophosphamide +Methotrexate+5-Fluorouracil (CMF)N=2, CMF+Tamoxifen (TMX)N=5, CMF+radiotherapy (RT)N=5,CMF+TMX+RT(N=3), 5-Fluo-rouracil+Adriamycin+Cyclophosphamide (FAC) N=11, FAC+TMX(N=17), FAC+RT(N=11), FAC+TMX+RT(N=14) and other combination (FAC+CMF+TMX+RT, RT+TAXOL, EMESET+5FU+ TMX, N=22)]. Minimum followup period considered was 5 years or death within that period and maximum followup period noted was 134 months with a median followup of 50 months. Thirty-nine percent (35/90) of patients relapsed and 21% (18/90) of patients died due to cancer within the study period. This work was approved by Scientific and Ethics committee of the institute and informed consent was obtained from the patients.

Immunohistochemical localization of Her-2/neu

It was performed on formalin fixed paraffin embedded (FFPE) tissues containing primary tumor evaluated by Hematoxylin and Eosin staining, on Ventana Benchmark XT autostainer using Ventana reagents (Ventana, USA). Four microns sections were cut and taken onto APES coated slides. Primary antibodies against Her-2/neu internal domain (Clone CB11, Biogenex, Dilution 1:30) and external domain (Clone SP3, DBS, Dilution 1:30) were used to detect Her-2/neu on individual sections. Immuno-histochemical procedure included following steps of deparafinization with EZ Prep (Ventana), antigen retrieval with CC1 (Ventana) for 30 minutes, incubation with 100 µl primary antibody for 32 minutes at 37°C (Her-2/neu CB11, or Her-2/neu SP3) and staining with Ultra View DAB Detection kit for 8 minutes (Ventana), counterstaining with hematoxylin for 8 minutes (Ventana) and mounting with DPX.

Using CB11 antibody against Her-2/neu internal domain, membranous and cytoplasmic staining pattern was observed (Figure 1a) while using SP3 antibody against Her-2/neu external domain pure membranous staining pattern was observed (Figure 1b). To confirm cytoplasmic staining as truncated form of Her-2/neu double staining immuno-histochemistry method was performed, using antibodies against Her-2/neu internal domain (CB11), Cytokeratin (polyclonal, and AE1/AE3) and Her-2/neu external domain (SP3) in three combinations. Tumor sections were stained with first combination of mouse monoclonal Her-2/neu internal domain antibody (Clone CB11, Biogenex) and Cytokeratin antibody (polyclonal, DBS) using double staining method. Double staining protocol including following steps of deparafinization with EZ Prep, antigen retrieval with CC1 for 30 minutes, incubation with Her-2/neu primary antibody (Clone CB11, Biogenex, Dilution 1:30) for 1 hour at 37°C, staining with Ultraview DAB detection kit for 8 minutes, denaturation of Her-2/neu CB 11 antibody at 95°C for 4 minutes, incubation with second primary Cytokeratin antibody (polyclonal, DBS, Dilution 1:30) for 40 minutes at 37°C, staining with Alkaline Phosphatase detection kit, counterstaining with hematoxylin and mounting with DPX. Her-2/neu was stained with chromogen DAB and Cytokeratin was stained with chromogen Alkaline Phosphatase red enhancer which showed brown colour and pink colour, respectively. Overlapping signal (brown and pink) in the cytoplasm of colocalization CB 11 Her-2/neu antibody with Cytokeratin antibody was

recorded (Figure 1c). It was further confirmed by using second combination of rabbit monoclonal Her-2/neu external domain antibody as first primary antibody (Clone SP3, DBS, Dilution 1:30) and mouse monoclonal Cytokeratin antibody (Clone AE1/AE3 , DBS, Dilution 1:30) as second primary antibody, and third combination of mouse monoclonal Her-2/neu internal domain antibody (Clone CB11, Biogenex, Dilution 1:30) as first primary antibody and rabbit monoclonal Her-2/neu external domain antibody (Clone SP3, DBS, Dilution 1:30) as second primary antibody. Using second combination pure membrane staining of Her-2/neu external domain and pure cytoplasmic staining of Cytokeratin depicted brown and pink in colour, respectively (Figure 1d). In third combination, overlapping signals of membranous staining of Her-2/neu internal domain and external domain along with pink cytoplasmic staining of Her-2neu internal domain was observed (Figure 1e). Immunohisto-chemical localization of estrogen receptors (ER, Clone SP1, Thermo Scientifc, Dilution 1:100) and progesterone receptors (PR, Clone SP2, Thermo Scientific, Dilution 1:100) was performed simultaneously to categorize patients according to molecular subtypes.

Scoring

For each of tumor section staining intensity and number of positive tumors cells were evaluated in area consisting abundant tumor cells. The scoring was done using the ASCO and CAP guidelines 2007 wherein the immunoreactivity scored as negative for 0 (no membrane staining), 1+ (faint or incomplete membrane staining), equivocal 2+ (10%-30% with strong membrane staining) and positive 3+ (>30%, tumor cells with complete membrane staining). Her-2/neu 0 and 1+ score interpreted as negative, and 2+ and 3+ interpreted as positive for membranous Her-2/neu internal and external domain expression. For cytoplasmic Her-2/neu internal domain scoring any degree of positivity was considered positive.

Statistical analysis

The data was statistically analyzed using the SPSS statistical software, version 15. Two tailed $\chi 2$ test was used to assess the association between two parameters. Correlation between two parameters was calculated using Pearson's correlation coefficient (r) method. Univariate and multivariate survival analysis for Disease Free Survival (DFS) and Overall Survival (OS) was done by Kaplan-Meier method and Cox-Forward Stepwise Regression method, respectively. P values $\leqslant 0.05$ was considered significant.

Results

Incidence

In this study breast cancer patients with Luminal A, Luminal B and Her-2 positive subtypes were included. In these patients, overexpression of membranous Her-2/neu internal domain was found in 70% (63/90) of patients {2+ (22%, 20/90) or 3+ (48%, 63/90)}. Of them, 30% (27/90) patients showed cytoplasmic expression along with membranous expression by CB11 antibody against internal domain {1+ (25%, 23/90) or 2+ (5%, 4/90)}. Overexpression of membranous Her-2/neu external domain was observed in 33% (30/90) of patients by SP3 antibody against external domain {2+ (6%, 5/90) or 3+ (27%, 25/90)}.

The cytoplasmic Her-2/neu internal domain expression as truncated form of Her-2/neu was confirmed by double staining immunohistochemistry method which showed an overlapping signal (brown and pink) of colocalization of CB 11 Her-2/neu antibody and Cytokeratin antibody. A pure pink cytoplasmic staining with colocalization of Her-2/neu external and internal domain antibodies was noted (Figures 1a-e).

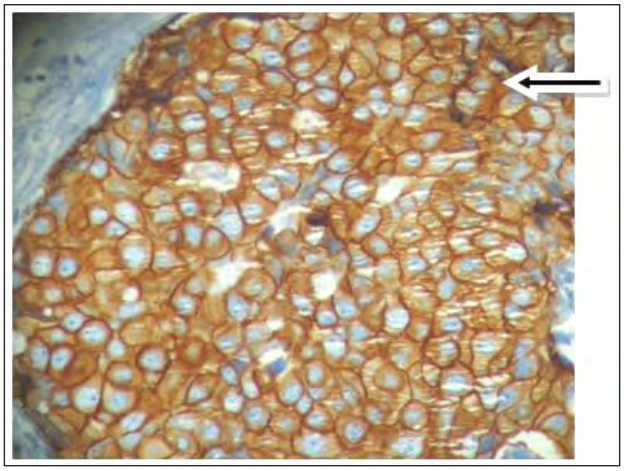

Figure 1a: Membranous and cytoplasmic expression of Her-2/neu internal domain was observed in a breast tumor using CB11 antibody

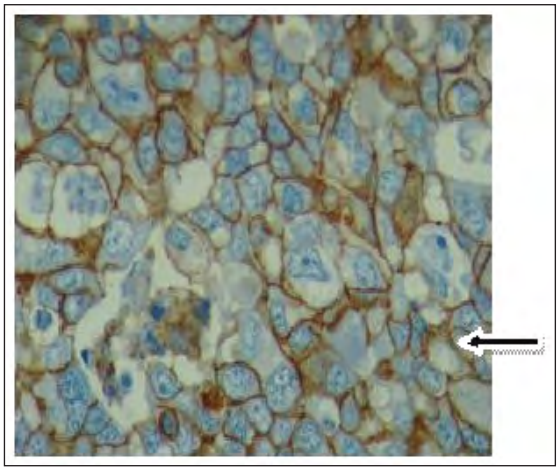

Figure 1b: Pure membranous expression of Her-2/neu external domain was observed in a breast tumor using SP3 antibody

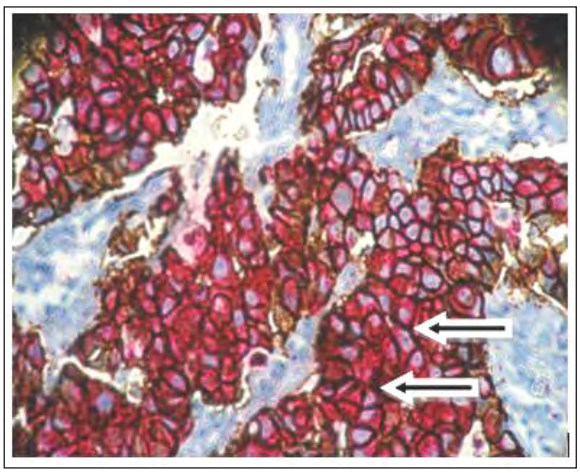

Figure 1c: Double staining immunohistochemistry with Her-2/neu internal domain antibody CB11 and polyclonal Cytokeratin showed colocalization of CB-11 antibody with Cytokeratin antibody as observed by overlapping signals of brown and pink colour confirmed cytoplasmic staining

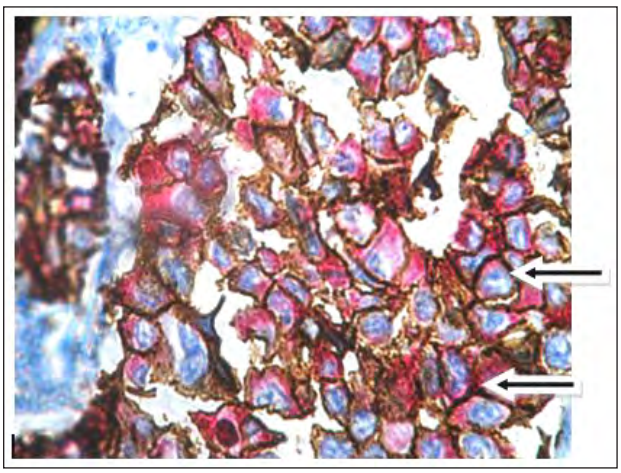

Figure 1d: Double staining immunohistochemistry with Her-2/neu external domain antibody SP3 and Cytokeratin AE1/AE3 showed pure membranous expression of Her-2/neu external domain (brown colour) and cytoplasmic expression of Cytokeratin (pink colour) in a breast tumor

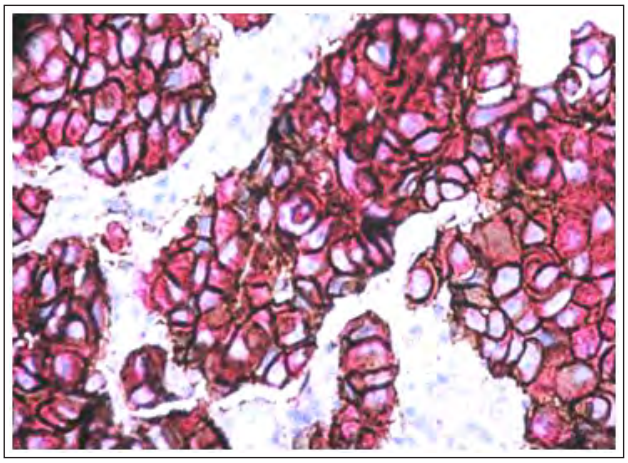

Figure 1e: Double staining immunohistochemistry with Her 2/neu external domain antibody SP3 and internal domain antibody CB11 showed overlapping signals of Her-2/neu internal domain and external domain (brown colour) and cytoplasmic staining of Her-2/neu internal domain (pink colour) which further confirmed cytoplasmic staining as truncated Her-2/neu

Correlation with clinicopathological parameters

Membranous Her-2/neu internal domain expression when correlated with clinicopathologic parameters, significantly higher incidence was noted in histologic grade (HG) III tumors (86%, 25/29, P=0.05), ER negative tumors (94%, 46/49, p=0.001) and PR negative tumors (85%, 45/59, p=0.001) as compared to their respective counterparts (Table 1).

Cytoplasmic Her-2/neu internal domain expression when correlated with clinicopathologic parameters, a trend of higher incidence was noted in T4 tumors (75%, 4/8), Nuclear grade (NG)III tumors (44%, 4/9), ER negative tumors (43%, 21/49, p=0.001) and PR negative tumors (38%, 20/53, p=0.05) tumors as compared to their respective counterparts. Further, none of the lobular carcinoma exhibited cytoplasmic Her-2/neu expression(Table 1).

Membranous Her-2/neu internal domain expression when correlated with clinicopathologic parameters, a trend of higher incidence was noted in NG III tumors (56%, 5/9), ER negative tumors (47%, 23/49, p=0.001) and PR negative tumors (42%, 22/53, p=0.05) as compared to their respective counterparts. Further with histological type, Her-2/neu external domain expression was observed only in IDC (Table 1).

Correlation between expression of membranous Her-2/neu internal domain, cytoplasmic Her-2/neu internal domain, and membranous Her-2/neu external domain

A significant positive correlation was noted between membranous Her-2/neu internal domain expression and cytoplasmic Her-2/neu internal domain expression (χ^2=16.53, r=+0.42, p=0.001). Forty-three percent (27/63) of patients with membranous Her-2/neu internal domain positivity showed cytoplasmic Her-2/neu expression while 57% (36/63) patients with membranous Her-2/neu internal domain positivity were negative for cytoplasmic Her-2/neu. None of the patients with membranous Her-2/neu internal domain negativity expressed cytoplasmic Her-2/neu.

Membranous Her-2/neu internal domain expression when correlated with Her-2/neu external domain expression, patients with membranous Her-2/neu internal domain negativity were negative for Her-2/neu external domain expression. Further, 48% (30/63) of patients with membranous Her-2/neu internal domain positivity showed Her-2/neu external domain expression while 52% (33/63) patients with membranous Her-2/neu internal domain positivity did not show external domain Her-2/neu expression. A

Table 1: Correlation of membranous Her-2/neu internal domain, cytoplasmic Her-2/neu internal domain, and Her-2/neu external domain expression with clinicopathological parameters

Parameters	N (%)	Membranous Her-2/neu internal domain expression		Cytoplasmic Her-2/neu internal domain expression		Membranous Her-2/neu external domain expression	
		Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)
Age in years							
⩽45	48 (53)	16(33)	32(67)	34(71)	14(29)	30(63)	18(38)
>45	42 (47)	11(26)	31(74)	24(69)	13(31)	30(71)	12(29)
Menopausal Status							
Pre Menopausal	43 (48)	15(35)	28(65)	32(74)	11(26)	27(63)	16(37)
Perimenopausal	01 (01)	00(00)	01(100)	00(00)	01(100)	00(00)	01(100)
Postmenopausal	46 (51)	12(26)	34(74)	31(67)	15(33)	33(72)	13(28)
Tumor size							
T1	13 (14)	04(31)	09(69)	09(69)	04(31)	08(62)	05(38)
T2	47 (52)	12(26)	35(74)	34(72)	13(28)	28(60)	19(40)
T3	26 (29)	10(39)	16(62)	19(73)	07(27)	21(81)	05(19)
T4	04 (05)	01(25)	03(75)	01(25)	03(75)	03(75)	01(25)
Lymph node							
Negative	52 (58)	15(29)	37(71)	35(67)	17(33)	38(73)	14(27)
Positive	38 (42)	12(32)	26(68)	28(74)	10(26)	22(58)	16(42)
Stage							
Ia	04 (04)	01(25)	03(75)	02(50)	02(50)	03(75)	01(25)
Ib	05 (06)	02(40)	03(60)	03(60)	02(40)	02(40)	03(60)
IIa	35 (39)	09(26)	26(74)	26(74)	09(26)	25(71)	10(29)
IIb	20 (22)	04(20)	16(80)	13(65)	07(35)	09(45)	11(55)
IIIa	18 (20)	08(44)	10(56)	14(78)	04(22)	14(78)	04(22)
IIIb	06 (07)	02(33)	04(67)	04(67)	02(33)	06(100)	00(00)
IV	02 (02)	01(50)	01(50)	01(50)	01(50)	01(50)	01(50)
Histology							
IDC	72 (80)	24(33)	48(67)	51(71)	21(29)	45(63)	27(37)
Lobular	02 (02)	00(00)	02(100)	02(100)	00(00)	02(100)	00(00)
Phylloid	00 (00)	00(00)	00(00)	00(00)	00(00)	00(00)	00(00)
Mucinious	01 (01)	00(00)	01(100)	01(100)	00(00)	01(100)	00(00)
Medullary	06 (07)	01(17)	05(83)	03(50)	03(30)	06(100)	00(00)
IDC+DCIS	05 (06)	02(40)	03(60)	04(80)	01(20)	03(60)	02(40)
IDC+Lobular	02 (02)	00(00)	02(100)	01(50)	01(50)	02(100)	00(00)
Comedo+DCIS	02 (02)	00(00)	02(100)	01(50)	01(50)	01(50)	01(50)
Histological Grade							
I	02 (02)	00(00)	02(100)	01(50)	01(50)	02(100)	00(00)
II	59 (66)	23(39)	36(61)	43(73)	16(27)	41(70)	18(30)
III	29 (32)	04(14)	25(86)a	19(66)	10(34)	12(59)	12(41)
Nuclear Grade							
I	01 (04)	00(00)	01(100)	01(100)	00(00)	01(100)	00(00)
II	14 (58)	05(36)	09(64)	11(79)	03(21)	09(64)	05(36)
III	09 (38)	04(44)	05(56)	05(56)	04(44)	04(44)	05(56)
BR Score							
3	00 (00)	00(00)	00(00)	00(00)	00(00)	00(00)	00(00)
4	02 (04)	01(50)	01(50)	02(100)	00(00)	02(100)	00(00)
5	02 (04)	01(50)	01(50)	02(100)	00(00)	02(100)	00(00)
6	13 (23)	03(23)	10(77)	07(54)	06(46)	07(54)	06(46)
7	18 (32)	05(28)	13(72)	13(72)	05(28)	11(61)	04(39)
8	21 (38)	05(24)	16(76)	14(67)	07(33)	13(62)	08(38)
9	00 (00)	00(00)	00(00)	00(00)	00(00)	00(00)	00(00)
Lymphatic Permeation							
Negative	29 (45)	08(28)	21(72)	21(72)	08(28)	19(66)	10(34)
Positive	35 (55)	10(29)	25(71)	21(60)	14(40)	22(63)	13(37)
Vascular Permeation							
Negative	58 (92)	16(28)	42(72)	38(66)	20(34)	37(64)	21(36)
Positive	05 (08)	02(40)	03(60)	03(60)	02(40)	04(80)	01(20)
Estrogen Receptor							
Negative	49 (54)	03(06)	46(94)	28(57)	21(43)	26(53)	23(47)
Positive	41 (46)	24(59)	17(42)b	35(85)	06(15)c	34(83)	07(17)d
Progesterone Receptor							
Negative	53 (59)	08(15)	45(85)	33(62)	20(38)	31(58)	22(42)
Positive	37 (41)	19(41)	18(51)e	30(81)	07(19)f	29(78)	08(22)g

(a: χ^2 = 6.75, r=+0.19, p=0.05, b: χ^2 = 29.20, r=-0.57, p=0.001, c: χ^2 = 8.46, r=-0.30, p=0.001, d: χ^2 = 8.96, r=-0.31, p=0.001, e: χ^2 = 13.64, r=-0.38, p=0.001, f: χ^2 = 3.67, r=-0.20, p=0.05 , g: χ^2 = 3.87, r=-0.20, p=0.05)

significant positive correlation was noted between membranous Her-2/neu internal domain expression and membranous Her-2/neu external domain expression (χ^2=19.28, r=+046, p=0.001, Table 2).

Among cytoplasmic Her-2/neu internal domain positive group, 59% (16/27) of patients showed Her-2/neu external domain expression while 41% (11/27) patients did not show Her-2/neu external domain expression. In cytoplasmic negative group, 22% (14/63) of patients showed Her-2/neu external domain expression. Further, H-score was also calculated to compare mean and median H-score of membranous Her-2/neu internal and external domain expression in cytoplasmic Her-2/neu internal domain positive group. The mean and median H-score of membranous Her-2/neu internal domain expression (mean = 73.85, median = 240) was found higher than the mean and median H-score of external domain expression (mean = 53.46, median = 225). The lower mean and median H-score of Her-2/neu external domain in cytoplasmic positive group suggested the shedding of external domain from the tumor to peripheral blood.

Univariate survival analysis

In relation to DFS and OS, a significantly higher incidence of relapse was observed in patients with cytoplasmic Her-2/neu internal domain positivity (61%, 14/23) than patients with cytoplasmic Her-2/neu internal domain negativity (33%, 19/58, Log Rank χ^2=3.70, df=1, p=0.05). A higher incidence of death was seen in patients with cytoplasmic Her-2/neu internal domain positivity (33%, 9/27) than patients with cytoplasmic Her-2/neu internal domain negativity (15%, 9/61, Log Rank χ^2=3.77, df=1, p=0.05). Further membranous Her-2/neu internal domain and external domain expression did not discriminate patients with better or worse DFS and OS (Table 3).

Table 2: Intercorrelation of markers and correlation with Her-2/neu allelic expression

	Membranous Her-2/neu internal domain (ID)		Cytoplasmic Her-2/neu internal domain		Membranous Her-2/neu external domain	
	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)
Membranous Her-2/neu ID						
Negative(27)	-	-	27(100)	00	27(100)	00(00)
Positive(63)	-	-	36(57)	27(43)	33(52)	30(48)
Cytoplasmic Her-2/neu ID						
Negative(63)	-	-	-	-	49(78)	14(22)
Positive(25)	-	-	-	-	11(41)	16(59)
Allelic subtypes						
Val 15 (21)	09(60)	06(40)	12(80)	03(20)	12(80)	03(20)
lle 15 (21)	04(27)	11(73)	10(67)	05(33)	09(60)	06(40)
Ile/Val 41 (58)	09(22)	32(78)	28(68)	13(32)	25(61)	16(39)
	χ^2 =7.60,r=+0.03,p=0.01		χ^2 =0.85,r=+0.08,p=0.46		χ^2 =1.93,r=+0.14,p=0.24	

Table 3: Univariate survival analysis for Disease Free Survival and Overall Survival of membranous Her-2/neu internal domain, cytoplasmic Her-2/neu internal domain, and Her-2/neu external domain expression

Disease Free Survival	Relapse N (%)	Remission N (%)	Overall survival	Alive N (%)	Death N (%)
Membranous Her-2/neu internal domain					
Negative (24)	13(54)	11(46)	Negative (25)	19(76)	06(24)
Positive (57)	35(61)	22(39)	Positive (63)	51(81)	12(19)
(Log rank χ^2=0.37, df=1, p=0.54)			(Log rank χ^2=0.44, df=1, p=0.50)		
Cytoplasmic Her-2/neu internal domain					
Negative (58)	39(67)	19(33)	Negative(61)	09(15)	52(85)
Positive (23)	09(39)	14(61)	Positive (27)	18(67)	09(33)
(Log rank χ^2=3.70, df=1, p=0.05)			(Log rank χ^2=3.77, df=1, p=0.05)		
Membranous Her-2/neu external domain					
Negative (57)	34(60)	23(40)	Negative (58)	48(83)	10(17)
Positive (24)	14(58)	10(42)	Positive (30)	22(73)	08(27)
(Log rank χ^2=0.00, df=1, p=0.97)			(Log rank χ^2=1.77, df=1, p=0.18)		

Multivariate analysis

Multivariate survival analysis was performed by Cox-Forward Stepwise Regression Method including clinicopathological variables ER, PR along with expression of membranous and cytoplasmic Her-2/neu internal domain and Her-2/neu external domain.

In relation to disease free survival, cytoplasmic Her-2/neu internal domain entered at step 1 (Wald=3.78, RR=5.10, 95% CI 0.98-26.43, p=0.05). The relative risk of relapse of patients with cytoplasmic Her-2/neu internal domain expression (truncated Her-2/neu) was approximately 5 times higher than the patients without cytoplasmic Her-2/neu internal domain expression. Regarding overall survival, none of the parameters entered the equation.

Disease free survival and overall survival in relation to treatment

Further correlation of Her-2/neu protein expression with DFS and OS in relation to treatment was evaluated in patients treated with FAC alone and FAC with adjuvant therapy as number of patients treated with CMF alone and CMF with adjuvant therapy or combination chemotherapy was small.

In relation to DFS, patients with cytoplasmic Her-2/neu internal domain expression treated with S+FAC+RT had a significantly low incidence of disease relapse (40%, 2/5, p=0.04) than patients treated with S+FAC (50%,1/2), S+FAC+TMX (67%, 2/3) and S+FAC+TMX+RT (100%, 4/4). No such correlation with treatment was obtained in patients with membranous Her-2/neu internal domain expression and external domain expression for DFS, however, a trend of low incidence of disease relapse was noted in patients with membranous Her-2/neu internal domain expression treated with S+FAC (22%, 2/9). In relation to OS, none of these markers showed significant correlation with treatment; however, a trend of low incidence of death was noted in patients with cytoplasmic Her-2/neu internal domain expression, and membranous Her-2/neu internal domain and external domain expression treated with S+FAC+RT (Table 4).

Correlation with allelic expression of Her-2/neu

In these patients, Ile and Val allele was found in 21% (15/71) each and heterozygous Ile/Val was found in 58% (41/71) of patients. Further, in patients with membranous Her-2/neu internal domain expression, a significant higher incidence of heterozygous allele (Ile/Val) (78%, 32/41) and Ile allele (73%, 11/21) was observed as compared to Val allele (40%, 6/21, χ^2=7.60, r=+0.03, p=0.01). However, in patients with cytoplasmic Her-2/neu internal domain expression and membranous Her-2/neu external domain expression no such significant correlation was observed (Table 2).

Discussion

In present study Her-2/neu internal domain and external domain expression by CB11 antibody and SP3 antibody respectively was evaluated, in a series of 90 breast cancer patients with luminal A, luminal B and Her-2 positive subtypes. Two types of staining pattern membranous and cytoplasmic were observed with CB11 antibody against Her-2/neu internal domain and only pure membranous staining was noted with SP3 antibody against Her2/neu external domain. The main goal was to detect truncated form (cytoplasmic) of Her2/neu which was confirmed by double staining immunohistochemistry method using three different antibodies, in a combination of Her-2/neu internal domain with Cytokeratin, Her-2/neu external domain with Cytokeratin, and Her-2/neu internal domain with external domain antibodies. Cytoplasmic expression of Her-2/neu was seen in 30% of the patients, membranous Her-2/neu internal domain expression in 70% of patients and Her-2/neu external domain expression in 33% of patients. Diagnostic immunohistochemistry considers only membranous staining (2+ or 3+ score) and not the cytoplasmic expression of Her-2/neu to term breast tumor as Her-2/neu positive. Ricardo et al stated CB11 clone against Her-2/neu internal domain shows unspecific cytoplasmic staining while SP3 clone against Her-2/neu external domain gives pure membranous staining and could be better predictor of patient's response to trastuzumab therapy than CB11 clone.[19] On the other hand, numerous recent reports have shown cytoplasmic staining as truncated form of Her-2/neu studied by different methodologies.[6,7,10,13] The initial study of Jose Baselga group used immunoblot method for detection of p95Her-2/neu, a truncated form of Her-2/neu. Further developed an immunofluoresence assay and confirmed cytoplasmic Her-2/neu expression as p95Her-2/neu by colocalisation of CB11 antibody and anticytokeratin antibody and compared with immunoblot. Of 25 tumors with p95Her-2/neu expression detected by immunoblot, 21 were positive for p95Her-2/neu expression by immunofluoresence assay. Also tumors that expressed only full-length p185Her-2/neu by immunoblot did not show cytoplasmic staining of Her-2/neu by immunofluoresence.[13] Till date immunoblot method for p95Her-2/neu detection may be considered as the gold standard, however it requires large amount of fresh-frozen tumor tissue and therefore immunofluoresence based assay was developed for FFPE tissue. Our study adopted double staining immunohistochemistry which is cheaper than immunofluoresence method and can be done along with diagnostic immunohistochemistry. The group of Sperinde et al has successfully generated an antibody

Table 4: Correlation of membranous Her-2/neu internal domain, cytoplasmic Her-2/neu internal domain, and Her-2/neu external domain expression with disease status in relation to treatment

Treatment	Disease Free Survival					
	Membranous Her-2/neu internal domain positive patients		Cytoplasmic Her-2/neu ID internal domain positive patients		Membranous Her-2/neu external domain positive patients	
	N	Relapse N (%)	N	Relapse N (%)	N	Relapse N (%)
S+FAC	9	2(22)	2	1(50)	6	1(17)
S+FAC+RT	13	6(46)	5	2(40)	9	1(11)
S+FAC+TMX	10	5(50)	3	2(67)	13	2(15)
S+FAC+TMX+RT	9	4(44)	4	4(100)	12	3(25)
	(Log rank χ^2=3.25, df =3, p=0.35)		(Log rank χ^2=8.32, df =3, p=0.04)		(Log rank χ^2=3.11, df =3, p=0.37)	
S+CMF	3	0(00)	2	0(00)	2	0(00)
S+CMF+RT	2	1(50)	1	1(100)	0	0(00)
S+CMF+TMX	3	1(33)	2	1(50)	1	1(100)
S+CMF+TMX+RT	2	0(00)	1	0(00)	0	0(00)
Others	6	2(50)	3	3(100)	3	1(33)
Treatment	Overall Survival					
	Membranous Her-2/neu internal domain positive patients		Cytoplasmic Her-2/neu ID internal domain positive patients		Membranous Her-2/neu external domain positive patients	
	N	Died N(%)	N	Died N(%)	N	Died N(%)
S+FAC	11	2(18)	3	1(33)	7	1(14)
S+FAC+RT	13	1(8)	5	1(20)	6	0(00)
S+FAC+TMX	12	4(33)	5	3(60)	5	3(60)
S+FAC+TMX+RT	9	3(33)	4	2(50)	4	2(50)
	(Log rank χ^2=5.69, df =3, p=0.12)		(Log rank χ^2=2.77, df =3, p=0.42)		(Log rank χ^2=5.79, df =3, p=0.12)	
S+CMF	4	1(25)	3	1(33)	3	1(33)
S+CMF+RT	3	0(00)	1	0(00)	1	0(00)
S+CMF+TMX	3	0(00)	2	0(00)	1	0(00)
S+CMF+TMX+RT	2	0(00)	1	0(00)	0	0(00)
Others	6	1(17)	3	1(33)	3	1(33)

that can specifically detect p95Her-2/neu and developed a Vera Tag assay for quantification of p95Her-2/neu expression in FFPE tumor specimens.[14] A study by Heriguchi et al demonstrated 34 of 1053 (4%) cases had cytoplasmic staining but lacked membranous staining with Hercep test and CB11 antibody correlated with neuroendocrine differentiation of breast carcinoma.[20] However, with TAB 250 and SV2-61V antibodies which recognizes Her-2/neu external domain showed no cytoplasmic reactivity in the same study.[20] All these findings support cytoplasmic staining of Her-2/neu by CB11 antibody as specific cytoplasmic staining and detect truncated form of Her-2/neu. Generation of truncated form p95Her-2/neu could be either by alternative splicing, missense mutations, or proteolytic shedding.[21,22] Experimental studies have shown p95 is a proteolytic product rather than product of an alternative transcript.[7,10] The protease responsible for the HER-2/neu shedding from breast carcinoma cells has not yet been identified but is likely a metalloprotease.[17,23]

In current study, cytoplasmic expression was observed in only those patients who showed membranous Her2/neu internal domain expression, and a significant positive correlation observed between cytoplasmic Her-2/neu internal domain with membranous Her-2/neu internal domain, and also with Her-2/neu external domain expression. Cytoplasmic Her-2/neu internal domain expression was noted in 30% of patients which was comparable with the studies of Saez et al, Sclariti et al and Molina et al (Jose Baselga group) where p95 expression was noted in 9%, 20% and 27% of the patients respectively.[6,13,10] Among membranous Her2/neu internal domain positive group, Her-2/neu external domain expression was observed in 48% of patients. In cytoplasmic positive group, Her-2/neu external domain positivity was noted in 59% of patients. It has been shown that external domain shed from tumor cells into peripheral blood which can be detected by ELISA. However, this was a retrospective study and peripheral blood was not available and therefore mean and median of H-score of membranous Her-2/neu internal domain and Her-2/neu external domain expression was evaluated in cytoplasmic Her-2/neu internal domain positive patients wherein the mean and median of H-score of Her-2/neu external domain (mean = 53.46, median = 225) was low as compared to that of membranous Her-2/neu internal domain (mean = 78.85, median = 240). In some of the patients, number of cells showing membranous positivity of Her-2/neu internal domain was higher than cells showing Her-2/neu external domain positivity. In a study by Pallaud et al lower concentrations of Her-2/neu external domain were constantly observed in tumors showing cytoplasmic staining.[24]

Further, membranous and cytoplasmic Her-2/neu internal domain and Her-2/neu external domain expression was correlated with clinicopathological parameters, disease status and treatment offered. Higher incidence of cytoplasmic Her-2/neu internal domain was noted in T4 tumors and NG III tumors. An inverse correlation of cytoplasmic Her-2/neu internal domain with ER, PR was in accordance with findings of Saez et al.[6] Unlike our study, Molina et al observed p95Her-2/neu was not differentially expressed in tumors <2cms versus large tumors, but noted an increasing incidence of p95Her-2/neu with an extent of node involvement.[10] Regarding membranous Her-2/neu internal domain expression, a positive correlation was noted with HG of the tumor and an inverse correlation with ER and PR. Similarly results were found with membranous Her-2/neu external domain expression. An important observation noted was that its expression was observed only in infiltrating ductal carcinoma and tumors with its component. The group of Pallaud et al observed higher incidence of Her-2/neu external domain positive cases in NG III tumors and in intraductal component, and also observed a significant correlation between Her-2/neu detected by IHC with serum Her-2/neu levels by ELISA.[24]

In univariate survival analysis, membranous Her-2/neu internal domain or membranous Her-2/neu external domain did not discriminate patients with better or worse OS. However, patients with cytoplasmic Her-2/neu internal domain positivity showed significantly reduced DFS and OS as compared to patients without cytoplasmic Her-2/neu internal domain expression in univariate analysis. It also emerged as significant prognosticator for DFS in multivariate analysis. Similar to our findings Saez et al also have reported p95Her-2/neu predicts worse outcome in Her-2/neu positive breast cancer.[6] The association of overexpression of p95Her-2/neu with reduced DFS could also be related to its biological properties which are distinct from p185Her-2/neu, such as increased signaling activity and enhanced oncogenic potential.[10] Singhai et al also found decreased survival in patients with elevated serum Her-2/neu external domain.[25] Other two studies evaluated p95Her-2/neu and correlated mainly with clinical outcome to identify response to anti-Her2/neu therapies.[13,14]

Patients with Her-2/neu amplification or over expression are eligible for treatment with trastuzumab, monoclonal antibody directed against Her-2/neu being used in metastatic breast cancer and also indicated in adjuvant therapy in primary breast cancer. Trastuzumab targets Her-2/neu receptor, binds to external domain and cause degradation, thereby inhibits signal transduction pathway. p95Her-2/neu

has often been cited as a likely determinant of trastuzumab resistance[14] because it lacks the Her-2/neu external domain, a trastuzumab binding domain. In the current study, there were only two patients treated with trastuzumab due to affordability of treatment cost and one of two expressed cytoplasmic Her-2/neu. Of two, patient with cytoplasmic Her-2/neu internal domain positivity developed liver metastasis whereas patient with cytoplasmic Her-2/neu internal domain negativity is disease free. In the study of Sclariti et al a series of patients with Her-2/neu positive advanced breast cancer treated with trastuzumab, presence of p95Her-2/neu was associated with clinical resistance to trastuzumab, whereas tumors expressing only full-length receptor exhibited a high response rate to trastuzumab. Within a cohort of trastuzumab treated metastatic breast cancer high levels of p95Her-2/neu were found to correlate with shorter progression free survival and OS who were Her-2/neu positive by Vera Tag Her-2/neu assay.[14] There are several other mechanisms responsible for trastuzumab resistance such as PTEN inactivation or loss and activation of IGF-IR.

With regards to treatment, Her-2/neu positive patients treated with S+FAC or S+FAC+RT showed a trend towards a reduced incidence of relapse and death as compared to addition of TMX in these treatment groups. These results were in accordance with Singhai et al who also observed hormonal resistance in patients with elevated Her-2/neu.[25] Colomer et al demonstrated elevated levels of Her-2/neu external domain adversely affected the efficacy of chemotherapy with biweekly paclitaxel and gemcitabine in cohort of metastatic breast cancer patients.[26]

In summary, cytoplasmic Her-2/neu internal domain expression, a truncated form identifies an aggressive phenotype of breast cancer. Double staining immunohistochemistry technique may provide a unique tool for evaluation of truncated Her-2/neu in breast tumors till antibody to detect p95Her-2/neu becomes commercially available. These laboratory findings are important to transfer to clinics for selection of treatment protocol for breast cancer patients.

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"There is no medicine like hope, no incentive so great, and no tonic so powerful as expectation of something tomorrow."

`

Orison Swett Marden

Effectiveness of Low Dose Rasburicase in Prevention and Treatment of Adult Tumour Lysis Syndrome: A Case Series Study

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Summary

Rasburicase, a recombinant urate oxidase product, is safe and effective in lowering serum uric acid. A schedule of rasburicase at a dose of 0.15-0.2 mg/kg given once daily intravenously for 5-7 days has been recommended in patients at risk of tumor lysis syndrome, but successful treatment with shorter duration of use has also been reported. The treatment is highly effective, but the cost of Rs 15,000 per 1.5 mg vial is very expensive. In year 2012 total four patients were treated with rasburicase at GCRI with a single dose of rasburicase prior to the initiation of chemotherapy, and the subsequent doses omitted as long as the serum urate levels remained below the upper limit of normal. Three of them died due to severe infection but there was drastic fall in serum uric acid even after single low dose of rasburicase, none requiring dialysis without any added drug related complication.

Keywords: Rasburicase, Tumor lysis syndrome, Acute leukemia, Acute lymphoma

Introduction

Patients with acute leukemia or lymphoma with a high tumor burden are at risk for tumor lysis syndrome, especially during the initial phase of induction chemotherapy.[1] This metabolic complication is characterized by hyperuricemia, hyperkalemia, hyperphosphatemia and hypocalcemia. Precipitation of urate, with or without precipitation of phosphate, in the renal tubules leads to acute renal failure. The severely affected patient often requires dialysis treatment, and death is not unusual. Further chemotherapy is inevitably delayed in the survivors.

Allopurinol, a xanthine oxidase inhibitor, has been the traditional preventive therapy for decades. It acts by preventing the breakdown of hypoxanthine to xanthine and the conversion of xanthine into urate. Unlike hypoxanthine, xanthine is poorly soluble in the renal tubules and the accumulation of xanthine in high-risk cases still puts the patient at risk for tumor lysis syndrome. However, allopurinol has no effect on uric acid. Urate oxidase, a naturally occurring compound in microbes that enzymatically degrades urate into highly soluble allantoin, became available for treatment in Europe in 1974.[2] Rasburicase, a recombinant urate oxidase product, became available more recently; its safety and effectiveness in lowering serum uric acid levels.[3,4] A schedule of rasburicase at a dose of 0.15-0.2 mg/kg given once daily intravenously for 5-7 days has been recommended in patients at risk of tumor lysis syndrome, but successful treatment with shorter duration of use has also been reported.[5]

The treatment is highly effective, but the cost of Rs15,000 per 1.5 mg vial is very expensive. As a result in year 2012 , total 4 patients were treated with a single dose of rasburicase at GCRI prior to the initiation of chemotherapy, serum urate levels was measured 4 hour after rasburicase administration in fresh collected blood sample transported in ice cold storage and the subsequent doses omitted as long as it remained below the upper limit of normal, 3 of them died due to severe infection but there was drastic fall in serum uric acid after single low dose of rasburicase, none requiring dialysis without any added drug related complication.

Case 1

An 18-year-old boy was diagnosed with acute lymphoblastic leukemia of PreB-cell subtype with a presenting white blood cell (WBC) count of 37x103/mm^3 (normal 3.9-10.7). He also had HBsAg positive status. Lactate dehydrogenase was 25063 units/L (normal <550 U/L). Serum urate was elevated at 11 mg/dL (normal 3.9-8.7 mg/dL), and renal function was impaired, with creatinine of 1.5 mg/dL (normal for age 0.5-1.2 mg/dL). Serum potassium and phosphate levels were not increased. The patient was thus at high risk for tumor lysis syndrome. Induction chemotherapy was commenced with oral prednisolone and Vincristine (1.4 mg/m^2). Oral allopurinol (100 mg TDS) and hydration without alkalinization of urine were started 24 hours earlier and continued throughout the first week of induction treatment. During second week of therapy second dose of vincristine (1.4 mg/m^2) was given with prednisolone with WBC count of 1x103/mm^3(normal 3.9-10.7x10^3/mm^3). Next day TLS profile was serum Uric acid of 12.46 mg/dL(normal 3.9-8.7mg/dL) , S. K+ of 5.6 meq/L (normal 3.5-5.5 meq/L), S Ca+2 of 3

meq/L (normal 7.8-10.5 meq/L) and renal function was impaired, with creatinine of 5.6 mg/dL (normal for age 0.5-0.8 mg/dL). So patient was in tumor lysis syndrome and allopurinol was stopped due to altered renal function and rasburicase 4.5 mg (0.17 mg/kg) was given intravenously. Serum urate levels dropped precipitously which was measured less than 3 mg/dl after 4 hour of rasburicase administration and remained below the lower limit of normal during the second week of induction chemotherapy. No additional dose of rasburicase was required. However, tumor lysis proceeded subclinically as evidenced by hyperphosphatemia, hypocalcemia, and hyperkalemia. Despite these changes, renal function improved gradually as measured with creatinine of 4.2 mg/dL . The full course of L-Asparginase proceeded normally but patient developed severe infection with septecemia, was treated with broad spectrum antibiotic and died after 14 days of giving rasburicase from septicemia. No adverse effects of rasburicase treatment were noted.

Case 2

A 15-year-old boy was diagnosed with acute lymphoblastic leukemia of the Burkitt's type, a notorious clinical entity predisposing to tumor lysis syndrome. Serum urate was elevated at 14 mg/dL(normal 3.9-8.7 mg/dL) and serum creatinine was 1.2 mg/dL (normal for age 0.5-1.2 mg/dL),WBC count of 23,800/mm^3(normal 3.9-10.7x10^3/mm^3), LDH-31,574 units/L (normal <550 units/L), while serum potassium and phosphate levels were normal. Induction with oral prednisolone was started 24 hours after commencement of oral allopurinol (100mgTDS) and hydration therapy without urinary alkalinization. After 2 days patients WBC count decreased to 12,000 /mm^3(normal 3.9-10.7x10^3/mm^3) and uric acid raised to 18 mg/dL(normal 3.9-8.7 mg/dL). So, rasburicase 1.5 mg (0.03 mg/kg) was given intravenously. Serum urate levels dropped rapidly and measured 2.7 mg/dl after 4 hour of rasburicase administration and maintained the lower limit of normal during next 3 day of induction. Subsequent doses of rasburicase were not needed. The patient developed extensive both lung consolidation and respiratory distress and so was kept on ventilatory support for 1 day patient died next day due to septecemia induced multiorgan failure. No adverse effects were noted with respect to rasburicase treatment.

Case 3

A 24-year-old female was diagnosed with FAB-5 acute Myeloid leukemia with a presenting WBC count of 245x103/mm^3, serum urate was 4.37 mg/dL, serum creatinine, potassium, and phosphate levels were not elevated. He was treated with hydroxyurea preceded by allopurinol (10 mg/kg/d) and hydration therapy for 24 hours. On day 3rd patients WBC count of 135 x 103/mm^3 with serum phosphate of 5.6meq/L (normal 2.5-4meq/L), serum Ca+2 of 5 meq/L (normal 7.8-10.5meq/L) and renal function was impaired, with creatinine of 2.3 mg/dL (normal for age 0.5-0.8 mg/dL) and serum urate was elevated at 9.37 mg/dL. A dose of intravenous rasburicase 1.5 mg (0.03 mg/kg) was given. Subclinical tumor lysis was evidenced by hyperphosphatemia and hypocalcemia, but serum uric acid levels measured less than 1 mg/dl after 4 hour of rasburicase administration during which allopurinol and hydration therapy continued. No additional dose of rasburicase was used. There was improvement in of serum creatinine levels which measured 1.6 mg/dl next day. Patient similar to case 2 developed bilateral lung consolidation and died of respiratory distress. No adverse effects were noted with respect to rasburicase treatment.

Case 4

A 30-year-old male was diagnosed as Stage IV diffuse Large B-cell Lymphoma with generalise lymphadenopathy and spine infiltration, serum urate was 4.37 mg/dL, serum creatinine, potassium, and phosphate levels were not elevated. Bone marrow biopsy and CSF cytology were negative for lymphomatous infiltration. Patient was diagnosed case of HIV positive started on ART. Patient received chemotherapy in form of cyclophosphamide, vincristine and prednisolone at low dose without Adriamycin due to low CD4 count. Patient on follow up of next cycle of chemotherapy had uric acid of 9.45 mg/dL. A dose of intravenous rasburicase 1.5 mg (0.03 mg/kg) was given. Serum urate levels dropped rapidly and was 2.1 mg/dl measured 4 hour of rasburicase administation and remained normal than after during which allopurinol and hydration therapy continued. No additional dose of rasburicase was used. Patient completed entire course of chemotherapy and was under completed remission. There was no adverse effects of rasburicase treatment.

Discussion

Urate oxidase is a proteolytic enzyme that degrades uric acid into highly soluble allantoin and hydrogen peroxide.[3] Therapeutically, it was first used in France as a purified microbial product.[2] Although randomized studies were lacking, the lower rate of French patients requiring renal dialysis during treatment of advanced B-cell malignancies compared with similar patients in the UK and the US suggested a superior role of urate oxidase over allopurinol in the management of tumor lysis syndrome.[2] Only 1.7% of these patients needed renal dialysis in the French national cohort, while 14.3% and 23% of the British and American patients, respectively, using allopurinol underwent dialysis. A nonrandomized study on the use

of nonrecombinant urate oxidase in 134 children with lymphoid malignancies also confirmed its superior clinical efficacy compared with historical controls.[6] In the latter study, none of the patients treated with urate oxidase required renal dialysis. The adverse effects were few and consisted mainly of allergic or anaphylactoid reactions. However, its use is contraindicated in patients with glucose-6-phosphate dehydrogenase deficiency.

Recently, urate oxidase became available as a recombinant product, rasburicase. Given as a daily injection of 0.15-0.2 mg/kg for 5-7 days in open-label clinical studies, rasburicase has been shown to have potent uricolytic effects and is highly effective in obviating the need for renal dialysis in patients at risk for tumor lysis syndrome.[3,4] In addition, hyperphosphatemia developed less readily compared with conventional management. As phosphate is more soluble in an acidic environment, this effect was thought to be an indirect benefit of rasburicase therapy because there was no need for alkalinization of urine.[3] In a randomized study reported by Goldman et al[7] twenty seven children were treated with rasburicase in the recommended dosage, while 25 children received allopurinol as control. The rasburicase-treated patients experienced a more rapid decline in serum urate levels and a much lower exposure to uric acid during the first week of treatment. Due to the limitation of sample size, however, a renal protective effect was not confirmed, although patients receiving rasburicase tended to have better serum creatinine profiles. At this recommended dosage, however, rasburicase treatment was 9000 times more expensive than conventional allopurinol treatment.[8]

Although the elimination half-life of rasburicase lies between 16 and 21 hours, 4 rapid and sustained reduction of serum uric acid levels for up to 96 hours were observed in healthy volunteers after a single injection during a Phase I study of the compound.[2] In addition, the results of a compassionate-use trial also suggest that a briefer course of rasburicase may be efficacious in patients with malignancy-associated hyperuricemia.[5] Two hundred forty-five children and adults were treated with rasburicase 0.2 mg/kg intravenously for 1-7 days (median 3) with or without concomitant chemotherapy. Although the reduction in serum urate was dramatic as a group, the clinical outcome for those patients who had received a single dose was not evaluated separately.

A single vial of rasburicase 1.5 mg costs Rs 15,000 locally. Given that rasburicase effects a lowering of serum urate rapidly, its use was modified according to the patient's prevailing serum urate levels. For the sake of convenience and avoidance of waste, a single 1.5-mg vial of the drug as a unified dose was used for each patient irrespective of body weight. All patient were tested for G6PD deficiency as sulfamethoxazole and trimethoprim is routinely used for prophalaxis for pneumocystis carni infection after ruling out deficiency of enzyme in patients with haematological malignancy. G6PD deficiency test was negative in all patients hence even rasburicase can be used without any risk of haemolysis. After this single dose, further injections were omitted if the urate levels remained low on daily monitoring. The serum urate levels remained low in all of the patients treated with a single injection of rasburicase except one patient, in spite of the ongoing tumor lysis at a subclinical level. One patient required 3 vial of 1.5 mg to normalize serum uric acid. None of the patient required dialysis. Though 3 patients died, none of the death was due to tumor lysis syndrome or drug hypersensitivity. All three deaths were due to underlying infection leading to septecemia and multi organ dysfunction. Thus, even though rasburicase effectively normalized uric acid level drastically, its impact on overall survival was not noticed. Although the potential accumulation of xanthine in the presence of allopurinol treatment might be deleterious to the kidneys, the phenomenon was not encountered in any of the patients treated. Feng X et al in his recent meta-analysis study of ten studies (eight retrospective and two prospective) evaluated the efficacy and cost savings of a single-dose rasburicase (SDR) regimen compared with the Food and Drug Administration-approved daily dosing of rasburicase (DDR) for 5 days or the traditional treatment with allopurinol in adult cancer patients with hyperuricaemia or at high risk for TLS. SDR response rate was not inferior to that of DDR, and the standard-dose SDR generates more cost savings compared with the DDR. It suggests that the single-dose rasburicase is clinically effective and cost efficient for the prophylaxis of high-risk TLS and the treatment of hyperuricaemia in adult patients with cancer.[9] Trifilio et al in a retrospective review conducted to determine the effect of a fixed 3 mg dose of rasburicase in 43 adult patients with cancer undergoing hematopoietic stem cell transplantation or receiving chemotherapy who had elevated or rising uric acid levels (6.4–16.8 mg/dl; median 9.6). Six patients received a second dose of rasburicase (3 mg in four patients and 1.5 mg in two patients) 24 h later. Uric acid levels declined by 6–95% (median 43%) within the first 24 h after rasburicase administration, and levels at 48 h were 9–91% (median 65%) lower than the baseline levels. Serum creatinine changed by 10% in 21 patients, increased by >10% in four patients and decreased by >10% in 18 patients. No significant renal dysfunction developed in any of the patients. And thus concluded that rasburicase is effective in lowering uric acid levels at a fixed dose of 3 mg, which

is much lower than the recommended dose10. Several other case series using fixed dose of 6 mg and 7.5 mg fixed dose rasburicase have also shown effictiveness though the number of patient were very small to make any recommendation. In our present case series, dose of 1.5 mg only was used which is lowest dose among any of the study mentioned above, still was found effective in 3 out of 4 patients.

Conclusion

Tumor lysis syndrome is an life threatening metabolic complication of rapid cell turnover disease like leukemia and lymphoma. Newer drugs such as rasburicase is available to treat hyperuricemia which is an important component of tumor lysis syndrome leading to renal failure and other metabolic complication. Drug rasburicase in its recommended dose is very expensive and not affordable to large section of patient in developing countries like ours. So, low dose of rasburicase 3, 6 and 7.5 mg has been used in many small series of patients seems equally effective. Our experience of rasburicase in 4 patients treated with further lower dose of 1.5 mg confirms its benefit and can be used in large section of patient due to its cost effectiveness.

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"Sometimes your medicine bottle has on it, 'Shake well before using.' That is what God has to do with some of His people. He has to shake them well before they are ever usable."

Vance Havner

Anaesthetic Management of Children with Moyamoya Disease:A Report of Three Cases

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Summary

Moyamoya disease is a condition that results from bilateral stenosis or obstruction of the intracranial arteries at the base of the brain that usually presents as recurrent strokes in children. Children present with cerebral ischemia, while adults with intraventricular haemorrhages.The surgical procedure EDAS (Encephalo-duro-arteriosynangiosis) is often complicated by cerebral ischemia, so goal in perioperative period is to maintain the balance between oxygen supply and demand in the brain. This report presents three cases of Moyadisease.

Keywords: Moyamoya disease, EDAS procedure, Anaesthetic management

Introduction

Moyamoya disease is a rare disease first described in 1960.[1] It is characterized by severe stenosis or occlusion of internal carotid arteries (ICA), minimal filling of the anterior and middle cerebral arteries (ACA/MCA) and the presence of a fine network of vessels around the basal ganglia. The name Moyamoya is a Japanese word meaning something like a "puff of cigarette smoke, drifting in the air".[2] Clinical experience would indicate that it is probably more common than the literature suggests. It has a peak incidence in childhood and early adolescence, females being more commonly affected than males. In paediatric cases the most common presentation is recurrent episodes of cerebral ischemia manifesting clinically as hemiplegia, monoplegia, paraesthesia, invoulantory movements and convulsions.[1]

The diagnosis is made principally from cerebral angiography, computerized tomography scanning and electroencephalography.[2] Looking at chronic nature of cerebral ischemia and the debilitating course of Moyamoya syndrome, various surgical procedures have been proposed to augment collateral cerebral blood flow.[3] Surgery for this condition is often complicated by cerebral ischemia. So, the goal in perioperative management is to maintain the balance between oxygen supply and demand in the brain.[4] This report describes anaesthetic management of three cases of Moyamoya disease in children.

Case Reports

Case 1: A 7 years old girl weighing 14 kilograms presented with right hemiplegia and ataxia after seizures since 2 months. Her angiography showed smooth arterial narrowing involving both ICA and proximal MCA/ACA with associated increased basal perforators. She was diagnosed as a Moyamoya phenomenon. After admission, syrup Phenytoin 30 mg twice daily and syrup Levotiracetam (100 mg) once a day were started. Patient was revascularised with EDAS, procedure lasted for 120 minutes and 100 ml blood loss was compensated.

Case 2: A 3 years boy weighing 10 kg presented with generalized tonic clonic seizures and unable to speak. He was treated with syrup Valproic acid, syrup Levotiracetam and syrup Phenytoin. Patient was diagnosed as Moyamoya disease on the basis of MR (magnetic resonance) angiography. Angiographic finding showed marked narrowing of intra-canalicular, intracavernous and supra-clenoid portion of left ICA with marked narrowing of M1 and M2 segment of left MCA, A1 and A2 segment of left ACA with multiple abnormal vascular channels in region of bilateral basal ganglia suggesting possibility of systemic vascular disease, more likely Moyamoya disease. Patient was revascularised with EDAS procedure lasted for 180 minutes with no significant blood loss.

Case 3: A 4 years girl weighing 12 kg presented with left hemiplegia and generalized tonic clonic seizures. She was treated with syrup Phenytoin. Tablet Metoprolol (25 mg) once daily was advised by cardiologist for sinus tachycardia. Her 2D echo was normal. Her MR angiography was showing occlusion of right MCA with narrowing of supraclenoid portion of bilateral ICA and left MCA artery, suggestive of possibility of Moyamoya disease. Patient was planned for bilateral EDAS first on left side and than on right side after five months. First EDAS lasted for 180 minutes and 100 ml blood loss was replaced. Second time EDAS lasted for 160 minutes without any significant blood loss.

Anaesthetic Management

All children underwent the EDAS procedure under general anaesthetia. On the day of operation patients were premedicated with oral Midazolam 0.5mg/kg two hours prior to surgery. Their regular dose of anticonvulsant and steroid were also given.

Before venous access, Xylocaine hydrocloride 2% local anesthetic cream was applied topically on puncture site to prevent excessive crying and hyperventilation. They were prepared for continuous monitoring with ECG, noninvasive blood pressure, SpO_2, body temperature and $EtCO_2$ (end tidal carbon dioxide).

Patients were induced with injection (inj.) Glycopyrrolate 0.01 mg/kg, inj. Fentanyl citrate 2 mcg/kg, inj. Thiopentone sodium 5mg/kg and inj. Vecuronium bromide 0.1 mg/kg IV bolus. After proper ventilation with O_2 and Sevoflurane for 3 minutes, patients were intubated with proper sized uncuffed tube. Patients were maintained with O_2+N_2O+Sevoflurane and continuous infusion of inj. Vecuronium bromide 0.1 mg/kg/hr. For brain relaxation inj. Mannitol 1 gm/kg IV(intravenous) was given 20 minutes before dura opening. Inj. Furosemide 1 mg/kg IV was given when needed. $EtCO_2$ was kept within normal limits in all patients by ventilator settings.

All patients were reversed with inj. Glycopyrrolate 0.02 mg/kg IV and inj. Neostigmine bromide 0.05 mg/kg IV and extubated. They did not develop new neurological deficits during initial postoperative period. Intraoperative fluid was administered according to fluid requirement. Post operative analgesia was given according to requirement in the form of suppository.

Discussion

Moyamoya disease is a rare cerebrovascular disease seen both in children and adults with variable progression and presentation. It is characterized by angiographic evidence of progressive stenosis or occlusion of terminal portion of the ICA and the proximal portion of the ACA and MCA. It is probably a genetically inherited, an autosomal dominant disease with low penetrance.[5]

In all three patients there were occlusion of supraclenoid portion of ICA, MCA and ACA. P N. Jaykumar et al noted that "stenosis and occlusion of the supraclenoid ICA and proximal part of ACA/MCA were the commonest angiographic finding".[6] MRI and MRA are safe and suitable for both diagnosis and follow up of Moyamoya disease particularly in pediatric patients.[1]

All our three patients exhibited the classical presentation of childhood disease, transient ischemic attack and strokes. Sulpicio et al studied 13 children of Moyamoya disease with same presentation.[3]

During preanesthetic assessment, special attention should be paid to neurological status, frequency of ischemic attacks, evidence of infarct and angiographic signs of low perfusion or cerebrovascular reactivity.[4]

It is important to prevent perioperative crying by proper premedication. All three patients were premedicated with oral midazolam. While securing venous access, local anesthetic cream was applied to minimize pain because excessive crying causes hyperventilation, hypocapnia and cerebral infarction.

All patients were induced with inj. Thiopentone sodium. It's very important to prevent hypotension, as inj. Thiopentone sodium decreases the already compromised cerebral perfusion. If it occurs, it should be treated promptly with vasoconstrictors. Ideal drug for induction is Thiopentone sodium or Propofol.[4] Fentanyl was administered for suppressing the cardiac response to induction and surgery. Sulpicio G et al used Isoflurane, N_2O and Fentanyl as maintenance drugs because these provided a stable hemodynamic state.[3]

Depolarizing muscle relaxant Vecuronium bromide, which does not cause any cardiovascular changes, histamine release and vasodilatation was used. Nigar et al in their studies used Thiopentone sodium, Remifentanil, Vecuronium bromide and Sevoflurane for induction and maintenance.[4] Some studies showed Ketamine as induction agent and maintenance of patients with O_2+N_2O and Halothane.[2]

Intraoperatively normocapnia ($EtCO_2$ between 25-35) was maintained. CO_2 is a potent modulator of cerebrovascular tone. Hypocapnia causes cerebral ischemia while mild hypercapnia can have undesirable effects on cerebral perfusion.[3] Cerebral ischemia, secondary to vasoconstriction induced by hypocapnia, is a likely cause of the neurological deterioration. So, the maintainance of normal or raised CO_2 levels is important to avoid such problems.[2] Some investigators have recommended relative hypercapnia state during vascular reconstruction.[7] During hyperventilation, hypocapnia decreased regional cerebral blood flow and so, caused hypoxia in the diseased hemisphere due to "steal" from the moyamoya collateral vessels to the dilated cortical vessels after the termination of hyperventilation.[3]

Normothermia (temperature between36-38^{o}c) was maintained by drapping the patients with the cotton pads and infusing warm fluids because rise in temperature causes ischemic attack and drop in body temperature induces vasospasm. Perioperative fluid balance was managed by crystalloids and blood loss by blood to keep normovolemia. Postoperative pain and stress free period is important, so adequate analgesia was given to our patients in the form of Diclofenac sodium/ Paracetamol suppository. Nigar et al used morphine (0.1 mg/kg) for children comfort.[4]

The goal for anaesthesia is to maintain the balance between oxygen supply and demand. This can be achieved by maintaining adequate cerebral perfusion pressure. It is essential to prevent ischemic complication during and after surgery. Main goals during anaesthesia are maintenance of normocapnia, normotension, normovolemia and normothermia.

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"Lost wealth may be replaced by industry, lost knowledge by study, lost health by temperance or medicine, but lost time is gone forever."

Samuel Smiles

Anaesthetic Management of a Case of Insulinoma

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Summary

Insulinoma is a rare neuroendocrine tumour of the pancreas, which is usually small, solitary and benign. It may be part of the multiple endocrine neoplasia type 1 syndrome. It is diagnosed by clinical, biochemical and imaging modalities. Surgical resection is the curative treatment with a high success rate. Intraoperatively, ultrasound and surgical palpation help to confirm the site of tumour. Intraoperatively, maintenance of optimum glucose level is of main concern because there may be severe hypoglycemia while handling the tumour. Here we report anaesthetic management of a case of insulinoma.

Keywords: Insulinoma, Anaesthetic management, Neuro-endocrine tumor

Introduction

Insulinoma is an adenoma of beta (β) cells of islets of Langerhans, and was first described by Harris in 1924. Insulinoma is usually small, solitary, benign and surgically curable.[1] The incidence is 1-4 per million and male to female ratio is 2:3 and usually presented in fifth decade.[2] Fasting hypoglycaemia in a healthy, well-nourished adult should raise the suspicion of insulinoma and trigger further investigation. These hypoglycaemic episodes may be non-specific, remain unrecognized and occasionally misdiagnosed. Hypoglycaemic symptoms appear when the plasma glucose falls below 50 mg/dL and neuroglycopenic symptoms appear at glucose levels below 45 mg/dL due to neuronal deprivation of glucose. Symptoms can be catecholaminergic response to hypoglycaemia (anxiety, tremor, nausea, hunger, sweating and palpitations) or neuro-glycopenic (headache, lethargy, dizziness, diplopia, blurred vision, amnesia, seizures, confusion and coma).[3] Whipple's triad is pathognomonic of insulinoma which includes symptoms of neuroglycopenia, hypoglycaemia(plasma glucose level less than 50 mg/dL) and relief of symptoms within 5–10 min following glucose administration.[4,5] Recently, the endocrine society clinical practice guidelines recommended the following criteria:[5]

1. Plasma concentrations of glucose less than 55 mg/dL (3.0 mmol/L)
2. Insulin level of at least 3.0 μIU/mL (18 pmol/L)
3. C-peptide of at least 0.6 ng/mL (0.2 nmol/L)
4. Proinsulin of at least 5.0 pmol/L

Medications such as diazoxide and somatostatin can be used to block the release of insulin. But the definitive treatment is surgical removal of the adenoma with or without subtotal or total pancreatectomy.6 Persistent hypoglycemia after surgery tends to occur in patients with multiple tumors. About 2% of patients develop diabetes mellitus after surgery. We describe the anaesthetic management of our patients with pancreatic insulinoma who underwent enucleation.

Case Report

A 55-year old male patient with BMI of 50kg/m2 was admitted with history of repeated attacks of headache, giddiness, restlessness since two years. This hypoglycemic attacks occurred 2-3 times in the night which relieved after eating 3-4 spoonful of sugar since last three months. He has to take small frequent meals throughout the day. His plasma fasting insulin level was 40.2 μIU/mL and plasma fasting glucose was 55 mg/dL. Lipid profile and ECG with other routine investigations were normal. USG abdomen, CT scan and MRI showed a 22x30 mm sized tumor situated at the head of pancreas.

Anaesthetic Management

Patient was scheduled for elective surgery for excision of insulinoma. He was allowed to take dinner containing high carbohydrate diet at 10.00 pm the night before operation and was premedicated with tab Lorazepam 1mg at previous night and tab Diazepam 5 mg in the morning. We used B Brawn glucometer for measuring blood sugar level in perioperative period. Intravenous infusion of dextrose 10% in saline was started at the rate of 30 drops min from midnight. Fasting blood sugar was 148 mg/dL. Pulse, NIBP, SpO_2, ECG were monitored. After preoxygenation for three minutes patient was induced with Inj. Thiopentone sodium and Inj. Vecuronium bromide. Anaethesia was maintained with O_2, N_2O, isoflurane, Inj. Fentanyl and Inj. Vecuronium. Dextrose 10% in saline was continued at the rate of 30 drops/min up to 30 min of removal of the tumour. With another IV channel ringer lactate solution started slowly. Capillary blood glucose measured every 15 minutes. Rapid infusion of 150 ml of dextrose 10% in saline during the handling of the tumour and thereafter it was stopped keeping short acting insulin ready for emergency use. A plum coloured, firm, well encapsulated tumour was removed. Blood sugar was maintained near normal as plotted in Figure 1. At the end of surgery patient was reversed from residual

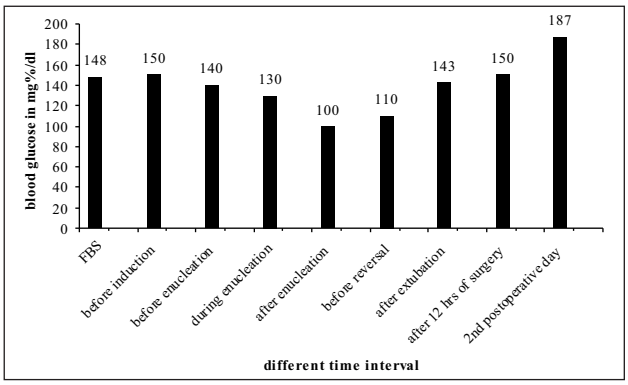

Figure 1: Blood glucose level mg/dl at different time interval

effect of muscle relaxant and was extubated. Patient was transferred to ICU. On the first postoperative day, 1000 ml of dextrose 5% in saline and 1000 ml of RL was administered. In ICU capillary blood glucose measured hourly for 24 hour and every two hourly on subsequent day, which were within normal limit. Post-operative analgesia was ensured with epidural catheter. On the third postoperative day patient was shifted to surgical ward where blood glucose level was closely monitored. Histological examination revealed pancreatic endocrine neoplasm. The patient was discharged from the hospital on 10th postoperative day with normal blood glucose level.

Discussion

Although enucleation is the treatment of choice for all benign insulinomas, intraparenchymal insulinomas may be missed and may require distal or partial pancreatectomy.[7] We did not encounter any difficulty in finding tumour. Frequent glucose monitoring is important to prevent plasma glucose level to fall below 40–50 mg/dL at any time.[8] The maintenance of peri-operative adequate blood glucose level is the prime importance in the anaesthetic management of a patient of Insulinoma. The patient can go into hypoglycaemic attacks during perioperative period if glucose is withheld over a long period of time or the patients are kept fasting for a long period.[3] Intravenous infusion of 10% dextrose should be started for the fasting period.[3]

Intraoperative maintenance of near normal glucose level is possible by frequent measurement of capillary blood glucose and administration of glucose accordingly in the operating theatre. Chari et al[8] has suggested perioperative steroid therapy in view of adrenocortical suppression. Its use is controversial as sometimes it may be harmful in view of hyperglycemia and increase risk of infection in the postoperative period. In our case, we avoided use of steroid and insulin was rarely required during the course of treatment. Insulin degrades at a rate of 2% per minute. Cryer et al[5] had showed a mean rate of increase in blood glucose of 40mg/100ml/hr occurred during the first half an hour after the tumour was removed which was similar to our case.

Conclusion

While the incidence of insulinoma is rare, a basic understanding of the tumor and its effect can facilitate safe intraoperative care of patients. A combination of clinical, biochemical and imaging tests is required to confirm the diagnosis. Surgical resection of the tumour is the treatment of choice. There may be a large swing in plasma glucose during handling of the tumour which should be carefully monitored and controlled.

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Summaries of Presentations at Clinical Meetings

01. Immunolocalisation of Wild Type EGFR, Exon 19 E746-A750 Frame Deletion and Exon 21 L858R Point Mutation in Triple Negative Breast Cancer

Patel Nupur
Immunohistochemistry and Flow-Cytometry Division

Summary

This study evaluated wild type EGFR, E746-A750 frame deletion in exon 19, and L858R point mutation in exon 21 by immunohistochemistry in patients with triple negative breast cancer. A retrospective study included 99 untreated early and advanced stage triple negative breast cancer patients. Immunohistochemical localization of wild type EGFR, EGFR E746-A750 deletion in exon 19, and EGFR L858R mutation in exon 21 was performed on formalin fixed paraffin embedded (FFPE) tissue blocks using mutation specific primary antibodies. EGFR protein expression was noted in 27% (27/99) of patients with 2+ or 3+ staining intensity in 7% (7/99) of patients. Significant correlation was of EGFR protein expression was not found with any of the clinic-pathological parameters. In univariate and multivariate survival analysis EGFR expression (2+ or 3+) emerged as significant prognostic factor for disease free survival. Respect to mutation status, exon 19 deletion was observed in 3% (3/99), exon 21 mutation in 1% (1/99) and both in 1% (1/99) of patients. One patient with exon 19 deletion having EGFR protein 2+ expression developed lung metastasis. Whereas the other patient with exon 19 deletion and one patient with both exon 19 deletion and exon 21 mutation had EGFR protein 1+ expression and remained disease free during study period. EGFR protein over expression was observed in less than one third of TNBCs with very low incidence of EGFR activating mutations in patients of western India.

02. Outcome of Liver Resection-5 Year Experience at GCRI, B.J.Medical College, Ahmedabad - January 2008- December 2012

Kumar Manish
Department of Surgical Oncology

Summary

Hepatocellular carcinoma (HCC) is the 6th most common malignancy worldwide and 3rd most common cause of death due to cancer. Surgical resection or liver transplant offers the only chance for a cure or even of long term survival. The main difficulty related to hepatic resection for treatment of HCCs is the high post-resectional tumor recurrence rate, with 5-year recurrence rates ranging from 42% to 70%. The results of liver surgery have improved dramatically in the last 20 years. There has been a major decrease in peri - operative mortality from 20% to <5% in high volume centres and this improvement has probably been due to a better understanding of hepatic anatomy, the use of hypotensive anaesthesia and better patient selection. Retrospective analysis of 21 cases of hepatic resections done for HCC in our institute from January 2008-December 2012 to define the risk factors associated with postoperative morbidity and mortality. Between January 2008 and December 2012, 21 hepatic resections were done for HCC in GCRI and recorded the details in a maintained database. All the patients underwent a thorough history and physical examination and any associated co morbid conditions were managed appropriately. Perioperative and postoperative findings, final HPE report, hospital stay and overall survival were analysed. As per protocol data was analysed. Our findings will be compared with one another study that was published in World Journal Of Surgery in 2003 and conclusion will be drawn.

03. Management of Hyperglycemia Pre- Peri- Post-Operative Management

Parekh Urvi
Department of Endocrinology

Summary

Presence of hyperglycemia pre, peri or postoperatively increases morbidity & mortality. It is important to control hyperglycemia as chances of dehydration & electrolyte abnormality increases. Surgical wound also weakens thereby increases complications. Blood sugar should be maintained < 180mg/dl in critically ill patients. Patients wellcontrol on oral hypoglycemic agent for minor surgery continue same treatment preoperatively. Type 1 diabetics or type 2 diabetics on insulin require intensive insulin therapy with onde basal and three bolus injections, patients nil by mouth sholud receive insulin infusion.sliding scale is outdated now. Post operatively, infusion sould be continued till patient can take full diet. Intensive insulin therapy is mandatory afterwards.

04. Retrospective Analysis and Outcome of Critical Ill Cancer Patients in Medical Intensive Care Unit

Raut Shreeniwas S,
Department of Medical Oncology

Summary

In a six month retrospective study of patients having admission in medical ICU at Gujarat cancer and research institute (GCRI) from November 2012 to April 2013, 132 admissions were documented, out of these 108 patients were eligible for data analysis. Paediatric age group comprised 18(16.66%), adults 82(75.92%) and geriatric 8(7.4%). The haematological malignancies were 66.66%, solid malignancies 33.33%. The intent of therapy in 67.59% patients was curative and in 32.4% patients it was palliative. Mean duration of ICU stay was 3.57 days (range up to 23 days). The most common reason for ICU admission was pneumonia and other major reasons were neurologic dysfunction, airway compression, septicemic shock, renal dysfunction, differentiation syndrome, cardiac failure, and tumour lysis syndrome. The ventilatory support was required in 56.48% patients. Inotropic support was required in 31.48% patients and both inotropic support and ventilation was required in 24.07%. Culture positivity was documented in 29.62%. Out of total 108 patients, effective ICU mortality was 37% and effective ICU beneficiary were 63%. The factors associated with mortality were haematological malignancies, reason for admission to ICU, requirement of either ventilation or inotrope. Actual outcome: Total deaths were 56(51.85%) while 40(37.03%) patients transferred out and 12 (11.11%) patients left the hospital against medical advice. There were 2(1.85%) readmissions.

05. Malignant Tumors of Head and Neck in Children - A Ten Year Single Centre Retrospective Study in GCRI

Jain Abhishek,
Department of Surgical Oncology

Summary

We attempted to analyze various clinical modes of presentation of malignant paediatric head and neck cancers as well as their management and prognosis. Our study aims at discussing the commonest clinical presentations, diagnosis, types of treatment and outcomes in cancers of the head and neck region in paediatric age group and to compare the results with other similar studies. It is a retrospective study of the last 10 years period between 2002 and 2012 conducted at our Institute-a tertiary regional cancer centre. All patients were investigated thoroughly according to the Standard protocols. Children under the age of 17 year were included. Lymphomas, primary brain tumors, primary ophthalmic tumours and benign head and neck tumors were excluded. The data collected were entered into MS-Excel spread sheets, and analysed. The procedures involved were preliminary data inspection, content analysis, and interpretation. This 10 year (2002-2012) retrospective review identified 59 children under the age of 17 years who presented with malignant head and neck tumors. Nasopharyngeal cancer was the most common followed by rhabdomyosarcoma. Nasopharyngeal cancer, parotid and thyroid cancers were most common in age group more than 10 year whereas rhabdomyosarcoma was common in 0-5 years .nasopharyngeal cancer and rhabdomyosarcoma were treated primarily with chemoradiotherapy and surgery used for salvage. Parotid and thyroid cancers were treated primarily with upfront surgery.Surgical management was done in 18/59 (30.5%) and the rest were managed with chemoradiotherapy. Most common surgeries done were parotidectomy (42.1%) followed by thyroidectomy (27.7%), craniofacial resection (5.5%).

06. Demographic Profile of Adolescent and Young Adult Females with Cancer in Urban Ahmadabad

Bharti Archana
Department Gynaecological Oncology

Summary

Adolescent and young adult (AYA) oncology patients belong to a distinct age group and, like pediatric, adult, and geriatric patients, have unique medical and psychosocial needs. Lack of demographic profile information for Indian AYA population led us to conduct this study. To study the cancer incidence and mortality rates and distribution of various cancers within the young female population of 15-39 year olds in Urban Ahmadabad. During 2010, data on female cancer patients aged 15-39 years from Gujarat Cancer and Research Institute Cancer Registry was analyzed. Cancer distribution and causes of cancer deaths were studied. Crude, Age specific and Age adjusted incidence and mortality rates were calculated using data on population size and its age structure for the corresponding year. Fifteen percent (N=266) new cancer cases and 13.43% (N=59) cancer deaths in female population occurred in AYA group. The incidence to mortality ratio was 4.5:1. Crude incidence and mortality rates were 24.36 and 5.4 per 100,000 populations. Age specific cancer incidence rates by five year age groups were function of age and, ranged 6.4- 57.4 % and 1.6-12.2% respectively. The leading cancer in rank was breast, myeloid leukemia, cervix, brain and nervous system cancers. Breast cancer was the leading cause of death followed by lymphoid leukemia and cancer esophagus. Adolescent and young adult female

population represents a *significant number* and is a distinct group with unique distribution of types of cancer and causes of death.There is a need to focus on leading cancers which are amenable to screening like cancer of breast and cervix. Awareness campaigns and screening programmes can markedly improve survival rates in adolescent and young adults.

07. Reconstruction of Buccal Mucosa Defects Using the Nasolabial Flap: Clinical Experience with 70 Patients

Thakar Krutarth D
Department of Surgical Oncology

Summary

Various reconstructive options are available after resection of Squamous cell carcinoma of buccal mucosa. Nasolabial flap is a very simple and useful alternative to other pedicle grafts or free flaps. The purpose of this study was to report the use of Nasolabial flaps in reconstruction of buccal mucosal defects, its indications, technique, complications and functional as well as aesthetic outcomes. This retrospective study was conducted at Gujarat Cancer and Research Institute between January 2010 and December 2012. We identified 70 previously untreated patients of T1 and T2 buccal mucosa Squamous cell carcinoma whose defect after surgical excision was reconstructed by inferiorly based nasolabial flap. Preoperative assessment included clinical stage and site of the lesion, flap design and general condition of the patients. The median follow up was 11 months (1 month-30 months). Of 70 patients we studied, mean age was 48 year (24-74years) which included 54 (77%) males and 16 (23%) females. 13 (18%) patients underwent only wide local excision and 57 (82%) patients had neck dissection (ligation of facial artery) along with primary resection. Reconstruction was done with inferiorly based nasolabial flap. Flap necrosis was present in 4(6%) and donor site infection in 1(1%). The functional outcome was satisfactory and aesthetic outcome was good in most cases.The Nasolabial flap is versatile, simple, quick and easy to harvest local flap which can be used to reconstruct small to medium size defects in buccal mucosa. It has high viability and low complication rate with satisfactory functional and cosmetic outcome.

08. Secondary Soft Tissue Sarcoma in Treated Case of Bilateral Retinoblastoma

Das Priyanka,
Department Medical Oncology

Summary

Retinoblastoma is a rare intraocular tumor of children which can occur spontaneously or be inherited as an autosomal dominant trait. Long-term survivors of childhood hereditary retinoblastoma, caused by a germline mutation in the RB1 gene, are at a 20-fold increased risk of developing and dying from a subsequent non-ocular cancer, primarily bone and soft tissue sarcomas, melanoma and brain tumor. The 50-year risk is approximately 50% for those treated with radiotherapy and 28% for those treated without radiotherapy. Those who received radiotherapy at less than 1 year of age are at highest risk. We present a case of a young patient with previous history of bilateral retinoblastoma that was treated by surgery and chemoradiation for his primary disease. Thirteen years after his initial treatment; he developed soft tissue sarcoma of the maxilla as the second primary lesion. CT (PNS + Neck) shows right maxillary mass extending to right nasal cavity, maxillary sinus, intraocular cavity abuting lateral rectus muscle.IHC suggestive of malignant fibrous histocytosis. There is convincing epidemiologic evidence linking past radiotherapy with sarcomas in hereditary patients.

"He who studies medicine without books sails an uncharted sea, but he who studies medicine without patients does not go to sea at all."

William Osler

Presentations at Clinical Meetings

(July 2013 to December 2013)

Sr. No.	Date	Speaker/Department	Title
1.	13.07.13	Patel Nupur Immunohistochemistry and Flow-Cytometry Division	Immunolocalisation of Wild Type EGFR, Exon 19 E746-A750 Frame Deletion and Exon 21 L858R Point Mutation in Triple Negative Breast Cancer
2.	10.8.13	Kumar Manish Surgical Oncology, Unit VI	Outcome of Liver Resection-5 Year Experience at GCRI, B.J.Medical College, Ahmedabad -January 2008- December 2012
3.	14.9.13	Parekh Urvi B Endocrinology	Management of Hyperglycemia in Pre- Peri and Postoperative Conditions
4.	26.10.13	Raut Shreeniwas S Medical Oncology, Unit-III	Retrospective Analysis and Outcome of Critically Ill Cancer Patients in Medical Intensive Care Unit
5.	09.11.13	Jain Abhishek Surgical Oncology, Unit-V	Retrospective Analysis of Head Neck Malignancies in Paediatric Patients
6.	30.11.13	Bharti Archana Gynecologic Oncology, Unit-II	Demographic Profile of Adolescent and Young Adults with Cancer in Urban Ahmedabad Agglomeration Area
7.	14.12.13	Thakar Krutarth Plastic Surgery	Reconstruction of Buccal Mucosa Defects Using Nasolabial Flap: A Clinical Experience of 70 Patients at GCRI
8.	28.12.13	Das Priyanka Medical Oncology, Unit-II	Secondary Osteosarcoma in Treated Case of Bilateral Retinoblastoma in Irradiated Area

Journal Club / Guest Lecture / Review Lecture Presentations

(July 2013 to December 2013)

Sr. No.	Date	Presenter/ Department	Topic	Authors	Citation
1.	13.07.13	Sharma Dinesh Surgical Unit III	The Methods of Reconstruction of Pancreatic Digestive Continuity after Pancreaticoduodenectomy : A Meta-Analysis of Randomized Controlled Trials	Yang Sh1, Dou KF, Sharma N, Song WJ.	World J Surg. 2011; 35: 2290-2297
2.	27.07.13	Shah Kinna Anaesthesiology	Vein Visualization, Patient Characteristic Factor and Efficacy of A New Infra-Red Vein Finder Technology	F.B.Chiao, Resta-Flater, J.Lesser, J.Ng. et al.	Br J Anaesthesia, 2013;110: 966-971
3.	10.8.13	Patankar Piyush Physiotherapy	Effectiveness of Early Physiotherapy to Prevent Lymphoedema after Surgery for Breast Cancer : Randomized Single Blinded, Clinical Trial	María Torres Lacomba , Alcalá de Henares	BMJ. 2010; 340:b5396
4.	24.8.13	Petkar Ritu Gynecologic Oncology	ACS, ASCCP and ASCP Screening Guidelines for the Prevention and Early Detection of Cervical Cancer	Saslow D, Solomon D, Lawson HW et al.	Am J Clin Pathol., 2012; 137: 516-542
5.	14.9.13	Dalal Esha N Cell Biology Division	Non-Small Cell Lung Cancer - Genetic predictors	Vladimira Koudelakova, Magdalena Kneblova, Radek Trojanec et al.	Biomed Pap Med Fac Univ Palacky Olomou Czech Repub. 2013;157:125 -136
6.	12.10.13	Patel Kartik Surgical Oncology Unit-1	Significance of Level V Lymph Node Dissection in Clinically Node Positive Oral Cavity SCC and Evaluation of Potential Risk Factors for Level V Lymph Node Metastasis	Parikh DG, Chheda YP, Shah SV et al.	Indian J Surg Oncol. 2013;4:275-279
7.	12.10.13	Nanavaty Angana Medicinal Chemistry & Pharmacogenomics	Frequent Genetic Alterations in EGFR- and HER2-Driven Pathways in Breast Cancer Brain Metastases	Ina Hohensee, Katrin Lamszus, Sabine Riethdorf, et al.	Am J Pathol. 2013;183:83-95
8.	09.11.13	Sharnangat Vijay Medical Unit-II	Point Break Trial: A Randomized Phase III study in Non Squamous Non Small Cell Lung Cancer	Jyoti D. Patel, Mark A. Socinski, Edward B. Garon et al.	JCO, 2013; 31:4349-4357
9.	14.12.13	Jivarajani Parimal Community Oncology	Geographic Pathology Revisited: Development of an Atlas of Cancer in India	Ambakumar Nandakumar, Prakash Chandra Gupta, Paleth Gangadharan et al.	Int. J. Cancer, 2005;116: 740–754

Case Presentations for Morbidity, Mortality at Clinical Meetings

(July 2013- December 2013)

Sr No	Date	Presenter/Department	Case discussion
1	27.07.13	Ruchi Barakhane Anaesthesiology	Mortality and Morbidity Data Presentation of Surgical and Medical Departments
2	27.07.13	Piyush Agrawal Surgical Oncology Unit-II	An Operated Case of Three Stage Oesophagectomy with Septicaemia and Postoperative Arrythmias-Morbidity
3	24.08.13	Devendra Prajapati Anaesthesiology	Mortality and Morbidity Data Presentation of Surgical and Medical Departments
4	24.08.13	Pinaki Mahato Medical Oncology Unit -II	A Case of Mediastinal Lymphoma Presenting as Spontaneous Tumor Lysis Syndrome
5	28.09.13	Devendra Prajapati Anaesthesiology	Mortality and Morbidity Data Presentation of Surgical and Medical Departments
6	28.09.13	Udaysingh Neuro Oncology	A Case of Foramen Magnum SOL-Mortality
7	26.10.13	Ruchi Barakhane Anaesthesiology	Mortality and Morbidity Data Presentation of Surgical and Medical Departments
8	26.10.13	Dinesh Sharma Surgical Oncology Unit-III	An Operated Case of TPLO with Flap Necrosis-Morbidity
9	30.11.13	Hardul Modi Anaesthesiology	Mortality and Morbidity Data Presentation of Surgical and Medical Departments
10	30.11.13	Kalpesh Medical Oncology Unit-III	Pulmonary TB in Case of ALL-Mortality
11	28.12.13	Hardul Modi Anaesthesiology	Mortality and Morbidity Data Presentation of Surgical and Medical Departments
12	28.12.13	Anju Khanna Gynecologic Oncology Unit-III	An Operated Case of Carcinoma Ovary with Septicaemia-Mortality

About the Journal and Instructions to Author

Gujarat Cancer Society Research Journal is a biannually (April and October), ISSN 2320-1150, peer-reviewed journal published by the Gujarat Cancer Society. **The journal is indexed with Index Coperinicus.**

The journal's full text is available online at http://www.cancerindia.org

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.

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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)
- References (separate page, Number references consecutively in the order in which they are first mentioned in the text. Identify references in the text in numerals in superscript and parenthesis; Font size: 12).
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Units and abbreviations

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
Molar	mol/L	Mililitre	ml
Percent	%		

Title Page

The title page should include

1. Type of manuscript (article/case report)
2. The title of the article, which should be concise, but informative; (Title case, not ALL CAPITALS, not underlined)
3. The name by which each contributor is known (Last name, First name and initials of middle name), with institutional affiliation;
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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

Summary and Keywords: Summary no more than **250 (150 for Case Report)** words. Should have following headings: **Introduction** (state the purposes of the study or investigation), **Materials and Methods** (selection of study subjects/patients, observational and analytical methods), **Results** (give specific data and their statistical significance, where ever possible), and **Conclusion** (succinct emphasis of new and important aspects of the study or observations). Do not use symbols in the summary; rather, spell out what they stand for in full. Three to eight keywords must be included below the summary.

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.

Materials and Methods: Describe precisely your selection of the observational or experimental subjects (patients, including controls). Identify the methods, apparatus (including manufacturer's name and address in parenthesis), and procedures in sufficient detail to allow others to reproduce the method. Give references to established methods, including statistical methods; provide references and brief descriptions for methods that have been published but are not well-known. For new or substantially-modified methods, describe and give reasons for using them and evaluate their limitations.

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.

Tables: Print each Table double-spaced on a separate sheet. Number Tables consecutively in Arabic numerals (e.g. 1, 2, 3) in the order of their first citation in the text and supply a brief title, which should be shown at the top of each table.

Illustrations (Figures) and Legends for Illustrations: All Illustrations must be submitted in JPEG finished format form that is ready for reproduction. Figures should be numbered consecutively in Arabic numerals (e.g. Figure 1, 2, 3) according to the order in which they have been first cited in the text. If photographs of persons are used, the subjects or patients must not be identifiable.
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
You CH, Lee KY, Chey RY et al: Electrogastrographic study of patients with unexplained nausea, bloating, and vomiting. Gastroenterology 1980; 79:311-314

Online journal article
Miyamoto O, Auer RN. Hypoxia, hyperoxia, ischemia and brain necrosis. Neurology [serial online] 2000; 54:362-71. Available at: www.neurology.org. Accessed February 23, 2000.

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
Lillywhite HB, Donald JA. Pulmonary blood flow regulation in an aquatic snake. Science. In press.

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Generally, submitted manuscripts are sent to one experienced referee from our panel. The contributor's may submit names of two qualified reviewers who have had experience in the subject of the submitted manuscript, but not associated with the same institution(s) as contributors nor have published manuscripts with the contributors in the past 10 years.

Department of Community Oncology and Medical Records

Cancer is a preventable disease. The fact that only 5-10% of all cancer cases are due to genetic defects and that the remaining 90-95% are due to environment and lifestyle provides major opportunities for preventing cancer. Prevention of cancer can take place on several different levels: primary prevention addresses the cause of cancer so disease does not occur, secondary prevention identifies disease before the onset of symptoms and keeps it from becoming more extensive and tertiary prevention reduces complications and progression of disease once it has become clinically apparent.

The Department is Working on Four Different Areas:
(A) Cancer registry programme (B) Cancer control programme (C) Cancer epidemiology and (D) Medical records.

(A) Cancer Registry Programme: Cancer registry plays a major role in ensuring good quality cancer data which can be helpful in cancer care, planning of health service and cancer control programmes. The department has been maintaining both hospital based cancer registry and population based cancer registries.
Hospital Based Cancer Registry: The hospital based cancer registry has been functioning since long.It is initiated and run by the institute as per the standards and norms prescribed by the National Cancer Registry Programme (NCRP) of the Indian Council of Medical Research (ICMR).
Population Based Cancer Registry: The department is working on two research projects for population based cancer registries in collaboration with NCRP and ICMR.
I Rural Cancer Registry - Ahmedabad district project: It was started from 1st January 2004 with the objective of assessing the magnitude and types of cancers in Ahmedabad rural areas and to calculate estimate of cancer incidence in Ahmedabad district. Annual reports from the year 2004 to 2010 have been published.
ii. Population Based Cancer Registry - Ahmedabad urban agglomeration area: It was started from the year 2007 with the objective of assessing the magnitude and types of cancers in Ahmedabadcity and to calculate estimate of cancer incidence in Ahmedabad urban area.Annual reports from the year 2007 to 2010 have been published.

(B) Cancer Control Programme: The department runs cancer control programme as per the aim and objectives laid down by National Cancer Control Programme, Government of India. The department organises various cancer awareness and detection camps for primary prevention of cancers by health education especially hazards of tobacco consumption and necessity of genital hygiene for prevention of cervical cancers and secondary prevention i.e. early detection and diagnosis of cancers.
i. Cancer detection camps: Department is involved in arrangement of cancer detection camps at various places in Gujarat.The objectives of these camps are to detect cancers in their early stage especially oral cancers in men, breast and cervical cancers in women. Suspicious cases are referred to GCRI for further diagnostic and therapeutic care.
ii. Mobile cancer screening van (Sanjeevanirath) camps: A high tech cancer screening mobile van (Sanjeevanirath) was launched in the year 2009 to detect the cancer patients in their early stages of the ailment, especially in rural areas. The expert team identifies suspected cases of possible cancer and if needed, refer them to GCRI for further evaluation. This mobile cancer screening van is equipped with advanced mammography machine to detect the breast cancer at an early stage.
iii. Cancer awareness camps: These camps are organised with the objectives of promoting awareness about the risks of common cancers and their curability if detected early in the community. During these camps, knowledge on cancer like its causes and natural history, warning signs and symptoms of cancer are explained to the visitors. Self-examination methods are emphasized particularly of oral cavity with need for quitting tobacco. Method of breast self-examination is also propagated among women visitors. Awareness about cancer is created by distribution of publicity material like pamphlets, posters, flip charts, exhibition and audio-visual programmes.

(C) Cancer Epidemiology:
Department helps doctors and researchers for statistical design and analysis of their studies. It provides various data to the doctors and researchers for their dissertation/research work. Also various statistical data and reports are provided to the administration and government as and when required.

(D) Medical Records:
Medical records are divided in two sections. 1. New Out Patient Department (New OPD) and 2. Old Out Patient Department (Old OPD)
New OPD: During the year around 29,000 new cases are registered.The documents under various schemes/categories such as Below Poverty Line (BPL) cards, Employees State Insurance Scheme (ESIS), State government employees/pensioners, Mukhyamantry Amrutam Yojana (MA Yojana), Lower Income Group category, Scheduled Castes (SC) and Scheduled Tribes (ST) categories etc. are verified. Some special radiological and laboratory investigation cards are issued. Year wise general statistics is also prepared.
Old OPD: It maintains the records of newly registered cases as well as old (follow-up) cases. An average 3,00,000 outdoor patients visit every year. Pathology reports and Traveling pass are issued. The work of indoor patient registration and medical reimbursement are also done. Patient's follow up information are also kept. Other departments are also referring case files for their study purposes. Computerised medical certificate are issued. Forms for Mediclaim policy and Life Insurance policy of expired patients are filled.

Departmental Research Projects: N=2
i. HPV Vaccine Project: The Gujarat Cancer and Research Institute (GCRI) is a collaborative research centre with International Agency for Research on Cancer (IARC), Lyon, France and working on a project named "Effectiveness and safety of 2 Vs 3 doses of HPV vaccine in preventing cervical: An Indian multi-centre randomized trial".
ii. INDOX project: GCRI is one of the participating centres for INDOX case control consortium study for breast cancer and colorectal cancers in India.

Day Celebration: Department is taking active participation in celebration of different days like World No Tobacco Day, World Cancer Day, National Cancer Awareness Day, Kite Flying Day, Holi, Navratri etc. The objectives of these celebrations are
I To create awareness about cancer among common people.
ii. To motivate people about screening of common cancers (Oral, Breast and Cervix)
iii. To sensitize people against tobacco hazards.

"Law and order are the medicine of the body politic and when the body politic gets sick, medicine must be administered."

B. R. Ambedkar

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Department of Community Oncology and Medical Records

Case registration new OPD

Old OPD - disbursing case files to the patients

Sanjeevani rath camp beneficiaries

Talk for cancer awareness

"World Cancer Day" rally

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