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"Rotationplasty" A Modified Amputation Surgery: A Beauty in the Eyes of Almighty

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Bone tumors constitutes around one percent of all malignant tumors and osteosarcoma and Ewing's sarcoma are observed in paediatric age group. The recent advances in imaging modalities, chemotherapy and surgical techniques have improved the chances of limb salvage surgery in majority of patients with malignant bone tumors. Amputation still plays important role in surgical management of malignant bone tumors. In young children with malignant bone tumor that require knee or hip resection; surgical treatment options includes reconstruction with an expandable megaprosthesis or amputation or modified amputation (rotationplasty).[1-4]

Despite expandable megaprosthesis provides good functional outcome it has its own limitation including limited availability of implant, costlier implant and repeated surgeries for limb lengthening.[4] Rotationplasty is a modified amputation surgery in which the ankle joint is converted into the knee joint following resection of the tumor and an 180 degree external rotation of the limb.[1,2] Rotationplasty is an excellent alternative procedure to amputation surgery in patients with malignant bone tumours of the femur and proximal tibia. The reversed or rotated ankle joint (modified "knee joint") is surgically placed at the level of the expected contralateral knee joint after growth completion, avoiding the need for revision surgery and lengthening.[4] Rotationplasty can also be used as a treatment modality in the management of congenital limb discrepancy, infected implant surgery and severe limb length discrepancy following trauma. This procedure converts high above amputation or hip disarticulation surgery into below knee amputation leading to an energy saving and better bio-mechanical procedure.[4] The important advantage of rotationplasty is patient experiences no phantom limb pain because the sole is the weight bearing area.

History of Rotationplasty (Table 1)

The rotationplasty surgery was popularized by Van Nes in 1927 and was performed mainly for congenital defects around knee joint.

Table 1: History of Rotationplasty

Author	Year	Contribution
Borggreve	1927	Performed rotationplasty for a patient with a fused knee joint and limb-length discrepancy due to tuberculosis
Van Nes	1927	Popularized this procedure in Congenital defect of Knee joint
Knahr and Salzer	1975	Alternative technique to above knee amputation in osteosarcoma of the distal femur
Winkelmann	1986	Classification of rotationplasty for malignant tumors of the proximal femur with or without involvement of the hip as well as of the lower pelvis

Table 2: Winkelmann classification of rotationplasty: Type A

Knee Rotationplasty (Type A)	Type A I	Type A II
	Tumors around Distal Femur	Tumors around Proximal Tibia

Table 3 : Winkelmann classification of rotationplasty: Type B

Knee Rotationplasty (Type B)	Type BT I	Type B II	Type B III
	Tumors around Proximal Femur without Hip joint involvement	Tumors around Proximal Tibia with involvement of lower pelvis	Tumors of complete Femur Type III a : Children Type III b: Adults

Classification

Modified Winkelmann classification of rotationplasty based on the level of tumor involvement.

Winkelmann classification of rotationplasty: Type A (Knee rotationplasty) and Type B (Hip rotationplasty).(Table 1, 2)

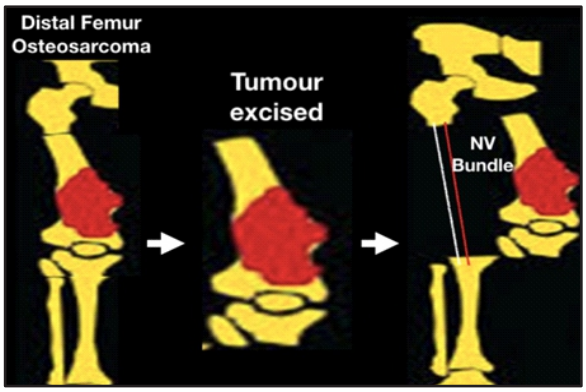

Figure 1: Key steps of rotationplasty in schematic format: Showing a osteosarcoma of distal femur, wide resection of tumor and isolation of neurovascular bundle

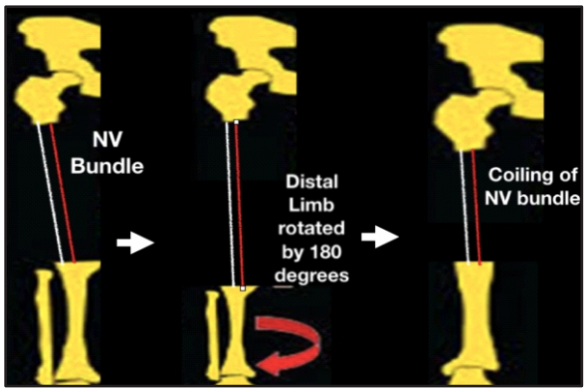

Figure 2: Key steps of rotationplasty in schematic format: Isolated neurovascular bundle, rotation of distal leg by 180 degrees and coiling of neurovascular bundle

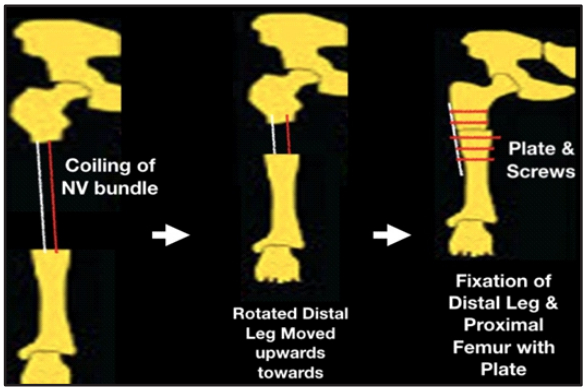

Figure 3: Key steps of rotationplasty in schematic format: Rotated leg is moved upwards and proximal femur is fixed with proximal tibia with help of a plate and screws

Figure 4: Plain radiograph of 9 year boy with osteosarcoma tibia, skin incision marked for rotationplasty and isolated neurovascular bundle following wide resection of tumor

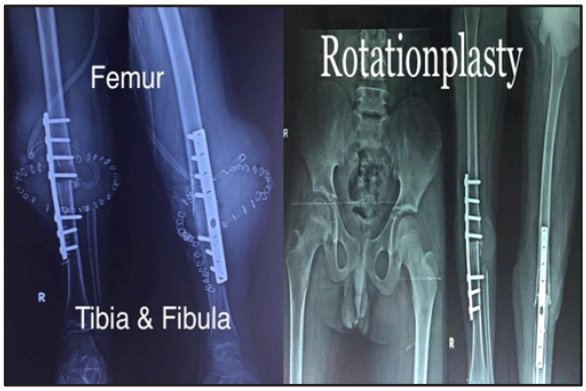

Figure 5: Immediate and follow-up radiograph of patient following rotationplasty surgery

Figure 6: Summary of rotationplasty with functional outcome of patient

Indications and Contraindications for Rotationplasty

The indications for rotationplasty includes
A sensate foot with functional ankle range of motion and adequate plantar flexion strength is prerequisite for a functional rotationplasty

- Primary bone tumors in paediatric age group involving femur bone or proximal tibia bone requiring reconstruction with expandable megaprosthesis: Expected growth of ipsilateral normal leg of more than 10 cm,
- Adult patients with bone tumors requiring hip disarticulation or high above knee amputation,
- Non oncologic indications: severe congenital limb discrepancy, infected implant surgery, non-reconstructive large bone defects following trauma.

The contraindications includes

- Patients with large size tumors in which sciatic nerve and common perineal nerve can't be saved with good oncologic margins,
- Tumor involving foot and ankle joint,
- Non willing patients for surgery and medially unfit for anesthesia.

Preoperative planning

- Preoperative work-up consist of plain radiograph of the affected extremity and a scannograph of both lower limb,
- MRI of the affected bone: Whole bone is screened and planning is done for the surgical resection of tumor, decide level of osteotomy and status of sciatic nerve and neuro-vascular bundle,
- Metastatic work-up: CT thorax, Bone scan and PET CT scan,
- Paediatric patients: The anticipated growth of the opposite leg is calculated: Contribution to growth by proximal femur, distal femur, proximal tibia and distal tibia. The ankle joint (new knee joint) following rotation pasty will be at different level.
- A growth calculator formula: Mosely's graph: Straight line graph can be used to estimate the leg length growth and the appropriate length of leg is calculated.

Key Surgical Steps (Figures 2 - 5)

- The skin incision are planned according to the prior biopsy scar and the affected skin are excised along with the resected specimen.
- The incision in the affected area are rhomboid shape or circular shape and the incision should be mirror image of planned distal limb.
- The sciatic nerve and its branches common peroneal nerve and posterior tibia nerve are dissected and separated from the tumor with oncological safe margin.
- The femoral vessels and popliteal vessels are dissected and separated from the tumor with oncological safe margin.
- The proximal osteotomy of femur bone or resection of femoral head is planned according to extent of bone sarcoma.
- The distal osteotomy or proximal tibia length are planned according to preoperative planning of anticipated growth of opposite leg.
- Neurovascular bundle are coiled.
- Type A rotationplasty: Fixation between proximal femur and tibia are performed with help of plate or intra-medullary nail after rotating the leg by 180 degrees.
- Type B: Distal femur or proximal tibia are placed in acetabulum after rotating the leg by 180 degrees.
- In cases with vascular involvement by tumor: resection of vessels and anatomizes are performed
- **Post operative radiograph:** To assess the bone union between osteotomy sites.

Complications

The early complications of rotationplasty includes neurovascular injury, thrombosis of artery and vein leading to amputation of the affected limb. Other complications includes altered wound healing which includes skin loss, flap necrosis and wound infection. The complications seen seen in later period includes nonunion, mal-union, limb length discrepancy and local tumor recurrence.

Recent advances about Rotationplasty

- In cases of vascular affection by the bone tumor; vascular anastomosis of artery and vein can be performed by a multidisciplinary team.
- Use of free flap and nerve repair can be doe in cases with soft tissue defect and nerve defects, respectively.
- The use of rotationplasty principle can be extrapolated and used in tumors around elbow joint to preserve hand function.

Conclusion

Management of bone tumors in pediatric patient is a great challenge to the clinicians. It would be optimal to say about Rotationplasty that **'Beauty in the eye of Almighty'**; it provides good function despite the limb it provides doesn't look cosmetic(Figure 6). Rotationplasty is an excellent surgical procedure that provides an optimal functional outcome in pediatric patients with malignant bone tumors. This surgery provides a permanent below knee amputation stump over which an artificial prosthesis is applied and provides unaided bipedal ambulation. Relatives of patient must be counselled about rotationplasty surgical steps and postoperative outcomes with its photos and videos. A good patient selection is important to achieve better functional outcomes following rotationplasty.

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Synchronous Dual Malignancies: An Observational Study of Clinicopathological Features Done at a Regional Cancer Centre

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Summary

A cancer patient survives for a longer period with current treatment protocols, compared to previously. As a result, patients have a higher chance of developing multiple primary malignancies. The chance of multiple primary malignancies is also exacerbated by common risk factors like addiction, genetic predisposition or environmental risk factors. The aim of this study is to observe the trend of synchronous malignancies, study their management and to review the relevant literature. A retrospective study of data collected prospectively of patients who presented with synchronous dual malignancies. The study was conducted over a period of one year, from January 2019 to December 2019, of patients with histologically proven second primary malignancy which satisfies Warren and Gates criteria. International Agency for Research on cancer (IARC) guidelines have been followed to define synchronous malignancies. Clinico-radiological, pathological and treatment related data were studied. Most common index primary site was the head and neck region followed by breast. Most common method of detection was Computed Tomography of thorax followed by clinical examination. Most common combination of malignancies was head and neck index primary with lung followed by bilateral breast primary.

Conclusions: The clinician needs to be alert to the possibility of a synchronous second malignancy and work up to rule out the same accordingly. Synchronous malignancies can be treated together, according to underlying disease biology and stage, and performance status, after discussion in multidisciplinary tumor board.

Keywords: Synchronous malignancy, second primary malignancy, dual malignancy

Introduction

The incidence of dual malignancies varies greatly among different sites of primary cancers. The incidence is higher for cancers with a genetic or hormonal basis or having a longer survival rate, such as breast or oral cavity.[1]The various epidemiological studies cite an incidence of dual malignancy between 2-17%. [2] The earliest study of dual malignancies was done by Bugher et al in 1934, who derived the equation for the probability of death from cancer during a given age with a coincidental second malignancy.[3]

With improved treatment options, leading to longer cancer survivorship, and more accurate diagnostic techniques, the rate of detection of second primary malignancies has increased[4]. With this, oncologists have to evolve their techniques in an attempt to address the multiple malignancies. Warren and Gates have given the criteria to define second primary malignancy, which was refined subsequently. (Table 1) [5,6]

Materials and Methods

A retrospective analysis of prospectively collected data was done, for patients presenting with pathologically proven double malignancy in a synchronous setting, over the period of one year from January 2019 to December 2019. Warren and Gates criteria [5,6] (Table 1) have been used to define second primary malignancy. International Agency for Research on Cancer (IARC) guidelines have been followed to define synchronous malignancies, which state that two malignancies are to be registered as synchronous if they occur within 6 months of each other.[2,7] The same has been previously used in multiple studies.[8-11] The patients with proven metastasis, or those presenting more than six months after index malignancy were excluded from the study. Also, patients with any one malignancy being hematological malignancy were excluded. Each patient was analyzed for type of malignancies, the time interval between detection of both malignancies, the method of diagnosis, the stage of each malignancy at presentation, histology of each malignancy and the treatment protocol given. Disease free survival and overall survival are not commented upon in the present study owing to the short duration of follow up.

The malignancy with which the patient presented first was considered as the index primary, and the malignancy which was detected subsequently during clinico-radiological evaluation, as the second primary malignancy.

Results

Over a period of one year, 17 cases of synchronous malignancies were included in the study,

Table 1: Warren and Gates Criteria

1	Histological confirmation of malignancy in both the index and secondary tumors.
2	There should be at least 2 cm of normal mucosa between the tumors. If the tumors are in the same location, then they should be separated in time by at least five years.
3	Probability of one being metastasis of the other must be excluded

Table 2: Details of Cases of Synchronous Dual Malignancies

No	Age/ Sex	Index Primary site	Addiction history	Stage of Index primary site	Second primary site	Stage of second primary	Method of detection of second primary	Time interval between detection of both primaries	Treatment	Status
1	67/M	SCC tongue	Tobacco chewing	pT2N1	Adenoca Left lung	pT1bN0	CT Thorax	Simultaneous	Hemiglossectomy+ MND + Left apicoposterior segmentectomy Adjuvant RT to head and neck region	Alive
2	65/M	SCC tongue	Tobacco chewing	pT2N0	SCC Right lung	cT3N2	CT Thorax	1 month	Hemiglossectomy+ MND + Palliative chemotherapy to lung	Alive
3	73/M	SCC Buccal mucosa	Nil	pT2N0	Adenoca Right lung	pT1b	CT Thorax	Simultaneous	Composite resection + Flap + Right upper lobectomy + Adjuvant RT to head and neck region	Alive
4	59/M	SCC Nasopharynx	Nil	cT2N2	Adenoca Prostate	cT2N1	Clinical examination	Simultaneous	Nasopharynx- Curative + RT + Chemotherapy to Nasopharynx + Hormonal therapy to prostate	Expired
5	41/F	SCC maxilla	Nil	pT4aN3b	SCC Tonsillo lingual sulcus	pT1	Intraoperative finding	Intra-operative finding	WLE of maxilla+ WLE of Tonsillo lingual sulcus growth +MND II Pt deferred adjuvant treatment.	Alive
6	54/M	Mucoepi-dermoid carcinoma Buccal mucosa	Tobacco chewing	pT2N1	Carcinoid Right Lung	pT1a	CT Thorax	4 months	Composite resection + Flap + Adjuvant RT to head and neck region + Right middle lobectomy	Alive
7	52/F	IDC breast Right	Nil	ypT4bN2	Papillary carcinoma thyroid	pT1a	Clinically	Simultaneous	Chemoport insertion + NACT + MRM + Total thyroidectomy +Post-operative RT to chest wall+ Hormonal therapy	Alive
8	60/F	IDC breast Left	Nil	pT2N0	Papillary ca breast Right	pT1N0	Mammography	Simultaneous	Bilateral Mastectomy+ Bilateral SLNB + Chemoport insertion + Adjuvant chemotherapy	Alive
9	39/F	IDC left breast	Nil	ypT4bN1	IDC breast right	ypT2N0	Mammography	Simultaneous	Chemoport insertion + NACT + Bilateral MRM + Adjuvant RT to right chest wall + Adjuvant chemotherapy	Alive
10	60/F	IDC breast Right	Nil	pT2N1	Tubular carcinoma breast Left	pT1N0	Mammography	Simultaneous	Right MRM + Left Mastectomy + Left SLNB + Chemoport insertion + Adjuvant chemotherapy + Adjuvant RT to right chest wall	Alive
11	48/F	IDC left breast	Nil	ypT3N3	Serous papillary ovarian	Stage Ib	USG Abdomen	Simultaneous	NACT + Debulking surgery for carcinoma ovary + left MRM +	Alive

No	Age/ Sex	Index Primary site	Addiction history	Stage of Index primary site	Second primary site	Stage of second primary	Method of detection of second primary	Time interval between detection of both primaries	Treatment	Status
					carcinoma				Adjuvant chemotherapy + Adjuvant RT to left chest wall + Hormonal therapy	
12	55/F	Serous papillary adenoca-ovary	Nil	Stage IIIA2	Squamous cell carcinoma cervix	Stage IB	Clinical examination	Simultaneous	Staging laparotomy + Adjuvant RT + Adjuvant chemotherapy	Alive
13	58/F	Serous papillary adenoca ovary	Nil	Stage IC1	ILC breast Left	pT2N0	Clinical examination	Simultaneous	Staging laparotomy + left Mastectomy + SLNB+Adjuvant chemotherapy	Alive
14	65/M	SCC lung Right	Cigarette smoker	ypT2bN0	Adenoca esophagus	ypT2N1	CT Thorax	Simultaneous	NACT + Right Middle+lower lobectomy + 3 stage esophagectomy	Expired
15	80/M	SCC lung Left	Cigarette smoker,	cT2N2	SCC esophagus	not feasible	CT Thorax	Simultaneous	Patient refused treatment	Lost to follow up
16	62/F	Clear cell RCC	Nil	pT2N0	Adenoca endometrium	stage IB	Contrast Enhanced CT(A+P)	Simultaneous	Left radical nephrectomy + Staging laparotomy For endometrial cancer	Alive
17	63/M	Adenocarcinoma esophagus	Nil	cT4Nx	Conventional RCC	cT2N0	CT Thorax	Simultaneous	Inoperable disease	Expired

Abbreviations used in table
1. SCC- Squamous Cell Carcinoma
2. CT scan- Computed Tomography
3. MND- Modified Neck Dissection
4. RT- Radiotherapy
5. WLE- Wide Local Excision
6. IDC- Invasive Ductal Carcinoma
7. ILC- Invasive Lobular Carcinoma
8. NACT- Neoadjuvant Chemotherapy
9. MRM- Modified Radical mastectomy
10. TAH- Total Abdominal Hysterectomy
11. BSO- Bilateral Salpingo-oopherectomy
12. RCC- Renal Cell Carcinoma

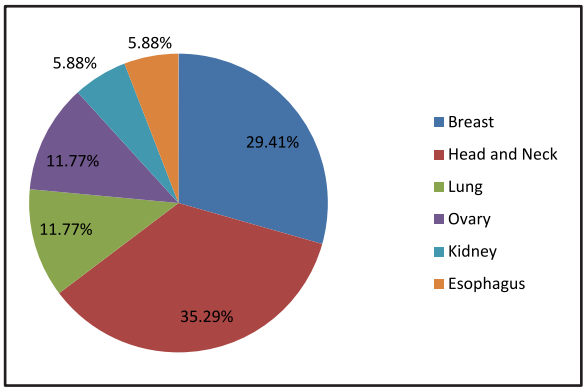

Figure 1: Distribution of Index primary

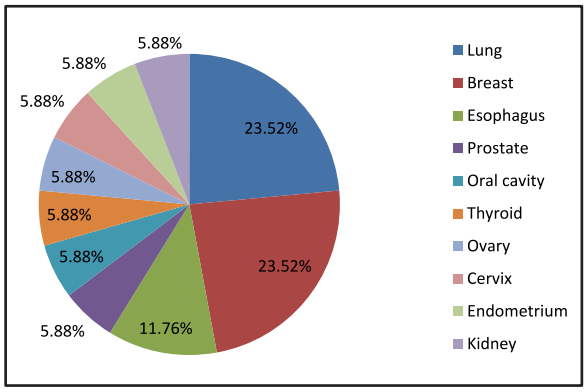

Figure 2: Distribution of second primary malignancy

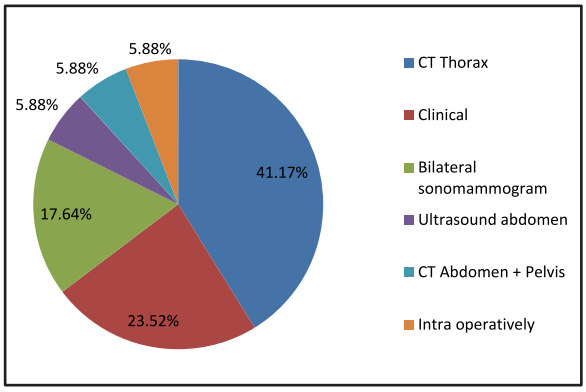

Figure 3: Methods of detection of second primary malignancy

according to the inclusion and exclusion criteria. (Table 2). The age range was 39-80 years, with median age of 60 years. There were eight male (47.05%) and nine female (53.94%) patients. In the region-wise distribution of index malignancy (Figure 1), the highest cases were of head and neck malignancy (6/17, 35.29%) followed by carcinoma of breast (5/17,29.41%). The region wise distribution of second primary malignancy (Figure 2), the most common sites were lung and breast (23.5%). Out of 17 patients in our study, five patients had significant history of addiction to tobacco chewing (three) and cigarette smoking (two). All three patients with

history of tobacco chewing had index primary in oral cavity. Two patients who had history of cigarette smoking had lung as index primary and esophagus as second primary.

Different diagnostic techniques were used to detect the second primary malignancy (Figure 3). In seven cases out of 17 (41.17%), the second primary malignancy was detected by Computerized Tomography (CT scan) of Thorax. In one case out of these seven, with index primary in tongue, CT scan of thorax was not done as a part of initial work up, but done after one month, when patient complained of persistent cough and hemoptysis, and the second primary malignancy in lung was detected. Four cases out of 17 (23.52%) were detected by clinical examination. Other methods of detection included bilateral sonomammogram (N=3), Ultrasound of abdomen (N=1) and CT of Abdomen and Pelvis (N=1). One case was detected intraoperatively.

Among the head and neck cancers, the site of second malignancy was lung in four cases out of six (66.67%). In one patient, with synchronous malignancy of maxilla and Tonsillolingual (TL) sulcus, the latter was detected intraoperatively. The lesion over TL sulcus was not seen on pre-operative magnetic resonance imaging, and missed on clinical examination due to the small size and posterior location. Endoscopic examination under general anesthesia was not done in the patient. One patient with index malignancy of nasopharynx had a second primary malignancy of prostate, detected clinically by per rectal examination while evaluating urological symptoms of the patient.

There were five patients with an index primary malignancy involving the breast (29.41%). Out of these five, three patients had the malignancy in bilateral breasts. Both lesions had different histopathology and receptor status in all three cases. One patient had synchronous breast and ovary cancer, with index primary in the breast. One patient had a second primary malignancy in thyroid. No patient had a significant family history. Genetic testing could not be done in the patients due to logistic issues.

Two patients had index primary malignancy in the ovary, and a second primary in breast and cervix, respectively. Two patients had synchronous malignancy in lung and esophagus, with lung as index primary. One patient had index primary in kidney, and a second primary in endometrium. One patient had index primary in esophagus and second primary in kidney.

In 14 patients out of 17 (82.35%), both primaries were detected simultaneously by clinical examination or radiological investigations during work up of index primary. Two cases were detected after the treatment of the index primary, within six months. One case was detected intra-operatively. Most common combination of malignancies was head and neck with Lung, with four cases out of 17 (23.52%).

Out of 17 patients, 10 underwent upfront surgery for both malignancies (58.82%). Three patients underwent neoadjuvant chemotherapy followed by definitive therapy. In one patient, the index primary was Carcinoma of tongue, which underwent upfront surgery, and the second malignancy in lung was detected after one month, and it was managed by palliative chemotherapy. One patient underwent non operative management, with curative radiation given to nasopharynx and hormonal therapy given for carcinoma of prostate since patient was not fit for surgery. One patient was inoperable and given palliative chemotherapy. One patient refused treatment. Out of 17 patients, three patients expired in the follow up (17.64%).

Discussion

With increasing survival of cancer patients, and increased prevalence of various addictions such as tobacco, or alcohol, incidence of multiple primary malignancies is increasing. Improved detection techniques such as Positron Emission Tomography with CT fusion (PET CT) or improved CT/MRI scans are also picking up multiple primary malignancies at an increasing rate. Genetic factors play an important role in synchronous malignancies as these patients are at an increased risk of cancer in multiple organs. Environmental factors such as exposure to asbestos, or long term radon exposure can also contribute to the same.[2]

The risk of development of second primary malignancy differs from site to site. In head and neck cancers, where there is 36% cumulative risk of a second primary malignancy over 20 years [11]. The oft-quoted reason for the same is the field cancerization theory [12] by Slaughter, where he observes, "From the foregoing observations it would appear that epidermoid carcinoma of the oral stratified squamous epithelium originates by a process of field cancerization, in which an area of epithelium has been preconditioned by an as-yet-unknown carcinogenic agent." In a study by Krishnatreya et al,[13] the incidence of a synchronous dual malignancy in a head and neck cancer is found to be 1.33%. The most common site of the second primary is lung with a 20 year cumulative risk of 13%. It was reflected in our study too, where four cases of synchronous malignancy involving head and neck region had lung as second primary. In a study by Morris et al [11], which was a population based study of head and neck cancer patient registered in SEER database, the risk of a second primary malignancy differed by subsite. It was highest in squamous cell carcinoma of hypopharynx, followed by oropharynx, oral cavity and larynx. The study also showed an

increase in incidence of esophageal cancer. Our study correlated with the above findings, as the highest incidence of synchronous dual malignancies is seen with head and neck cancer, with six cases out of 17 demonstrating index primary malignancy in head and neck region (35.29%).

As elucidated in a study by Warnakulasuriya et al that addiction plays a strong role in causation of head and neck cancer, particularly tobacco.[14] It was further supported by Muwonge et al[15] who studied the role of tobacco and alcohol in causation of oral cancer. Our study reflects these findings, as five patients were found to have a significant history of tobacco chewing and cigarette smoking. Notably, all three patients with tobacco addiction had a primary lesion in head and neck region and both the patients with cigarette smoking had synchronous malignancy in lung and esophagus.

Keeping the increased incidence of synchronous malignancy in head and neck cancers, the initial evaluation of a head and neck cancer should include a through clinical examination, supported by an office procedure like nasal endoscopy or an indirect laryngoscopy, especially in smokers. These patients have an increased rate of detection of "silent" second primary malignancies, and curative therapy can be attempted in a single sitting in such patients.[16] In a study by Loh et al, [17] CT Thorax was shown to have a detection rate of 10.8% of synchronous lung malignancy or pulmonary metastasis in head and neck malignancies. CT thorax should be added to imaging of head and neck. When the primary lesion is locally advanced, PET-CT can be considered instead of CT thorax, if logistically feasible to detect synchronous second primary as well as distant metastases. If detected early, these cases can be taken up for upfront surgery, if operable, or can be planned for neoadjuvant treatment followed by definitive surgery, or definitive chemo-radiation, based on patient's performance status and associated co morbidities, after planning the same in a multi-disciplinary tumor board discussion.

Another common site for synchronous malignancy is the breast. Owing to multiple genetic factors, such as BRCA or p53 gene mutation, or common risk factors, there is a high prevalence of synchronous breast cancers. In a study by Londero et al, [18] the prevalence of synchronous breast cancers was 3%. The meta-analysis in the study demonstrated a lower overall survival (OS) for patients of synchronous and metachronous breast cancers, compared to unilateral breast cancers. Synchronous breast and ovary cancers need to be differentiated from breast cancers metastasizing to ovary. A metastatic breast cancer is positive on immunohistochemistry for gross cystic disease fluid protein 15 (GCDFP15), Mammaglobin and GATA 3 and negative to PAX 8, CA125 and WT1. A serous cystadenocarcinoma is positive for PAX 8, CA 125 and WT1, and negative for Mammaglobin, GATA 3 and GCDFP15.[19] Present study demonstrated two patients with synchronous malignancy of breast and ovaries, and three patients with synchronous breast cancers. Due to common risk factors between breast and ovarian cancer, and increased incidence of bilateral breast cancers, screening should always be done to rule out these synchronous malignancies. A simple and cost-effective method for screening of ovarian malignancies in a carcinoma breast is an ultrasound of abdomen. Similarly, bilateral sonomammogram should be a standard practice in a carcinoma ovary patient, to detect synchronous breast cancer. For other sites, the incidence of genitourinary synchronous tumors has been reported to be 2.8-6.3% in different studies [20-22] In our study, we reported two patients having synchronous malignancies with genitourinary cancers, both diagnosed incidentally.

Management of such patients with synchronous malignancies requires discussion in a multidisciplinary tumor board, taking into consideration the underlying disease biology. If two malignancies are present with different disease biology, the more aggressive disease is managed first. As in present series, one patient had a index malignancy involving breast, which was locally advanced, and a second primary in the form of a papillary carcinoma of thyroid. Neoadjuvant chemotherapy of breast was given first, keeping in mind the aggressive biology of breast carcinoma relative to thyroid malignancy, and later both sites were operated simultaneously. If a preoperative chemotherapy is planned, it should be planned to be effective against both the malignancies. Before planning treatment, the underlying co-morbidities and performance status also needs to be taken care of, especially when dealing with a major organ resection, such as esophagectomy. As shown in the current study, dual malignancies with esophagus as one primary had a poor outcome (Table 2), and hence, a judicious approach needs to be taken for consideration of surgery in such cases.

Conclusion

With rising incidence of synchronous malignancies, it is important to diagnose them early. A through clinical examination is a must, especially in malignancies with a high incidence of second primary, such as oral cavity and breast. The radiological investigations are supplementary to clinical examination and should be ordered according to the site of index primary. For head and neck malignancies, we recommend a screening CT scan of thorax to rule out synchronous lung or esophageal malignancies. Similarly, for breast malignancies, we recommend

bilateral mammosonography and an ultrasound of abdomen in all. These methods are easily available, easy to interpret and are not as resource and cost-intensive as a PET-CT scan, which is limited by availability and cost.

The treatment decisions should be undertaken by a multidisciplinary tumor board in accordance with tumor biology, stage of the disease and patient's performance status. Aggressive and locally advanced primaries should be given priority for treatment. Chemotherapeutic agent, which is effective in both malignancies should be used if feasible, for neoadjuvant or adjuvant therapy.

Conflicts of interest: None
Work is attributed to: Department of Surgical Oncology

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Blood Stream Infections in Immunocompromised Cancer Patients Detected by Automated Blood Culture System - A Retrospective Analysis

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Summary

For the diagnosis of bacteremias/ fungemia's automated blood culture system like BACTEC 9050 / FX40 is helpful in quick identification of the causative agent and further help treatment of these critically ill patients. Aim was to retrospectively analyze the growth of the pathogenic organisms causing blood stream infections in immunocompromised cancer patients and performing the antibiotic sensitivity testing using automated Identification(ID) and antibiotic sensitivity testing(AST). Standard bacteriological methods were followed for the diagnosis of etiological agent. Automated blood culture system flagged positive and negative cultures. Further the isolated organisms were followed up for the identification and susceptibility testing using automated Vitek-2 Compact. The results were entered in WHO Net system and data was analyzed. Out of the total 1590 blood cultures received, 18.3%were blood culture positive. Growth of Gram-Negative bacilli outnumbered than the gram-positive cocci. Out of the non – lactose fermenting bacilli (NLF), Acinetobacter baumanii showed 20-100% resistance to different antibiotics tested. Burkohlderia showed 19.6-100% resistance to different antibiotics whereas pseudomonas sp. showed around 50 % resistance to all antibiotics. Amongst the Lactose fermenter , E. Coli showed 33.3-100% resistance to most of the antibiotics. The antibiotic sensitivity of gram-positive cocci showed that 0% of Enterococcus faecalis were resistant to Vancomycin, 75% of Enterococcus faecium were resistant to vancomycin. Other gram-positive Cocci showed variable resistance to the panel of different antibiotics. Almost all species of staphylococci are beta Lactamase producers and were resistant to penicillin and cephalosporins. S. *capitis* showed 100% resistance to most of the antibiotics, and Vancomycin resistance was also seen in S. Xyloses (100%), S. epidermidis.(15.4%) and S. aureus (8.3%). It was also observed that Candidemia was caused by C. albicans and glabrata. In Conclusion, we can say that gram negative bacilli are the predominantly isolated bacteria which caused mortality, complications and late recovery from the disease due to antibiotic resistance. The automation in microbiology helped in early reporting and thus early institution of antibiotics to the patients.

Keywords: Immunocompromised Cancer Patients, Automated blood Culture system, GPC, GNB.

Introduction

Blood stream infections are threatening and cautionary leading to morbidity and mortality in cancer patient. It is very crucial and important to detect Bacteremia and Fungemia on an urgent basis and use methods that rapidly detect the presence of clinically important bacteria/Fungi.[1]

Earlier conventional technique was used which comprised of biphasic media to detect the growth of aerobic bacteria. After inoculation of blood, the broth is tilted on the slant twice on the first two days and once daily until day seven. They were tedious and time consuming. Whereas automated methods to detect bacterial growth from patients suffering with bacteremias is by detecting liberated CO_2 by fluorescence at the bottom of the bottles.[2] The aim of this retrospective analysis of bacteremia / fungemia is to generate a meaningful data to know the trends of the causative agents and their antibiotic/ antifungal resistance and to design antibiotic policy.

Materials and Methods

The data analysis was done from November 2018 to April 2019 (a period of 6 month) in the Microbiology laboratory of the Gujarat Cancer Research Institute, Ahmedabad. The subjects were patients suffering with different types of cancers and they had signs and symptoms of blood stream infections like persistent fever, chills, rigors and other systemic symptoms in alignment with septicemia.

As per routine laboratory set up, patients 8-10 ml of blood from adult and pediatric patient was collected aseptically in blood culture (BC) bottle from Becton Dickinson. The bottles were incubated for five days until negative as per set protocol. Total number of days was noted for positive culture bottle. Follow up from the positive culture bottle was done as per the laboratory policy. The organisms causing bacteremia were identified and further processed for antibiotics susceptibility testing by automated ID and AST system (Vitek-2 compact).The results were noted and dispatched through WHONET and laboratory information system (LIS).

Results

Automated blood culture system really helped in hastening up the yield of bacteria / fungi as an early and rapid diagnosis. Table-1 shows that the number of days of incubation of blood culture bottles was done in the automated BC system. It was observed that the detection of bacteria /fungus was in one day (24 hours) which accounted to 30.66 average events. Blood stream infections were 27.90% (1590/5697) of total culture/sensitivity samples received in the laboratory. They were requested by the clinicians in those patients

Table 1 : Blood culture positive by automated BACTEC automated system

Sr. No.	Positive cultures in days	Events Positive -2018 (6 Months)						
		November	December	January	February	March	April	Average events
1	One day	27	22	26	25	48	36	30.66
2	Two days	11	14	12	07	17	14	12.5
3	Three days	09	01	02	02	03	01	3.00
4	Four days	05	01	02	00	01	04	2.16
5	Five Days	02	00	02	06	00	03	1.16

Table 2: Blood Cultures requested in patients suffering with different Cancer (n=1591)

Diagnosis	Number of Blood cultures	(%)
CNS cancer	0001	0.06
GI cancer	0044	2.76
Gynec cancer	0035	2.19
Head & Neck cancer	0037	2.32
Leukemia	1027	64.5
Lymphoma	0046	2.89
Musculoskeletal	0004	0.25
Other tumor	0377	23.6
Respiratory cancer	0019	1.19

who were critical and were suffering with continuous fever and not relieved by empiric antibiotic therapy. Table-2 shows the clinical diagnosis of the patients whose blood cultures were received. 64.5% (1027/1591) of leukemia patients were critically ill and were immune compromised. Out of 1591 blood cultures, 20.6% (329/1591) were categorized as others which included breast cancer, neonatal mass, perineal mass, pancytopenia, pelvic mass, glioma, teratoma, meningioma etc. It was observed that the blood cultures from adults (862) were more than paediatric (729) patients. There was male preponderance in both positive cases like 1.55:1 and 1.49 : 1, respectively. (Figure 1) The study analysis showed that 17.43% blood culture were positive, 78.79% were sterile and in 2.45% there was contamination which was less than 3% as cut off value as per national reporting.

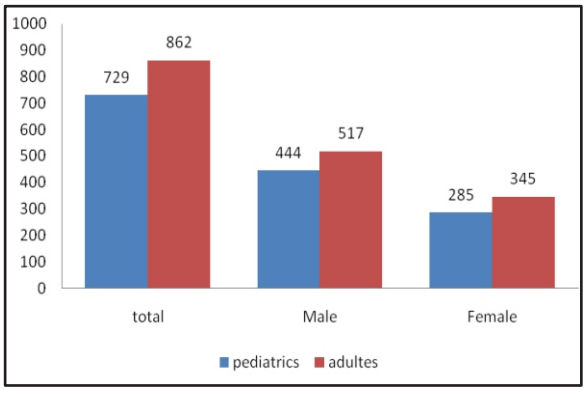


Figure 1: Blood Cultures received (1591) in the laboratory in paediatric and adults Patients

As per Table 3, around 18.30% (293/1598) bacteremias were due to gram positive cocci, gram negative bacilli and fungemia due to Candida spp. Maximum infections (66.21%) were due to gram negative bacilli, followed by gram positive cocci (32.42%) and then due to Candida species(1.36%).

Table 3: Isolates causing Blood Stream Infection (n=1598)

Sr. No.	Type of organism grown	Number	%
1	GPC	95	32.4 %
2	GNB	194	66.21%
3	Fungus (Candida)	04	1.36%
	Total	293	18.30%

Amongst the gram negative bacilli, non-lactose fermenting (NLF) bacilli were 57.21% (111/194) and the lactose fermenting bacilli were 42.78%. Out of the 57.21% of the NLFs isolated, 26.28% were Burkholderia *cepecea*, 17.01% were Pseudomonas *sp* and 6.18% were Sphingomonas *paucimobilis*. Other NLF isolates were Acinobacter (5.6%), Proteus *mirabilis*(0.51%) and Salmonella (1.03%). Lactose fermenting (LF) Gram negative bacilli isolated were Enterobacter *cloacae*(1.54%), E. *Coli* (23.91%) and Klebsiella *pneumonae*(18.04%). (Table-4)

Table 4 : Isolated Gram-Negative Bacilli from Blood Culture (n=194)

NLF Organism	Number of isolates	(%)
Acinetobacter sp.	11	5.6
Burkholderia cepacia	51	26.28
Proteus mirabilis	1	0.51
Pseudomonas sp.	33	17.01
Salmonella Sp.	2	1.03
Sphingomonas paucimobilis	12	6.18
Leclercia adecarboxylata	1	0.51
Total	**111**	**57.21**
LF Organism	**Number of isolates**	**(%)**
Enterobacter cloacae	03	1.54
E Coli	45	23.19
Klebsiealla Pneumonie	35	18.04
Total	**83**	**42.78**

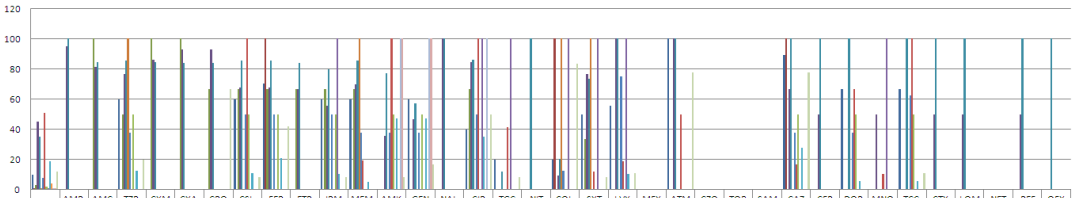
Out of the 95 isolates of gram positive cocci, we found that 25.26% Staphylococcus *hominis ss. hominis*, 21.05% were Staphylococcus *haemolyticus*, 16.84% were Staphylococcus *aureus ss. aureus*. Total methiciline resistance amongst Staphylococci was 85.33%. It was surprisingly noted that other species of Staphylococci also showed methyciline resistance. (Table 5)

Table 5: Gram positive cocci (n=95)

Organism	Number of isolates	(%)	MRSA	
			TOTAL	%
Enterococcus faecalis	1	01.05	-	-
Enterococcus faecium	4	04.21	-	-
Enterococcus gallinarum	1	01.05	-	-
Enterococcus sp.	10	10.50	-	-
Staphylococcus aureus ss. aureus	16	16.84	08	12.5
Staphylococcus capitis ss. capitis	1	01.05	01	1.5
Staphylococcus epidermidis	14	14.73	12	18.7
Staphylococcus haemolyticus	20	21.05	19	29.6
Staphylococcus hominis ss. hominis	24	25.26	21	32.8
Staphylococcus xylosus	1	01.05	01	1.5
Staphylococcus, coagulase negative (CONS)	3	3.157	02	3.1
TOTAL	**95**		**64**	**85.33**

Antibiotic resistance was reported based on the minimum inhibitory concentration (MIC) of the drugs. The Acinetobacters isolated belonged to baumanni sp. and general species. A. *baumanni* showed 100% resistance to aztreonam, 88% resistance to ceftazidime. There was a range of resistance from 50 to 70% to levofloxacin, sulfamethoxazole/trimethoprim, piperacillin/tazobactam, ticarcillin/clavulanic acid, and carbapenems. There was one Acinetobacter *sp*. which was 100% resistance to colistin, cefipine and ceftazidime. E *coli*, Klebsiealla *pneumonia*, Enterobacter *cloacae* showed 50 to 100% resistance to most of the antibiotics. *Leclercia adecarboxylata* is an aerobic gram-negative rod-shaped bacterium of the Enterobacteriaceae family and causes bacteremia's and respiratory tract infections. This is an unusual finding in our study and its antibiotics sensitivity testing showed that it is 100% resistance to piperacillin/tazobactam, meropenem, colistin and trimethoprim /sulfamethoxazole. The Pseudomonas sp. were sensitive to most of the antibiotics in the panel. Pseudomonas *aeruginosa*, Pseudomonas *fluorescens*, Pseudomonas *stutzeri* showed around 50% resistance. Burkholderia *cepacia* was 100% resistance to cefoperzone/sulbactam, amikacin, ciprofloxacin, ticarcillin/clavulanic acid and carbapenems. But it differed in showing only 12-19% resistance to meropenem, levofloxacin, and ceftazidime. Surprisingly, Proteus *mirabilis* was 100% resistance to imipenem, ciprofloxacin tigecycline, colistin, levofloxacin and minocycline. Isolated Salmonella *typhi* and Salmonella *para typhi* A were 100% resistance to amikacin, gentamicin and ciprofloxacin. (Table-6)

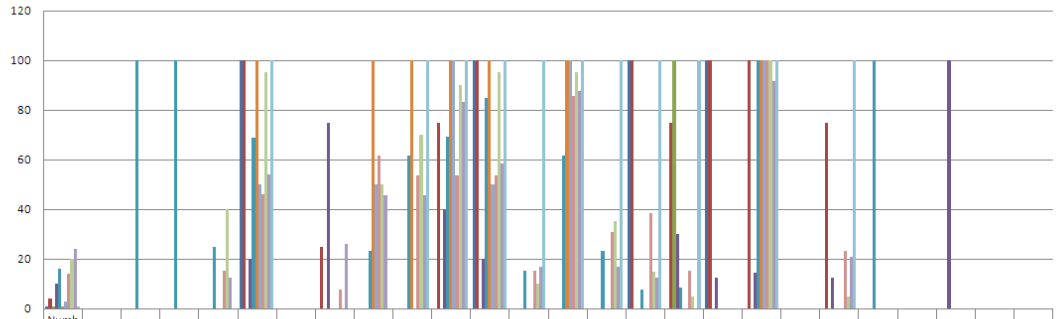
Table - 6 : NLF-LF Antibiotic Resistance



No:	AMP %R	AMC %R	TZP %R	CXM %R	CXA %R	CRO %R	CSL %R	FEP %R	ETP %R	IPM %R	MEM %R	AMK %R	GEN %R	NAL %R	CIP %R	TGC %R	NIT %R	COL %R	SXT %R	LVX %R	MFX %R	ATM %R	CZO %R	TOB %R	SAM %R	CAZ %R	CFP %R	DOR %R	MNO %R	TCC %R	CTX %R	LOM %R	NET %R	PEF %R	OFX %R
Acinetobacter baumannii	10			60			0	60	70		60	60		60		40	20		20	50	55.6		100				88.9		66.7	0	66.7					
Acinetobacter sp.	1			0				0	100		0	0	0	0		0	0		100	0	0						100		0	0	0					
Enterobacter cloacae	3		100	50	100	100	66.7	66.7	66.7	66.7	66.7	66.7	0	0	0	66.7	0	0	0	33.3																
Escherichia coli	45	94.9	81	76.7	86	92.5	92.5	67.4	67.4	66.7	55.8	69.8	35.6	46.7	100	84.4	0	0	9.3	76.7	100		100				66.7	50	0	50	0	50	50	0	50	0
Klebsiella pneumoniae ss. pneumoniae	35	100	84.4	85.3	84.4	83.9	83.9	85.3	85.3	83.9	79.4	85.3	77.1	57.1	100	85.7	11.8	100	20.6	73.5	100		100				100	100	100	0	100	100	100	0	100	100
Leclercia adecarboxylata	1			100				0	0		0	100	0	0		0	0		100	100	0														
Pseudomonas aeruginosa	8			37.5				50	50		50	37.5	37.5	37.5		50			12.5		75						37.5		37.5		62.5				
Burkholderia cepacia	51							100			0	19.6	100			100	41.2		0	12	19.1		50				17		66.7	10.6	100				
Pseudomonas fluorescens	2			50				50	50		50	0	50	50		0	0			0	0						50		50	0	50				
Proteus mirabilis	1			0				0	0		100	0	0	0		100	100		100	100	100		0				0			100	0				
Pseudomonas sp.	19			12.5				11.1	21.1		10.5	5.3	47.4	47.4		35.3	0		0	0	10.5				0		27.8		5.9	0	5.6				
Pseudomonas stutzeri	4			0				0	0		0	0	0	0		0	0			0	0						0		0	0	0				
Salmonella Paratyphi A	1			0				0	0		0	0	100	100		100	0		0	0	0		0				0		0	0	0				
Salmonella Typhi	1			0				0	0		0	0	100	100		0	0		0		0	0	0	0	0		0		0	0	0				
Sphingomonas paucimobilis	12			20			66.7	8.3	41.7		8.3	0	8.3	16.7		50	8.3		83.3	8.3	11.1		77.8				77.8		0	0	11.1				

AMP Ampicillin	CXM-Cefuroxime	AMK-Amikacin	GEN-Gentamicin	CIP-Ciprofloxacin	TGC-Tigecycline	OXA-Oxacillin	RIF - Rifampin	TCY-Tetracycline	VAN-Vancomycin	GEN-Gentamicin	PEN-Penicillin G
NIT-Netilmicin	SXT-Trimethoprim/Sulfamethoxazole	CLI-Clindamycin	ERY-Erythromycin	LVX - Levofloxacin	LNZ-Linezolid	DAP-Daptomycin	TEC - Teicoplanin	AZM-Azithromycin	NOR-Norfloxacin	FOS-Fosfomycin	SPX-Sparfloxacin

Table - 7 :GPC Antibiotics Resistance



	Number of isolates	AMP %R	CXM %R	AMK %R	GEN %R	CIP %R	TGC %R	NIT %R	SXT %R	CLI %R	ERY %R	LVX %R	LNZ %R	OXA %R	RIF %R	TCY %R	VAN %R	GEH %R	PEN %R	DAP %R	TEC %R	AZM %R	PRI %R	NOR %R	FOS %R	SPX %R
Enterococcus faecalis	1					100	0	0			0	100	0			100	0	100	0	0	0					
Enterococcus faecium	4					100	0	25			75	100	0			100	75	100	100		75					
Enterococcus gallinarum	1					0	0	0			0	0	0			0	100	0	0		0					
Enterococcus sp.	10	0			0	20	0	75			40	20	0			0	30	12.5	14.3		12.5		0	100	0	
Staphylococcus aureus ss. aureus	16	0	100	100	25	68.8	0	0	23.1	61.5	69.2	84.6	15.4	61.5	23.1	7.7	8.3		100	0	0	100				0
Staphylococcus capitis ss. capitis	1				0	100	0	0	100	100	100	100	0	100	0	0	0		100		0					
Staphylococcus, coagulase negative	3				0	50	0	0	50	0	100	50	0	100	0	0	0		100	0	0					
Staphylococcus epidermidis	14				15.4	46.2	0	7.7	61.5	53.8	53.8	53.8	15.4	85.7	30.8	38.5	15.4		100	0	23.1					
Staphylococcus haemolyticus	20				40	95	0	0	50	70	90	95	10	95	35	15	5		100	0	5					
Staphylococcus hominis ss. hominis	24				12.5	54.2	0	26.1	45.8	45.8	83.3	58.3	16.7	87.5	16.7	12.5	0		91.7	0	20.8					
Staphylococcus xylosus	1				0	100	0	0	0	100	100	100	100	100	100	100	100		100		100					

AMP-Ampicillin	AMC-Amoxicillin/ Clavulanic acid	TZP-Piperacillin/ Tazobactam	CXM-Cefuroxime	CXA-Cefuroxime axetil	CRO-Ceftriaxone	CSL-Cefoperazone/ Sulbactam	FEP-Cefepime	ETP-Ertapenem	IPM-Imipenem	MEM-Meropenem	AMK-Amikacin
GEN-Gentamicin	NAL-Nalidixic acid	CIP-Ciprofloxacin	TGC-Tigecycline	NIT-Netilmicin	COL-Colistin	SXT-Trimethoprim/ Sulfamethoxazole	LVX-Levofloxacin	MFX -Moxifloxacin	ATM-Aztreonam	TOB-Tobramycin	SAM -Ampicillin -Sulbactam
CAZ-Ceftazidime	CFP-Cefoperazone	DOR-Doripenem	MNO-Minocycline	TCC-Ticarcillin/ Clavulanic acid	CTX-Cefotaxime	LOM-Lomefloxacin	NET-Netilmicin	PEF-Pefloxacin	OFX-Ofloxacin		

Resistogram of Gram-Positive Cocci to different antibiotics is given in Table 7. Enterococci species were resistant to ciprofloxacin, nitrofurantoin, erythromycin, levofloxacin, gentamycin, penicillin and teicoplanin ranging from 0-75%. Enterococcus *faecium* (75%) and Enterococcus *gallinari* (100%) were resistant to vancomycin (VRE). Different species of staphylococcus identified were Staphylococcus *capitis*, CONs, Staph.*epidermidis*, Staph.*haemolytics*, Staph.*hominis* and Staph.*xylosus*. Staphylococcus *capitis* and *xyloses* were resistant to almost all antibiotics like ciprofloxacin, trimethoprim/ sulfamethoxazole, clindamycin, erythromycin, levofloxacin, oxacillin (MRSC,MRSX) and penicillin, but Staphylococcus *xyloses* was also resistant to tigecycline and vancomycin.

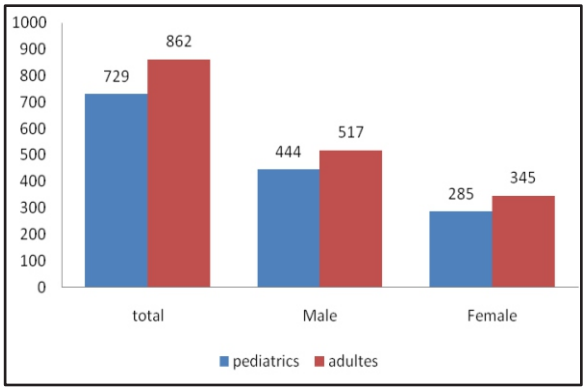


Figure 2: Sensitivity pattern of Candida sp.(n=3)

Candidemia was there in four patients and in three patients it was due to Candida *albicans* and in one patient it was due to C.*glabrata*. Both the species of Candida were sensitive to amphotericin, capsofungin, flucytocine, microfungin. C.*grabrata* was sensitive to Voriconazole and while C.*albicans* was resistant. (Figure 2)

Discussion

Approximately 85% of patients diagnosed as acute leukemia undergoing intensive anti-leukemia treatment developed infections and fever during neutropenic phase; and in 50% of these patients clinico-mcrobiological evidence of infection can be obtained specifically in cases of bacterimias.[3] This is basically due to the long-term placement of I.V. central line /peripheral catheters. The automated blood culture system from Becton Dickinson is a boon to the fast diagnosis of bacterimias and hastens up antibiotic treatment.

In the present retrospective analysis, it was observed that in 30.66 events the blood cultures were positive in one day (18-24 hours) for the detection of bacterial isolates which is much similar (19.33 hours) to the study conducted by Kaur et al (2004) from MM Institute of Medical Sciences & Research, Ambala.

Blood cultures are the strong supporting modality to guide the clinicians for better and timely antibiotic treatment in cases of febrile neutropenia.

There were 64.5 % requests from clinicians for leukemia patients with symptoms of septicemias & there has always been male preponderance in pediatric as well as adult patients. Out of which 66.2% of bacteremias were due to gram negative bacilli (GNB) and 32.4% due to gram positive cocci (GPC) unlike the study of Kaur et al (2014), who reported 19.06% GNB & 19.33 GPC isolated.

In a review of epidemiology and antibiotic resistance the recent changes in bacteremia in patients with cancer found that gram negative bacteria were the most frequent pathogens isolated. They also found the extensive emergence of antimicrobial resistant strains associated with increased risk of morbidity. This increasing incidence of antibiotic resistance was reported in both types of organism.[2] A similar picture was highlighted in our data analysis too, where in non–lactose fermenting bacilli showed 50-100% resistance to cephalosporins, aminoglycosides and beta lactam and beta lactamase inhibitors.

Some of the un-usually isolated bacteria like *Leclercia adecarboxylata* were flagged in the blood cultures by the automated Vitek ID and AST system to identify the kind of organism. This bacterium was resistant to beta lactam and beta lactamase inhibitor combination unlike the reports published in the literature. Unlike the study by Kaur et al[3] where they found 29.92% CONS, our observation showed Staphylococcus *Hominis sp Hominis* were highlighted more. Staphylococcus *hominis* is a human skin contaminant. It causes infections in people with abnormally weak immune system and majority of our patients are immunocompromised.

The methicillin resistant Staphylococcus *species*(MRSsp) identified were 85.53% of which Staphylococcus *hominis* showed 32.8%, Staphylococcus *haemolyticus* showed 29.6% and Staphylococcus *epidermis* showed 18.7% resistance to methicillin. Staphylococcus *aureus* showed 12.5% resistance to methicillin(MRSA). Our study differed a lot from the one conducted by Olson et al[5] where they communicated that methicillin resistant S. epidermidis(MRSE) accounted for 47% and methicillin resistant S. aureus was 29.5%. Majority of the isolation was in patients with ophthalmic disease. Their study was limited to two species of Staphylococci, epidermidis & aureus. In recent study by Asai *et al* (2020)[6] where they characterized relevance of coagulase-negative Staphylococcus epidermidis by automated blood culture system from BD, and identified using MALDI-TOF instrument. They found that most common isolates were Staphylococcus *hominis* (38%), followed by S. *capitus* (24%) and S. *caprae* (16%). Their study is approximately comparable to current study where S. *hominis* isolation was almost similar.

Conclusion

In conclusion it was found that automated blood culture system is convenient, simple to use and rapid method for the diagnosis of septicemias, bacteremia and fungemia. In this study analysis, it is observed that gram negative bacteria especially the *Enterobacteriaceae* are the major cause of bacteremia in hematology and other oncology patients in our institute. The high antibiotic resistance among the gram negative and gram-positive microorganisms is seen, for which combination therapy of aminoglycosides with cephalosporins and piperacillin/tazobactam can be given. For the gram-positive cocci antibiotic vancomycin must be spared to treat critically ill patients and linezolid is to be given for resistant gram-positive infection. The emerging trends in antibiotic resistance, and the spread of methicillin resistance to oppurtunistic staphylococci infections is a major concern and calls for vigilence in choosing antibiotics for gram positive cocci to treat patients and further the multi drug resistance of gram negative bacilli is again a concern and requires to formulate empirical therapy and warns us to create antibiotic policies for the hospital and should be made available in consultation with the clinicians where patients are getting infected with superbugs.

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Co-occurrence of ATRX and IDH Mutations Identify Subgroup of Glioma Patients for Better Survival

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Summary

In gliomas, with IDH mutational status, ATRX loss of protein classified diffuse astrocytic low grade and primary and secondary glioblastoma (GBM). The current study we sought to explore the clinical impact of ATRX and IDH in glioma patients. A total of 47 astrocytoma tumors of glioma were included and loss of ATRX protein expression and IDH1/2 mutations were detected using immunohistochemistry and real-time PCR, respectively. Data was evaluated by SPSS software. Loss of ATRX protein was noted in 46.7% and mutation in IDH1/2 was detected in 42% of glioma tumors. Further, significantly high incidence of loss of ATRX protein was noted in patients with frontal lobe of tumors compared to temporal and parietal locations. ($\chi 2$=10.473, r=+0.482, p=0.003). This is indicating that the incidence of ATRX mutation is significantly varied based on location of the glioma tumors. With marginal statistical significance, we observed a positive correlation between ATRX loss and IDH mutation in astrocytoma lineage tumors ($\chi 2$= 3.59, r = +0.283, p=0.060). In survival analysis, multivariate survival analysis demonstrated that patients whose tumors showed co-occurrence of mutations of ATRX and IDH together have significantly better progression free (HR=0.234, 95% CI=0.085-0.641, p=0.005) and overall survival (HR=0.447, 95% CI=30.224-0.897, p=0.024) compared to patients with either absent of both genes mutations and/or presence of any one mutation. Thus, our results indicated that though glioma is single entity, ATRX behaves biologically different in different location of glioma tumors. Also, co-detection of ATRX and IDH has more clinical impact in predicting for better progression free survival and longer overall survival than analyzing any one marker mutational status.

Keywords: Glioma, ATRX, IDH, Co-detection, Multivariate analysis

Introduction

Gliomas are the most frequent primary malignant brain tumors, characterized by complex biological behavior with a heterogeneous molecular background. Fortunately, for this devastating neoplasm, more and more research on the intra-tumoral heterogeneity has been specified over the years. Currently, mutational classification of brain tumors have led to a more targeted management of gliomas tailored to individual patients' mutations[1] , however, the molecular picture is still not clear and therefore, till date the targeted therapy didn't reach to the clinic for glioma patients management!

The revised version of 2016 updated WHO classification of CNS tumors has had a particular impact on the diffuse low and high grade astrocytoma tumors. Accordingly, the three molecular markers utilized for diffuse astrocytomas are absence/presence of isocitrated dehydrogenase (IDH) mutations, TP53 mutation, and α thalassemia/mental retardation syndrome X-linked (ATRX) loss.[2] ATRX, is a transcriptional regulator, originally it was discovered in patients with the X-linked mental retardation syndrome (ATRX syndrome).[3] However, since two decades, the significance in cancer is rising. In gliomas, with IDH mutational status, ATRX loss of protein classified diffuse astrocytic low grade and primary and secondary glioblastoma (GBM). Thus, a tight bonding between IDH mutations and ATRX mutations has been noted.[4] They differentiate astrocytic lineage of glioma tumors. For glioma patients, though, IDH is one of the most common only molecular prognostic factor; no novel therapeutic targeted therapy still translated at clinic, based on IDH status. For ATRX also, deciphering a comprehensive role in gliomas is still in its infancy. Therefore, in the current study, we aim to evaluate the clinical impact of ATRX and IDH mutations for glioma patients using immunohistochemistry and real-time PCR, respectively.

Material and Method

Patients

A total 47 untreated histologically confirmed glioma patients with astrocytoma tumors registered at The Gujarat Cancer & Research Institute from January 2017 to January 2020 were enrolled in the current study. The study was approved by the institute's ethics committee board and written consent forms were obtained from all the patients prior to treatment administration. Detailed clinical and pathological history of the patients was obtained from the case files maintained at the Medical Record

Table 1: Patient and Tumor Characteristics

Characteristics	N	%
Total Patients	**47**	
Age		
≤45	26	55.3
>45	21	44.7
Gender		
Female	22	46.8
Male	25	53.2
Tumor Location		
Frontal	23	49.0
Temporal	11	23.4
Parietal	9	19.1
Occipital	4	8.5
Treatment		
Only Surgery	23	48.9
Followed by		
Radiotherapy and/or Chemotherapy	24	51.1
Histological Grade		
Grade II	16	34.0
Grade III	8	17.1
Grade IV	23	48.9
GBM-based on IDH status		
Primary GBM	26	57.8
Secondary GBM	19	42.2
Progression free survival (n=37)		
Norecurrence	16	43.2
Recurrence	21	56.8
Overall survival (n=37)		
Alive	20	54.1
Died	17	45.9

Table 2: IDH1/2 Mutations Detection using Therascreen Mutation Detection Assay

Gene	Mutation	Amino Acid Change
IDH1	c.395G>A	R132H
Codon132	c.394C>T	R132C
	c.394C>A	R132S
	c.394C>G	R132G
	c.394G>T	R132L
	c.394_395CG>GT	R132V
Codon100	c.299G>A	R100Q
IDH2	c.515G>A	R172K
Codon172	c.515G>T	R172M
	c.514A>T	R172W
	c.516G>T	R172S
	c.514A>G	R172G

Table 3: Lab Established ΔCT Cut-Off value for Mutation Detection

Mutations	Lab established Cut-off ΔCT
ΔCTIDH1R132Mut	≤4.25
ΔCTIDH1MutR132H	≤4.40
ΔCTIDH1MutR132C	≤5.80
ΔCTIDH1R100Mut	≤4.22
ΔCTIDH2R172Mut	≤4.00
ΔCTIDH2MutR172K	≤5.70

Department of our institute. The clinico-pathological characteristics of the enrolled patients are enlisted in Table 1.

In the present study, more than 50% of patients were <45 years. All patients underwent for surgery as primary treatment. Fifty-one percent of patients had taken radiotherapy and/or chemotherapy as adjuvant therapy. Out of a total of 47 patients, 37 patients could be followed for a minimum period of 24 months or until their death within that period. Progression-free survival (PFS) and overall survival (OS) was evaluated. Within 24 months, 56.8% (21/37) patients had developed recurrent disease and 45.9% (17/37) of patients died within 24 months. (Table 1)

Also, according to tumor location site, majority of patients had tumors in temporal (49%) and frontal (23%) sites of the brain. According to histological grade of tumors, 34% patients had grade II tumors and 17% patients had grade III tumors and 49% of patients had grade IV astrocytoma tumors. Based on IDH mutational status, Glioblastoma patients were categorized into primary and secondary GBM. In the present study, 57.8% patients had primary GBM, whereas, 42.2% patients had secondary GBM tumors. (Table 1)

Immunohistochemistry

ATRX protein expression was studied using immunohistochemistry described previously (5). Formalin-fixed paraffin embedded tissue blocks retrieved from the tissue repository of our institute's Pathology Department. The blocks were cut into 4 µm sections and mounted on 3-amino propyl triethoxy silane (APES)-coated slides. The staining was performed using HRP/DAB (ABC) Detection IHC kit (Abcam, Cambridge, UK) according to manufacturer's protocol. Briefly, antigen retrieval treatment was given by heating the sections in 10 mM sodium citrate buffer (pH-6.0) in a pressure cooker. Then after, sections were incubated overnight at 4^0 C with the primary monoclonal antibody from Boster Bio; anti-ATRX clone RAD54 with 1:100 dilution in TBS. Similarly, for IDH1 R132H, the primary antibody used was anti-human IDH1 R132H mouse monoclonal antibody DIA clone H09 (Dianova, Germany) at a dilution of 1:100. The stained sections were mounted with DPX and observed under the light microscope. Sections with intense staining for IDH1 R132H were used as positive control, whereas negative control was obtained by omission of primary antibody. IDH R132H using mutation specific clone of DO9 is recommended method for detection of IDH mutational status for brain tumors. Therefore, here for validation of real-time PCR method, we evaluated cytoplasmic staining pattern of IDH1 R132H in more than 10% of patients.

Assessment of ATRX expression

For ATRX, only nuclear staining was considered for evaluation. Loss and retention of nuclear expression was noted for each patient. If nuclear staining was present in >10% of area, then considered retention for ATRX (no loss of expression) (Liu et al 2019).

DNA Extraction

Genomic DNA was extracted from histopathology confirmed astrocytoma FFPE blocks retrieved from histopathology department of our institute. DNA isolation was done using the AuPreP GENbt DNA extraction Kit, according to the manufacturer's instructions. The concentration, purity and quality of the extracted DNA were determined by Qubit 2.0 Fluorometer (Invitrogen, USA) and 0.8% gel agarose electrophoresis, respectively.

Real-time PCR for IDH1/2 mutation detection

IDH1/2 mutations was detected using ARMS PCR using therascreen IDH1/2 RGQ PCR kit following manufacturer's instructions (Qiagen). Qualitative detection of 6 mutations within IDH1 codon 132, one within IDH1 codon 100 (R100Q) and 5 within IDH2 codon 172 was noted (Table 2). PCR was performed using the Rotor-Gene Q 5-plex HRM instrument (Qiagen). Quality control was seen using CT values of controls. With each assay, we run positive, negative and no template control to ensure that acceptable Ct values were met and that the reactions were performed correctly. The PCR condition used was: 95°C Time: 10 min Cycling 40 times 95°C for 15 sec 60°C for 60 sec with an acquisition of FAM™ fluorescence in channel Green: Single. Sample ΔCt values were calculated as the difference between the mutation assay Ct and respective total assay Ct from the same sample. Samples were classified as mutation positive if the ΔCt value was less than or equal to the ΔCt cut-off value of the respective mutation assay. (Table 3)

Statistical Analysis

The data was analyzed statistically using SPSS Inc. version 20 software. The correlation between the loss or retention of ATRX protein with clinicopathological parameters of glioma patients was determined by two-tailed chi square test ($\chi 2$) and spearman's correlation. Survival analysis was performed using Kaplan-Meier survival function and the differences in survival were tested for statistical significance using log-rank statistic. Multivariate survival analysis was performed using Cox forward stepwise proportional hazard regression model. $p \leq 0.05$ was considered to be statistically significant.

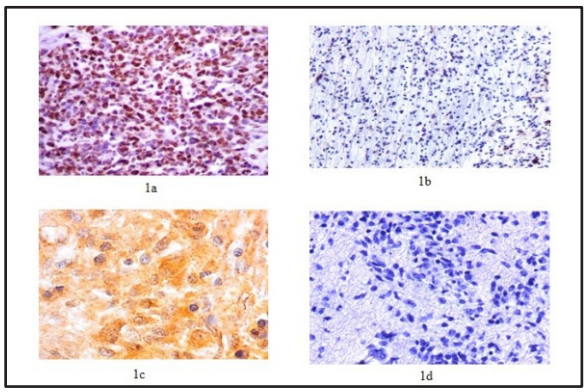

Figure1: Representative immunohistochemical staining of ATRX and IDH1 R132H in glioma patients

Figure 1a: Retention of ATRX in astrocytoma tumors
Figure 1b: Loss of ATRX expression in astrocytoma tumors
Figure 1c: Cytoplasmic expression of IDH1 R132H (clone: HO9) in astrocytoma tumors
Figure 1d: Negative control for IDH1 R132H

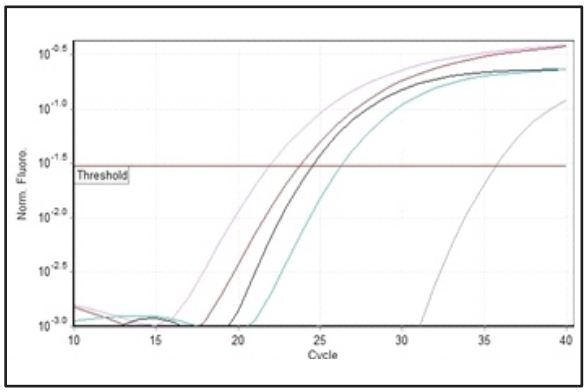

Figure 2: Representative real-time PCR threshold values curve of IDH1/2 mutation of glioma patients

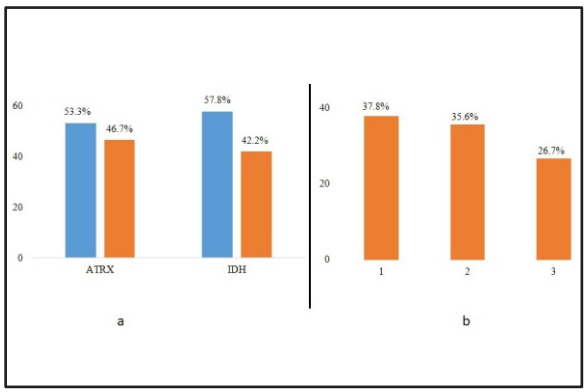

Figure 3: Representative image of incidence of ATRX and IDH mutations in glioma patients

a. ATRX: Retention and loss of expression of ATRX protein
IDH: Absent and present of IDH mutations in glioma tumors
b. Absence of mutations in ATRX and IDH1/2 genes
Mutations present in any one gene (ATRX or IDH)
Mutations are present in ATRX and IDH1/2 genes

Table 4: Correlation between ATRX and Tumor Location

Variables	N	ATRX Expression		p value	r	$\chi 2$
TumorLocation	**45**	**Retention N(%)**	**Loss N(%)**	**0.003**	**-0.482**	**10.473**
Frontal	22	8(36.4)	14(63.6)			
Temporal	10	5(50)	5(50)			
Parietal	9	9(100)	0(0)			
Glioblastoma						
Primary	26	17(65.2)	9(34.6)	0.060	+0.283	3.59
Secondary	19	7(36.8)	12(63.2)			

Table 5: Univariate survival analysis for PFS and OS using Kaplan-Meier Analysis

Univariate survival		PFS			OS		
Parameters		**Patients relapsed**	**p value**	**Log rank**	**Patients died**	**p value**	**Log rank**
	N	**N(%)**			**N(%)**		
Grade of tumors							
Grade II	10	03(30)	NS	-	02(20)	0.063	5.53
Grade III	07	04(57)			01(14)		
Grade IV	20	14(70)			14(70)		
ATRX expression							
Retention	20	16(80)	0.002	9.99	12(60)	0.024	5.10
Loss	17	05(29)			05(29)		
IDH1/2mutations							
Absent	22	03(14)	0.062	3.49	14(64)	0.035	4.44
Present	15	05(33)			03(20)		
ATRX and IDHmutations							
Bothare absent	16	12(75)	0.003	11.75	10(63)	10(63)	6.97
Anyone present	10	08(80)			06(60)		
Bothare present	11	01(09)			01(09)		

Table 6: Multivariate survival analysis using all parameters for PFS and OS

Survival	Step	Parameter	HR	Lower	Upper	p value
PFS	1	ATRXmutation	0.234	0.085	0.641	0.005
OS	1	ATRX & IDH mutations together	0.447	0.224	0.897	0.024

Results

Incidence of ATRX and IDH ½ mutations in glioma patients

Loss of nuclear staining of ATRX indicates the presence of ATRX mutation phenotype in glial tumors. The incidence of loss of ATRX protein in glioma tumors was 46.7%, (21/45) and retention of ATRX was observed in 53.3% (24/45) of tumors (Figure 1). The IDH1/2 mutations using qPCR was detected in 42% (20/47) of glioma tumors (Figure 2). Mutation of either ATRX or IDH1/2 was noted in 36% of patients. We also observed mutations of ATRX and IDH1/2 both together in 27% of patients. (Figure 3)

Relation of ATRX loss with clinicopathological parameters

A significantly high incidence of loss of nuclear ATRX was observed in tumors from frontal lobe of brain compared to tumors from temporal and parietal locations. ($\chi 2$=10.473, r=-0.482, p=0.003). This is indicating that the incidence of ATRX mutation is significantly varied based on location of the glioma tumors. Also, this result indicates different biological behavior of glioma tumors based on sites of brain from where they are located (Table 4).

Similar difference in ATRX protein expression we observed between primary and secondary GBM tumors. The incidence of loss of

ATRX protein was significantly high in patients having presence of IDH mutation (secondary GBM) as compared to patients with absence of IDH mutation (primary GBM), however, we found marginal statistical significance in this correlation ($\chi 2 = 3.59$, r = +0.283, p= 0.060). (Table 4)

Univariate Survival Analysis

Univariate survival analysis for PFS and OS was performed using Kaplan-Meier survival analysis for all clinicopathological parameters and ATRX and IDH mutational status.

Progression free survival

Univariate Kaplan-Meier survival analysis for PFS demonstrated that patients with retention of ATRX protein in their tumors showed significantly high incidence of relapsed in comparison to patients with loss of ATRX expression (p=0.002, df=1, Log rank=9.99). This finding indicates that patients with presence of ATRX mutation had significantly longer PFS compared to their respective counterparts. However, with IDH mutational status, marginal significance was observed with PFS (p=0.06, df=1, Log rank=3.49). In line of this, most striking result we noted was that patients with presence of both mutations in their tumors had significantly low incidence of relapsed in comparison to patients with either any one mutation present or absent of both mutations together in their tumors (p=0.003, df=2, Log rank=11.75). (Table 5)

Overall survival

Univariate Kaplan-Meier survival analysis for OS indicated that patients with retention of ATRX protein in their tumors showed significantly high incidence of death in comparison to patients with loss of ATRX expression (p=0.024, df=1, Log rank=5.10). This finding indicates that patients with presence of ATRX mutation had significantly better OS compared to their respective counterparts. Similar result we noted for IDH mutation status. The incidence of death was significantly high in patients whose tumors showed absent of IDH mutations in comparison to patients with presence of IDH mutations (p=0.035, df=1, Log rank=4.44). Also, we noted significantly low incidence in death rate in those patients whose tumors showed presence of ATRX and IDH mutations together as compared to patients with either any one mutation present or absent of both mutations (p=0.037, df=2, Log rank=6.97). (Table 5)

Multivariate Survival Analysis

Progression-free survival

To assess the dependence of the predictive value of ATRX and IDH on other known prognostic factors (age, gender, location of tumors, histologic grade), a multivariate Cox forward stepwise proportional hazard regression analysis was performed. We observed that for PFS, only presence of ATRX mutation that is loss of ATRX nuclear protein expression entered the equation at step 1. Thus, ATRX remained a significant risk factor for recurrence of disease (HR=0.234, 95% CI=0.085-0.641, p=0.005; Table 6). This indicated that loss of nuclear ATRX protein could serve as an independent prognostic factor for predicting progression-free survival.

Overall survival

Multivariate survival analysis using the Cox forward stepwise proportional hazard regression model demonstrated that for OS, presence of both ATRX and IDH mutations together entered the equation at step one (HR=0.447, 95% CI=30.224-0.897, p=0.024; Table 6). Thus, for glioma patients, overall survival remains better if their tumors showed presence of ATRX and IDH both mutations together.

In addition, we would like to add that in our study, out of 37 patients, only 50% of patients had completed planned treatment. Therefore, PFS and OS analysis based treatment subgroups was not done due to small sample size.

Discussion

Currently, the updated 2016 WHO classification for CNS tumors has incorporated molecular aberrations that might help to resolve the discrepancy between classification and clinical outcome of astrocytic glioma tumors. For glioma patients, IDH mutation is emerged as prognostic and predictive parameter independent of the WHO grade of tumors.[1] However, based on IDH status, till date, no novel therapeutic targeted therapy is translated at clinic. On the contrary, in many cases, WHO grade II or III IDH-wild-type infiltrating astrocytoma patients have worse outcomes than IDH-mutant glioblastomas (grade IV), reflecting that their tumors are likely to behave in a manner similar to IDH-wild-type glioblastoma. This is creating a significant problem in the current grading criteria. Moreover, in addition to IDH mutations, ATRX mutation has just been discovered in gliomas and have been the subject of numerous studies on the classification and prognosis of glioma. Keeping this in mind, in the current study, we evaluated the clinical significance of ATRX alongwith IDH mutations for glioma patients with astrocytic tumors.

Many types of tumor cells, including glioma tumor cells maintain telomere length via telomere activation, while some types of tumors elongate telomere length by telomere independent manner, which is known as "ALT" and this ALT phenotype was significantly correlated with ATRX loss.[8, 9] To

detect ATRX mutation phenotype in glial tumors, loss of nuclear expression of ATRX protein using immunohistochemistry is used.[10] This loss of nuclear protein may occur due to mutations, deletions, or gene fusions and correlates with ALT phenotype. In the current study, loss of ATRX protein expression was observed in 46.7% of glioma tumors indicating presence of ATRX gene mutations. In grade II astrocytoma tumors, the loss of ATRX was noted in 60% and in secondary GBM it was found in 43%. There are many reports demonstrating ATRX mutation or loss in multiple tumors, including low and high grade astrocytomas. This is indicating an imminent "driver" role of ATRX in cancer. Also, Wiestler et al (2013) have found 41% ATRX loss in astrocytoma tumors. However, Jiao et al (2012) described a significantly higher mutation rate of ATRX mutation with 73% anaplastic astrocytomas tumors. This difference of percentage in ATRX mutation and loss of expression, is probably due to the different techniques used.

In the present study, we found statistically significant difference in incidence of loss of ATRX protein with different locations of glioma tumors. We noted that patients with frontal glioma tumors had significantly high incidence of ATRX protein loss compared to patients with tumor in temporal followed by parietal tumors. Similar to our findings, Ebrahini et al (2016) have observed significant loss of ATRX in frontal lobe tumors. This is further corroborated by the relatively high frequency of seizures in the frontal lobe attributed to IDH mutant gliomas.[12] In addition, Debajyoti et al (2018) also noted ATRX loss of expression most frequently seen in frontal region of the brain. This is indicating that the incidence of ATRX mutation is varied based on location of the glioma tumors. Thus, ours and others findings demonstrated that though glioma is a single entity, the biological behavior may differed based on location of primary sites.

In addition, we also noted a significant difference in incidence of loss of ATRX protein between primary and secondary GBM. With marginal significance, a high incidence of loss of ATRX protein in patients having secondary GBM than patients with primary GBM. This is indicating that mutation of ATRX is more frequent in secondary GBM compared to primary GBM. Similarly, Ebrahimi et al (2016) also noted frequent loss of ATRX expression in secondary GBM compared to primary GBM. In addition, Haase et al (2018) have noted that expression of ATRX is varied with respect to GBM. According to the mutator hypothesis of oncogenesis, early mutations in "caretaker genes" can drive further tumor development.[15] ATRX has role for NHEJ DNA repair pathway. It is possible that the genetic instability in ATRX-deficient GBM drives proliferation by affecting cell cycle control or differentiation, as has been shown in other genetically unstable tumor models. Additionally, impaired apoptotic signaling through defective DNA-PKcs phosphorylation and/or concurrent TP53 mutations could provide an additional proliferative advantage to ATRX-mutated tumors.[15]

Recently, Hu et al (2020) have shown significant correlation between ATRX loss and presence of IDH1/2 mutations in grade II gliomas. Also, Mukherjee et al (2018) have shown how expression of mutant IDH1 initiates telomeric dysfunction and alters DNA repair pathway preferences at telomeres, cooperating with ATRX loss to defeat a key barrier to gliomagenesis. This is suggesting new therapeutic options to treat low-grade gliomas. In the current study, we also have noted positive correlation between ATRX loss of protein and presence of IDH with marginal statistical significance (p=0.066), probably due to less sample size. However, most striking result we noted when we analysed univariate and multivariate survival analysis using co-detection of ATRX and IDH mutations. Patients with presence of both genes mutations together emerged at step 1 for PFS and OS indicating their significance in predicting survival and early recurrence for glioma patients. In astrocytoma tumors of glioma patients, presence of both mutations together showed better OS than any one mutation or absent of both mutations. This invariable co-occurrence of ATRX with IDH mutations support a cooperative pathogenic mechanism by which dysfunction in both proteins is required for oncogenesis in a large subset of diffuse glioma tumors. Also, overlap of IDH1/2 mutations and ATRX alterations argues for a specific role of ATRX in IDH-driven gliomagenesis. Additionally, multiple studies have shown that as a consequences of ATRX loss, genomic instability caused, and these same functional relationships recapitulate in IDH-mutant glioma tumors too! Also, Kanan et al (2012) have reported high frequency of ATRX gene mutation which was entirely restricted to IDH-mutant low grade gliomas of astrocytic lineage-astrocytoma. Further, a better prognosis for patients with ATRX mutations has been suggested in a retrospective cohort by Noushmehr et al (2010). Further, Jiao et al (2012) have experimentally proven that loss of ATRX caused by siRNA induced apoptotic cells increasing, reduced tumor cell proliferation and repressed the cell migration in glioma cells. Moreover, Cai et al (2015) reported that decreased expression of ATRX can cause inhibition of migration, promotion of apoptosis and reducing of proliferation in glioma cells.

Conclusion

ATRX loss of protein expression is present in glioma patients having tumors of astrocytic lineage.

We concluded that co-occurrence of ATRX and IDH mutations in glioma tumors has more clinical impact in predicting PFS and OS of glioma patients than studying any one molecular marker. Thus co-detection of ATRX and IDH mutations could identify subgroup of glioma patients with better clinical outcome. However, as only half of our patients completed planned treatment and due to overall low number of patients studied, we cannot conclusively confirm that. Therefore, validation of this data is recommended in larger sample size.

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Evaluation of Serum LDH, p53 and BCL2 in Lung Cancer Patients

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Summary

To evaluate the role of serum lactate dehydrgenase (LDH) and the expressions of p53, BCL2 in non small cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC) patients and correlate their expressions with clinicopathological parameters and diagnostic lung cancer markers panel, prognostic role and disease outcome. Total 100 lung cancer patients having NSCLC and SCLC were enrolled in the study. p53 and BCL2 expression were studied by Immunohistochemistry method and serum LDH level were analyzed in Cobas 6000 analyzer.p53 expression was significantly higher in males, smokers, LN3 nodal status and in squamous cell carcinoma patients (SCC). A significant inverse correlation was noted with adenocarcinoma markers. Higher BCL2 expression was seen in patients with smoking habits, T4 and T2 tumor size and stage III disease. A positive correlation was noted in adenocarcinoma markers and an inverse correlation with SCC markers. In SCLC, a significant positive correlation was noted with chromogranin. A higher abnormal LDH level was noted in T4 and T3 tumor size, without lymph node involvement, stage III disease and in SCC patients. A higher incidence of death was observed in patients with abnormal LDH level. In SCLC, a significant higher abnormal LDH level was noted in patients with ≤56 years of age. No significant correlation was found with survival. In inter-marker correlation between p53, BCL2 and LDH showed that patients with p53 and BCL2 positive had high LDH level. In conclusion, higher expression of mutant p53 and BCL2 in smokers suggests that higher tobacco consumption increased the risk of mortality or poor survival by inducing the altered metabolism of p53 and BCL2 by effect of carcinogens. A positive correlation of mutant p53 and BCL2 expression with LDH in adenocarcinoma as well as impact of LDH on survival suggests that LDH plays an important role in cancer cell metabolism.

Keywords: p53, BCL2, LDH, NSCLC, IHC, SCLC

Introduction

Lung cancer is the leading cause of cancer incidence and mortality worldwide. In India, the incidence of lung cancer is 5.9% and mortality is 8.82%.[1] At Gujarat Cancer & Research Institute a regional cancer center of Western India, incidence of lung cancer is accounted for 5.8% according to hospital-based cancer registry.

Lung cancer is broadly divided into small cell lung carcinoma (SCLC) and non small cell lung carcinoma (NSCLC), with a rapid frequency of proliferation in both smokers and non-smokers.[2] The variation in the rates of lung cancer unfold the maturity of the tobacco epidemic and differentials in the historic patterns of tobacco exposure, including intensity, the time period of smoking, type of cigarettes, degree of inhalation and environmental pollution.[3-4] Besides, tobacco consumption, other factors such as genetic susceptibility, poor diet, occupational exposures and air pollution may act autonomously in shaping the illustrative epidemiology of lung cancer.[5]

Apart from the above parameters, one of the major causes which induce various types of cancers including lung cancer is altered metabolism which is an emerging and potential hallmark of cancer and plays an important role in the cancer cell progression. It promotes the tumor formation by triggering various oncogenes such as Ras, RAF, EGFR, MIC, MYB, ABL2, BCR and tumor suppressor genes such as p53, FLT3, BRCA1, BRCA2, IDH1 and many more. Different intracellular metabolic enzymes like LDH (Lactate dehydrogenase) are released by tumor cells, due to intracellular machinery alteration and apoptosis deregulation.[6] The signaling pathways perturbed in cancer regulates metabolism with some metabolic enzymes functioning as tumor suppressors genes and oncogenes.[7]

In lung cancers, altered metabolic genes are ubiquitously either over expressed or under expressed. The mechanism of altered metabolism was first explained by Otto Warburg who observed an abnormal characteristic of cancer cell energy metabolism in which only 2 ATP molecules are produced by enzyme named Lactate dehydrogenase (LDH) which interconverts pyruvate to lactate at the end of the glycolytic pathway using NAD+ as a cofactor. Down regulation of LDH can lead to an inhibition of cancer cell proliferation.

Uncontrolled proliferation of cancer cells induce the expression of p53 which is known as the guardian of genome regulates many different aspects of metabolism. The function of p53 in regulation of metabolism includes the regulation of glycolysis, pentose phosphate pathway, mitochondrial oxidative phosphorylation and lipid metabolism. p53 gene is frequently mutated in maximum numbers of human tumors. p53 lost its tumor suppressive function and tumor associated mutant p53 proteins often gain new tumorigenic activities termed as gain-of-function (GOF) of mutant p53.[8] It has been reported that mutant

p53 proteins and wild-type p53 proteins frequently regulate similar cellular biological processes with contradictory effects.[8-9] For example, in metabolic regulation, wild type p53 inhibits the initiation of glycolysis whereas; mutant p53 promotes glycolysis through different mechanisms.

BCL2 is the anti-apoptotic protein that localizes to the mitochondria and blocks the recruitment and activation of pro-apoptotic proteins such as Bax. p53 is proposed to activate cell cycle check points, whereas anti-apoptotic gene BCL-2 has shown to inhibit cell death. The widen roles of BCL-2 proteins in energy metabolism come up with its additional ways in which this molecule alters normal metabolism beyond its well-recognized role in regulation of apoptosis.[10]

So, the present study aimed to evaluate the role of p53, BCL2 and serum LDH in Lung cancer patients to predict the role of altered metabolism and correlate its activity p53 and BCl2 and to evaluate its individual and combined efficacy in predicting prognosis of NSCLC and SCLC patients.

Material and Method

In this retrospective study, 100 (80 patients with NSCLC and 20 patients with SCLC) lung cancer patients who had been diagnosed and treated at Gujarat Cancer & Research Institute (GCRI)in the duration of 2015 to 2017 were included. The detailed clinical history such as patient's age, gender, habit (smoking or tobacco), histopathological finding, and treatment offered and disease status were recorded in the division from the case file maintained at the Institutional Medical Record Department. Paraffin embedded tissue block of these lung cancer patients were archived from Histopathology Department of GCRI. The study was approved by the Institutional Scientific Review Board and Ethics Committee.

Immunohistochemical Localization

Immunohistochemical localization of p53 and BCL2 were evaluated on formalin fixed paraffin embedded (FFPE) tissue blocks containing primary tumor evaluated by Hematoxylin and Eosin (H&E) staining, on Ventana Benchmark XT autoimmunostainer using Ventana reagents (Ventana, USA). The commercially available antibodies used were p53 (Clone SP5, Thermo Scientific. 1:50) and BCL2 (Clone 124, Cell Marque. 1:100). Briefly, 3-4 µm thin sections were cut on microtome (Leica, Germany) and taken on to 3-Aminopropyltriethoxysilane (APES) coated slides. Briefly the protocol included following steps of deparafinization using EZ prep solution, antigen retrieval for 30 minutes for BCL2 and 90 minutes for p53 using retrieval solution CC1 and incubation with ultra view DAB inhibitor for 4 minutes, addition of 100µL of p53 and BCL2 antibody at 37ºC for 120 minutes 32 minutes respectively, followed by incubation with ultra view HRP multimer for 8 minutes, ultra view DAB Detection kit for 8 minutes. The sections were counterstained with hematoxylin for 8 minutes and bluing reagent for 4 minutes and mounted with DPX.

Scoring

Two individual observers scored the sections. Cytoplasmic staining pattern for BCL-2 and nuclear staining pattern was observed for p53. Histoscore (H-score) was evaluated by multiplying percentage of positive cells with the staining intensity. H-score from 0 to 300 was evaluated where score of less than or equal to 50 (≤50) was scored as negative and that of more than 50 (>50) was scored as positive for p53 as well as BCL2.

Evaluation of Serum LDH

Evaluation of lactate dehydrogenase in human serum was done on Roche/Hitachi Cobas C systems: Serum collected using standard sampling tubes. Reagents are ready to use and packed in closed cassettes it makes reagent handling fully automated. This method has been standardized against the original IFCC formulation using deionized water as zero calibration pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity. The COBAS 6000 system automatically calculated the LDH activity of each sample.

Statistical Analysis

Statistical analysis was carried out using SPSS statistical software version 20 (SPSS Inc, USA). Mean, standard error (SE) of mean and median were calculated. Pearson's Chi-square test with Pearson's correlation coefficient (r) was used to assess correlation and significance between two parameters. Survival analysis was performed using Kaplan-Meier survival function and the differences in survival were tested for statistical significance using log-rank statistic. P values ≤ 0.05 were considered to be significant.

Results

Expression of p53, BCL2 and Serum LDH in lung cancer patients

In NSCLC, 39% (31/80) showed nuclear expression of p53 where as 61% (49/80) were negative for p53 expression (Figure: 1a and 1b). Cytoplasmic expression of BCl2 was observed in 35% (28/80) of patients and 65% (52/80) of patients were negative for BCL-2 expression (Figure: 2a and 2b). Out of 80 patients 56% (45/80) patients showed abnormal LDH level, while 44% (35/80) patients showed normal (normal range 100-190 IU/L) LDH level.

In SCLC, all the patients showed positive nuclear expression of p53 hence 100% positivity was noted, Cytoplasmic expression of BCL2 found positive

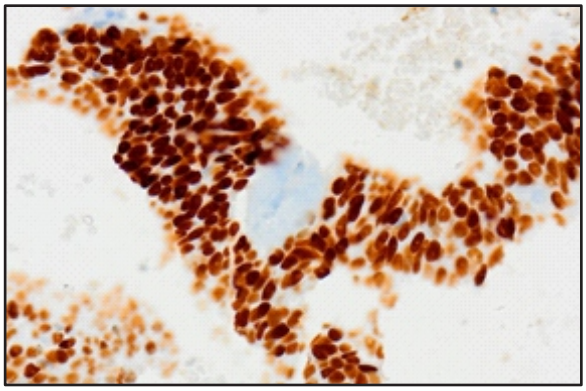

Figure 1(a): p53 positive expression in adenocarcinoma patients (Nuclear staining)

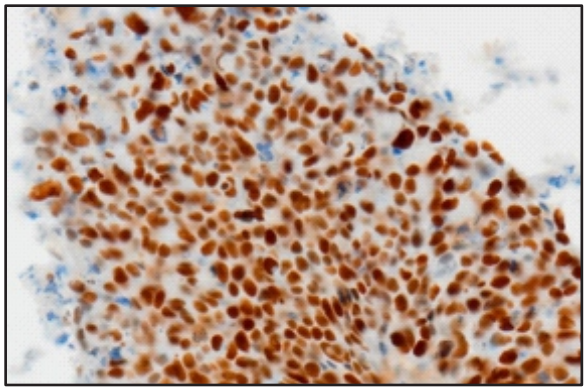

Figure 1(b): p53 positive expression in squamous cell carcinoma patients (Nuclear staining)

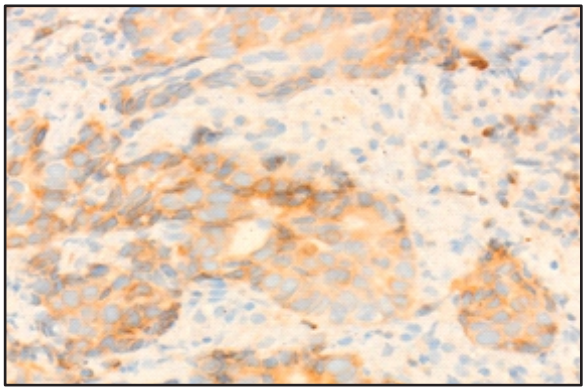

Figure 2(a): BCL-2 positive expression in adenocarcinoma (cytoplasmic staining)

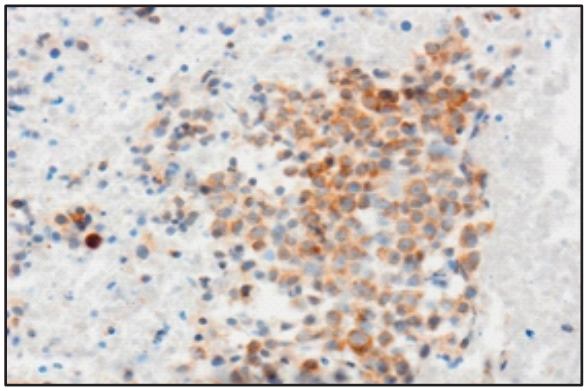

Figure 2(b): BCL 2 positive expression in squamous cell carcinoma (cytoplasmic staining)

in 45% (9/20) of patients and 55% (11/20) of patients showed negative BCL2 expression. Out of 20 patients 70% (14/20) patients showed abnormal LDH level, while 30% (6/20) patients showed normal LDH level.

Correlation of p53, BCL2 and Serum LDH with Clinical and Pathological parameters

In relation with clinical parameters, a trend of higher p53 expression was noted in patients with >60 years of age (49%, 18/31). A significant higher p53 expression was observed in male patients (43%, 31/31; p=0.018) and in smokers (55%, 26/31) compared to non-smokers (15% 5/31; p=0.0001). Correlating p53 expression with pathological parameters, a significant higher expression of p53 was noted in patients with LN3 (nodes represent contralateral mediastinal or contralateral hilar lymphadenopathy or scalene or supraclavicular nodes)nodal status (85% 11/31) as compared to patients without lymph involvement (43%, 3/31), LN2 (nodes represent ipsilateral mediastinal or subcarinal lymphadenopathy) (29%, 16/31) and LN1 (nodes are ipsilateral nodes within the lung up to hilar nodes) (20%, 1/31; p=0.002) involvement. Further, higher p53 expression was noted in patients with stage III (39%, 29/31) disease as compared to patients with stage II (20%, 1/31) disease. There was only one patient with stage I (1/31) disease who showed p53 expression. With histological sub types a significant higher p53 expression was observed in patients with squamous cell carcinoma (60%, 24/31) as compared to patients with adenocarcinoma (17% 7/31; p=0.0001). (Table 1a)

No significant correlation of BCL2 was noted with any clinical parameters however, higher BCL2 expression was observed in smokers (40%, 19/28). A trend of higher BCL-2 expression was noted in patients with stage III (35%, 26/28) disease as compared to patients with stage II (20%,1/28) disease, There was only one (1/28) patient was in stage I disease and showed BCL-2 expression. (Table 1a)

No significant correlation of LDH was noted with clinical or pathological parameters. However, higher LDH level was noted in patients with stage III (57% 42/45) disease as compared to patients with stage II (40%, 2/45) disease. There was only one patient with stage I disease whose LDH level was found to be abnormal. A higher trend of abnormal LDH level was noted in patients without Lymph Node involvement status (71% 5/45) as compared to patients with LN2 (nodes represent ipsilateral mediastinal or subcarinal lymphadenopathy) (58%, 32/45), LN3 (nodes represent contralateral mediastinal or contralateral hilar lymphadenopathy or scalene or supraclavicular nodes) (46%, 6/45), and LN1 (nodes are ipsilateral nodes

Table 1 (a): Correlation of p53, BCL-2 and Serum LDH with clinical and pathological parameters in NSCLC patients (N=80)

Parameters	N (%)	p53 expression		BCL-2 expression		LDH level	
	N (%)	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	Normal N (%)	Abnormal N (%)
Age(years)		49(61%)	31(39%)	52(65%)	28(35%)	35(44%)	45(56%)
≤60	43(54)	30(70%)	13(30%)	26(61%)	17(39%)	21(49%)	22(51%)
>60	37(46)	19(51%)	18(49%)	26(70%)	11(30%)	14(38%)	23(62%)
Gender		49(61%)	31(39%)	52(65%)	28(35%)	35(44%)	45(56%)
Male	72(90)	41(57%)	**31(43%)** [a]	47(65%)	25(35%)	32(44%)	40(56%)
Female	8(10)	8(100%)	0(0.0%)	5(63%)	3(37%)	3(37%)	5(63%)
Habit		49(61%)	31(39%)	52(65%)	28(35%)	35(44%)	45(56%)
Non-smoker	33(41)	28(85%)	5(15%)	24(73%)	9(27%)	14(42%)	19(58%)
Smoker	47(59)	21(45%)	**26(55%)** [b]	28(60%)	19(40%)	21(45%)	26(55%)
Tumor size		49(61%)	31(39%)	52(65%)	28(35%)	35(44%)	45(56%)
T1 (≤3cm)	32(40)	20(63%)	12(37%)	25(78%)	7(22%)	19(59%)	13(41%)
T2 (>3cm to ≤5cm)	17(21)	12(71%)	5(29%)	9(53%)	8(47%)	7(41%)	10(59%)
T3 (>5cm to ≤7cm)	11(14)	7(64%)	4(36%)	8(73%)	3(27%)	3(27%)	8(73%)
T4 (>7cm)	20(25)	10(50%)	10(50%)	10(50%)	10(50%)	6(30%)	14(70%)
Nodal status		49(61%)	31(39%)	52(65%)	28(35%)	35(44%)	45(56%)
N0	7(9)	4(57%)	3(43%)	4(57%)	3(43%)	2(29%)	5(71%)
N1	5(6)	4(80%)	1(20%)	5(100%)	0(0.0%)	3(60%)	2(40%)
N2	55(69)	39(71%)	16(29%)	35(64%)	20(36%)	23(42%)	32(58%)
N3	13(16)	2(15%)	**11(85%)** [c]	8(62%)	5(38%)	7(54%)	6(46%)
Stage		49(61%)	31(39%)	52(65%)	28(35%)	35(44%)	45(56%)
I	1(1)	0(0.0%)	1(100%)	0(0.0%)	1(100%)	0(0.0%)	1(100%)
II	5(6)	4(80%)	1(20%)	4(80%)	1(20%)	3(60%)	2(40%)
III	74(93)	45(61%)	29(39%)	48(65%)	26(35%)	32(43%)	42(57%)
Histological subtypes		49(61%)	31(39%)	52(65%)	28(35%)	35(44%)	45(56%)
Adenocarcinoma	40(50)	33(83%)	7(17%)	24(60%)	16(40%)	20(50%)	20(50%)
Squamous cell carcinoma	40(50)	16(40%)	**24(60%)** [d]	28(70%)	12(30%)	15(37%)	25(63%)

Note: p value: a$\chi 2$ =5.624, r= 0.265, p= 0.018, b $\chi 2$= 13.179, r= 0.406, p=0.0001, c $\chi 2$= 14.475, r= 0.215, p= 0.002, d $\chi 2$= 15.221, r= 0.436, p=0.0001

within the lung up to hilar nodes) (40% 2/45), lymph node involvement. With histological subtype higher abnormal LDH level was observed in squamous cell carcinoma (63%, 25/45) patients as compared to adenocarcinoma (50% 20/45) patients. (Table1a)

In SCLC, as all the patients were positive for p53 expression no correlation could be performed with clinical and pathological parameters higher BCL2 expression was observed in male patients (47%, 9/9) as only one female patient included in this study, and in smokers (50%, 7/9) compared to non-smokers (33% 2/9). Correlating BCL2 expression with pathological parameters, higher expression of BCL-2 was noted in patients with LN2 (nodes represent ipsilateral mediastinal or subcarinal lymphadenopathy) nodal status (50% 8/9) as compared to patients with LN3 (nodes represent contralateral mediastinal or contralateral hilar lymphadenopathy or scalene or supraclavicular nodes) nodal status (25%, 1/9). With disease stage, all the patients were of stage III disease and forty five (45%, 9/20) of patients showed positive BCL2 expression. A significant higher abnormal LDH level was noted in patients with younger age group ≤56 years of age (92%, 11/14; p=0.010), and a higher trend of abnormal LDH level was observed in T2 tumor size (88%, 7/8) followed by T4 (75%, 3/4), T1 (50%, 3/6) and T3 (50%, 1/2) tumors. As all the patients were of stage III disease and of them seventy (70%, 14/20) percent of patients had abnormal LDH level. (Table 1b)

Correlation of p53, BCL2 and Serum LDH with other diagnostic lung cancer panel

Further p53, BCL2 and Serum LDH expression in NSCLC were correlated with diagnostic lung cancer panel which included TTF-1(Thyroid transcription factor), CK7 (cytokeratin 7), CEA (carcinoembryonic antigen), p63 and CK5/6. In relation to p53 expression a significant inverse-correlation was noted with adenocarcinoma markers TTF –1 (p=0.003), CK7 (p=0.026) and CEA (p=0.033), with no significant correlation with squamous cell carcinoma markers. Also no significant correlation of

Table 1 (b): Correlation of BCL2 and Serum LDH with Clinical and pathological parameters in SCLC patients (N=20)

Parameters	N (%)	BCL-2 expression		LDH level	
	N (%)	Negative N (%)	Positive N (%)	Normal N (%)	Abnormal N (%)
Age(years)		11(55%)	9(45%)	6(30%)	14(70%)
≤56	12(60)	6(50%)	6(50%)	1(8%)	11(92%)a
>56	8(40)	5(63%)	3(37%)	5(63%)	3(37%)
Gender		11(55%)	9(45%)	6(30%)	14(70%)
Male	19(95)	10(53%)	9(47%)	6(32%)	13(68%)
Female	1(5)	1(100%)	0(0.0%)	0(0%)	1(100%)
Habit		11(55%)	9(45%)	6(30%)	14(70%)
Non-smoker	6(30)	4(67%)	2(33%)	2(33%)	4(67%)
Smoker	14(70)	7(50%)	7(50%)	4(29%)	10(71%)
Tumor size		11(55%)	9(45%)	6(30%)	14(70%)
T1 (≤3cm)	6(30)	4(67%)	2(33%)	3(50%)	3(50%)
T2 (>3cm to ≤5cm)	8(40)	3(37%)	5(63%)	1(12%)	7(88%)
T3 (>5cm to ≤7cm)	2(10)	2(100%)	0(0%)	1(50%)	1(50%)
T4 (>7cm)	4(20)	2(50%)	2(50%)	1(25%)	3(75%)
Nodal status		11(55%)	9(45%)	6(30%)	14(70%)
N0	0(0)	0(0%)		0(0%)	
N1					
N2	16(80)	8(50%)	8(50%)	5(31%)	11(69%)
N3	4(20)	3(75%)	1(25%)	1(25%)	3(75%)
Stage		11(55%)	9(45%)	6(30%)	14(70%)
I	0(0)	0(0%)		0(0%)	
II					
III	20(100)	11(55%)	9(45%)	6(30%)	14(70%)
Note: p value: a $\chi 2$ =6.706, r= -0.579, p= 0.010					

BCL2 expression and LDH level was noted with any of the adenocarcinoma and squamous cell carcinoma markers. (Table 2a)

In SCLC, besides NSCLC the diagnostic panel also included synaptophysin and chromogranin. In relation to BCL2 a significant positive correlation was noted with chromogranin, and higher trend was observed with synaptophysin. With LDH a significant inverse correlation of abnormal LDH was noted with CEA whereas, no significant correlation was noted with other markers. (Table 2b)

Univariate Survival Analysis

Disease free survival was not evaluated since majority of the patients had persistent disease. According to Kaplan-Meier univariate survival analysis, for overall survival (OS) a similar incidence of death was noted in patients with and without p53, BCL-2 expression A trend of higher incidence of death was noted in patients with abnormal LDH level as compared to patients with normal LDH level. (Table 3a)

However, Kaplan-Meier univariate survival analysis in adenocarcinoma patients, with respect to overall survival (OS) a higher incidence of death was noted in patients with positive p53 and BCL2 expression as compare to patients with negative expressions. In LDH a higher incidence of death was noted in patients with abnormal LDH level as compared to patients with normal LDH level. (Table 3b) In squamous cell carcinoma, a similar incidence of death was noted in patients with p53 positive and negative expression. A higher incidence of death was noted in patients with negative BCL-2 expression as compare to patients with positive BCL2 expression. In LDH a higher incidence of death was noted in patients with abnormal LDH level.(Table 3c)

In SCLC, since the patients had persistent disease, disease free survival was not evaluated. Out of 20 patients, 9 patients died and remaining 11patients were lost to follow-up with a median survival of 10 months. Hence, survival analysis was not evaluated. However, all the patients who died had abnormal LDH level.

Inter-marker correlation between p53, BCL2 and LDH

When intermarker correlation was performed among p53, BCL2 and abnormal LDH level, similar abnormal LDH levels were noted among patients with p53 positive (61%, 19/45) and p53 negative (53%,

Table 2 (a): Correlation of p53, BCL2 and Serum LDH with other diagnostic lung cancer panel (NSCLC)

Parameters	p53 expression		BCL-2 expression		LDH level	
	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	Normal N (%)	Abnormal N (%)
TTF – 1	N=71		N=71		N=71	
	42(59%)	29(41%)	45(63%)	26(37%)	29(41%)	42(59%)
Negative	20(45%)	24(55%)	30(68%)	14(32%)	18(41%)	26(59%)
Positive	**22(82%)**[a]	5(18%)	15(56%)	12(44%)	11(41%)	16(59%)
CK7	N=71		N=71		N=71	
	41(58%)	30(42%)	46(65%)	25(35%)	31(44%)	40(56%)
Negative	10(40%)	15(60%)	18(72%)	7(28%)	9(36%)	16(64%)
Positive	**31(67%)**[b]	15(33%)	28(61%)	18(39%)	22(48%)	24(52%)
CEA	N=54		N=54		N=54	
	29(54%)	25(46%)	32(59%)	22(41%)	22(41%)	32(59%)
Negative	9(37%)	15(63%)	16(67%)	8(33%)	10(42%)	14(58%)
Positive	**20(67%)**[c]	10(33%)	16(53%)	14(47%)	12(40%)	18(60%)
P63	N=54		N=59		N=59	
	34(58%)	25(42%)	39(66%)	20(34%)	24(41%)	35(59%)
Negative	13(65%)	7(35%)	12(60%)	8(40%)	10(50%)	10(50%)
Positive	21(54%)	18(46%)	27(69%)	12(31%)	14(36%)	25(64%)
CK5/6	N=46		N=46		N=46	
	25(54%)	21(46%)	27(59%)	19(41%)	19(41%)	27(59%)
Negative	14(67%)	7(33%)	12(57%)	9(43%)	8(38%)	13(62%)
Positive	11(44%)	14(56%)	15(60%)	10(40%)	11(44%)	14(56%)
Note: p value: aχ2 =8.988, r= -0.356, p= 0.003, b χ2= 4.981, r= -0.265, p=0.026, c χ2= 4.562, r= -0.291, p= 0.033						

Table 2 (b): Correlation of BCL2 and Serum LDH with other diagnostic lung cancer panel (SCLC)

Marker	BCL2 expression		LDH level	
	Negative N (%)	Positive N (%)	Normal N (%)	Abnormal N (%)
TTF - 1	N=16		N=16	
	9(56%)	7(44%)	4(25%)	12(75%)
Negative	3(75%)	1(25%)	1(25%)	3(75%)
Positive	6(50%)	6(50%)	3(25%)	9(75%)
CK7	N=15		N=3	
	9(60%)	6(40%)	0(0%)	3(100%)
Negative	2(33%)	4(67%)	0(0%)	3(100%)
Positive	7(78%)	2(22%)	0(0%)	0(0%)
CEA	N=9		N=9	
	4(44%)	5(56%)	2(22%)	7(78%)
Negative	2(33%)	4(67%)	0(0%)	**6(100%)**[b]
Positive	2(67%)	1(33%)	2(67%)	1(33%)
P63	N=7		N=7	
	5(71%)	2(29%)	2(29%)	5(71%)
Negative	5(83%)	1(17%)	2(33%)	4(64%)
Positive	0(0%)	1(100%)	0(0%)	1(100%)
CK5/6	N=3		N=3	
	1(33%)	2(67%)	0(0%)	3(100%)
Negative	1(33%)	2(67%)	0(0%)	3(100%)
Positive	0(0%)	0(0%)	0(0%)	0(0%)
Synaptophysin	N=20		N=20	
	11(55%)	9(45%)	6(30%)	14(70%)
Negative	3(100%)	0(0%)	0(0%)	3(100%)
Positive	8(47%)	9(53%)	6(35%)	11(65%)
Chromogranin	N=20		N=20	
	11(55%)	9(45%)	6(30%)	14(70%)
Negative	**6(100%)**[a]	0(0%)	1(17%)	5(83%)
Positive	5(36%)	9(64%)	5(36%)	9(64%)
Note: p value: a χ2= 7.013, r= 0.592, p= 0.008, b χ2 =5.143, r= -0.756, p= 0.023				

26/45) expression. Similar BCL2 expression was observed among p53 positive (39%, 12/35) and p53 negative (33%, 16/35) tumors. Also, in correlation of BCL2 with abnormal LDH levels similar abnormal LDH level was noted in patients with BCL2 positive (61%, 17/45) and BCL2 negative (54%, 28/45) tumors. (Table 4)

Discussion

The present study evaluated 100 lung cancer patients in which, 80 patients of NSCLC (40 patients were of adenocarcinoma and 40 patients were of squamous cell carcinoma) and 20 patients of SCLC. In this study and in most of the studies majority of patients are presented with advanced disease at the time of diagnosis like stage III/IV.[11] So, despite of significant developments in the oncological management, the survival of late stage lung cancer over recent years remains poor.

The major causes which induce various types of cancers including lung cancer is altered metabolism which is further added as an emerging and potential hallmark of cancer and plays an important role in the cancer cell progression. LDH is considered relevant in all cancers due to its role as a metabolic check point in cancer glycolytic pathway and also plays a role in activation of some proto-oncogene and the maintenance of invasiveness and metastatic potential. Mutant p53 regulates various metabolisms by mediating metabolic changes such as alteration in morphology of gene and alteration of cellular metabolism that promotes tumor cell survival and growth.[8] Alteration in the expression and the function of

Table 3 (a): p53, BCL-2 and Serum LDH expression in relation to Overall survival. (NSCLC)

Markers Expression	N	OS in months Mean ± SE	Alive N (%)	Dead N (%)
p53 Expression				
Negative	49	21.33±2.12	21(43)	28 (57)
Positive	31	21.81±3.47	13 (42)	18 (58)
Log rank=0.03, df=1, p=0.957				
BCL-2 Expression				
Negative	52	19.99±2.16	22 (42)	30 (58)
Positive	28	23.86±3.19	12 (43)	16 (57)
Log rank=0.785, df=1, p=0.375				
LDH Level				
Normal	35	20.54±2.63	17 (49)	18 (51)
Abnormal	45	22.52±2.47	17 (38)	28 (62)
Log rank=0.540, df=1, p=0.462				

Table 3 (b): p53, BCL-2 and Serum LDH expression in relation to Overall survival in adenocarcinoma

Markers Expression	N	OS in months Mean ± SE	Alive N (%)	Dead N (%)
p53 Expression				
Negative	33	22.46±3.00	14 (42)	19 (58)
Positive	7	34.71±6.92	2 (29)	5 (71)
Log rank=0.997, df=1, p=0.318				
BCL-2 Expression				
Negative	24	21.86±3.48	11 (46)	13 (54)
Positive	16	27.62±5.88	5 (31)	11 (69)
Log rank=0.691, df=1, p=0.406				
LDH Level				
Normal	20	22.15±4.16	9 (45)	11 (55)
Abnormal	20	25.78±4.63	7 (35)	13 (65)
Log rank=0.819, df=1, p=0.366				

Table 3(c): p53, BCL-2 and Serum LDH expression in relation to Overall survival in Squamous cell carcinoma

Markers Expression	N	OS in months Mean ± SE	Alive N (%)	Dead N (%)
p53 Expression				
Negative	16	21.17±2.68	7(44)	9 (56)
Positive	24	19.19±3.29	11 (46)	13 (54)
Log rank=0.015, df=1, p=0.902				
BCL-2 Expression				
Negative	28	18.71±2.66	11 (39)	17 (61)
Positive	12	21.37±3.86	7 (58)	5 (42)
Log rank=0.319, df=1, p=0.572				
LDH Level				
Normal	15	18.89±3.36	8 (53)	7 (47)
Abnormal	25	21.04±2.91	10 (40)	15 (60)
Log rank=0.484, df=1, p=0.487				

Table 4: Intercorrelation between p53, BCL-2 and LDH

Parameters		LDH		BCL-2	
		Normal N=35(44%)	Abnormal N=45(56%)	Negative N=52(65%)	Positive N=28(35%)
p53	Positive (N=31)	12(39%)	19(61%)	19(61%)	12(39%)
	Negative (N=49)	23(47%)	26(53%)	33(67%)	16(33%)
		X2 = 0.522		X2 = 0.306	
		r = -0.081		r =0.062	
		P = 0.470		P = 0.580	
BCL-2	Positive (N=28)	11(39%)	17(61%)		
	Negative (N=52)	24(46%)	28(54%)		
		X2 = 0.349			
		r = -0.066			
		P = 0.555			

BCL2 contributes to the progression of human cancers. High BCL2 expression has been reported in many different tumors types including lung cancer, breast cancer and ovarian cancer.

p53 is frequently mutated in human tumors in the present study expression of p53 was seen in 39% of patients. The results were in accordance with Halvorsen et.al who observed p53 expression in 47% of patients and in discordance with the study of Mattioni et.al who observed p53 positive expression in 20% NSCLC patients which was lower compared to present study.[12-13] Over expression of p53 can induce circulating p53 antibodies in patients of various types of cancer, including lung cancer, because the altered conformation of p53 produced by mutations which may trigger an auto immune response once the protein has been released from tumor cells.[13-14] Cytoplasmic expression of BCL2 was found in 35% patients. In the study of Gryko et.al BCL2 positive expression was noted in 56% patients and which was higher compared to present study.[15] Fifty-six percent of patients showed abnormal LDH level, whereas 44% of patients showed normal range of LDH. (Normal range 100-190 IU/L) Similar to our study Lee et.al study showed 57% of patients with abnormal LDH level in lung cancer patients.[16]

Further, when p53 expression was correlated with the clinical parameters a significant higher p53 expression was seen in male patients, because majority of patients enrolled in the study were male. Also a significant higher p53 expression was observed in smokers. Our results indicate that smoking may play a critical role in promoting NSCLC progression via modulation of p53 protein expression which may be due to metabolic changes. As the risk of lung cancer increases with the age higher p53 expression was found in patients with >60 years of age which was in

accordance with the study of Xie et al.[17] In the study of Mattioni et al no significant difference with p53 expression was found with regard to age and gender.[13] In correlation of pathological parameters, a significant higher p53 expression was observed in patients with LN3 nodal status, similar to the study of Zhou et al that showed a significantly higher positive p53 expression in patients with lymph node metastasis.[18] When p53 expression was correlated with histological subtype, significant higher p53 expression was noted in patients with squamous cell carcinoma, suggesting that p53 mutations are usually gain before clonal expansion in SCC including those without histological evidence of precursor lesion.[19] Higher p53 expression was observed with advancement of disease stage and majority of patients enrolled in the study were of advance disease stage. However, study of Halvorsen et al showed that no significant correlation of p53 expression with tumor size and stage.[12]

Further BCL-2 expression when correlated with the clinical parameters higher BCL-2 expression was observed in smokers. No significant correlation was found between BCL-2 expression and gender or age as similar in the study of Anagnostou et al.[20] These results suggest that Bcl2 may be a primary target of carcinogens in tobacco smoke. With diseases stage, higher BCL-2 expression was noted in patient with stage III diseases as compared to patients with stage II. However, the study of Anagnostou et al showed no significant correlation of BCL-2 expression with stage.20 The study of Tsamandas et al observed that BCL2 expression in advanced-stage and high-grade gastric carcinomas indicate that BCL2 is involved in early stage of tumor development and might be playing a role in metabolic dysfunction.[21]

A higher LDH was noted in patients without lymph node involvement which was in discordance with study of Kayser et al which showed LDH level in correlation with lymph node metastasis revealed a statistically significant difference in regard to the intensity score between tumors with no or positive hilar lymph node metastases and mediastinal lymph node metastases.[22] In correlation with histological subtype of NSCLC, a higher expression of abnormal LDH level was found in patients with squamous cell carcinoma as compared to patients with adenocarcinoma. However, the study of Lee et al found that serum LDH levels were not significantly different according to histology.16 Higher LDH level was noted in patient with stage III diseases as compared to patients with stage II and there was only one patient with stage I diseases who showed abnormal LDH level. In advance disease stage induction of increased LDH level could be probably because of high amount of glucose uptake by cancer cells in hypoxic condition.

In SCLC, patients with ≤56 years of age show significant correlation with abnormal LDH which is discordance with the study of Lui et al who found that the LDH level was not related to clinical characteristics in SCC patients.[23]

Further p53 expression when correlated with lung cancer panel which showed a significant inverse-correlation with adenocarcinoma markers (Thyroid transcription factor) TTF-1, (cytokeratin 7) CK7 and CEA. The results were in discordance with study of Myong (2003)[24] who noted that there was no significant correlation between TTF-1 expression and over expression of p53. However, the study of Zhan et al study observed that TTF-1 over expression is associated with a favorable prognosis in patients with NSCLC.[25] whereas p53 over expression is associated with poor prognosis.24 No significant correlation of BCL-2 and Serum LDH was noted with adenocarcinoma markers TTF-1, CEA and CK7 as well as squamous cell carcinoma markers CK5/6 and p63.

A significant positive correlation of BCL-2 was noted with chromogranin, and a trend of higher expression was observed with synaptophysin. The study of Li et al, demonstrated that the expres¬sions of BCL-2 are more valuable than the highly spe¬cific markers such as synaptophysin, chromogranin.[26] With LDH a significant inverse correlation of abnormal LDH was noted with CEA. No significant correlation was noted with other markers.

No significant correlation was found between overall survival of patients and expression of p53, BCL-2 and abnormal serum LDH level. In this study when overall survival was correlated with respect to histological subtypes it was noted that patients with positive expression of p53, BCL-2 and Abnormal LDH and with adenocarcinoma histology shows increased incidence of death and poor survival.

In inter-marker correlation between p53, BCL-2 and sLDH showed that patients with higher p53 positive and BCL-2 positive expression had high LDH level. This result suggests that LDH might play an important role in apoptosis regulation. No significant correlation was noted between p53 and BCL-2. Similar results were obtained by the study of Yoo et al and suggested that there may be other oncogene products or additional factors that regulate apoptosis in vivo.[27]

Conclusion

In conclusion, higher expression of mutant p53 and anti-apoptotic protein BCL-2 in smokers suggests that higher tobacco consumption increased the risk of mortality or poor survival by inducing the altered metabolism of p53 and BCL-2 by effect of carcinogens. Higher p53 expression associated with squamous cell carcinoma.

A positive correlation of mutant p53 expression and BCL-2 expression with LDH in adenocarcinoma as well as impact of LDH on survival suggests that LDH plays an important role in cancer cell metabolism. The efficacy of drugs against LDH may be effective, however are under clinical trials.

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Moods and Shadows of Life

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The *red glowing sun* seen from between the branches of neem tree,
Rising slowly while I was walking, through a ray of light, through a ray of hope.
The destruction around us; The pain and agony shading moods.
The darkness spreading!
The darkness covering the humans.
The red glowing sun seen, grew into the yellow golden fire ball.

The *chirping of the birds* awakens and trickles the consciousness.
The "Tehu" "Tehu" of the peacock,
the "Chirp" "Chirp" of the chirping birds.
The croaking of the crow, the melody of the koel.
All Simply Vanished!

The *gloominess* spread around.
Man is engulfed in 'life and death struggle'
Whole life inevitably culminates to end in death!!
Could it be "COVID" or Other?
It can happen today or can happen tomorrow!
The yellow golden fire ball grew.
The day brightened.
I completed normal work-out.

The *road* *showed movement* of activities.
A two-wheeler passed and a rickshaw fellow on his peddles.
A Thela (ठेला) person came with fruits and sold.
And that red bus passed-by.

The *gloominess of death* lingered.
People around were careless, fearless, and busy with their activities.

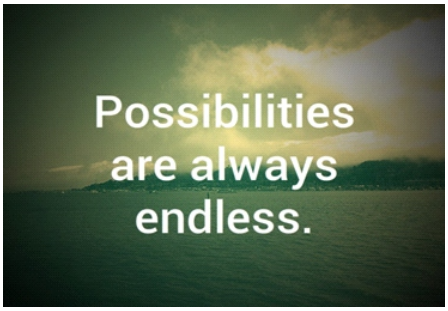
That *yellow golden fire ball* declined - Hid behind the clouds.
Left a message " Life is up and down"
Participate! Participate!! and Participate!!!
Get back to those chirping birds.
The sparrow, the parrot, the bulbul, and the peacock, on the branches of that neem tree.
Be busy with life.

Life goes on - our mind gets stuck in fear, agony, pain, and apprehension.
All created and generated by us.
Otherwise, life is that of "that morning" where that red glowing sun is seen, seen between the branches of "That neem tree".

And that *rising sun*, leaves an imprint, endowed with wisdom, understanding the beginless and endless life!
That life continues through the phenomenon of ceaseless, endless, birth and death!!
Sweetly the Koyal (कोयल) says believe in "Dheerta (धीरता)", with total self-restrain, with willingness to undergo and deal any miserable state of mind.

Let us face the world engulfing
"Ultra Micro Enemy" the COVID-19.
The world is the same!

Same is that *red glowing sun*.
Those chirping birds, that golden fire ball declining behind the clouds, leaving behind innumerable possibilities in front of us.



Luteoma of Pregnancy - Mimicking a Malignant Ovarian Mass

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Summary

Luteoma of pregnancy (LP) are uncommon, benign, tumor like condition of the ovary, believed to be developed as the consequences of pregnancy induced hormonal changes and spontaneously regress postpartum. They are asymptomatic and incidental finding during surgery or imaging. They can pose a challenge for diagnosis and management plan because most of them present as malignant tumors of ovary. To avoid unnecessary surgery during pregnancy, if there is clinically high index of suspicion of tumor being pregnancy luteoma, can manage it conservatively during antepartum period and radiologically follow-up these suspicious masses during postpartum. Management of LP can vary from case to case and depends mainly on clinical situation. They usually present in the second and third trimesters of pregnancy. They are usually solid masses and frequently bilateral. Some patients may have elevated serum testosterone levels and display features of virilization in mother and female fetus. However, in some cases which present atypically or acutely due to the complications like rupture, torsion or haemorrhage into the mass lesion which requires immediate surgical exploration for diagnosis as well as timely management.

We present a 24-year-old woman, primigravida with 27 weeks of gestation, who was referred to the hospital with sudden distension of abdomen, abdominal discomfort and vomiting since 1month. Sonography and MRI showed large cystic lesion arising from left ovary, gross ascites and mild pleural effusion. The patient underwent surgery to remove this mass considering the imaging findings were suspicious for neoplasia. Histology and immunohistochemistry revealed pregnancy luteoma.

Keywords: Luteoma of pregnancy (LP), Mass mimicking ovarian malignancy, Ovarian tumors during pregnancy, Antepartum exploratory laparotomy, Virilizing tumors of pregnancy.

Introduction

The American College of Obstetrics and Gynecologists (ACOG) has released guidelines that describe the diagnostic approach and management of adnexal masses occurring outside of pregnancy. However, guidelines that dictate physician's approaches to females with incidental adnexal masses during pregnancy remain vague. Having to consider both the pregnant and fetus when making decisions regarding the management plan makes it more complicated. The main concerns with pregnant female who develop adnexal masses are - pregnancy complications and malignancies; timely management in this case is essential, without jeopardizing the health of the fetus. According to a recent study, adnexal masses are discovered in 1 per 76–1 per 2328 deliveries.

Luteoma of pregnancy (LP) was first described by Sternberg and Barclay in 1966. In general, luteomas are asymptomatic, and they are incidentally found during imaging and peripartum surgeries like cesarean section or tubal sterilization. Luteoma of pregnancy is a benign, hyperplastic tumor-like condition of the ovary. It may be unilateral or bilateral, 1/3rd is bilateral.[1] Hyper secretion of androgens occurs in about 25% of women with pregnancy luteoma, among them 10% to 50% of the patients may display some clinical findings associated with hyperandrogenism and when the masculinized mothers gave birth to female babies they showed features of virilization (approximately 60% to 70%).[2] LP can pose a challenge for diagnosis and management plan because most of them present as malignant tumors of ovary.[3]

Case Report

Hereby presenting a case report of 24 years old, primigravida with 27 weeks of gestation, who was referred to the hospital with sudden distension of abdomen, abdominal discomfort and vomiting since 1 month. Patient had a past history of vaginal reconstructive surgery- Z plasty for transverse vaginal septum at the age of 13 years, which got failed and again she underwent surgery for the same after marriage, following which she conceived spontaneously. On per abdomen examination ascites was present, uterus 26 weeks size, relaxed, FHS heard. Mass felt at left flank extending towards left hypochondriac area. Ultrasonography(USG) shows a single live intrauterine gestation of 27 weeks and 10.2x11.4x10.9 cm heterogeneous echo texture lesion at left lumbar region extends into left iliac region. Few cystic areas with thin septation and solid component shows vascularity. Moderate ascites and mild right sided pleural effusion was noted. Magnetic resonance imaging (MRI) of pelvis shows 16x12x14.8 cm heterogeneous signal intensity lesion with internal necrotic / haemorrhagic areas in left lumbar region up to inferior pole of spleen and splenic flexure. Moderate to gross ascites was present. Tumor markers were - CA 125 (1259 U/ml), AFP (129.1 ng/ml),

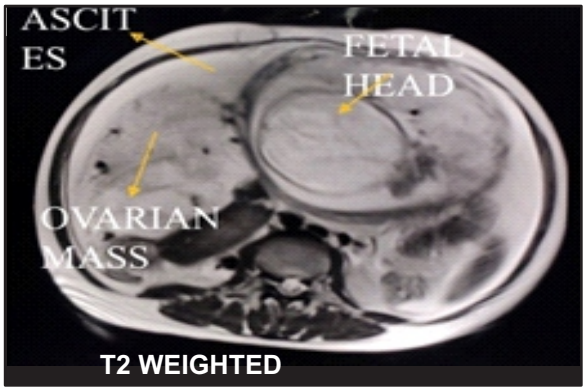

Figure 1: T2 weighted image showing left ovarian mass in relation to gravid uterus

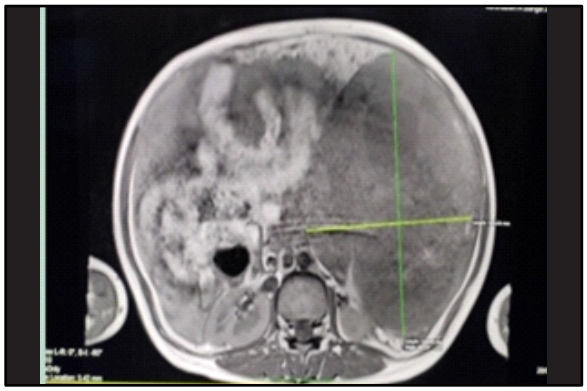

Figure 2: T2 weighted MRI showing left ovarian 16x12x14.8 cm solid cystic, well encapsulated mass with moderate to gross ascites. Right ovary is normal.

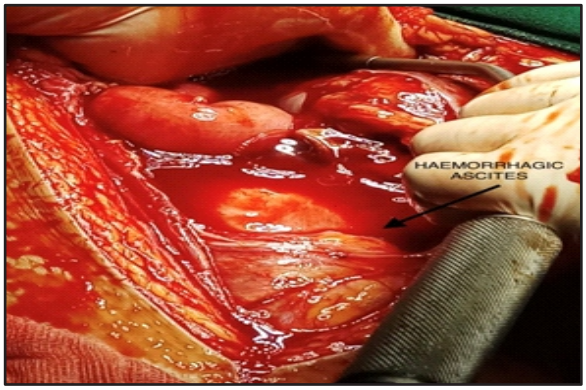

Figure 3: Intra operative image showing hemoperitoneum

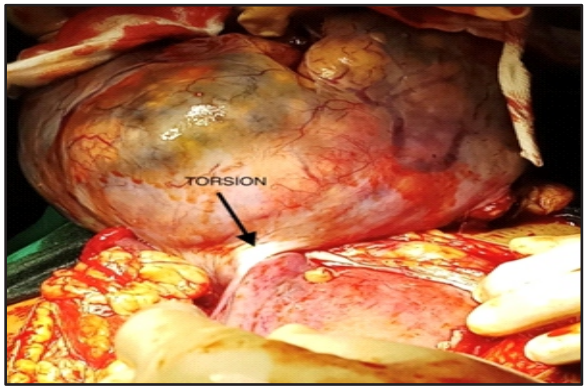

Figure 4: Intra operative image showing torsion of left ovary

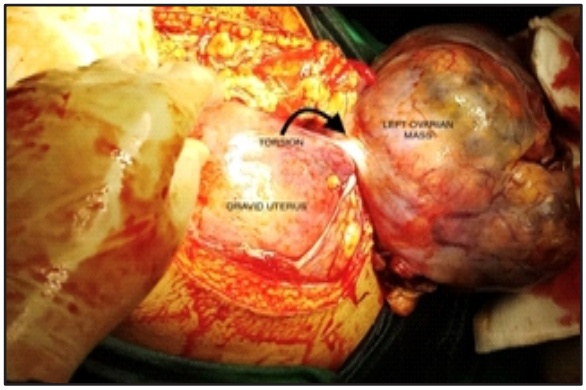

Figure 5: Intra operative image showing ovarian mass in relation with gravid uterus

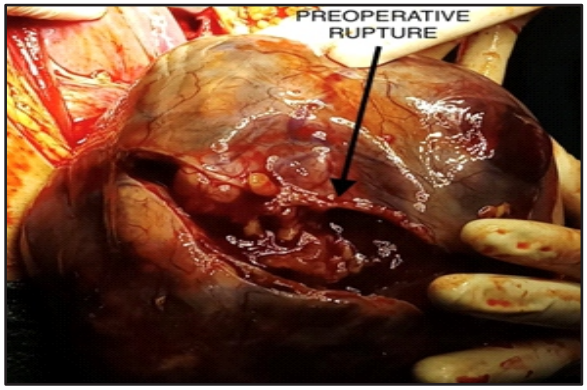

Figure 6: Intra operative image showing preoperative rupture of the ovarian mass

bhCG(16367 IU/ml), CEA(0.66 ng/ml), He4 (62.72pM). ROMA (Risk of Malignancy Algorithm) was 15.4%-high risk. Ascitic fluid cytology was suggestive of reactive mesothelial cells and negative for malignancy. Patient had hemoglobin 7.6 gm/dl and received two packed cells preoperatively.

Since there was radiologically, serologically and clinically high index of suspicion of malignancy, the decision was taken for surgery. On exploratory laparotomy 500 ml haemorrhagic ascites was drained out. Uterus was 26 weeks sized, relaxed with visible fetal movements. Approximately 25x15 cm mass, which had 360 degrees of torsion and preoperative rupture, was seen arising from the left ovary. Mass was removed along with adherent omentum and sent for frozen section. Rest of the abdomen was normal on gentle exploration.

Frozen section report was suggestive of luteoma of pregnancy. Postoperatively FHS monitoring was done and patient was managed with adequate analgesia, tocolytics(isoxsuprine) and progesterone (Duphaston). Fetal well being assessed by USG on 2nd post operative day.

Final histopathology and immunohistochemistry confirmed the diagnosis of LP. Patient was discharged on 6th post operative day

with good fetal and maternal condition. She delivered a full term baby of 2.8 kilograms by elective caesarean section. There were no signs of virilization in the newborn.

Discussion

LP is a rare condition, which resemble malignant tumors of ovary. They are believed to be a result of luteinized stromal cell hyperplasia. Approximately 50% of the times it is multinodular and in one third of the patients it is bilateral.

Luteoma of pregnancy have variable size at presentation, ranges from microscopic to over 20 cm in maximum diameter in literature and our patient had luteoma of 25 cm. Luteomas are most often clinically indolent and discovered incidentally. However, can rarely present as acute abdomen due to ovarian torsion, rupture or hemorrhage into the mass. They may be hormonally active and secrete androgen, which is responsible for virilization of both mother and female fetus. Rodriguez et al reported a case with gross ascites and elevated CA125 level in pregnancy.[4] Massive ascites and an elevated CA125 level in these type of cases resemble malignant tumor. These phenomena have been rarely reported in pregnancy luteoma cases. The increased expression of CA125 may be induced by mechanical stimulation/irritation of the mesothelium.[4] The solid ovarian tumor could physically irritate the peritoneum and stimulate the overproduction of peritoneal fluid. Rubinstein et al., suggested that the ascites results from a discrepancy between the arterial supply to a large tumor mass tissue and the venous and lymphatic drainage of the same mass, leading to stromal edema and transudation.[5] Tan ML et al reported torsion of tumor leading to its rupture and intra-peritoneal bleeding which required blood transfusion and immediate surgical exploration.[6] Even our patient presented similarly. Thus, decision regarding appropriateness of surgical management should be made by the clinician.

In a review of 11 cases of adnexal masses in pregnancy, most common tumor being mature cystic teratoma, found in 40 patients. Others are serous cystadenoma/cystadenofibroma (11 cases), mucinous cystadenoma (16 cases), and corpus luteal cyst (3 cases). Malignant or potentially malignant tumors constitute 6.1% of tumors, including a serous cystadenocarcinoma, serous borderline tumors (19 cases), an immature teratoma, a Sertoli-Leydig cell tumor and a Juvenile granulosa cell tumor. In general the malignant adnexal masses encountered during pregnancy is 3–6%.

Management of LPs varies and it depends mainly on clinical presentation, the character and the size of the tumor, period of gestation at presentation and choice of the patient.[7] Non-obstetric surgery during pregnancy posts additional concerns to anaesthesiologists. The chief goals are to preserve maternal safety, maintain the pregnant state and achieve the best possible foetal outcome. The choice of anaesthetic technique and the selection of appropriate anaesthetic drugs should be guided by indication for surgery, nature and site of the surgical procedure. Anaesthesiologist must consider the effects of the disease process itself and inhibit uterine contractions and avoid preterm labour and delivery. Foetal safety requires avoidance of potentially dangerous drugs and assurance of continuation of adequate uteroplacental perfusion. Until date, no anaesthetic drug has been shown to be clearly dangerous to the human foetus. The decision on proceeding with surgery should be made by multidisciplinary team involving anaesthesiologists, obstetricians, surgeons and perinatologists. Indications for surgery are clinical and radiologic suspicion of malignancy and patient presenting as acute abdomen due to ovarian torsion, rupture, haemorrhage into the mass. If the doubt arises regarding the diagnosis of pregnancy luteoma, then surgical exploration to be performed antenatally or post delivery and most frequent procedure being a unilateral salpingo-oophorectomy. Whenever the surgery is done intra operative frozen section for histological assessment is compulsory. This is because in case frozen section report is inconclusive and if diagnosis of malignancy is questionable, we can proceed with conservative management rather than more aggressive radical surgery. If final histopathology suggestive of malignancy, surgical staging should be performed postpartum.[8] Our patient underwent exploration because clinical and radiological features were suspicious of malignancy, patient had sudden abdominal distension due to ascites, abdominal discomfort and raised tumor markers.

Whenever the clinical judgment and radiological features are in favour of a luteoma, our management option should be conservative during antepartum and follow-up with imaging during postpartum, as there is spontaneous resolution after delivery.

Conclusion

Luteomas due to its clinical resemblance with malignant neoplasms, complicates its diagnosis and treatment. With high index of clinical and radiological suspicion for LP, conservative management during antepartum period and follow-up with imaging during postpartum period is an acceptable management strategy which will avoid unnecessary surgical intervention leading to pregnancy complications. However, in some instances with atypical and acute presentations, surgical exploration may be necessary to rule out malignancy and to provide timely treatment.

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Role of Paediatric Palliative Care in a One - Year Old Patient with Yolk Sac Tumour of Vagina and Uterus: A Case Report

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Summary
Yolk Sac Tumour (YST) is a rare and highly malignant germ cell tumour of childhood. Diagnosis is based on histopathological, immune-histochemical studies and highly elevated alfa fetoprotein level. Pediatric oncology population in India does not receive palliative care and only few who do are in late stage disease. Early integration of palliative care improves the quality of life of patients and caregivers throughout the course of illness. We describe role of palliative care in a child with rare tumor.
Keywords: Pediatric Palliative Care, Yolk Sac Tumour

Introduction

Malignant Germ Cell Tumour (MGCT) is uncommon tumour of childhood and accounts around 3% of paediatrics malignancies, yolk sac tumour (YST) is the most common histological subtype of MGCT and usually involves Gonads (testes and ovaries).[1] Affected individual are almost exclusively infants, and all are younger than three year of age at presentation.[1] Extra- pineal gonadal site are rare and involves mediastinum, endometrium, cervix, vagina, gland and sacrococcygeal area.[1] Early integration of palliative care with standard oncology care provides adequate symptoms control and total care of patients and caregivers.[2-4] Paediatric Palliative care differs from adult palliative care and requires special attention to physical, developmental, psychosocial, ethical, spiritual issues that are unique to each child. In this study we describe a case of rare site uterus and vagina YST in one-year old child with vaginal bleeding causing psychosocial distress in family. A child's life-threatening illness profoundly impacts family both emotionally and financially. Early palliative care in paediatric patients reduces the suffering of child and parents.

Case report

A one-year old female patient was brought in January 2018 with history of vaginal bleeding for twenty-five days. Radiological studies (USG and MRI) showing well defined lesion involving uterus and vagina. Elevated level of serum alfa fetoprotein, normal B-human chorionic gonadotropin and Immunohistochemical studies favoured the diagnosis of Yolk Sac Tumour. The patient received three cycles of JEB regime (Carboplatin + Etoposide and Bleomycin) from 9th January to 19th February 2018 which resulted in radiological resolution of lesion and decreased AFP level (19.39ng/ml). After four months, there was recurrence of lesion posterior to urinary bladder at upper vagina with lung metastasis and raised AFP (625.50ng/ml) for which patient received six cycle of VeIP regime (Cisplatin + Ifosfamide + Vinblastin) from 24th July 2018 to 21st February 2019 resulting in resolution of both vaginal and lung metastatic lesion. Patient received six cycle of TIP regime (Paclitaxel + ifosfamide + Cisplatin) from 6th June to 26th August 2019 for recurrent lesion at vaginal wall. Further chemotherapy was planned, but patient lost for follow up. After five-months patient was brought with vaginal bleeding and radiological studies showed tumour metastasized to lung and liver. Patient was planned for palliative radiotherapy to primary site but parents refused. At this point they were referred to Department of Palliative Medicine.

When they visited palliative medicine department…

While parents brought the child to our OPD, she was continuously, inconsolably crying. On quick examination, she was found having abdominal pain (Wong baker faces scale 9/10). On history taking, her condition was described same for one week. She was on syrup paracetamol 12mg/kg three times a day. She had history vaginal bleeding and was on Tab Tranexamic acid 20 mg/kg in three divided doses a day. They were coming for treatment from another state, 1000 kms away. She had two elder sisters staying with her grandparents. During communication with parents, we found that parents were educated, social, spiritual, co-operative, and economically belonged to middle class.

Parents were emotionally drained due to unacceptable physical suffering of their child, psychologically stressed due to worries of other

daughters being left alone to be cared by grandparents. Economically, also it was burdensome to travel frequently or to stay in vicinity of hospital for long-time. They were confused and in spiritual dilemma about refusing the radiotherapy, an unfruitful treatment at this stage.

Patient was quiet and comfortable after receiving 1mg Morphine IR orally. Tab Morphine IR 10 mg was dissolved in 10ml purified water and precise 1ml was given orally by syring.[5] We have followed this practice of Morphine administration as Morphine suspension is not commercially available. Parents were taught to prepare 1 mg oral morphine in similar way at home. They were advised to give 1ml every four hours and half ml for breakthrough pain if required, along with other supportive treatment.[6]

Parents were counselled for disease status and prognosis, round the clock use of medications, end of life care, general nursing care and place of care preference (hospital, hospice or home). All their questions were answered empathetically.[7]Parents chose home care and took the child home to her sisters and grandparents with satisfaction of receiving proper guidance and relief from guilt. Palliative care team remained in constant contact with them through phone calls, and found that she expired peacefully after two weeks.

Discussion

Vaginal YST are rare and clinically present with history of bloody vaginal discharge which is often accompanied by polypoid mass protruding from the vagina.[8] Vaginal YST are both locally aggressive and capable of metastasis via hematogenous and lymphatic pathway. The serum AFP level remains a useful marker for diagnosis and monitoring the recurrence of vaginal YST in infants. Combined chemotherapy and surgical resection remain the mainstay of treatment.[9] Patients and caregivers are not routinely screened for psychosocial-emotional distress.[6] Also paediatric patients are not prescribed strong analgesic for optimum pain relief because of lack of knowledge regarding its use in children.[6,7] Paediatric palliative care is also less utilised in our country and still palliative care references are done late in the disease trajectory.[8]

In paediatric palliative care[10] the transition from health to ill health may occur in four distinct ways.

1. Have a potentially curable illness but treatment fail
2. May receive intensive treatment that can be expected to prolong the life but child is likely to die before adulthood

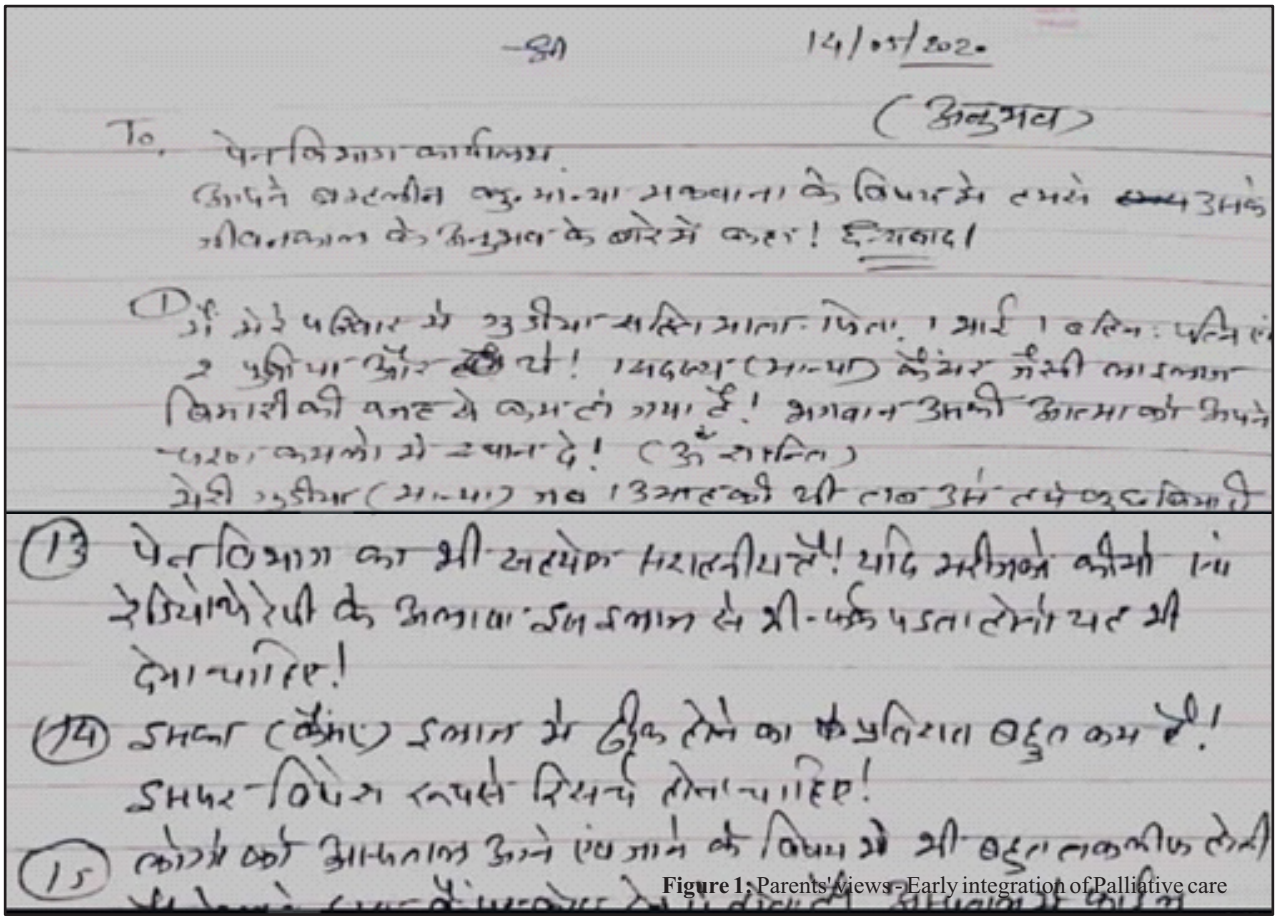

Figure 1: Parents' views - Early integration of Palliative care

3. Are diagnosed with progressive condition for which no curative treatment exists
4. Have a progressive condition but are vulnerable to early death as a result of general debility and morbidity such as respiratory infection.

In our case, YST falls in category one and received intensive treatment. For most children with malignancy cure is probability or possibility at the time of diagnosis. At that time child and family need support in living with uncertainty. This is called upstream palliative care in which the seriousness of condition is revisited and discussed at regular interval during the child's illness.[10] Paediatric patients and their families make multiple transitions during the course of illness. The goal of care should be discussed regularly and renegotiated so that they reflect on the child's care in changing circumstances. This can be possible only if palliative care is integrated early.

In this case, due to pain and symptoms burden of the child, parents had lots of suffering throughout the disease trajectory, which includes emotional distress, lack of communication, financial issues, transportation problem etc. Early palliative care improves quality of life by adequate symptom relief, good emotional, spiritual, and psychosocial support to the patients and caregivers.[2,3]

Conclusion

In this case, though patient received only one palliative care consultation during the last visit to hospital, parents appreciated adequate control of physical symptoms, pain management and guidance in decision making by giving realistic prognostic information and emotional support in end of life care (Figure 1). In India where parents remain the sole decision makers for the child, it is utmost importance for bridging the gap in cancer care by providing both cancer treatment and psychosocial-spiritual support to parents in paediatric patients.

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Presentations at the Clinical Meetings (January 2020 to June 2020)*

Sr No.	Date	Speaker/Department	Title
1	11.01.2020	Pareek Ananya Medical Oncology	Medical Oncology in India: Workload, Infrastructure, and Delivery of Care
		Jain Khushboo Radiotherapy	Cyber Knife : The Cutting Edge Technology
2	08.02.2020	Gupta Nidhi Gynaec Oncology	Pathological Chemotherapy Response Score is Prognostic in the Tubo-Ovariean High-Grade Serous Carcinoma: A Systematic Review and Meta-Analysis of Individual Patient Data
		Patel Kinjal Molecular Oncology	Development and Validation of an Individualized Immune Prognostic Signature in Early-Stage Non-squamous Non-Small Cell Lung Cancer

Panel Discussion at the Clinical Meetings

Sr No.	Date	Moderator/Department	Panelist/Department	Title
1	25.01.2020	Patel Shailesh Surgical Oncology	Parikh Sonia Medical Oncology	GE Junction Tumors and Ca. Stomach
			Warikoo Vikas Surgical Oncology	
			Mehta Maitrik Radiotherapy	
			Rajpura Hitesh Radiology	
			Shah Ashini Pathology	
2	22.02.2020	Panchal Harsha Medical Oncology	Sharma Mohit Surgical Oncology	Current Treatment in HER-2 Negative Metastatic Breast Cancer
			Parikh Ankita Radiation Oncology	
			Shah Kajal Medical Oncology	
			Rajvik Kruti Cancer Biology	

Data Presentation for Morbidity, Mortality at Clinical Meetings

Sr. No.	Date	Presenter/ Department	Data Presentation
1.	25.1.2020	Maru Bhumi Anaesthesiology	Morbidity and Mortality Data presentation of Surgical and Medical Departments
2.	22.2.2020	Talukdar Jupi Anaesthesiology	Morbidity and Mortality Data presentation of Surgical and Medical Departments

***Meetings onwards March 2020 had not been held due to Covid-19 Pandemic**

About the Journal and Instructions to Authors

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Gujarat Cancer Society Research Journal is a biannually (April and October) peer-reviewed journal published by the Gujarat Cancer Society (formerly published as GCS Research Bulletin). The journal's full text is available online at http://www.cancerindia.org

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The Journal intents to cover basic, clinical, clinico-basic research and medical education carried out by the staff of the Gujarat Cancer Society and Gujarat Cancer and Research Institute related to human well being including ethical and social issues in the field of Oncology. The Journal gives preferences to original scientific papers, case reports, anecdotal reports and mini reviews. It may comprise invited review articles, publish oration speeches and work presented in the clinical meetings and the journal clubs. Hence it will continue to serve as an academic-research bridge between the basic sciences and the applied sciences, viz. various disciplines of medicine within and outside GCS-GCRI.

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Introduction

The Gujarat Cancer and Research Institute (GCRI) "Library and Information Services Department", covers an area of approximate 186.2 square meter, with two floors present old building area. It started functioning since 1972 with professional library staff. GCRI Library services is not only restricted to print books, journals and other related print documents but also provides facility of online databases, e-resources, bibliographic record, resource sharing, indexing, archival repository, automation, scientific publication resources, e-library facilities etc, for easy accessibility to the users.

The Gujarat Cancer and Research Institute's library is an academic nerve for the faculty members, resident doctors, medical scientists, post-graduate, PhD students, researchers and experts. The library is fully automated with barcoding facility and all hardcopy of books are laminated with plastic cover. Books and library materials are arranged in open access user-friendly system and DDC classification is used for classification all the library books and other materials. Bibliographic database and library house-keeping operation of GCRI library is managed by automation SOUL 2.0 software with Web OPAC (Open Public Access catalogue) facility. Through the feature of Web OPAC facility users can specify author details, titles, subject area, publisher, issue/return status, availability of library materials, etc.

GCRI Library and Information Services Department plays a vital role for the Institute

GCRI library is providing value added services to their users that means "Right Information to Right Person in a Right Way." The main purpose of GCRI library is to support medical education, including teaching and research with special focus on improved patient care and academic credit. To fulfill all the expectation of user groups, GCRI library is providing various services viz digital library, digital notice board, audio-video facility, high speed internet and Wi-Fi facility, email service, borrowing facility, current awareness service, SDI, alert services, staff library of non-medical books, computer labs, reading room facility, etc.

1. GCRI Digital Library facility

Digital medium is one such media for preserving and maintaining information and resources on internet and E-format. It is very user friendly and easily accessible for the storage of information and users can retrieve information anywhere and anytime. Keeping this modern trend, GCRI Library and Information Services initiated GCRI digital library service since 12th August 2015 onwards. GCRI digital library is focus on the collection of digital objects that includes institutional repository, online database, and other E-resources. Library bibliographic database is available on Digital library with Web OPAC facility. The Institutional repository has been created to collect, preserve, and disseminate the scholarly output of GCRI staffs and resident doctors like PhD thesis, DM, MCH, MD, MS and MSc dissertations, Fellows-logbooks, Articles, Scientific papers, Annual reports, GCSMCH quarterly newsletters, GCS Research Journals, Bibliographic information, Monographs, GCRI

2. Digital Notice Board facility

In present days, many academic and medical institute libraries rely on wooden notice board hanging on the wall to display announcements and other circulars in Gujarat. GCRI Library has initiated the first step to move wooden notice board to digital notice board for disseminating the relevant and quick information to its users. The GCRI Library and Information Services Department inaugurated the Digital Library Notice board on 17th February 2018 to commemorate and disseminate all the upcoming events, academic updates, important messages, multimedia etc to their users.

3. E-mail, Current Awareness Service and Alert Services

The department plays an important role to disseminate, circulate, and update various important information through e-mail communication service.

a) New arrival books, Journals and E-databases e-mail to all the library users.
b) E-mail for all office circular and all academic events.
c) Handling email outlook portal for all the e-mail group of Academic faculties, Clinical doctors group, Resident doctors, Research, and Office staffs.

4. Reprography services

GCRI Library provides reprography service for photocopying, scanning, and printing at minimum price.

5. Library membership facility

All faculties, GCRI staff and resident doctors are automatically members of the GCRI Library and Information Services Department. Outstation membership facility is also available for ex-employee, ex-students and those related/engaged with GCRI.

Borrowing right for library members

Resident doctors /Diploma students / other students can issue 03 library items for 15 days. Faculties/Officers can issue 05 library items for 30 days, HOD/ Departmental issue can be permitted for 12 Library items for 12 months and Visitors and Outstation members can avail only reference and reprography facility.

6. Audio-Video /CD & DVD facility

GCRI library e-books and e-journals has 89 DVD collections, 71 special collection on conference report of Live surgery SELSICON DVDs, 11 HPB Workshop Audio-Video collections and 57. Live OT Procedures UGICON Audio-Video collections .

7. Database Services

- **UpToDate anywhere online database Access**

  To enhance the usage utilities, doctors, students and other academic staffs, can access UpToDate Medical Database through user ID/Password in off campus or through remote login. For remote access users can use their mobiles, Tablets and laptops. Users can also access the UpToDate Database through hospital IP address in the campus use only.

- **EBSCO Oncology E-Database**

  Total 115 Oncology E-Journals can be accessed in hospital campus by the library users of GCRI and Off Campus/Remote access through mobile phones, tablets and laptops.

- **OVID and Clinical Key Databases access services through NCG**

  Library users can access OVID Wolters Kluwer & Clinical Key Elsevier database by forming part of National Cancer Grid Program of Government of India.

8. Reference Service

GCRI Library assists user for locating or searching required information from the physical library collection as well as digital collection and databases. GCRI Library has initiated the internet-based reference services for their users.

9. Article on request and Literature Search Service

Library staffs are well versed in carrying out literature search, and providing full-text article to their users. The search results are delivered either as hardcopy or as an electronic copy sent to user's e-mail or desktop.

10. Computer Lab and Internet facility

GCRI library has well equipped air-condition computer lab having 17 nodes connected with high speed broadband internet facility. Wi-Fi facility is also installed in the library premises.

11. Reading room facility

The library has individual air- condition reading area for resident doctors as well as faculty members.

12. Staff Library of non-academic books

Apart from academic course and medical books, this department also provides facility for non-academic books categorized into novels, story books, personality development books, spiritual books, Gujarati, Hindi, and English literature books etc to all the academic and non-academic staffs of GCRI.

13. Research hub

a) The Department plays an important role in collection of Thesis/Dissertation/Logbook from the author/creator and keep them organized in proper way, which will be ready reference to upcoming students.

b) Every year all scientific, clinical and research publication works are collected, compile and organised in a form of book.

c) For promotion of academic integrity through NCG platform, GCRI library supports to check plagiarism activity through use of anti-plagiarism software 'iThenticate'.

14. Training program and Academic Activities

a) Training and live demo sessions are organized on regular basis for PG teachers and residents doctors to give them guidelines for searching and using remote login access of EBSCO, UpToDate, Clinical Key, OVID, and PubMed online Medical Database.

b) Induction Program is conducted by GCRI Library & Information services department for the newly joined GCRI Staff members and Students.

c) Organize training sessions on "iThenticate Plagiarism software" use for verifying the originality of any written research work to all researcher, academicians and students.

d) The library department provides Question Bank facility by collecting all the old university question paper for MD, MS, MCH, DM, MSc & Other courses.

e) The library department keeps a record for displaying recent publications, upcoming conference, and meeting on the Digital notice board. CME, Clinical meeting and other academic events are informed to all academic staff and Resident doctors by library through an email or displaying notice board.

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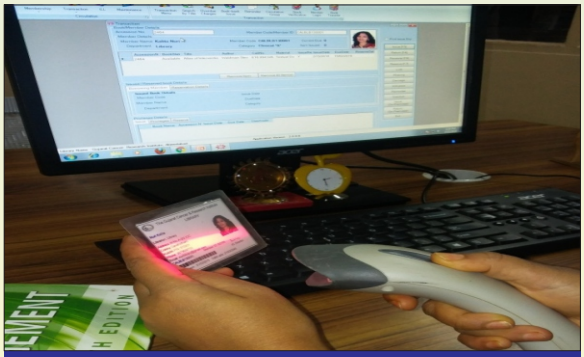
Library and Information Services Department

Activities of Library and Information Service Department

Digital Notice Board

Computer Lab & Digital Library



Library Automation SOUL 2.0 Software

Training Session of 'i-Thenticate' Anti-plagiarism Software

Live Demo Session on Access & Use of UpToDate and other Medical e-databases