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Editorial

The last issue of February, we had good articles especially of FNACS (that too of tertiary care hospital), we had articles on thyroid diseases spectrum (FNACS). Thyroid and it co-relations with histopathology by Bethsaida system, signification of FNACS scoring of breast lesions with histopathology co-relation, HER-2 gene amplification using insitu hybridization in breast cancer, spectrum of Benign breast diseases at a tertiary hospital of Karnataka. FNACS of head and neck lesions in HIV ve+ cases, FNACS of salivary gland lesions and its challenges with histopathology, spectrum of leprosy in a teaching institute of coastal Andhra Pradesh.

Other articles like on hematology, leucocytes and tubercular lymph node FNACS and so on were also remarkable.

Weather is changing rapidly, it is so hot in late April (as discussed earlier also global climatic changes) with problems of sun stroke (heat stroke), dehydration, summer diarrhea, vomiting to name a few disorders, so take precautions.

R.K. Bhatnagar
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Original Research Article

Histopathological Pattern Analysis in Non Neoplastic Skin Biopsies: A Prospective Study of 142 Cases

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Abstract

Background: Non neoplastic skin lesions form the majority of morbidity from skin diseases, however majority of them are often diagnosed as non specific dermatitis. The objective of this study was to analyse and classify non neoplastic skin lesions based on histopathological pattern and to study the relative frequency of various lesions along with age and sex distribution.

Materials and methods: This was a prospective study comprising of 142 cases; from March 2016 to February 2017, done at BGS Global Institute of Medical Sciences. Routine staining with H&E and special stains and Immunofluorescence, wherever needed, were performed. Microscopic findings were studied by two pathologists independently.

Results: A total of 142 cases were studied of which papulosquamous disorders (38 %) were the most common lesions followed by dermatitis (27%) and pigment disorders (14%).

Conclusion: The high frequency of non specific dermatitis as a final diagnosis may reflect the common reaction pattern of many skin lesions; however detailed evaluation of skin lesions and a pattern based classification, can reduce the frequency of nonspecific diagnosis.

Keywords: Skin Lesions; Non Neoplastic; Histopathology.

Introduction

There are about 2000 known skin diseases; seen across various age groups [1]. Of which non-neoplastic skin lesions form the majority of the morbidity from skin diseases [2]. Although some of these lesions are easy to diagnose clinically, a substantial number fall under the "non specific dermatitis" group. Often, a good clinical history along with an adequate skin biopsy is helpful in making a correct histopathological diagnosis [3]. The present study is an attempt to classify non neoplastic skin lesions based on histopathological pattern; and to study the relative frequency of various lesions along with age and sex distribution.

Materials and Methods

The material for the present study comprised skin biopsies from patients received in the Department of Pathology, BGSGIMS Medical College, Bengaluru; from March 2016 to Feb 2017. This was a prospective study comprising of 142 cases. All non neoplastic skin lesions were included; and all neoplastic skin lesions and oral mucosal biopsies were excluded from our study. Specimens were fixed in 10% formalin, processed and 3-5μ thick sections were cut. Routine staining with H&E and special stains (PAS, AFB, Verhoeff-van gieson, Alcian blue and Fite faraco stain) and Immunofluorescence,

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wherever needed, were performed. Serial sectioning and step deeper were taken where ever necessary. Microscopic findings were studied by two pathologists independently. Relative frequency of various lesions along with age and sex distribution of the lesions were analysed.

Results

A total of 142 cases were studied of which papulosquamous disorders were the most common lesions (38 %) followed by Dermatitis (27%) and pigment disorders (14%), (Chart 1) with 30 % of the patients presenting in the age group of 31-40 years (Table 1). Of the 142 cases, 87 cases (61%) were females and 55 cases (39%) were males. (Chart 2). In both males and females, the most common lesion was papulosquamous disorders (Chart 2).

Papulosquamous lesions were more common in the older age group with a slight female predilection. The most frequently encountered tissue reaction pattern was the psoriasiform pattern, with psoriasis constituting the highest percentage of cases (34% of the number of papulosquamous lesions).

There were 6 cases (4%) of granulomatous lesions. The most common etiology of granuloma in our study was Leprosy accounting for 5 cases. Fite faraco stain was done to demonstrate Lepra bacilli. There were 6 cases of viral infection and 2 cases of fungal infection.

There were 7 cases of bullous diseases; of which 3 cases were diagnosed to be bullous pemphigoid, one case of Dermatitis Herpetiformis and one case of Linear Ig A disease, one case of Pemphigus foliaceus and one case of Pemphigus vulgaris. Immunofluorescence was done on all cases for confirmation.

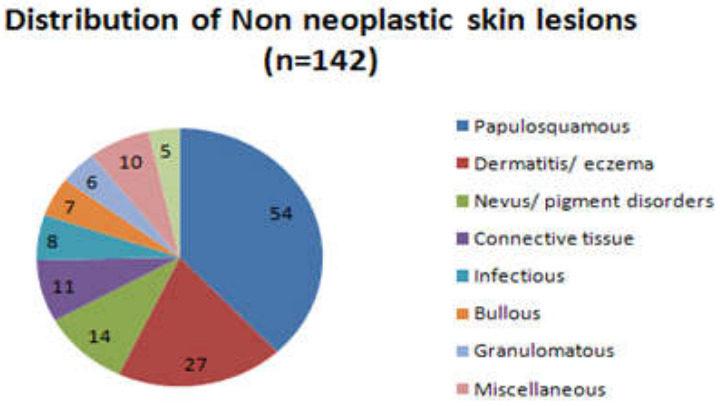

Chart 1: Distribution of number of cases (n=142)

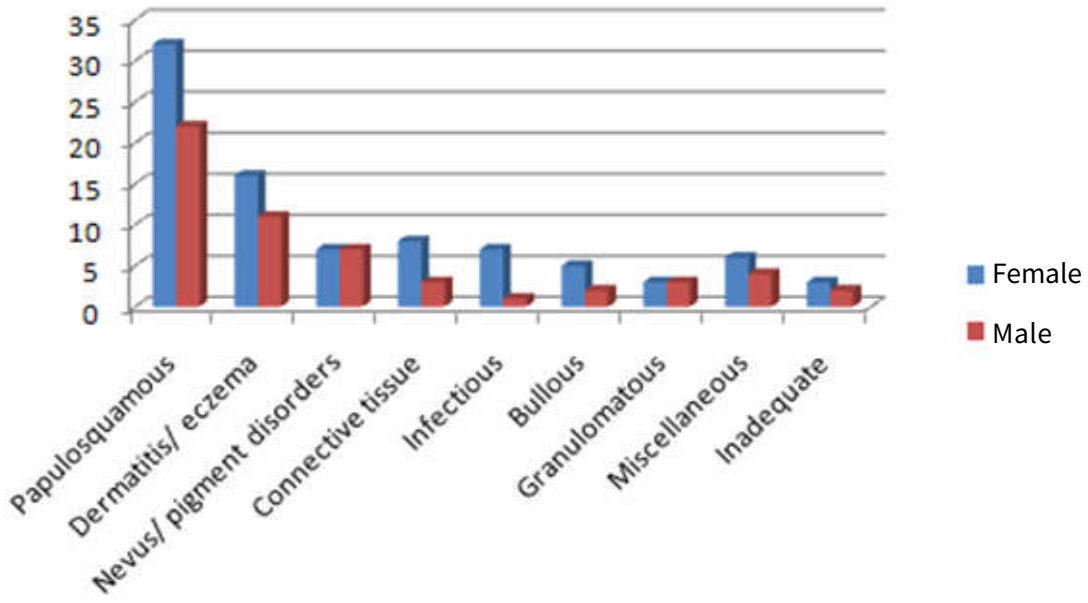

Chart 2: Sex distribution of the cases

Table 1: Age group distribution of various lesions

Skin lesion	<21	21-30	31-40	41-50	51-60	61-70	71-80	>81	Grand Total
Papulosquamous	8	8	9	15	8	6			54
Dermatitis/ eczema	5	3	3	5	7	3		1	27
Nevus/ pigment disorders	2	1	5	3	3				14
Connective tissue	1	4	5	1					11
Infectious	2	3	2	1					8
Bullous		1	2	1		1	2		7
Granulomatous	1		1	1	1	2			6
Miscellaneous	4	1	2		1	2			10
Inadequate	1	2	1	1					5
Grand Total	24	23	30	28	20	14	2	1	142

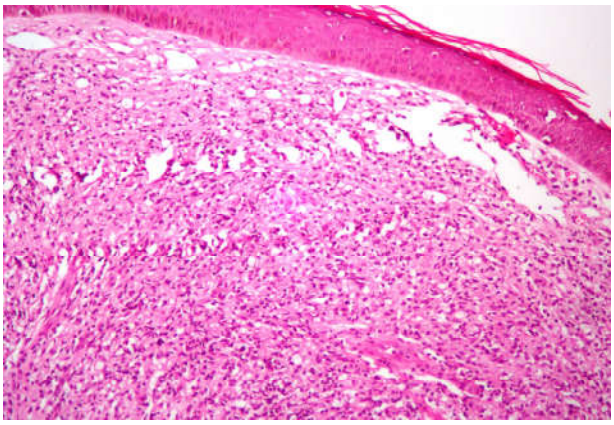

Fig. 1: Histoid leprosy

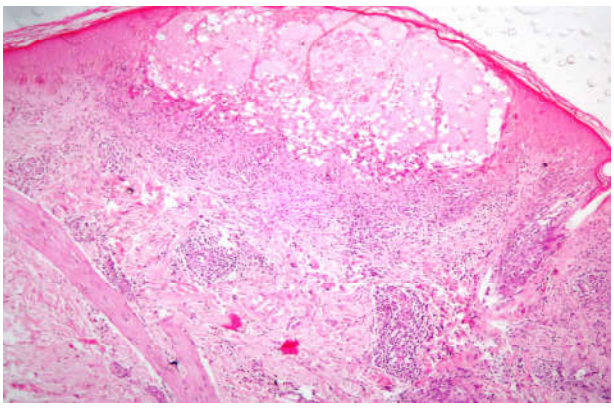

Fig. 2: Viral (Herpes simplex) Dermatitis

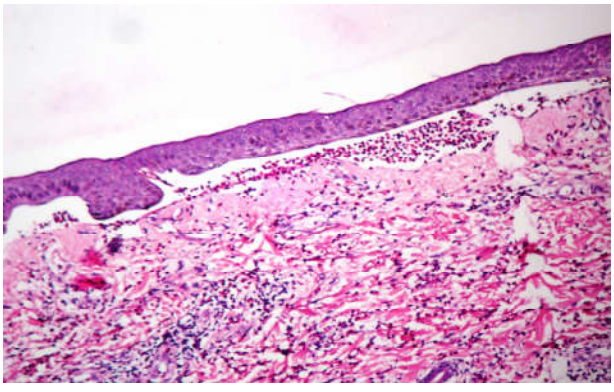

Fig. 3: Bullous pemphigoid

Discussion

Histopathological diagnosis helps dermatologists, in grouping patients into various disease groups that share similar outcome, and a common set of responses to therapy. It helps to prognosticate and manage the outcome of patients. Although histopathology remains the gold standard, not all lesions are amenable to definitive specific histologic diagnosis [3]. The limitation of histopathology in arriving at a specific diagnosis may be due to (1) inadequate sample size, (2) inappropriate biopsy site, (3) overlapping clinical and histological features, (4) inadequate or often absent clinical history, (5) treatment effect [4]. However, a histologic image represents a true synthesis of all molecular events acting in a given microscopic scene and thus, the best approximation to the goal of improving diagnostic accuracy can be achieved by correlation of findings at the molecular, histologic, and gross anatomic levels with patient's physical findings and clinical history [3].

Skin has the same components at different anatomical sites, but in varying proportions; so that the skin has different morphology at different sites. Understanding of the normal histology of skin is central to recognising cutaneous pathology. The epidermal layer is composed primarily of keratinocytes along with a small population of langerhans cells, melanocytes, merkel cells, and unmyelinated axons. Langerhans cells originate in the bone marrow and are functionally and immunologically related to the monocyte-macrophage series. They have antigen processing and presenting capacity. Merkel cells thought to arise from stem cells and have neurosecretory function, are specialized epithelial cells that express cytokeratin, desmosomal proteins and neuroendocrine markers. Separated from the epidermis by a structurally and chemically complex basement membrane zone, the dermis consists of endothelial and neural cells, fibroblasts, monocyte/macrophages and mast cells enveloped within a matrix of collagen and glycosaminoglycan. Adnexae extend from the epidermis into the dermis and consist of specialized cells for hair growth, epithelial renewal (stem cells), and temperature regulation. Adnexal epithelium

also appears to provide a safe haven for certain precursor cells (e.g., for melanocytes and dendritic cells) in an environment sequestered from deleterious environmental influences at the skin surface. The dermal microvascular represents an ensemble of cells responsible for cutaneous nutrition, immune cell trafficking, regulation of vessel tone, and local hemostasis [3].

Modern pathology emphasizes on pattern recognition. Pattern recognition helps to identify a small list of possible differential diagnosis. Further, observation of the sections aided by ancillary techniques will help in making a more definitive diagnosis [3]. Classification of skin lesions are either pattern based or pathogenesis based. We have used pattern based classification to make a diagnosis and integrated them in the pathogenesis based classification according to the ICD-10 of the disorders of skin and subcutaneous tissue.

Papulosquamous disorders constituting 38% of the cases was the largest disease group in our study followed by dermatitis/ eczema (19%). The persistent irritating and poor cosmetic effects of these lesions explains the willingness of these patients to undergo biopsy, and thus may be the reason for their high frequency [2]. Papulosquamous lesions were more common in the older age group with a slight female predilection. The most frequently encountered tissue reaction pattern was the psoriasiform pattern, with psoriasis constituting the highest percentage of cases (34% of the number of papulosquamous lesions).

Often in the differential diagnosis of Psoriasis; Chronic eczematous dermatitis, superficial fungal infections, Lichen Simplex chronicus and Pityriasis rubra pilaris should be considered. Chronic eczematous dermatitis shows more prominent spongiosis, with more irregular psoriasiform hyperplasia. It doesn't tend to have parakeratotic mounds. It is often associated with dermal eosinophilia. Superficial fungal infections can be confirmed by the presence of fungus using special stains such as PAS or GMS stain. Lichen simplex chronicus is characterised by irregular acanthosis, wedge shaped hypergranulosis and superficial dermal fibrosis. Munro microabscesses are less frequent. Pityriasis rubra pilaris is characterised by alternating parakeratosis and orthokeratosis in checkerboard pattern. Parakeratosis is seen shouldering the hair follicle and follicular keratin plugs are common [3].

The high frequency of dermatitis/ eczema group is attributable to the common reaction pattern of many non neoplastic lesions [2]. Since the pathogenesis of most dermatitis is unknown, they are best classified morphologically rather than based on etiology [5]. Leprosy (figure 1) was the most common lesion followed by fungal dermatitis and viral dermatitis (figure 2). Leprosy presents as macules or nodules affecting both skin and peripheral nerves. Histologically in lepromatous leprosy there is extensive inflammatory infiltrate in dermis separated by a narrow grenz zone. Five cases showed positive fite faraco stain with globi of lepra bacilli within foamy macrophages. Tuberculoid leprosy on the other hand showed epithelioid cell granulomas in the dermis with evidence of neural invasion [5]. Cutaneous tuberculosis is an infection caused by mycobacterium tuberculosis and occurs by three routes; direct extension from underlying tuberculosis focus, inoculation and through blood. Lupus vulgaris was the most common form of cutaneous tuberculosis in our study. Lupus vulgaris is generally seen in patients with good immunity and isolation of acid fast bacilli is often uncommon in these cases [6,7,8].

Among the pigment disorders, the most common lesion was intradermal nevus followed by compound nevus and junctional nevus. Nevus had equal sex predilection in our study and was observed more commonly in 31-40 years age group.

Vesiculobullous lesions constituted 4.93 % of our cases. Autoimmune bullous diseases of skin and mucous membrane are uncommon, disabling and potentially lethal diseases. For a quick and reliable diagnosis, histology along with immunofluorescence is needed. It is important to subclassify them because prognosis and therapy vary among variants of autoimmune bullous diseases [9]. All our cases were confirmed with immunofluorescence findings and substantiated (figure 3). In the differential diagnosis of Pemphigus vulgaris; Grave's disease, Bullous impetigo, Hailey-Hailey Disease, Darier disease, Acantholytic acanthoma and Warty Dyskeratoses have to be considered.

Bullous pemphigoid should be differentiated from Epidermolysis Bullosa Acquisita, which on indirect immunofluorescence with salt split skin shows linear deposition of antibodies on the floor of the blister. Linear IgA Bullous Dermatosis shows abundant neutrophils, and Direct Immunofluorescence shows linear deposition of IgA at the dermoepidermal junction. Cicatricial pemphigoid is characterised by subepidermal bulla associated with dermal fibrosis. Porphyria cutanea tarda should be differentiated from other causes of pseudoporphyria such as chronic renal failure (in dialysis), medication (tetracycline, NSAIDS, Cephalosporins) where in urinary porphyrin levels are normal. Porphyria cutanea tarda should also be differentiated from Bullous pemphigoid, Toxic epidermal necrolysis, Bullous amyloidosis and bullous lupus erythematosus [3].

Granulomatous diseases comprised about 4.23%. Connective tissue disorders comprise about 7.7%. Granulomatous diseases and connective tissue disorders will usually have a biopsy performed no matter how confident the clinical diagnosis is. This is based on the fact

that the patient might have to use therapy for prolonged period of time or with the attended possible complication of therapy, and to rule out an infectious etiology before administering steroids [2].

Sarcoidosis characterised by naked granulomas, asteroid body and schaumann bodies have to be differentiated from foreign body granulomas, cutaneous tuberculosis (tuberculin skin test, AFB culture and Quantitative TB gold, ZN stain on biopsy), Tuberculoid leprosy, lupus vulgaris and Granuloma rosacea. Granuloma annulare histologically presents in two patterns: Interstitial pattern and the Palisaded pattern. Interstitial pattern should be differentiated from Interstitial Granulomatous Dermatitis, and interstitial Mycosis fungoides.

Palisaded pattern of Granuloma annulare should be differentiated from Necrobiosis lipoidica, Rheumatoid nodule and Granulomatous dermatitis. Interstitial Granulomatous dermatitis is a subtype of Reactive Granulomatous Dermatitis presenting as linear cords or annular erythematous plaques usually on lateral upper trunk and proximal limbs. It has to be differentiated from Palisaded Neutrophilic Granulomatous Dermatitis where there is more prominent neutrophilia with basophilic degenerated collagen, palisading histiocytes, neutrophils with nuclear debri and focal leukocytoclastic vasculitis. Cutaneous Crohn also known as metastatic Crohn disease is a granulomatous lesion and is lymphocyte rich with ulceration and typically presents with abundant eosinophils [3].

Conclusion

Histopathologic examination serves as the Gold standard for arriving at a diagnosis, in majority of skin disorders, provided a good clinical history is available. Step deeper sectioning often provides valuable information towards making a diagnosis. The high frequency of non specific dermatitis as a final diagnosis may reflect the common reaction pattern of many skin lesions; however detailed evaluation of skin lesions and a pattern based classification, can reduce the frequency of nonspecific diagnosis.

Acknowledgement

We thank the Department of Dermatology for providing us the biopsies that were used in the study.

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Original Research Article

Seroprevalence of HIV among Voluntary Blood Donors in a Tertiary Care Hospital in and Around Koodapakkam, Puducherry, India

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Abstract

Background: Human immunodeficiency virus (HIV) infection rates higher in India when compared with world population. Nearly 3 million people have been affected with HIV in the world. The screening for HIV and other transfusion transmitted infections among blood donors before transfusion is much important in preventing risk of infections among patients and extending their life. HIV-2 cases also been reported even in blood donor population in some parts of India. This study was carried out to observe the HIV-1 and HIV-2 prevalence among voluntary blood donors from in and around koodapakkam, puducherry, India.

Methods: A total of 790 voluntary blood donors sample were collected over the period of 3 years from (August 2014 to July 2017). All the voluntary blood donors (790) blood was screened for both HIV-1 and HIV -2 using card test and Enzyme Linked Immunosorbent Assay (ELISA) test.

Results: Out of 790 voluntary blood donors, only one donor (0.126%) was found to be HIV-1 positive using card test. And again using ELISA kit same donor was found to be positive for HIV -1. There is no prevalence of HIV-2 among voluntary blood donors in and around koodapakkam, puducherry,India.

Conclusions: The prevalence of HIV was 0.125 per cent among the voluntary blood donors from koodapakkam area. No HIV-2 case was found among the studied blood donor population. This study concludes that the HIV threat is meagre in this area among the voluntary blood donors.

Keywords: Voluntary Blood Donor Screening; HIV-1-HIV-2- Koodapakkam; Seroprevalence.

Introduction

Acquired immuno-deficiency Syndrome (AIDS) is a major public health threat among the world population. Human immunodeficiency virus(HIV) infection rates higher in India when compared with world population. Nearly 3 million people have been affected with HIV in the world. The screening for HIV and other transfusion transmitted infections among blood donors before transfusion is much important in preventing risk of infections among patients and extending their life. HIV-2 cases also been reported even in blood donor population in some parts of India. One of the most preventive measure in the spread of AIDS is screening of blood and its products among the voluntary blood donors. India ranks third globally among the highest number of HIV affected individuals. There are multiple

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methods available for the detection of anti -HIV antibody or antigen or both [1-5]. This study was carried out to observe the HIV-1 and HIV-2 prevalence among voluntary blood donors from in and around koodapakkam, puducherry, India.

Materials and Methods

All voluntary blood donors who donated blood at the blood bank of Sri Lakshmi Narayana Institute of Medical Sciences located at koodapakkam, were taken in this study. The voluntary blood donors sample were collected over the period of 3 years from (August 2014 to July 2017). All the voluntary blood donors (790) blood was screened for both HIV-1 and HIV -2 using card test and Enzyme Linked Immunosorbent Assay (ELISA) test after getting consent from the donor.

Results

Out of 790 voluntary blood donors, only one donor (0.126%) was found to be HIV-1 positive using card test (Table 1). And again using ELISA kit same donor was found to be positive for HIV -1. There is no prevalence of HIV-2 among voluntary blood donors in and around koodapakkam, Puducherry, India. Gender wise, only male voluntaries were participated shown in Table 2.

Table 1: HIV positive among voluntary blood donors

No. of voluntary blood donors in blood bank	HIV Positive	HIV Negative
790	1	789

Table 2: Sex ratio

No. of voluntary blood donors in blood bank	Male	Female
790	790	Nil

Discussion

Blood transfusion plays an important role in prophylactic and therapeutic management of several critical health problems. The screening of blood among the donors provides wide range of protection against various diseases like HIV, HbSAg, HCV, etc. These transfusion transmitted infections can be avoided by routine screening on all donated blood.

HIV infection in India is more common when compared to other countries and India ranks third among HIV affected individual. It was first noted in tamilnadu in 1986, since then there is steady increase in HIV affected people across the country. In India it is more common in coastal regions among the commercial sex workers [4-10].

The Seroprevalence of HIV infection reported in Indian blood donors ranges from 0.084-3.87 percent [8-10]. According to our study the Seroprevalence of HIV infection is about 0.125 percent.

Among these HIV-1 is 100 percent. No HIV-2 cases had been reported in our study. This is in concordance with makroo et al [9,10]study in which HIV -1 prevalence was around 0.249 percent in north india and no HIV-2 cases had been reported. But as per Thakral et al and Singh et al study showed, the seroprevalence of HIV -2 was around 0.16 percent and 0.54 percent respectively [11,12].

Conclusion

The prevalence of HIV was 0.125 per cent among the voluntary blood donors from koodapakkam area which is of HIV-1 infection. No HIV-2 case was found among the studied blood donor population. This study concludes that the HIV threat is meagre in this area among the voluntary blood donors.

Note: There is no conflict of interest.

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Original Research Article

Role of Fine Needle Aspiration Cytology in Diagnosis of Subcutaneous Cysticercosis: An Analysis on Clinico-Pathological Profile in A Tertiary Care Hospital

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Abstract

Background: Parasitic infestation remains a leading serious health problem with worldwide distribution especially in developing like India with wide range of morbidities. The fact that cysticercosis infestation caused by Taeniasolium remains a major health hazard with variable clinical manifestation. However, presentation of Cysticercosis infestation as lymphadenopathy with subcutaneous manifestation is a rare entity as observed in our present study which often mis-interpretated formesenchymal soft tissue tumour. The effective pick up of the infestation on routine cytology screening showers limelight on the diagnostic modalities.

Materials and Methods: All patients with lymphadenopathy and subcutaneous masses with various clinical differentials were subjected for routine Fine needle aspiration Cytology procedure with standard cytological stains. All relevant clinical information including socio economic status and procedural data were documented with ethical clearance. Demonstration of parasitic infestations and its association was based on personal knowledge on previously published articles on the disease.

Results: Among 153 cases examined, 15 cases demonstrated features of Cysticercosis infestation, 5 of which showed classical larval stages of proglottids on cytology smears. 4 cases demonstrated cellulose acetate material and 2 cases with cystic fragments in reactive lymphoid background which was confirmed on subsequent histopathology. Rest 4 cases shows fragmented parasites. All the cases were started on prompt appropriate treatment and well recovered.

Conclusion: Cysticercosis infestation should be included in priority as one of the differential diagnosis by Pathologists in patients presenting with lymphadenopathy & painless subcutaneous masses especially in endemic tropical regions. The study emphasizes the judicious and early diagnostic utility of cytology in detection of Cysticercosis infestation in initiating prompt treatment.

Keywords: Cysticercosis; FNAC; Histopathology; Subcutaneous Swelling; Lymphadenitis.

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Introduction

Parasitic infestation remains one of the very serious endemic health problem in developing countries like India especially in endemic regions and among low socioeconomic groups with poor hygienic habits [1]. Majority of the parasitic infestations are symptomatic presenting with classical clinical manifestations. However, in few exceptional instances presentation with superficial palpable nodules and subcutaneous manifestations are

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encountered [2,3] often leading to clinical misdiagnosis of mesenchymal soft tissue tumour.

Human Cysticercosis is a common parasitic infestation caused larval stage of Cestode Taenia Solium [Cysticercus cellulose] usually affecting brain, muscles, ocular region, heart, liver and occasionally subcutaneous tissue. Hence the condition is rightly termed as 'Biological Marker' of the social and economic development of a community [4]. Humans are the only definitive host [reservoirs] of T. Solium harboring larvae of adult taeniasis and pigs acting as an intermediate host with an incubation period ranging from months to several years. Cysticercosis is an acquired disease in humans as a sequelae of incidental ingestion of eggs excreted in feces by human carrier [5]. The incidenceis more prevalent in endemic areas like pig husbandry and poor hygienic pork consumption practices[6]. The transmission and life cycle of T. Solium is of several stages and few cases with retrograde infections. Interestingly, subcutaneous swelling or lymphadenitis is an uncommonmode of presentation of Cysticercosis infestation in India, which often goes misdiagnosed as tuberculous lymphadenitis or soft tissue tumour including Lipoma clinically [6]. The differential diagnosis of subcutaneous presentation includes wide spectrum of diseases with varied implication on management. However, Cysticercosis with subcutaneous manifestation did not receive much attention in the past decades and only few epidemiological data and published articles are available on paper [7,8].

The diagnosis of Cysticercosis is largely based on excision biopsy of mass in conjunction with radiological imaging like MRI and CT scans showing classical 'ring enhancing lesion'. Serology studies like ELISA, Immunoblast assay and cytology are applied as ancillary diagnostic methods only with varied specificity.

The diagnostic role of Fine needle aspiration cytology in identifying Cysticercus infestation presenting as lymphadenopathy and subcutaneous masses was first emphasized by Kung etal., in 1989 [9]. Fine Needle Aspiration Cytology (FNAC) provides a good platform for assessment of any sub-cutaneous lesions. The spectrum of Cyto-morphological picture of Cysticercus lymphadenitis covers a wide range from viable cysts, larval forms of necrosis and calcified lesions with many differentials. The morphological identification of larvae on cytology smears by many eminent researchers had improved the diagnostic utility of FNAC in superficial nodular manifestation of the disease [10]. In the present prospective study, we report a series of 15 cases of Cysticercus infestation presented as subcutaneous mass/ lymphadenitis manifestation emphasizing the role of FNAC as simple interpretative and definite diagnostic aspect carrying a significant practical value especially in endemic regions and areas with limited diagnostic resources.

Materials and Methodlogy

Cytology being a simple day-care procedure in our hospital, more than 800 FNAC procedures of all foci are performed annually. The present Cross-sectional research study included, cases presented with subcutaneous swelling and lymphadenitis with various clinical differentials during a period of 3 years 2014-2016 in our tertiary care hospital.

All Palpable nodules are screened at different sites majority being cervical region which were clinically interpreted as tuberculous and reactive lymphadenitis, lymphoma, soft tissue mesenchymal tumour. Swelling from intraoral and thyroid was excluded from the study. All the clinical details were entered in the proforma sheet and the data acquisition and presentation were obtained based on the criteria of the published articles on the disease [1,2]. The aspirations were performed in patients using 22 Gauge needle attached to 5 and 10 cc syringe as per proper protocol in reference to standard textbooks [1]. Aspiratesranging from 0.5 ml to 2 ml in volume and appearance (clear, purulent, necrotic) was noted. The aspirates were smeared on a glass slide fixed in 95% alcohol and stained with cytological stain as per guidelines of Standard operating procedure in reference to standard textbooks [1].

Totally six smears were made, 3 for dry Giemsa stain and 3 for special stains including Zeihl-Neelsen, Papanicolau and Grams stain. In cases with purulent aspirates, under sterile precaution the part of the aspirate was transferred for culture sensitivity study test. Excision biopsy for histopathological evaluation was suggested in conditions where no definite could be arrived on cytology or with poor treatment outcomes.All the observed data are entered and parameters are correlated with previous research studies.

Results and Observations

The present prospective study included patients of varying age group from 20 to 50 years of age with an average being 35 years with male preponderance was observed compared to females with a ratio of 4:1 as tabulated in Table 1.

Site Distribution

Cervical region was the predominant site [10 cases] followed by trunk region including supra-sternal region as given in Table 2.

Nature of Aspirate

The nature of aspirate was clear fluid on 12 cases with grey white chalky appearance indicating parasitic infection and 3 showed frank purulent aspirate indicating underlying infective etiology.

Clinico-Pathological Profile

Clinical presentation varied with pathological findings with equal proportions. All the patients presented with painless, slow growing swelling with firm, mobile consistency especially Head & neck region, extremities, back (Figure 1 & 2) and were interpreted to be either one the diagnosis as tabulated in Table-3.

Cyto-Morphology

In 5 cases with clear aspirate, actual parasitic structures were demonstrated on the smears (4 suckers & hooklets)(Figure 3) with subsequent biopsy confirmation in 3 cases. Two cases with clear aspirate showed cellulose acetate with fibrillary background. No actual parasites could be seen but the subsequent biopsy showed the parasites. In rest of the cases with purulent aspirates, only mixed inflammatory infiltrates comprising polymorphs, eosinophils, histiocytes are noted in varying proportions with cellulose acetate material (Figure 4). ZN stains were negative in all cases. Aspirates from lymphadenitis showed reactive lymphoid background which was confirmed on subsequent histopathology (Figure 5). Gram stain from purulent aspirates showed gram positive organisms in 10 cases indicating super-added infections.

Table 1: Age & gender distribution of cases

Age (years)	Gender	
	Male	Female
0-20	4	1
21-40	5	2
41- 60	2	-
>60	1	

Table 2: Site Distribution of cases

Site	Number of Cases
Head & Neck/ cervical	9
Extremities[upper limb] Truncal region[Suprascapular&suprasternal]	4
Abdominal region	1
Thigh	1

Table 3: Correlation of Clinico- Pathological profile with Nature of aspirate

Clinical profile	Nature of aspirate & volume	Pathological Findings [Cysticercosis infection]	Inflammatory cell component
Tuberculous Lymphadenitis (n=20)	Clear granular material- <0.5ml	Fragmented larval forms in suppurative background (n=2)	Polymorphs, lymphocytes, plasma cells, histiocytes
Soft tissue tumour [Mesenchymal] (n=10)	Blood stained clear material- <0.5 ml	Hooklets& proglottids -larval forms. (n=5)	Lymphocytes , macrophages, eosinophils
Suppurative lymphadenitis /abscess (n=15)	Purulent material - <1ml	Cellulose &fibrillary background (n=3)	Polymorphs, histiocytes, eosinophils
Reactive lymphadenitis (n=30)	Clear fluid -< 1.5ml	Cellulose acetate material (n=5)	Lymphocytes

Fig. 1: Cervical lymph node swelling with suppuration mimicking tuberculous lymphadenitis

Fig. 2: Sub–cutaneous swelling in extremities mimicking mesenchymal soft tissue tumour

Fig. 3: Cytomorphology showing classical Cysticercus forms with proglottids and hooklets. Inset: shows lymphoid population of background. Pap-40X

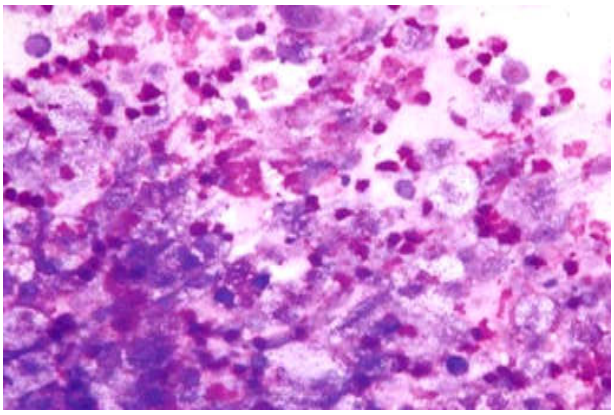

Fig. 4: Cytomorphology from purulent aspirate showing mixed inflammatory infiltration and bluish fibrillary appearance cellulose material. H&E-40X

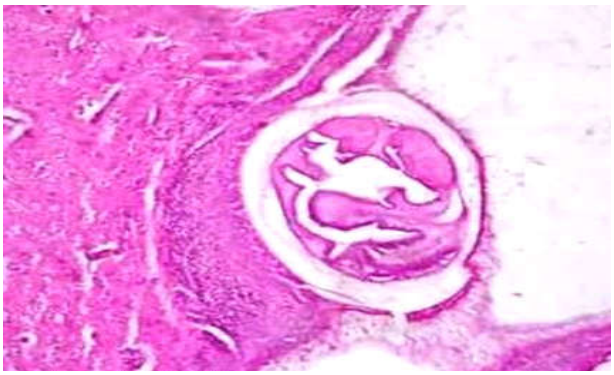

Fig. 5: Lymphnode biopsy showing cysticercus larvae with capsulation. H&E-40X

Discussion

Human Cysticercosis is an endemic disease in Tropical often termed as "Tods-Ready Disease" [2]. According to historical Literature and World Health Organization data, Cysticercosis in humans is an age old ancient disease well known from the Egyptian buried mummies by Paleo-Parasitologist [3]. Majority of the cases reported are due to consumption of uncooked food and unhygienic customs, poor socio-economic causes [2,3].

The term "Neurocysticercosis" being an usual entity, Lymphadenopathy and subcutaneous presentation of cysticercosis is relatively a less common prevalence in this entity without much Research works especially in the diagnosis modality [3]. However recent data from tropical areas reveal the fact that the incidence of cysticercosis is more commoner than previously assumed with high case reports of lymph node and sub-cutaneous nodular presentations [4,5].

With humans being the only definite hosts of TaeniaSolium diagnostic modalities employed to detect cysticercosis includes radiology, serology and histopathological evaluation with excision biopsy being gold standard method [6]. Cysticercusnodules are challenging to distinguish from mesenchymal tumors and infectious lymphadenitis often warranting excision biopsy as observed in our present study [7]. FNAC is a simple minimally invasive procedure with varied sensitivity and specificity established in late 1990's often obviating the need for excision biopsy. While Cytomorphology of Cysticercosis warrantsdemonstration of actual parasites which is rare to pick up on smears, presence of hooklets and fragments holds good for reporting Cysticercus even in absence of viable forms as proved in the present study with concordance to previous Researchers.

In the present study majority of case are reported with male preponderance in cervical region correlating with prior studies [7,8]. Four cases with purulent aspirate showed bluish fibrillary, granular background demonstrating cellulose acetate with eosinophils, plasma cells, lymphocytes histiocytes and subsequent excision biopsy also showed parasitic fragments. The inflammatory reaction is attributed to immune response due to super added infection. This alerts the Pathologists that granular dirty background with metachromasia on dry smears should raise the suspicion of parasites especially cysticercosis as observed in present previous studies as well [9,10].

It is proved from the present study that aspiration of clear fluid should be viewed with high index of suspicion for parasitic infestation even in the absence of straightforward parasitic fragment. Hence the clear fluid must be correlated and processed without discarding as non-specific. From the present study it is evident that presence viable forms doesn't elicit any inflammatory reaction unless it is secondarily infected

Conclusion

Cysticercus infestation presenting as sub-cutaneous swelling and lymphadenitis manifestation is more common than usually assumed. In instances with clear fluid aspirates cysticercosis infection should be tabled as

priority differentials. FNAC is simple minimally invasive procedure and has high sensitivity and specificity in picking up subcutaneous cysticercosis often obviating the need for invasive surgical procedures**.**

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Original Research Article

Diagnostic Utility of Body Fluid Cytology

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Abstract

Background: Cytological examination of various body fluids like ascitic, pleural, synovial, CSF, urine etc, plays an important role in diagnosing various non- neoplastic and neoplastic conditions. Hence, this study is conducted to evaluate the utility of this simple procedure and to understand the newer techniques that can be applied in further confirming our diagnosis.

Materials and Methods: It is a retrospective study conducted on all the body fluids which came for cytological analysis. The fluids were centrifuged, the supernatant was discarded and the sediment was used to make three smears which were stained with Papanicolaou, Hematoxylin and Eosin stains (H&E) and with leishman stain. All the smears were then thoroughly examined.

Results: The most common sample received was pleural fluid (55%) with ascitic fluid being the second most common. Chronic inflammation was the most common pathology noted in both the pleural as well as the ascitic fluid. A total of 8 cases showed metastatic deposits which were from adenocarcinoma. The cells showed typical features of malignancy.

Conclusion: Cytological analysis of body fluids helps us in differentiating non-neoplastic and neoplastic lesions. Further typing of the neoplastic lesions can be done using special stains and immunohistochemical stains

Keywords: Body Fluids; Cytology; Mesothelial Cells; Neoplastic Cells.

Introduction

Cytological analysis of body fluids plays a pivotal role in the diagnosis of various lesions and aids the clinicians in the appropriate management of them [1-2]. A major contribution is in detection of the cancer cells accurately and then typing of the tumor [3]. Apart from this, fluid examination also reveals other pathological conditions, most commonly inflammation, infection with bacteria, fungi or viruses and parasitic infestation [4].

The main serosal body cavity fluids comprise of peritoneal, pleural, cerebrospinal and pericardial fluids. Cytological analysis has gained increased acceptance to such an extent that a positive diagnosis is often considered as a definitive diagnosis. In our study we try to understand the cytology of various body fluids, categorize effusions and study their implications in the proper diagnosis of various clinical conditions [5].

Materials and Methods

This study was conducted in the Department of Pathology, Dr D.Y. Patil Medical College, Pimpri. It was a

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retrospective study of one year duration from October 2016 to September 2017. All the body fluids which came for cytological analysis were included in the study. Relevant clinical information regarding age, sex, symptoms and accompanying signs which were previously available were obtained. These fluids were analyzed for physical properties like the volume of fluid received, colour and odour. For cell count, a drop of fluid was mixed with a drop of toluidine blue and cell counts were done on the improved Neubauer counting chamber. All the cases were analyzed for protein, sugar, cytology and microbiological study.

Later, these fluids were centrifuged at 2000rpm for five minutes, the supernatant was discarded and the sediment was used to make three smears. Two were fixed in 95% methyl alcohol, and then stained with Papanicolaou and Hematoxylin and Eosin stains (H & E). Other was air dried and stained with leishman stain.

Results

Cytological analysis was done on 273 cases of body fluids. The age ranged from 15 - 85 yrs. Maximum number of patients was in the age range 46 - 60 yrs (Figure 1). The male to female ratio was 1.7:1 with 171 males and 102 females. The most common sample received was pleural fluid (55%) with ascitic fluid being the second most common (36%). The other less frequent fluids that were received were urine for cytology, testicular fluid, CSF and synovial fluid (Table 1).

Chronic inflammation was the most common (60%) pathology noted in the pleural fluid. The smears showed chronic inflammatory infiltrate composed of lymphocytes and macrophages on a proteinaceous background (Figure 2). Reactive mesothelial cells with no pathology were seen in 24 cases (16%). Acute suppurative inflammation was seen in 23 cases (15.3%) (Figure 3). Eleven cases of tuberculous inflammation were diagnosed with smears showing epithelioid cells and lymphocytic infiltrate. Only 2 cases (1.3%) of malignancy (Figure 4) were noted, and were diagnosed as positive for adenocarcinoma (Figure 5). The most come pathology noted on ascitic fluid cytological analysis was also chronic inflammation. Forty eight cases (48.4%) showed smears with chronic inflammatory cells like lymphocytes and macrophages. Reactive mesothelial cells were noted in 34.3% of the cases (Figure 6). Eight cases (8%) showed neoplastic cells in the fluid. Many large tumor cells with hyperchromatic nuclei scanty cytoplasm arranged in clumps depicting adenocarcinoma, were seen (Figure 7). Five cases of bacterial peritonitis and 2 cases of tuberculous peritonitis were also noted (Figure 8).

Out of the 9 cases of urine samples received, predominantly to rule out malignancy, only 1 case showed dysplastic cells with large nuclei and scant cytoplasm and was reported as suspicious of malignancy (Figure 9).

Table 1: Showing the type and percentage of various body fluids

	Type of body fluid	No of samples	%
1	Pleural fluid	150	54.9
2	Ascitic fluid	99	36.3
3	Urine	9	3.3
4	Testicular fluid	6	2.2
5	CSF	6	2.2
6	Synovial fluid	3	1.1
	Total	273	

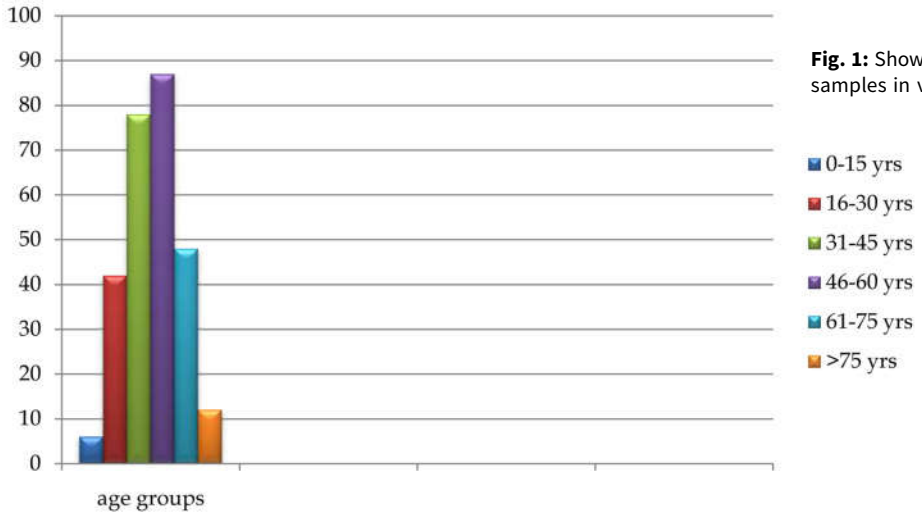

Fig. 1: Showing the distribution of cytological samples in various age groups

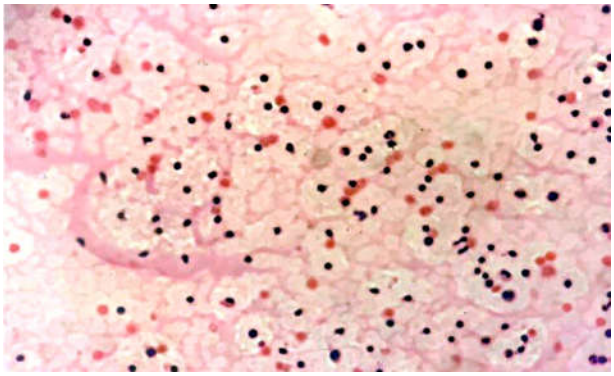

Fig. 2: Chronic inflammatory infiltrate showing predominantly lymphocytes

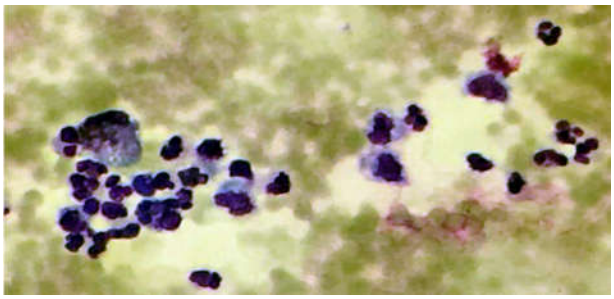

Fig. 3: Acute suppurative inflammation showing numerous polymorphs

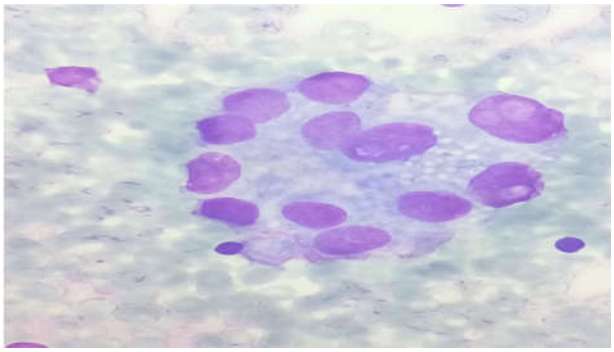

Fig. 5: Showing spectrum of lesions on pleural fluid cytology

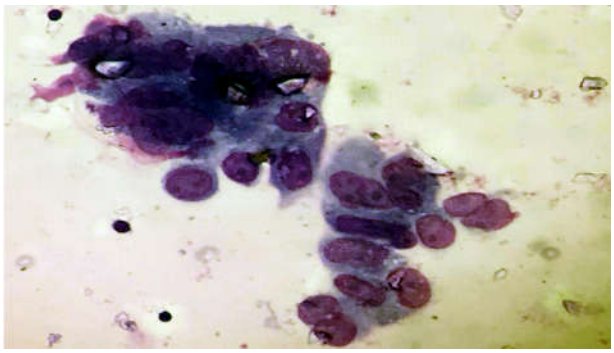

Fig. 6: Showing reactive mesothelial cells

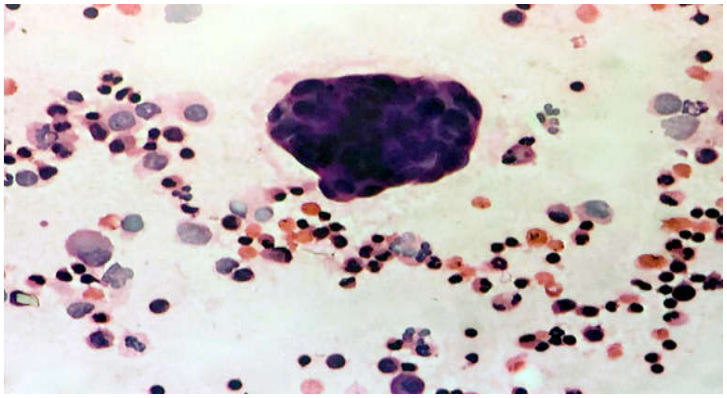

Fig. 4: Showing a cluster of large pleomorphic cells with hyperchro-matic nucleui on a background of mixed inflammatory infiltrate

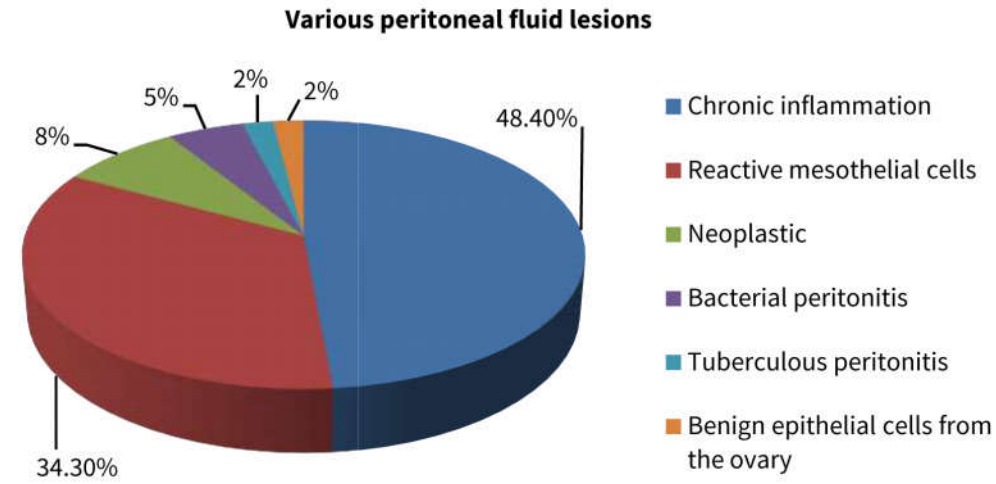

Fig. 7: Showing metastatic deposits of adenocarcinoma

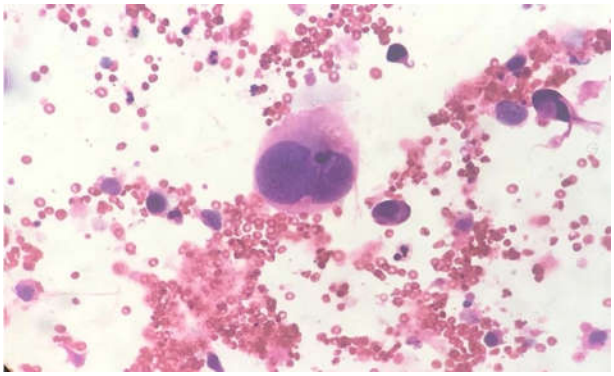

Fig. 8: Showing the various pathological lesions seen on ascitic fluid

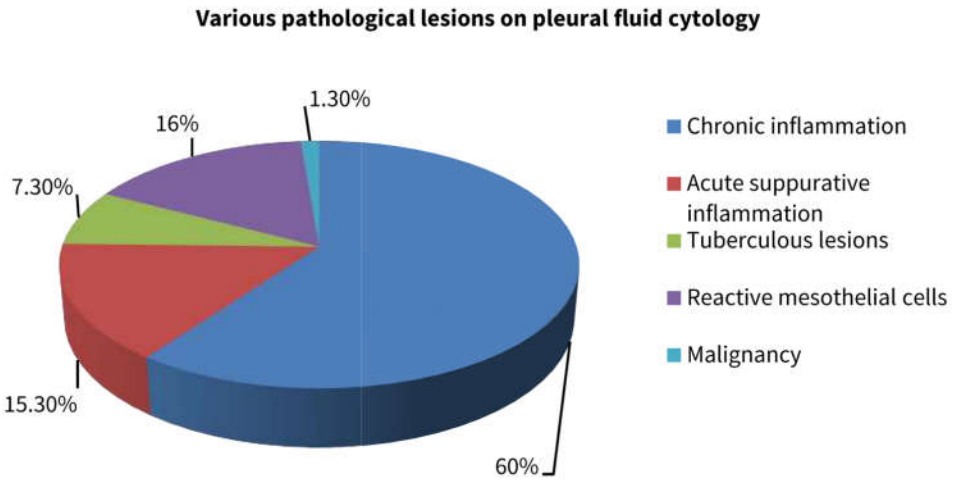

Fig. 9: Dysplastic cells in urine

Discussion

In 1867, Lucke and Kiebs first observed lymphocytes and other larger round cells with large clear nuclei in a milky ascitic fluid but an authenticated description of cancer cells in pleural and peritoneal fluids appeared only in 1882. In that year Quincke published a detailed description of cancer cells originating in ovarian and pulmonary cancer. And even now analysis of the body fluids is considered as a simple and an important method of diagnosing many non- neoplastic and neoplastic conditions [1].

In our study a total of 273 fluids were received. Males were more than females. Most of the patients were in the age group of 46-60 yrs. Pleural fluid was the most common fluid received in our study. This correlates well with studies conducted by others [4,6].

Among the non- neoplastic causes, chronic inflammation was the most commonly seen pathology in both pleural and peritoneal fluids. These smears showed predominantly lymphocytes and few of them also showed macrophages along with lymphocytes on a proteinaceous background. The cause for such chronic inflammatory exudates is usually infection of the organs enclosed by the serous membranes and sometimes because of the presence of tumors in these organs. Hence, it is advisable that those cases that are reported as chronic inflammation need a repeat sample analysis atleast for three consecutive times to exclude a suspicion of malignancy [7]. Suppurative lesions were lesser in number which showed abundant neutrophils along with very few lymphocytes [8]. Most of the synovial fluids were transudate in nature with majority of them showing neutrophilic predominance. Majority of CSF fluid showed lymphocytic predominance. No metastatic deposits were noted in CSF.

Reactive mesothelial cells were seen in 34 cases of ascitic fluid and in 24 cases of pleural fluid. These cells can often be confused with malignant cells. The mesothelial cells are arranged in mono layered sheets in clusters or singly in contrast to the malignant cells that are usually arranged in papillary or glandular pattern. They are large pleomorphic cells with hyperchromatic nuclei and scanty cytoplasm [9]. Few mesothelial cells exhibit a typical window between two cells because of the brush border villi seen in them. Also, abnormal mitosis noted in the cells on cytology warrants a careful study to rule out an underlying malignancy. Special stains play an important role in distinguishing reactive mesothelial cells from adenocarcinoma deposits. Periodic acid Schiff stain can be used to demonstrate the PAS positive granules in the cytoplasm of the mesothelial cells and only a diffuse red blush occasionally in the malignant cells where as mucicarmine , a mucin stain shows positivity only in adenocarcinoma and never in mesothelial cells [10].

Immunohistochemistry can also be used to distinguish reactive mesothelial cells from adenocarcinoma. CK, EMA,CEA, Leu M1, B72.3, BerEP4, CA 19-9, HMB45 are the panel of markers that are used. Desmin and calretinin are positive in mesothelial cells while cytokeratin is positive in adenocarcinoma [11].

Neoplastic cells were noted in 8 cases of ascitic fluid and in 2 cases of pleural fluid. All the cases showed deposits of adenocarcinoma cells. The tumor cells showed features of adenocarcinoma, i.e., the tendency to form smoothly contoured cohesive groups composed of large cells with eccentric, malignant-appearing nuclei, prominent nucleoli and vacuolated cytoplasm. However, at times, adenocarcinoma cells in effusions were seen to exhibit

great morphologic variation. Adenocarcinoma is the most common type of tumor to produce metastasis in the serous cavities, with primary most often from breast, lung, ovary and GIT [12,13,14].

Studies conducted by Wong JW et al and Shulbha et al showed that metastatic deposits were noted more in pleural fluids than ascitic fluid [8,15].

The cytological examination of fluids by means of smears, even though carefully prepared, leaves behind a large amount of residual fluid, that is not further investigated but that might contain valuable diagnostic material. This residual material can be evaluated by treating it as cell block in a simple and expedient fashion, and examined in addition to the routine smears.

The cell block technique will increase the positive results, and will help to demonstrate morphological details by preserving the architectural patterns, which could be of great help in making correct diagnosis of the primary site. The cell block technique has an added advantage that multiple sections of the same material can be obtained for special stains [16,17]. Study conducted by Hathila et al mentioned that conventional smear method was useful for all types of specimen with different cellularity. Cytospin method allowed concentrating the scanty cellular sample but was not quite useful for highly cellular specimen in which it may lead to confusion in interpretation [18].

Conclusion

Cytological analysis of body fluids helps us in diagnosing various pathological conditions and differentiating non-neoplastic from the neoplastic lesions. Further typing of the neoplastic lesions can be done using special stains and immunohistochemical stains.

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NIL

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Original Research Article

Distribution of ABO and Rhesus (D) Blood Groups among Blood Donors in Tertiary Care Rural Hospital of Central India: A Retrospective Study

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Abstract

Introduction: A lot of diversity is present in a vast country like India in relation to race, religion and creed. Similar to it the geographical distribution of ABO and Rhesus blood groups varies across the country.

This study was done to examine the distribution of blood groups among voluntary blood donors in a rural hospital in central India.

Methods: A retrospective study was performed over 5 years (from January 2012 to December 2016) at Blood Bank of Mahatma Gandhi Institute of Medical Sciences, Sewagram, Distt. Wardha, Maharashtra, India to determine and compare the distribution of ABO and Rh blood groups. The ABO and Rhesus (D) blood grouping was done by tube method using venous blood samples.

Results: The predominant donors belonged to age group between 26-35years (50.69%). Male donors were more than female donors (98.78%). The commonest ABO blood group was O (34.46%) followed by B (32.50 %), A (24.98 %) and AB (8.05%) respectively. The prevalence of Rhesus positive and negative distribution in the studied population was 96.76% and 3.23% respectively.

Conclusion: The commonest ABO blood group in our study was O followed by B, A, and AB respectively. The knowledge of the distribution of blood group in a particular geographical area is very important for blood banks. Transfusion service play an important role in the patient's health care.

Keywords: ABO Rhesus (D) Blood Groups; Blood Donors; Blood Bank.

Introduction

The ABO blood group system was first discovered by Karl Landsteiner in 1900 [1] and later he discovered Rh blood group in 1941 with Weiner [2]. Together these two systems have proved to be a breakthrough in the history of blood transfusion and immunohematology. Red blood cells contain a series of glycoproteins and glycolipids on their surface which constitutes the blood group antigens. Production of blood group antigens is genetically controlled. On the basis of presence or absence of A and B surface antigens ABO blood group system is divided into four blood types namely A, B, AB and O [3-4].

ABO blood group system is important as the A and B antigens are strongly antigenic and anti-A and anti B are naturally occurring antibodies present in the serum of persons lacking the corresponding antigen.

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In case of incompatible blood transfusions, these antibodies are capable of producing intravascular hemolysis [5].

Rh (rhesus) blood group system is the second most important blood group system and classified as Rh positive and Rh negative depending upon whether Rh antigen is present on red cells or not [6].

Rh antigens are highly immunogenic. D antigen is most significant out of 49 Rh antigens identified till now. The Rh status is routinely determined in blood donors, transfusion recipients, and in a pregnant woman [7] as the D negative individuals produce anti-D if they encounter the D antigen through transfusion or pregnancy and causes hemolytic transfusion reaction, or hemolytic disease of the fetus and newborn [6].

It is important to have the knowledge of ABO and Rh blood group distribution [8-9] for the effective blood banks inventory management.

It is also important for clinical studies, for reliable geographical information, reducing maternal and premature mortality rate [10], for genetic studies and for doing research in population migration patterns in addition to resolving certain medico-legal issues particularly in case of disputed paternity [11]. This study reports the distribution of ABO and Rhesus blood groups among blood donors.

Material & Methods

A retrospective study of the distribution of ABO and Rhesus (D) blood groups among blood donors was carried out at our Blood Bank, in a tertiary care health institute, in a rural area in Maharashtra. This study was done for five years from January 2012 to December 2016. Total 22,255 medically fit donors in the age group of 18 – 60 years donated blood during this period. After blood donation, ABO blood grouping & Rh typing was done by test tube agglutination method by commercially available standard antisera i.e Anti A, Anti B, Anti AB and Anti D. Both forward (cell grouping) & reverse grouping (serum grouping) was carried out for determination of ABO blood groups. Final blood group was confirmed only if both forward & reverse groups were identical Those who tested positive with anti-sera D were considered to be Rh positive and those who did not be were considered to be Rh negative. The donor blood group data were analyzed on specially prepared proforma and compiled.

Results

The total donors examined from January 2012 to December 2016 were 22255 out of which 21964 were male donors and 291 were female donors. Most of the donors belonged to the age group 26-35 years (50.69%) and least were from age group 60 and above. The commonest ABO blood group was O (34.46%) followed by B (32.50 %), A (24.98 %) and AB (8.05%) respectively (Table 1). The distribution of Rhesus (Rh) D factor was as follows, 21535 (96.76 %) Rh D positive and 720 (3.24 %) Rh D negative. (Table 2). Out of 21964 male blood donors, 21282 were Rh D positive, 682 were Rh D negative. Out of 291 female blood donors, 253 were Rh D positive, 38 were Rh D negative. The

Table 1: Sex-wise distribution of ABO blood groups over a period of five years

Sex	A	B	AB	O
Male	5495	7131	1762	7576
Female	65	102	29	95
Total (%)	5560(24.98 %)	7233(32.50 %)	1791(8.05%)	7671(34.46%)

Table 2: Sex- wise distribution of Rh blood groups over a period of five Years

Rhesus blood group	Male (%)	Female (%)
Rh +ve	21282(96.9%)	253(86.9%)
Rh –ve	682(3.1%)	38(13.1%)
Total	21964(100%)	291(100%)

Table 3: Sex-wise distribution of RH positive and negative blood groups over a period of five years

Sex	Blood Groups							
	A+	A-	B+	B-	AB+	AB-	O+	O-
Male	5341	154	6928	203	1706	56	7307	269
Female	52	13	91	11	23	6	87	8
Total (%)	5393(24.23 %)	167(0.75%)	7019(31.53%)	214(1%)	1729(7.77%)	62(0.28%)	7394(33.22%)	277(1.2%)

distribution of ABO Rh-positive blood groups was as follows, blood group A positive 24.23 %, B positive 31.53 %, O positive 33.22 % and AB positive 7.77%, the most common being blood group is O positive. Among total 720 (3.24 %) Rh D negative blood donors, O negative (1.2%) was most common followed by B negative (1%), A negative (0.75%), and AB negative (0.28%) (Table 3).

Discussion

There is marked variation in incidence of ABO and Rh groups in different races, ethnic groups, and socioeconomic groups in a densely populated country like India. There is variation in frequencies of ABO and Rh blood groups from one population to another and time to time in the same region.

The present study showed a large number of male donors compared to female donors which are comparable with other studies within India [12-13].

The most common age group donating blood is 26–35 yrs. This is comparable with other studies [14-15]. Above 60 yrs of age, people suffer from various ailments like hypertension, diabetes mellitus, low hemoglobin and ischemic heart diseases and hence restricted from donating blood or are considered unfit.

We compared our results with other studies carried out in different geographical areas. A study was done in Eastern India, by Nag et al [16], southern India by S Periyavan et al [17] Mallikarjuna et al [18] and Das et al [19] found that the commonest blood group was 'O', followed by B, A, and AB.

The studies done in Northern India by Tulika et al [20], and by Sidhu et al [21], showed blood group B to be the commonest, followed by O, A, and AB. The studies done in Western India by Wadhwa et al [22], Patel et al [15] and by Mehta et al [23], showed blood group B is the commonest followed by O, A, and AB. Geographical distribution of Blood Groups in India shows that in Eastern and Southern part, O is the most frequently occurring blood group, similar findings are seen in our study, where as in Northern & Western part of India, B is the commonest blood group contrary to our study.

Outside India, the studies done in USA by Mollison et al [24], in Ethiopia by Kassahun et al [25], the commonest blood group was O similar to our study. The study done in in Pakistan by Rehman et al [26], observed that the commonest blood group was B. Study done at Nepal by Pramanik et al [27] found that the commonest blood group was A.

The incidence of Rh D positivity blood group in most of Indian studies varies from 94 to 98 % and Rh D negativity from 2 to 6% [28] whereas in our study 96.78 % were Rh D positive and 3.22 % were Rh D negative.

So to frame better National Transfusion Policy and efficient delivery of services regional Blood Grouping studies are the need of hour especially for management during emergency situations.

Conclusion

The knowledge of the distribution of blood group is very important for blood banks. Transfusion services plays an important role in the patient's health care. Access to safe and sufficient blood supply will help to reduce the morbidity and mortality rates. Every transfusion centre should have a record of frequency of blood group system in their population. It not only provides scientific data but also serves to enable insight into possibilities of the future burden of blood group associated diseases.

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Permission from IEC: Yes

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Original Research Article

Histopathological Study of Surface Epithelial Tumours of Ovary at a Tertiary Care Hospital

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Abstract

Background: Ovarian cancer accounts for 3% of all cancers in females. Ovarian Tumours are most common tumours of female genital tract. Ovarian cancer is leading cause of deaths among gynaecological cancers. The aim of the study was to find the incidence of surface ovarian tumor at the Department of Pathology, S Nijalingappa Medical College, Bagalkot.

Materials and Methods: This prospective study comprised of 82 primary ovarian tumours diagnosed in the department of pathology at S N Medical college, Bagalkot during the time period of 1st January 2016 to 30th June 2017. Records were retrieved from the departmental registers and were analyzed.

Results: Total 82 cases were studied in this duration of study period. Out of 82 cases majority were benign tumours (81.75), followed by malignant tumours (15.9%) and borderline tumours (2.4%) were found.

Conclusion: Most of the ovarian tumours in this study were surface epithelial tumours followed by germ cell tumours. Clinical features are variable. Histopathological examination is needed for proper diagnosis, treatment and for prognosis of the disease.

Keywords: Ovary; Benign; Malignant; Surface Epithelial Tumor.

Introduction

Ovaries, a paired organ of female reproductive system, are vital part of a woman's body. Shushruta has described ovarian tumours in his book "Shushruta and Ayurveda" [1]. Tumors of the ovary are amazingly diverse pathologic entities due to the three cell types that make up the normal ovary: the multipotential surface (coelomic) covering epithelium, the totipotential germ cells, and the multipotential sex cord/stromal cells.

Each of these cell types gives rise to different ovarian tumors [2]. Tumours of the ovary are common forms of neoplasia in women [3]. Such tumours can occur in all age groups, beginning from intrauterine age to postmenopausal [4].

Surface epithelial tumours are the most commonly found type of ovarian tumors. Ovary being a common area of primary malignancy, metastasis may occur [5]. Among cancers of the female genital tract ovarian tumours are the third most common site of malignancy [6].

Ovarian carcinomas are considered to be the sixth most frequent cause of death from all cancers in women; 70% of female patients with ovarian tumors present during advanced stage disease [7,8].

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The purpose of the study was to find the incidence of surface ovarian tumors at S. Nijalingappa Medical College, Bagalkot.

Materials and Methods

The materials for the present study were ovarian tumour specimens received at the Department of Pathology, Shri S Nijalingappa Medical College, Bagalkot, diagnosed over a span of one and half year from January 2016 to June 2017 prospectively. Specimens were sent from the obstetrics and gynecology department of S Nijalingappa Medical College, Bagalkot. All the specimens were received at the histopathology laboratory in Department of Pathology. Clinical details were taken from the medical records of patients from HSK Hospital. Bagalkot. The specimen were received in the form of oophorectomy/ salphingoophrectomy (unilateral/ bilateral) / pan hysterectomy. Representative bits were taken and routinely processed with paraffin embedding. Thin sections were taken and were stained by hematoxylin and eosin. Specimens sent in 10% formalin were fixed and subjected to thorough gross examination according to the protocols. Slides were then studied microscopically. All details of the cases were collected from medical records.

Results

A total of 82 cases of ovarian tumours were studied over a period of one and half years duration (1st January 2016 to 30th June 2017). Among 82 cases of ovarian tumours 45 (54.7%) cases were surface epiterial tumours, 23 (28.1%) cases of germ cell tumour, 14 (17.2%) cases of sex cord stromal tumours were noted Histomorphological features were studied and the tumours are classified according to WHO 2014 classification of ovarian tumours.

We included varied age group from 2nd decade to 7th decade patients. In the study we found ovarian tumour incidence was seen more in the age groups of 21-30 and 31-40 years. Among all the 45 cases of tumors, 38 were benign, 2 were borderline and 5 were malignant cases. Thus the benign surface epithelial tumors were the commonest with 84.44% followed by 11.11% malignant tumors, and 4.44% of borderline tumors. Out of the 45

Table 1: Age distribution of ovarian tumours

Age	Total number of tumors	Benign	Nature of Tumor Borderline	Malignant
11-20	3	3	0	0
21-30	13	12	1	0
31-40	13	11	1	1
41-50	11	7	0	4
51-60	4	4	0	0
61-70	1	1	0	0
Total	45	38	2	5

Table 2: Clinical presentation of ovarian tumor

Symptoms	Total	Benign	Type of tumor Borderline	Malignant
Mass p/a	23	18	0	5
Pain	31	26	2	3
Menst.Dist	12	11	1	0
Bowel Compl	2	1	0	1
Bladder Compl	1	1	0	0
Ascites	12	8	0	4
others	5	3	0	2

Table 3: Unilateral/ bilateral ovarian tumours

Side	No. of cases	Percentage (%)
Unilateral	43	95.55
Bilateral	2	4.44

Table 4: Type of ovarian tumours

Nature of Tumor	Frequency	Percentage (%)
Benign	38	84.44
Malignant	5	11.11
Border line	2	4.44
Total	45	100.0

Fig. 1: Serous cystadeno carcinoma Gross picture – Unilateral tumour with smooth external surface

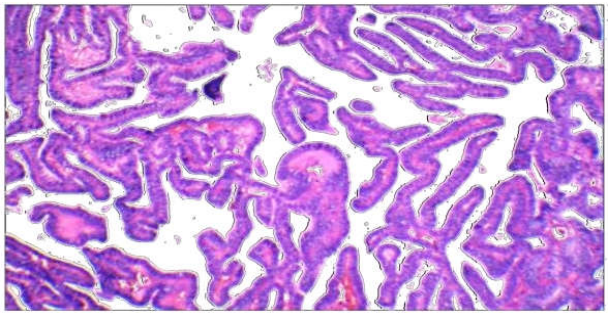

Fig. 2: Microscopy- Serous cystadeno carcinoma showing papillary projections lined by atypical cell. H&E 100x

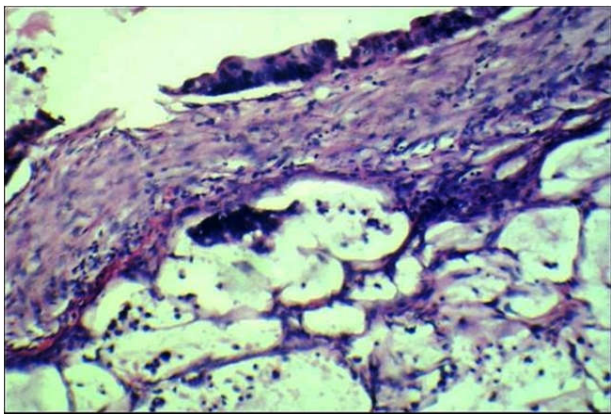

Fig. 3: Mucinous cystadeno carcinoma Microscopy showing tumour cells floating in large extracellular mucin lakes.H&E 100x

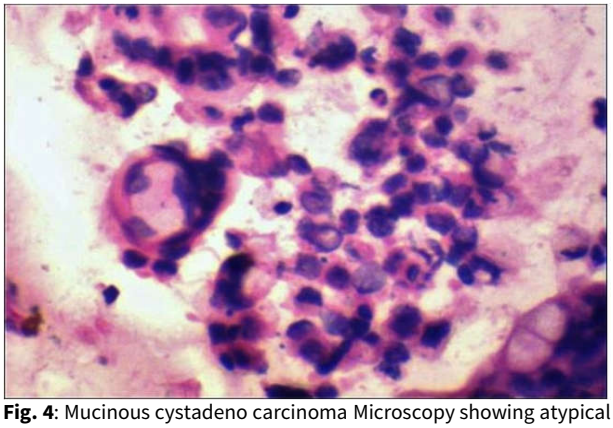

Fig. 4: Mucinous cystadeno carcinoma Microscopy showing atypical cells. H&E 400x

Fig. 5: Benign brenner tumour Gross picture of Benign Brenner tumour

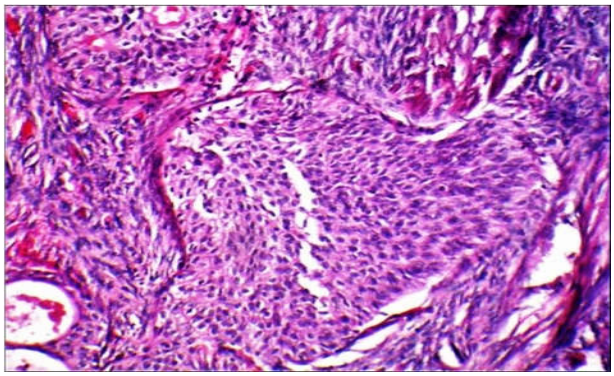

Fig. 6: Benign brenner tumour Microscopy showing nests of transitional cells and fibrous stroma. H&E 100x

patients 29 were of <40 years of age, while the remaining 16 were above the age of forty. Among the all patients, 95.55% had unilateral ovarian tumors and only 4.44% had bilateral ovarian tumors. Out of 45 surface epithelial tumors 22 cases (26.8%) were serous cystadenomas. Majority of them were unilateral , all were unilocular with serous fluid.

Discussion

On the basis of four major types of tissue, primary ovarian neoplasm can be broadly classified as surface epithelialstromal tumors, germ cell tumors and sex cord – stromal tumors [2]. Surface epithelial tumors are the most common neoplasm of the ovary. They originate from the ovarian surface epithelium or its derivatives and occur in women of reproductive age and beyond. In the diagnosis of ovarian tumor, clinical data and gross features and detail microscopic examination is required. One of the most important clinical features is the age of the patient. About 80% of ovarian tumors are benign and occur in age between 20-45 years, the remaining 20% are malignant and are more common in older women between 45-65 years [2]. Surface epithelial tumors comprise 58% of all ovarian neoplasms and about 90% of malignant tumors in the Western world. But their

frequency is less in the East [7]. Surface epithelial tumors form the main histological group not only in this analysis but also in other international studies [9].

More than one cell type is often seen. If the secondary cells type involve less than 10% of the tumor, itis classified according to the predominant cell type, while if it is more than 10% , it is classified as mixed epithelial tumor [4]. Among all the subtypes, only Brenner tumor shows a predominant stromal component. A total of 82 cases were studied in our present study. Out of which 67 (81.7%) were benign, 2 (2.4%) were borderline malignancy and 13 (15.9%). A study by Selvi et al also showed 72% benign, 2.5% borderline and 25.5% malignant which was in concordance with our study.

Histologically all patients who presented with ovaran tumors were studied. The tumors were classified according to WHO classification. Surface epithelial tumors are the commonest variety constituiting 54.7%.

Conclusion

In our study, surface epithelial tumors comprised 54.7% of all ovarian tumors. The most common symptopms were abdominal pain and mass per abdomen, followed by menstrual irregularities Most of the ovarian tumors were unilateral. Most of the benign tumors were cystic.

Histopathological study is still gold standard to diagnose most of the primary ovarian tumors. However it may be supplemented by the newer techniques like IHC, morphometric analysis, flow cytometry for appropriate diagnosis.

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Original Research Article

Fine Needle Aspiration Cytology of Liver Mass Lesions with Cytoradiological Correlation

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Abstract

Background: Introduction of Guided fine needle aspiration allowed accurate positioning of the needle when lesion were small or deep.

Aim: The present study was undertaken to know the pathological spectrum of hepatic mass lesions and to correlate the cytoradiological findings.

Methods and Materials: This study was performed on admitted and OPD patients of SNMC, Bagalkot over a period of six months. Image guided fine needle aspiration cytology was carried out and aspirates were examined, and compared with radiological diagnoses.

Results: The present study included 51 cases of hepatic lesion masses. Presenting age ranged from 23 to 84 years with most common predisposition in the seventh decade of life. Males were more commonly affected than females. Metastatic tumours were more common than hepatocellular carcinoma. The most common metastatic hepatic lesion was Adenocarcinoma followed by squamous cell carcinoma, urothelial carcinoma and lymphoma. Multiple SOL was more common radiologically for both primary malignancy and metastatic lesions. Cytoradiological correlation was found to be 95.91%.

Conclusion: The study concludes that fine needle aspiration cytology of the hepatic lesion is an effective and safe method for a quick, early and accurate diagnosis without any serious complications related to the procedure.

Keywords: Guided FNAC; Liver; HCC; Metastasis.

Introduction

The evaluation and management of discrete hepatic masses is common clinical problem. Appropriate clinical management of these masses depends on obtaining accurate diagnosis [1]. A diagnostic modality such as fine needle aspiration [FNA], which offers accuracy without significant complications and which requires minimal intervention at low cost, warrants consideration early in the investigative sequence [4]. Introduction of ultrasonography and computed tomography allowed precise localization of the lesion and accurate positioning of the needle when lesions were small or deep [3]. The present study was undertaken to know the pathological spectrum of hepatic mass lesions and to correlate the cytoradiological findings.

Methodology

Image guided FNAC was performed on a total of 51 cases, in which lesion/pathology in the liver was suspected and hepatic mass was confirmed on radiological

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examination. This study was performed on admitted and OPD patients of Shree Nijalingappa medical college and HSK hospital from Jan 2017 to June 2017. Ultrasound guidance was usually preferred for its simplicity, real-time monitoring and flexible needle placement. Computed Tomography (CT) guidance was reserved for lesions that were not demonstrated by ultrasound. Proper aseptic care was taken by cleaning the skin surface with povidone iodine before every FNAC. Aspiration was done using 22 gauge, disposable spinal needle attached to a 10mL disposable syringe which was introduced into the lesion under radiological guidance. The cytological material obtained were smeared on clean glass slides as quickly as possible, wet-fixed or air-dried and stained by Papanicolaou (Pap), May-Grunwald Giemsa (MGG), and haematoxylin and eosin stains. The smears were thoroughly screened. Various cytological features of the smears were recorded and cytological diagnoses was offered which was correlated with radiological diagnosis.

Results

Diagnostic yield of image guided FNA in our study was 96.07% and out of total 51 cases two aspirates were inadequate for cytological evaluation and were excluded. Age of the patient ranged from 23 to 84 years with maximum cases in the seventh decade of life. There was a male predominance with a male to female (MF) ratio of 2.2:1. The most common clinical presentations were abdominal pain, appetite loss, abdominal mass, breathlessness and weight loss.

Radiological findings revealing single space occupying lesion (SOL) were 19 and multifocal lesions were seen in 32 cases. Fourteen out of 19 single SOL were malignant hepatic masses and two were benign. All the multifocal lesions were malignant lesions radiologically (Table 1). Cytological examination revealed that non malignant lesions comprised minority of the three cases (5.9%) and included one case each of haemangioma, fatty change and cirrhotic nodule. Smears of haemangioma showed few scattered spindle cells with bland nuclei in the background of hemorrhage. Smears of fatty change showed clusters of benign looking hepatocytes with vacuolated cytoplasm and eccentrically placed nuclei and smears of cirrhosis showed individually scattered and loose cohesive clusters of hepatocytes, mild anisonucleosis, cohesive clusters of bile duct epithelial cells and strands of fibrous tissue.

Malignancy was the most common liver lesion with 46 cases (90.2%). Metastatic tumours with 23 cases (50%) were the most common malignant lesion followed by 19 cases (41.3%) of hepatocellular carcinoma (HCC) and one case (2.17%) of cholangiocarcinoma. Three cases (6.52%) were rendered cytological diagnosis suspicious of HCC. Among the metastatic tumours, the most common was adenocarcinoma with 20 cases (86.95%) , One case (4.35%) each of squamous cell carcinoma, urothelial carcinoma and lymphoma (Table 2). The diagnosis of metastatic lesion was made in correlation with the clinical and radiological evidences of primary lesions.

The smears from metastatic adenocarcinoma showed

Table 1: Radiological findings in cytologically diagnosed malignancy

Radiology	Primary	Metastsis	Total
Single SOL	8	6	14
Multiple SOL	15	17	32
Total	**23**	**23**	**46**

Table 2: Spectrum of lesions in the present study

		Cytological Dignosis	Number	Percentage
Non Neoplastic		Fatty change	1	2
		Cirrhosis	1	2
Benign		Haemangioma	1	2
Malignant	HCC	HCC grade I	4	8.2
		HCC grade II	14	28.6
		HCC grade III	1	2
		Suspicious of HCC	3	6.1
		Cholangiocarcinoma	1	2
	Metastasis	Adenocarcinoma	20	41.1
		Squamous cell carcinoma	1	2
		Urothelial Carcinoma	1	2
		Lymphoma	1	2
Total			49	100

Table 3: Cytoradiological correlation of liver lesions

		Cytological Diagnosis		
		Malignant	**Benign**	**Total**
Radiological Diagnosis	Malignant	46	1	47
	Benign	0	2	2
	Total	46	3	49

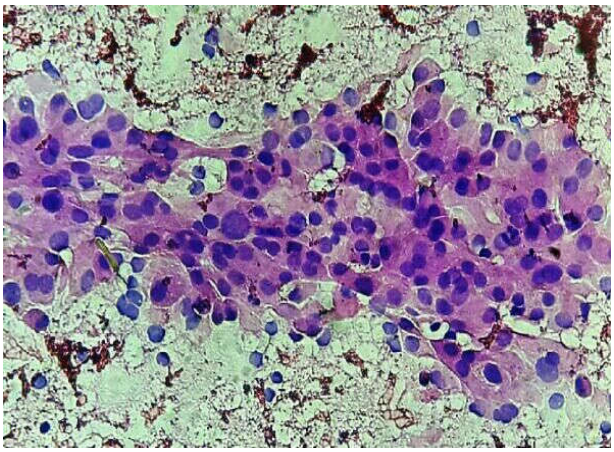

Fig. 1: Hepatocellular carcinoma: thickened trabeculae and cells showing anisonucleosis. (H&E, 400x)

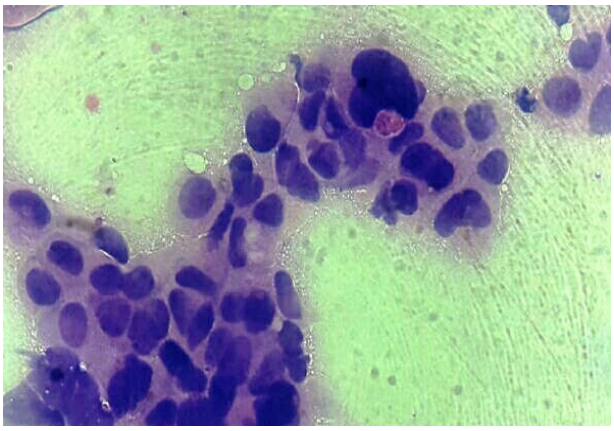

Fig. 2: Hepatocellular carcinoma grade III: pleomorphic cells showing anisonucleosis. (H&E, 400x)

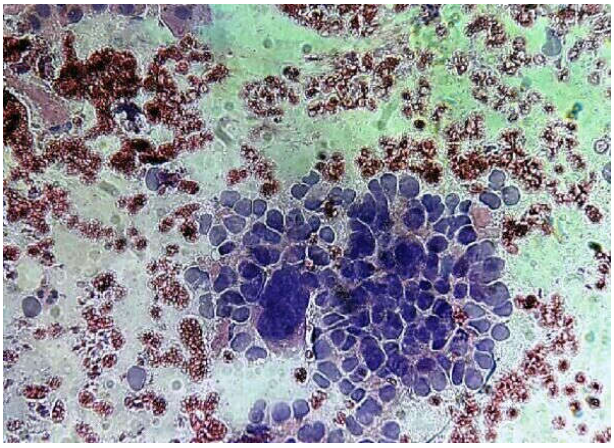

Fig. 3: Cholangiocarcinoma: Pleomorphic cells arranged in clusters showing nuclear crowding. (H&E, 400x)

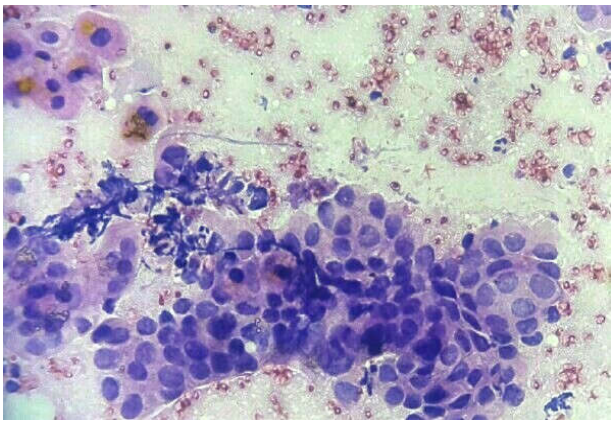

Fig. 4: Metastatic adenocarcinoma: Tumour cells arranged in clusters and microacinar pattern. Few normal looking hepatocytes in the left top corner. (H&E, 400x)

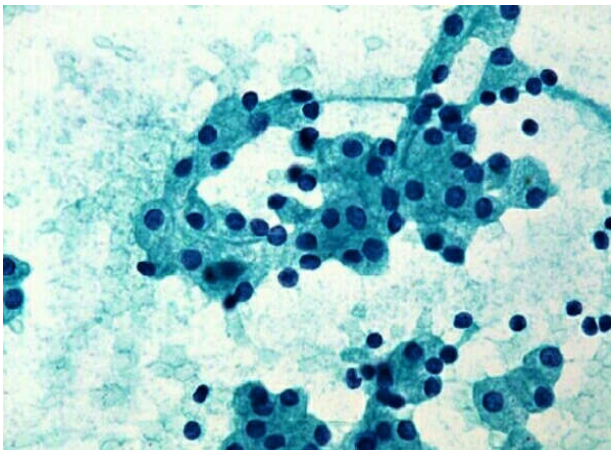

Fig. 5: Metastatic Urothelial carcinoma: Cells with eccentrically placed nucleus, abundant cytoplasm and cytoplasmic tailing. (PAP, 400x)

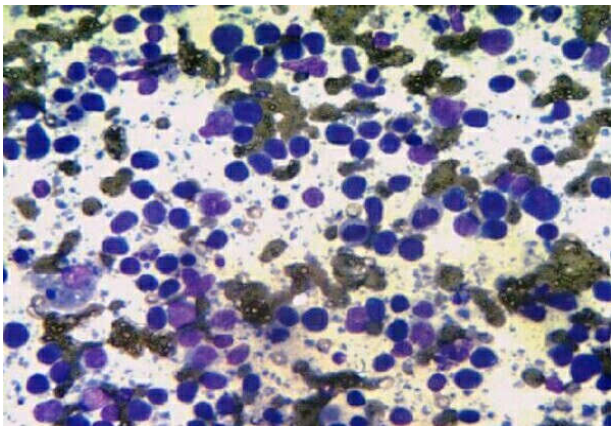

Fig. 6: Metastatic Lymphoma: Dispersed large lymphoid cells with scant cytoplasm.(MGG, 400x)

clusters and sheets and microacinar pattern of round to cuboidal cells with enlarged hyperchromatic, prominent nucleoli and moderate amount of eosinophilic or vacuolated cytoplasm. The cells showed increased nuclear cytoplasmic (NC) ratio and anisonucleosis . Twenty cases diagnosed as metastatic adenocarcinoma showed primaries most commonly from gall bladder with eight cases followed by six cases from the colon, two cases from the lungs, two from the pancreas, one case each from rectum and stomach. The smears from the metastatic squamous cell carcinoma showed individually scattered and clustered round to polygonal cells with hyperchromatic pleomorphic nuclei and abundant cytoplasm. Few tadpole like cells and individual cell dyskeratosis are seen. One case of squamous cell carcinoma had primary in gall bladder. Smears studied from the metastatic urothelial carcinoma showed individually scattered, clusters and sheets of round to polygonal cells with eccentrically placed hyperchromatic nuclei and eosinophilic cytoplasm. Few cells were showing cytoplasmic tails with bulbous ends. Few multinucleated cells were also seen. One case of urothelial carcinoma had primary in bladder. Smears from the metastatic lymphoma showed monotonous population of large lymphoid cells and bare nuclei with few hepatocytes and scattered lymphocytes in the background of necrotic debris. One case of metastatic lymphoma had its primary from the pancreas.

The commonest primary hepatic lesion was HCC. HCC was further differentiated into three grades depending upon the differentiation. Four cases (21.05%) showed grade I (well differentiated), 14 cases (73.68%) showed grade II (moderately differentiated) and 1 case (5.27%) showed grade III (poorly differentiated). The smears from HCC grade I showed hepatocytes individually scattered, in clusters and in thickened trabeculae with endothelial rimming. Hepatocytes show increased NC ratio with centrally placed round to oval nucleus. Bare nuclei and transgressing of the vessels are seen.

The smears from the HCC grade II were cellular and showed hepatocytes arranged in singles, clusters and thickened broad trabeculae. Cells were showing high NC ratio and many bare nuclei were seen. The smears from the HCC grade III showed cells arranged in singles, clusters and sheets. Cells showed anisocytosis, anisonucleosis, hyperchromasia, prominent nucleoli. Large bizarre bare nuclei was also seen.

Smears studied from the cholangiocarcinoma showed individually scattered, clusters and microacinar pattern of cuboidal to columnar cells with enlarged, hyperchromatic nucleus, prominent nucleoli and moderate amount of cytoplasm. Cells also showed anisonucleosis. Cytoradiological correlation in our study was 95.91% with two discordant cases.

Discussion

The use of fine needles to obtain tissue for cytological diagnosis was first used by Lucatello in 1895. In 1935 Flora in France started using a needle which measured 0.5mm in diameter to reduce the complications. In 1972 Rasmussen et al described a method of FNA under the direct guidance of ultrasonic scanning. In 1976 Haaga et al described a method for precise localization of the lesion by CT. This allowed accurate positioning of the needle when the lesion was very small and deep. Over the last 15 to 20 years of the twentieth century, it became increasingly clear that percutaneous FNA of single or multiple focal lesions of liver demonstrated by palpation, nuclear scan, ultrasonography, CT scan and at laparoscopy is both accurate and safe.

Focal hepatic lesions range from cysts and inflammatory processes to neoplasms, be they benign or malignant, primary or metastatic. FNA under image guidance offers accuracy at a low cost without major complications. The contraindications of FNAC are hemorrhagic diathesis, prolonged prothrombin time, vascular structure in the path and suspected extrahepatic obstructive jaundice. The main advantage of FNAC is the possibility of multiple passes, sampling a wide area or multiple sites, which increases the chances of obtaining adequate viable cells. Adequacy of the sample can be checked immediately and the procedure causes the patient very little discomfort.

The diagnostic yield in our study was 96.07% which was as high as the study done by Khanna et al [4] which recorded 98.4%. This is because the study employed the technique of immediate cytological assessment by means of rapid staining procedures and joint presence of both radiologists and pathologists which is recommended. Various causes of inadequacy are hemorrhagic, necrotic smears, or smears from non- representative area. In present study causes of inadequacy were hemorrhagic and non- representative smears. An aspirate that obtains material only from surrounding tissue of the tumour may show reactive and proliferative changes, whereas an aspirate from the center of a large tumour may sample only degenerative and necrotic material. Hence, for the aspirate to be representative the needle should pass through entire mass which can be achieved in ultrasound guided FNAC.

Present study showed age range from 23 to 84years and a male predominance with MF ratio of 2.2:1 which was comparable to the study done by Meena SP et al [5], which recorded age range of 23 to 90 and MF ratio of 1.2:1.

Malignancy was the most common hepatic lesion with 90.2% was comparable to the other studies done by agarwal et al [2] (91.09%) and Khanna et al [4] (88.7%). Metastatic deposits were the most common malignancy

with 50% than HCC with 47.82%. The study done by Agarwal et al [2] showed an incidence of metastatic deposits as high as 93.2% of all liver malignancies. The liver is one of the most frequent site of metastases. Malignant tumour originated in any site in the body may metastasize to liver by lymphatic, venous or arterial routes. Present study showed adenocarcinoma as the most common metastatic hepatic lesion with 82.6% which was comparatively high when compared to other studies done by Balani et al [1] with 52% and Agarwal et al [2] with 56.16%. In the present study the most common site of primary in case of metastatic deposits in the liver were gall bladder followed by the colon. This observation was identical to the observations of Balani et al [1].

Among the HCC cases Grade II was the most common with 73.68% which was comparable to 56.3% recorded by Khanna et al [4]. Important cytological criteria useful in distinguishing HCC from non neoplastic liver are increased nucleocytoplasmic ratio, arrangement of tumour cells in trabecular pattern and atypical naked hepatocytic nuclei. Other secondary criteria useful in differentiation are nuclear features i.e., irregularly granular chromatin, uniformly prominent and multiple nucleoli [6]. The salient features separating HCC from metastatic adenocarcinoma given by Greene et al [7] were tumour cells in HCC are polygonal or polyhedral, whereas cells are usually columnar or cuboidal in adenocarcinoma; cells in HCC have abundant eosinophilic and granular cytoplasm with one or two macronucleoli, whereas adenocarcinoma cells may show mucin secretion, and presence of more than two nucleoli is common. Three cases was cytologically diagnosed as suspicious of HCC. All the three cases were scanty cellular and showed individually scattered round to polyhedral cells with increased NC ratio, enlarged hyperchromatic nuclei with prominent nucleoli and abundant eosinophilic cytoplasm. Few atypical bare nuclei were also seen in a hemorrhagic background.

Present study also showed one case (1.96%) of cholangiocarcinoma. The study done by Balani et al [1] recorded four cases (7.7%) of cholangiocarcinoma. It is very difficult to differentiate cholangiocarcinoma from metastatic adenocarcinoma. Although subtle differences in the cytological presentation of certain metastatic cancers (such as of the colon) may be obvious to very experienced observers, the diagnosis of cholangiocarcinoma is a diagnosis of exclusion [8]. The presence of proliferating ductules, in particular more than ten ductular clusters has been suggested as a cytological feature to distinguish cholangiocarcinoma from metastatic adenocarcinoma. The diagnosis of cholangiocarcinoma is made only after ruling out other primary sites clinically [9].

Present study showed 65.21% of all primary hepatic lesions were radiologically multiple SOL and 73.91% of all metastatic deposits in the liver were radiologically multiple SOL. One case which was radiologically diagnosed as HCC was given as cirrhosis in cytology (Table 3) and one case which was diagnosed as HCC radiologically was diagnosed cytologically as Mets SCC. Radiologically it showed a single SOL in liver. Two cases which was diagnosed as HCC produced only hemorrhagic materials and rendered as inadequate for opinion on cytology. Both the cases showed very small single SOL radiologically. Cytoradiological correlation in the study done by Khanna et al [4] was 92% which was comparable to the present study 95.91%.

Conclusion

The study concludes that FNAC of the hepatic lesion is an effective and safe method for a quick, early and accurate diagnosis without any serious complications related to the procedure. Close collaboration of the pathologist and the radiologist and correlation with the clinical history, yield the best results.

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Original Research Article

Assessment of Diagnostic Accuracy of Endometrial Aspiration Technique and Comparison of the CYTO-Morphology of Aspirated Smears with Histopathology

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Abstract

Background and Aim: Endometrial aspiration cytology is a diagnostic modality for studying cyto-harmonal changes and for evaluating endometrial pathology ranging from inflammatory changes, hyperplasia and endometrial malignancies. Present study was performed to assess the diagnostic accuracy of endometrial aspiration technique and to compare the cyto-morphology of aspirated smears with histopathology.

Materials and Method: Present study was carried out at a department of pathology GMERS Medical College, Gotri, Vadodara, Gujarat. Endometrial aspiration was performed using Karman's cannula in 100 cases who presented with abnormal uterine bleed, infertility and postmenopausal bleed. The endometrial specimens obtained by D & C were processed and evaluated histologically. The endometrial aspirated smears were correlated with histopathology findings for diagnostic accuracy.

Results: The study cases included 48 patients in reproductive age group and 52 patients in the perimenopausal and postmenopausal age groups. The diagnostic accuracy on cytology was 90% when compared to histology. The smears were inadequate for reporting in eight cases. The sensitivity of endometrial cytology was 93% and specificity was 85.7%.

Conclusion: Endometrial aspiration technique with Karman's cannula is convenient, simple, safe and less invasive procedure. The endometrial aspiration cytology proved to be effective diagnostic modality in the interpretation of normal and abnormal endometrium. The present study emphasizes the need for early screening to diagnose pre-invasive lesions and endometrial malignancies in peri-menopausal and post-menopausal patients.

Keywords: Cytology; Endometrial Aspiration; Histopathology; Malignancies.

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Introduction

Endometrial aspiration technique is less invasive efficient diagnostic procedure for screening the endometrial status and for diagnosing gynecological disorders when compared to endometrial biopsy or curettage. In 1943 Cary developed a metal cannula for the aspiration technique and the method was described by Papaniculoau and Marchetli. The aspiration techniques are less popular due to unsatisfactory smears and difficulty in interpretation by pathologist [1,2,3].

Endometrial aspiration does not require cervical dilatation or anesthesia. It is a safe, simple and reliable technique without any complication and can be used as a out-patient procedure with minimum discomfort to the patient [3].

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Materials and Method

Present study was carried out at a department of pathology GMERS Medical College, Gotri, Vadodara, Gujarat. The study included 100 cases in the reproductive, menopausal and postmenopausal age groups, who presented with complaints of abnormal uterine bleeding, infertility and post-menopausal bleeding. Thorough clinical history, general brief systemic and local examination was done for each case. Consent of the patient was obtained before the diagnostic procedures.

Endometrial aspiration was done using a plastic disposable Karman's cannula measuring 4mm. This was inserted into the endometrial cavity and connected to 20cc disposable syringe. Negative pressure was created and maintained, while the entire mucosa was uniformly aspirated. The material is squirted on a clean glass slide and smeared and immediately fixed in a fixative and then stained by Papaniculoau stain.

After endometrial aspiration, diagnostic dilatation and curettage was done for histopathological study. The smears of endometrial aspiration cytology were compared with histopathology. Patients diagnosed with acute inflammatory disorders of the Genital –tract, Pregnancy and with gross evidence of cervical malignancy were excluded.

The criteria for assessment of endometrium on cytology was based on the studies done by Bistolleti and Hjerpe et.al and Koss, diagnostic cytology [4,5,6,7].

Except in women with complete atrophy of endometrium, the smears should contain at least five or six clusters of endometrial epithelial cells to be judged as adequate.

The epithelial cells are arranged in flat monolayer sheets. The size of the cells varies from 45μ to 75μ. The nuclei of the epithelial cells are closely packed with smooth contours and dispersed chromatin. Mitoses of normal configuration may be seen.

The epithelial cells are arranged in clusters or sheets. In early secretory phase, the epithelial cells are larger due to increased volume of cytoplasm, often vacuolated. In late secretory phase the endometrial cells occur in thick clusters sometimes in tubular or glandular configuration. Some nuclei are small, shrunken and give a burnt out appearance. The background is hemorrhagic with neutrophils,

Simple Hyperplasia

There is increase in cellularity, with cells in sheets and tight clusters. The cells are round with scanty cytoplasm mild to moderate anisonucleosis, fine granulation and even chromatin.

Complex Hyperplasia

Smears are cellular with many clusters. Piling up of cells with crowding and overlapping of nuclei with mild pleomorphism, fine granular chromatins with prominent nucleoli in a few cells are seen.

Atypical Hyperplasia

Smears are hypercelluar with glandular cells in clusters and papillary fronds. Individual cells exhibit increased nuclear cytoplasmic ratio, granular chromatin and nuclear crowding, disturbed nuclear polarity and presence of nucleoli.

Endometritis: [8,13]

Non-specific Endometritis: Smears showed few clusters of endometrial cells infiltrated with numerous inflammatory cells. 2 cases were reported as endometritis on cytology.

Tubercular endometritis: The smear shows sheets and clusters of endometrial cells. The cells show mild atypical changes with epitheloid cells and chronic inflammatory cells. Langerhan's giant cells and granular eosinophillic material, suggestive of caseation may be seen.

Endometrial Adenocarcinoma: Dyscohesive and three-dimensional clusters are seen with anisonucleosis. Loss of nuclear polarity, hyperchromasia, macronuclei, increased nuclear chromatin granularity and abnormal mitotic figures. The cancer cells may be dispersed or occur singly or may form clusters, that are either flat or multilayered, the latter of papillary configuration.

Results

Endometrial aspiration was done in a total of 100 patients presenting with various gynecological complaints and infertility. The relevant clinical history and findings were noted. Following endometrial aspiration D & C was done in 75 patients, whereas in 25 patients hysterectomy was done (2 Cases of Carcinoma, 1 atypical hyperplasia, 1 complex and 5 simple hyperplasia). Findings on Cytology and histopathology were studied and documented.

Among 100 patients studied 48 cases belonged to reproductive age group, 25 cases in postmenopausal and the rest belonged to peri-menopausal age group. Patients presented with symptoms of menorrhagia, Polymenorrhea. These patients belonged to reproductive and premenopausal age group, the age of who ranged from 25-55yrs. Out of 2 malignancies, one presented with post menopausal bleeding and the other with menorrhagia. Of the 100 cases studied a clinical diagnosis of DUB was made in 65 cases and infertility in 6 cases.

Cytological Analysis

Endometrial aspirate was satisfactory only in 95 patients due to endometrial atrophy and technical failure. Although inadequate sample was obtained on aspiration, endometrial yield was sufficient on curettage (Table 1).

Cytology of 48 cases in reproductive age group showed 33 cases in proliferative phase, 9 cases in secretory phase, 3 cases with simple hyperplasia and 3 cases were inadequate for diagnosis.

In perimenopausal and postmenopausal age group, 13 out of 52 cases were in proliferative phase, 27 cases in secretory phase, 2 cases had endometrial hyperplasia, of which 1 case was diagnosed as atypical hyperplasia, 2 cases as endometrial adenocarcinoma and 5 cases were inadequate for interpretation. 3 cases were reported as non-specific endometritis of which 1 case was confirmed as tubercular endometritis.

The endometrial aspiration cytology results were correlated with histopathological findings. Out of 50 cases reported as proliferative phase on cytology in the reproductive age group, 44 cases correlated well with histopathology, giving accuracy of 88%, other 6 cases were diagnosed on histopathology as simple hyperplasia, 2 cases showing both proliferative and secretory endometrium due to hormonal therapy and early secretory phase. 25 aspirated smears were reported as secretory phase, of which 24 were confirmed on histopathology, giving 92% accuracy and the misinterpreted case showed proliferative phase on histopathology. 7 cases were reported as endometrial hyperplasia, of which 5 cases were confirmed by histology, 3 cases were reported as simple hyperplasia, and 1 case was reported as complex hyperplasia, the other as atypical hyperplasia. The 2 cases, which did not correlate with histology, were reported as proliferative phase. The accuracy for simple hyperplasia was 50% and atypical hyperplasia was 100%. All 3 cases of endometritis, including 1 case of tubercular endometritis correlated well with the histopathological findings (Table 2).

The diagnostic accuracy in different lesions ranged from 60-100%.

Discussion

Diagnostic dilatation and curettage is a commonly performed Gynecological surgery. It requires hospitalization, anesthesia and cost is significant. Some complications are faced by the patients. For these reasons, various endometrial sampling techniques with endometrial brush, membrane filtration method, endometrial Gravlee- jet washers, Isaacs's endometrial cell sampler, Mi-Mark Helix, lippies loop method, Endocyte, Endo-pap sampler, disposable plastic cannula, Vabra aspirator have been used [7].

Table 1: Cytology Findings among study participants

Cytology	No. of Cases	Percentage
Proliferative phase	50	50%
Secretory phase	25	25%
Simple hyperplasia	5	5%
Complex hyperplasia	1	1%
Atypical hyperplasia	1	1%
Endometritis:		
Non specific	2	2%
Specific	1	1%
Atrophic	5	5%
Malignancies	2	2%
Inadequate	8	8%

Table 2: Correlation of cytological and histological findings

Cytological Findings	No. of Cases	Histological Findings	
		Consistence	Inconsistent
Proliferative phase	50	44	6
Secretory phase	25	24	1
Simple hyperplasia	5	3	2
Complex hyperplasia	1	1	-
Atypical hyperplasia	1	1	-
Endometritis	3	3	-
Endometrial Carcinoma	2	2	-
Metastatic Carcinoma	-	-	-
Atrophic Endometrium	5	3	2
Inadequate samples	8	-	-

Uterine aspiration curettage under negative pressure has been reported to give excellent endometrial specimens both for cytology and histological examination. The curettage has failure rate of 12-29%. With experience, a cytopathologist can make an accurate diagnosis on aspiration cytology.

In the present study, 20cc syringe (disposable) with 4mm Karman's cannula was used for endometrial aspiration. This is easy technique, an outpatient procedure with only mild to moderate discomfort in most of the patients. The procedure was rapid, taking around two minutes, and technically was just like inserting an IUCD. There was no hemorrhage, infection or perforation following the procedure and also risk of anesthesia was not there. Aspiration cytology was done in 100 patients, the age ranged from 20-60yrs. This was followed by Dilation and curettage in 75 cases. In remaining 25 cases hysterectomy was done. Cytology smears and histopathology sections were compared and correlated [8].

A variety of direct endometrial sampling techniques, which are modifications of Cary's original method, have been tried with varying degrees of success over last few decades. In studies of Liza et al., the insemination cannula used for intrauterine aspiration, had sensitivity of 81.6% and specificity of 83.3%. Recently, Malik et al. used insemination cannula of 0.5mm diameter for aspiration sampling, in 2008 with successful sampling of 96% [9].

The adequacy of the samples for cytology was 92% in the study. Inadequate samples for interpretation were 8% of cases, which was comparable to Malik et al .The causes being atrophic endometrium and technical failure.

The diagnostic accuracy of endometrial aspirates was 100% for malignancies, endometritis and atypical hyperplasias. 92% for secretory phase, 88% for proliferative phase and 72.2% for endometrial hyperplasia, similar to that of Schachter et al of 77%. The cytological appearances of simple hyperplasia represent an exaggeration of the appearance of proliferative phase and features may be due to response of the endometrium to exogenous estrogen administration. On cytology the degree of hyperplasia is difficult to interpret. Slight nuclear enlargement and the presence of small nucleoli may indicate hyperplasia. The cytological pattern of atypical hyperplasia and endometrial adenocarcinoma is difficult to differentiate. The background of these smears show aggregates of nuclear debris, leucocytes and degenerated tumor cells [10,11,12].

Dilation & Curettage followed endometrial aspiration in 75 cases. In remaining 25 cases, hysterectomy was done. These hysterectomy patients included prolapse, leiomyoma, and malignancies. Majority of the age group belonged to 25– 45yrs. The commonest presenting complaint in this study was menorrhagia in 45% of cases.

In the present study, proliferative phase had 88% correlation with histology which is similar to studies of Ambiye et al and Malik et al. The accuracy of secretory phase observed in the present study, was comparable to Malik, et al. In the present study, accuracy of 73% was observed in diagnosis of hyperplasia, including simple hyperplasia, complex and atypical hyperplasia., similar to that of Schachter et al of 77%. There is always a tendency to over diagnose simple hyperplasia on cytology. The cytological appearances of simple hyperplasia represent an exaggeration of the appearance of proliferative phase. The features may be due to response of the endometrium to exogenous estrogen administration.

On cytology the diagnosis of hyperplasia could be made but the degree of hyperplasia is difficult to interpret. Slight nuclear enlargement and the presence of small nucleoli may indicate hyperplasia. The cytological pattern of atypical hyperplasia and endometrial adenocarcinoma is difficult to differentiate. Hence the diagnostic criteria of well- differentiated lesion are very subtle [13,14].

In the present study 2 cases were showing, both proliferative and secretory phase on aspiration smears. Both the cases had received hormone therapy earlier, and they correlated well with histology. 3 cases were reported as endometritis on aspiration cytology and all of them including a case of tubercular endometritis were confirmed by histopathology giving an accuracy of 100%. The present study showed 100% accuracy to Padubidri et al for the diagnosis of tubercular endometritis [13].

In the present study, 2 cases of malignancy were diagnosed as endometrial adenocarcinoma on cytology and were confirmed by histology. The accuracy was 100%, giving a correlation of 100% similar to the study of Malik et al [2,3].

The sensitivity and specificity obtained by all methods were 68.2-97% and 79-100% and the sensitivity and specificity in the present study using Karman's cannula (4mm) is 93% and 85.7% respectively.

Conclusion

Endometrial aspiration is a well-tolerated safe simple outpatient procedure. It is a cheap and efficient diagnostic technique for reporting endometrium of patients of all ages.

It is an efficient technique for screening postmenopausal patients in diagnosing uterine as well as extra-uterine metastatic malignancies. The Karman's cannula used in this study is inexpensive and alternate endometrial sampling method. This study suggests that endometrial aspiration cytology gives good correlation with histopathology, having high sensitivity and specificity.

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Original Research Article

Correlation between Mean Platelet Volume and the Cause of Thrombocytopenia: A Hospital based Study

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Abstract

Background and Aim: Thrombocytopenia (TCP) is a common clinical problem and its etiology varies widely. Automated hematology analyzers have contributed to more accurate, precise, and faster results. Platelet indices including mean platelet volume (MPV) offer valuable information about the morphology and maturity of platelets. But MPV has not been used commonly for diagnosing the cause of TCP. Thus we undertook this study to ascertain the correlation between MPV and the cause of TCP.

Methods: 510 cases of thrombocytopenia and 500 cases of Control group with normal platelet count were included in the study. TCP was defined as platelet counts below 1.5 lacs/cumm. Hematological analysis was done on Mindray BC-3000 plus automated hematology analyzer with blood collected in K2 EDTA blub. All cases were reevaluated by peripheral examination. Only those cases were included in the study which showed platelet count and platelet volume parameters with graph both in cases and control group.

Result: Group A with hyperdestruction showed increased value of MPV (10.46 fl) when compared with hypoproduction Group B (8.7 fl) and abnormal pooling Group C (8.15 fl) and with the control group (9.5 fl). All the three groups showed statistically significant difference in comparison to control. There was no significant difference between the mean platelet count between the control group and the three groups.

Conclusion: MPV is a sensitive and reliable indicator for diagnosis of thrombocytopenia due to various causes. More attention should be paid to MPV along with other platelet indices to differentiate between hyper destructive TCP from hypo productive and abnormal pooling TCP.

Keywords: Control Group; Mean Platelet Volume; Platelet; Thrombocytopenia.

Introduction

Thrombocytopenia (TCP) is a fairly common condition with diverse disorders with varying etiology. It is the most common cause of abnormal bleeding. Thrombocytopenia may be defined as a subnormal number of platelets in circulating blood [1]. It is important to know whether TCP is due to accelerated platelet destruction, decreased production or spleni pooling. The diagnosis of TCP requires bone marrow examination, platelet-associated immunoglobulin G (Paige) and reticulated platelets. These methods are either invasive &/or costly and not available routinely. Automated hematology analyzers are now routinely available in laboratories and institutes. These analyzers now provide platelet indices like mean platelet volume (MPV), platelet size deviation width (PDW), and

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platelet large cell ratio (P-LCR) routinely without any extra cost. But these parameters were overlooked and their clinical usefulness was not recognized in determining cause of thrombocytopenia. MPV and other indices are increased in TCP [1,2]. Many studies on these parameters have shown that they can be used to determine the cause of TCP and they have sufficient sensitivity and specificity in the diagnosis of TCP [3,4,5,6]. MPV is the average platelet volume & it provides evidence to differentiate category of TCP & may be interpreted in the same way as MCV of red blood corpuscles (RBC). We have thus undertaken this study to find the utility of MPV in diagnosing various categories of TCP.

Material and Methods

The present study was carried out for one year in the department of pathology. During this period 510 cases of thrombocytopenia were studied. Control group included 500 cases with normal platelet count, RBC count and WBC count. Detailed history was obtained regarding age, sex, clinical diagnosis and other hematological parameters form the case records of the patients. Hematological analysis was done on Mindray BC-3000 plus automated hematology analyzer with blood collected in K2 EDTA blub within 30 minutes to 5 hours of collection. Only those cases were included in the study which showed platelet count and platelet volume parameters with graph both in cases and control group. Each case of TCP was studied by peripheral examination and cases with different results by these methods were excluded from the study. Proportion, mean and standard deviation was calculated. TCP was defined as platelet counts below 1.5 lakhs/ul. Clearance was obtained from institutional ethical committee. Data was analyzed and ANOVA test was applied as a test of significance. P value <0.001 was considered as highly significant.

Results

Five hundred ten cases of TCP were studied for the present study. 318 (62.35%) were males and 192 (37.65%) were females with a male to female ratio of 1.6. A slight male preponderance was seen for the whole study and also in all age groups. Age ranged from 4 months to 87 years. Most number of cases for TCP was seen between 11-20 years accounting for 92 (18.03%) cases. 75 cases (14.70%) were seen in the age group of 31-40 years. 13 % cases belonged to age group of 21-30, 41-50, 51-60 & 61-70 years (Table 1).

All cases were grouped into three groups based on the predominant mechanism of thrombocytopenia. Group A – Hyperdestructive TCP, Group B – Hypoproductive TCP and Group C – Abnormal pooling. Group A constituted majority of 352 (69%) cases. This group was further subdivided into varies categories based on the clinical diagnosis. In Group A, bacterial infections constituted the most number of cases 100 (28.40%). Cardiac cases, Dengue, malaria and renal diseases constituted 25%, 13.06%, 6.8% & 5.3% respectively. While sepsis, viral infections & pregnancy constituted 1.9% each. All the categories in Group A had variable mean MPV with highest mean MPV in ITP (11.9±0.4) followed by dengue (10.90±0.4) and renal diseases (10.75±0.55). MPV was lowest in pregnancy (9.7±0).

Table 1: Distribution of patients according to Age & Gender

Age	No of Cases	Male	Female
< 1 year	1	1	0
1-10 year	48	35	13
11-20 year	92	63	29
21-30 year	70	48	22
31-40 year	75	39	36
41-50 year	70	51	19
51-60 year	68	37	31
61-70 year	69	31	38
71-80 year	11	9	2
81-90 year	6	4	2
91-100 year	0	0	0

Table 2: Statistical significance among variables

Group A Vs Control	Z = 8.57 (p<=0.01*)
Group B Vs Control	Z = 17.005(p=0.05*)
Group C Vs Control	Z = 46.63 (p=0.03*)
Group B Vs Group A	Z = 15.98(p=0.001*)
Group C Vs Group A	Z = 22.80 (p<=0.05*)
Group B Vs group C	Z = 12.86 (P=0.02*)

* indicates statistically significance pdŠ0.05

Group B constituted 30% of cases which included anemia 23 (15.03%), aplastic anemia 3 (1.96%), leukemia 42 (27.45%) and solid malignancy on chemotherapy 85 cases (55.55%). In this group also MPV was variable with highest in Leukemia (12±0.8) followed by Aplastic anemia (10.45±0.95) and solid malignancies on chemotherapy (9.5±1.1). Group C included 5 cases (1.42%) with splenomegaly suggesting abnormal pooling. The mean MPV was 8.15±0.35.

Control group included 500 cases of normal platelet counts with 280 (56%) males & 220 (44%) females. The mean platelet count in control group was 1.72±0.12 lacs/cumm, in Group A it was 0.755±0.05 lacs/cumm, in Group B- 0.468±0.09 lacs/cumm while in Group C it was 0.55±0.1 lacs/cumm.. Mean MPV for the 3 groups A, B & C. In the control group the MPV was 9.5±0.55 while in hyperdestruction group (group A) it was 10.46±0.15, in hypoproduction group (group B) 8.7±0.8 and in Abnormal pooling group (Group C) it was 8.15±0.35. Thus it can be seen that Group A shows higher value of MPV than group B and group C.

To know the statistical significance, Z Test was applied on all groups and also the control group. In all groups the values were highly significant (P<0.0001) when compared with control group and also among the three groups. (Table 2). There was no significant variation in the mean platelet counts among the three groups.

Discussion

Thrombocytopenia (TCP) can be due to hyperdestruction of platelets, hypoproduction of platelets or abnormal spleenic pooling. Platelet destruction may result from both intracorpuscular defects and extracorpuscular abnormalities. One of the major causes of increased destruction of platelets is by immunological mechanism in which antibodies against platelets cause their premature destruction. Hypoproduction of platelets may be due to bone marrow suppression like aplastic anemia, leukemias, infiltration by malignancy, chemotherapy and irradiation. Abnormal pooling or abnormal in vivo distribution of an essentially normal total platelet mass may also produce thrombocytopenia. This type of TCP is seen in various disorders associated with splenomegaly [1,2].

Bone marrow (BM) examination is the gold standard test in discriminating between the types of thrombocytopenia. However, this procedure is invasive and not necessary as first-line diagnostic procedure. Other tests like reticulated platelets and PAIgG can be used but PAIgG and bone marrow aspiration was not recommended as a diagnostic measure in recent guidelines [3,5,7]. Thus a simple, inexpensive and non-invasive method needs to be evaluated for the differential diagnosis of thrombocytopenia.

MPV has evoked much interest of all the platelet indices and many studies have suggested that MPV and other platelet indices are potentially useful markers for the early diagnosis and categorization of TCP and can play a role in the rapid evaluation of bone marrow activity of patients with platelet-associated disorders. These studies have yielded variable results [6,7,8,9,10]. Mean platelet volume (MPV) is a platelet index that has been available since the 1970s. Since then, other indices of plateletshave been introduced, including platelet volume distribution width (PDW), plateletcrit (PCT), and platelet large cell ratio (PLCR) [11]. While MCV for red cells have been widely accepted and used for anemia classification, platelet indices have not been routinely used by clinicians and their clinical usefulness has been elusive. With the widespread availability of automated hematology analyzers platelet parameters are now routinely available and thus can be applied in the clinical setting.

In our study bacterial infections constituted the majority of cases for TCP (28.40%) followed by cardiac diseases (25%) and solid malignancies on chemotherapy (55.55%). While Alam M et al found malaria (43.2%) and other infections as major causes for TCP [12], Ross C et al found anemia(38.2%) as the major cause of TCP followed by ITP(10.9%) [13]. Solid malignancy on chemotherapy and leukemia constituted a higher percentage of cases in our study when compared with other studies [4,14]. This difference is due to the fact that this hospital caters more to oncology patients.

The present study revealed that mean MPV was significantly higher in hyper destructive group (Group A) as compared to hypoproductive group (Group B), abnormal pooling (Group C) and control group. Numbenjapon et al [5] also evaluate MPV in discriminating hyperdestructive from hypoproductive thrombocytopenia and found that a cut-off MPV value of 7.9fl would have a sensitivity of 82.3% and a specificity of 92.5% and concluded that MPV is a reliable diagnostic test to differentiate between these two conditions. Similarly Kaito et al [3], Ntaios et al [6] Khaleel et al [8] and shah et al [14] also reported that MPV was higher in ITP patients when compared with hypoproductive thrombocytopenic patients, which reflected an increase in the production rate and they established cut off values ranging from 9 fl to greater than 11 fl. According to Elsewefy et al [2] and Sridhar et al [4] these differences in the cut off value could be due to difference in selection of patients and the difference in the type of hematology analyzer used as older automated analyzers, which could have been used in these studies, cannot discriminate platelets from other similarly sized particles such as fragmented red or white blood cells, cell debris, and immune complexes. The high

MPV in platelet destruction could be explained by the fact that newly produced platelets are larger than circulating platelets, which tend to decrease in size with age in the circulation similar to reticulocytes with increased mean volume. As a result, in patients with thrombocytopenia secondary to peripheral destruction the MPV is increased, reflecting active bone marrow compensation with release of young platelets [5,8].

Although the above studies found MPV reliable in differentiating the categories of TCP, Elsewefy et al [2] and Borkataky et al [15] found no significant difference in the MPV between the destructive thrombocytopenia group and the control group or the hypoproductive thrombocytopenia group. Borkataky et al also demonstrated that platelet distribution width (PDW) has discriminating property to determine the etiology of thrombocytopenia. Khanna R et al [9] and Xu RL et al [10] also mentions that MPV have low sensitivity and specificity to predict the presence of bone marrow diseases in thrombocytopenic patients. The present study has its limitations as we did not analyze other platelet indices like PDW & P-LCR other than MPV. This was done intently as we wanted to test only MPV as a discriminating factor since this factor has been put to test by researchers in the recent years. Further studies by combining the platelet indices may provide promising results.

Some studies suggest that it is not always possible to record platelet indices in severe TCP and in the presence of red cell fragmentation, a platelet histogram cannot be adequately drawn, and the indices cannot be recorded [3,4,8]. Babu E and Basu D also mentioned the difficulties in getting these parameters and discarded those cases lacking these parameters [16]. We avoided this problem by discarding cases without indices and histogram and selected only those cases which had platelet indices and provided a histogram. In control group all cases had showed values and histogram.

The mean platelet volume (MPV) reflects the average platelet size and platelet function better than the platelet count itself. Young platelets are larger than old platelets. Increased number of young platelets indicates increased platelet production. In fact, the MPV is inversely proportional to the degree of platelet maturity [11]. Larger platelets are functionally, metabolically, and enzymatically more active than smaller ones [17]. Nelson et al in their study mentioned that, patients with thrombocytopenia due to loss or destruction of platelets have larger platelets, whether the loss is due to infection, hemorrhage, or immune destruction and when thrombocytopenia was due to lack of production, the platelet volume was similar to that seen in patients with normal blood cell counts [18]. In our study the mean MPV in hyperdestructive group was significantly higher than in hypoproductive group and abnormal pooling group. The difference between these values was highly significant statisticall. Vinholt PJ et al in their review article observed that MPV can aid in diagnosing the cause of thrombocytopenia, but a clear cut-off cannot yet be provided, while Islam S et al concludes that MPV along with other indices could help to distinguish hyper-destructive thrombocytopenia and hypo-productive thrombocytopenia very easily and it is also cost effective [20]. Though the present study included all age groups, study by Aponte-Barrios NH et al in pediatric age group found that MPV can distinguish between thrombocytopenia due to an increase in the destruction of platelet and thrombocytopenia due to a reduction in platelet production [21].

Conclusion

The widespread availability of automated hematology analyzers in hospitals and laboratories now permit accurate measurement of MPV and other indices. Although bone marrow examination remains the gold standard for TCP, MPV is definitely a useful and reliable test to differentiate between hyperdestructive TCP form the hypoproductive and abnormal pooling TCP. The results of our study for differentiating cause of TCP are statistically significant. More attention should be paid to these indices for causes of TCP. MPV should be combined with other platelet indices for more accurate results and further studies with combining these indices will definitely provide promising results.

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Original Research Article

An Autopsy Study of Liver Pathology in Cases of Maternal Deaths

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Abstract

Background: Liver diseases cause significant morbidity and mortality in both pregnant women and infant. Early diagnosis and timely intervention will help us to achieve reduction in maternal and infant mortality.

Aim: To study histopathological changes in liver in cases of maternal deaths and to correlate with biochemical profiles.

Material and Methods: A retrospective analysis of 101 cases of maternal deaths received in the year 2016 were studied. The histopathological changes in liver were reviewed and were correlated with liver function test if available. A total of 99 cases were available for study.

A Result: Liver disease related to pregnancy was seen in 31% of cases, with pre eclampsia/ eclampsia leading the list. In 3% of cases liver disease developed concurrently with pregnancy, while in single case pregnancy was seen in pre existing liver disease. Incidental findings were seen in 35% of cases, while in 29% of cases no changes were seen.

Conclusion : Autopsy helps us to understand the pathogenesis and presentation of liver pathology in maternal deaths. The study helps us to diagnose liver diseases in pregnancy with varied presentation in early stage. This will help in achieving our national goal of reducing maternal and foetal

Keywords: Pre eclampsia/Eclampsia.

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Introduction

Pregnancy is a clinical state, which causes physiological changes in the organs of the body. However the liver appears normal or near normal on light microscopy as well as the biochemical profiles are also normal [1]. Alteration in biochemical profiles and histopathological changes in liver will help us to identify liver disease in pregnancy. Timely management of liver diseases in pregnancy is essential to reduce the maternal and foetal mortality [2].

The present study was undertaken to study the histopathological changes in liver from the autopsies performed in case of maternal death and to correlate these findings with the antemortem biochemical profiles.

Material and Method

The present study is a retrospective study performed in department of pathology over a period of a year from January to December 2016. Our medical college being

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the only tertiary centre in North Maharashtra region, the department of pathology receives the viscera for histopathological examination from the autopsies performed in civil hospitals and health centres in north Maharashtra. Each viscera is accompanied with history, clinical findings and investigations along with post mortem report.

Total 555 viscera (cases) were received in the 2016, for histopathological examination. Out of these 555 cases 101 (18.19%) cases were of maternal death.

The age of mother, trimester and method of delivery were noted in all the maternal deaths. The received visceral organs were examined grossly and sections were taken for histopathological examination. Special stains were performed where ever needed. Out of these 101 cases two viscera were completely autolysed. Thus 99 cases of maternal deaths were reviewed. Sections from liver were reviewed along with their special stains by two pathologists. The findings were correlated with the trimester of death, laboratory investigations and changes seen in other organs.

Result

The histopathological findings in liver were classified into (Table 1).

Liver diseases related to pregnancy.	Total No of cases – 31 (31%)
Pre eclampsia/ eclampsia	17
Acute Fatty Liver	5
HELLP syndrome	4
Intrahepatic cholestasis	2
Hepatic rupture	1
Jaundice with no injury	1
Submassive hepatic necrosis	1
Liver disease developing concurrently with pregnancy	3
Acute hepatitis	3
Pregnancy in pre-existing liver disease	1
Cirrhosis	1
Incidental findings in liver	Total No of cases – 35 (35%)
Anaemia	20
Sickle cell anaemia	6
Tuberculosis	2
Poisoning	2
Puerperal Sepsis	2
Dengue	1
Typhoid	1
Malaria	1
No Abnormality seen in Liver:	29 cases

1. Liver diseases related to Pregnancy.

A) Pre eclampsia/ Eclampsia (17 cases)

Presented – Second and Third Trimester

Liver Function Tests-	Median value
S Bilirubin (0-1.2 mg/dl)	1.6 mg/dl
S AST (8-33 U/L)	94 U/L
S ALT (4-36 U/L)	190 U/L
S ALT (4-36 U/L)	242 U/L

HPE-(Figure 1 and Figure 2)-Peri- portal hepatocytic necrosis, flame shaped necrosis, subcapsular haemorrhage, cavernous haemangioma, fibrin thrombi in space of Disse.

B. Acute Fatty Liver- 5 cases

Presented- Third Trimester.

Liver Function Tests-	Median value
S Bilirubin (0-1.2 mg/dl)	8 mg/dl
S AST (8-33 U/L)	160 U/L
S ALT (4-36 U/L)	130 U/L
S ALT (4-36 U/L)	370 U/L

HPE-Micro and Macro fatty change, portal triadits, focal areas of necrosis.

C. HELLP-4 cases

Presented- Third Trimester.

Liver Function Tests-	Median value
S Bilirubin (0-1.2 mg/dl)	5.8 mg/dl
S AST (8-33 U/L)	832 U/L
S ALT (4-36 U/L)	394 U/L
S ALT (4-36 U/L)	530 U/L

Platelet count-45000/cmm.

HPE-Sub massive to massive hepatic necrosis, Deposition of fibrin in space of Disse (Figure 3).

D. Intra hepatic Cholestasis -2 cases.

Presented- Second Trimester.

Liver Function Tests-	Median value
S Bilirubin (0-1.2 mg/dl)	3 mg/dl
S AST (8-33 U/L)	180 U/L
S ALT (4-36 U/L)	310 U/L
S ALT (4-36 U/L)	200 U/L

HPE- Hepatocytes Normal, Hepatocytes and bile canaliculi shows bile thrombi.

E. Submassive hepatic necrosis.

Clinically presented has post partum haemorrhage.

LFT- Not available.

F) Hepatic Rupture.

Presented- second trimester, pain in abdomen, vomiting.

LFT-not done.

Suspected poisoning

HPE- Cavernous haemangioma.

G. Jaundice with no distinct clinical injury.

Presented- Third trimester.

Liver Function Tests-	Median value
S Bilirubin (0-1.2 mg/dl)	3 mg/dl
S AST (8-33 U/L)	28 U/L
S ALT (4-36 U/L)	34 U/L
S ALT (4-36 U/L)	122 U/L

HPE- Liver normal.

1. Liver disease developing concurrently with pregnancy

Acute hepatitis Presented in –Third trimester (1 case), post partum haemorrhage (2 cases). Clinically presented with altered sensorial and jaundice.

Liver Function Tests-	Median value
S Bilirubin (0-1.2 mg/dl)	5 mg/dl
S AST (8-33 U/L)	432 U/L
S ALT (4-36 U/L)	348 U/L
S ALT (4-36 U/L)	262 U/L

HPE-Focal areas of Hepatocytes necrosis, portal triaditis, and sinusoids infiltrated with inflammatory cells, Kupfer cell hyperplasia.

1. Pregnancy in pre-existing liver disease-Cirrhosis

Presented with worsening of portal hypertension in post partum period.

4. Incidental findings in liver-

A. Anaemia – Liver shows changes of chronic passive venous congestion.

B. Sickle cell anaemia-HPE-Liver, sinusoids filled with sickle shaped RBC's. Focal areas of necrosis seen.

C. Tuberculosis- HPE-Liver shows tubercular granuloma.

D. Poisoning- HPE- Marked congestion seen.

E. Puerperal Sepsis-HPE- Liver- Sinusoids show inflammatory infiltrate, focal abscess seen.

F. Dengue- HPE- Liver- Sinusoids show dilatation with marked congestion.

G. Typhoid- HPE- Liver-Focal collection of lymphocytes seen in liver parenchyma (Typhoid nodule).

H. Malaria-HPE-Liver, sinusoids filled with parasitized RBCs and kupfer cells contain hemazoin pigment.

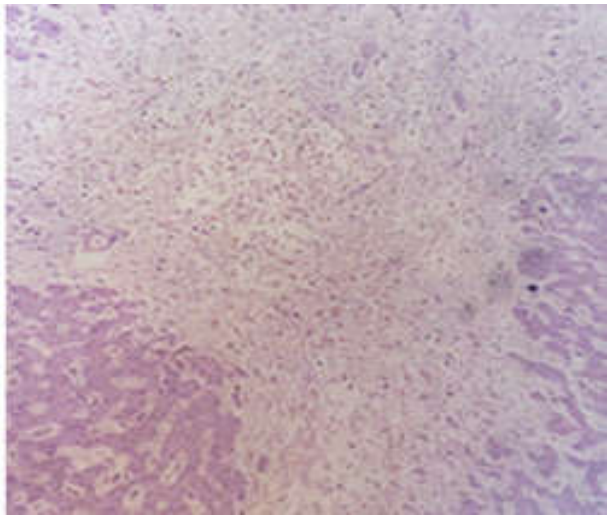

Fig. 1: Periportal hepatic necrosis seen in eclampsia

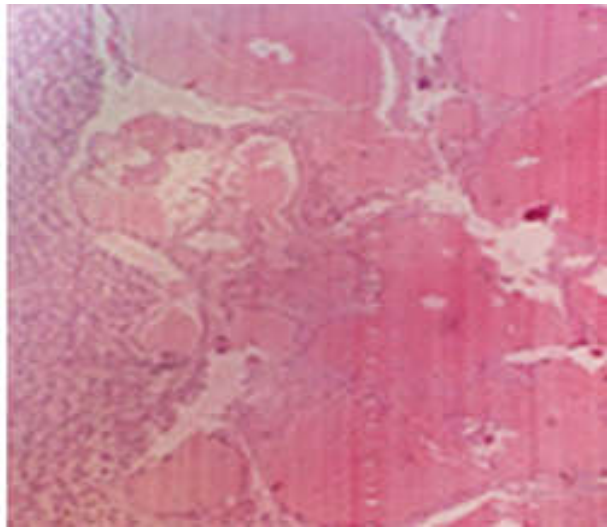

Fig. 2: Cavernous hemangioma

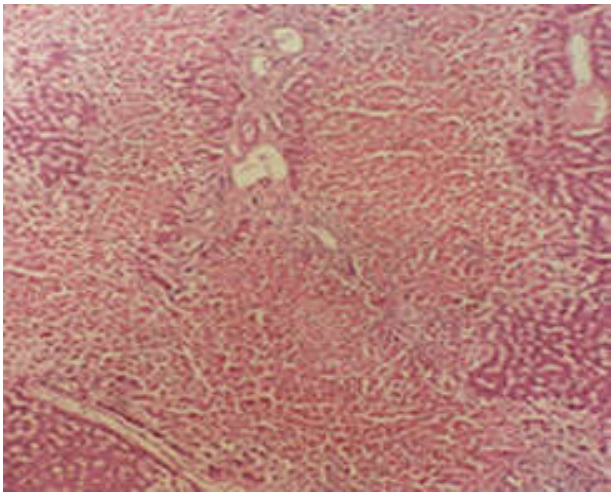

Fig. 3: Submassive hepatic necrosis

Discussion

Liver is called as "The Custodian of milieu interior". It is vulnerable to metabolic, toxic, microbial and circulatory insult.

In 31% of our cases the cause of maternal death was due to liver diseases related to pregnancy, with maximum cases being preeclampsia/ eclampsia (17 cases). Our findings correlated with a study by Kavita Khedkar et al (3), where 31% of maternal deaths were due to liver diseases related to pregnancy with maximum cases being pre eclampsia/ eclampsia.

Pre eclampsia/ eclampsia are seen in second or third trimester, it is associated with normal serum Bilirubin with moderately increased serum aminotransferase activity, along with deposition of fibrin in sinusoids. The deposition caused hepatic ischemia, haemorrhage and rupture due to vasoconstriction of hepatic vascular bed which was due to dysfunction of endothelium [4]. We too had similar findings.

Acute Fatty liver presents with medical and obstetric emergency. It presents in third trimester. To begin with the symptoms are non specific like abdominal pain, nausea, vomiting and may worsen to those of acute liver failure or jaundice [5]. Our cases of acute fatty liver succumbed to liver failure and jaundice with characteristic changes of fatty degeneration accompanied with focal areas of necrosis and minimal inflammation around portal triad.

HELLP is a severe variant of pre-eclampsia, but it can develop in women who do not have pre-eclampsia [6]. Three of our cases were hypertensive. Raised Bilirubin and Jaundice were seen in all cases. 5% of cases show evidence of jaundice [7].

Intrahepatic cholestasis is a diagnosis of exclusion. Our case presented in second trimester. On histopathological examination there was no evidence of viral hepatitis. There was marked elevation of serum alkaline phosphatase which is unique to intrahepatic cholestasis [8].

There was a single case of hepatic rupture and submassive hepatic necrosis; there was no other relevant clinical history or investigations available in these cases.

The course of most viral hepatitis infection is unaffected by pregnancy, however severe course of viral hepatitis in pregnancy has been observed in patient with Hepatitis E virus infection. The severe outcome in pregnant women is due to increased concentration of Th2 cytokines present in pregnancy [9].

There was a single case of cirrhosis, which succumbed to death due to worsening of portal hypertension. In pregnancy 40-50% increase in circulatory volume and systemic vasodilatation causes worsening of portal hypertension and increased risk of variceal bleeding [10]. This leads to worsening of liver failure, with mortality rate of 10-18% [11].

Incidental findings in liver were also seen in cases where the cause of death was not due to liver aetiology.

India accounts for 15% of world's maternal death as of 2015 [12]. Liver diseases in pregnancy have serious consequences. The overall maternal mortality of liver disease is 19.7% [13]. The clinical presentation is varied and clinical jaundice is seen in few cases. It is essential to be aware of the liver diseases in pregnancy. So prompt diagnosis and early management will help us in reducing foetal as well as maternal mortality. Hence in this review we have focussed on changes seen in liver in cases of maternal deaths.

Thus it once again proves Autopsy remains the Gold standard in understanding the diseases, there are very few studies of liver pathology in pregnancy; hence the present study was undertaken.

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Original Research Article

Clinico Histopathological Study of Ovarian Tumors in a Tertiary Care Hospital: A Two Year Retrospective Study

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Abstract

Background: Ovarian tumors are the common forms of neoplasms in women. Ovarian cancers has the worst prognosis, because they are asymptomatic in early stages and are often detected at later stages.

Aim: To determine the incidence, age wise distribution of ovarian neoplasms and also to classify the ovarian neoplasms based on the histomorphological pattern.

Materials and Methods: This study was done in the Department of Pathology, Narayana medical college and Hospital, Nellore, AndhraPradesh, India for a period of two years from January 2016 to December 2017.Total 110 cases of ovarian neoplasms were studied.

Results: Out of total 110 cases, 90 cases (81.81%) were benign, 16 cases (14.54%) were malignant and 4 cases (3.63%) were borderline. Ovarian tumors were more common in 31-40 years age group. The most common presenting symptom was abdominal pain (46.5%). Surface epithelial tumors were the commonest, accounting for 80 cases (72.72%). Most of the ovarian tumors were cystic (75.45%). About 94.3% of tumors were unilateral.

Conclusion: In our study, most common ovarian tumors are surface epithelial tumors. Benign tumors are common in the young age, whereas malignant tumours are common in the post menopausal age group. As most of the tumors are diagnosed in late stages, early diagnosis of ovarian tumors is necessary, so as to decrease the morbidity and mortality.

Keywords: Benign; Classification; Ovarian Tumor; Prognosis; Surface Epithelial Tumors.

Introduction

Ovarian tumors are the common forms of neoplasms in women[1]. In gynaecological malignancies, ovarian cancers has the worst prognosis [2] and is responsible for mortality and in countries like Europe, united states and eastern India, it is the fourth leading cause of death [3].

Ovarian tumors are often detected at later stages and by the time they are diagnosed they are larger in size [4], because most often the ovarian tumors are asymptomatic in early stages [5]. Serum HCG, CA 125, Alphafetoprotein and placental alkaline phosphate are the useful tumor markers to diagnose ovarian tumors[1].

Nulliparity, family history of ovarian and breast cancers [1], hormone replacement therapy, tobacco consumption and mutation in BRCA1 and BRCA2 genes are the risk factors for ovarian tumors. Whereas the protective factors are multiparity and use of oral contraceptive pills[6,7]. As the diagnosis and prognosis of ovarian tumors depend

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on its histological type, classification of ovarian tumors is very important [2].

Our study was done to determine the incidence, age wise distribution of ovarian neoplasms, symptomatology and also to classify the ovarian neoplasms based on the histomorphological pattern and also to assess its benign and malignant nature.

Materials and Methods

This study was done in the Department of Pathology, Narayana Medical College and Hospital, Nellore, AndhraPradesh, India. It was a retrospective study done for a period of 2 years from January 2016 to December 2017. Total 110 cases of ovarian neoplasms were studied.

Non neoplastic lesions of ovary were excluded from our study. The Specimens were received from the Department of Gynaecology. All the details like age, clinical history and symptomatology were recorded.

The specimens were first fixed in 10% formalin followed by grossing. During grossing all the features of the neoplasms were noted. Later followed by processing and embedding. Paraffin embedded blocks were cut into 4 microns thin sections and were stained with haematoxylin and eosin. Special stains were done whenever necessary.

Histopathology reporting was done by two pathologists, separately to reduce observer bias. All the neoplasms were classified using WHO guidelines.

Results

Out of total 110 cases, 90 cases (81.81%) were benign, 16 cases (14.54%) were malignant and 4 cases (3.63%) were borderline. The incidence of ovarian tumors was studied as shown in Table 1.

Ovarian tumors were more common in 31-40 years age group accounting for 45 cases (40.90%). The youngest age of the patient was 15 years, oldest age of the patient was 65 years. The age distribution of ovarian tumors was shown in Table 2.

The most common presenting symptom was abdominal pain (46.5%) followed by abdominal lump (29.5%), menstrual irregularities (11.4%), infertility (5.6%), postmenopausal bleeding(5%), ascites(4%) and weight loss(3%). About 5% of cases were diagnosed incidentally during gross and microscopic examination of hysterectomy and salpingoophorectomy specimens.

Among all the ovarian tumours, surface epithelial tumors were the commonest accounting for 80 cases (72.72%), followed by germ cell tumors 20 cases (18.18%), sex cord stromal tumors 9 cases (8.18%)and metastatic tumor 1 case (0.9%).

The distribution of ovarian tumors according to the WHO classification was studied as shown in Table 3. Most of the ovarian tumors were cystic 83 cases (75.45%), solid 22 cases (20%), both solid and cystic consistency 5 cases (4.54%). The size of the ovarian tumors, ranged from 3 cms to 28 cms. About 94.3% of tumors were unilateral, showing right sided predominance and 5.7% of tumors were bilateral.

Table 1: Incidence of ovarian tumors

Age group	Benign	Borderline	Malignant	Total
11-20	3	-	1	4
21-30	17	1	1	19
31-40	41	2	2	45
41-50	17	1	4	22
51-60	10	-	5	15
61-70	2	-	3	5
Total	90	4	16	110

Table 2: Age distribution of ovarian tumors

Age group	No. of Cases	Percentage
11-20	4	3.63%
21-30	19	17.27%
31-40	45	40.90%
41-50	22	20%
51-60	15	13.63%
61-70	5	4.54%
Total	110	

Table 3: Distribution of ovarian tumors according to WHO classification

Histological type		Cases	Percentage
Surface epithelial tumors			
Serous	Benign	40	36.36%
	Borderline	2	2.2%
	Malignant	8	7.27%
Mucinous	Benign	12	10.90%
	Borderline	3	2.72%
	Malignant	3	2.72%
Endometroid	Malignant	2	1.81%
Transitional	Benign	2	1.81%
Clear cell	Malignant	1	0.9%
Germ cell tumors			
Teratoma	Mature	18	16.36%
	Immature	1	0.9%
	Monodermal	1	0.9%
Dysgerminoma		2	1.81%
Yolksac tumor		1	0.9%
Sexcord stromal tumors			
Granulosa cell tumor		4	3.63%
Fibroma		5	4.54%
Fibrothecoma		1	0.9%
Thecoma		1	0.9%
Sertoli leydig cell tumor		2	1.81%
Metastasis		1	0.9%

Table 4: Comparision of Histopathological types of ovarian tumors in different studies

Histopathological type	Ahmad et al [9]	Tejaswini [10]	Jha et al [11]	Present study
Surface epithelial tumors	63.50%	85.25%	52.2%	72.72%
Germ cell tumors	27.13%	9.72%	42.2%	18.18%
Sexcord stromal tumors	5.84%	3.95%	3.1%	8.18%

Discussion

Ovaries are the paired intrapelvic organs of the female reproductive tract [1]. Ovarian neoplasms are usually not diagnosed till advanced stage because of their anatomical location [2] and they are asymptomatic in majority of cases [7]. Ovarian tumors are complex in view of its histogenesis, clinical behaviour and malignant potential. It is the sixth most common tumor in females [8].

Benign ovarian tumors may occur in any age group but most common in the age group between 20 to 45 years, where as malignant tumors are more common in old age group between 45-65 years [8].

In our study, most of the tumors were benign (81.81%) of cases, whereas malignant accounts for 14.54% of cases and borderline 3.63% of cases. In a study done by Vinithawillis et al [5], benign tumors were the commonest (91.1%),followed by malignant (7.1%)and borderline (1.8%). Nirali et al [1] study also showed benign tumors as the commonest (84.5%), followed by malignant (13.2%) and borderline (2.3%). Our study was consistent with these two studies where benign tumors are the commonest.

In our study, most of the ovarian tumors were common in the age group between 31-40 years (40.90%). In a study done by Prakash et al [4], ovarian tumors were common in the age group between 20-39 years (53.4%) and Sheikh s et al [8] study showed maximum number of cases in 21-30 years (43.5%). Our study, Prakash et al[4] and Sheikh s et al[8] studies were similar showing maximum number of cases in child bearing age group.

Benign tumors were more common in the age group between 31-40 years. Out of total 90 benign cases, 41 cases were in this age group. Malignant tumors were more common in the age group between 51-60 years. Out of 16 malignant cases, maximum number of 5 cases were in this age group.

The WHO classification of ovarian tumors is based on their tissue of origin either surface epithelium or germ cells or stroma of ovary. So, they are classified as surface epithelial tumors, germ cell tumors and sex cord stromal tumors of ovary [8].

Surface epithelial tumors were the commonest in our study accounting for 72.72% followed by germ cell tumors (18.18%), Sex cord stromal tumors (8.18%) and metastatic tumor (0.9%). Table 4 showing comparision of histopathological types of ovarian tumors in different studies. In all these studies, surface epithelial tumors were the commonest type.

In our study, serous tumors were the commonest with maximum no of cases diagnosed as serous cystadenoma of ovary (36.36%) followed by mature cytic teratoma (16.36%) and mucinous cystadenoma of ovary (10.90%). In a study done by Vinitha willis et al [5] serous cystadenoma of ovary was the commonest (46.4%) followed by benign cystic teratoma (21.4%) and mucinous cystadenoma of ovary (17.8%).

However, Sheikh S et al study [8] constituted benign cystic teratoma as the commonest (30.6%), followed by serous cystadenoma (26.9%) and mucinous cystadenoma (15%). Our study was closer to the study done by Vinitha willis et al [5] in which serous cystadenoma was the most commonest type. Serous cystadenocarcinoma was the commonest malignant tumor in our study constituting for about 7.27% of cases, which was similar to the study done by Sheikh s et al [8], in their study serous cystadenocarcinoma was diagnosed in 7.8% of cases.

In Germ cell tumors, benign mature cystic teratoma was the commonest. The youngest patient of age 15 years in our study was diagnosed with mature cystic teratoma. The results of our study were comparable to the study done by Nirali Thakkar et al [1]. However, Immature teratoma and Monodermal teratoma was accounting for 0.9% of cases each in our study. Dysgerminoma (1.8%) and yolksac tumor (0.9%) were the other malignant germ cell tumors diagnosed in our study.

In sexcord stromal tumors, fibroma (4.54%) was the commonest tumor. The oldest patient in our study was diagnosed with fibroma. The other sexcord stromal tumors diagnosed in our series are Granulosa cell tumor (3.63%), sertoli leydig cell tumor (1.81%), fibrothecoma (0.9%) and thecoma (0.9%). In the studies done by Vinithawillis et al [5] and Nirali Thakkar et al [1], fibroma was the most commonest sexcord stromal tumor, similar to that of our study. One case of metastatic tumor to the ovary (0.9%) was diagnosed in our study with primary from colonic cancer.

In our study, abdominal pain was the commonest symptom (46.5%) seen in about half of the cases followed by abdominal lump (29.5%). Our results were similar to the studies of Agarwal et al [7] and Wasim et al [12]. Menstrual irregularities and post menopausal bleeding were noted in few cases. Some of the ovarian tumors were diagnosed incidentally while doing infertility checkup (5.6%) of cases. Ascites and weight loss were seen in malignant ovarian tumors.

Most of the ovarian tumors in our study were cystic (75.45%), followed by solid (20%) and combined solid and cystic consistency 4.54%, which was similar to the study done by kancherla et al [3] with predominant cystic consistency (78%), followed by solid (16%), both solid and cystic consistency (6%).

The size of the ovarian tumors ranged from 3cm to 28cms. The largest ovarian tumor was mucinous cystadenoma of ovary. Smallest ovarian tumor was fibroma of ovary in our study.

In our study, most of the ovarian tumors were unilateral (94.3%) most commonly right side ovary was involved. Bilateral involvement was seen in 5.7% of cases . Bilateral involvement was seen in serous cystadenoma and metastatic tumor of ovary. In a study done by Kancherla et al [3], unilateral involvement was seen in 76% and bilateral involvement was seen in 24% of cases. The incidence of bilateral involvement in this study was high when compared to that of our study.

Conclusion

In our study, most common ovarian tumors are surface epithelial tumors. Benign tumors are common in the young age group, where an malignant tumors are common in the post menopausal age group. Differentation of ovarian tumors into benign and malignant is important for prognosis and treatment. As most of the cases are diagnosed in late stage, early diagnosis of ovarian tumors is necessary, so as to decrease the morbidity and morality. Hence, effective screening should be done in those people who have the risk factors for malignancy.

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Original Research Article

Mast Cell Count in Atopic and Contact Dermatitis

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Abstract

Context: Mast cells are the major effector cells in immediate hypersensitivity through activation via the high affinity IgE receptor and FcξRI. Given the broad array of proinflammatory mediators secreted from FcξRI – activated mast cells and IgE elevation is seen in a majority of atopic and contact dermatitis patients. Mast cells are believed to be involved in the pathogenesis of atopic and contact dermatitis.

Aims: The present study aimed at evaluating the mast cell count with respect to morphology, distribution and quantity in atopic and contact dermatitis cases.

Settings and Design:Retrospective study in an urban tertiary care hospital.

Material and methods: The study included a total of 50 skin biopsies and mast cell count was assessed in skin biopsies from patients with atopic and contact dermatitis. The tissues were fixed in 10% formalin, processed by routine paraffin embedding technique. The sections were stained with freshly prepared toluidine blue stain. The toluidine blue stained sections were evaluated for qualitative and quantitative aspects of mast cells. Mast cells were counted per field at 40 X magnification.

Statistical Analysis Used: The test results were evaluated by student T test.

Results: Mast cells were significantly increased in skin biopsies from patients with atopic and contact dermatitis with mean mast cell count 104.94/10 high power fields.The test results were statistically significant and the p value was (<0.005).

Conclusions: This study has revealed the role of mast cells as effector cells in development of atopic and contact dermatitis.

Keywords: Mast Cells; Toluidine Blue; Atopic Dermatitis; Contact Dermatitis**.**

Introduction

Mast cells are the hematopoietic cells that originate from the progenitor cells in the bone marrow .

Mast cell progenitors enter the circulation and become mature mast cells after entering destination tissues under the influence of local microenvironment [1]. The mast cells are present in small numbers in normal dermis, generally concentrated about blood vessels, especially postcapillary venules [2].

Mast cells are dynamic cells playing central role in allergic inflammation, protective immune response and other inflammatory responses.

They are found in all levels of skin including dermis, around blood vessels, nerves, appendages, at dermo epidermal junction (DEJ) and also in subcutaneous tissue

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[3]. We decided to quantify the mast cells to understand their significance and role as the effector cells in the pathogenesis of dermatitis. Mast cells occur in normal dermis in small numbers as oval to spindle shaped cells with centrally located granules in the cytoplasm that do not stain with H & E stain. They are identifiable when stained with Toluidine blue, Cresyl violet, Azure A and Methylene blue due to the presence of metachromatic granules in their cytoplasm.

In the present study, we have evaluated mast cell infiltration with respect to morphology, distribution and quantity in skin biopsies of patients with atopic and contact dermatitis using simple histologic techniques.

Materials and Methods

The study was conducted in the department of Pathology M.S Ramaiah Medical college. This retrospective study included a total of 50 skin biopsies taken for diagnostic purposes from patients with atopic and contact dermatitis after informed consent. Control tissues were obtained from normal skin of 50 patients, skin which was removed as a part of radical surgery for malignancy of breast ,oral malignancy etc.Lack of pathology in these control specimens was confirmed by routine histology. The tissues were fixed in10% formalin, processed by routine paraffin embedding technique. The sections were stained with freshly prepared Toluidine blue stain. The toluidine blue stained sections were evaluated for qualitative and quantitative aspects of mast cells. Mast cells were counted at 40x magnification, in ten consecutive high power fields and the total number of mast cells was summated and expressed/10 HPF. The areas selected included dermoepidermal junction (DEJ), perivascular, peri-adnexal and areas of cellular infiltration.

Results

A total of 50 skin biopsies were obtained from patients of atopic and contact dermatitis. Control tissues were obtained from normal skin of 50 patients, skin which was removed as a part of radical surgery for malignancy of breast , oral malignancy etc. In normal skin biopsies taken as control, mast cells were seen predominantly in perivascular and peri- adnexal location. They were sparse around DEJ and in dermis.Mean Mast cell count in the control skin biopsies was 18.86/10HPF (Table 1). The age distribution of these cases ranged from 12yrs-57 yrs. Male:female-1:2.3 ,female sex predilection was seen.

In the cases with dermatitis, mast cells were found throughout the dermis and were concentrated in the vicinity of vessels and skin appendages (Figures 1,2).

They had a blue rounded nucleus, and their granules were metachromatically stained reddish purple with toluidine blue. Mast cells were significantly increased in the skin biopsies from patients with atopic and contact dermatitis and mean mast cell count in these cases were 104.94/10HPF ($p<0.005$). In majority of the cases mast cells were degranulated(70%).

Table 1: Mean mast cell count in control and test skin biopsies

Skin lesions	Mean mast cell count/10high power field
Control	18.86/10hpf
Atopic and contact dermatitis	104.84/10hpf

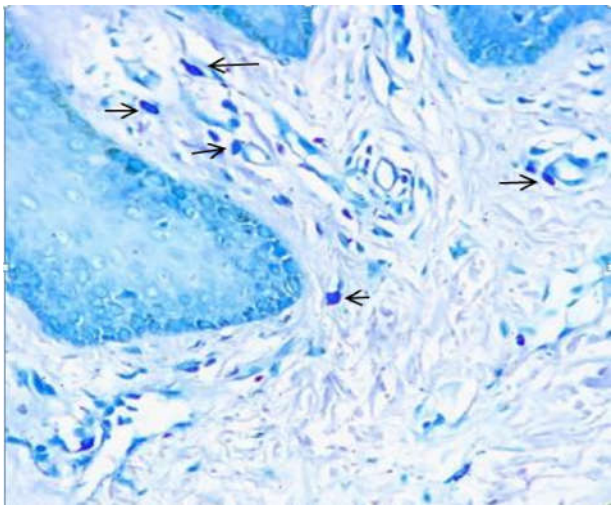

Fig. 1: Magnification 40X, Toluidine blue stained section, arrows (black) shows increased mast cells in upper dermis and around the blood vessels.

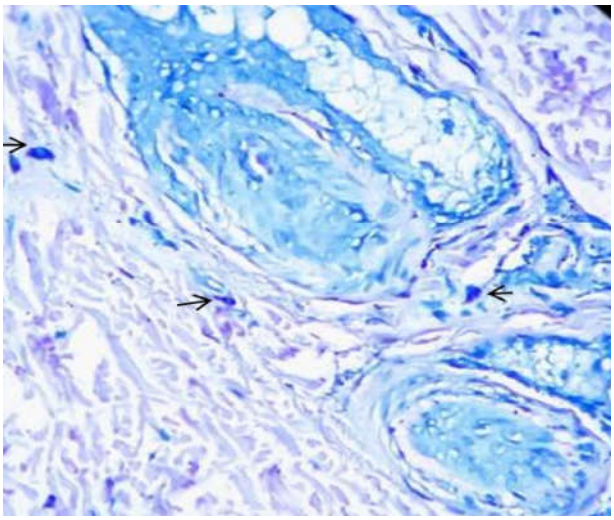

Fig. 2: Magnification 40X,Toulidine blue stained section, arrows (black) degranulated mast cells in periadnexal location

Discussion

Mast cells are well known as effector cells of IgE-mediated allergic reactions.

There is evidence of increase in the number of mast cells with degranulation in atopic and contact dermatitis, hence we decided to quantify the mast cells to understand their significance and role as the effector in the pathogenesis of dermatitis.

Mast cells are identifiable on H&E staining, but because of comparable morphology with other mononuclear cells, such as monocytes, histiocytes, lymphocytes, and melanocytes, they may require special staining for verification [4].

The high-affinity receptor for IgE (FcξRI) expressed on mast cells consists of four subunits (αβγ2): an IgE-binding α chain, a signal-amplifying and receptor-stabilizing β chain, and two disulfide-bonded γ chains that are the main signal transducers .

Upon encounter with multivalent antigen, IgE-bound FcåRI on mast cells become aggregated or crosslinked, leading to cell activation. Activated mast cells secrete three classes of substances: (1) preformed chemical and protein mediators, such as histamine, serotonin, heparin and chondroitin sulfates, proteases, major basic protein, acid hydrolases, cathepsin, (2) lipid mediators, such as prostaglandins, leukotrienes, and platelet-activating factor (PAF), (3) preformed and/or de novo synthesized growth factors, cytokines, and chemokines, such as tumor necrosis factor (TNF)-α , TGF-β, MIP-1α , MCP-1, VEGF, IFN-α /β/γ, GM-CSF, IL-1α /β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-16, IL-18, IL-25, etc.

Allergic Contact Dermatitis

Allergic contact dermatitis is a common inflammatory dermatosis caused by exposure to a variety of haptenic antigens in sensitive individuals. In the sensitization phase the inflammation is initiated by the antigen being taken up by the skin antigen-presenting Langerhans cells, which then migrate to lymph nodes and present the antigen to naive T cells and activate these cells. During the elicitation phase T cells subsequently migrate to the affected skin site and are re-activated by the antigen presenting cells. This leads to a cascade of inflammatory responses, representing a type IV allergic reaction.

There are only a few studies addressing the function of mast cells in allergic contact dermatitis in humans. An early work examined allergic contact dermatitis reactions to dinitrochlorobenzene and urushiol (the antigen responsible for poison ivy contact dermatitis) and noted the presence of mitotic mast cells three days after the application of the allergens, as well as an increase in the number of these cells. In addition, mast cells noted to have undergone degranulation [5]. Most of our knowledge on the role of mast cells in allergic contact dermatitis is derived from studies of mouse models, in which skin reactions are induced by topical application of haptens. A number of studies demonstrated an increase in the mast cell density at the reaction sites. For example, in one study the investigators topically applied picryl chloride on days 4, 11, 18, and 25, and after the 4th application noted a significant increase in the number of mast cells [6]. These additional cells could originate from the bone marrow or from division of cells originally residing in the skin.

Degranulation of mast cells is also a regular finding in the lesional skin in the mouse models.This is observed at the sites of allergic contact sensitivity induced by a variety of haptens, including oxazolone, picryl chloride, trinitrochlorobenzene [7-8]. In a time course study, Kerdel et al [8], noted there was modest degranulation of mast cells between 1 and 6 h and extensive degranulation at 12 h, after antigen challenge, in mice sensitized with 0.1% tinitrochlorobenzene (TNCB) and then challenged with 1% TNCB. In summary, allergic contact dermatitis in humans is associated with an increase in the number of mast cells and activation of these cells and these findings are noted also in mouse models. In addition, it would be of interest to test whether mast cell inhibitors are useful for treatment of allergic contact dermatitis, in conjunction with other modes of therapies.

Atopic dermatitis (AD)

Atopic dermatitis is a relatively common chronic inflammatory skin disease characterized by intense pruritus and eczema, and is often associated with allergic asthma, rhinitis and food allergies. Various studies indicated that AD has a complex etiology, with involvement of multiple immunologic and inflammatory pathways. About 80% of AD patients show high levels of total and specific IgE antibodies to a variety of allergens, especially those in food and inhalant antigens, such as chicken egg, dust mites, pollens, and molds. Serum IgE levels in AD patients tend to be higher than those in other allergic diseases. Previous studies have shown a strong correlation between the serum. IgE levels and severity of AD. IgE-dependent allergic reactions are therefore thought to contribute to the development of AD. However, the involvement of IgE in the pathogenesis of AD remains controversial. Mast cells have also been shown to represent

key effector cells of acute AD lesions and contribute significantly to chronic AD.In acute AD lesions, mast cell population is normal in number but shows degranulation [8]. In contrast, there is a significant increase in their number, especially in areas of lymphocytic infiltration in the papillary dermis, in chronic AD lesions [10]. Elevated concentrations of histamine have been detected in the skin and plasma of patients with AD [11].

In AD skin, both IL-4 and IL-13, key cytokines for the development of the Th2 response, are increased. Mast cells have been shown to be the major source of these cytokines [12]. A recent report revealed that IL-4 and IL-13 are expressed by 66% and 20% of mast cells in AD skin, respectively [13]. Mast cells can contribute to Th2 polarization in the skin of AD patients through these cytokines. Mast cells also produce IL-5, which is likely to contribute to eosinophil infiltration in the AD skin [14]. Moreover, mast cells are found to be in close association with endothelial cells in the lesional skin and there is evidence suggesting that they stimulate vascular proliferation, probably via the release of proangiogenic factors [15]. Thus, these cells can promote inflammation indirectly through increasing the vasculature at the inflammatory sites. The current view is that AD, like other allergic disorders, results from complex interactions between a number of genetic and environmental factors.

There have been several reports about a significant association between the polymorphism of mast cellrelated genes and AD. One such report has demonstrated a strong association between gene polymorphism in the β chain of high-affinity IgE receptor (FcξRI) and AD [16]. Such polymorphism can result in increased surface expression of this receptor as well as amplification of intracellular signaling, resulting in increased IgE-dependent mast cell activation. Chymase, which is a chymotrypsin-like serine protease stored in mast cellgranules that hydrolyzes a variety of substrates, such as angiotensin I, metalloproteases, lipoproteins, and procollagen, is increased in the AD skin [17]. A recent discovery is a drug targeting IgE, Omalizumab, which has shown promise in the treatment of AD. Omalizumab is a humanized monoclonal antibody that binds to IgE at the same location recognized by FcξRI and it is thus able to effectively inhibit IgE binding to mast cells.In view of information supporting mast cells as critical effector cells in both acute and chronic AD lesions, therapies targeting mast cells might prove useful for treatment of AD.

Conclusion

Mast cells are found to be increased in number in many immunological skin diseases, including contact dermatitis, atopic dermatitis.This study has revealed the role of mast cells as effector cells of allergic reactions, which might also be true in the development of skin lesions in atopic and contact dermatitis.However, little is known about their potential role in modulating the course of atopic and contact dermatitis disease progression. Mast cell density, distribution and morphology may be useful in identifying various allergic skin lesions, understanding their evolution and modifying treatment protocols.

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Conflict of Interest: Nil

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Original Research Article

Papillary Lesions of the Breast: Experience from a Tertiary Care Center

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Abstract

Introduction: Papillary breast lesions are a group of lesions that are characterized by presence of papillae supported by fibrovascular cores lined by epithelial cells with or without myoepithelial cell layer.

Aims: This study was conducted to analyse the clinicopathological characteristics of papillary lesions of the breast.

Materials and Methods*:* A retrospective and prospective analysis of 12 cases of papillary lesions of breast received over a period of 2 years was done. The patient's clinical details were collected from medical archives and the histopathological findings were reviewed. The lesions were classified into benign, atypical and malignant categories.

Results: During the study period, 12 cases of papillary lesions of breast were reviewed. The mean age was 43 years. The central quadrant was the most common location (66.6%). The most common presenting complaint was lump (75% cases), more commonly as solitary lump (83.3%) rather than multifocal disease (16.7%). Benign papillary lesions (58.3%) were more common than the malignant lesions (41.7%). The most common papillary lesion was intraductal papilloma (50% of the cases), followed by invasive micropapillary carcinoma and intracystic papillary carcinoma constituting 25% and 16.7% cases respectively.

Conclusion*:* Diagnosis of papillary lesions is challenging and its classification includes different entities that have specific diagnostic criteria. Due to their heterozygosity in morphology with benign, atypical and malignant subtypes, morphological features such as type of fibrovascular core and continuity of myoepithelial layer along with immunohistochemical stains for myoepithelial cells should be considered for accurate diagnosis and proper management.

Keywords: Fibro Vascular Core; Myoepithelial; Papillary Lesions.

Introduction

Papillary lesions comprise of a distinct spectrum of breast lesions and their diagnosis continues to be a challenge due to their heterozygosity in morphology, with benign, atypical and malignant subtypes, the differentiation of which may sometimes be difficult even for an experienced pathologist. They are rare and constitute less than 10% of benign breast lesions and less than 1% of malignant breast neoplasms [1–3]. There is

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limited data in the literature that discusses their clinical presentation, detailed histopathological features including the presence of atypia or associated ductal carcinoma in situ and prognosis [4]. On the other hand, high frequency of coexistence of several lesions in presence of a papillary lesion and different terminologies used for the same papillary lesion sometimes make clinicians confused on decision making.

This study aimed at discussing the diagnostic difficulties of papillary lesions along with presence and absence of few critical histopathological findings. The objectives of the study were to analyze the different histopathological findings, both epithelial and stromal, in various papillary lesions and to identify those histopathological findings that can differentiate between benign, atypical and malignant papillary lesions. Immunohistochemical analysis was also conducted for myoepithelial cells, which aided in identifying benign, atypical and malignant papillary lesions and in analysis of hormonal status in all malignant cases.

Materials and Methods

A retrospective and prospective study of 12 papillary lesions of breast was conducted over a period of 2 years in the Department of Pathology of a tertiary health care hospital. Breast core biopsy specimens were excluded from the study and those cases, which fulfilled the definition of papillary lesions, were included.

The clinical history and radiological findings were retrieved from medical archives. Macroscopic findings were noted and microscopic findings were reviewed. While reviewing, importance was given to histopathological findings such as type of lesion whether infiltrating or non-infiltrating, presence of papillary pattern, architectural complexity, presence of fibrovascular core, whether broad and sclerotic or thin and arborizing fibrovascular cores, associated benign changes in the adjacent breast tissue. The lesions were classified according to WHO classification of papillary lesions (2012) [5,6].

Further, Immunohistochemical (IHC) staining was performed for myoepithelial cells, which aided in identifying benign, atypical and malignant papillary lesions and also to know the hormonal status in all malignant cases.

Various histopathological features were analysed for their frequency and were compared with the final diagnosis using cross tabs and Chi-square value (Ç2) with one-degree freedom, wherever appropriate. A p-value of <0.05 was considered significant for all the performed tests. All tabulations and statistical analysis was done using IBM SPSS 20.0 data software.

Results

A total of 12 cases of papillary lesions were seen during the study period. The mean age of the patients was 43 years (age range: 18-70 years). The central quadrant was the most common location (66.6%). The right side breast was most commonly involved (66.6%), followed by left side (16.7%) and bilateral breast (16.7%). The most common presenting complaint was lump (75% cases), followed by nipple discharge (16.7% cases), mastalgia and heaviness in the breast (8.3% cases). Ten cases (83.3%) presented as solitary lesion while two were multifocal. Further, these lesions were categorized into benign, atypical and malignant [Table 1].

Papillary lesions were categorized on the basis of fibrovascular cores as broad sclerotic type or the thin arborizing type. In 71.4% cases of benign lesions, broad sclerotic fibroepithelial cores were observed [Figure 1 (A-C)] and the remaining 28.6% of cases had a thin arborizing fibrovascular core [Figure 1(D-F)]. In comparison, 100% of malignant lesions predominantly showed thin, arborizing fibrovascular cores.

We observed a higher degree of association of epithelial proliferation and architectural complexity in malignant lesions (60-100% cases) compared to benign tumours (0-40% cases). The histopathological findings associated with papillary lesions are tabulated in [Table 2].

Table 1: Distribution of papillary lesions of the breast

Type of Papillary lesions	No.	%	Clinical Diagnosis	No.
Benign	7	58.3		
Intraductal papilloma- Solitary	6	50	Fibroadenoma	3
			Fibrocystic disease	2
			Duct ectasia	1
IntraductalPapillomatosis	1	8.3	Fibrocystic disease	1
Malignant	5	41		
Invasive micropapillary carcinoma	3	25	Carcinoma of breast	2
			Benign adnexal tumour	1
Intracystic papillary carcinoma	2	16.7	Carcinoma of breast	2

Table 2: Histopathological findings of papillary lesions of the breast

Histopathological findings		Benign	%	Malignant	%
Fibrovascular cores	Broad	5	71.4	0	
	Thin	2	28.6	5	100
Location	Central	6		2	
	Peripheral	1		3	
Number	Solitary	6		4	
	Multifocal	1		1	
Epithelial changes:					
Epitheliosis		2	28.6	5	100
Epithelial hyperplasia, usual type		2	28.6	4	80
Sclerosingadenosis		2	28.6	3	60
Comedo necrosis		0	0	3	60
Stromal changes:					
Fibrosis		3	42.8	3	60
Hyalinization		3	42.8	3	60
Sclerosis		3	42.8	3	60
Osteoclast giant cell		0	0	3	60
Calcification		1	14.3	3	60
Haemorrhagic/hemosiderin		1	14.3	4	80
Lymphocytic infiltration		2	28.6	3	60
Blood vessel proliferation		2	28.6	4	80

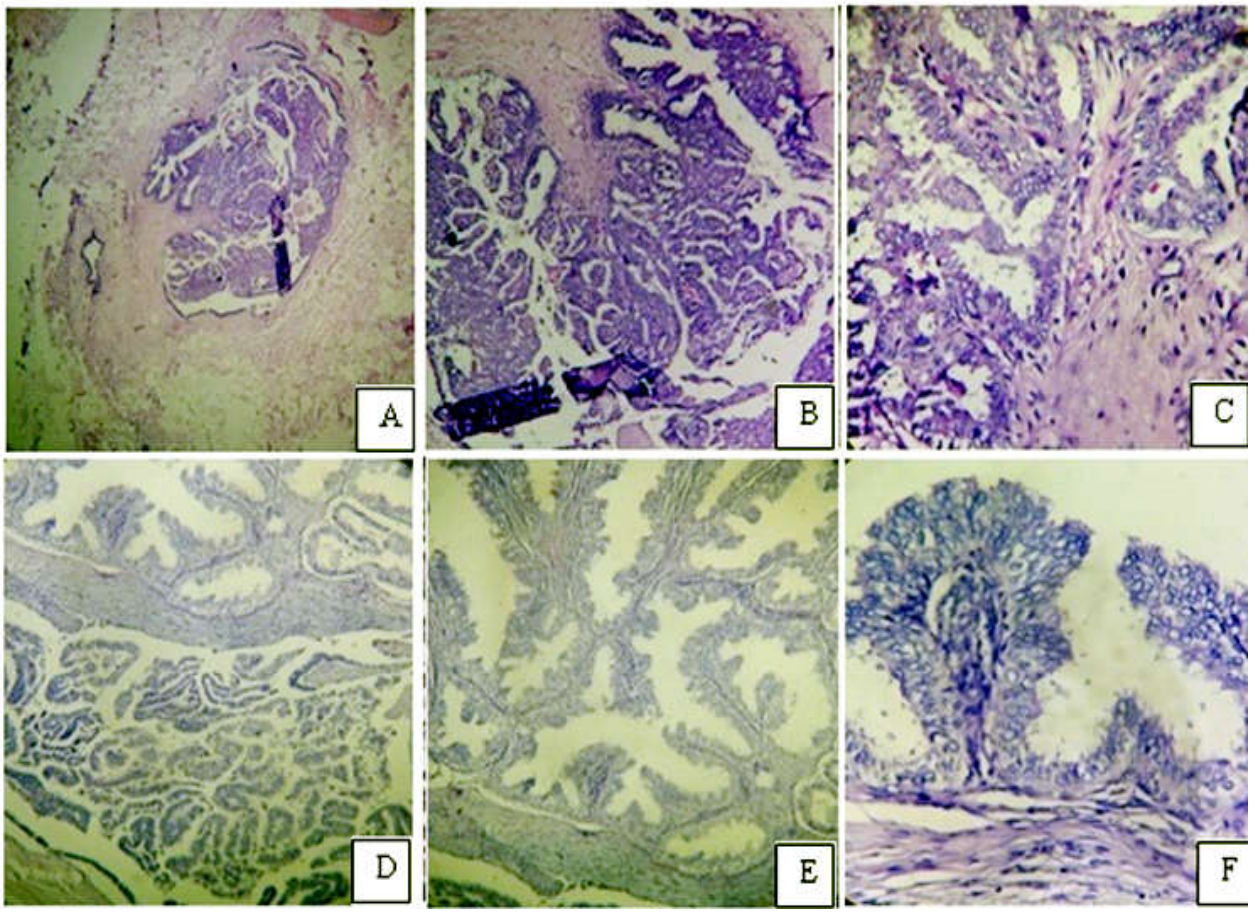

Figure 1: A-F: Intraductal benign papilloma (H&E): [A, B]: Papilloma with surrounding sclerotic stroma. (4x, 10x) [C]: Papilloma exhibiting broad sclerotic fibrovascular core. (40x) [D-F]: Papilloma with arborising papillary fronds and thin fibrovascular cores. (D, E: 10x; F: 40x)

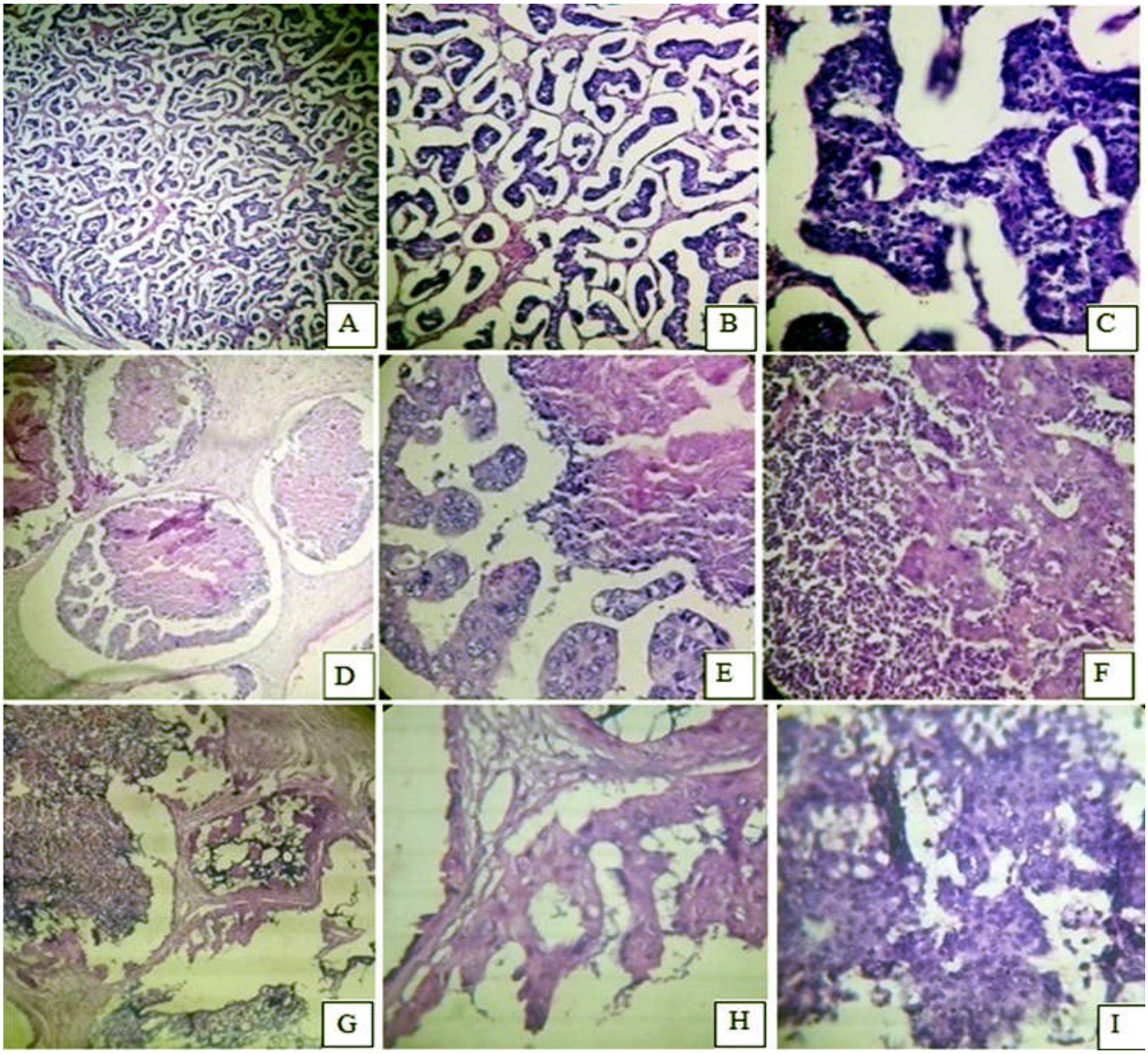

Fig. 2: A-C: Invasive Micropapillary Carcinoma (H&E): Tumour cells in micropapillary pattern lying in empty stromal lacunae and lacking true fibrovascular cores. (A, B, C: 4x, 10x, 40x respectively), D-E: Invasive Micropapillary papillary carcinoma (H&E): D, E: Micropapillary lesion lacking true fibrovascular cores with comedo neecrosis (D: 10x, E: 40x) F: lymph node with metastasis from micropapillary carcinoma. (40x), G-I: Intracystic Papillary carcinoma (H&E): Tumour composed of papillary fronds projecting into the lumen with absence of myoepithelial cells. (G: 4x; H, I: 40x)

The most common papillary lesion accounting for 50% of the cases was intraductal papilloma, seen in 6 cases with age of presentation ranging from 18-50 years. This benign tumour was usually solitary and was mostly well-circumscribed, nodular lesion that was located more commonly in the subareolar region. Intraductal papillomas exhibited various types of metaplasias most common being apocrine (57%) followed by mucinous (28.8%) and clear cell (14.2%) types.

A single case of papillomatosis was encountered in an 18-year-old female. Histopathological findings were similar to intraductal papilloma, but the attachment to the duct wall was discerned only focally.

Three cases of invasive micropapillary carcinoma with age of presentation 35, 58, 65 years respectively, were seen. [Figure 2 (A-C), Figure 2 (A-C)] Apocrine metaplasia was noted along with associated lesions like microcalcification, hyalinization, osteoclast like giant cells haemorraghe and hemosiderin pigment.

An interesting case of a 65-year-old female presented with mass in upper outer quadrant of right breast since 1 year, with exophytic growth above skin level, blackish, 9x8

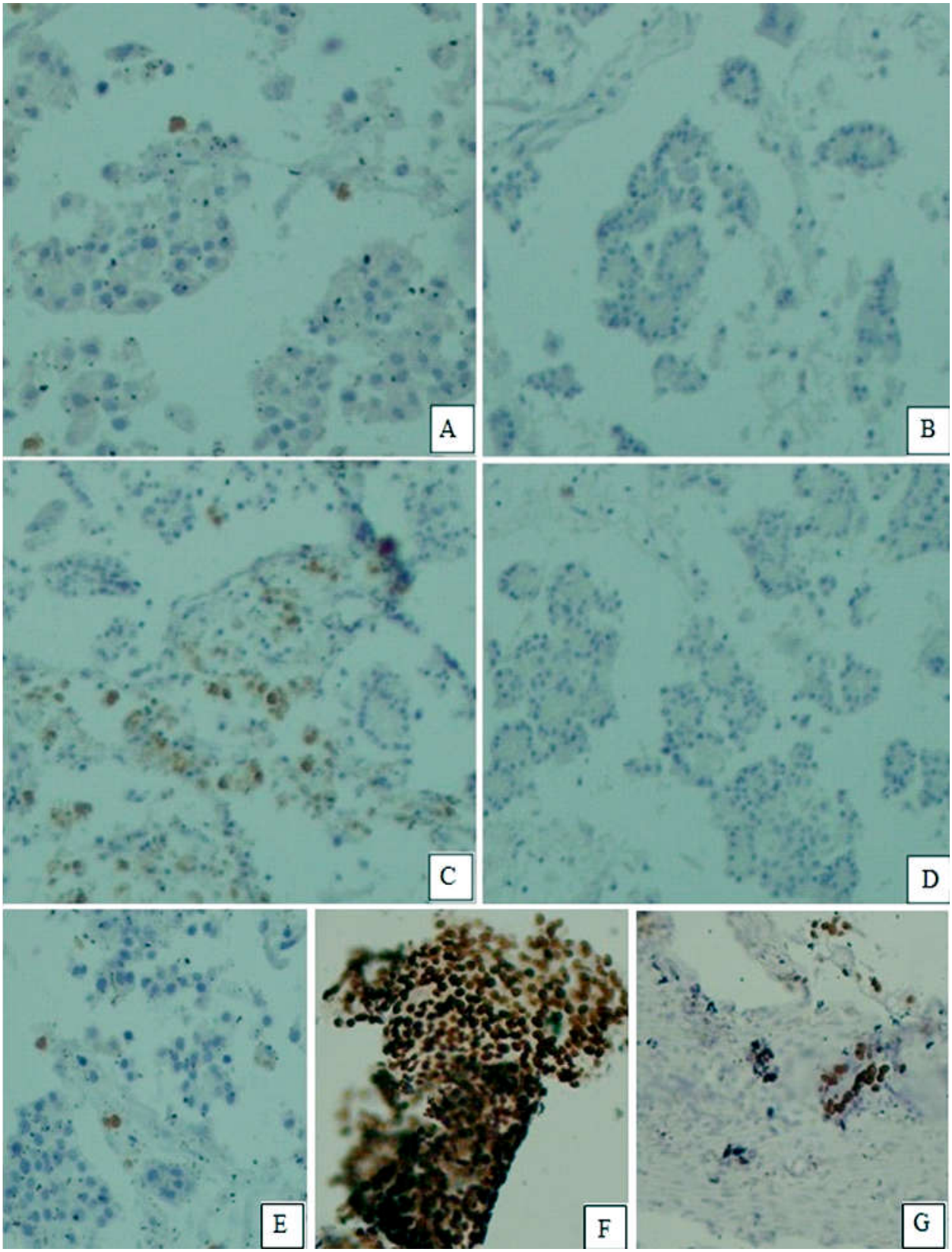

Fig. 3: A-D: Invasive Micropapillary carcinoma with absent myoepithelial cells (IHC): A: negative for p63 (40x) B: negative for CK6 (40x) C: negative for synaptophysin (40x) D: negative for chromogranin (40x), E-G: Intracystic Papillary carcinoma with absent myoepithelial cells (IHC): E: negative for p63 (40x) F: Positivite for ER (40x), G: focal Positivite for PR (40x)

cm, and firm, fixed. Cilinical diagnosis and FNAC was suggestive of benign adnexal tumor. A histological diagnosis of Invasive micropapillary carcinoma of breast was given and Immunohistochemical (IHC) staining was done to rule out neuroendocrine tumour. It was negative for p63, CK- 6, synaptophsin and chromoganin, thus confirming our diagnosis [Figure 3 (A-D)].

Intracystic papillary carcinoma also called, as encapsulated papillary carcinoma is a variant of intraductal papillary carcinoma. These lesions exhibit cystic spaces that surround papillary proliferation and lie within a dilated duct. They accounted for 16.6% of total number of cases [Figure 2(G-I)]. The age of presentation of these lesions were 50 and 62 years respectively. Apocrine metaplasia was noted along with associated lesions like microcalcification, hyalinization, comedo necrosis haemorraghe and hemosiderin pigment.

On statistical analysis the variables such as age, location, laterality, focality, epithelial change, stromal change, nuclear pleomorphism and presence or absence of broad sclerotic fibrovascular cores, only nuclear pleomorphism and presence or absence of broad sclerotic fibrovascular cores were found to be statistically significant with a p-value of <0.05. These two variables were also considered the reliable parameters on histopathology review of the slides.

Discussion

Papillary lesions of the breast include different entities based on clinical and morphological evaluation. They are characterized by the presence of papillary, arborizing epithelial proliferation, which is supported by fibrovascular stalks with or without an intervening myoepithelial layer [4]. Its diagnosis continues to be one of the most challenging aspects. Identification of whether the lesion is benign or malignant has a great impact on therapy [4]. In this study, the diagnostic issues in various types of papillary lesions encountered in our hospital are discussed.

Papillary lesions of the breast include benign forms comprising of intraductal papilloma (central, peripheral, or atypical- intraductal papilloma with ADH/DCIS/LCIS); juvenile papillomatosis and malignant lesions comprising of intraductal papillary carcinoma, encapsulated papillary carcinoma (without/with invasion); solid papillary carcinoma (in situ/invasive), invasive papillary carcinoma and invasive micropapillary carcinoma [5].

Intraductal papillomas are the most common type of papillary lesions and presents as solitary lesions in the subareloar region in most of the cases [7,8]. The most common clinical finding is the presence of palpable mass followed by presence of nipple discharge that can be either bloody, serous or serosanguinous in nature [9]. The diagnosis is usually straightforward with lesion characterized by arborizing papillae exhibiting fibrovascular stalks that are covered by myoepithelial cells [10]. In cases with florid epithelial hyperplasia or atypical ductal hyperplasia the diagnosis is difficult as these findings obscure the papillary nature of the lesion [4]. But, generally these changes are focal. Stromal changes such as diffuse sclerosis can also create confusion mimicking invasion. These papillomas are usually subjected to various morphological changes such as torsion with secondary haemorrhagic infarction of the papillae [7]. We encountered various epithelial and stromal changes like sclerosing adenosis, epitheliosis, fibrosis, hyalinization and haemorrhagic infarction. In difficult cases, immuno-histochemistry for identifying the myoepithelial cells proves the benign nature of these lesions [4].

Multiple papillomas or papillomatosis is a disease of younger age group. They most commonly affect the peripheral terminal duct-lobular units or distal portions of the terminal ducts unlike their solitary counterpart that affects almost always-central ducts [9-10]. The morphology of papillomatosis is similar to that seen in intraductal papillomas. Ueng et al., describes that papillomatosis generally have focal attachment to the duct wall [4]. However, there is enough evidence of multiple points of attachment of several papillae in papillomatosis [4-10]. WHO states the term papillomatosis is confusing and should be avoided as this term has also been used for usual ductal hyperplasia and multiple papillomas [1].

Most of the benign papillary lesions pose less diagnostic problems. Atypical epithelial proliferations (ADH and DCIS) may occur in papillomas, and are usually of low grade. Diagnosis of these low-grade papillary lesions such as intraductal papilloma with atypical ductal hyperplasia (ADH) or papillomas with Ductal Carcinoma Insitu (DCIS) also termed as atypical papillomas requires expertise [8]. Atypical ductal papillomas are defined as presence of a focal proliferation of atypical epithelial cells with low nuclear grade (WHO). Ueng et al. termed such lesions as low grade Ductal Intraepithelial Neoplasia (DIN) and described that these changes are seen only focally [4]. Different authors use different terminologies and criterias to identify these lesions [4,11,12]. Page et al. termed a lesion as papilloma with low-grade DCIS when it had morphology similar to non-comedo DCIS with a size greater than 3mm and intraductal papilloma with ADH when the atypical epithelial proliferation is < 3 mm [11]. However, the same authors term those papillomas that are more than 3mm in size with epithelial proliferation as papillomas with atypia. This definition replaces alternative terminologies that were focused on the proportion of atypical cells (30 or 90%) within a papilloma. In contrast, Collins et al. stated that the extent or the size of atypical epithelial proliferation in the lesion is not required for diagnosis of atypical

papillomas [13]. An intermediate- or high-grade DCIS within a papilloma can be diagnosed regardless of the extent of atypia. However, the diagnosis is made when there is morphological evidence such as architectural and cytological features of atypical proliferation in these lesions [13,14].

Atypical papillomas exhibit decreased number of myoepithelial cells that can be proved by immunohistochemistry. The management of both intraductal papillomas with ADH/DCIS is by complete excision and a stringent follow-up.

Papillary carcinoma constitutes less than 1-2% of breast carcinomas. They are generally non-invasive. However, some papillary carcinomas can have stromal invasion that displays morphological features of infiltrating ductal carcinoma [4]. The invasive nests can be either microinvasive or grossly evident [17].

Intraductal papillary carcinomas are rare and accounts for 2% of all breast cancers. They affect women in their fifth and sixth decade of life [15]. It is distinguished by other types of intraductal carcinoma by the presence of a true fibrovascular stalk of the lesion [16]. Morphologically, these lesions are similar to DIN and show near complete or complete absence of myoepithelial cells in the papillae [4].

The diagnostic term for larger intracystic papillary carcinomas has changed to encapsulated papillary carcinoma because the fibrous capsule that surrounds these lesions usually lacks a myoepithelial cell layer [4]. The question of whether an encapsulated papillary carcinoma represents an in situ lesion or an indolent form of invasive papillary carcinoma has not been resolved. However, the diagnosis of frankly invasive carcinoma can only be made if invasion is present outside the fibrous capsule. If not, encapsulated papillary carcinoma should be staged as papillary carcinoma in situ, and has an excellent prognosis. Regional lymph-node metastases occur only rarely without evidence of invasion. These occur in elderly patients (average age of 65 years). The tumor often presents as a solitary, retroareolar well-defined mass on mammography, and a cystic lesion with solid components on ultrasound.

On gross examination, it commonly appears as a circumscribed friable mass within a cystic cavity, and hence is also termed intracystic papillary carcinoma. Histologically, one or more well-circumscribed nodular masses within a cystically dilated duct surrounded by a thick fibrous capsule are typically evident. The duct spaces are filled by slender fibro vascular stalks, which are devoid of myoepithelial cells. They behave indolently and are best regarded as intraductal papillary carcinomas for management purposes, particularly if persistence of a basement membrane around the duct can be ascertained with collagen IV or laminin immunostains [4].

Another form of sharply circumscribed, a variant of intraductal papillary carcinoma and frequently non-invasive papillary carcinoma is solid papillary carcinoma, but, in contrast to encapsulated papillary carcinoma, this tumor is usually composed of multiple adjacent nodules, and the papillary nature may not be readily apparent because of its predominantly solid growth patterns. Diagnostic criteria include the presence of small, monotonous cells with hyper-chromatic nuclei with significant mitotic activity with often at least focal granular eosinophilic cytoplasm that may show neuroendocrine or mucinous differentiation [5]. Generally, the prognosis is good, and when a surrounding myoepithelial cell layer is present, the tumor is classified as an in situ carcinoma.

Invasive Micropapillary Carcinoma (IMPC) is an aggressive yet rare form of breast cancer with a propensity for lymphovascular invasion and regional lymph node metastasis. It is characterized by micropapillary clusters of neoplastic cells, lacking true, central, fibrovascular cores and lying within fairly prominent clear empty stromal lacunae [4]. These tumours are of higher grade and exhibit higher nuclear pleomorphism. Often they show lymphocytic infiltration along with inverse polarity of tumour cell clusters with exposed basal layer. Foci of intraductal carcinoma are seen almost in all cases of micropapillary carcinoma [18]. This should not be confused with micropapillary DCIS; wherein the ducts are dilated and lined by very small tumour cells. Micropapillary DCIS is often multifocal and multicentric in contrast to IPMC, which often presents as palpable mass.

Invasive papillary carcinoma of the breast is regarded as a specially differentiated adenocarcinoma of the breast with papillary morphology, but otherwise no distinguishing clinical, genetic, or prognostic features [4].

Myoepithelial cell continuity was noted in all the benign papillary lesions, while there was complete absence of the myoepithelial cells. Morphology of the papillae differed in benign and malignant lesions. The papillae had broad and sclerotic fibrovascular cores in benign lesions, and all malignant cases had typical thin, arborizing fibrovascular cores. This morphological difference was of great help in most of the difficult cases to arrive at the diagnosis and was found statistically significant (p-value <0.05). Similar findings, were also described by Pathmanathan et al [19].

Limitations

An important gap in the literature related to papillary lesions of the breast is the lack of universality in classifying the various lesions. This is due to use of various terminologies and criteria to categorize them. To the best of our knowledge, we have incorporated WHO classification to define these lesions, however, the classification is limited by certain overlapping features. The limitations of the present study were the small size of

population and lack of follow up. Larger studies and a stringent follow up especially in those cases where only lesional excisions were performed may throw a better insight into the various prognostic factors of papillary lesions.

Conclusion

To conclude, diagnosis of papillary lesions of the breast is challenging due to its varied clinical, radiological and pathological features that has direct impact on management. The continuity of myoepithelial layer and presence of broad and sclerotic fibrovascular cores are consistent features in benign papillary lesions. In this review, we have provided guidelines to distinguish and accurately diagnose the various papillary lesions based on H&E morphology. The diagnostic dilemma in papillary lesions of the also occurs in core biopsies where under-sampling can be a problem. Immunohistochemical work-up to identify myoepithelial cells is useful in distinguishing papillary carcinoma from atypical papilloma. Distinguishing papillary carcinoma from other papillary lesions of the breast is extremely important; therefore excision biopsy of the entire lesion is necessary in all suspected papillary lesions.

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Original Research Article

A Study of Histopathological Spectrum in Non-Neoplastic and Neoplastic Lesions of Kidney

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Abstract

Objective: We conducted three years retrospective study in tertiary care hospital in India catering to both urban as well as rural fraternity to see the various pattern of renal diseases.

Methods: The present study comprised of studying all the biopsy (core needle as well as nephrectomy) specimens coming to pathology department, GMC Aurangabad.

Results: A total of 72 cases over a period of three years were studied. Data in this study showed that non-neoplastic lesions comprised of 48 cases (66.7%) & neoplastic lesions were 24 cases (33.3%). Chronic pyelonephritis predominated in the non-neoplastic group & in the neoplastic group renal cell carcinoma was the commonest.

Conclusion: A wide range of lesions are encountered on histopathology, many of which may be misdiagnosed clinically and radiologically; therefore, it is mandatory that every renal biopsy specimen be subjected to detailed histopathological examination for a clinico-morphological correlation to ensure proper management.

Keywords: Renal Diseases; Chronic Pyelonephritis; Renal Cell Carcinoma (RCC).

Introduction

The kidney can be involved in a number of pathological conditions, some of them require nephrectomy. Although Simple Nephrectomy is a common procedure in surgical practice, it is the ultrasound guided renal core biopsy (histopathological examination) which in the field of nephrology remains the gold standard for diagnosing various renal disorders [1]. By this procedure, it is possible to establish an accurate diagnosis, to know the prognosis of disease process and develop the rational approach to treatment of renal disorders. Radical nephrectomy is the treatment of choice for patients with renal cell carcinoma [2].

Objective of this study was to review all cases of renal biopsy (core needle as well as nephrectomy) specimens received in the department of pathology and to evaluate the different histomorphological lesions, observe variations if any from the conventional pattern, along with clinic-morphological correlation for proper postoperative management.

Aims and Objectives

To study the histopathological spectrum of non-neoplastic and neoplastic lesions in tertiary care hospital.

Material and Methods

The present study was conducted in the Department of Pathology Government Medical College and Hospital,

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Aurangabad over a period of three years. (January 2015 to December 2017) All renal biopsy (core needle as well as nephrectomy) specimens submitted to the Department of Pathology were considered in the study. The age, sex, clinical history, IVP, MRI, CT and any other relevant investigation findings were noted from patient's clinical records. Nephrectomy specimens were examined and gross sections from representative areas were taken and processed. Slides were stained by H&E and special stains as and when required.

Results

A total of 75 renal biopsies were examined over a period of three years (from January 2015 to December 2017) out of which 3 biopsies were inadequate so we studied 72 cases in detail. Data in this study showed that non-neoplastic lesions comprised of 48 cases (66.7%) & neoplastic lesions were 24 cases (33.3%) [Table 1] . From 72 cases, there were 47 males (65.3%) and 25 females (34.7%). The male:female ratio was 1.88:1. The highest number of patients belonged to the age group 61-80 years (22 cases) followed by those in the age group 21-40 years (21 cases). The youngest patient was 5 years old and the oldest patient was 95 years old. A majority of cases of chronic pyelonephritis were seen in the age group of 41-60 years. All cases of renal cell carcinomas fell into the age group of 61-80 years. All cases of Wilms tumor were in patients below the age of 15 years. Majority of the cases of hydronephrosis belonged to the age group of 21-40 years [Table 2].

A wide range of lesions were found on histopathological examination. Maximum number of cases were of chronic pyelonephritis (45.1%), followed by renal cell carcinoma [Figures 1-3] (19.7%). Other renal lesion encountered were Xanthogranulomatous pyelonephritis, Tuberculous pyelonephritis, Pyonephrosis, Hydronephrosis, Wilms tumor, Squamous cell carcinoma, Urothelial carcinoma, Angiomyolipoma and Ganglioneuroma [Table 2].

Out of the 48 cases of non-neoplastic lesions, chronic pyelonephritis was the commonest histopathological condition observed with a total of 32 cases. There were 10 cases of hydronephrosis and three cases of xanthogranulomatous pyelonephritis, and two cases of pyonephrosis and one case of tuberculous pyelonephritis.

Out of the 22 cases of malignant tumours the incidence of renal cell carcinoma was highest with a total of 14 cases, followed by Wilms tumour 4 cases and 2 cases each of urothelial carcinoma and squamous cell carcinoma. There were two benign tumours one of each angiomyolipoma [Figure 4] and ganglioneuroma. From 14 cases of renal cell carcinoma, 9 Cases (64%) were male and 5 Cases (36%) were female. Grossly, growth in size was in the range of 4-12 cm. The growths were solid having variegated appearance with necrosis, hemorrhagic, and yellowish areas. In the maximum number of cases (57%), the upper pole of the kidney was involved grossly. Microscopically, majority of the cases (71%) were of clear cell type of renal cell carcinoma.

All four cases of Wilms tumor were characterized by a grossly enlarged kidney with hemorrhagic and nodularappearance. Microscopically, three of the cases were of biphasic Wilms tumor, one case of triphasic Wilms.

Table 1: Percentage incidence of Non-neoplastic and Neoplastic lesions

Conditions		No of Cases	% Percentage
Non-neoplastic lesions		48	66.7%
Neoplastic lesions	Benign tumours	02	2.7%
	Malignant tumours	22	30.5%

Table 2: Age-wise distribution of various histopathological lesions of kidney

	Histopathological diagnosis	0-20 yrs	21-40 yrs	41-60 yrs	61-80 yrs	>80 yrs	No. of cases	% of all lesions
1	Chronic pyelonephritis	04	13	12	03	00	32	44.4
2	Xanthogranulomatous pyelonephritis	00	02	00	01	00	03	4.2
3	Tuberculous pyelonephritis	00	01	00	00	00	01	1.4
4	Hydronephrosis	01	05	02	01	01	10	13.8
5	Pyonephrosis	00	00	00	02	00	02	2.7
6	Renal Cell Carcinoma	00	00	00	14	00	14	19.4
7	Wilms tumour	04	00	00	00	00	04	5.6
8	Urothelial Carcinoma	00	00	01	01	00	02	2.7
9	Squamous cell carcinoma	00	00	02	00	00	02	2.7
10	Angiomyolipoma	00	00	01	00	00	01	1.4
11	Ganglioneuroma	01	00	00	00	00	01	1.4
	Total	10	21	18	22	01	72	100

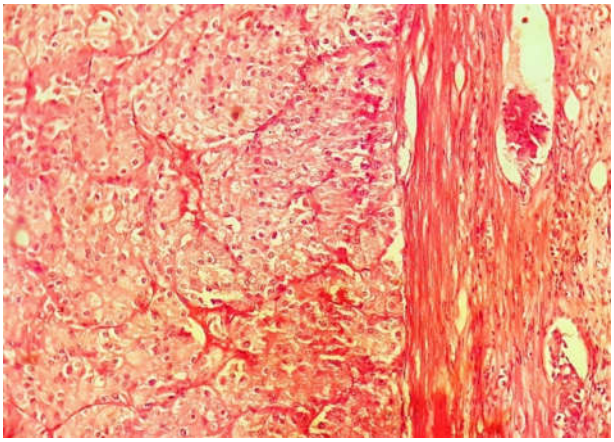

Fig. 1: Chromophobe renal cell carcinoma showing a solid growth pattern and perinuclear cytoplasmic clearing. (H & E: 10x)

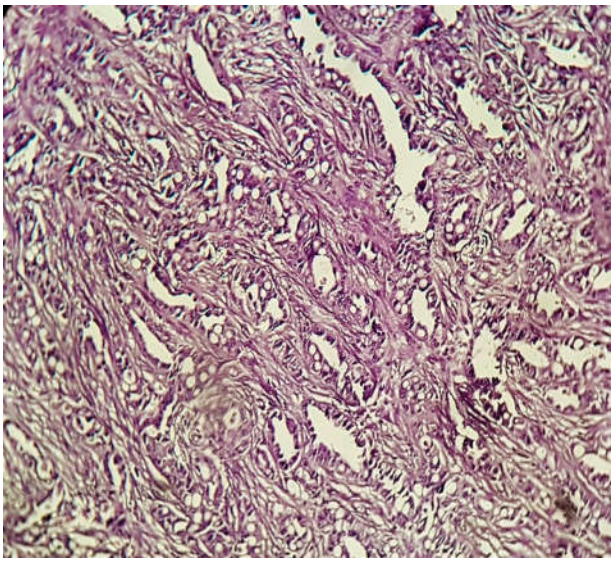

Fig. 2: Collecting duct renal cell carcinoma showing tubular structures are lined by tumour cell. (H & E: 10x)

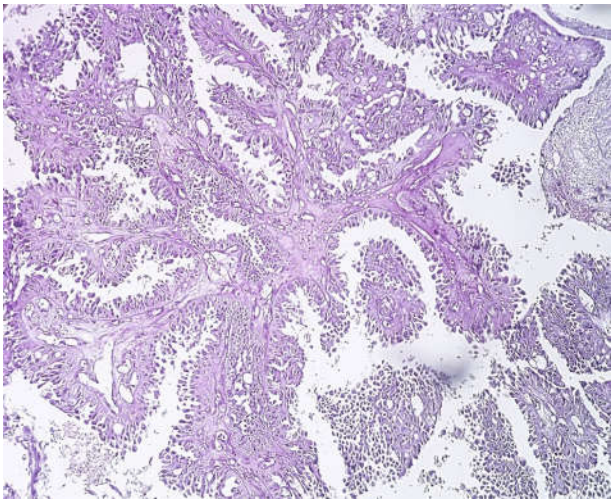

Fig. 3: Papillary renal cell carcinoma showing papillary structure lined by tumour cell with fibrovascular cores. (H & E: 10x)

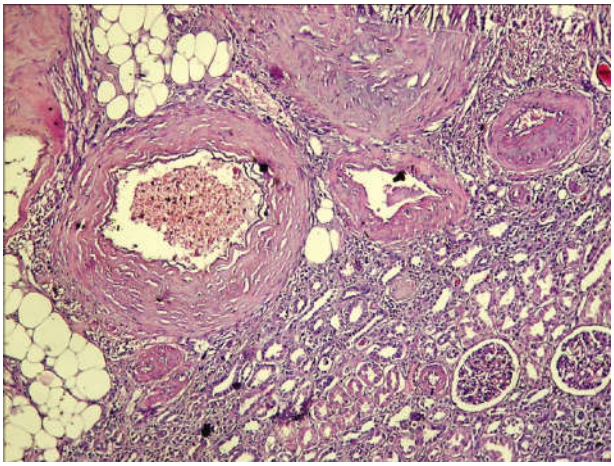

Fig. 4: Angiomyolipoma composed of mature fat cells, irregular thick and thin walled blood vessels, and smooth muscle proliferations. (H & E: 10x)

The most common clinical presentation observed was flank pain, followed by fever and abdominal lump. Least common clinical presentation was burning micturition.

Discussion

Out of the 72 cases of renal biopsies during the study period, 48 cases (66.7%) were non-neoplastic and 24 cases (33.3%) were neoplastic. There were 65.3% males and 34.7% females. The male: female ratio was 1.88:1. This was inconcordance with El malik et al who reported 61% in males and 39% in females with M:F = 1.9:1 [3]. Aiman et al also reported a male preponderance in their study [4].

Among the non-neoplastic conditions, chronic pyelonephritis was the commonest lesion our study. This was similar to the findings by El malik et al 3]. Among 32 cases of chronic pyelonephritis (CPN), grossly majority of cases shows dilatation of pelvi-calyceal system (23 cases) and loss of cortico-medullary junction (09 cases), which is concordance with study conducted by Datta et al.[5] Out of 32 cases of CPN, microscopically 2 cases were diagnosed to CPN with nephrolithiasis and other associated findings include pyonephrosis, granulomatous reaction.

In the present study, 3 (4.2%) cases of xanthogranulomatous pyelonephritis were seen. Popat *et* al [6] observed 2 cases (2.5%) and El Malik et al [3].[3] 6 cases (1.1%), of xanthogranulomatous pyelonephritis. Genitourinary tuberculosis comprises 20% of all extra pulmonary tuberculosis [7]. In present study we found one case of tuberculous pyelonephritis in 35 year old male presented with flank pain and hematuria.

Renal cell carcinoma is a group of malignancies arising from the epithelium of the renal tubules. Renal cell carcinoma (RCC) is a tumour of adults with an average

age of diagnosis 55 to 60 years. The clear cell renal cell carcinoma is the most common and constitutes 70-80% of all renal cancer. Papillary carcinomas account for 10% to 15% of renal cancers. Chromophobe carcinomas represent 5%, collecting duct (Bellini duct) carcinoma represents 1% or less of renal epithelial neoplasms. Approximately 5% to 10% of primary renal tumours originate from the urothelium of the renal pelvis, which range from apparently benign papillomas to invasive urothelial (transitional cell) carcinomas. Sarcomatoid renal cell carcinoma makes up about 1% of all renal tumours in adults [8].

In this study, among the malignant cases, renal cell carcinoma was the commonest malignancy with an incidence of 63.6%. This was similar to the studies of Popat et al who found in their study that 70% of malignant lesions were renal cell carcinoma [6]. Similar findings were reported by Rafique M [9] who observed that majority of malignant neoplasms of the kidney (97%) were renal cell carcinomas. In our study, out of 14 cases of RCC, clear cell type was the commonest 10 cases (71.4%) followed by 1 case (7.1%) of each that is papillary, sarcomatoid, chromophobe type and collecting duct carcinoma. This was consisting with R.C. Mohanty et al [10]. Study who found 24 cases of RCC, clear cell variant was most frequent i.e 18 cases (75%), two cases (8.33%) of RCC with chromophobe cells, One sarcomatoid RCC (4.16%), One was diagnosed as carcinoma of collecting ducts of Bellini (4.16%).

There were 4 cases of Wilms tumor one was a 5 year old child another was of 8 year old and yet another were of two 12 and 14 years old child confirming it as the commonest childhood tumor.

Primary squamous cell carcinoma is very rare only two cases were reported in our study. The incidence of renal squamous cell carcinoma among malignant renal tumors is in the range of 0.5-0.8%, as reported by Lie et al [11] and Blacher et al [12]. There were 2 cases (8.3%) of urothelial carcinoma observed in our study. In different studies renal pelvis tumors accounted for approximately 10% of all renal tumors.

In benign tumours we found two cases, from that one of angiomyolipoma and other of ganglioneuroma. Renal angiomyolipoma (AML) is an uncommon benign mesenchymal tumour of the kidney. It has a incidence of 0.3-3% among kidney tumors [13]. Here, we report a case of renal AML in a 57-year-old female.Renal ganglioneuroma is a rare form of benign tumour involving neural crest cells and kidney tissues [14]]. Renal ganglioneuromas in pediatric patients are even more uncommon with only three other reported cases [15-17]. Our case was forth case of a 4 year-old girl with an incidental finding of a right renal mass after ultrasound examination due to frequent urinary tract infections. Histopathological examination is required for diagnosis, there are no other diagnostic methods to identify ganglioneuromas.

The histopathological analysis correlated well with the clinical diagnosis; however, few benign lesions like xanthogranulomatous pyelonephritis may be misdiagnosed clinically as malignant. Similarly, cases of angiomyolipoma, ganglioneuroma and renal squamous cell carcinomas were confirmed on histopathological analysis only.

Conclusion

Renal cell carcinoma - Clear cell type is the most common malignant tumor. Chronic pyelonephritis (CPN) is the most common non-neoplastic lesion. In conclusion, a wide range of lesions are encountered on histopathology, many of which may be misdiagnosed clinically and radiologically; therefore, it is mandatory that every renal biopsies specimen be subjected to detailed histopathological examination for a clinico-morphological correlation to ensure proper management.

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Original Research Article

Immunohistochemical Analysis of Vascular Endothelial Growth Factor Receptor in Human Uterine Leiomyomas in Relation to Endometrial Hormonal Pattern

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Abstract

Aim: To analyze the expression of VEGF and find the significance of upregulation of VEGF in uterine leiomyomas in different phases of endometrium.

Materials and Methods: A total of 50 leiomyoma specimens were selected of which 25 cases showing proliferative phase in endometrium, 25 cases showing secretory phase in endometrium and 10 cases of normal myometrium used as control. Haematoxylin and Eosin slides of all the tissues were evaluated. The formalin fixed, paraffin embedded blocks were sliced in 3-4 µm thickness for IHC. The Avidin Biotin complex (ABC) detection system was used. Immunoreactivity was regarded as positive when brown staining was localized in the cytoplasm of leiomyoma cells.

Results: The intensity of immunostaining in leiomyoma and matched myometrium in both phases of menstrual cycle was observed and graded as 0,1+,2+ and 3+.

Conclusion: VEGF expression in leiomyoma was abundant relative to the normal myometrium of the same individual uterus and that VEGF protein expression in leiomyoma cells predominated in the secretory phase than the proliferative phase. Therefore, both oestrogen E2 and progesterone P4 seemed to induce the tumour growth. VEGF expression was significantly higher in those with myomas compared to those with a healthy myometrium suggesting that VEGF may play a significant role in pathogenesis of uterine myomas. These suggest that VEGF and other angiogenic factors may represent potential targets for the treatment and prevention of uterine fibroids.

Keywords: VEGF Protein; Leiomyoma; Immunohistochemistry.

Introduction

Uterine leiomyoma is the most common benign smooth muscle cell tumor of the myometrium, occurring in as many as 30% of women over 35 years of age [1]. Prevalence rate for uterine fibroids is approximately 1 in 20 or 5 % or 13.6 million people globally. As per the Country/Region Extrapolated Prevalence Population Estimated to have uterine fibroid in India is 53 million in a total population of 1.2 billion [2]. Leiomyomas are a frequent cause of menorrhagia, dysmenorrhea, pelvic pain, reduced fertility, and recurrent pregnancy loss.A growing body of evidence suggests that the action of sex steroids may be mediated in part by local growth factors produced by the target cells [3,4,5]. However, the mechanisms of ovarian steroid hormone actions in the regulation of leiomyoma growth are not well defined yet.

Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulates vasculogenesis

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and angiogenesis. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate.

Serum concentration of VEGF is high in bronchial asthma and diabetes mellitus [6]. VEGF's normal function is to create new blood vessels during embryonic development, new blood vessels after injury, muscle following exercise, and new vessels (collateral circulation) to bypass blocked vessels. When VEGF is overexpressed, it can contribute to disease. Solid cancers cannot grow beyond a limited size without an adequate blood supply; cancers that can express VEGF are able to grow and metastasize.

VEGF mRNA and protein expression have been identified in the smooth muscle cells of both normal myometrium and leiomyomas [7,8,9]. The VEGF receptors, VEGFR-1 and VEGFR-2 are also expressed in myometrium [10], leiomyomas and in cellular leiomyomas [9]. A stronger VEGF expression was found in leiomyomas than in adjacent myometrium, indicating that local angiogenesis may be important for the development and growth of these tumours [11] Hong et al. (2001) reported that VEGF is significantly expressed in leiomyosarcoma compared with leiomyoma. VEGF stimulates angiogenic activity which is responsible for actively growing tumours and may enhance the growth of fibroids and disease progression in many carcinomas [12,13,14]. Both E2 end P4 induce VEGF expression in the rodent uterus[15].

Materials and Methods

This study was conducted at the Meenakshi Medical College and Research Institute Hospital, a rural tertiary care hospital. The Institutional Medical Ethics Committee approved this study. Fifty cases were selected from August 2015 until July 2017 and it was diagnosed by a single histopathologist to avoid inter-observer variations.

Uterine leiomyomas and adjacent normal myometrial tissues were obtained from women with regular menstrual cycles who underwent abdominal hysterectomy for medically indicated reasons at Meenakshi Medical College and Hospital. The mean age group was 39 years, and none had received hormonal therapy for at least three cycles before surgery. Informed consent was obtained from each patient before surgery for the use of uterine tissues for the present studies. Each uterine specimen was examined by a pathologist for histological examination and dating of the endometrium. Endometrial tissues were obtained from the extirpated uteri, and the day of the menstrual cycle was determined by endometrial histological dating according to the method of Noyes et al. and the patient's last menstrual period. Histological features of all cases studied with hematoxylin and Eosin. A total of 50 uterine leiomyomas and myometrial tissues were collected of which 50% were from proliferative phase and 50% from secretory phase of menstrual cycle.

Inclusion Criteria

Cases from August 2015 to July 2017 were selected.

Exclusion Criteria

Samples were excluded if accurate menstrual cycle dates could not be assigned, if unexpected pathology was found eg - endometrial hyperplasia and leiomyoma variants.

Immunohistochemical Study

Haematoxylin and Eosin slides of all the tissues were evaluated, and for each case the best paraffin block with highest tumour content were chosen in order to prevent artefacts staining.

These formalin fixed, paraffin embedded blocks were sliced in 3-4 µm thickness for IHC. The Avidin Biotin complex (ABC) detection system was used on specimens of formalin fixed, paraffin embedded tissue section.

Scoring System

Immunoreactivity was regarded as positive when brown staining was localized in the cytoplasm of leiomyoma cells. The intensity of immuno staining was evaluated by repeated staining of the same specimens.

Grading

(-) : No immunostaining

(1+) : Weak but definitely detectable immunostaining

(2+) : Moderate immunostaining

(3+) : Intense immunostaining

The extent was semiquantitatively estimated with a range of 0% to 100%. Percentages were estimated by counting at least 50 cells and then establishing the ratio of immunoreactive cells to total number of cells multiplied by 100; percentages were rounded to the nearest 10%. When less than 10% of cells were positive a score of 0 was used, 10 % to 30% cell positivity was scored as 1 , 31% to 60% positivity was scored as 2 , and more than 60% positive cells was labelled as 3.

Statistical Analysis

Statistical analysis was carried out using SPSS version 19.0 (IBM SPSS, US) software with Regression Modules installed. All data was entered into a Data Collection Proforma Sheet and were entered into Excel (MS Excel 2010). Results are represented in the form of tables and bar charts.

Results

A total of 50 uterine leiomyomas and myometrial tissues were collected of which 25 were from proliferative phase and 25 from secretory phase of menstrual cycle. Hysterectomy specimen with endometrial hyperplasia, leiomyoma variants, ovarian pathology and cervical pathology were not included in the study. Immunohistochemical study was done using VEGF antibody on all 50 cases selected.

Leiomyomas are seen in the women of child bearing age, most commonly occurring in the 4thdecade. The mean age being 39 years. The youngest patient in our study was 25 years old, and the oldest was 54 years old.

Though leiomyoma is a disease of low parity, in our study we have noted it to be more common in para-II women.

In our study, most of the patients presented with menstrual disturbances followed by pain abdomen and dysmenorrhea. Rare symptoms included vaginal mass and infertility. In our study it was noted that the size of the fibroid uterus varied from a few centimeters to 30 weeks of gravid uterus. It is seen that, about 38% were of the size of 16 weeks gravid uterus, 42% were of the size between 16-20 weeks, and huge fibroids of >20 weeks were encountered in 20% of the patients. The heaviest fibroid weighed 3.5 kg.

All the leiomyomata were corporeal, no extrauterine fibroids were encountered. Among the uterine about 98% were in the body & 2% were in cervix. Intramural fibroid was the commonest variety comprising about 74% of the cases, 10% submucous, 14% subserous and 2% cervical fibroid was encountered

In our study we selected our cases in accordance to proliferative and secretory phases of normal menstrual cycle. All other pattern of endometrium was excluded. For our case we selected 50% of each proliferative and secretory phase.

Table 1: VEGF Expression in Leiomyoma & Myometrium (Proliferative phase)

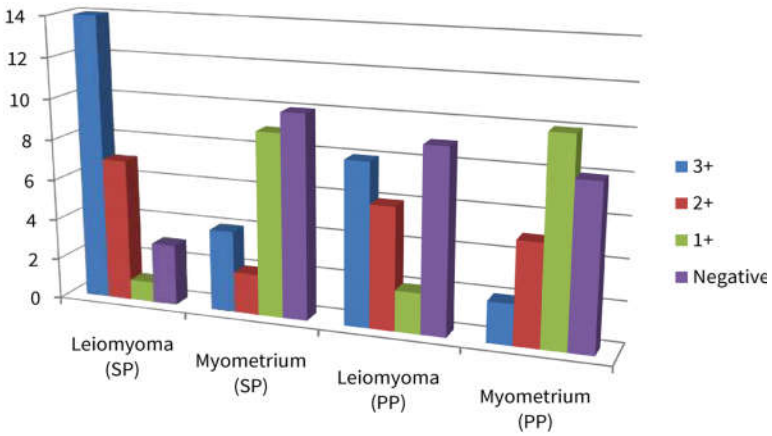
S. No	Cases	VEGF Antibody	
		Positive	Negative
1	Leiomyoma	65%	35%
2	Myometrium	70%	30%

Table 2: VEGF Expression in Leiomyoma & Myometrium (Secretory phase)

S. No	Cases	VEGF Antibody	
		Positive	Negative
1	Leiomyoma	90%	10%
2	Myometrium	68%	32%

Table 3: Comparison of intensity of VEGF expression in Leiomyoma and Myometrium in Secretory phase

S. No	Intensityof VEGF antibody	Proliferative phase	
		Leiomyoma	Myometrium
1	3+	8	2
2	2+	6	5
3	1+	2	10
4	Negative	9	8



Graph 1: Comparison of intensity of VEGF staining in leiomyoma and myometrium in both phases of menstrual cycle

Table 4: Comparison of intensity of VEGF expression in Leiomyoma and Myometrium in Secretory phase

S. No	Intensity of VEGF Antibody	Secretory phase	
		Leiomyoma	Myometrium
1	3+	14	4
2	2+	7	2
3	1+	1	9
4	Negative	3	10

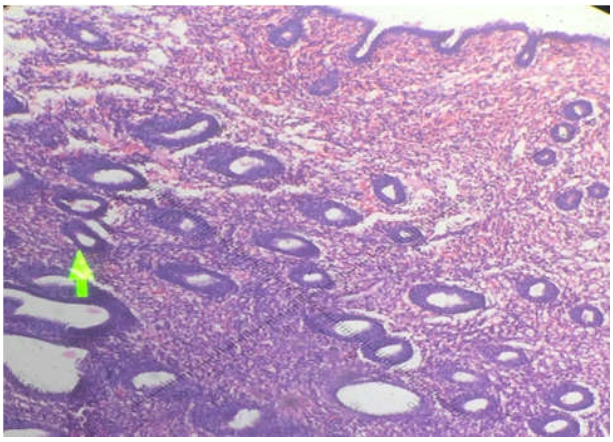


Fig. 1a: Photomicrograph of Proliferative phase (H&E, magnification 400X)

Fig. 1b: Photomicrograph of VEGF expression in leiomyoma in proliferative phase (IHC, magnification 400X)

Fig. 1c: Photomicrograph of VEGF expression in myometrium in proliferative phase (IHC, magnification 400X)

Leiomyoma cases showed 65% positivity while normal myometrium showed 70% of positivity (Table 1),(Figure 1a,1b,1c). Thus indicating that VEGF expression remained unchanged in leiomyoma and myometrium.

Leiomyoma cases showed 90% positivity while normal myometrium showed 68% of positivity in secretory phase (Table 2),(Figure 2a, 2b, 2c). Thus indicating that VEGF expression was more prominent in leiomyoma than myometrium.

Comparison of immunohistochemical staining for VEGF protein showed that leiomyoma cells in the secretory phase of the menstrual cycle showed more predominant immunostaining than the leiomyoma cells in the proliferative phase. However, there was not much difference in the intensity of immunostaining for VEGF protein in myometrial smooth muscle cells between the proliferative phase and the secretory phase of the menstrual cycle (Table 3, 4) (Graph 1).

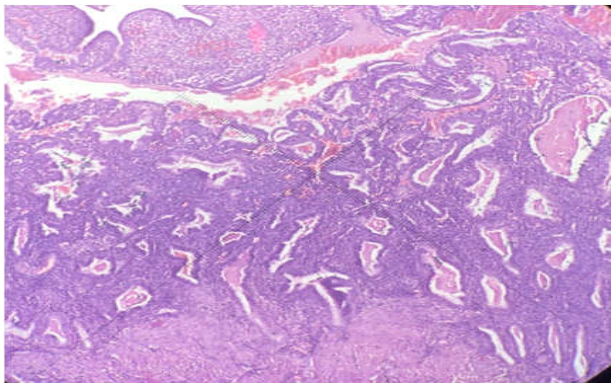


Fig. 2a: Photomicrograph of secretory phase (H&E, magnification 400X)

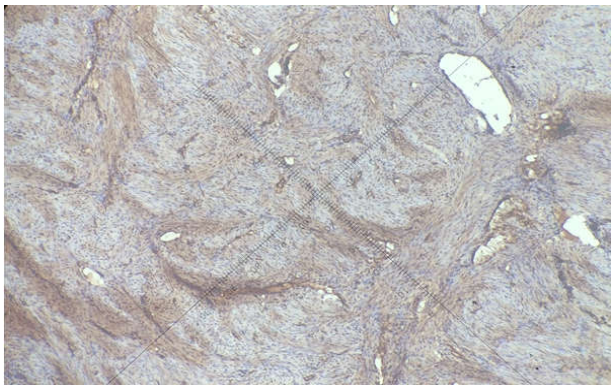


Fig. 2b: Photomicrograph of VEGF expression in leiomyoma in secretory phase (IHC, magnification 400X)

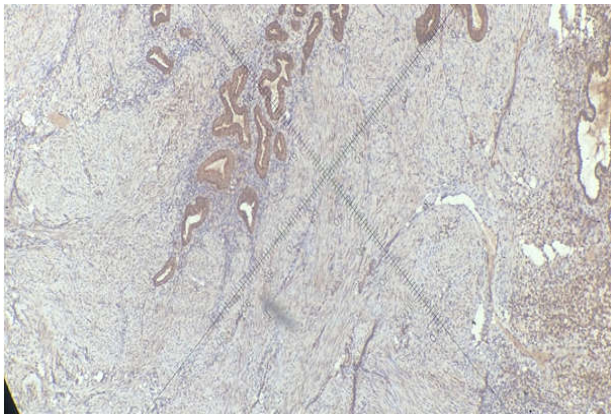


Fig. 2c: Photomicrograph VEGF expression in myometrium in secretory phase (IHC, magnification 400X)

Discussion

Immunohistochemical staining comparison of VEGF showed that leiomyoma cells showed intense immunostaining during secretory phase than during the proliferative phase. However there was no difference in the immmunostaining pattern of VEGF protein in the myometrial smooth muscle cells between the proliferative and secretory phase of the menstrual cycle.

Lewicka et al, found that VEGF expression was found to be increased in the leiomyoma cells in patients with endometrium in the secretory phase compared to patients with endometrium in proliferative phase. It was also revealed that VEGF expression was 2.03 fold higher in those with myomas than those with healthy myometrium. This suggested that VEGF may play a significant role in the pathogenesis of uterine leiomyomas.

In a study by Gentry et al, it was found that VEGF was expressed in 14 out of 18 leiomyoma sections from women without GnRH pretreatment, and in 15 out of 18 of those women with prior treatment. VEGF expression in the adjacent myometrium was much lower being noted in 2 out of 18 sections from women without prior GnRH treatment and 1 out of 18 in women with prior GnRH pretreatment. Moreover, when VEGF expression was present, expression was strong in leiomyomas, but not in adjacent myometrium. The differential expression of VEGF antigen in leiomyomas compared with adjacent myometrium indicates that local angiogenesis may be important in the development and growth of these tumours. GnRHa therapy does not appear to alter this pattern of VEGF expression.

Studies done by Dixon et al, showed that VEGF protein was seen in the cytoplasm of smooth muscle cells of leiomyomas and matched myometrium. In a study by Hyder et al, it was found that VEGF was expressed more during the oestrogenic phase than the progesterone phase of the menstrual cycle. In a study by Sanci et al, it was found that EGFR had mild to moderate immunoreactivity in leiomyoma cells compared to a lesser staining pattern in cellular leiomyoma but a strong immunoreactivity was noted in cells of leiomyosarcoma.

In the same study, it was found that VEGF had moderate immunostaining pattern compared to a lesser staining pattern in cellular leiomyoma in contrast to a stronger immunoreactivity in leiomyosarcoma.

All these results suggests that in both leiomyoma and leiomyosarcomas, angiogenesis factors such as VEGF and its receptors may be involved in tumour angioigenesis.

Conclusion

Based on our data on VEGF staining in leiomyoma and normal myometrium in relation to secretory and proliferative phase of menstrual cycle and the results from the literature on the expression of VEGF we conclude there is an abundance of VEGF expression in leiomyoma relative to the normal myometrium of the same individual uterus and that VEGF protein expression in leiomyoma cells predominated in the secretory phase than the proliferative phase, though there was not much of significant difference in number was noted. Therefore, both oestrogen E2 and progesterone P4 seemed to induce the tumour growth. VEGF expression was significantly higher in those with myomas compared to those with a healthy myometrium suggesting that VEGF may play a significant role in pathogenesis of uterine myomas. These suggest that VEGF and other angiogenic factors may represent potential targets for the treatment and prevention of uterine fibroids.

The limitations of our study using immunohistochemical analysis alone should be acknowledged; for completion, it may require further validation using other methods including cell culture, immunoblotting, molecular and cytogenetic methods for understanding the underlying molecular mechanisms that determine VEGF over-expression and other pathways in development of uterine smooth muscle tumors. It is worth mentioning that, our results may contribute to clarify the sequential evolution in the development and treatment of uterine leiomyoma.

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Original Research Article

An Immunohistochemical study of Human Papilloma Virus 16 (HPV 16) Expression in the Various Types of Carcinoma Cervix in the Rural Population of Tamilnadu

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Abstract

Objective: To Study and observe the immunoexpression of HPV 16 in the various types of carcinoma cervix (Ca Cx) diagnosed in the rural tertiary care hospital in Tamilnadu.

Materials & Method: The biopsies 243 cases of carcinoma cervix which was diagnosed in the Division of Pathology for the period of 5 years from January 2011 and December 2016were included for the study. A Total of 43 cases were selected for the immunohistochemical staining (AVIDIN-BIOTIN Method) to find the immunoexpression for HPV 16. A positive immunoexpression of 48.8% was observed for HPV 16 out of which 51.3% are Squamous cell carcinoma and 1 case of Carcinosarcoma of cervix. 6 cases of Adenocarcinoma and 1 case of small cell carcinoma was negative for HPV 16 immunoexpression.

Conclusion: Regular and periodic screening for all women above 30 years for cervical cancer and Screening of the most common high-risk HPV types should be done. Cervical cancer awareness programs should have regular follow up.

Keywords: Carcinoma Cervix; High Risk Human Papilloma Virus; HPV 16; Immunoexpression.

Introduction

Carcinoma cervix is one of the leading malignant neoplasms in the world as well as in Indian subcontinent. It is one of the major cause of morbidity and mortality which disproportionately affects the poorest population of developing countries [1,9]. Carcinoma cervix relatively affects young female population. The association of several risk factors which include promiscuity, multiple sexual partners, and intercourse at an early age, oral contraceptives, and smoking was demonstrated by epidemiological studies. However, the association of viral infection was considered an important factor for over a century. The present study was undertaken to analyse the histopathologic pattern of carcinoma cervix and to study the association of HPV 16 immunoexpression in selected cases in the rural population of Tamilnadu.

Materials and Method

A total of 243 biopsies, diagnosed as carcinoma of cervix in the Department of Pathology, Rajah Muthiah Medical College and Hospital, Annamalai University, Chidambaram, for the period of 5 years from January 2011 and June 2016 were included in the study. A 4 micrometre thickness sections from paraffin embedded blocks were subjected to routine Haematoxylin and Eosin staining procedure. HPV 16 immunostaining with Streptavidin Biotin Conjugate Method (ABC Method) was

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carried out in selected cases. The data was analysed for the histopathological types of carcinoma cervix and positive immunostaining. The positive control was provided by Biogenix.

Observation

Two hundred sixty nine cases of Female Genital Tract (FGT) malignancy cases were diagnosed in the study period. In that, carcinoma cervix accounted for 243 cases (90.3%). The commonest histopathological diagnosis of Carcinoma cervix was Squamous cell carcinoma, 235 cases (95.3%) followed by adenocarcinoma of cervix 6 cases (2.5%) & 1 case of carcinosarcoma (0.41%)

Out of the 43 cases of Ca cx selected for the immunohistochemical (including all variants) 48.8% showed positive immunoexpression for HPV 16 (The positive control was provided by Biogenix). The most common positive staining for HPV 16 was observed in Squamous cell carcinoma of cervix (51.3%). Carcinosarcoma and Small cell carcinoma of cervix showed negative immunoexpression for HPV 16.

Table 1: Incidence of Malignancies in Women

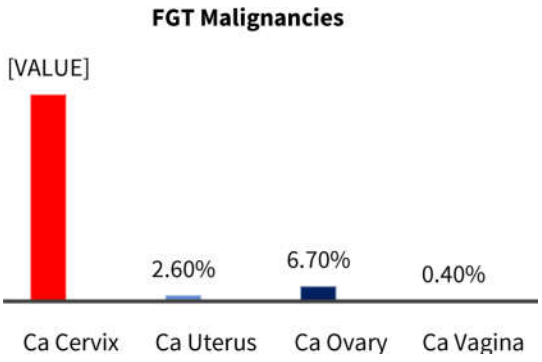
1	Total number of biopsies	26,026
2	Total number of malignancies	1511 (5.8%)
3	Malignancies in women	758 (50.1%)
4	Malignancies of Female Genital Tract	269 (35.5%)
5	Malignant tumors of breast	162 (21.4%)
6	Malignant tumors of other systems	327 (43.1%)

Table 2: Incidence of FGT Malignancies

1	Malignant tumors of cervix	243 (90.3%)
2	Malignant tumors of uterine body	7 (2.6%)
3	Malignant tumors of ovary	18 (6.7%)
4	Malignant tumors of vagina	1 (0.4%)

Table 3: Positive immunoexpression for HPV 16 in the Carcinoma cervix cases

S. No.	Histopathological type	Number of Cases of Ca Cx	Positivity for HPV 16
1	Squamous cell Carcinoma	39	20 (51.3%)
2	Adenocarcinoma	1	1
3	Small cell carcinoma	2	0
4	Carcinosarcoma	1	0
	Total	43	21 (48.8%)



Graph 1:

Discussion

Carcinoma of cervix (Ca Cx) is the second leading cause of death in female malignancy [1,2]. Although Human Papilloma Viral (HPV) infection proved 100% to be the cause of cancer cervix [3]. HPV 16 & 18 is responsible for about 70-80% of the cervical cancer worldwide [4]. A uniquely lethal character of the Ca Cx by HPV infection is it strikes the women a very early and relatively young age [5]. The distribution of HPV 16 is around 50 – 60% in India and various parts of the world [6,7]. In the present study we tried to estimate the prevalence of the HPV 16 in the specimen of carcinoma cervix in the rural region medical college hospital. The prevalence of HPV 16 in the present study is around 51% which is comparable with the other incidence rate [8,9]. Although multifactorial causes are implicated in CaCx, early age exposure to HPV virus is attributed to almost all cases of the carcinoma cervix [10,11]. The screening program which is already in existence like Visual Inspection with Acetic acid (VIA) along with PAP smear should include the detection of HPV strains, at least the high-risk strains HPV 16 & 18 [11]. Since HPV 16 prevalence in asymptomatic is also high [12]. Easy accesses should be available to the awareness programs in the rural and underdeveloped areas. Awareness program essentially in the native language about cervical cancer and the screening modalities along with personal hygiene. Follow-up in positive cases of screened. There

are very good model projects which uses cost effective HPV screening, self-sampling and effective and result-oriented low-cost treatment modalities [13], which we can adapt to decrease the burden of Ca Cx and to give effective treatment for the HPV positive cases.

Conclusion

The Carcinoma cervix, although showed an alarming rate of increase in Indian population and with more than substantial evidence of the major causative factor, Human Papilloma Virus (HPV 16). There are no nationwide screening programs for detection of HPV in cervical cancer, almost 80% of Ca Cx is by HPV infection. It is suggested to screen the at least the most common high-risk Human Papilloma Virus, namely HPV 16 along with the routine screening modalities at the level of rural medical college hospitals and try to adapt cost effective screening and treatment model projects.

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Original Research Article

Cytomorphological Spectrum of Lymph Node Lesions on FNA with Special Reference to Acid Fast Bacilli Positivity in Tuberculous Lymphadenitis: A 3-Year Study in a Medical College Hospital in Mandya, Karnataka

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Abstract

Background: Evaluation of lymphadenopathy is of clinical significance as the underlying disease may range from a treatable infectious etiology to malignant neoplasm.Peripheral lymphadenopathy is common in all age groups and management of cases depends on lymph node pathology, which can be evaluated by fine-needle aspiration or excision biopsy.

Objectives: The study was undertaken to assess Cytomorphological spectrum of lymph node lesions on FNA with special reference to Acid Fast Bacilli positivity in TuberculousLymphadenitis.

Materials and Methods: A 3 years retrospective analysis of the data was carried out on 944 patients of lymphadenopathy in the Department of Pathology, Mandya Institute of Medical Sciences, Mandya from January 2013 to December 2015. Fine needle aspiration cytology (FNAC) was done and smears were stained with Hematoxylin and Eosin, May-Grunwald-Giemsa and Papanicolaou (PAP) stains. Other stains like Ziehl-Neelson (ZN), Periodic Acid Schiff (PAS), etc were done wherever required.

Results: The spectrum of various lymph node lesions on cytomorphological findings in this study showed 560 cases (59.32%) of reactive nature, 178 cases (18.85%) were tubercular , 34 cases (3.6%) were of acute lymphadenitis and 8 cases (1%) were of chronic lymphadenitis. AFB positivity was found in 39.8% of tuberculous lymphadenitis patients. The remaining 164 cases (17.37%) were of malignant lymphadenopathy, consisting 15 cases (1.58%) of primary malignancies i.e. lymphomas and 149 cases (15.78%) of metastasis to lymph nodes. On correlation of FNAC findings with histopathology overall sensitivity, specificity and diagnostic accuracy as 97.9%, 99.7% and 99.3% respectively.

Conclusion: Fine needle aspiration study is an important tool for evaluation and management of various lymphoid disorders. It is a safe, simple, cost effective and reliable technique for establishing the diagnosis. Demonstration of AFB helps to establish the diagnosis of tuberculous lymphadenitis

Keywords: FNAC; Tuberculosis; AFB; Ziehl Neelson Stain.

Introduction

Lymphadenopathy is one of the most common clinical finding in outpatient department [1]. It may be due to infections, autoimmune diseases, metabolic disorders, malignancy etc [2]. Most often it may be the only manifestation of underlying occult malignancy [2].

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Fine needle aspiration cytology is a simple, rapid, safe, cost effective procedure and unlike a surgical biopsy, it does not produce any residual scar [3]. It generally does not require anaesthesia or hospitalization, and can be used as an alternative investigation technique to excision biopsy [4]. It can be used for superficial lymphadenopathies as well as deep seated lymphadenopathies in abdominal cavity and thorax with the help of CT scan and ultrasonography [1]. It is used as diagnostic tool in the management of patients presenting with lymphadenopathy and should be considered before more invasive and costly procedures particularly in developing countries [5].

Tuberculous involvement of lymph node is the most common manifestation of extrapulmonary tuberculosis [6]. The incidence of mycobacterial lymphadenitis has increased in parallel with the increase in the incidence of mycobacterial infection worldwide [7]. Tuberculous lymphadenitis is seen in nearly 35 per cent of extrapulmonary tuberculosis and constitutes about 15 to 20 per cent of all cases of tuberculosis [7].

Acid fast staining of FNAC smears is highly valuable for routine diagnosis of tuberculosis [8]. In cases of atypical presentation of tuberculosis such as aspiration of purulent material by FNAC, they may be misdiagnosed as acute suppurative lymphadenitis in the absence of granuloma. Demonstration of Acid Fast Bacilli helps to diagnose such cases as Tuberculosis [9].

FNAC of lympnodes is very helpful to diagnose primary and secondary malignancy. Malignancies in lymph node are predominantly metastatic in nature [10]. A sentinel lymph node may be the first sign of underlying malignancy in the thoracic cavity, abdominal cavity or pelvic region [11].

Materials and Methods

A 3 year retrospective analysis of the data was carried out on 944 patients of lymphadenopathy in the Department of Pathology Mandya Institute of Medical Sciences, Mandya from January 2013 to December 2015. All patients who had presented with palpable lymphadenopathy to the Cytopathology laboratory of the Department of Pathology, MIMS, Mandya during the study period were included in the study irrespective of age and sex.

Data was retrieved from the records of the Department of Pathology. FNAC was performed by a cytopathologist , using a 23 G needle attached to 10ml syringe and Franzen handle.

Smears of FNAC of lymph node done during the study period and stained with Haematoxylin and Eosin and/or Papaniculaou stain, and May- Graunwald Giemsa stain were retrieved and reviewed under the microscope. In all cases where cytological diagnosis was of tuberculous lymphadenitis, smears stained with Ziehl Neelson stain were reviewed for presence or absence of Acid fast bacilli.

Records of lymph node biopsy specimens received to Histopathology Laboratory during the study period were reviewed and details regarding their gross appearance are recorded. Hisopathological sections stained with Hematoxyllin and Eosin stain of all such cases were retrieved and reviewed under the microscope.

Results

A total of 944 FNACs were studied. The age of the patients ranged from <1year to 80 years with a male to female ratio of 1.4:1. The nodal site sampled most frequently was cervical group in 793 cases (84%),followed by inguinal group in 74 cases (8%), axillary groups in 60 cases (6%) , generalized lymphadenopathy in 12 cases(1.27%), epitrochlear in 2 cases and femoral in 1 case (Table 1).

Maximum cases sampled were between 1-10 years (177), while 151 cases were in the group 11-20 years, 172cases were in the group 21-30 years, 153 cases were in the group 31-40 years, 110 cases were in the group 41-50 years,81 cases belonged to age group 51-60 years,72 cases were in the group 61-70 years and 28 cases were in the group 71-80 years. Reactive hyperplasia was most common in age group 1-10 years, tuberculous lymphadenopathy was commonly seen in age group 21-40 years, most of the lymphoma cases was seen in more than 40 years of age and metastatic carcinoma was most commonly seen in the age of more than 60 years (Table 2).

The patients were evaluated into two broad categories of benign and malignant disorders. 780 cases (82.62%) were of benign lymphadenopathy, of which 560 cases (59.32%) were of reactive nature, 178 cases (18.85%) were tubercular, 34 cases were of acute lymphadenitis and 8 cases were of chronic lymphadenitis.

The remaining 164 cases (17.37%) were of malignant lymphadenopathy, consisting 15 cases (1.58%) of primary malignancies i.e. lymphomas and 149 cases (15.78%) of metastasis to lymph nodes. Thus, it was noted that metastatic tumour accounted for 91% cases of malignant lymphadenopathy, Non-Hodgkin's lymphoma for 11cases (6.57%) and Hodgkin's lymphoma for 4 cases (2.43%). Out of total 149 cases (15.78%) of metastasis to lymph node, 97 (65%) were of metastatic squamous cell carcinoma, 14 (9.4%) were of metastatic ductal carcinoma from breast, 5 (3.35%) were of metastatic malignant melanoma, 4 (2.68%) were of metastatic adenocarcinoma, 4(2.68%) were suspicious of malignancy, 3 (2%) were of metastatic papillary carcinoma of thyroid and 22(14.7%) were of metastatic poorly differentiated carcinoma.

Table 1: Site wise distribution of lymph node lesions

Cytological diagnosis	Total cases	Cervical	Axillary	Inguinal	Femoral	Epitrochlear	Generalised
BenignLymphadenpathies							
Reactive hyperplasia	560	468	26	52	01	02	11
Acute lymphadenitis	34	27	01	06	-	-	-
Chronic lymphadenitis	08	07	-	01	-	-	-
Tuberculous lymphadenitis	178	158	18	02			
Malignant Lymphadenopathies							
1. Lymphoma	15	10	01	03	-	-	-
Hodgkin	04	03	-	01	-	-	-
Non Hodgkin	11	07	01	02	-	-	01
2. Metastatic tumor	149	123	14	12	-	-	-
Squamous Cell Carcinoma	97	92	05				
Infiltrating Ductal Carcinoma	14	03	11				
Poorly differentiated carcinoma	22	15	02	02			
Adenocarcinoma	04	04					
Malignant melanoma	05	01		04			
Papillary carcinoma Thyroid	03	03					
Suspicious of malignancy	04						
Total	944	793	60	74	01	02	12

Table 2: Distribution of lymph node lesions in relation to Age

Age (yrs)	Reactive hyperplasia	Acute lymphadenitis	Chronic lymphadenitis	TB lymphadenitis	Lymphoma	Metastatic carcinoma	Total cases (%)
1-10	152	08	01	14	01	01	177(18.75)
11-20	120	06	01	22	01	01	151(16)
21-30	110	05	02	53	02	-	172(18.22)
31-40	88	03	02	53	01	06	153(16.20)
41-50	54	03	-	17	04	32	110(11.65)
51-60	26	03	01	10	03	38	81(8.58)
61-70	08	06	01	05	03	49	72(7.62)
>70	02	-	-	04	-	22	28(2.96)
Total	560	34	08	178	15	149	944(100)

Table 3: Distribution of various lymph node lesions

Sl. No	Cytological diagnosis	No. of cases	Percentage (%)
1.	Reactive hyperplasia	560	59
2.	Acute lymphadenitis	34	3.6
3.	Chronicnon specific lymphadenitis	08	1
4.	Tuberculous lymphadenitis	178	19
5.	Lymphoma	15	1.5
6.	Metastatic tumor	149	16

Table 4: Cytomorphological features in tuberculouslymphadenitis with AFB positivity

Cytomorphological features	Cases (%)	AFB Positive (%)
Epithelioid granuloma without caseous necrosis	51(28.65%)	06(11.76%)
Epithelioid granuloma with caseous necrosis	72(40.44%)	52(72.22%)
Caseous necrosis without granuloma	55(30.89%)	13(23.63%)
Total	178	71(39.88%)

Table 5: cytohistopathological correlation of lymph node lesions

Cytological Diagnosis	Histopathological Diagnosis					
	No. of cases	Reactive hyperplasia	Tuberculous lymphadenitis	Hodgkin lymphoma	Non Hodgkin lymphoma	Metastatic carcinoma
Reactive hyperplasia	12	11	01	-	-	-
Tuberculous lymphadenitis	08	-	08	-	-	-
Hodgkin lymphoma	03	-	-	03	-	-
NonHodgkin lymphoma	08	-	-	-	08	-
Metastatic carcinoma	23	-	-	-	-	23

Most of the squamous cell carcinoma metastasized to cervical lymph node, ductal carcinoma of breast to axillary lymph node, malignant melanoma to inguinal lymph node and metastatic carcinoma, metastatic adenocarcinoma, metastatic papillary carcinoma thyroid, metastatic poorly differentiated metastasized to cervical lymph nodes (Table 3).

The criteria by which a diagnosis of reactive lymphadenopathy was established included high cellularity, polymorphous pattern of cells without malignant features, mitotic activity and a significant number of tingible bodies (Figure 1a & 1b).

Acute suppurative lymphadenitis cases showed predominantly polymorphonuclear leukocytes, necrotic debris and other lymphoid cells.

The aspirates from lymph nodes were diagnosed as tubercular lymphadenopathy based on the presence of epitheloid cell granuloma and caseous necrosis with or without Langhan's giant cells in a background of lymphoid cells or epitheloid cell granuloma without caseation or only necrotic material consisting of diffuse granular debris. All cases were stained with Ziehl Neelson staining, among these only 39.88% cases were positive for acid fast bacilli (Figure 2a, 2b, 2c, 2d). Predominant cytomorphological pattern was epitheloid cell granuloma with caseous necrosis (40.44%). AFB positivity was seen in 71 cases (39.8%) and was maximum with epithelioid cell granulomas with necrosis (72.22%) followed by necrosis without granulomas (23.63%) (Table 4).

Non Hodgkin's lymphoma showed predominantly monomorphic population of lymphoid cells.(Figure 3a & 3b). Hodgkin's lymphoma showed a mixed cell population with characteristic Reed-sternberg giant cells (Figure 4a & 4b).

Metastatic squamous cell carcinoma showed tumor cells showing squamoid nature and atypia (Figure 5).

A cytohistopathological correlation was done in 54 cases. Out of 54 cases, 12 were of reactive hyperplasia, 11 of which were true positive and 01 were false positive which turned out to be tuberculous lymphadenopathy on histological examination .

In 178 cases of tuberculous lymphadenitis, histological correlation was seen in 08 cases that were exactly correlated. A total 54 malignant lymphadenopathy cases were correlated in which 11 were lymphomas and 23 were of metastatic carcinoma. Out of 11cases, 03 were of Hodgkins lymphpoma and 08 were of nonHodgkins lymphoma (Table 5).

Table 6: Comparison of AFB positivity on ZN staining

Study	AFB positivity on Z-N Staining (%)
S shamshad et al	46.4%
Adhikari et al	41.6%
Hirachand S et al	50%
Hemlatha et al	52%
Patel MM et al	31%
Present study	39.9%

Table 7: Statistical analysis of lymphnode lesions

Cytological diagnosis	No of cases	Histopathological diagnosis				Sensitivity (%)	Specificity (%)	Diagnostic accuracy (%)
		Reactive	Tuberculosis	Lymphoma	Metastasis			
Reactive	12	11	01			91.6%	99%	97.3%
Tuberculosis	08		08			100%	100%	100%
Lymphoma	11			11		100%	100%	100%
Metastatic tumor	23				23	100%	100%	100%
Total	54	11	09	11	23			
Overall						97.9%	99.75%	99.32%

Table 8: Comparison of statistical analysis

Studies	Sensitivity	Specificity	Diagnostic accuracy
Vijaya bharathi et al	97.18%	98.74%	98.26%
Shamshad s et al	94.6%	98.50%	97.6%
Present study	97.9%	99.75%	99.32%

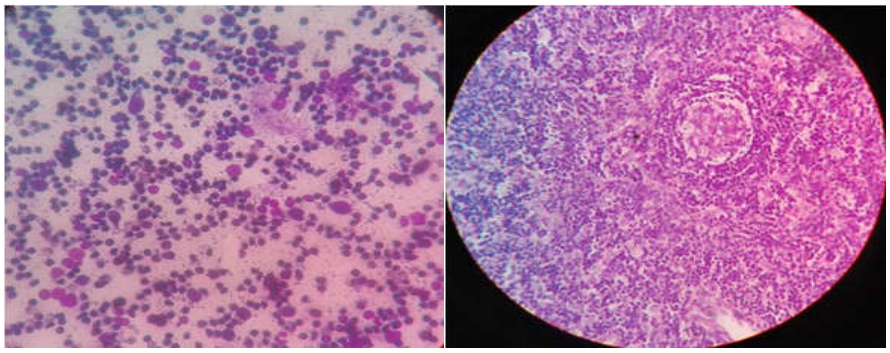

Fig. 1a: Reactive Hyperplasia:FNAC smear showing polymorphous population of lymphoid cells.MGG 40X. **Fig. 1b:** Reactive Hyperplasia:Section shows lymphoid follicle with germinal centre. H&E 40X

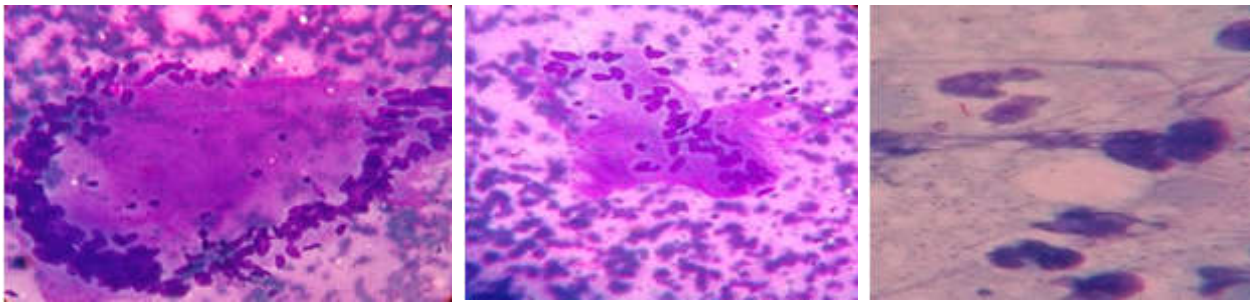

Fig. 2a: Tuberculous Lymphadenitis.:FNAC smear showing granuloma(MGG). **Fig. 2b:** Langhans giant cell(MGG) and Fig 2c: AFB positivity. ZN 40X

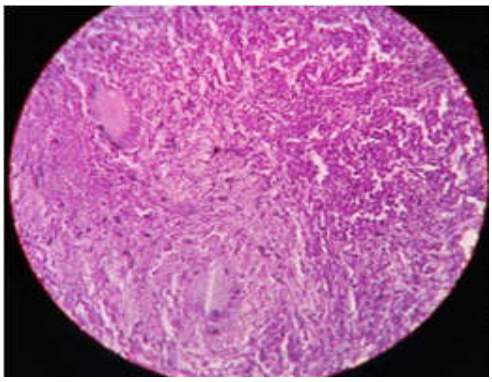

Fig. 2d: Tuberculous Lymphadenitis.Biopsy section showing granuloma,Langhans giant cell .H&E 40X

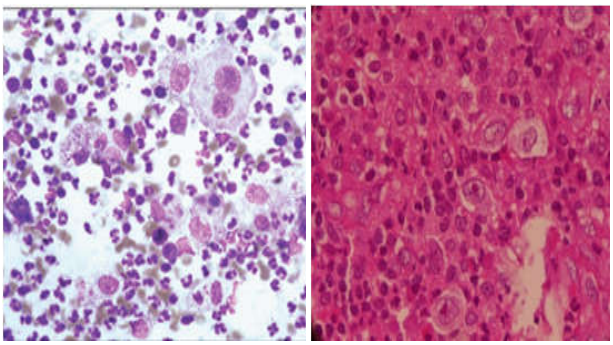

Fig. 4a: Hodgkins lymphoma..FNAC smear showing Reedsternberg cells and reactive cells. MGG 40X . **Fig. 4b:** Hodgkins lymphoma.Biopsy sectionshowing Reed Sternberg cells H&E 40X

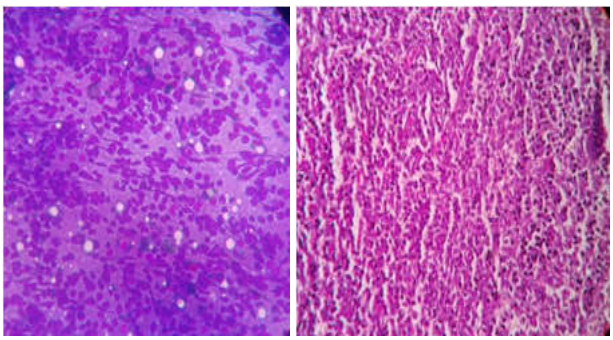

Fig. 3a: Non Hodgkins Lymphoma: FNAC smear showed predominantly monomorphic population of small lymphoid cells. MGG40X. **Fig. 3b:** Non Hodgkins lymphoma:Section shows effacement of lymphnode architecture by small lymphoid cells.H&E 40X

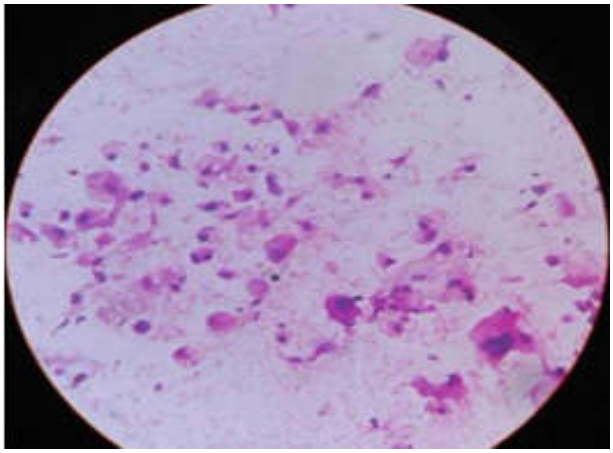

Fig. 5: Metastatic Squamous Cell Carcinoma.FNAC smear showing malignant squamous cells. H&E 40X.

Discussion

Fine needle aspiration cytology is a simple, safe, inexpensive and reliable investigative procedure .With the recent advances in ultrasound and CT scan technologies, deep lesions can be aspirated using these procedures and helps in preventing surgical intervention [12].

The lesions arising in the lymph node can be found in patients ranging from early to advanced age.In our study youngest patient was less than 1year old and oldest was 80 years of age. These figures come in close comparison to S.S. Ahmad et al study of 1000 patients where the youngest patient was 2years old and oldest was 95 years old.

In our study, a male preponderance was noted with a male to female ratio of 1.4:1 which correlated with Sumyra K.Q et al study which showed a M:F ratio 1.5:1

Cervical region was the most frequent site of lymphadenopathy in our study (84%). Many other studies also found [(Mandakini M.P et al (83.9%), Sumyra K. Q et al(76%), S.S. Ahmed et al (73.5%)] cervical region to be the most common site of involvement.

In our study most of the cases were benign (82.62%) and 17.37% were malignant. Our findings were similar to findings of Arjun singh et al study (85.4% and 14.6%).

Out of benign lesions, reactive lymphadenopathy was most common (59.32%) and 18.85% were tubercular which correlates closely with findings of Sumyra K.Q et al (36.9% and 9.1% respectively).

We reported 15 cases (1.58%) of lymphomas and 149 cases (15.78%)of metastasis to lymph nodeswhich correlate with findings of S.S.Ahmed et al (16.6%). Majority of the metastatic nodes sampled were squamous cell carcinoma (65%).

In the present study, the second most common cytological diagnosis was tuberculous lymphadenitis (178 cases). Predominant cytomorphological pattern was epitheloid cell granuloma with caseous necrosis (40.44%) which is comparable to findings of Mandakini M.P et al (64.85%).

AFB positivity was seen in 71 cases (39.8%) and was maximum with epithelioid cell granulomas with necrosis (72.22%) followed by necrosis without granuloma pattern (23.63%) which is comparable with the findings of Patel MM et al (31%), Adhikari et al (41.6%), S shamshad et al (46.4%), Hirachand S et al [13] (50%), Hemalatha et al study (52%) (Table 6).

A cytohistopathological correlation was possible in 54 cases. Out of 54 cases, 12 were of reactive hyperplasia, 11 of which were true positive and 01 was false positive which turned out to be tuberculous lymphadenopathy on histological examination .

In 178 cases of tuberculous lymphadenitis, histological correlation was possible in 08 cases that were exactly correlated. A total 34 malignant cases were correlated in which 11 were lymphomas and 23 were of metastatic carcinoma. Out of 11cases, 03 were of Hodgkins lymphpoma and 08 were of non Hodgkins lymphoma and were correlated with histopathology .

In the present study overall sensitivity, specificty and diagnostic accuracy of FNAC 97.9%, 99.7% and 99.3% respectively (Table 7).

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Original Research Article

Evaluation of Role of Touch Cytology in Diagnosis of Helicobacter Pylori in Non-Ulcer Dyspepsia Cases

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Abstract

H. pylori has emerged as an undisputed cause of chronic active gastritis, however the relationship between H. pylori and non- ulcer dyspepsia is still a matter of controversy. Various tests are available for diagnosis of H. pylori. Research in several directions has brought forward no general consensus about the most suitable method to diagnose H. pylori . Hence this study was done to evaluate the role of touch cytology diagnosis of H. pylori infection in patients of non -ulcer dyspepsia and to compare it with histology and urease test.

***Material And Methods*:** Touch smears were made from antralbiopsies obtained from patients (125) undergoing upper GI endoscopy. Rapid urease test was performed. Touch smears were stained with Giemsa stain and histological sections with Haemtoxylin and Eosinstain. The gastric biopsies were classified as per Sydney system.

Observations and Results: In this study touch cytology was positive for H pylori in 53 out of 83 cases (62.8%). Organisms were better appreciated on smears than on the biopsies, smears took lesser time to screen and report was available to the patient and the clinician the same day. Urease test was positive in 57.8% cases making it a reliable and rapid test for diagnosis. The percentage positivity ofit is comparable to touch cytology results (62.8%) in our study. Thus touch smear cytology appears to complement histo-pathology.

Conclusions: Touch cytology is a quick and simple sensitive test for diagnosis of H. pylori infection in patients of non- ulcer dyspepsia. It complements histology and urease test.

Introduction

The discovery of Helicobacter pylori from gastric biopsies by Marshall and Warren in 1982 opened the flood gates to the new era of understanding of gastro-duodenal pathology [1]. Studies have shown that the presence of H.pylori is associated with a variety of gastrointestinal diseases including gastritis, gastric and duodenal ulcers, gastric adenocarcinomas and lymphomas [2,3]. However, its role in non-ulcer dyspepsia remains controversial [4]. The removal of the organism by antimicrobial therapyis correlated with the resolution of symptoms and cure of disease [5].

The tests available for the diagnosis of H. pylori can be categorized as direct and indirect. Cytology, histo-pathology, rapid urease test and culture method

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constitute direct evidence of its presence in a biopsy. Indirect tests include serological diagnosis ,stool antigen test and urea breath test (UBT), PCR, and even in situ hybridization. A test should be sensitive and specific, inexpensive, easy, rapid with good patient acceptance and minimally invasive. One such test is touch cytology [6].

Role of touch cytology is already established in diagnosis of various benign and malignant lesions of lymph nodes [7,8], its use in diagnosing non- neoplastic lesions of GIT is increasing.

The characteristic histological appearance of H. pylori is 3x0.5um, spiral rod, haematoxophilic on H&E stain .It is located adjacent to the gastric epithelium. Since these organisms browse on the mucosa, mucosal samples can be taken through endoscope, touch imprints can be made and the same biopsy be subjected for histo-pathological examination. Because histologic gastritis may be present in the absence of mucosal abnormalities, histological examination permits better correlation between presence of bacteria and its pathological result.

An important characteristic of H. pylori is its ability to produce urease [9]. The presence is indicated by color change. Urease test is dependent on density of bacteria and therefore most sensitive when performed on antral tissue.

The prevalence of H. pylori infection varies between developed and developing countries, being 30% and more than 80% respectively [10]. A recent report from India indicates almost 80% of the population is infected with H. pylori [10].

The discovery of H.Pylori and its close association with gastritis has led to speculation that it may have a role in causing symptoms "non- ulcer dyspepsia". It is defined as upper abdominal pain or epigastric pain, discomfort, heartburn, nausea ,vomiting or other symptoms referable to the upper GIT and lasting for more than four weeks, unrelated to exercise and for which no foal lesion or systemic disease can be found responsible [11].

Helicobacter pylori is found in 43 to 87% of subjects of NUD. Wide variation in subject's frequency represent both differences in criteria to diagnose NUD and different populations evaluated [12].

With this background the present study was done to evaluate the role of touch cytology in diagnosis of H. pylori in patients of non-ulcer dyspepsia and compare it with histology and urease test.

Material and Methods

The present study was conducted in department of pathology and endoscopy room of the gastro enter ology department of the hospital, over a period of 1 year. All the patients presenting with symptoms of dyspepsia like nausea, vomiting, belching, epigastric pain, requiring upper GI endoscopy were included. The total number of patients were 125. The biopsy was obtained with help of punch biopsy forceps (Olympus). 2-4 biopsies were taken from the antrum. The first biopsy was put in 10% W/V of the urease solution to detect for the presence of H.pylori. It was considered positive if the color changed to pink in one hour.

The second and the third biopsies were used to make touch smears. The biopsies were gently rolled on the two slides and were stained with MGG stain. The biopsies were then put into 10% buffered formalin for histological correlation, stained with H and E. They were graded according to The Sydney system 1994 [13].

Results

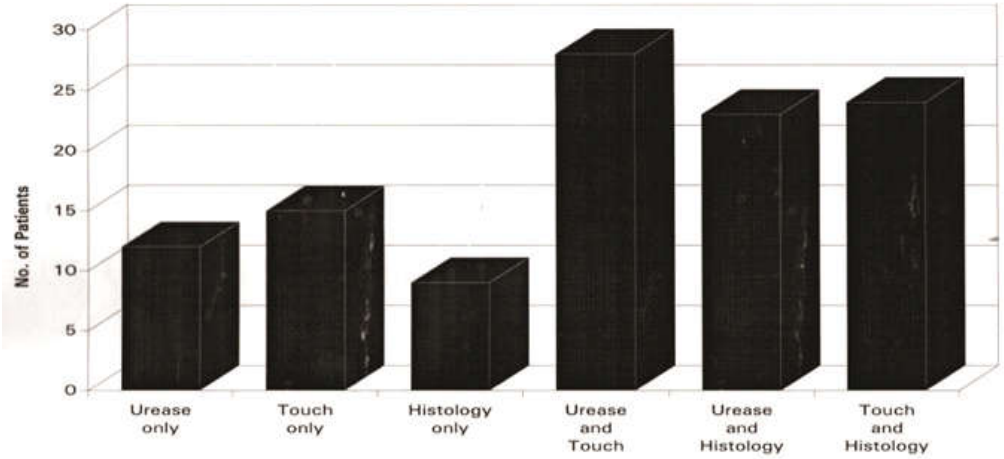
The study group comprised of 125 patients with 31-40 years as the most common age group (40%)(50). (Table 1). Out of total 83 cases positive for H. pylori by either urease test, touch cytology or histology, maximum cases were positive in age group of 31-40years (40.9%)(34) (Table -2).72%(90)were males and 28% were females (35). Most common symptom was pain (26.4%), followed by pain and belching (21.6%).

Out of 83 positivecases 26n experienced only pain (31.3%) while 18 patients (21.6%) had both pain and belching. The most common finding on endoscopy was antral gastritis 60n (48%). 29.6% (37) had normal study, while 10 (8%) had antral hyperemia, 7(5.6%) had erosive antral gastritis, 8 (6.4%)had duodenitis, 3 had antral ulcer (Table 3). Maximum percentage positivity fell in the group of antral gastritis 42 out of 60 (50.6%) and out of 37 patients with normal study, 23 were positive for H.pylori. All 3 patients with antral ulcer on endoscopy were positive for H. pylori by either of the methods.

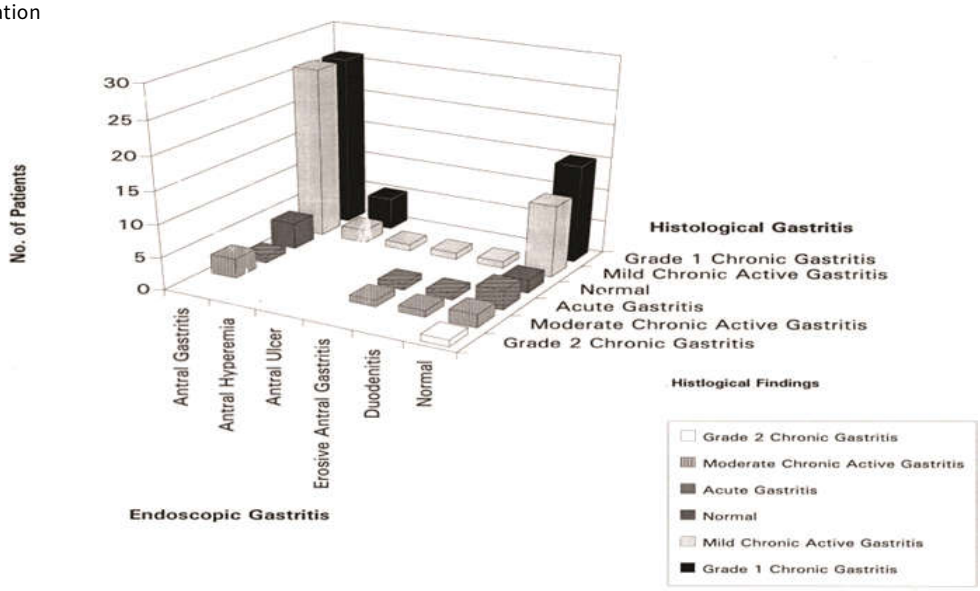
On correlating endoscopic diagnosis with histologic gastritis as per Sydney classification, out of 125 cases only 6 had normal histology others had evidence of acute, chronic and chronic active gastritis.

In our study, out of 83 positive cases, 53 were positive by touch smears (63.8%) 48 by urease test (57.8%) and 41 by histology (49.3%). Positive concordance rates was 12.8% (16) and negative concordance rate was 33.6% (42) (Table 4).

Inour study 50.9% (28 out of total 55) were positive by cytology and urease test. 52.2% (23 out of total 44) were positive by histology and urease test. Out of 48 cases 24 showed positivity by both histology and touch cytology (50%). The range of percentage positivity by two tests was in range of 50 to 52.2% (Table 5).



Graph 1: Respective contributions of there Different test for Diagnosis of Helicobacter pylori alone and in combination



Graph 2: Correlation endoscopic gastri with histologic gastri in patients of non-ul dyspepsia

Table 1: Helicobacter pylori positive in various age groups

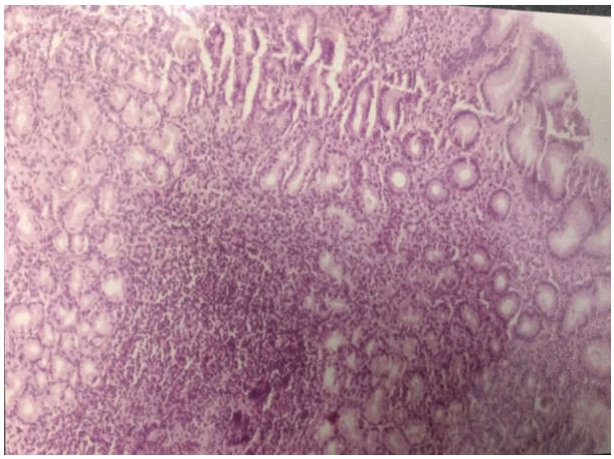
S. No	Age in Years	Number	H. Pylori Positive	Percentage
1	0--10	0	0	0
2	11—20	4	2	2.6
3	21—30	49	30	36.1
4	31—40	50	34	40.9
5	41—50	19	14	16.8
6	51--60	3	3	3.6
	Total	125	83	100

Table 2: Association of symptoms with helicobacter pylori infection

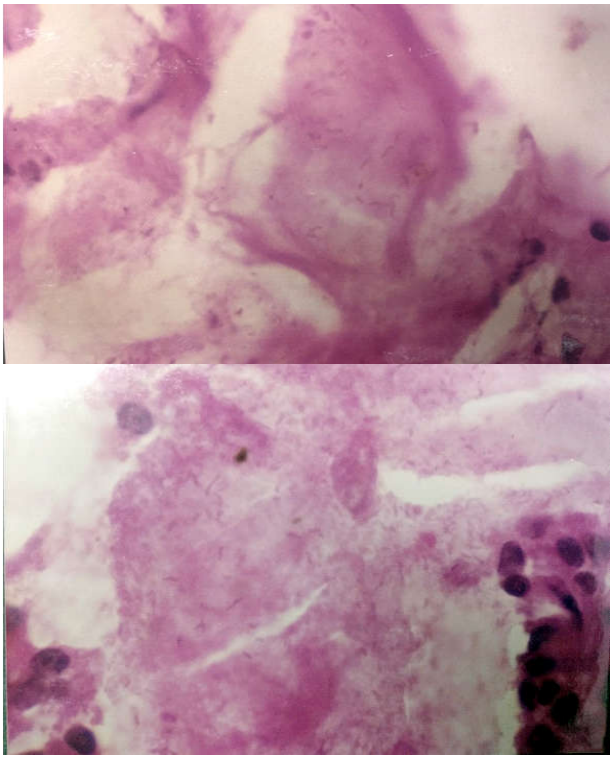
S. No	Symptoms	Number	Percentage	H. Pylori Number and %	
1	Pain and Belching	27	21.6	18	21.6
2	Pain and Nausea	21	16.8	11	13.2
3	Pain Only	33	26.4	26	31.3
4	Belching Only	3	2.4	3	3.6
5	Belching Pain And Nausea	16	12.8	10	12
6	Belching and Nausea	15	12	10	12
7	Nausea,Vomiting and Pain	4	3.2	2	2.4
8	Nausea And Vomiting	2	1.6	2	2.4
9	Others	4	3.2	1	1.2
	Total	125	100%	83	100%

Table 3: Endoscopic findings in patients of non ulcer dyspepsia and correlation with helicobacterpylori

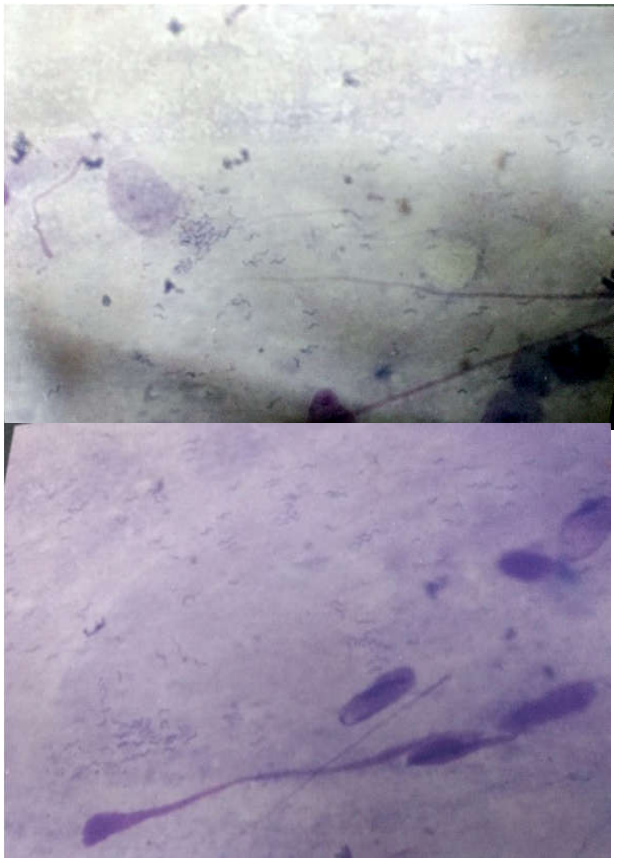
Endoscopic Findings	Number	Percentage	H. Pylori Positive	Percentage
Antral Gastritis	60	48	42	50.6
Antral Hyperemia	10	8	7	8.4
Antral Ulcer	3	2.4	3	3.6
Erosive Antral Gastritis	7	5.6	4	4.8
Duodenitis	8	6.4	4	4.8
Normal	37	29.6	23	27.8
Total	125	100%	83	100%



Photomicrograph 1: Showing lymphoid follicles in a biopsy H &E



Photomicrograph 4,5: Showing H.pyloriin H&E sections



Photomicrograph 2,3: Showing presence of spiral shaped H.pylori in imprint ,touchsmearsMGG stain

Fig. 1: Helicobacter pylori on touch smear

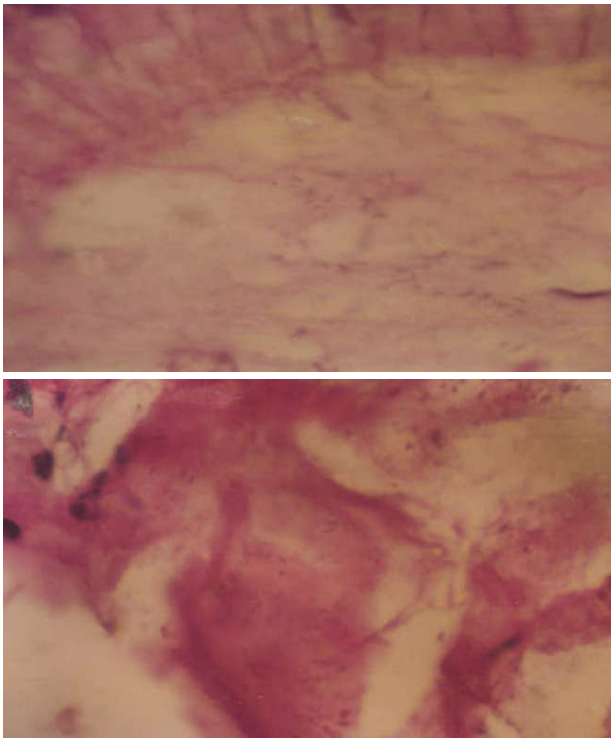


Fig. 2: Helicobacter pylori in gastric pits on H & E

Lymphoid follicleswere seen in 22 out of 125 patients of non- ulcer dyspepsia, 12 were positive for H.pylori infection accounting for 54.5%.

Discussion with Fast Forward 21 years

The association of H.pylori with gastrointestinal pathology has generated intense interest in developing methods for diagnosis of H.pylori infection.

Various stains have been recommended by different authors from time to time. These include H & E by Taylor, Giemsa by Gray, acridine orange by Walters, War thin starry stain by Marshall and Brown, Hop's stain by Westblom. Genta RM suggested the use of anew stain for simultaneous visualization of H.pylori andgastric morphology [14].

More refined methods areimmuno-histological methods based on monoclonal or polyclonal antibodies against H.pylori by Perez [15]. Cerqueira L proposed the use of in situ to identify the bacteria when the conventional methods pose a difficulty [16]. Clayton et al found PCR to be useful method, its sensitivity being comparable to histology an culture method [17]. Luzza F used salivary specific immunoglobulinG in diagnosis of H.pylori infection in dyspeptic patients [18]. However, the above methods have not gained popularity owing to their, cost, special requirements and non-availability at every center.Zullo analyzed the clinical practice view point to culture or not to culture H.pylori, and found culture to be time consuming, low sensitivity test and it added significantly to the cost when antibiotics are tested [19]. Thus culture has its limitations viz. requirement of proper standard, reduced viability of H pylori, and proper specimen transport. H.pylori is afastidious organism and not all microbiological labs are well equipped for its isolation.

Calvet reappraised the accuracy of diagnostic test for H.pylori, he found 94% sensitivity of rapid Urease test [20]. Similar results of reliability of RUT have been in the literature from time to time. Masahiro Kawanishi, found rapid urease test for identification of *Helicobacter pylori* in comparison with histological and culture studies to be better and very rapid test [21]. Redeen et al also found urease test to be a reliable test for diagnosis of H. pylori , and Tokuanga et al found MRU test has high sensitivity, specificity, low cost, shorter incubation time and combination with Giemsa stain for Touch cytologyis cost efficient in clinical settings [22,23]. Roy A, suggests use of RUT to confirm H. pylori and recommends it for early diagnosis and treatment of H.pylori associated diseases [24].

Saxsena et al on evaluation of endoscopic based diagnostic methods found the specificity, sensitivity of urease test, touch cytology and histopathology, concluded that when single test is to be used touch cytology is best [25]. In our present study also, the highest number of positive cases were seen with touch cytology alone 63.8%, and combination with urease test (57.8%) and or histology (49.3%) gave comparable result. Similar studies by Fakhjou. A, et al however found diagnostic value of RUT to be lower than Touch cytology and histopathology. He found Touch cytology to be most sensitive and histology to be most specific in outpatient setting [26]. L Trevisani et al found Touch cytology to be a reliable and cost effective method for diagnosis of H.pylori infection. He suggested that rapid urease test is cheapest, touch cytology is faster and cheaper than histology [27].

Cubukcu A, et al studied Imprint cytology in diagnosis of H.pylori and found it to be simple rapid, cheap method and imprints do not affect the quality of biopsy [28]. Yamamoto T et al evaluated the usefulness of touch smear cytology for diagnosis of H.pylori infection and cytology is a very accurate, convenient rapid, low cost test and has stability [29].

Kaur G et al studied diagnosis of H.pylori infection in gastric imprint smears and found out sensitivity, specificity, and PPV and NPV of Touch cytology and compared it with histology, best results are seen when complemented with histology [30].

Hashemi MP et al Touch cytology in diagnosing H.pylori infection, comparison of four staining methods RUT should still be acknowledged as primary test in diagnosis of

H.pylori. If RUT is negative, Wright stained TC is a safe substitute [31]. Rahabar M et al, Adlekha S et al studies also found imprint cytology to be fast and reliable [32,33].

Ina study by Rami I, who evaluated diagnostic methods in gastric biopsies of non- ulcer dyspepsia, found that in comparison to histology and urease test, the RUT and PCR is 100%sensitve and RUT with culture is 100% specific [34]. Khalifghogli M compared five diagnostic methods for H.pylori; according to his study, the accuracy of the tests for H. pylori diagnosis can be arranged in order as follows: RUT >PCR>histology> stool antigen test> serology, however he suggested that utilization of biopsy-based and non-invasive methods is useful for H. pylori infection confirmation [35].

Lee JY et al recommended that histology is an excellent method for detecting H. pylori as it provides additional information on the mucosa [36]. In our study also histology was helpful as it provided the status of the mucosa as per Sydney system of classification, but combined with touch cytology the results were superior to just histology, 50% as compared to histology alone in 18%.Uotani also recommends that urease test can be used for initial screening, but for confirmation at least two tests should be done [37].

On reviewing the vast literature on invasive and non-invasive tests for diagnosis of H. pylori, we found touch cytology and rapid urease test to be the most practical, rapid and cheap methods among all for the diagnosis of Helicobacter pylori infection; histopathology complements these two tests as is seen in our study too.

It is surprising that the gastroenterologists are not using this simple technique as an office procedure to detect H.pylori infection. It calls upon to ponder the reasons why this test has not gained popularity, when the report is available within few hours in the endoscopy room itself.

Conclusions

Various tests are available for diagnosis of Helicobacter pylori ranging from simple urease test to FISH and PCR. Research in several directions has brought no general consensus about the best method for its diagnosis.

With this view the study was carried out and we found Touch cytology to be simple, rapid cost effective method for diagnosis. The organisms are better appreciated morphologically in smears than biopsies, smears took lesser time than histology, t hey were even positive in cases of fragmented biopsies, however histology gives additional information on gastric mucosa, hence we recommend touch cytology as simple fast screening test and report can be given on the same day. We suggest its role complementary to histological examination.

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Original Research Article

Utility of Fine Needle Aspiration Cytology in Nonneoplastic Lesions of Lymphnodes

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Abstract

Introduction: The goal of fine needle aspiration cytology of lymph node is essentially to distinguish benign from malignant lesions. Nonneoplastic disorders that can manifest in lymph nodes include those with specific diagnosis and nonspecific causes, reactive hyperplasia being the most common diagnosis. The role of fine needle aspiration cytology (FNAC) is of paramount importance in determining the etiology of lymphadenopathy, so that appropriate management can be instituted.

Aims and Objectives: To identify, to categorise and to study incidence of various nonneoplastic lesion of lymphnodes.

Materials and Methods: FNAC'S were done using a 22 guage needle with ten cc syringe for superficial lesions and lumbar puncture needle for deep seated lesions under ultrasound guidance. In this study patients were divided into three groups based on age i.e group 1 (0-20 years), group 2 (21-50 years) and group 3 (>50 years).

Observation and Results: Out of 6411 FNAC cases during one and half years study, 1117 cases were enlarged lymphnodes. A major proportion of lymphadenopathies in this study were due to benign conditions 809 cases (72.42%). Malignant cases accounted for 308 cases (27.57%). Among 1117 cases, maximum number of cases were recorded in age group 2:480 cases. (42.97%), group 1: had 334 cases (29.90%) followed by group 3:303 cases (27.12%). Out of 809 nonneoplastic cases the most common cause of lymphadenopathy was found to be reactive lymphadenitis with 517 cases (63.91%), followed by tuberculosis with 241 cases (29.78%), also seen were BCG lymphadenitis in 4 cases (00.49%).

Conclusion: In our study we concluded that different etiological factors play a role in causation of lymphadenopathy. So FNAC provides a reliable, safe, rapid and economical method of screening these patients.

Keywords: Fine Needle Aspiration Cytology; Reactive Lymphadenitis; Nonneoplastic Lymphadenopathy.

Introduction

FNAC is a commonly used diagnostic approach in the investigation of enlarged lymphnodes. It is an OPD procedure with little trauma to patient at a lower cost compared to biopsy. Lymphadenopathy is manifested clinically by the enlargement of lymphnodes. The vast majority of enlarged lymphnodes are nonneoplastic.

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Therefore their recognition and differential diagnosis are of great impoartance to rule out the neoplastic diseases .

Needle aspiration of lymphnodes is one of the oldest applications of the technique in the diagnosis of human disease. In 1904, two British military surgeons, Greig and Gray, working in Uganda, published a paper describing the diagnosis of sleeping sickness by recognizing mobile trypanosomes in lymph node aspirates. In 1921, Guthrie of Johns Hopkins described the application of needle aspiration to the diagnosis of tumors. In 1930, Martin and Ellis of Memorial Hospital for Cancer (now the Memorial Sloan-Kettering Cancer Centre) included tumors that had metastasized to the lymph nodes among the targets of aspiration biopsy [1].

As a result of the pioneering work of Franzén et al (1960) and the widespread current acceptance of the technique, aspiration of lymph nodes has become a standard laboratory procedure [1].

The spectrum of applications of fine needle aspiration (FNA) biopsy in diagnosing disease has become ever wider. The technique has been found most usefull for the selection of a representative node for biopsy, for the diagnosis of recurrent lymphoma, for staging the extent of the disease, and for monitoring treatment [2].

Hence in order to make the most rational use of fine needle aspiration cytology (FNAC), clinicians and pathologists alike must understand and accept that the aims and purpose of fine needle biopsy (FNB) of peripheral lymph nodes at the 'primary' or 'community' level are different from those at the 'secondary' or 'specialist' level.

At the primary level, FNAC is used as a triage to distinguish between cases of lymphadenopathy with a high or a low level of suspicion of significant disease by the simplest, least invasive and least costly method. This preliminary assessment is based on routine cytologic smears only. Depending at the specialist/secondary level, the role of FNB is to provide material for further cytomorphologic analysis and for ancillary studies [3]. Here we studied the spectrum of nonneoplastic lesions of lymph node based on routine cytologic smears.

Aims and Objectves

1. To identify the cause of enlarged lymphnode and to rule out neoplasia.
2. To categorise various nonneoplastic lesions of lymphnodes .
3. To study the incidence of various nonneoplastic lesions.
4. To study site wise distribution of nonneoplastic lymphadenopathy.

Materials and Methods

This is a combined prospective and retrospective study of all lymphnodes FNAC'S reported over a period of 18 months from January 2016 to June 2017. This study was carried out in the department of Pathology. In this study patients were divided into three groups based on age i.e group 1 (0-20 years), group 2 (21-50 years) and group 3 (>50 years). All FNAC'S were performed using a 22 guage needle for conventional, superficial lesions. For deep seated lesions lumbar puncture needle was used with ultrasound guidance. The smears were routinely stained after air drying with Geimsa, alcohol fixed smears were stained with PAP, H & E and observations made. Ziehl-Neelsen stain was done in suspected tubercular lesion.

Results

The total number of cases studied was 1117. The age of the patients ranged from one month to 93 years. Among these lesions 809 (72.42%) cases were nonneoplastic. Neoplastic lesions constituted 308 cases (27.57%).

Maximum number of cases were recorded in group 2: 480 cases (42.97%) . The age of the patient is a key factor in diagnosis. In younger patients enlarged lymphnodes are mostly due to nonneoplastic conditions.

Out of 809 cases, cervical group of lymphnodes were the most aspirated 654 cases (80.84%) . Followed by axillary lymphnodes in 65 cases (08.03%). Supraclavicular in 36 cases (4.45%), inguinal in 34 cases (4, 20%), abdominal in 2 cases (0.25%), occipital in 10 cases (1.24%), left arm in 2 cases (0.25%), and generalized lymphadenopathy in 4 cases (0.49%). We also had one case (0.12 %) of enlarged parotid lymphnode .

Among the diagnosis of aspirated nonneoplastic lymphnode lesions, the most common cause of lymphadenopathy was found to be reactive lymphadenitis in 517 cases (63.91 %) out of 809 (72.42%). Among the infectious cause of lymphadenopathy, tuberculous lymphadenitis was the most common accounting to 241 cases (29.78%).

Followed by granulomatous lymphadenopathy in 26 cases (03.22%), and suppurative pathology in 20 cases (02.47%). We had four (00.49%) cases of BCG lymphadenitis. One case (00.13%) of calcification was noted. This study revealed higher incidence of reactive lymphoid hyperplasia in group 2 (21-50 years).

It was observed that the cases of granulomatous and suppurative lesion was highest in group 2. Whereas BCG lymphadenitis and calcification was seen in only group 1(0-20) patients. Comparison of distribution of nonneoplastic lymphnode diagnosis of present study with different studies showed similar results.

Table 1: Age wise distribution of lymphadenopathy patients (n=1117)

Groups	Age Group (years)	No of cases	Percentage
Group1	0-20	334	29.90%
Group2	21-50	480	42.97%
Group3	>50	303	27.12%
Total		1117	100 %

Table 2: Result of FNAC showing frequency distribution of various nonneoplastic conditions in each group. n =809

Diagnosis	Group 1 No. of	% cases	Group 2 No. of	% cases	Group3 No. of	% Cases
Reactive	178	22.00%	216	26.70%	123	15.20%
Tuberculosis	65	08.03%	112	13.84%	64	7.91%
Granulomatous	05	0.61%	13	1.60%	08	0.99%
Suppurative	04	0.49%	09	1.11%	07	0.86%
BCG lymphadenitis	04	0.49%	_		_	
Calcification	01	0.13%	_		_	
Total	257		350		202	

Table 3: Distribution of sites of lymph node involvement(n=809)

Sites	No of Cases	%
Cervical	654	80.84%
Axillary	65	08.03%
Supraclavicular	36	04.45%
Inguinal	34	04.20%
Occipital	10	01.24%
Generalized lymphadenopathy	04	00.49%
Left arm	02	00.25%
Intraabdominal	02	00.25%
Parotid	01	00.12%
Retroperitoneal	01	00.12%
Total	809	100%

Table 4: Cytological diagnosis of Nonneoplastic lymph node aspirations(n=809)

Diagnosis	No. of Cases	Percentage
Reactive	517	63.91 %
Tuberculosis	241	29.79 %
Granulomatous	26	03.21 %
Suppurative	20	02.47 %
BCG lymphadenitis	04	00.49
Calcification	01	00.12 %
Total	809	100 %

Table 4: Comparison of Distribution of Nonneoplastic Lymphnode diagnosis with Other Studies

Diagnosis	Present Study	Biradar et al	Gayatri et al	Ripunjaya et al
Reactive	63.91 %	55.62 %	44.70 %	34.36 %
Tuberculosis	29.78 %	23.75 %	14.65 %	00.78 %
Granulomatous	03.22 %	00.37 %	14.65 %	20.28 %
Suppurative	02.47 %	00.37 %	03.94 %	11.26 %

Discussion

FNAC is a commonly used diagnostic approach in the investigation of lymphadenopathy. It is an OPD procedure with little trauma to a patient at a lower cost compared to biopsy. Lymphadenopathy can be due to nonneoplastic conditions or neoplastic lesions. The ratio of nonneoplastic to neoplastic was 2.6:1 in our study. The ratio of nonneoplastic to neoplastic varies according to the kind of medical practice in which they present. In general practice malignancy represent 1.1% of lymphnode lesions, whereas at referral centres their frequency is 40-60% [4,5,6].

Lymphadenitis are acute and chronic inflammatory process of lymphnodes, that occur in response to a variety of pathogenic agents. Whereas lymphadenopathies are reactive process of lymph node in response to a variety of exogenous and endogenous stimulants . The etiology and pathogenesis of many lymphadenopathies are still unknown. Possible causes of lymphadenopathies include microorganisms not yet identified, autoimmune diseases, immune deficiency and dysregulation, foreign bodies, medical procedures, and tumours [7]. In cases of reactive lymphadenopathy, FNAC has made the diagnosis easier and the number of surgical biopsies has reduced. The causative organisms can be identified with FNAC in infectious cases.

The present study was done over a period of one and half year. One year retrospective and six months prospective study. The total number of FNAC during the period was 6411 . Out of these 1117 cases presented with lymphadenopathy to department of pathology.

The anatomic location of localized adenopathy will sometimes be helpful in narrowing the differential diagnosis. For example cat scratch disease typically causes cervical or axillary adenopathy, infectious mononucleosis causes cervical adenopathy and a number of sexually transmitted diseases are associated with inguinal adenopathy. Parotid, submaxillary and epitrochlear lymphnodes are frequently encountered in HIV infections [8,9]. In our study the most common group of enlarged lymph nodes were cervical lymphnodes 654 cases (80.84%), followed by axillary in 65cases (8.03%), supraclavicular in 36 cases (4.45%), inguinal in 34 cases (4,20%), abdominal in 2 cases (0.25%), occipital in 10 cases (1.24%), left arm in 2 cases (0.25%), and generalized lymphadenopathy in 4 cases (0.49%). Cervical lymphnodes were commonly involved in our study which was comparable with the findings of other workers [10,11,12,13]. We also had 1 case (0.12%) of parotid lymph node enlarged . The parotid gland is the only salivary gland to contain lymphoid tissues within its capsule. Because of their close association, pathologic changes within the two tissues are often combined thus raising questions about their pathogenesis and presenting problems of differential diagnosis [14].

Among the lesions, 809 (72.42%) cases were nonneoplastic and 308 (27.57%) cases were neoplastic which is similar to study done by Kim [15]. To recall the main etiologic categories, some authours suggest using acronyms like MIAMI (malignancies, infections, autoimmune, miscellaneous and iatrogenic [4]. Nonneoplastic conditions had various lesions like reactive lymphadenopathy, tuberculosis, BCG lymphadenitis, granulomatous lymphadenopathy, acute suppurative lymphadenitis. Granulomatous lymphadenitis can be seen, not only in infectious process such as tuberculosis, atypical mycobacterosis, brucellosis or infections caused by fungi, but also in sarcoidosis, foreign body reactions, non – Hodgkin lymphoma, Hodgkin lymphoma. BCG lymphadenitis is a well known complication of BCG vaccination occurring in 0.013%-23% [16]. Our study showed four cases(0.35%) of BCG lymphadenitis. In other studies results are high [17]. We also had one case (0.08%) of lymphnode calcification. Nodal calcification suggest differential diagnosis, with prior granulomatous disease being the most common etiology . Benign causes of lymph node calcification are infectious etiologies such as tuberculosis [18], histoplasmosis, coccidiodomycosis and aspergillosis, and non infectious causes such as silicosis [19]. It is also rarely reported in amyloidosis [20]. Radiologically malignant causes of calcified lymph nodes are lymphoma, metastatic thyroid carcinoma [18,21], mucinous adenocarcinoma and rarely squamous cell carcinoma [22], which can be confirmed by cytology. A calcified firm neck mass could suggest malignancy [23]. Our case of nodal calcification was due to tuberculosis.

In our study the most common cause of lymphadenopathy was reactive lymphadenopathy with 517/809 cases (63.91%). It was also the most frequent cause in other studies [12,24,25] .

Conclusion

Lymphadenopathy is a commonly encounterd condition in medical practice requiring prompt and accurate diagnosis so that a proper treatment protocol can be started. Lymphadenopathies are mostly nonneoplastic process, so their recognition and differential diagnosis are of great importance to rule out the neoplastic diseases. FNAC technique is useful for initial screening of lymphadenopathy at primary level or community level and further workup at specialist/secondary level for cytolomorphologic analysis and ancillary studies.

In our study we concluded that different etiological factors play a role in causation of lymphadenopathy and that aspiration cytology provided a reliable, safe, rapid and economical method of screening the patients.

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Case Report

Adenosquamous Variant of Metaplastic Carcinoma of Breast

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Abstract

Introduction: Metaplastic carcinoma of breast belongs to a diverse class of neoplasm constituting the rarest histological variant of invasive ductal carcinoma. Adenosquamous carcinoma of breast is a rare tumour which is a variant of metaplastic carcinoma, comprising 0.3% of breast carcinomas.

Case Report: Here, we report a case of adenosquamous variant of metaplastic carcinoma of breast in a 61 years old female who presented with a lump in the right breast.

Conclusion: The present case highlights that although adenosquamous carcinoma of breast is rare, we should be aware of this possibility and include it in the differential diagnosis whenever appropriate.

Keywords: Metaplastic; Adenosquamous; Carcinoma.

Introduction

Metaplastic carcinoma of breast belongs to a diverse class of neoplasm consisting of mixture of adenocarcinoma and areas of squamous cell, spindle cell and/or mesenchymal differentiation, accounting for less than 1% of all invasive carcinomas [1].

Adenosquamous carcinoma of breast is a rare tumour included in WHO classification of breast cancer, as a subtype of metaplastic carcinoma, constituting 0.3% of all breast carcinomas [2,3]. Adenosquamous carcinoma is characterized by areas of well-developed tubule/gland formation intimately admixed with widely dispersed solid nests of squamous differentiation [4].

Adenosquamouscarcinoma are divided into low grade and high grade. Low grade adenosquamous carcinoma has less nuclear anaplasia, do not metastasize and have an overall good prognosis [3]. In contrast, high grade adenosquamous are quite aggressive and show lymph node metastasis at the time of diagnosis.

Case Report

A 61 years old female, presented with a lump in the right breast for 8 months. Physical examination revealed a lump which was hard, measured 6x5 cm with nipple retraction and palpable ipsilateral axillary lymph nodes. The contralateral breast and axillary nodes were normal.

Sonomammography revealed an ill-defined lesion in the upper right quadrant with axillary lymphadenopathy (Figure 1). Trucut biopsy confirmed the diagnosis of invasive ductal carcinoma NOS following which she underwent modified radical mastectomy and the specimen was sent for histopathological examination.

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On gross examination, radical mastectomy specimen measured 15x13x4 cm. Cut surface revealed a pearly white lesion in upper outer quadrant (Figure 2).

Microscopic examination showed foci of architecturally confluent glandular formation with an adjacent desmoplastic stroma (Figure 3). Also seen were tumor composed of nests, jagged islands of mild to moderately

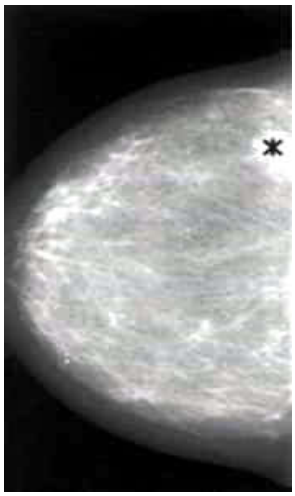

Fig. 1: Mammogram of breast showing radiodense lesion shown by *which has speculated margins and microlobulations suggestive of carcinoma breast

Fig. 2: Modified radical mastectomy specimen measuring 15x13x4 cm. Cut surface revealed pearly white lesion in upper outer quadrant (arrow)

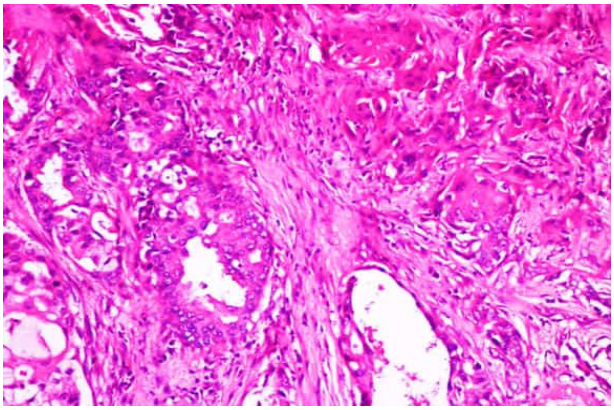

Fig. 3: Photomicrograph showing foci of architecturally confluent glandular formation with desmoplastic stroma (H&E; 10x)

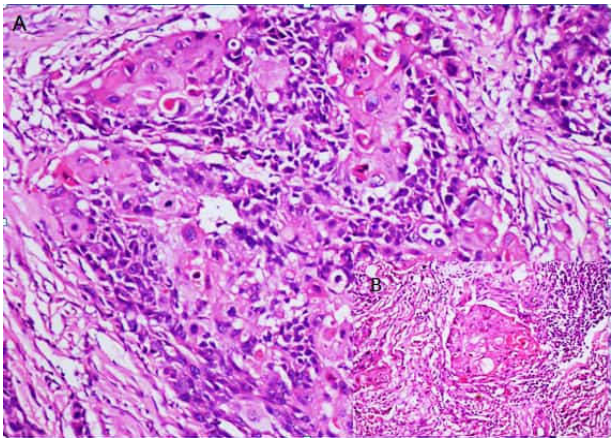

Fig. 4(A): Photomicrograph showing parts of a tumor composed of nests, jagged island of mild to moderately pleomorphic cells with a squamoid appearance (H&E; 10x);] (B) – [Inset: Photomicrograph showing foci of keratin pearl formation with a few dyskeratotic cells (H&E; 10x).

pleomorphic cells with a squamoid appearance (Figure 4A). There were foci of keratin pearl formation with a few dyskeratotic cells (Figure 4B). Ductal carcinoma insitu with solid and cribriform growth pattern was also seen. The diagnosis of adenosquamous carcinoma was given.

The immunohistochemical staining showed triple negative for ER, PR, and Her2 neu expression and showed strong positive for cytokeratin.

Discussion

Adenosquamous breast carcinoma was first described by Rosen in 1987 and later in a follow up study by Van Hoeven in 1993 [5]. Adenosquamous carcinoma presents as a palpable mass and has been found in women whose age ranges from 31 to 87 years [3].

Adeosquamous carcinoma is difficult to diagnose from other benign and invasive tumors on noninvasive investigations. On imaging only the benign nature of the lesion is observed. These tumors do no exhibit much cytological atypia, despite the infiltrative nature of these tumors, so making it difficult to diagnose on cytology.

On trucut biopsy, the infiltrative nature of the tumor cannot be observed. So, diagnosis is usually made histologically on excision biopsy specimen [6]. At gross examination, adenosquamous carcinoma tends to display a stellate or infiltrative configuration, with poorly defined borders. Microscopically, the carcinomatous component is characterized by small glandular structures, with rounded rather than angulated contours, and solid cords of epithelial cells, which may contain squamous cells, squamous pearls or squamous nests formation. The invasive neoplastic component typically shows long, slender, extensions at the periphery and infiltrate in

between the normal breast structures, features which have been associated with inadequate local excision and high incidence of recurrence.

Adenosquamous carcinoma is consistently negative for ER, PR Her2-neu expression, hence may be a useful diagnostic tool. Myoepithelial and cytokeratin stains are positive, but the extent of staining is highly variable. SMA, p63, calponin and CD10 show variable degree of positivity [2,7].

The study conducted by Khatib et al., who reviewed one case of low-grade adenosquamous carcinoma of breast, showed triple negative for ER, PR, Her2 neu expression. SMA and calponin were positive and highlighted the myoepithelial cells, but p63 showed focal positivity [8]. Similarly, our case showed triple negative for ER, PR, Her2 neu expression but showed strong positivity for cytokeratin expression.

The study conducted by Geyer et al., who observed five cases of adenosquamous carcinoma of breast, all of them belonged to 54 to 76 years of age [2]. Similarly, our case was 61 years old.

The overall prognosis of adenosquamous carcinoma is good but it has a tendency to locally recur depending on the adequacy of local excision. So, complete local excision or mastectomy is usually recommended. Adenosquamous carcinoma should always be differentiated from tubular carcinoma, infiltrating syringomatous adenoma of the nipple and adenomyoepithelioma [8].

Conclusion

Adenosquamous carcinoma is a rare entity, has risk of local recurrence after incomplete excision and has low metastatic potential. In conclusion, adenosquamous carcinoma should always be kept in the differential diagnosis whenever appropriate.

Acknowledgement

Nil

Conflict of Interest: Nil

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Include summary of key findings (primary outcome measures, secondary outcome measures, results as they relate to a prior hypothesis); Strengths and limitations of the study (study question, study design, data collection, analysis and interpretation); Interpretation and implications in the context of the totality of evidence (is there a systematic review to refer to, if not, could one be reasonably done here and now?,

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