Original Research Article

A study of diagnostic accuracy of fine needle aspiration cytology in salivary gland lesions based on the milan system for reporting salivary gland cytopathology

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Abstract

Background: Fine needle aspiration cytology is accurate, simple, rapid, inexpensive, well tolerated and with minimal complications for the patient. Fine needle aspiration cytology of suspected salivary gland lesions has now been accepted as an excellent, though challenging, primary method in preoperative diagnosis and management of patients. But value of fine-needle aspiration cytology for diagnosis of salivary gland lesions is controversial because of low sensitivity, wide variation in reporting, and the common belief that most parotid masses require surgery. The aim of the present study was to evaluate the diagnostic accuracy of FNAC for salivary gland lesions. **Material and Methods:** Present study was prospective, observational type conducted in patients whom both cytological and histological studies for salivary gland swelling were done. The cytological features were evaluated and classified based on the Milan system for reporting salivary gland pathology; which is a recent system providing guide for diagnosis and management according to the risk of malignancy. **Results:** Total 90 patients were included in this study. The most affected age group was 41-50 years (28.89 %). 22.22 % patients were belonged to 31-40 years age group. 57 patients were male (63.33%) and 33 cases were females (36.67%). Most common benign tumour was pleomorphic adenoma (31.11 %) and most common malignant tumour was mucoepidermoid carcinoma (6.67 %). The present study demonstrated sensitivity as 90.91% and specificity as 100 %. The positive and negative predictive value of salivary gland lesions by FNAC was 100 % and 96.87 % respectively. **Conclusion:** FNAC is the best method for initial assessment of salivary gland lesions. Initial results give valuable information to the surgeon in preoperative diagnosis of various salivary gland lesions, which helps in planning of operative procedure and post-operative management. Milan system categorizes salivary gland cytology into well defined categories; limiting the false negative and false positive cases.

Key Words: Salivary gland lesions, Fine needle aspiration (FNAC), Parotid gland, The Milan system.

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INTRODUCTION

The oral cavity is affected by a wide variety of pathologic lesions, those need an accurate diagnosis for further management[1]. The estimated global annual incidence of salivary gland tumors varies from 0.4-13.5 cases per 100,000 population[2]. Pleomorphic adenoma of salivary glands is the most frequently encountered benign tumor and Squamous cell carcinoma is the most common malignancy reported in the oral cavity[3].Oral and oropharyngeal mass lesions are commonly diagnosed by biopsy. The procedure may require anesthesia and may have diagnostic difficulties especially for trans-mucosal lesions4. Fine needle aspiration cytology (FNAC) is

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accurate, simple, rapid, inexpensive, well tolerated technique and with minimal complications for the patient. Fine needle aspiration cytology (FNAC) of suspected salivary gland lesions has now been accepted as an excellent, though challenging, primary method in preoperative diagnosis and management of patients. It has acquired an edge over incisional biopsy and frozen section[5].FNAC provides information that informs two key decisions in patient management. First, FNAC differentiates between neoplastic and nonneoplastic lesions. Neoplastic lesions usually are managed surgically, whereas nonneoplastic lesions are managed conservatively. Second, in case of a neoplastic lesion, FNAC determines whether the lesion is malignant or benign, which inturn helps to determine the extent of surgery and whether the facial nerve can be spared during surgery6. But value of FNAC for diagnosis of salivary gland lesions is controversial because of low sensitivity, wide variation in reporting, and the common belief that most parotid masses require surgery. The aim of the present study was to evaluate the diagnostic accuracy of FNAC for salivary gland lesions.

MATERIAL AND METHODS

Present study was prospective, observational study conducted in the Department of Pathology, at a tertiary hospital. Study duration was from Jan 2016 to Dec 2018. Ethical committee approval was taken. Patients who underwent both cytological and histological studies for salivary gland swelling were included in this study. Patients with inconclusive cytology reports due to scanty, inadequate aspirate on FNAC were excluded from present study. Total 90 cases were included in this study, with cytohistological correlation. All patients were evaluated with detailed history, clinical examination, and relevant hematological and radiological investigations. Initial evaluation was done with FNAC. Fine needle aspiration cytology was performed from different sites of the salivary gland swelling without local anaesthesia. FNA air-dried smears were stained with Giemsa stain and wet smears fixed in 95% ethyl alcohol were stained with haematoxylin and eosin stain. Histological examination was done with paraffin embedded tissue sections obtained from salivary gland tissue and stained with haematoxylin and eosin (H and E). The overall diagnostic accuracy and the sensitivity and specificity were calculated with the help of statistical data by using the SPSS software.

RESULTS

The age of the patients with salivary gland lesions ranged from 18 to 76 years with a mean age of 46 years. The most affected age group was 41-50 years (28.89 %). 22.22 % patients belonged to 31-40 years age group. 14.44 %, 13.33 % and 10 % patients were from age group 21-30 years, 51-60 years and 61-70 years respectively. From total 90 cases studied, 57 patients were male (63.33%) and 33 cases were females (36.67%). The male to female ratio was 1.73:1.

Table 1: Patient distribution

Age Group	No of Patient		Total	Percentage
	Male	Female		
< 20	3	2	5	5.56
21-30	8	5	13	14.44
31-40	14	6	20	22.22
41-50	19	7	26	28.89
51-60	6	6	12	13.33
61-70	5	4	9	10
71-80	2	3	5	5.56
> 80	0	0	0	0
Total	**57**	**33**	**90**	**100**

We noted that most common lesions were non-neoplastic, accounting for 53.33% (48 patients), followed by 35.56% benign neoplastic lesions (32 patients) and 11.11% (10 patients) had malignant tumours. Ratio of benign to malignant tumours is 3.2:1.

Table 2: Distribution of salivary gland lesions as cytology diagnosis

Cytological diagnosis	No. of patients	Percentage (%)
Non-neoplastic	48	53.33%
Benign	32	35.56%
Malignant	10	11.11%

Most common benign tumour was pleomorphic adenoma (31.11%) and most common malignant tumour was mucoepidermoid carcinoma (6.67 %).

Table 3: FNAC report using Milan grading

Diagnostic category	No. of patients	Percentage (%)
I. Non-diagnostic	0	0
II. Non-neoplastic	44	48.89
III. Atypia of undetermined significance (AUS)	4	4.44
IV. Neoplasm		
Neoplasm: Benign	28	31.11
Neoplasm: Salivary gland neoplasm of uncertain malignant potential (SUMP)	4	4.44
V. Suspicious for malignancy (SM)	3	3.33
VI. Malignant	7	7.78

Table 4: FNAC report

Cytologic diagnosis	No. of patients	Percentage (%)
Non-neoplastic lesions		
Chronic sialadenitis	29	32.22%
Cystic lesions	10	11.11%
Acute on chronic sialadenitis	6	6.67%
Chronic granulomatous inflammation	3	3.33%
Benign neoplasms		
Pleomorphic adenoma	28	31.11%
Warthin's tumour	4	4.44%
Malignant lesions		
Mucoepidermoid carcinoma	6	6.67%
Acinic cell carcinoma	2	2.22%
Carcinoma-ex pleomorphic adenoma	1	1.11%
Adenoid cystic carcinoma	1	1.11%

The present study demonstrated sensitivity as 90.91% and specificity as 100 %. The positive and negative predictive value of salivary gland lesions by FNAC was 100 % and 96.87 % respectively.

DISCUSSION

FNAC has an important role in the preoperative diagnosis of salivary gland lesions. Cyto-diagnosis helps in formulating the treatment plan especially in recurrent and inoperable malignancies without undergoing open biopsy. The age and sex distribution of salivary gland lesions has been well established in literature with male preponderance. We noted maximum patients from age group 31-50 years (51.11%). The increased incidence of malignant neoplastic lesions in the late 40s and 50s with benign lesions affecting the late 30s and early 40s was noted in earlier studies. Our findings are consistent with them[7,8.] Increased incidence of benign neoplastic lesions as compared to malignant lesions was noted in this study. Ratio of benign and malignant tumours in our study was 3.2:1. Vaishali *et al* [9] noted 4.9:1 ratio and Shilpa Gandhi *et al*10 noted 3.5:1 ratio of benign and malignant tumours in their study. Most lesions in the minor salivary glands were benign in nature, which is contrary to common belief; which could be attributed to the small sample size in the present study. In our study most common benign and malignant tumours were pleomorphic adenoma and mucoepidermoid carcinoma respectively. These findings are similar with other authors[9, 10, 11]. The American Society of Cytopathology and International Academy of Cytology recently proposed a tiered international classification scheme called the "Milan System for Reporting Salivary Gland Cytopathology" (MSRSGC), providing guide for clinical management according to ROM in different categories[12,13]. Table 5 The Milan system for Reporting Salivary Gland Cytopathology: Implied risk of malignancy and recommended clinical management

Table:

Diagnostic category	Risk of malignancy (%)	Management
I. Non-diagnostic	25	Clinical and radiologic correlation/repeat FNAC
II. Non-neoplastic	10	Clinical follow-up and radiological correlation
III. Atypia of undetermined significance (AUS)	20	Repeat FNAC or surgery
IV. Neoplasm		
Neoplasm: Benign	<5	Surgery or clinical follow-up
Neoplasm: Salivary gland neoplasm of uncertain malignant potential (SUMP)	35	Surgery
V. Suspicious for malignancy (SM)	60	Surgery
VI. Malignant	90	Surgery

MSRSGC is a newer system for reporting salivary gland lesions according to risk stratification with an objective to provide a better communication between clinicians and cytopathologists so as to improve overall patient management. It is an evidence based six tiered system, which provides risk of malignancy and clinical management strategies for each category.[14,15] We noted risk of malignancy in our patients similar to that of given in above table. The present study noted sensitivity as 90.91% and specificity as 100%. In one case false negative diagnosis was made on FNAC examination. A case of low grade mucoepidermoid carcinoma was initially diagnosed as pleomorphic adenoma on FNAC. Pleomorphic adenoma is a biphasic neoplasm and no two pleomorphic adenomas look alike. Epithelial metaplasia, mainly squamous and oncocytic, and significant cytologic atypia may at times be worrisome. Careful evaluation and follow up is needed in such patients. Different populations and studies had a wide variation in the sensitivity and specificity of FNAC for salivary gland swelling. Pastore *et al*. found a sensitivity and specificity of 83% and 93% respectively in their study with 357 cases of salivary gland lesions16. Jain *et al.* revealed 92.8% sensitivity and 93.9% specificity in a study involving 80 cases of salivary gland swellings, out of which 14 cases were of malignant salivary gland neoplasms.[17] Kim *et al*. noted a diagnostic accuracy of FNAC to be 92% in differentiating malignant from benign salivary gland tumors18. Our findings are consistent with these studies. Other studies noted lower sensitivity and specificity values of FNAC for salivary gland lesions. Zerpa *et al*. in their study with 93 cases of parotid gland tumors, noted a sensitivity and specificity of 57% and 95% respectively.[19] Fakhry *et al.*[20] evaluated 249 parotid tumors, sensitivity and specificity to detect malignancy was assessed to be 80% and 89.5% respectively. They found 16 false positive results, among which warthin's tumor and pleomorphic adenoma were most common, while false negative diagnosis was given in cases of lymphomas and mucoepidermoid carcinomas. The diagnostic accuracy for benign and malignant tumors was 16% and 44% respectively.

CONCLUSION

FNAC is simple, reliable, low cost, least harmful diagnostic procedure. FNAC is the best method for initial assessment of salivary gland lesions. Initial results give valuable information to the surgeon in preoperative diagnosis of various salivary gland lesions, which helps in planning of operative procedure and post-operative management. MSRSGC is a recent method for classification of salivary gland cytology into six categories. Inspite of the heterogeneity and morphological overlap between the various salivary gland lesions, this system will surely help in assessing the risk of malignancy and deciding on the treatment modality.

REFERENCES

1. Saleh HA, Clayman L, Masri H. Fine needle aspiration biopsy of intraoral and oropharyngeal mass lesions. Cytojournal 2008; 5(4): 4.
2. Scher RL, Oostingh PE, Levine PA, Cantrell RW, Feldman PS. Role of fine needle aspiration in the diagnosis of lesions of the oral cavity, oropharynx, and nasopharynx. Cancer 1988; 62(12): 2602-6.
3. Tessy PJ, Jayalekshmy PS, Cicy PJ, Poothiode U. Fine needle aspiration cytology of salivary gland lesions with histopathological correlation:A two year study. Int J Healthc Biomed Res 2015; 3(4): 91-9.
4. Stewart CJR, MacKenzie K, McGarry GW, Mowat A. Fine-needle aspiration cytology of salivary gland: A review of 341 cases. Diagn Cytopathol 2000; 22(3): 139-46.
5. Kotwal M, Gaikwad S, Patil R, Munshi M. Fnac of salivary gland- a useful tool in preoperative diagnosis or a cytopathologist'sriddle. Journal of cytology. 2007;24(2):85-8.
6. Robert L. Schmidt, Brian J. Hall, Andrew R. Wilson, Lester J. Layfield, A Systematic Review and Meta-Analysis of the Diagnostic Accuracy of Fine-Needle Aspiration Cytology for Parotid Gland Lesions Am J Clin Pathol 2011;136:45-59.
7. Choudhury AA, Sultana T, Siddique BH, Amin AS. Diagnosis of parotid gland mass by the Fine needle aspiration cytology (FNAC) and it's histopathological correlation-2 Years Study in BSMMU, Dhaka. Bangabandhu Sheikh Mujib Med Univ J. 2011;4(2):65-9.
8. Shishegar M, Ashraf MJ, Azarpira N, Khademi B, Hashemi B, Ashrafi A. Salivary gland tumour in maxillofacial region- a retrospective study of 130 cases in a southern Irian population. Pathol Res Int. 2011
9. Vaishali H Anand, Dipesh Prajapati, kalpana K Dave: FNAC and Histopathology of Salivary Gland Tumors. SEAJCRR Jan-Feb 3 (1) page 609-618. 2014
10. Shilpa H Gandhi *et al*: FNAC Diagnosis of Salivary Gland Lesions with Histopathological Correlation NJIRM 2013; vol. 4 (3). May-June.
11. Wahiduzzaman M *et al*: Major Salivary gland tumors: A Clinicopathological study. J. Shaneed Suhrawardy med Coll, June 2013, Vol. 5, No. 1.
12. Faquin WC, Rossi ED, editors. Cham: Springer; 2018. The Milan System for Reporting Salivary Gland Cytopathology. [Google Scholar]
13. Rossi ED, Faquin WC, Baloch Z, Barkan GA, Foschini MP, Pusztaszeri M, *et al*. The Milan system for reporting salivary gland cytopathology: Analysis and suggestions of initial survey. Cancer Cytopathol. 2017;125:757–66.
14. Wei S, Layfield LJ, LiVolsi VA, Montone KT, Baloch ZW. Reporting of fine needle aspiration (FNA) specimens of salivary gland lesions: A comprehensive review. DiagnCytopathol. 2017;45:820–7.
15. Rossi ED, Wong LQ, Bizzarro T, Petrone G, Mule A, Fadda G, *et al*. The impact of FNAC in the management of salivary gland lesions: Institutional experiences

leading to a risk-based classification scheme. Cancer Cytopathol. 2016;124:388–96.
16. Pastore A, Borin M, Malagutti N, Di Laora A, Becati D, Delazer AL, *et al*. Preopertive assisment of salivary gland neoplasm with fine needle aspiration cytology and echography: a retrospective analysis of 357 cases. Int J Immunopathol Pharmacol. 2013;26(4):965–71.
17. Jain R, Gupta R, Kudesia M, Sing S. Fine needle aspiration cytology in the diagnosis of salivary gland lesions: a study with histologic comparison. Cytojournal. 2013;10:5.
18. Kim BY, Hyeon J, Ryu G, Choi N, Baek CH, Ko YH, *et al*. Diagnostic accuracy of fine needle aspiration cytology for high grade salivary gland tumors. Ann Surg Oncol. 2013;20(7):2380–7.
19. EstellesFerriol E, Dalmau GJ. Diagnostic accuracy of fine needle aspiration cytology in parotid tumors. Acta Otorrinolaringol Esp. 2014;65(3):157–61.
20. Fakhry N, Antonini F, Michel J, Penicaud M, Mancini J, Lagier A, *et al*. Fine needle aspiration cytology in the management of parotid masses: evaluation of 249 patient. Eur Ann Otorhinolaryngol Head Neck Dis. 2012;129(3):131–5.

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Original Research Article

A study of red cell indices and anaemia in adults at a tertiary care center

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Abstract

Background: Anemia is a condition that occurs when the blood hemoglobin (Hb) concentration falls below the lower limit of the normal reference interval stated for the age, sex, race, and altitude of a particular person. Iron deficiency, vitamin B12 and folic acid deficiency, malaria, hookworm infestations, schistosomiasis, renal diseases and other chronic infections are the common causes of anemia. The aim of the present study was to determine the correlation between red cell indices and type of anaemia on peripheral blood smear. **Material And Methods:** This prospective, observative study was carried out in the Department of Pathology, at a tertiary care hospital. Patients above 18 years of age, with hemoglobin concentration for the diagnosis of anaemia according to World Health Organization were included in the study. **Results:** After applying the inclusion and exclusion criteria, a total of 260 patients were included in the present study. Most common age group was between 19-30 years and most common type was moderate anaemia (8-11 g/dl). Males constituted around 38 % while females were 62 %. Male to female ratio was 1:1.6. On peripheral blood smear most common type of anemia was microcytic hypochromic type, which was seen in 157 patients. MCV corresponded to the peripheral blood smear findings in 132 patients and Red cell distribution width (RDW) corresponded to the peripheral blood smear findings in 211 patients. **Conclusion:** Red cell parameters and peripheral blood smear plays an essential role in the differential diagnosis of anaemia and in its treatment. Microscopic examination of peripheral blood smear samples is as important as automatic hemogram.

Key Words: Anaemia, RBC indices, MCV, MCHC, MCH, peripheral smear examination,

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INTRODUCTION

Anaemia is a condition that occurs when the blood hemoglobin (Hb) concentration of a person is below the lower limit of the normal reference interval stated for his /her age, sex, race, and altitude. The World Health Organization defines anaemia as hemoglobin level less than 13g/dl in adult males and less than 12 g/dl in adult females and less than 11g/dl in pregnant females[1]. Iron deficiency, vitamin B12 and folic acid deficiency, malaria, hookworm infestations, schistosomiasis, renal diseases and other chronic infections are common causes of anaemia. Nutritional anaemia is a worldwide problem and its prevalence is highest in developing countries[2]. Peripheral blood smear examination and hemogram are common routine hematological investigations done in laboratories, yielding significant results. RBC indices are calculated parameters that are part of an automated blood count report. They provide important guidelines for the diagnosis and classification of anemia and also in monitoring the treatment.[3] Automated hematology cell counters have the advantages of improved accuracy and precision, have decreased subjective errors and are safe in handling blood specimen.

RBC indices include:

1. Mean cell (or corpuscular) volume (MCV) refers to the average size of the RBCs. Reference interval for adults is typically 80 - 100 femtoliters (fL).

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2. Mean cell hemoglobin (MCH) refers to the average weight of hemoglobin in the RBCs. The reference interval for adults is typically 26 – 32 picograms (pg).
3. Mean cell hemoglobin concentration (MCHC) refers to the average concentration of hemoglobin in the RBCs. Reference interval for adults is typically 32 - 36 g/dL.

The study of RBC indices is an effective method for the quantitative assessment of RBC. Since MCHC is independent of RBC count and size, it is considered to be of greater clinical significance as compared to the other red cell indices. Along with peripheral blood smear examination (PBS), it allows a broad differential diagnostic impression that provides directions for further specific investigations[4]. Anemia is an important cause of morbidity and mortality in all age groups. Considering the high prevalence of anaemia all over the world and especially the high burden it carries in developing countries like India, the present study was done to determine the correlation between red cell indices and the type of anemia on the peripheral blood smear.

MATERIAL AND METHODS

Present study was a prospective, observational type carried out in the Department of Pathology, at a tertiary care hospital, for a period of six months. Study approval was taken from institutional ethics committee. Written informed consent for participation in the study was obtained from patients. Study samples were collected from both inpatient and outpatient departments.

Inclusion criteria

Patients aged more than 18 years, with a low hemoglobin concentration as per the World Health Organization guidelines were included in the study.

Exclusion criteria

Patients aged less than 18 years, pregnant females and patients with chronic illness (heart diseases and kidney diseases) were excluded from the study. 2 ml samples were collected from the patients in Ethylene diamine tetra acetic acid (EDTA) tubes. Samples were processed for CBC in cell counter. The cell counter generated parameters: mainly red blood cell (RBC) indices (MCV, MCHC, and MCH), RDW (Red cell distribution width) and platelet counts were analyzed. Peripheral Smears were prepared from the same samples and were stained by standard protocol with Leishman stain. After preparation, each peripheral smear was examined by a single faculty from the department, for the morphological abnormalities associated with different types of anemia and the results were noted. The results obtained by peripheral smear examination and the cell counter generated parameters were correlated.Statistical analysis was done using descriptive statistics.

RESULTS

After applying the inclusion and exclusion criteria, a total of 260 patients were included in the study. For the assessment of severity, anemia is classified based on the hemoglobin value into mild (11 g/dL to normal), moderate (8 g/dL to 11 g/dL), and severe (less than 8 g/dL) types in adult males and adult non pregnant females[1]. In this study, the most common age group affected was 19-30 years and the most common type was moderate anaemia (8-11 g/dl).

Table 1: Age wise distribution of grading of anaemia

Age (yrs.)	Mild	Moderate	Severe	Total	Percentage (%)
19-30	31	68	23	122	47%
31-40	19	28	11	58	22%
41-50	15	11	8	34	13%
51-60	13	9	3	25	10%
>60	11	8	2	21	8%
Total	**89**	**124**	**47**	**260**	

Males affected were 38 % while females were 62 %. Male to female ratio was 1:1.6.

Table 2: Gender distribution and Grading of anaemia

Anaemia severity	Males (%)	Females (%)	Total
Mild	37 (38%)	52 (32%)	89
Moderate	45 (46%)	79 (49%)	124
Severe	16 (16%)	31 (19%)	47
Total	**98 (38%)**	**162 (62%)**	**260**

On peripheral blood smear, the most common morphological type was microcytic hypochromic anemia, seen in 157 patients.

Table 3: Morphology of RBC

Types	Male (%)	Female (%)	Total
Microcytic hypochromic	61 (62%)	96 (59%)	157
Normocytic normochromic	27 (28%)	55 (34%)	82
Normocytic hypochromic	7 (7%)	10 (6%)	17
Microcytic normochromic	2 (2%)	1 (1%)	3
Macrocytic normochromic	1 (1%)	0	1
Total	**98**	**162**	**260**

MCV was corresponding to peripheral blood smear findings in 132 patients.

Table 4: Mean Corpuscular Volume (MCV) vs distribution of type of anaemia

Types	Normal	Low	High	Total
Microcytic hypochromic	25	126	6	157
Normocytic normochromic	68	5	9	82
Normocytic hypochromic	0	0	17	17
Microcytic normochromic	2	1	0	3
Macrocytic normochromic	1	0	0	1
Total	**96**	**132**	**32**	**260**

Red cell distribution width (RDW) was corresponding to peripheral blood smear findings in 211 patients.

Table 5: Red cell Distribution Width (RDW) vs distribution of type of anaemia

Types	Normal	Low	High	Total
Microcytic hypochromic	6	3	148	157
Normocytic normochromic	25	15	42	82
Normocytic hypochromic	0	0	17	17
Microcytic normochromic	0	0	3	3
Macrocytic normochromic	0	0	1	1
Total	**31**	**18**	**211**	**260**

DISCUSSION

The cases having anaemia were classified by peripheral blood smear examination into five major groups as microcytic hypochromic, normocytic normochromic, normocytic hypochromic, microcytic normochromic, macrocytic normochromic. Morphological classification of anaemia shows three types of anaemia. The most common type is microcytic hypochromic anaemia which shows reduced MCV (mean corpuscular volume) <80 fl and reduced MCHC (mean corpuscular hemoglobin concentration) <32 gm/dl. Normocytic normochromic anaemia has normal MCV (82- 100 fl) and macrocytic anaemia has MCV >100 fl and normal MCHC[5,6]. In our study, anemia was more prevalent among females (62%) than males (38%). Studies done in different parts of India showed similar finding with more prevalence of anemia in the adults among females.[7, 8, 9]. In our study, the most common type was microcytic hypochromic anaemia. Several studies had shown that microcytic hypochromic anaemia was common and etiology was suggestive of iron deficiency.[7, 8, 10]. We had only 1 case of macrocytic anaemia. Macrocytic anaemia is common among older adults and comprises almost 14% of all anaemia cases[11]. Macrocytosis is most commonly associated with vitamin B12, folic acid deficiency followed by alcoholism and liver disease[11]. Choudhary *et al*[12] showed that most of the cases of anaemia showed abnormal red cell distribution width (RDW). There was increase in RDW level with microcytic anaemia. We also noted similar findings in our study. Since iron deficiency anaemia is the most common type of anaemia, RDW can be used as an effective tool for the diagnosis. RDW determination had high sensitivity and specificity for early diagnosis of microcytic (iron deficiency) anaemia. RDW is usually not done as a part of CBC in developing countries. The use of RDW can be promoted for early detection and starting treatment of iron deficiency anaemia[12]. In iron deficiency (ID), a normocytic normochromic anaemia along with anisocytosis precedes the development of anisochromia, hypochromia and microcytosis. Using automated full blood counters, the earliest evidence of iron deficiency is an increase in the red cell distribution width (RDW). This is an indicative of anisocytosis which precedes anaemia. Thalassemia trait is considered to be an important differential diagnosis of iron deficiency anaemia. The RDW is usually elevated in iron deficiency anemia, but is often normal in thalassemia trait. The poor bioavailability of nonheme iron present in plant-based local diets like rice, pulses and vegetables that are high in phytic acid and fiber, could inhibit iron absorption. It is likely to be an important factor in the development of anaemia and iron deficiency[13]. The benefit of manual scan of peripheral smears is to detect clinically significant morphological abnormalities (like pencil cells, sickle cells, tear drop cells, schistocytes etc.) that are not quantifiable by the automated cell counters. The results of hematology analyzer should be validated with manual scan of peripheral blood smear and both the results must be correlated. It is important to understand the underlying pathophysiology of the disorder affecting red cell production and destruction. This can be effectively done with critical and careful evaluation of clinical findings and investigations. Simple and common investigations like peripheral blood smear and hemogram can definitely provide initial guidance for further workup.

CONCLUSION

Red cell parameters and peripheral blood smear play an essential role in the differential diagnosis of anaemia and in its treatment. Microscopic examination of peripheral blood smear samples is as important as automated hemogram.

REFERENCES

1. World Health Organization. Hemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and mineral nutrition information system. Geneva, WHO, 2011.
2. Balarajan Y, Ramakrishnan U, Ozaltin E, Shankar AH, Subramanian SV. Anaemia in low-income and middle-income countries. Lancet. 2011 Dec 17;378(9809):2123-35. \
3. Brugnara C, Mohandas N. Red cell indices in classification and treatment of anaemias: from M.M.Wintrobes's original 1934 classification to the third millennium. Curr Opin Hematol. 2013; 20(3):222-30. doi:
4. Glader B. Anaemia: General considerations. In: Greer JP, Rodgers GM, Paraskevas F, Glader B, eds. Wintrobe's clinical Haematology. 11th ed. Philadelphia: Wolters Kluwer Company, 2004: 947-978.
5. Jadhav SU, Khaparde S. Study of the red cell indices, hemogram and platelet variations in anemic (10 gm %) patients by automatic cell counter in a tertiary care centre, Ahmednagar, Maharastra, India. Int J Res Sci 2017; 5: 1582-8.
6. Qureshi NA, Chauhan MAZ, Goswami AP, Suri Sk. Study of anaemia and its correlation with hematological parameters in patient of various age group. J Dental Med Sci 2015; 14: 29-35.

7. Uria GA, Naik PK, Midde M, Yalla PS, Pakam R. Prevalence and severity of anaemia stratified by age and gender in rural India. Hindawi 2014; 10: 1-5.
8. Jadhav MV, Agarwal SA, Kadgi NV, Khedkar SS, Kulkarni KK, Kudrimoti JK. utility of automated RBC parameters in evaluation of anaemia. Intl J Healthcare Biomed Res 2015; 3: 170-81
9. Gupta SK, Agarwal SS, Kaushal R, Jain A, Gupta VK, Khare N. Prevalence of anaemia among rural population living in and around of rural health and training center, Ratua village of Madhya Pradesh. Muller J Med Sci Res 2014; 5: 15-8.
10. Verma P, Singh S, Ghildiyal A, Kumar A, Krishna A. Prevalence of anaemia in adults with respect to sociodemographic status, blood groups and religion in north Indian population. Int J Biol Med Res 2012; 3: 2422-8.
11. Maruyama S, Hirayama C, Yamamoto S *et al*. Red blood cell status in alcoholic and non alcoholic liver disease. J Lab Clin Med 2001; 138: 332-7
12. Choudhary M, Sharma D, Shekhawat DS, Dabi D. Significance of red cell distribution width in the diagnosis of iron deficiency anaemia: an observational study from India. J Pediatr Neonatal Care 2015; 2; 102-6
13. Rao N. Anaemia and micronutrient deficiency. Natl Med J India. 2003;16(suppl 2):46-50.

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A Study of Clinicopathologic Spectrum of Carcinoma Breast

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Abstract

Introduction: Traditional models of breast cancer development are based on morphological studies and suggest the transition from a normal epithelial cell via hyperplasia and atypical hyperplasia to ductal carcinoma in situ. The risk for carcinoma increased with the rate of proliferation and atypia in breast biopsies. *Methodology:* In this study, collection of family history and interrogation with the patient forms an integral part. This was done by direct communication with the patient at the Pathology and Surgery departments, collection of details from the radiotherapy department records and by collecting filled up proforma from the patients by post. *Results:* It is clearly seen that nearly 78% of the tumours belonged to the higher grade –Grade 2 and 3, whereas low-grade tumours constituted only 22%.*Conclusion:* The lymph node positivity is a strong indication to make the public aware of this disease and to intensify the screening and surveillance programmes

Keywords: Breast Carcinoma; Clinical Profile; Pathological Profile.

Introduction

Breast cancer is one of the leading causes of cancer deaths worldwide. It accounts for 23% of all carcinomas in women and 14.1% of female cancer deaths. Incidence rates have continued to increase world wide, with an overall increase of 0.5% since 1990 [1]. Changes in the incidence rates are greatest in the developing countries.

In India an average of 80,000 women are diagnosed with carcinoma breast and 40,000 die every year, of this disease [2]. Although it is currently the second most common carcinoma among Indian women (19%), after carcinoma cervix (30%), in the urban registries like Delhi, and Bombay, carcinoma breast has overtaken carcinoma cervix in frequency. These data not only demonstrate the current health problem associated with carcinona breast in the Indian population, but also indicate that socioeconomic trends will lead to rapid increase in its distribution to the overall health burden.

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The frequency of this disease has prompted an invasive study of the risk factors involved and the morphological spectrum of the disease, so as to gain clues to its etiology as well as to identify modifiable risk factors that would be helpful for prevention strategies.

Traditional models of breast cancer development are based on morphological studies and suggest the transition from a normal epithelial cell via hyperplasia and atypical hyperplasia to ductal carcinoma in situ. The risk for carcinoma increased with the rate of proliferation and atypia in breast biopsies. But the only intraductal proliferation that can be considered as an obligate precursor to every breast cancer is carcinoma in situ [3,4]. This does not imply that very in situ case will progress to invasive cancer. There is no direct evidence that epithelial hyperplasia and atypical hyperplasia are precursors to carcinoma neither from histopathological nor epidemiological or molecular biological studies.

Methodology

Study was done on the mastectomy specimens

received in the histopathology division of Department of Pathology, MCH, during the study period.

In this study, collection of family history and interrogation with the patient forms an integral part. This was done by direct communication with the patient at the Pathology and Surgery departments, collection of details from the radiotherapy department records and by collecting filled up proforma from the patients by post. A detailed family history was collected with a standard proforma.

Only mastectomy specimens were included in the study. They were fixed in 10% formalin. Sections were obtained from the tumour proper nipple& areola, adjacent areas of the tumour, surgical margins and lymph nodes. The sections are stained with Haematoxylin and Eosin. These were studied in detail under light microscope. Pathological interpretation of the specimen was done as to the tumour size histological type and grade, presence of DCIS, associated fibrocystic disease, skin involvement, involvement of surgical margins and lymph node status.

Results

Table 1: Age distribution

Age	Cases	%
< 20 years	0	0
20-30	4	3
31-40	19	13
41-50	58	40
51-60	42	29
>60 yrs	22	15

Table 2: Morphological Sub typing

Histology Type	No. of cases
Infiltrating duct Carcinoma (NOS)	136
Lobular Carcinoma	1
Medullary Carcinoma	2
Mucinous Carcinoma	1
Squamous Carcinoma	1
Metaplastic Carcinoma	4

Table 3: *Classification according to grade*

Tumour size	No. of Cases	%
< 2 cm	5	4
2 to 5 cm	118	81
More than 5	22	15

Table 4: Tumour Size

Grade	No. of Cases	%
Grade 1	32	22
Grade 2	67	46
Grade 3	46	32

Table 5: Other associated features

Features	No. of cases
Ductal Carcinoma in situ	
Grade 1	11
Grade 2	6
Grade 3	15
	Total 32 (22%)
Fibrocystic disease of Breast	
Without atypia	16
With atypia	2
	Total 18 (12%)
Paget's Disease	5
Lymph node metastasis	64 (45%)

Discussion

The maximum number of cases is in the 41 – 50 age group - 40%. 29% of the tumours were in the 51 -60age group, 16% in the below 40 age group and 15% above 60 years. Statistical data for the previous 5 years of this department clearly shows that there is a steady increase in the incidence of breast cancer every year. It also appears that there is a shift in the age composition of these patients from >50 age group, to < 50 age group. The number of cases in the above 60-age group is declining. These findings show that the pattern of age distribution reaches maximum in the 4th and 5th decades and remains constant or declines thereafter. There are two reasons for such a pattern.

The first and foremost is the acceptance of the newer screening measures by the general public. The young females are aware of the importance of early detection. They do self-palpation and seek medical advice as soon as they detect a lump. The triple assessment method – clinical examination, mammography and FNAC, provides rapid diagnosis without invasive procedures. The second reason is the difference in pre and postmenopausal hormonal status. Estrogen induces proliferation of the ductal epithelium. Both endogenous and exogenous estrogen excess can result in carcinoma. After menopause, there is a quick fall in the estrogen level. This may reduce the cancer risk in postmenopausal women.

The Histological Classification

The largest single group was Invasive Ductal Carcinoma (NOS), which constituted 94% of the study group. This is comparable to the findings in other studies. Many of the subtypes like tubular carcinoma, papillary carcinoma etc was not seen in this study. There were 4 cases of metaplastic carcinoma. This constituted a considerably larger group (3%) when compared to other studies [5,6].

Histological classification has prognostic significance. IDC variants like tubular carcinoma, mucinous carcinoma, medullary carcinoma, secretory carcinoma and papillary carcinoma has good prognosis. It has been proved recently that the production of gel forming secretory mucins like MUC-2 and MUC-6 is responsible for the better prognosis of mucinous carcinoma [7]. This mucin acts as a barrier against cancerous extension.

The Grade of the Tumours

Grading was done following the Modified Bloom Richardson method, taking into account tubule formation, nuclear pleomorphism and mitotic counts.

It is clearly seen that nearly 78% of the tumours belonged to the higher grade –Grade 2 and 3, whereas low-grade tumours constituted only 22%. This data is clearly significant because grading is a powerful prognostic information and most of the studies so far done have shown a significant association between grade of the tumour and patient survival [8]. Survival worsens with increasing grade.

Classification Based on Tumour Size

Size of the tumour is an easily measurable, strong predictor of tumour dissemination and prognosis. It is also a criterion for the classification of 'Minimal breast carcinomas' which includes all in situ carcinomas irrespective of the size and all invasive carcinomas less than or equal to 1 cm in diameter. These patients have 75% 10-year survival rate in node negative cases [9,10].

In the present study, tumour size of majority of patients was between 2-5 cm (81%). While in 4% of the patients the tumour size was <2 cm, only 15% of the patients had tumour size more than 5 cm. This shows that most of the lesions are detected before they attain very large size. Patient has a considerable survival advantage, if the tumour is diagnosed before attaining a large size.

Lymph Node Metastasis

45% of the tumours of this study group were node positive. Lymph node metastasis is the single most important prognostic parameter.

The presence of lymph node metastasis alters the stage of the disease irrespective of the tumour size. The 10-year survival rate of node negative patients is around 70%. This falls to 25 -30% in node positive cases[11,12]. Small node negative tumours can be cured by less vigorous treatment.

In this study majority of the patients have a favorable tumoursize. But irrespective of that, the high percentage of lymph node positivity places them in the higher stage, poor prognostic group.

Associated Proliferative Lesions

In this study, 22% of the cases showed associated DCIS and The opinion regarding considering in situ lesions, as prognostic factors are variable. Some studies say that prominent DCIS around an invasive tumour conveys a better prognosis [15,16]. In the modern era,

breast conservation surgeries are getting more popularity. So, the detection of in situ lesions in the resected margins of wide excision specimens determines the recurrence rates. Fibrocystic disease, which is considered as a forerunner of malignancy, was present in only 12% of the patients in the present study group.

Early Onset Carcinoma Breast Cases

Age Group

The age limit below which a carcinoma should be called as an early onset carcinoma varies in different studies. It has not been clearly defined till now. In a study by SunitaSaxenaet al [17], all cases less than or equal to 40 years is taken as early onset cases.

In this study, all cases less than or equal to 40 years are included in the early onset category. 16% of the cases were of early onset type. The youngest age detected was 25 years. The mean age calculated is 35 years. Sunita Saxena [17] et al got 59% of the cases in their study group, as early onset type. When compared to that, the percentage of early onset carcinoma cases in our population is low. Geographic risk factors may alter the epidemiology of carcinoma breast cases in different parts of the country.

The Histological Characteristics

All the 23 cases were histological invasive ductal carcinoma (NOS) type. Other variants like medullary carcinoma, secretory carcinoma which are usually described in younger age group, were not identified in this study. No significant difference in histological patterns is described between early onset carcinomas and those in older age group. IDC (NOS) is the most common type described in both groups.

Majority of the cases were in the grade 2 category. This observation is similar to that seen in the older age group.

Associated in situ carcinoma is seen in 56% of the cases. Majority are high grade DCIS. According to Claus EB et al [18], an inverse relation is seen between the age of onset and carcinoma in situ risk. He found out that, those cases < 49 years of age had 2.1 times risk than controls. Cases older than 49 years had 1.5 times the risk of controls. In this study, the association of more cases with high grade DCIS, may be the result of progression from florid epitheliosis through in situ carcinoma into invasive carcinoma.

Histologically all the 5 cases were invasive duct carcinomas (NOS) type. It is the most common histological type described in all forms of hereditary case [19]. A higher percentage of medullary carcinomas are also described. But no other histologic type was seen in this study. According to HannealinaErolaet al [20], the distinct pathologic features of hereditary carcinomas are found only in patients less than 50 years of age. These distinct pathologic features are high incidence of medullary carcinomas, high-grade tumours, ER/PR negativity and p 53 mutations.

2 cases showed in situ carcinomas (grade 1 and 2) and in 2 cases fibrocystic disease was present. The results vary in different studies. In the pre BRCA era [21], a higher prevalence of proliferative lesions (35%) was reported in hereditary cases. According to Claus EB, the family history of breast cancer is an important risk factor for carcinoma in situ. Cases with DCIS or LCIS are more likely to have a history of breast cancer in first degree relative.

Recent studies suggest that familial breast cancer is associated with a different set of initiating events when compared to sporadic carcinomas. A lower prevalence of precursor lesions is seen hereditary cancers [22]. This is due to the acceleration of tumourigenesis in these cases. The environmental carcinogenesis step is bypassed and there is a quick progression of tumour cells through a low-grade phase into a higher grade.

Conclusion

- Comparatively younger age group females are also affected by this disease with much frequency than previous years.
- Nearly half of the study group has lymph node positivity at the time of diagnosis.

References

1. Parkin D M, Bray F, Ferlay J, Pisoni P. Global cancer statistics, 2002.CA Cancer J Clin 2005; 55:74-108.
2. Kumar, Abbas, Fausto.Robbins and Cotran. Pathologic Basis Of Disease.7th edition, page 1131
3. Dupont W D,.Risk factors in women with proliferative breast disease. N Engl J Med 1985; 312:146 -151.
4. Fitzgibbons PL, Weaver D, Thor AD, Allred DC, LichterA .Prognostic factors in breast cancer. Arch Pathol Lab Med 2000; 124:966 -978.
5. Dixon JM et al. Long term survivors after breast cancer. Br J Surg 1985; 72; 445.
6. WHO Classification of tumours, Pathology And Genetics, Tumours of The Breast and Female Genital

Organs. Edited by Fattenah A Tavassoli and Peter Devilee, page 18.

7. S Matsukita, M Namoto, S Kitazima, S Tanaka. Expression of Mucins MUC 1, MUC 2, MUC 5AC and MUC 6 in invasive carcinoma of breast; comparison with invasive ductal carcinoma. Histopathology 2003; 42: 26-36.
8. Hensen DE, Ries L, Freedman LS, Carriaga M. Relationship among outcome, stage of disease and histologic grade for22,616 cases of breast cancer; the basis for a prognostic index. Cancer 1991; 68: 2142-2149.
9. Rosai and Ackermann's Surgical Pathology, 9th Edition, Volume 2, page 1824.
10. Saigo P, Rosen PP. Prognostic factors in invasive mammary carcinomas 1cm or less in diameter. Am J ClinPathol 1980; 73: 303-304.
11. Elston CW, Gresham GA, Rao GS et al. The Cancer Research Campaign (Kings/Cambridge). Trial for early breast cancer – pathological aspects. Br J Cancer 1985; 45: 655-669.
12. Fergasion DJ, Meier P, Karrison T et al. Staging of breast cancer and survival rates; an assessment based on 50 years of experience with radical mastectomy. J Am Med Assoc 1982; 248; 1337-1341.
13. Silverberg SG, Chitala AR. Assesment of the significance of the proportion of intraductal and infiltrating tumour growth in ductal carcinoma of the breast. Cancer 1973; 32: 830-837.
14. Matsukuma A, Enjoji M, Toyoshima S. Ductal carcinoma of the Breast. An analysis of the proportion of intraductal and invasive components.Pathol Res Pract 1991; 187: 62-67.
15. Fourquet A, Campana F, Zafrani B et al. Prognostic factors of breast carcinoma reccurence in the conservative management of early breast cancer. A 25 year follow up. Int J Rad OncolBiolPhys 17; 719-725.
16. Jacqueimer J, Kurtz JM, Amalric R et al. An assessment of extensive intraductal component as a recurrence after breast conserving therapy.Br J Cancer 1990; 61: 873-876.
17. SunitaSaxena, AnurupaChakraborthy, Mushy Marshal, SanjeevKotwal, Dinesh Bhatnagar,Ravindar S, Veena K Sharma, Gilbert Lenoir. Contribution of germline BRCA 1 and BRCA 2 sequence alterations to breast cancer in Northern India. BMC Medical Genetics 2006; 7: 75.
18. Claus EB, Stowe M, Carter D. Family history of breast and ovarian cancer and the risk of breast carcinoma in situ. Department of Epidemiology and Public Health, Yale University School of Medicine, New Heaven, CT 06520-8034, USA. Claus @ biomed. Med. Yale.edu
19. Chappuis PO, Nethercort V, Foulkes WD. Clinicopathological characteristics of BRCA 1 and BRCA 2 related breast cancer. SeminSurgOncol 2000; 18: 287-295.
20. HannaleenaEerola, PaiviHeikkila, AnittaTamminau, Kristina Aittomaki, HeliNevanlinna. Relationship of patient's age to histopathological features of breast tumours in BRCA 1 and BRCA 2 and mutation negative breast cancer families. Breast cancer Research 2005; 7: R465-R469.
21. Skolnick MH, Cannon Albright LA, Goldger DE et al. Inheritance of proliferative breast disease in breast cancer kindreds. Science 1990; 250: 1715-1720.
22. Camilo Adam, Carol Reynolds, Charyl L Soderborg, Jeffrey M Slezak, Shannon K Mc Donnell, Thomas J Sebo, Robert B Jenkins. Pathologic characteristics of breast parenchyma in patients with hereditary breast carcinoma- including BRCA 1 and BRCA 2 mutation carriers. Cancer 2000; 97(1): 5-15.

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A Study on Laboratory Profile of Patients with Viral Illness (Influenza Like)

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Abstract

Introduction: Influenza-like illness (ILI) is defined by the CDC as fever (temperature ≥100°F or 37.8°C) and either cough or sore throat in the absence of another known cause. *Methodology:* The patients with clinical features of Influenza like illness were enrolled. A complete clinical examination was carried out and relevant investigations done and documented in the proforma. *Results:* Fever was the most common symptom and was seen in all cases (100%), followed by cough (94.3), sore throat (74.3%), nasal catarrh (22.9%), difficulty in breathing (8.6%), vomiting (11.4%), diarrhea(2.8%). *Conclusion:* The clinical manifestations of influenza A H1N1 virus infection range from self- limited uncomplicated subfebrile respiratory illness to severe and even fatal respiratory disease and extrapulmonary disease. Gastrointestinal symptoms include vomiting, diarrhea and abdominal pain.

Keywords: Influenza; Laboratory Profile; Clinical Manfestations.

Introduction

The first case of the flu in India was found on the Hyderabad airport on 13th May 2009.As of 24th may 2010, 10193 cases of swine flu have been confirmed with 1035 deaths [1].

In India the state of Maharashtra is the worst affected followed by Karnataka. As of 8th august 2010 in Karnataka a total of 2899 confirmed cases and 198 deaths have been reported. It is difficult to estimate precisely the number of people infected by the H1N1 viruses as many may not have developed symptoms while others with mild illness may not have sought medical care [2].

Influenza-like illness (ILI) is defined by the CDC as fever (temperature ≥100°F or 37.8°C) and either cough or sore throat in the absence of another known cause. A confirmed case of 2009 H1N1 infection is defined by ILI with positive test results for the 2009 H1N1 virus by either realtime reverse transcriptase polymerase chain reaction (rRT- PCR)or viral culture. A probable case is ILI with positive influenza A test results but negative results for seasonal H1 and H3 by rRT-PCR. A suspected case does not meet either definition but is either a person younger than 65 years hospitalized for ILI or a person of any age with ILI and an epidemiological link to a confirmed or probable case within 7 days of illness onset [3].

Laboratory findings at presentation in patients with severe disease typically include normal or low-normal leukocyte counts with lymphocytopenia and elevations in levels of serum aminotransferases, lactate dehydrogenase, creatinekinase, andcreatinine. Myositis and rhabdomyolysis have occurred in severe cases. A poor prognosis is associated with increased levels of creatine kinase, creatinine, and perhaps lactate dehydrogenase, as well as with the presence of thrombocytopenia and metabolic acidosis [4].

Methodology

The patients with clinical features of Influenza like illness were enrolled. A complete clinical examination was carried out and relevant investigations done and documented in the proforma.

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Inclusion Criteria

Proved cases of influenza A H1N1 and negative cases, where no other specific diagnoses were clinched.

Exclusion Criteria

All other Influenza like illness patients where specific diagnosis other than influenza A H1N1 was clinched were excluded from the study group.

Results

Age of the patients ranged from 1month to 75 years and majority of the caseswere in the age range of 16-30yrs. Mean age was 32.72yrs.

Fever was the most common symptom and was seen in all cases (100%), followed by cough (94.3),sore throat(74.3%), nasal catarrh (22.9%),difficulty in breathing (8.6%), vomiting (11.4%), diarrhea(2.8%). The clinical manifestations favour the diagnosis of influenza A H1N1 but the rRT- PCR was negative for H1N1 in these cases.

Hematocrit values ranged from 17.1% to 43.3%. Majority of the patients had their hematocrit in the range of 36-42 %. The mean hematocrit value was 40.65%.

Table 1: Age wise distribution of negative cases

Age range in yrs	Number of cases	Percentage of cases
1-15	06	17.1
16-30	13	37.1
31-45	08	22.9
46-60	06	17.1
61-75	02	5.8
total	35	100

Table 2: Clinical features of negative case

Symptom	Number of cases	Percentage
Fever	35	100
Cough	33	94.3
Sore throat	26	74.3
Nasal catarrh	8	22.9
Difficulty in breathing	3	8.6
Vomiting	4	11.4
Diarrhea	1	2.8

Table 3: Haematocrit values of negative cases(36%-50%)

HCT	Number of cases	Percentage
<24	1	2.9
24-30	1	2.9
30-36	6	17.1
36-42	21	60
42-48	6	17.1
Total	**35**	**100**

Table 4: Total Leucocyte count values of negative cases(4-11 $\times 10^3/mm^3$)

TLC(10^3/cumm)	Number of cases	Percentage
<4	4	11.4
4-8	15	42.9
8-12	9	25.7
12-16	4	11.4
16-20	3	8.6
Total	**35**	**100**

Mean Corpuscular Volume (78-98fl)

MCV values ranged from 68fl-92fl. Mean value of MCV was 84.14 fl. Majority of patients had MCV within normal range.

Mean Corpuscular Hemoglobin(27-32pg)

MCH values ranged from 24.2 pg-32.6 pg. Mean value of MCH was 27.86 pg. Majority of cases had values within normal range.

Mean Corpuscular Hemoglobin Concentration (32-36 g/dl)

MCHC values ranged from 28.2-35.8 g/dl. Mean value was 32.2g/dl. Majority of cases had values within normal range.

Red Blood Cell Count

The RBC count ranged from 1.5- 6.2 x10v / mm³.Normal value (3.80-5.8 x10v /mm³). Mean value was 4.69x10v /mm³.

Red Cell Distribution Width

The RDW ranged from 10.9-14.9%. Normal values(10.0 – 15.0%). Mean value was 13.04%

Total Leucocyte count ranged from 2.6 $\times 10^3$ to 19.8 $\times 10^3$/ mm^3. Majority of the patients had their count in the range of 4×10^3 -8 $\times 10^3/mm^3$. Mean leucocyte count was 8.58 $\times 10^3/mm^3$. Referring to the normal values for the age, 4 cases had leucopenia and 8 cases had leucocytosis.

Lymphocyte percentage ranged from 6.2%-42.8%. Mean lymphocyte percentage was 25.73%

Table 5: Relative Lymphocyte count values in negative cases(20-45%)

Lymphocyte %	Number of cases	Percentage
<20	6	17.1
20-30	18	51.4
30-40	10	28.6
>40	1	2.9
Total	**35**	**100**

Table 6: Absolute Lymphocyte count values of negative cases(1-3.5$\times 10^3/mm^3$)

Lymphocyte	Number of cases	Percentage
<1	8	22.9
1-2	8	22.9
2-3	12	34.2
>3	7	20
Total	**35**	**100**

Table 7: Platelet count values of negative cases(150-450 $\times 10^3$/cumm)

Platelet	Number of cases	Percentage
<150	2	5.7
150-250	29	82.9
250-350	2	5.7
>350	2	5.7
Total	**65**	**100**

Table 8: Age comparison study of negative cases

Study	Age range	No of cases	Percentage
Zarogoulidis P et Al[5] n =60	16-86	27	45
Present n=100 study	.08-75	35	35

Table 9: Comparison of Hemoglobin values of negative cases

Study	Mean Hemoglobin value (g/dl)
Chan WL et al[6](2011)	13.7
Present study	12.6

Table 10: Comparison of Total leucocyte count of negative cases

Study	Mean TLC (× 10^3/ mm^3)
Chan WL et al[6](2011)	7.2
Zargoulidis et al[5](2011)	12.8
Present study	8.6

Table 11: Platelet comparative study of negative cases

Study	Mean platelet count(10^3/dl)
Chan WL et al[6](2011)	207
Present study	197.9

Lymphocyte count ranged from 0.4×10^3-7.2×10^3/ mm^3. Mean lymphocyte percentage was 2.24×10^3/ mm^3. With reference to normal values for the age 8 cases had lymphopenia.

Relative Granulocyte Count

Granulocyte percentage ranged from 53%-91.3%. Normal values 40-70%. Mean granulocyte percentage was 69.3%

Absolute Granulocyte Count

Granulocyte count ranged from 1.8×10^3-14.1×10^3/ mm^3.Normal values $1.5\text{-}7.0\times10^3/mm^3$. Mean granulocyte count was $5.92\times10^3/mm^3$.

Relative Monocyte Count

Monocyte percentage ranged from 2-10.4%. Normal values 2-10%.Mean monocyte percentage was 4.6%

Absolute Monocyte Count

Monocyte count ranged from $0.1\text{-}1.7\times10^3/mm^3$. Normal values $0.2\text{-}1.0\times10^3/mm^3$. Mean monocyte count was $0.58\times10^3/mm^3$.

Liver function tests were done in 4 cases. All cases had their bilirubin and protein levels within normal range. One case had elevated SGOT levels, and one case had elevated ALP levels.

Serum electrolytes like serum sodium and serum potassium were done in 3 cases. No abnormalities seen.

Discussion

In a study done by Zargoulidiset al [5] 27 cases were Influenza A H1N1 negative and the age range of the cases was 16yrs to 86 yrs

In our study 35 cases were Influenza A H1N1 negative and the age range was 8 months to 75 yrs.

In our study mean hemoglobin value was 12.6g/dl when compared to 13.7g/dl in study done by Chan et al [6] in Singapore.

The mean leucocyte count in our study was 8.6×10^3/ mm^3, similarly the mean leucocyte count in a study done by Chan et al [7] was within normal range 7.2×10^3/ mm^3 but the mean leucocyte count in a study done by Zargoulidis et al[8] was slightly elevated 12.8×10^3/ mm.

Comparison of Absolute Lymphocyte Count

In our study 22.9% had lymphopenia while 16% cases had lymphopenia in a study done at Singapore by Onget al [7].

In our study the mean platelet count was 197.8 $\times10^3$/dl when compared to 207×10^3/dl in study done by Chan et al [6] Thrombocytopenia was seen in 2 cases in our study.

Conclusion

Influenza A/ H1N1, like seasonal influenza, is more likely to cause serious illness in individuals at risk with underlying chronic illness or in those who are in the paediatric age group or pregnant women. Public health efforts should focus on providing appropriate support and preventive services, including vaccination, to these groups.

References

1. Fauci AS, Kasper DL, Longo DL, Braunwald E, Hauser SL, Jameson JL, Loscalzo J. Harrison's principles of Internal Medicine. 17th ed. USA: Mcgraw Hill: 2008.
2. Narain JP, Kumar R, Bhatia R. Pandemic (H1N1) 2009: Epidemiological, clinical and prevention aspects. Natl Med J India 2009; 22: 242-7.
3. Sullivan SJ, Jacobson RM, Dowdle WR, Poland GA. 2009 H1N1 Influenza. Mayo Clin Proc. 2010; 85(1): 64-76.
4. Hayden FG. Clinical Aspects of Pandemic 2009 Influenza A (H1N1) Virus Infection. N Engl J Med 2010; 362:1708-19.
5. ZarogoulidisP,Constantinidis T, SteiropoulosP, PapanasN, Zarogoulidis K, Maltezos E. Are there any differences in clinical and laboratory findings on admission between H1N1 positive and negative patients with flu-like symptoms. BMC Research Notes 2011, 4:4.
6. Chan W L, Goh H K, Vasu A, Lim B L, Seow E. Experience of a screening centre for influenza A/ H1N1: the first 50 days. Emerg Med J 2011; 28:18-24.
7. Ong AK, Chen MI, Lin L, Tan AS, Nwe NW, Barkham T, Tay SY et al., Improving the clinical diagnosis of influenza—a comparative analysis of new influenza A (H1N1) cases. PLoS One. 2009 Dec 29; 4(12): 8453.
8. Duque V, Cordeiro E, Mota V, Vaz J, Morais C, Rodrigues F et al., The early days of pandemic (H1N1) 2009 virus infection in the central region of Portugal. Rev Port Pneumol. 2010 Nov; 16(6):870-9.