Original Article

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Pathology Section

Utility of Microwave Processing in Plasma-Thromboplastin Method of Cell Block Prepared from Fine Needle Aspirates

MANIYAN PRAKASH SUMITHA[1], SHANKARAN RUKMINI NIVEDITHA[2], THEJASVI KRISHNAMURTHY[3]

ABSTRACT

Introduction: Cell Blocks (CBs) in cytology complement routine smears and increase diagnostic accuracy. However, routine processing of CBs on an average varies between 12-18 hours, thereby delaying the additional information it can provide. Though application of microwaves in histopathology is well known, its use in cytology has been sparse. In an attempt to decrease the turnaround time microwave processing of CBs have been attempted.

Aim: To assess the utility of microwave processing of Plasma Thromboplastin Method of Cell Block (PTCBs) from Fine Needle Aspirates (FNAs).

Materials and Methods: All aspirates (80) from routine FNA procedure done over a period of two months in the Department of Pathology, in a Tertiary Care Hospital were included in the study. The aspirates were clotted by Plasma Thromboplastin (PT) method (slid onto Whatman filter paper, wrapped) and fixed in 10% formalin. Dehydration, clearing and wax impregnation were carried out in a domestic microwave and blocks were ready within 10 minutes for embedding.

Results: The PTCBs processed by microwave technique were available for section cutting and staining within 10 minutes. Majority of the aspirates (27.5%) were from breast followed by thyroid (25%), lymph node (13.75%), parotid (10%) and lipoma (10%). Females constituted 75% of study population with adequate material in 70% of all cases. The quality of the sections, staining, architecture and morphology were comparable to routine paraffin sections. IHC was possible on PTCBs.

Conclusion: Microwave processing of PTCBs is possible which hastens the turnaround time and assist in planning therapeutic modalities.

Keywords: Cytology, Diagnostic accuracy, Turnaround time

INTRODUCTION

Cell Block (CB) refers to the collecting of sediment, blood clots or grossly visible pieces of tissue from cytologic specimens that are processed into paraffin blocks and stained with H&E stain [1,2]. The CB preparation for microscopic evaluation was first introduced by Bahrenburg in 1896 [3-5]. In the words of Koss L "The CB technique should be used for processing all residual material remaining after completion of cytological preparations. This material often contains useful information" [1].

Various methods of CB preparation has been described since ancient times which include the one without any holding media which is the simplest of all (Fixed sediment method) [1], while the other methods require holding media like bacterial agar [1,2], plasma thromboplastin method [1,2,6], simplified cell block method [1,2], compact CB method [1,2], vapour fixation method [7] and histogel method to name a few [1,2,8]. Among these, the Plasma Thromboplastin method of Cell Block (PTCB) is simple, less time consuming, reproducible and economical as this can be done by using in-house raw materials like plasma and thromboplastin commonly found in blood bank and haematology laboratory [1,7,9].

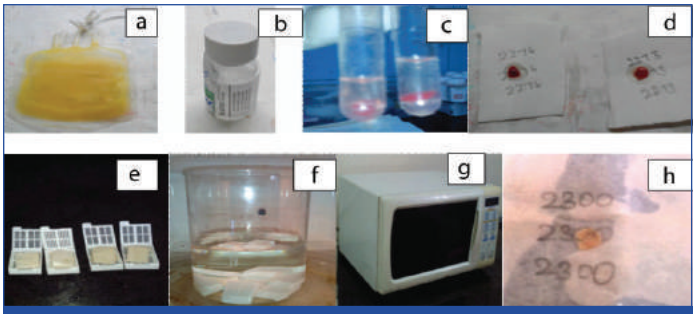
Routine processing of CBs on an average, be it either with Histokinette varies between 12-24 hours [1] or manual processing would require atleast 5-6 hours (for small biopsies) [1,8]. This causes a delay in the additional information CBs can provide, one of the important reasons why CBs are attempted. Hence techniques like microwave processing are the need of the hour for faster processing especially in this era of neoadjuvant and targeted therapies [2,8]. Furthermore, we are now in the age of personalised medicine, where metastases are targeted for diagnosis mainly by Fine Needle Aspiration (FNA) [2,10].

MATERIALS AND METHODS

This observational study was undertaken in the Cytopathology section, Department of Pathology, Kempegowda Institute of Medical Sciences, Bengaluru, Karnataka, India. Over a period of two months, all the aspirates, (eighty (80) from routine FNAs conducted in the cytopathology section were included. After obtaining informed consent, an exclusive needle pass was made for CB preparation in co-operative patients. In rest of the cases, material remaining after making routine FNA smears was used.

FNA material (separately collected for CB or remaining after making smears) was rinsed with phosphate buffer solution and subjected to centrifugation @1500 rpm for 5 minutes (REMI CENTRIFUGE). The supernatant was discarded and 3 drops of outdated plasma [Table/Fig-1a], (obtained from blood bank) was added to the sediment and mixed well. This was followed immediately by adding 3 drops of thromboplastin [Table/Fig-1b], quickly to the plasma-sediment mixture and waited for 5-10 minutes for the formation of clot [Table/Fig-1c], in the same plastic test tube. Later, the clot thus formed was slid into the Whatman filter paper (No.52, WR BALSTON LTD., 11 cm disc) [Table/Fig-1d], held securely, wrapped and placed in routine histokinette plastic tissue cassettes [Table/Fig-1e] and transferred to a Borosil beaker [Table/Fig-1f] for microwave processing. A modified Bellotti's technique [11] was used for microwave processing. A domestic microwave oven with low (20°C-30°C), medium (40°C-55°C) and high (70°C-90°C) temperature settings [Table/Fig-1g] was used for processing the samples as follows:

1. Fixation-10% buffered formalin for 1 minute @ medium heat (55°C) setting [11]
2. Dehydration with 50% ethyl alcohol for 1 minute @ low heat and 70% ethyl alcohol for one minutes @ low heat (30°C) setting [11]
3. Clearing with xylene for 1 minute @ low heat (30°C) →2 cycles [11]
4. Wax impregnation for 2½ minute @ high heat (70°C) →2 cycles [11].



[Table/Fig-1a-h]: The various stages of PTCB preparation and processing (refer text).

The following precautions were taken while carrying out microwave processing:

1. A well ventilated room with exhaust fan.
2. Compulsory Personal Protective equipment which includes hand gloves, mask, goggles and cap.
3. Handling the reagents and glass beakers with utmost care to avoid inhalation of fumes.

Thus, the material from routine FNA's from PT method of CB were ready for embedding after this short processing cycle of less than 10 minutes [Table/Fig-1h]. After this, tissues were embedded in paraffin wax. They were given a unique identification number, and the sections were cut at 4-5 microns and stained with Haematoxylin-Eosin (H&E) for microscopic examination. Special stains and IHC were attempted in required cases. Thus, both the FNA smears and PTCB processed through microwave processing were available on the same day and almost the same time for examination.

With all the available clinical data, the diagnosis on smears was compared to that with PTCB. The adequacy of material on PTCB was assessed and whether it contributed in reforming the diagnosis by performing additional ancillary tests like IHC in feasible cases. The microsections and final diagnosis on microwave processed PTCB was compared with respective histopathology sections wherever available.

RESULTS

In this study, 80 cases from routine FNA's were included. Among these, FNA'S from Breast lump constituted the majority of cases with 27.5% (22/80) [Table/Fig-2]. Females constituted 75% (60) and males 25% (20) [Table/Fig-3].

Site of FNA	Frequency (n)	Percentage (%)
Breast	22	27.5%
Thyroid	20	25%
Lymph node	11	13.75%
Parotid glands	08	10%
Lipoma	08	10%
Lung mass	01	1.25%
Axillary swelling	02	2.5%
Epidermal cysts	02	2.5%
Testicular swelling	02	2.5%
Miscellaneous	04	05%
Total	80	100%

[Table/Fig-2]: Distribution of cases (n=80).

Of the 80 cases, material was adequate in 56 of them i.e., in 70%, while in the remaining 24 cases material was not present in PTCBs. All the breast aspirates had adequate material in PTCBs while majority of the thyroid aspirates (65%) did not show contributable material. All (100%) aspirates from Lipomas did not show any material in PTCBs [Table/Fig-4].

Of these 56 cases, 32 (58%) of them contributed in the diagnosis and helped in faster diagnosis [Table/Fig-5] whereas in the remaining 24 cases (42%), the contribution was not commendable. Cellular aspirates of lymph node, breast and tumourous tissue were processed well with PTCB and microwave processing. The quality of the sections, staining, morphology of cells and architecture were comparable to routine paraffin sections.

Site of FNA	Female (n)	Male (n)	Total (n)
Breast	20	02	22
Thyroid	20	00	20
Lymph node	08	03	11
Parotid glands	03	05	08
Lipoma	05	03	08
Lung	00	01	01
Axillary swelling	02	00	02
Epidermal cysts	00	02	02
Testicular swelling	00	02	02
Miscellaneous	02	02	04
Total	60 (75%)	20 (25%)	80

[Table/Fig-3]: Sex distribution.

Site	Material present (n)	Material not present (n)	Total (n)
Breast	22	00	22
Thyroid	07	13	20
Lymph node	10	01	11
Parotid swelling	07	01	08
Lipoma	00	08	08
Lung mass	01	00	01
Axillary swelling	02	00	02
Epidermal cysts	02	00	02
Testicular swelling	02	00	02
Miscellaneous	03	01	04
Total	56 (70%)	24 (30%)	80

[Table/Fig-4]: FNA and material present on PTCB.

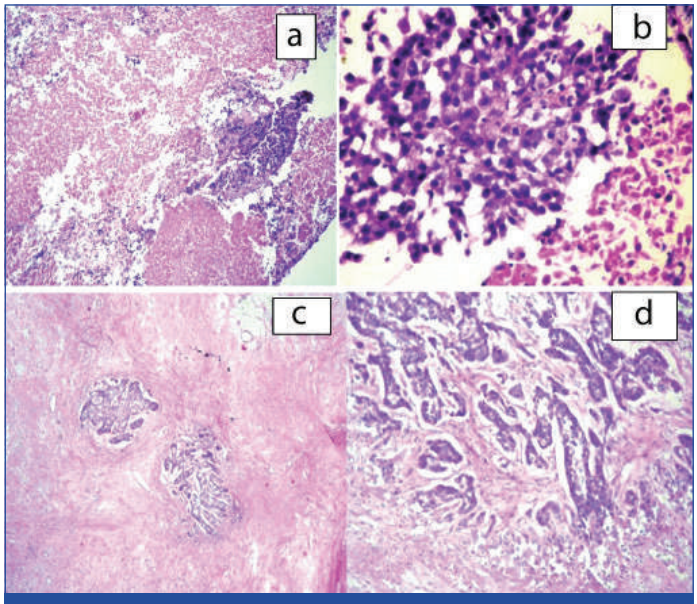
In 2 of the cases, with lump in the breast routine FNA suggested malignancy while on PTCB a definite diagnosis of infiltrating ductal carcinoma [Table/Fig-6] could be given which was confirmed by histopathology. IHC was attempted on the PTCB and was comparable with the IHC on routine HPE blocks [Table/Fig-7]. Two cases of swelling in the parotid region were given a diagnosis of smears positive for malignancy on routine FNA. However, PTCB upgraded the diagnosis to suspicious for mucoepidermoid carcinoma in one case and the other as carcinoma ex pleomorphic adenoma. Both these diagnoses were confirmed on histopathology [Table/Fig-8]. One case of axillary swelling in an elderly female patient was reported as positive for malignancy on FNA, whereas glandular and tubular arrangement of malignant cells was obtained on PTCB [Table/Fig-9]. One case of lower lobe lung mass was given suspicious for malignancy on CT guided FNA smears which were upgraded to suspicious of squamous cell carcinoma/poorly differentiated carcinoma on PTCB. On CT guided biopsy, the same case turned out to be squamous cell carcinoma [Table/Fig-5].

DISCUSSION

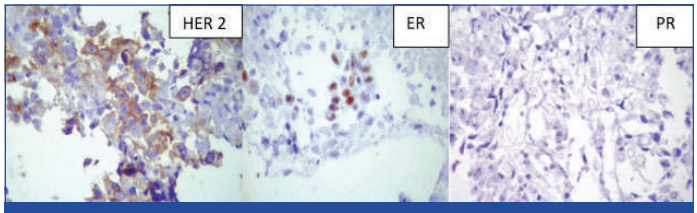
The conventional smear technique on body fluids surpassed the CB method after worldwide acceptance of Papanicolaou's methods, though CBs were in use since the 1900s [1]. One of the constraints of conventional FNA smear is limited material available for adjuvant diagnostic investigations [1,12,13]. The CB technique has been applied to aspirated material [9] and has been used increasingly to improve the diagnostic accuracy of FNA.

Sl. No.	Site	FNA	PTCB	HPE
1	Breast	Positive for malignancy	Intraductal carcinoma IHC	IDC-NOS
2	Breast	Positive for malignancy	Intraductal carcinoma IHC	IDC-NOS
3	Breast	Breast abscess	Breast abscess	---
4	Breast	Fibroadenoma	Fibroadenoma	Fibroadenoma
5	Breast	Benign breast disease	Fibroadenoma	Proliferative breast disease without atypia
6	Breast	Subareolar abscess	Breast abscess	---
7	Breast	Fibrocystic disease	Fibrocystic disease	Fibrocystic disease
8	Breast	Fibroadenoma	Fibroadenoma	---
9	Breast	Lactational changes	Breast abscess	---
10	Breast	Fibroadenoma	Fibroadenoma	Fibroadenoma
11	Breast	Fibrocystic disease	Fibrocystic disease	---
12	Breast	Fibrocystic disease	Fibrocystic disease	---
13	Parotid swelling	Acute suppurative process	Acute suppurative process	---
14	Parotid swelling	Positive for malignancy	Mucoepidermoid carcinoma	Mucoepidermoid carcinoma
15	Parotid swelling	Positive for malignancy	Carcinoma ex pleomorphic adenoma	Carcinoma ex pleomorphic adenoma
16	Parotid swelling	Pleomorphic adenoma	Pleomorphic adenoma	Pleomorphic adenoma
17	Parotid swelling	Sialadenosis	Sialadenosis	---
18	Lymph node swelling	Reactive node	Reactive lymph node	---
19	Lymph node swelling	Reactive node	Reactive lymph node	---
20	Lymph node swelling	Granulomatous lymphadenitis	Granulomatous lymphadenitis	Tuberculous lymphadenitis
21	Lymph node swelling	Reactive node	Reactive lymph node	---
22	Lymph node swelling	Reactive node	Reactive lymph node	---
23	Lymph node swelling	Reactive node	Reactive lymph node	---
24	Lymph node swelling	Reactive node	Reactive lymph node	---
25	Axillary Lymph node swelling	Positive for malignancy	Intraductal carcinoma -breast	---
26	Swelling axillary region	Suggestive of abscess	Acute suppurative inflammatory process	---
27	CT guided FNA lung mass	Suspicious for malignancy	Suspicious of squamous cell carcinoma/poorly differentiated carcinoma	CT guided biopsy suggestive of squamous cell carcinoma
28	Swelling on forehead	Epidermal cyst	Epidermal cyst	Keratinous cyst
29	Swelling on neck	Epidermal cyst	Epidermal cyst	Keratinous cyst
30	Thyroid	Colloid goiter	Colloid goiter	---
31	Thyoid	Colloid goiter with cystic change	Colloid goiter	---
32	Thyroid	Multinodular goiter	Haemorrhage	---

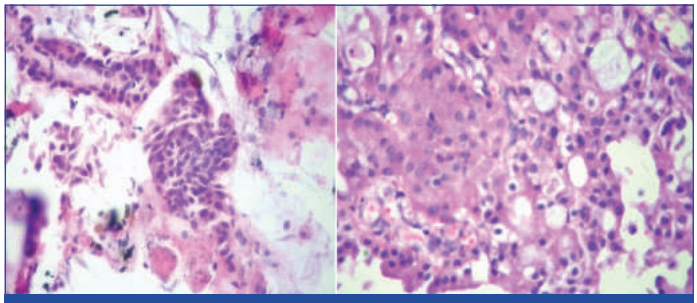
[Table/Fig-5]: Distribution of cases contributing in the diagnosis.



[Table/Fig-6]: PTCB microsections from a case of IDC breast, H&E-10X (a) and 40X (b); corresponding histopathological microsections, H&E 10X (c) and 40X (d).

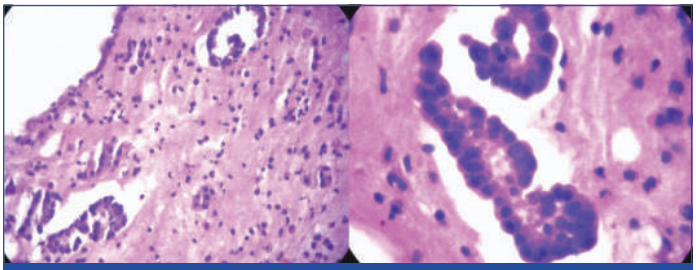


[Table/Fig-7]: IHC on PTCB microsections in a case of IDC breast (IHC, DAKO).



[Table/Fig-8]: PTCB microsections from a case of MEC (H&E-10X) corresponding histopathological microsection (H&E 40X).

Karnauchow PN et al., were the first to emphasise that CB technique could be used in thick tissue particles aspirated by FNA, which could provide sufficient material for a good section, special stains and IHC [9]. The CB technique helps in retrieval of small tissue fragments [1,3,4,8], improving cellular yield and diagnostic accuracy and provides scope for ancillary studies like IHC and special stains [1,2,4,5].



[Table/Fig-9]: PTCB microsections from a case of metastatic IDC breast in an axillary lymph node, (H&E-10X) and corresponding histopathological microsection (H&E-40X).

PTCBs for FNA and serous effusions by routine processing have been studied by Kulkarni MB et al., and Karnauchow PN et al., [6,9]. They concluded that PTCB method complements routine smears and thus aids in diagnosis with morphology comparable to routinely processed CB and an additional advantage of ability to do IHC.

We chose PTCB, as the method is simple and can be performed with minimal reagents which are readily available from the pathology laboratory.

Some of the disadvantages encountered were waiting time to bring the plasma to room temperature and difficulty in differentiating necrosis from proteinaceous material but routine processing of PTCB would require 18 to 24 hours/block.

The application of microwaves in histopathology has gained acceptance in last 2-3 decades [8]. It has been virtually used for all procedures in histopathology like stabilising tissues, fixation, processing, staining, frozen techniques, immune techniques, electron microscopy and antigen retrieval [8,14,15]. Rarely tried in cytology, microwave processing has been studied in preparing CB from sputum [16], direct FNA samples [11] and cervico vaginal smears [5].

As early as in 1986, Kok LP et al., used microwave technique for preparation of that CB from sputum which proved that CB could be prepared in approximately or even less than one hour [16]. A study by Bellotti MS et al., used a rapid method for processing of materials collected directly by FNA using microwave technology [11]. Microwave processing in cytology using routine CB in cervico-vaginal smears is done by Gangane N et al., [5].

Routine processing of PTCB would vary between 5-6 hours (manual processing) to 18-24 hours/block (automatic tissue processing using histokinette) while microwave processing decreases the turnaround time drastically to less than an hour [1,8,11].

In our study, the microwave processing of CBs on an average was 10-15 minutes/block (however inclusion of the time taken for PT method of CB preparation-1.5 to 2 hours) This is in concordance with the study by Prabhala S et al., who have combined PTCBs with microwave processing [17].

In our study, the quality of the sections, staining, morphology of cells and architecture are comparable to routine paraffin sections similar to other studies in addition to being both laboratory personnel and environment friendly [5,16-19]. The microwave processed CBs had better cytoplasmic and nuclear details with good erythrocyte integrity than the conventional method in concordance with Mathai AM et al., [18]. Kok LP et al., opined there is homogenous distribution of immersion fluids on the cells in microwave processing and hence the sections were comparably of superior quality [16]. CB technique provides archival material for future use. Also, various studies opine that antigenic epitopes are likely to be preserved better in microwave processed tissue. [5,8]. The material in CBs get concentrated in one small focal plane, which helps in faster and better evaluation [5] Overall, there was drastic reduction of time [8,13,16-18] and the microwave processed PTCB were available in few hours for comparison to the routine FNA smears. IHC was possible in microwave processed PTCBs in our study similar to other studies [5,8,12,14,15]. Skov BG et al., successfully tested Programmed Death ligand 1 IHC on PTCB method [20].

Cellular aspirates of lymph node, breast and tumourous tissue processed well with PTCB and microwave processing. In aspirates from thyroid, fibrous/neural and lipomatous lesions, PTCB did not yield material. Paucity of aspirate on routine FNA could be a factor in fibrous/neural tissue. In thyroid lesions, 24G needle is used, as it is a vascular organ and hence could lead to sparse cellularity. Also, needling is preferred to aspiration in anticipation of intra-organ haemorrhage leading to inadequate material for CBs. Majority of the thyroid cases which did not have material on PTCB were benign lesions like colloid goiter, some with cystic change on FNA smear examination. In lipomatous lesions, none of them showed material on PTCB possibly because adipocytes would have floated during centrifugation and pipetted off along with the supernatant.

In this era of molecular pathology and targeted therapy, many laboratories use CB's for molecular techniques such as polymerase chain reaction-based sequencing, in situ hybridization and quantitative polymerase chain reaction to detect oncogene mutations, gene fusions, gene copy number gains, and protein overexpression. Despite the fact that, suboptimal cellularity is a common limitation of CBs, they are now feasible for next generation sequencing with the recommended cell content of minimum 500 tumour cells for various molecular techniques [2,21-24]. Various studies and research are being carried out for diagnosis and staging of metastases by FNA [2,10] and microwave processed PTCB could be a boon. In addition, CBs are of paramount importance for the bio-bank storage of cytological material in this bio banking millennium [2].

LIMITATION

- Expertise and precision is required.
- The process is labor intensive because the solutions are manually manipulated.

CONCLUSION

Microwave processing is possible on PTCBs and morphology of cells and architecture are comparable to that of routinely processed CB. IHC is possible in microwave processed PTCBs. Microwave processing decrease the turnaround time significantly and complemented smears in diagnosis. These could help in planning and strategising neoadjuvant therapy in cases of malignancy and hence decrease morbidity of extensive surgery.

FUTURE RECOMMENDATION

Studies in large numbers are essential.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Pathology, Rajarajeswari Medical College and Hospital, Bengaluru, Karnataka, India.
2. Professor, Department of Pathology, Kempegowda Institute of Medical Sciences, Bengaluru, Karnataka, India.
3. Associate Professor, Department of Pathology, Kempegowda Institute of Medical Sciences, Bengaluru, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Maniyan Prakash Sumitha,
#27/2, Shri Krishnaprabhuvan, 7th Main, Near Canara Bank Ashoka Pillar, Jayanagar 2nd Block,
Bengaluru-560011, Karnataka, India.
E-mail: sumi1939@gmail.com

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Extramedullary Plasmacytoma of the Jugular Bulb- A Rare Case Report

Authors
Dr Syeda Sarah[1*], Anwaruddin Ahmed[2], Vamseedhar Annam[3], Sharmila P Surhonne[4]
[1,2]Post Graduate Student, [3]Professor, [4]Professor and Head
Department of Pathology, Rajarajeswari Medical College and Hospital, Bangalore, Karnataka, India-74
*Corresponding Author
Dr Syeda Sarah
6 E block Flat no.703, Provident sunworth apartment, Behind Rajarajeswari Medical College, Mysore road, Bangalore-560060, India
Mob: 9742498685, 9108089726

Abstract
Introduction: *Extramedullary plasmacytoma (EMP) of jugular bulb is a very rare plasma cell proliferative disorder arising outside the bone marrow.EMP represents less than 1%of head and neck malignancies. More than 90% of EMPs are diagnosed in the head and neck with the soft tissues and upper aero digestive tract being the most common sites. EMPs should be distinguished from other plasma cell dyscrasias for prognosis and treatment. There are very scarce previous documented reports on plasmacytoma arising from jugular bulb. Hence,we present here a rare case report of EMP arising from the jugular bulb masquerading as an extradural space occupying lesion*
Case Report: *A 56 year old man presented to our hospital with history of neck stiffness, torticollis and progressive nuchal pain since 3 months. Radiological findings identified an extradural expansile mass lesion in the left jugular foramen bulb causing bony erosion. Intraoperative squash cytology suggested a differential diagnosis of small round blue cell tumor. Surgical excision of the mass was performed and sent for histopathological evaluation, which showed features of plasmacytoma. Immunohistochemistry was positive for CD 138 confirming the diagnosis of EMP.*
Keywords: *Extramedullary Plasmacytoma, Squash Cytology, IHC.*

Introduction

EMP is an extremely rare B-lymphocytic plasma cell dyscrasia originating from soft tissue accounting for <1% of all head and neck malignancies which was first described by Schridde in 1905.[1,2] Plasma cell neoplasms can present as a solitary plasmacytoma or multiple lesions can present as a bone lesion or soft tissue lesion.[3] It is known that SPB and EMP arise from the analogous cell types confined to a single area, the former has high tendency for multiple myeloma (MM), while the latter consist of plasma cell infiltration with no sign of MM and hence, the pathogenesis of these two diseases are not alike.[4] EMP represent for 3% of all plasma cell neoplasia. Plasmacytomas are more common in males, with a male-to-female ratio of 3:1 occurring in fourth to sixth decades in life.[5] Almost 80% of EMP occur in submucosal lymphoid tissue in head and neck, commonly

affecting the nasal cavity, paranasal sinus, tonsillar fossa and oral cavity but may also occur in the gastrointestinal tract, urinary bladder, gland, lymph node and skin.[6]

Here, we present a rare case of EMP arising from the jugular bulb masquerading as an extradural space occupying lesion.

Case Report

A 56 year old man, farmer by occupation, visited our hospital with chief complaints of torticollis (positive towards the left side), neck stiffness and nuchal pain since 3 months. The neck pain and stiffness was gradually progressive. There was no history of trauma or any other associated systemic illnesses.

Radiological Investigation with computed tomography (CT) scan of brain revealed a well defined enhancing mass lesion in the left jugular bulb causing bone erosion. (Figure.1a)

Magnetic resonance imagining (MRI) showed a large expansile mass lesion in the left jugular foramen with erosion and widening of the jugular foramen extending to the C2 vertebral body at the anteroinferior aspect (Figure.1b).

A diagnosis of extradural space occupying lesion (SOL) was made and patient was planned for excision of the mass. Intraoperative squash cytology with Hematoxylin and Eosin(H&E), Giemsa and Papanicolaou (PAP) stain was performed (Figure.2a,b,c) which revealed cellular smears of round to oval tumour cells with eccentrically placed nucleus, condensed chromatin & scant cytoplasm arranged in sheets in a haemorrhagic background. The differentials considered were small round blue cell tumor-plasmacytoma, meningioma, and poorly differentiated carcinoma.

The excised SOL specimen was sent for histopathological evaluation.

Macroscopy consistent of multiple grey white to grey brown soft tissue bits approximately amounting to 2 cc.

Microscopy revealed small round to oval cells with mild pleomorphism having eccentric round to oval nucleus arranged in sheets, cords and clusters separated by thin fibrovascular core. At places many dilated vascular spaces surrounded by these tumor cells with intervening fibrocollagenous tissue was observed (Figure.3). The diagnosis was narrowed down to differentials of Meningioma & Plasmacytoma.

IHC

Immunohistochemistry was strongly positive for CD138 & kappa (Figure.4a&b). Focal positivity was seen for lambda (Figure.4c) which confirmed the monoclonal nature of plasma cells.

Multiple systemic and radiological studies were performed in order to rule out systemic plasmacytoma. Serum protein electrophoresis, beta-2 microglobulins were normal. Urine examination for Bence Jones proteins and for light chain assay was negative. Full blood count and levels of serum glucose, calcium, liver function tests and renal function tests were within normal limits. Bone marrow aspiration from the iliac crest was normal and showed no evidence of plasma cell infiltration. A radiological skeletal survey was performed which showed no evidence of lytic skull lesions in the skull, vertebral column or bones of chest wall or pelvis.

Diagnosis

After ruling out the possibility of Multiple myeloma (MM), a final diagnosis of Extramedullary plasmacytoma of jugular bulb was given.

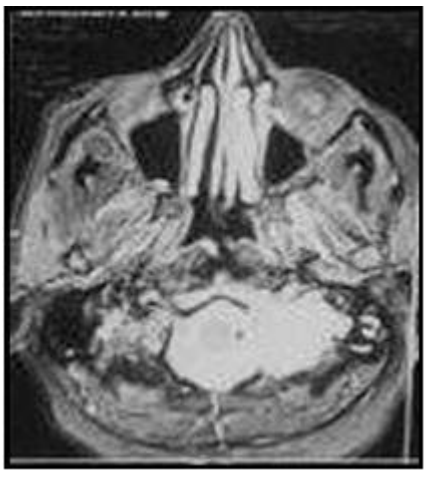
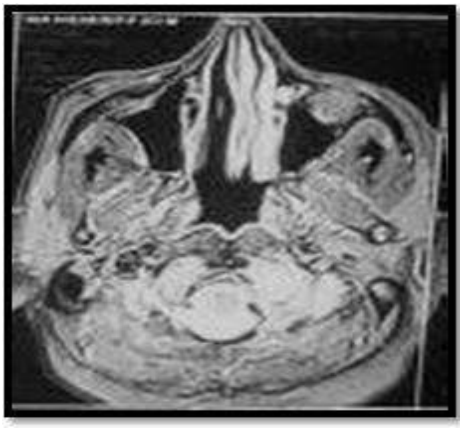


Fig 1: (a) CT Scan



(b) MRI

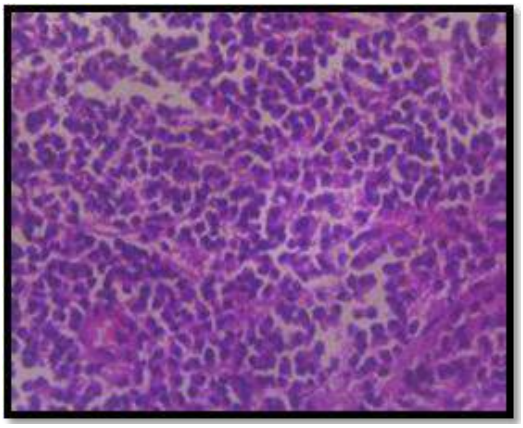


Fig 3: H&E Section

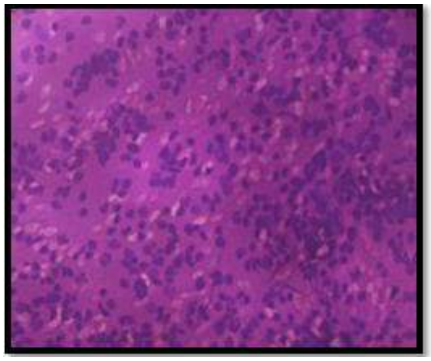


Fig 2 Squash cytology: (a) H&E Stain

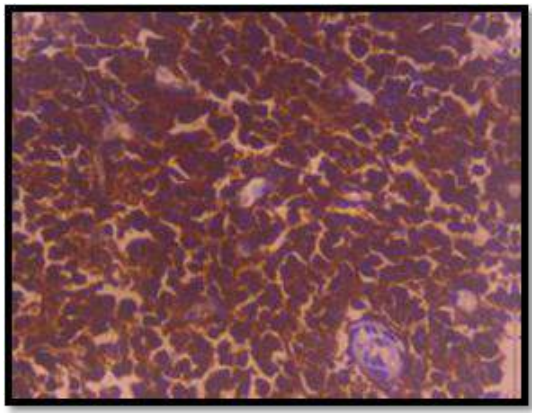
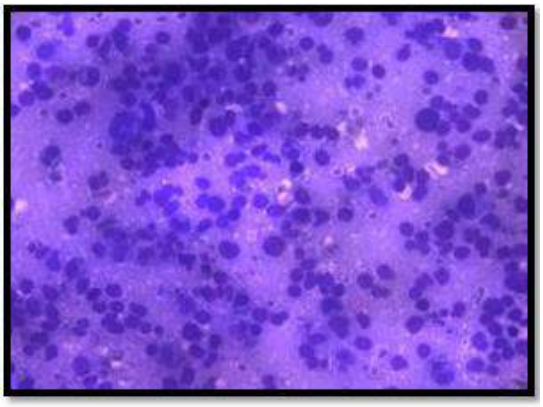
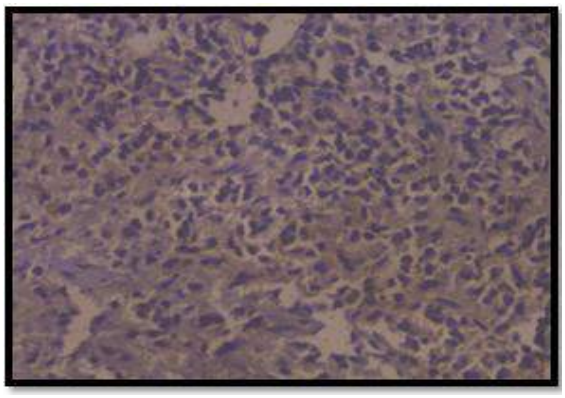


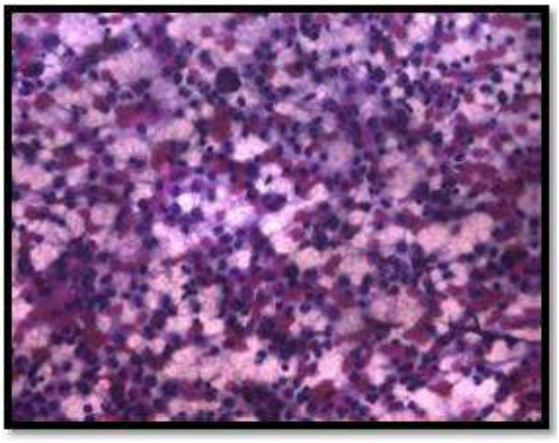
Fig 4 IHC: (a) CD138



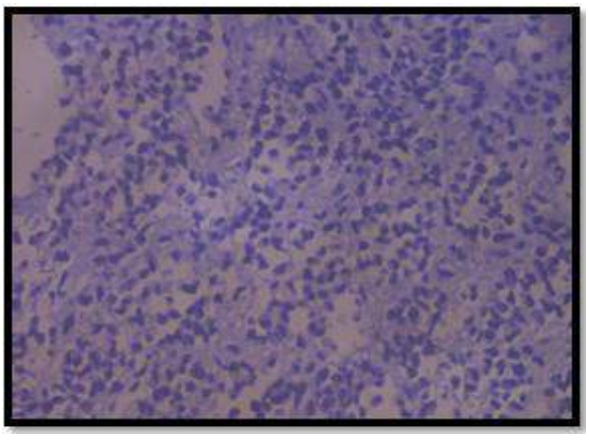
(b) GIEMSA Stain



(b) KAPPA



(c) PAP Stain



(c) LAMBDA

Discussion

Solitary plasmacytoma is a rare neoplasm of monoclonal plasma cells.EMP originates from plasma cells with a single class of heavy and light chains in a monoclonal proliferation of B cells.[7] EMPs occur in men approximately three times more than in women.[2,8] The diagnosis of EMP depends on histopathological examination. The clinical symptoms are usually in relation to the specific location of lesion than to the nature of tumor. We hereby report a case of extramedullary plasmacytoma arising from Jugular bulb, rarely reported in world literature.

EMP of the jugular bulb clinically presented as torticollis and progressive neck stiffness in our case. Other patients can also have cranial nerve palsy and cervical lymphadenopathy.[7]

No lymph node involvement was found in our patient. The aetiology of this disease remains unknown, but factors such as viral pathogenesis have been previously indicated.[2]

The recommended diagnostic criteria of EMP of soft tissue are[6] : (i) Pathological tissue evidence of monoclonal plasma cells involving a single extramedullary site.(ii) no bone marrow involvement (iii) no anemia , hypercalcemia or renal impairment caused by plasma cell dyscrasias (iv) negative skeletal survey results and (v) low serum or urinary levels of monoclonal immunoglobulin.

For diagnosis of an EMP through set of investigations are required. These include blood count serum and urinary protein electrophoresis, peripheral blood smear, renal function test, liver function test, tissue biopsy, radiological survey, CT and MRI scan of the affected area and bone marrow biopsy.Since the morphologic characteristics of EMP may resemble those of extramedullary invasion of a well differentiated MM, the possibility of MM should be omitted before confirming the diagnosis of EMP.

Wiltshaw classified soft tissue plasmacytoma into 3 clinical stages as follows: [8]

Stage 1	Limited to an extramedullary site
Stage 2	Involvement of regional lymph nodes
Stage 3	Multiple metastasis

The Differential diagnosis for EMP includes Lymphoma, Poorly differentiated carcinoma, Meningioma. Squash cytology with histopathology aids in diagnosis of EMP. Histopathology showing monoclonal plasma cells and Igs on IHC can confirm the diagnosis.CD 138 is a specific marker to confirm the plasmacytic nature of the cells which differentiates it from a carcinoma which stains for CK and CD 40.[9] Light chain positive for Kappa and focal positive for lambda helps us in determining the process is monoclonal.

The mainstay of treatment for EMP of head and neck as described by United Kingdom Myeloma Forum (UKMF) recommends radiotherapy with surgical excision while EMPs in other sites recommends surgery excision initially.[10] The most important prognostic factor determining the outcome post therapy is progression to MM. The reported conversion rate of EMP to Multiple Myeloma is 15-30%.[6]

Conclusion

Extramedullary plasmacytomas are a rare neoplasm of the head and neck but should be included in the differential diagnosis of Extradural space occupying lesions for early detection, favourable prognosis and outcome. Squash cytology and routine histopathological examination helps in detection of these lesions. Immunohistochemistry is of vital significance to establish clonality and neoplastic nature of these tumors and definitative diagnosis.

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ORIGINAL ARTICLE

Inflammatory Dermatoses of the Superficial Cutaneous Reactive Unit—Study of Morphological Features with Clinical Correlation

Preethi Dinesh[1], Jyothi A Raj[2], Yadalla Harikishan Kumar[3]

Abstract

Background and objectives: Inflammatory dermatoses of the superficial cutaneous reactive unit are a common and complex variety of clinical conditions. This study was undertaken to perform a detailed morphological evaluation of lesions classified in this category, and to correlate the clinical details to arrive at the most appropriate diagnosis.

Methodology: Skin biopsies of clinically diagnosed/suspected cases of inflammatory dermatoses were processed and stained with hematoxylin and eosin (H&E), followed by microscopic examination.

Results: A total of 160 skin biopsies of superficial inflammatory dermatoses were studied. Lesions were categorized into papular/non-vesiculobullous (non-VB) (142 cases/88.75%) and VB (18 cases/11.25%) lesions. Papular lesions were frequent in males, with a peak incidence in the fourth decade. Patients presented mostly with pigmented plaques and papules over the extremities. Papular lesions were categorized based on epidermal changes: 8 cases without epidermal changes and 134 with epidermal changes. Lesions with epidermal changes were further categorized into interface dermatitis (60 cases), psoriasiform dermatitis (58 cases), and spongiotic dermatitis (16 cases). Commonly reported lesions were lichen planus (LP) with its variants followed by psoriasis vulgaris. VB lesions were common in the third and fourth decades, predominantly in females. These patients presented mostly with generalized vesicles. Common lesions reported were erythema multiforme (seven cases) and pemphigus vulgaris (six cases). Of the 160 cases, clinicopathological concordance was seen in 156 cases (97.5%) and discordance in 4 cases (2.5%).

Interpretation and conclusion: The incidence of superficial inflammatory dermatoses in our study was comparable with those reported in other studies. Despite advances in molecular techniques, morphology remains the gold standard for the diagnosis and prognosis of many inflammatory dermatoses. This study emphasizes the importance and utility of a systematic approach to superficial inflammatory dermatoses which is relevant from the treatment perspective.

Keywords: Inflammatory dermatoses, Morphology, Papules, Plaques, Vesiculobullous.

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Introduction

The skin is the single largest organ that protects against mechanical trauma, radiation, and infection. It is concerned with thermoregulation, sensory reception, conservation, and excretion of fluid. As a sophisticated organ, it has important endocrine roles, particularly in the synthesis of vitamin D, powered by sun exposure.[1]

The first compartment of skin (epidermis, papillary dermis, and superficial vascular plexus) reacts together in many dermatological conditions and was termed the superficial cutaneous reactive unit by Clark.[2]

Superficial inflammatory dermatoses are very common and comprise a wide, complex variety of clinical conditions. The immune system within the skin has limited ways in which it reacts to an antigenic stimulus and many inflammatory diseases do not show specific histological features. Accurate diagnosis can sometimes be difficult to establish, although it is essential for clinical management. A systematic approach during histological evaluation is essential to narrow the differential diagnosis, thereby achieving the most appropriate diagnosis.

The cases of superficial inflammatory dermatoses are categorized based on the approach of Elder et al.,[2] which was also followed by Alsaad and Ghazarian.[3]

[1]Bhagavan Mahaveer Jain Hospital, Girinagar, Bengaluru, Karnataka, India

[2,3]RajaRajeswari Medical College & Hospital, Mysore Road, Bengaluru, Karnataka, India

Corresponding Author: Jyothi A Raj, RajaRajeswari Medical College & Hospital, Mysore Road, Bengaluru, Karnataka, India, Phone: +91 9900570122, e-mail: jyoki255@yahoo.co.in

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Conflict of interest: None

Aims and Objectives

- Morphological evaluation of skin biopsies of inflammatory dermatoses involving the superficial cutaneous reactive unit.
- To correlate the pathological features with clinical findings.

Materials and Methods

This study was undertaken over a period of 2 years. Skin biopsies of all cases clinically diagnosed as one of the variants of superficial

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inflammatory dermatoses, received for histopathological evaluation, were included in the study. Biopsies were processed routinely and stained with hematoxylin and eosin (H&E), followed by microscopic examination. Relevant clinical details were correlated with histopathological findings.

Inclusion and Exclusion Criteria

All skin biopsies clinically diagnosed as one of the inflammatory dermatoses of the superficial cutaneous reactive unit were included in the study. Granulomatous diseases and vasculitides were excluded.

Results

One hundred and sixty skin biopsies clinically diagnosed or suspected to be lesions of superficial inflammatory dermatoses were included in the present study. They were categorized as papular/non-vesiculobullous (non-VB) [*n* = 142 (88.75%)] and VB [*n* = 18 (11.25%)] lesions. Non-VB lesions were classified into those with epidermal changes and those without epidermal changes. Lesions with epidermal changes were further categorized into interface, psoriasiform, and spongiotic dermatitides.

Clinical Presentation

Of the 160 cases, 95 (59.37%) were males and 65 (40.63%) were females. Most cases (40) occurred in the third decade. Common presentation was in the extremities in 78 cases. Lesions were generalized in 29 cases. Most cases had overlapping clinical features: 80 patients had plaques and 79 had hyperpigmentation. Papules and scaling were seen in 49 and 40 cases, respectively.

Categorization of Papular/Non-VB Lesions

Papular/non-VB lesions were categorized into lesions without epidermal changes and lesions with epidermal changes. Lesions without epidermal changes were eight in number; these were categorized based on the type of papillary dermis and perivascular inflammation (Table 1).

The 134 papular/non-VB lesions with epidermal changes were further categorized as interface dermatitis (*n* = 60), psoriasiform dermatitis (*n* = 58), and spongiotic dermatitis (*n* = 16).

Interface Dermatitis

Vacuolar changes, lichenoid infiltrate, and both these features in combination were used as criteria to categorize this group. LP and its variants were the most common type (Tables 2 to 4). The most common clinical pattern was pigmented plaques and papules (Fig. 1).

Table 1: Categorization of papular lesions without epidermal changes (*n* = 8)

Type of inflammatory infiltrate	*Histologic types*	*No. of cases*
Lymphocytic infiltrate	Polymorphous light eruption	1
	Pityriasis lichenoid chronica	1
Lymphoeosinophilic infiltrate	Prurigo nodularis	1
	Urticaria	1
Lymphoplasmacytic infiltrate	Discoid lupus erythematosus (DLE)	1
	Urticaria	2
Lymphohistiocytic infiltrate	Prurigo nodularis	1

Table 2: Interface dermatitis with lichenoid infiltrate (*n* = 23)

Lesions	*No. of cases*
LP	9
LP pigmentosus	6
Post-inflammatory hyperpigmentation	5
Hypertrophic LP	2
LP striatus	1
Total	23

Table 3: Interface dermatitis with vacuolar changes (*n* = 17)

Lesions	*No. of cases*
Discoid lupus erythematosus	4
Pityriasis lichenoides chronica	4
Lichen sclerosus et atrophicus	2
LP	2
LP pigmentosus	2
Hypertrophic LP	2
LP striatus	1
Total	17

Table 4: Interface dermatitis with vacuolar changes and lichenoid infiltrate (*n* = 20)

Lesions	*No. of cases*
LP	14
Hypertrophic LP	3
LP pigmentosus	2
Drug-induced LP	1
Total	20

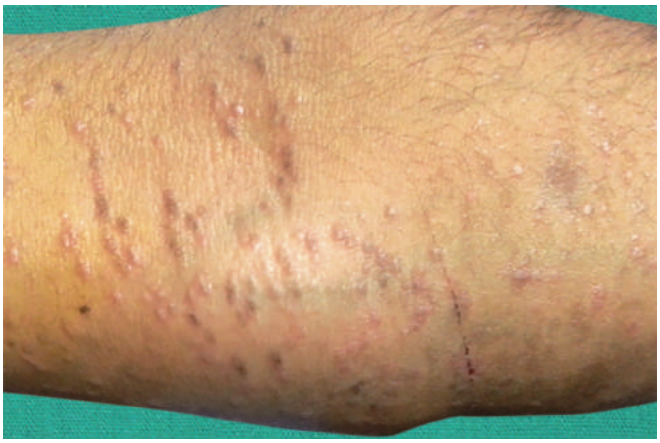

Fig. 1: LP: Small, shiny, flat-topped violaceous papules on the flexor surface of forearm

The predominant histological features in the epidermis in interface dermatitis were hyperkeratosis, acanthosis, hypergranulosis, spongiosis, and orthokeratosis. Features at the dermoepidermal junction were lichenoid infiltrate and vacuolar changes. Twenty cases had overlapping features of vacuolar changes and lichenoid infiltrate. Pigment incontinence, lymphohistiocytic infiltrate, and dilated blood vessels were seen in the dermis (Fig. 2).

Psoriasiform Dermatitis (Table 5)

Most lesions under this category were psoriasis vulgaris (25 cases). Common clinical patterns were plaques, pigmentation, and scaling. Predominant histological features observed in the

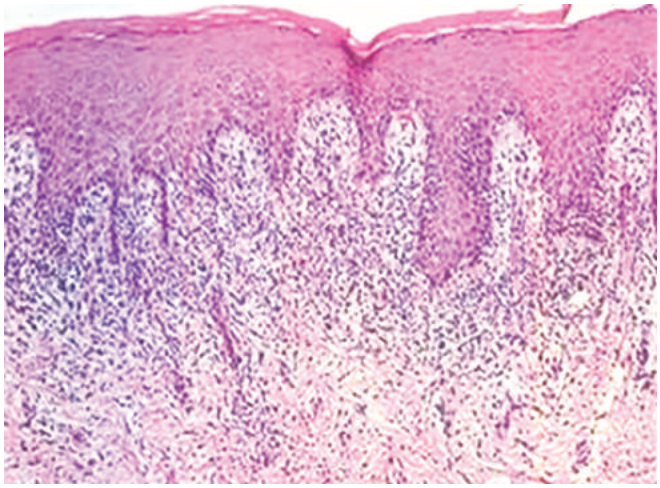

Fig. 2: LP: Photomicrograph shows hyperkeratosis, acanthosis, and basal cell degeneration in the epidermis. Dense band-like lymphohistiocytic infiltrate seen at the dermoepidermal junction (H&E, 10×)

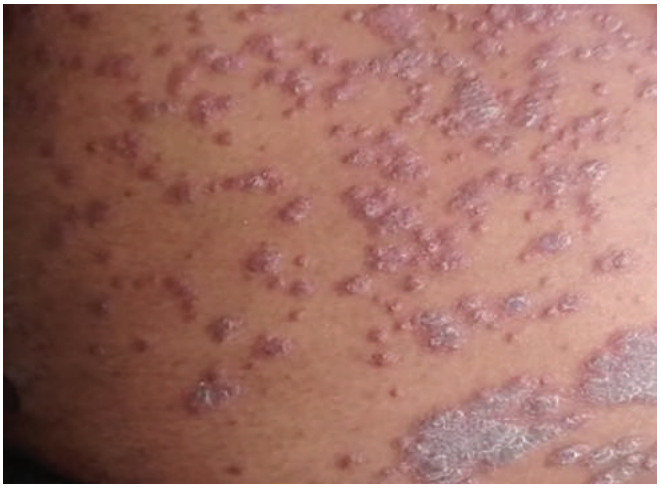

Fig. 3: Psoriasis vulgaris: Sharply demarcated, dry papules of variable sizes, covered with layers of silvery scales on the trunk

Table 5: Variants of psoriasiform dermatitis

Lesions	*No. of cases*
Psoriasis vulgaris	25
Prurigo nodularis	13
Lichen simplex chronicus	9
Parapsoriasis	3
Seborrheic dermatitis	3
Pustular psoriasis	2
Pityriasis rubra pilaris	2
Pityriasis versicolor	1
Total	58

epidermis were hyperkeratosis in all cases, elongated rete pegs, and acanthosis. Spongiosis, keratotic plugging, and Munro's microabscesses were less common. Predominant dermal findings were perivascular inflammation and dilated/congested blood vessels. A clinicopathological correlation was seen in all cases (Figs 3 to 5).

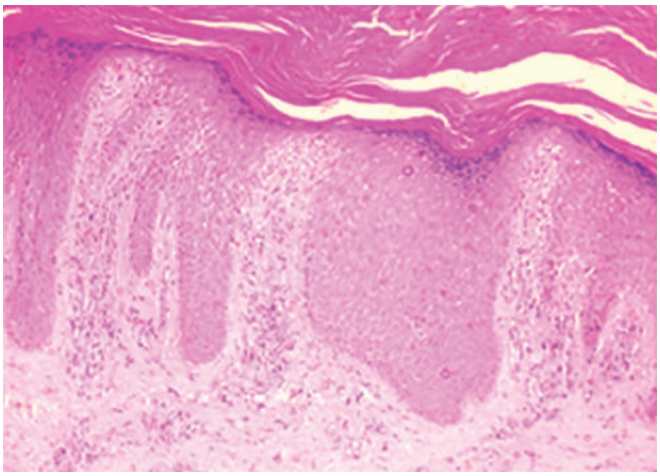

Fig. 4: Psoriasis vulgaris: Photomicrograph shows elongated rete ridges and supra papillary thinning of epidermis. Chronic inflammatory infiltrate noted in the dermis (H&E, 10×)

Spongiotic Dermatitis

Under this category, seven cases of eczema, four cases of pityriasis rosea, three cases of polymorphous light eruption, and two cases of irritant contact dermatitis were reported.

Most cases presented as plaques and scales. Spongiosis, acanthosis, and hyperkeratosis were the common epidermal features. Predominant dermal findings were inflammation, vascular changes, and dermal edema (Fig. 6).

VB Lesions

Most of the VB lesions had overlapping clinical features: 11 patients had vesicles and 8 cases had erythema. Papules and bullae were seen in five and four cases, respectively (Fig. 7).

Histopathological Findings of VB Lesions (Tables 6 and 7)

All cases of pemphigus vulgaris showed split/blister at the suprabasal level and those of erythema multiforme, dermatitis herpetiformis, and bullous pemphigoid showed split at the subepidermal level. Hailey–Hailey disease showed split at the

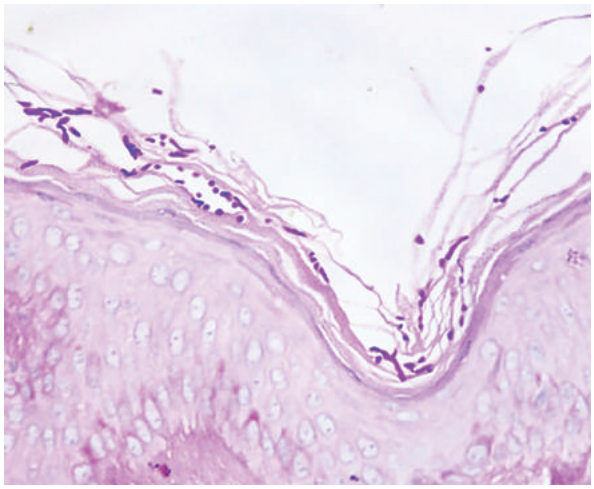

Fig. 5: Pityriasis versicolor: PAS-positive fungal hyphae in the stratum corneum (Periodic acid–Schiff (PAS), 40×)

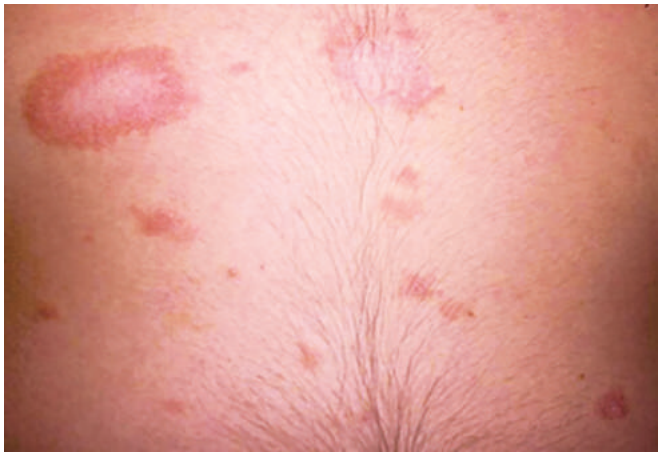

Fig. 6: Pityriasis rosea: Erythematous plaques and macules over the trunk

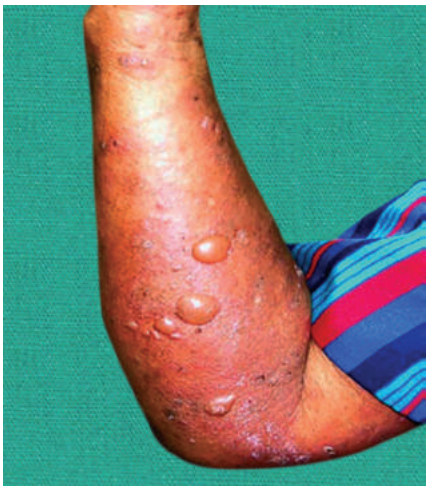

Fig. 7: Pemphigus vulgaris: Fluid-filled blisters on the forearm

Table 6: Variants of VB lesions (n = 18)

Lesions	*No. of cases*
Erythema multiforme	7
Pemphigus vulgaris	6
Dermatitis herpetiformis	3
Bullous pemphigoid	1
Hailey–Hailey disease	1
Total	18

subcorneal level. Bulla contents were acantholytic cells, eosinophils, and neutrophils in most cases (Figs 8 to 10).

Clinicopathological Correlation of Lesions of Superficial Inflammatory Dermatoses

Of the 160 cases, clinicopathological concordance was seen in 156 cases (97.5%) and discordance in 4 cases (2.5%).

Discussion

Pinkus was the first to describe tissue changes in the form of spongiotic, psoriasiform, and lichenoid tissue reactions.[4] Interpretation of skin biopsies requires identification and integration of morphological features with their clinical diagnosis.

Table 7: VB lesions—histopathological features

Histopathological features	*No. of cases*	*Percentage*
Level of split		
Suprabasal	6	33.3
Subepidermal	10	55.6
Subcorneal	2	11.1
Blister content		
Acantholytic cells	9	50
Eosinophils and neutrophils	7	38.9
Acellular (only fluid)	2	11.1
Other features		
Hyperkeratosis	3	16.7
Spongiosis	8	44.4
Basal cell degeneration	5	27.8

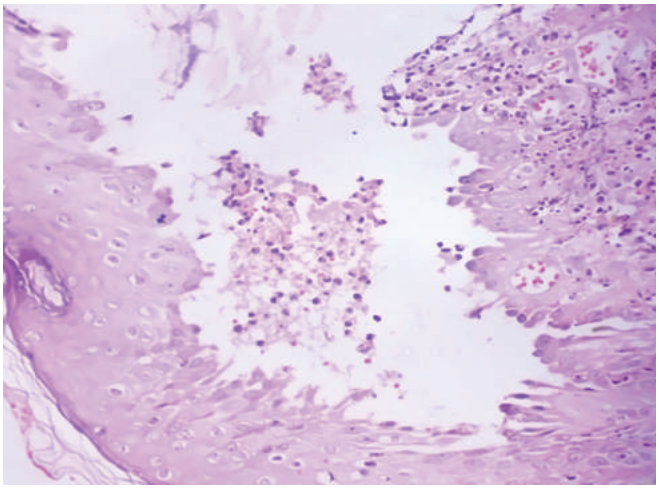

Fig. 8: Pemphigus vulgaris: Photomicrograph shows intraepidermal blister containing acantholytic cells, neutrophils, and eosinophils. Dermal papillae form villi lined by a single layer of basal keratinocytes (H&E, 20×)

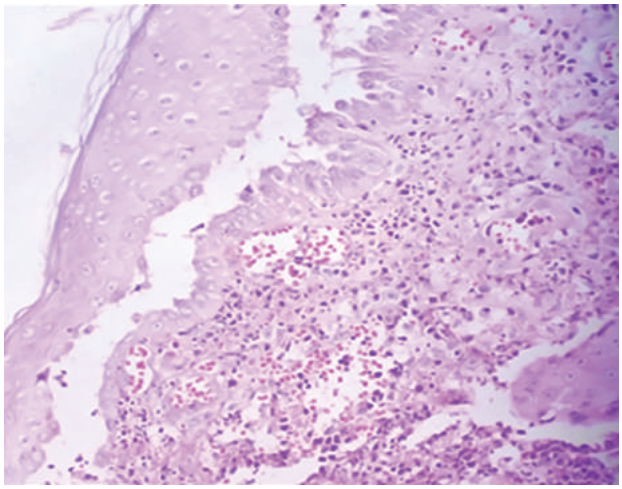

Fig. 9: Dermatitis herpetiformis: Photomicrograph shows subepidermal blister. Neutrophils, eosinophils, and acantholytic cells are seen within the blister (H&E, 20×)

In our study, categorization of lesions is majorly based on the approach followed by Alsaad and Ghazarian.[3] Of the 160 cases of

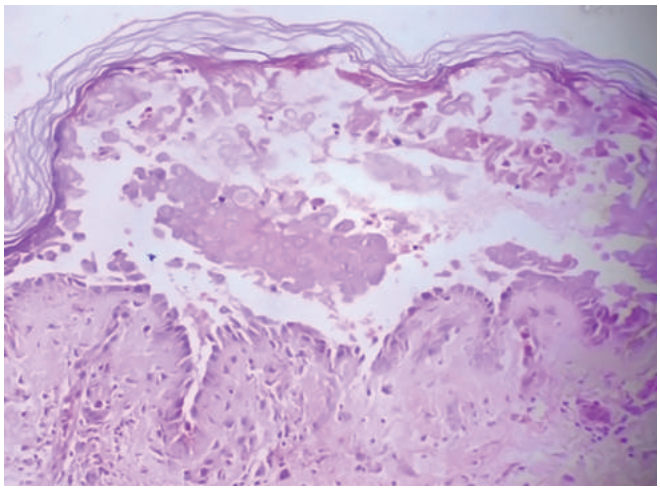

Fig. 10: Hailey–Hailey disease: Photomicrograph shows suprabasal blister containing acantholytic cells, neutrophils, and eosinophils. Basal cells protrude into the blister (H&E, 20×)

superficial inflammatory dermatoses, 142 were papular/non-VB lesions and 18 were VB lesions.

Papular/Non-VB Lesions

These lesions were categorized based on the presence/absence of epidermal changes. Eight lesions were without epidermal changes and 134 were with epidermal changes. Among the lesions without epidermal changes, lymphocytic infiltrate in the papillary dermis was common in all histological types, similar to the observations by Alsaad and Ghazarian[3] in their cases. Lesions with epidermal changes were categorized into interface dermatitis (60 cases), psoriasiform dermatitis (58 cases), and spongiotic dermatitis (16 cases).

Chaudhary et al.[5] and D'Costa et al.[6] reported a similar incidence of interface dermatitis to our study. The predominant histological features of hyperkeratosis, acanthosis, lichenoid infiltrate, and pigment incontinence were reported in this study, corroborated with the observations made by Hegde et al.[7] In psoriasiform dermatitis, most histological features in the epidermis were similar to the findings reported by Raghuveer et al.[8] Munro's microabscesses are not found in early lesions of psoriasis and were reported only in 6.9% cases in the present study. Raghuveer et al. noted microabscesses in 20.5% cases. Dominant histopathological findings in spongiotic dermatitis were spongiosis, acanthosis, and hyperkeratosis. Dermal inflammation showed predominance of lymphoeosinophilic infiltrate followed by lymphohistiocytic infiltrates in our study and these observations were comparable with the findings of Younas et al.[9] Prasad et al.[10] reported more number of cases with lymphohistiocytic infiltrates.

Clinicopathological Correlation of Papular/Non-VB Lesions (Table 8)

In our study, clinicopathological concordance of papular/non-VB lesions was better than other studies. Of the 60 cases of interface dermatitis in the present study, 57 cases (95%) were concordant with clinical diagnosis. Hegde et al.[7] reported 87.2% concordance in their study of 125 cases of interface dermatitis. Psoriasiform dermatitis showed the clinicopathological correlation in all the cases (100%) in the present study. This was comparable with the study done by D'Costa et al.[6] Spongiotic dermatitis showed a concordance of 93.75% (15 of 16 cases) in the present study. Kaler et al.[11] in a study of 25 cases of spongiotic dermatitis observed 92.3% clinical correlation.

Table 8: Clinicopathological correlation of papular/non-VB lesions

Clinicopathological correlation	*D'Costa et al.*[6] *(%)*	*Kaler et al.*[11] *(%)*	*Present study (%)*
Concordance	149 (92.5)	137 (90.6)	138 (97.2)
Discordance	12 (7.5)	14 (9.3)	4 (2.8)
Total	161 (100)	151 (100)	142 (100)

There is considerable overlap of lesions among psoriasiform, spongiotic, and lichenoid dermatitis. Drug reactions and discoid lupus erythematosus have psoriasiform features with interface changes.[11] Eczematous dermatitis and psoriasis may have significant clinical overlap. Eczema has more spongiosis, less uniform hyperplasia, and a retained or thickened granular layer which are the differentiating features from psoriasis.[12] In partially treated psoriasis cases, the granular layer may be present which is a feature of spongiotic dermatitis; hence, clinical details are important for accurate diagnosis.[3,11]

Categorization of papular/non-VB lesions into three distinct entities is a useful approach in reaching the diagnosis. This categorization is also relevant from the treatment perspective. Systemic steroids are to be avoided in psoriasiform lesions, while it can be used as a mode of treatment in lichenoid lesions. Similarly, cyclosporine or azathioprine is used as a last resort in severe cases of spongiotic dermatitis, while it can be used as a first line of treatment in psoriasiform and lichenoid lesions.[13] Hence, appropriate placement of the lesions in each category will aid in differentiating the lesions sooner and help in management.[3,11]

VB Lesions

VB skin diseases represent a group of dermatoses with protean manifestations, having varied etiopathogenesis, but common clinical presentation of vesicles, pustules, or bullae. Among them, the pemphigus group is the commonest.[14,15] Histopathological diagnoses are based on the level of blister separation, inflammatory infiltrate, and altered keratinocytes, such as acanthocytes.[14,16] In the present study, the distribution of VB lesions was generalized. Most cases presented as vesicles. These patterns were comparable with the study done by Arundhathi et al.[14] Subepidermal blister was the predominant feature in our study. Blister contents of inflammatory cells were reported in about half the cases, comparable to the study done by Deepti et al.[17]

Clinicopathological Correlation of VB Lesions

All the 18 cases were concordant with clinical diagnoses (100%) in our study. Krishnamurthy et al.[15] reported 64.8% concordance, while Arundhathi et al.[14] reported 72.2% concordance with clinical diagnosis.

Conclusion

Inflammatory dermatoses of the superficial cutaneous reactive unit are very common and comprise a wide, complex variety of clinical conditions. Morphology remains the gold standard for their diagnosis. In view of their complexity, knowledge of clinical details, detailed microscopic evaluation to recognize their myriad histological patterns, and a schematic approach for categorization

and diagnosis, all contribute to arriving at the most appropriate diagnosis and patient management.

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Original Research Article

A comparison of direct microscopy and culture with periodic acid schiff staining in the diagnosis of onychomycosis

Vishwajith[1], Ankitha Ravishankar[2], S A Lakshminarayana[2,*], S Sangeetha[2]

[1]*Dept. of Microbiology, RajaRajeswari Medical College and Hospital, Bengaluru, Karnataka, India*
[2]*RajaRajeswari Medical College and Hospital, Bengaluru, Karnataka, India*

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ABSTRACT

Introduction and Objectives: Onychomycosis, or fungal infection of the nail apparatus by dermatophytes or nondermatophytes, is more than just a cosmetic problem. Dermatophytes are known to cause 90% of toenail and atleast 50% of fingernail onychomycosis. Our study offers insight into the unique current epidemiological aspects of onychomycosis in our region, including the less known nondermatophytemolds;. Since histopathology of nail clippings using Periodic Acid Schiff is clearly an invaluable tool in diagnosing onychomycosis, we integrated and comparatively evaluated it with the tests of routine mycology for the same.

Materials and Methods: Patients in Rajarajeswari Medical College and Hospital (a tertiary care hospital) presenting with clinically apparent onychomycosis were included in this study. Each specimen of subungual debris and nail clippings was divided into two portions- one for direct microscopy and the other for culture. Nail clippings alone were used for PAS staining.

Results: The present study was carried out on 40 clinically suspected cases of onychomycosis in the Department of Microbiology, Rajarajeshwari Medical College and Hospital over a period of one year. Out of 40 samples, 14(35%) samples showed fungal elements in 20% KOH, in 12(30%) samples fungal culture was positive and 19(47.5%) were PAS stain positive. Out of 12 isolates, 5(41.6%) were dermatophytes and 7(58.4%) were nondermatophytes.

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1. Introduction

Onychomycosis, or fungal infection of the nail apparatus by dermatophytes or nondermatophytes, is more than just a cosmetic problem.[1,2] Approximately 10% of the general population, up to 50% of people aged above 70 years and upto one third of diabetic individuals have onychomycosis.Onychomycosis is not cured unless treated.[3] It can cause psychosocial issues, apart from physical constraints on movement and pain from thickened nails.[4] Also, the nails serve as a constant reservoir of fungi for infecting other parts of the skinand contacts. Life-threatening sequels – foot ulcers in uncontrolled diabetics and cellulitis in diabetics and immunocompromised patients may result.[4]

Several steps are recognised in the pathogenesis of onychomycosis: contact with arthroconidia, adherence and inv asion of stratum corneum.[5] Depending on the pattern of nail invasion, clinical presentation is categorised into the following subtypes: Distal lateral subungual (DLSO), proximal subungual(PSO), endonyx subungual, superficial (SO), mixed, and secondary onychomycosis. Eventually, the common result is total dystrophic onychomycosis.[6]

Dermatophytes are known to cause 90% of toenail and atleast 50% of fingernail onychomycosis.[4] These are keratinolyticfungi[7] DLSO is the most frequent pattern. Candida yeasts are implicated in immunocompromised individuals and in occupations involving f requent contact with moisture.[4] Nondermatophyte moulds (NDM) lack the enzymes required to digest keratin, and a primary breech by dermatophytes or nail trauma is thought to be necessary for their invasion, however, a recent study

* *Corresponding author*.
E-mail address: lakshmi.kims@gmail.com (S. A. Lakshminarayana).

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involving Fusarium species suggests otherwise.[7,8] They are the main pathogens in immunocompromised individuals, including HIV positive patients, exclusively in whom they cause PSO.[6] Diffuse or deep SO and periungual inflammation are also suggestive.[4] However, there is increasing recent evidence of clinically indistinguishable NDM onychomycosis in the absence of predisposing factors; suggesting the importance of geographical variables including climate, and socioeconomic factors. The prevalent pathogen in each region could vary with time.[1,7,9]

Onychomycosis accounts for about 50% of all nail disorders.[4] Many of the gross features are nonspecific, thus laboratory techniques supporting clinical suspicion are needed to establish the diagnosis.[10] Nail plate thickening and the inherently slow growth of nail, makeonychomycosis difficult to treat.[11] Recurrence and relapse are common.[12] Fungal etiology along with clinical subtype and severity must guide choice of treatment as efficacy of systemic antifungal drugs varies with implicated fungi.[13] Delay in diagnosis and treatment may lead to total nail dystrophy.[14]

Direct microscopy of potassium hydroxide mount and fungal culture are the routine techniques of lab diagnosis. KOH mount provides a rapid, simple, inexpensive screening tool.[11] Fungal culture remains the indisputable gold standard, being the only method that identifies the precise identity of causative viable fungi.[15] and the most important test to determine the course of therapy, prognosis and for epidemiology.[7]

The relative difficulty in diagnosing nondermatophyte onychomycosis lies in the wide variety of roles- atleast six ecological subtypes- an isolated nondermatophyte may have. Out of these, only primary and successional invader deserve attention for their implication in treatment.[16] Hence, repeated isolation is recommended along with positive direct microscopy in nondermatophytemolds.[17]

The inclusion of a third diagnostic method- PAS staining of nail sections- has many advantages. It is considered as the gold standard in terms of sensitivity,[14] and is more economical than GMS, which has comparable sensitivity.[18] It is least dependant on sampling methods-distal clipping suffices. Cases treated prior to diagnosis and efficacy of antifungal treatment can be evaluated[11]. Fungal morphology is better demonstrated than on direct microscopy, and provides results comparable to punch biopsy. Histopathological demonstration of nail plate invasion suffices to positively confirm the pathogenic role of a nondermatophyte repeatedly isolated on culture[19]. Hence, there is a need to comparatively evaluate PAS staining of nail sections against the routinely used methods.

Our study offers insight into the unique current epidemiological aspects of onychomycosis in our region, including the less known nondermatophytemolds. Since histopathology of nail clippings using Periodic Acid Schiff is clearly an invaluable tool in diagnosing onychomycosis, we integrated and comparatively evaluated it with the tests of routine mycology for the same.

2. Objectives

1. To isolate and identify causative dermatophytes and nondermatophytes in clinically suspected cases of onychomycosis.
2. To compare the efficacy of direct microscopy and culture with histologic examination using Periodic Acid Schiff in the diagnosis of onychomycosis.

3. Materials and Methods

3.1. Type of study

Laboratory Investigation

3.2. Study design

Comparative Cross-sectional study

3.3. Study population

Patients in Rajarajeswari Medical College and Hospital (a tertiary care hospital) presenting with clinically apparent onychomycosis were included in this study.

3.4. Sample size

N= 40

3.5. Subject selection criteria

3.6. Inclusion criteria

All clinically suspected cases of onychomycosis were be included in the study, who gave consent to participate in the study.

3.7. Exclusion criteria

Fungal infections other than onychomycosis were excluded from the study.

3.8. Duration of study

The duration of study was 8 weeks.

3.9. Data collection procedure

Data was collected using a specially designed Case Report Form.

1. Demographic data-Name, age, sex, occupation, address, phone number, urban/rural
2. History: duration of dystrophic nail(s), nail trauma, excessive sweating of hands/feet whichever affected, previous and family history of onychomycosis Disease

data- Presence of HIV, Diabetes mellitus, peripheral vascular disease Treatment data- oral or topical antifungal drugs and their duration of treatment, immunosuppressants Personal history- Smoking.

3. Dystrophic nails shall be classified based on appearance into the five basic types of onychomycosis: distal subungual, proximal subungual, white superficial, Candidial and total dystrophic.

3.10. Sample collection

Nail area was thoroughly cleansed with alcohol to remove contaminants like bacteria. Nail clippings will be collected using nail clippers. Specimens like subungual debris and other scrapings were collected using a sharp curetor a no.15 scalpel blade based on the site of maximum localization of the infecting fungi as determined from clinical appearance.[16]

3.11. Processing of specimens

Each specimen of subungual debris and nail clippings was divided into two portions- one for direct microscopy and the other for culture. Nail clippings alone were used for PAS staining.

3.12. PAS staining and Histologic examination

Nail clippings were fixed in 10% formalin and treated with 4% phenol for softening. The processed specimens were embedded in paraffin blocks, and about 3 micron thin slices will be prepared and mounted on glass slides. PAS staining was then performed. Stained slides were examined microscopically for magenta coloured fungal elements.[16]

3.13. Direct microscopy

All specimens were subjected to direct microscopy in 40% KOH solution and examined for the presence of fungal mycelia and spores.

3.14. Fungal culture

Nail scrapings and clippings were inoculated on antibiotic containing Sabouraud Dextrose Agar (SDA) with and without cycloheximide. SDA with cycloheximide were incubated at 37°C, and SDA without cycloheximide at 25°C and at 37°C, for 3 weeks aerobically. Isolates were identified by standard laboratory procedures.

3.15. Criteria for nondermatophyte onychomycosis

A nondermatophytemold /yeast isolated on culture had to show corresponding findings- atypical hyphae or yeasts and pseudohyphae respectively- on direct microscopy or PAS stained nail sections to be considered significant. In addition, no dermatophyte must have been concurrently isolated in case of nondermatophytemolds if PAS stained nail sections were negative for fungal elements.

3.16. Statistical tools

SPSS software was used for statistical analysis of the final data.

3.17. Ethical considerations

Only patients who give informed consent were included in the study. Institutional Ethical Committee approval was obtained prior to starting the study.

4. Results

The present study was carried out on 40 clinically suspected cases of onychomycosis in the Department of Microbiology, Rajarajeshwari Medical College and Hospital over a period of one year to compare the efficiency of KOH, culture and PAS stain in the diagnosis of onychomycosis.

The results are analysed as follows

Table 1: Results by different diagnostic methods

Results	n(%)
KOH	14 (35)
Fungal Culture	12(30)
PAS	19(47.5)

5. Results

Out of 40 samples, 14(35%) samples showed fungal elements in 20% KOH, in 12(30%) samples fungal culture was positive and 19(47.5%) were PAS stain positive.

Table 2: Culture results

Culture results	n(%)
Dermatophytes	5(41.6)
Non-Dermatophytes	7(58.4)
Total	12(100)

Out of 12 isolates, 5(41.6%) were dermatophytes and 7(58.4%) were nondermatophytes.

6. Discussion

The routine diagnostic tests in onychomycosis are direct microscopy of KOH mount and fungal culture. KOH mount is a rapid, simple, inexpensive screening test. Potassium hydroxide is used to digest, soften and clear the keratin of nail plate and subungual debris; thus making fungal elements more visible. However, its sensitivity is low, as fungal elements require experience to be identified. Cotton fibres may stimulate fungal elements. The sensitivity of KOH mount can be improved by performing centrifugation

Table 3: Organisms isolated

Organism	Number
Dermatophyte	**5**
Trichophyton rubrum	3
Trichophyton mentagrophytes	1
Epidermophytonflocosum	1
Non-dermatophyte	**7**
Fungi	**6**
Curvularia	2
Alternaria	2
Fonscesea	1
Epicoccumpurpurascens	1
Yeast	**1**
Candida albicans	1

of KOH treated nail clippings followed by staining with chitin specific Chlorazol Black E, fluorescent brightener or PAS. The class of pathogenic fungi can be delineated through morphology. However, these procedures may not be feasible for routine use, and fluorescent brightener requires a special microscope to be visualized.

Fungal culture is the only test that can identify the causative agent at a genus and species specific level. However, up to 4 weeks may be required, and culture does not distinguish pathogens from contaminants. The major disadvantage of these two tests is their high false negative rates. Delayed or false negative diagnosis may result in total nail dystrophy due to inadequate or delayed treatment.

Mayer et al in 2012, evaluated HPE-PAS as a second line diagnostic tool in onychomycosis by subjecting 100 direct microscopy and fungal culture negative nail samples to HPE-PAS. 38% of these turned out positive. They also observed parakeratosis and globules of plasma significantly more when fungal elements were present, indicative of ongoing inflammatory reaction.[20]

M.Shenoy et al noted a significantly higher sensitivity for PAS staining of nail sections (90%) over KOH mount (64%) and fungal culture (42%).[11] Jeelani et al observed a similar sensitivity for HPE-PAS(91.6%), while fungal culture (88%) was found to be more sensitive than KOH mount (77%), both showing considerably higher sensitivities.[14]

In a similar study in 2011, Wilsmann-Theis et al evaluated nail samples from a total of 851 clinically suspected patients and found HPE-PAS to be most sensitive (82%), followed by fungal culture (53%) and direct microscopy (48%). In our study KOH showed 14(35%) sensitivity, culture 12(30%) sensitivity and PAS showed 19(47.5%) sensitivity.[21]

7. Conclusion

Both dermatophytes and nondermatophyticmolds are causative agents of onychomycosis and sometimes yeasts are encountered. KOH mount and fungal culture are routinely used for diagnosing onychomycosis which has lesser sensitivity compared to PAS. PAS staining helps in rapid identification and helps in s tarting treatment early.

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10. Conflict of Interest

None.

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Author biography

Vishwajith Assistant Professor

Ankitha Ravishankar Final Year Student

S A Lakshminarayana Associatre Professor

S Sangeetha Professor and HOD

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Original Research Article

Ventilator associated pneumonia: An enduring hitch in intensive care units!! A study from a tertiary care center

Vishwajith[1], Archana Rao K[1,*], Sangeetha S[1], Lakshminarayana SA[1]

[1] *RajaRajeswari Medical College and Hospital, Bengaluru, Karnataka, India*

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ABSTRACT

Introduction: Ventilator-associated pneumonia (VAP) is a serious health care-associated infection. It prolongs hospital stay and drives up hospital costs reporting high morbidity and mortality. VAP is defined as pneumonia that occurs 48h or more after endotracheal intubation or tracheostomy, caused by infectious agents not present or incubating at the time mechanical ventilation.VAP requires rapid diagnosis and initiation of the appropriate antibiotics.

Materials and Methods: The present study was done in the department of Microbiology, Rajarajeswari Medical college, Bangalore. All the clinically suspected cases of VAP from intensive care units over a period of one year were included in the study. Endotracheal aspirate (ETA) and bronchoalveolar lavage (BAL) samples were collected from all patients and processed . Identification was carried out according to standard biochemical tests. Sensitivity pattern was determined using Kirby-bauer disc diffusion according to CLSI guidelines.

Results: Out of 160 patients, who were on mechanical ventilation, 7 patients fulfilled the clinical and microbiological criteria. Incidence of VAP in our study is 4.4 and incidence density is 10.5 for 1000 ventilator days.57% of bacterial isolates were found to be *Acinetobacter* spp. followed by *Pseudomonas aeruginosa* 29% and *Klebsiella pneumoniae* 14%. Among 7 cases, 3(43%) were Early onset, 4(57%) were late onset VAP.

Discussion and Conclusion: Even in the era of advanced medical care VAP remains a major challenge. The risk of developing VAP can be reduced by VAP prevention care bundles. Timely diagnosis is a major step to initiate appropriate antibiotics for better outcomes. Both patients and units are at risk of developing multidrug–resistant organisms and therefore appropriate antibiotic stewardship is essential. Better knowledge of local patterns of pathogens causing VAP can help facilitate treatment choice, in turn reducing the ventilator days and hospital stay.

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1. Introduction

Nosocomial infection in the intensive care units remain a major threat. The patients in the intensive care units would fall as a prey not only for their critical illness but also for the nosocomial infections. According to the reports, 27% of the critically ill patients suffer from pneumonia and it stands as the second most common nosocomial infection in critically ill patients.[1] Eighty-six percent of nosocomial pneumonias are associated with mechanical ventilation and are termed ventilator-associated pneumonia (VAP).[2] VAP is defined as pneumonia that occurs 48h or more after endotracheal intubation or tracheostomy, caused by infectious agents not present or incubating at the time mechanical ventilation.[3]

Ventilator-associated pneumonia (VAP) is a serious health care-associated infection. It causes prolong hospital stay (ranging from 4 days to 14 days) and ride up hospital costs reporting high morbidity and mortality. It also leads to increased antibiotic pressure. It accounts for the physical, psychological, financial burden to the patient as well to the family.[2] International Nosocomial Infection Control Consortium suggest that the overall rate of VAP is 13.6 per 1000 ventilator days.[4] However, the individual rate varies according to patient group, risk factors, and hospital setting.

* *Corresponding author*.
E-mail address: archanaswaroop79@gmail.com (A. Rao K).

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On the average VAP develops after 5-7 days of mechanical ventilation, with a mortality rate between 24% and 76%.[5] Early-onset VAP, is defined as that occur within the first four days of mechanical ventilation.[6] This is usually attributed to the antibiotic-sensitive community-acquired bacteria such as Haemophilus and *Streptococcus*. Whereas VAP that develops after 5 days of mechanical ventilation is termed as late onset sepsis, caused by multidrug – resistant bacteria such as *Pseudomonas aeruginos*a.

VAP appeals early diagnosis and initiation of appropriate antibiotics. Over the past several decades, our knowledge of VAP has grown significantly regarding risk factors, pathogenesis, microbiological profile and its prevention. This study was done to detect the microbiological profile, incidence and incidence density of VAP in our centre.

2. Objective

1. Determine the incidence of VAP
2. Determine the incidence density
3. Identify various bacterial pathogens causing VAP.

3. Materials a nd Methods

3.1. Inclusion criteria

All the clinically suspected cases of VAP (Fever, leucopenia, change in respiratory secretions, respiratory distress and bradycardia or tachycardia) from intensive care units.

3.2. Exclusion criteria

Cases other than VAP

3.3. Procedure

The present study was done in the department of Microbiology, Rajarajeswari Medical college, Bangalore, Karnataka, India. Endotracheal aspirate (ETA) was collected with a mucus extractor by deep suctioning in a patient who was intubated. Bronchoalveolar lavage (BAL) was collected by wedging a bronchoscope or catheter into a bronchus and isolating the distal airway. A volume of saline is instilled and the fluid is aspirated back from the airway using gentle suction. The smears are made from secretions in the sputum cup and smears are stained with both Gram's stain and acid fast staining. Endotracheal tips(ET) were transferred to sterile centrifuge tubes. ET tips were rinsed with 1 ml normal saline, so that washed fluid collects within the centrifuge tube. centrifuge tube was vortexed for dislodging collection within the ET tip and to disperse organisms into the saline. Calibrated 2 mm loop that holds 5 μL was taken and inoculated on to blood agar (BA), and MacConkey agar(MA) plates. The sample was inoculated in the thioglycollate tube. The media was incubated at 37^0 c for 24 hours Plate s were examined after 24hrs. If there is no growth on the plates they are reincubated for another 24 hours. Thioglycollate tubes were incubated for 7 days if plates do not show growth. Examined daily for turbidity. If thioglycollate media shows turbid ity, smear and Gram stain was done and subcultured. Identification was carried out according to standard biochemical tests. Antibiotic sensitivity tests were done by Kirby-Bauer disc diffusion method. The following antibiotics were used for susceptibility testing: ampicillin, amoxyclav, cefuroxime, ceftazidime, cefepime, ciprofloxacin, gentamicin, amikacin, piperacillin, piperacillin+tazobactam, meropenem, imipenem, aztreonam, netilmycin, tigecycline, colistin, and co-trimoxazole. All the discs were procured from [Hi-media laboratories limited]. The diameter of the zone of inhibition was measured and interpreted according to the CLSI guidelines. Incidence and incidence density are calculated.[1,7–9]

3.4. Statistical analysis

The statistical analysis was performed using standard tests. Fisher's exact test was applied. $P < 0.05$ was considered to be statistically significant.

4. Results

Out of 160 patients, who were on mechanical ventilation, 7 patients fulfilled the clinical and microbiological criteria. Out of 7 VAP cases five were males and two were females. The mean age of the patient was 40years and showed male preponderance. Gender description is as shown inTable 1. Out of 7, 2 patients were admitted in respiratory intensive care unit (RICU), 4 in medical intensive care unit (MICU) and one patient was admitted in surgical intensive care unit (SICU). The distribution of patients is as shown in Table 2. Among seven culture positive cases, *Acinitobacter* species was grown in four (57%) samples, *Pseudomonas aeruginos*a in 2(29%),and *klebsiella pneumonia*e in 1(14%) sample. The bacteriological distribution is as shown in Table 3. The growth of *Pseudomonas, acinitobacter* and *Klebsiella* is as depicted in Figures 1, 2 and 3 respectively. Antibiotic susceptibility testing done on Kirby-baur disc diffusion method is as shown in Figure 4. All the isolates were sensitive to colistin and tigecycline, 97% were sensitive to imipenem, 90% to meropenem, 85% to tobramycin, 85% to netilmycin, 66% ceftazidime, 66% to ofloxacin, 85% aztreonem. Among 7 cases, 3(43%) were early onset 4(57%) were late onset VAP. Incidence of VAP in our study is 4.4 and incidence density is 10.5 for 1000 ventilator days.

Table 1: Gender distribution of the VAP cases

Gender	Number of cases
Male	5
Female	2
Total	7

Table 2: Distribution of VAP cases in various critical care units

Name of critical care unit	Number of cases
MICU	4
RICU	2
SICU	1
Total	7

Table 3: The bacteria distribution in VAP samples

Name of the bacteria	Number of samples	Percentage
Acinetobacter species	4	57%
*Pseudomonas aeruginos*a	2	29%
Klebsiella pneumoniae	1	14%
Total	7	100%

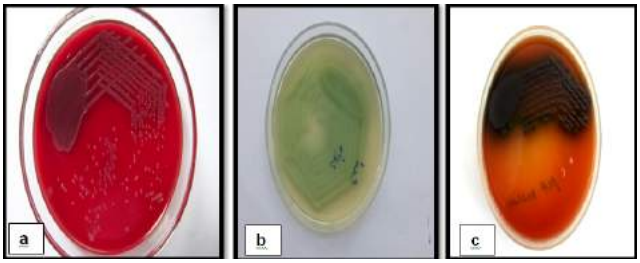

Fig. 1: Growth of *Pseudomonas aeruginos*a on culture media; **a):** Blood agar: large, irregular, beta-hemolytic colonies with iridescence; **b):** Nutrient agar : large, irregular colonies with greenish diffusible pigmen; **c):** Mac Conkey agar: large, irregular, non lactose fermenting colonies

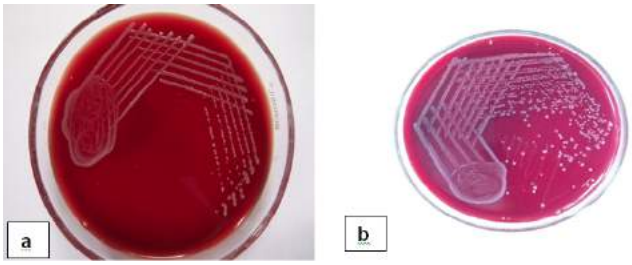

Fig. 2: Growth of *Acinetobacter species* on culture media; **a):** Blood Agar: translucent to opaque, smooth raised colonies; **b):** Mac Conkey Agar: Lactose non-fermenting colonies with faint pink tint

5. Discussion

Even with the implementation of strict hospital infection control practices VAP remain as an enduring hitch in the critical care units. 28% of patients who receive mechanical ventilation in the critical care units are at risk of developing

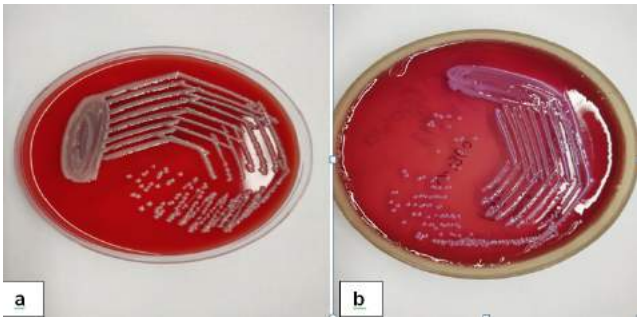

Fig. 3: Growth of *Klebsiella pneumoniae* species on culture media ; **a):** Blood Agar: Opaque, smooth raised, mucoid colonies ; **b):** Mac Conkey Agar: Lactose fermenting,mucoid colonies

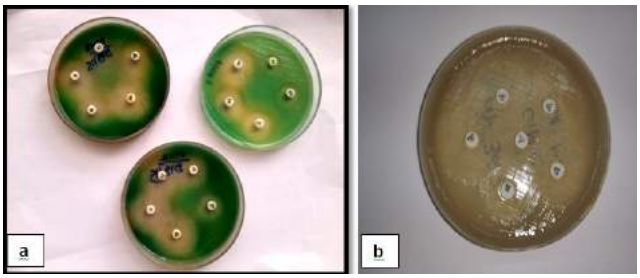

Fig. 4: Antibiotic sensitivity plate ; **a):** Pseudomonas aeruginosa; **b):** Klebsiella pneumoniae

VAP. The incidence of VAP is directly proportional to the duration of mechanical ventilation. Estimated rates are 3% per day for the first 5 days, 2% per day for days 6-10, and 1% per day after

day 10.[10] Knowledge on local pathogens and their sensitivity patterns causing VAP is a preliminary requisite to facilitate treatment choice, thus reducing the ventilator days and hospital stay.

In our study, the mean age of the patient was 40years and showed male preponderance. This is in concordance with the study done by Hina Gadani et al.[11] The incidence and incidence density in our study was 4.4 and 10.5 for 1000 ventilator days. This is lower when compared to the same done by Hina gadina et al (incidence 37%)[11] and Neelima R et al(incidence 57.14, 31.7/1000 incidence density).[12] This could be because of strict vigilance on infection control practices, adequate nursing staff and stern convenance of VAP prevention care bundles. In our study growth from VAP samples yielded *Acinetobacter* species, *Pseudomonas aeruginos*a and *klebsiella* species, this is in correlation with the bacteriological profile of study done by Neelima R et al.[12] *Pseudomon as or Acinetobacte*r pneumonia when compared to other organisms are associated with higher mortality. Studies have shown that mortality risk significantly escalates with delay in starting appropriate and adequate dose of antibiotics. Reports state that, antibiotic use prior to the onset of ventilator-associated pneumonia

(VAP) would substantially increase the probability of infection with multidrug-resistant (MDR) pathogens.[10] Hence prompt and early diagnosis is required to incite appropriate an tibiotics for improved outcomes.[13]

Endotracheal tube or tracheotomy interferes with the normal anatomy and physiology of the respiratory tract, which acts as main culprit for the development of VAP. The level of consciousness is impaired in intubated patients that hinders the voluntary clearance of secretions. This leads to the macro aspiration and micro aspiration of contaminated oropharyngeal secretions that are rich in harmful pathogens. They eventually reach the lower airways leading to the development of a pneumonia.[13] Several studies reported Decontamination of the digestive tract reduces the incidence of VAP by decreasing colonization of the upper respiratory tract. Methods used include antiseptics, such as chlorhexidine in the oropharynx. The aim of this method is to eradicate potentially harmful pathogens like aerobic gram-negative microorganisms and *methi cillin-sensitive Staphylococcus aureus* from oropharyngeal or gastrointestinal tract. Practice of regular oral care and habitual maintainance of basic hygiene minimize dental plaque and colonization with aerobic pathogens thus bring down the mortality and lessen the antibiotic resistance in ventilated patients from cririical care units

An effective strategy should be adopted which aims at infection control at various perspectives like education of the medical staff, universal hand hygiene, use of personal protective equipment and a protocol for microbiological surveillance, prompt reporting, implementing the preventive care bundles in the critical care unit. Basic preventative measures include minimizing time on a ventilator via the implementation of an early weaning protocol, providing regular sedation breaks[13] Utilization of appropriate laboratories facilities and procedures and an immediate implementation of necessary infection control measures also help in restrain the spread of infection right at its source. Knowledge about the local factors leading to VAP and the microbiologic milieu of a given unit is statuary nip the bud.

6. Conclusion

VAP remains a significant risk to the critically ill ventilated patient. Simple and effective preventive measures should be followed. Strict vigilance on early diagnosis, treatment and prevention should be adopted to bring down the VAP rates.

7. Source of Funding

None.

8. Conflict of Interest

None.

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Author biography

Vishwajith Assistant Professor

Archana Rao K Assistant Professor

Sangeetha S Professor and HOD

Lakshminarayana SA Associate Professor

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