Original Article

DOI: 10.7860/NJLM/2020/45120:2396

Pathology Section

Thyroid Lymphomas versus Lymphocytic Thyroiditis: A Diagnostic Challenge on Fine Needle Aspiration Cytology

DEEPTI MISHRA[1], VALLI PRIYA[2], NIRAJ KUMARI[3], NARENDRA KRISHNANI[4]

ABSTRACT

Introduction: The diagnosis of Primary Thyroid Lymphoma (PTL) and its distinction from Florid Lymphocytic Thyroiditis (FLT) is often difficult on Fine Needle Aspirate (FNA). The sensitivity is low for Low Grade Lymphoma (LGL) and the distinction of PTL is important as they are treated by chemotherapy rather than surgery. We studied the efficacy of FNA for thyroid lymphoproliferative disorders which includes thyroid lymphoma and lymphocytic thyroditis and detection of cytomorphological features differentiating between thyroiditis and lymphoma.

Aim: To find out interobserver variation in cytological diagnosis of thyroid lymphomas and thyroiditis with evaluation of the cytomorphological pointers of lymphoma on thyroid cytology.

Materials and Methods: Thirty-three cases of FNA thyroid with lymphoid proliferation in which histological confirmation with Immunohistochemistry (IHC) was available were retrieved and studied for various cytomorphological features by three pathologists. The findings were correlated with the final histopathology diagnosis.

Results: Of the total 33 cases that had significant lymphoid infiltrate on thyroid FNA, 28 cases were confirmed as Non-Hodgkin's lymphoma, three cases as thyroiditis and two case of carcinoma on histology. All the lymphoma cases were positive for CD20 antibody on histology. The percentage of cases of thyroid lymphoma was high in present study because only selected type of cases were included (FNA thyroid with lymphoid proliferation) and it does not refer to true incidence of thyroid lymphoma. Among three observers, two observers (observer 1&2) diagnosed 18 cases of HGL, two cases of FLT and one observer (observer 3) diagnosed 15 cases of HGL and one cases of FLT correctly on FNA, five cases of LGL were correctly diagnosed by all the three observers. The diagnostic accuracy was 76.1% with high specificity (80%) and sensitivity (94.7%) for HGL and high concordance among the three pathologists (Kappa= 0.8). LGL and FLT had low sensitivity (55.6% and 66.7%) and least interobserver correlation (kappa – 0.21). Monomorphism, large cells, absent plasma cells, macrophages were significantly associated with diagnosis of lymphoma.

Conclusion: Cytological features of thyroid lymphoma are diagnostic in HGL, however the features are more non-specific in LGLs. High cellularity, relatively monomorphic population and frequent occurrence of lymphoglandular bodies were common in LGL compared to FLT. It is challenging to diagnose PTLs, especially low grade on FNA. For this reason, it should be kept in mind that patients with chronic lymphocytic thyroiditis should be evaluated carefully and possibility of LGL should not rule out on cytology. The role of FNA is limited but it is simple safe and cost effective for initial workup in centres lacking facility of flow cytometry and immunocytochemistry.

Keywords: Interobserver variation, Lymphoid proliferation, Monomorphism, Thyroid cytology

INTRODUCTION

Lymphoma of thyroid is an uncommon malignancy accounting for 1% to 3.5% of all malignant lymphomas and 5% of all thyroid malignancies [1]. Women are affected more frequently than men (2.5-8.4:1), however the age of onset is earlier in men [2-6]. Diffuse Large B-Cell Lymphoma (DLBCL) is the commonest PTL with an incidence of 70% followed by marginal zone lymphoma occurring in 30% of cases. The other lymphomas are follicular lymphoma (3-5%), Hodgkin's lymphoma (2%) and Small Lymphocytic Lymphoma (SLL) (2%) [7-9]. PTL arise in an immunologically abnormal gland, usually one which is affected by chronic lymphocytic thyroiditis with incidence being 40-80 times more in patients with Hashimoto's Thyroiditis (HT) and >80% of PTL patients having HT [6,10,11]. PTLs usually occurs between 10-30 years of onset of HT. The diagnosis of PTL and its distinction from FLT poses a diagnostic challenge on FNA. The presence of large monotonous cells favours DLBCL. However, the presence of heterogeneous population of cells in FLT and marginal zone lymphoma makes their distinction difficult. Certain features such as abundance of lymphocytes, centrocyte like cells and absence of plasma cells, macrophages favour LGL [12]. The sensitivity and specificity of FNA for diagnosis of lymphoma varies from 65%->90% [13-15]. Swart GJ et al., described increased sensitivity (97%) and specificity (86%) of FNA for diagnosing thyroid lymphomas combined with flow cytometery [16]. Similarly, IHC improves sensitivity and specificity of FNA. Core needle biopsy with immunophenotyping is considered superior and often needed before management [14]. The diagnosis and their distinction is crucial because almost all PTL do not require surgery. Also, marginal zone lymphoma usually requires only chemotherapy, while DLBCL and mixed subtypes require combined chemotherapy and radiotherapy [1].

In the present study, cytomorphological features of thyroid lymphoproliferative disorders which had histological confirmation were reviewed with an aim to find the interobserver variation in cytological diagnosis of thyroid lymphoproliferative disorders and also to evaluate the cytomorphological pointers of lymphoma on thyroid cytology.

MATERIALS AND METHODS

The present study was a retrospective study of cytology and histology review. Thirty-three cases of FNA thyroid with lymphoid proliferations which includes thyroid lymphomas, thyroiditis and atypical lymphoid proliferation with histology done in each case were retrieved for review. These cases were received in the Department of Pathology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India from year 2000 to 2015. Cytological smears were blindly reviewed by three pathologists {observer1 (O1), observer 2 (O2) and observer 3 (O3)}. To perform thyroid FNA, the thyroid nodule was identified by palpation and a 22- to 25-gauge needle was used to procure cell samples from at least three different

areas of a thyroid nodule. The slides were originally stained with May Grunwald Giemsa, Haematoxylin-eosin and Papanicolaou stains. The aspirated material was quickly smeared into glass slides, wet fixed in 95% ethyl alcohol for subsequent Papanicolaou staining and air-dried smears for staining with one of the Romanowsky modified methods (May-Grunwald-Giemsa). All the lymphoma cases were positive for CD20, a B cell marker on histology.

Clinical information was recorded from the Hospital Information System (HIS). Following cytological features were evaluated – cellularity, cell size, cell monomorphism, plasma cells, macrophages, follicular cells, lymphoglandular bodies and lymphoepithelial lesions. These subjective features were correlated by chi-square test. The histological diagnosis was compared with cytological diagnosis and diagnostic accuracy for the various diagnoses which includes lymphoma and FLT was obtained.

STATISTICAL ANALYSIS

The interobserver variation for each diagnosis was also calculated by kappa co-efficient. Statistics was done using SPSS software, version 16 (IBM, USA).

RESULTS

There were 33 patients including 20 females and 13 males (M:F = 0.65:1) and age range of 22-69 years (median 60 years and mean 55.1 years). All patients had neck swelling in the form nodular or diffuse thyroid enlargement. Thyroperoxidase Antibody (TPO) was available for 10 patients and it was raised in seven patients (101 to >2300 IU/ ml, Normal range <20 IU/ml). Triiodothyronine (T3), Thyroxine (T4) and Thyroid Stimulating Hormone (TSH) levels were available for 29 patients. The thyroid function tests were deranged in eight patients while 21 patients were euthyroid [Table/Fig-1].

Of the total 33 cases that had significant lymphoid infiltrate on thyroid FNA, 28 cases were confirmed as Non-Hodgkin's lymphoma, three cases as thyroiditis and two cases as carcinoma on histology. One case that had trucut biopsy was inadequate for diagnosis because of small fibrotic tissue. The number of cases of thyroid lymphoma was high because this study has selected particular type of cases (FNA thyroid with lymphoid proliferation) only. The cytological and histological diagnosis of all cases is shown in [Table/Fig-2].

The diagnosis of HGL had the highest sensitivity as 94.7% and specificity of 80%. The diagnosis of LGL and FLT had high specificity of 95.8% and 96.6%, respectively; however the sensitivity was much lower (55.6% and 66.7%, respectively). The sensitivity, specificity and predictive values of HGL, LGL and FLT on FNA are mentioned in [Table/Fig-3].

Among three observers, two observers (observer 1&2) diagnosed 18 cases of HGL, two cases of FLT and one observer (observer 3) diagnosed 15 cases of HGL and one cases of FLT correctly on FNA, five cases of LGL were correctly diagnosed by all the three observers with overall accuracy of 78.1% among all thyroid lymphoproliferative lesions. Among nine LGL cases, four cases by observer 1, two cases by observer 2 and three cases by observer 3 were falsely negative (misdiagnosed as FLT) and among 3 thyroiditis cases, one false negative case (misdiagnosed as LGL) by two observers (observer 1 & 2) and two cases were falsely negative (misdiagnosed as LGL) by observer 3 , respectively [Table/Fig-4].

The interobserver variation was least for HGL with excellent agreement and kappa co-efficient of 0.81, 0.87, and 0.81 among O1 & O2, O2 & O3 and O3 & O1 respectively. The agreement between O1&O2 was moderate for LGL and FLT (0.72, 0.62). However, the agreement is even lower for the subgroups of FLT and LGL between O2 & O3 and O3 & O1. The interobserver variation of cytological diagnosis with respect to histological diagnosis is shown in [Table/Fig-4].

Correlation between cytological features with cytological diagnosis showed monomorphism (p=0.04), large cell size (p=0.008), absence of plasma cells (p=0.01), presence of nucleoli (p=0.02) and absence of macrophages (p=0.006) to be significantly associated with a cytological diagnosis of lymphoma. Features favouring HGL are monomorphism, cell size three times that of small lymphocyte, presence of nucleoli and absence of macrophages and plasma cells (p<0.05) [Table/Fig-5]. No single cytological feature was significant in differentiating LGL [Table/Fig-6] from FLT [Table/Fig-7]. Lymphoepithelial lesions, presence of colloid and necrosis were

S. no.	Average age	Male (M)/Female (F) F-n-20, M-n-13	Histological diagnosis NHL-28 (19-high grade, low garde-9), FLT-3, Carcinoma-2
1	65	F	High grade lymphoma
2	66	M	Low grade lymphoma
3	60	M	High grade lymphoma
4	68	F	Low grade lymphoma
5	49	M	High grade lymphoma
6	57	F	Low grade lymphoma
7	61	F	High grade lymphoma
8	65	F	High grade lymphoma
9	44	F	High grade lymphoma
10	65	M	Low grade lymphoma
11	65	F	High grade lymphoma
12	69	F	High grade lymphoma
13	56	F	High grade lymphoma
14	49	M	High grade lymphoma
15	62	M	Low grade lymphoma
16	50	M	High grade lymphoma
17	69	F	High grade lymphoma
18	39	F	Low grade lymphoma
19	60	F	High grade lymphoma
20	47	M	High grade lymphoma
21	62	M	Florid lymphocytic thyroiditis
22	36	F	High grade lymphoma
23	67	M	Low grade lymphoma
24	46	F	Florid lymphocytic thyroiditis
25	53	F	High grade lymphoma
26	22	M	Undifferentaited carcinoma
27	23	F	Florid lymphocytic thyroiditis
28	68	M	High grade lymphoma
29	64	F	High grade lymphoma
30	61	F	Low grade lymphoma
31	55	F	Low grade lymphoma
32	63	M	Carcinoma
33	65	F	High grade lymphoma

[Table/Fig-1]: Demographic details.

Histological diagnosis (n)	Cytological Diagnosis		
	Florid lymphocytic thyroiditis	Low grade lymphoma	High grade lymphoma
High grade NHL (19)	1	0	18
Low grade NHL (9)	4	5	0
Thyroiditis (3)	2	1	0
Carcinoma (1)	0	0	1
Carcinoma (1)	0	1	0

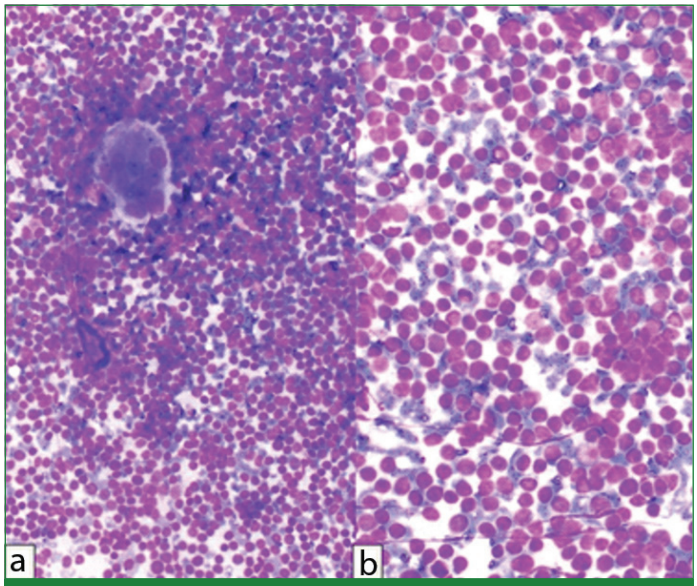
[Table/Fig-2]: Correlation of cytological and histological in relation to atleast one observer.

Diagnosis	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
FLT (3)	2/3 (66.7)	29/30 (96.6)	2/3 (66.7)	29/30 (96.6)
LGL (9)	5/9 (55.6)	23/24 (95.8)	5/6 (83.3)	23/27 (85.2)
HGL (19)	18/19 (94.7)	12/15 (80.0)	19/20 (95.0)	12/13 (92.3)

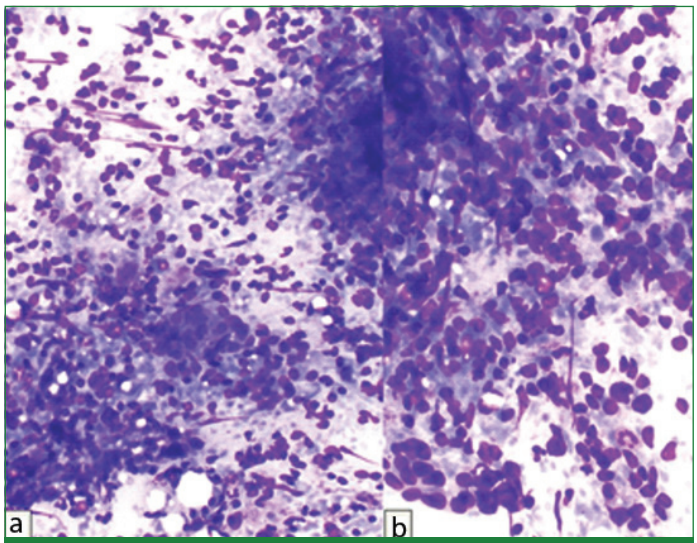
[Table/Fig-3]: Sensitivity and specificity of diagnosis.

Histological diagnosis	Cytological diagnosis			Interobserver agreement (kappa co-efficient)		
	Observer 1(O1)(n)	Observer 2(O2)(n)	Observer 3(O3)(n)	O1&O2	O2&O3	O3&O1
Thyroiditis (n=3)	Thyroiditis (2) LGL (1)	Thyroiditis (2) LGL (1)	Thyroiditis (1) LGL (2)	0.62	0.21	0.28
Low grade lymphoma (n=9)	FLT (4) LGL(5)	Thyroiditis (2) LGL (6) HGL(1)	Thyroiditis (3) LGL (6)	0.72	0.24	0.41
High grade lymphoma (n=19)	Thyroiditis (1) HGL (18)	Thyroiditis (1) HGL (18)	Thyroiditis (1) LGL (3) HGL (15)	0.81	0.87	0.81

[Table/Fig-4]: Interobserver variation in cytological diagnosis of thyroid FNAC with lymphoid infiltrate.



[Table/Fig-5]: : a) Highly cellular smear with monomorphic large atypical lymphoid cells along with intact thyroid follicle in HGL (200X, May-Grunwald-Giemsa); b) Large atypical lymphoid cells with coarse chromatin, small nucleoli and scant cytoplasm in HGL (400X, May-Grunwald- Giemsa).

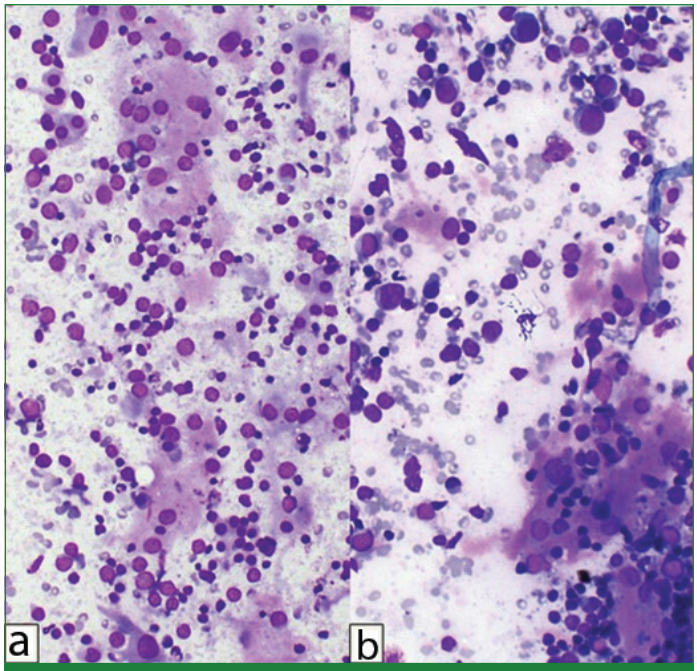


[Table/Fig-6]: a) Polymorphous population of lymphoid cells in LGL (200X, May-Grunwald-Giemsa); b) Moderately high cellularity, predominantly polymorphous population of lymphoid cells with atypia, lympho-glandular bodies in case of LGL. (400X, May-Grunwald-Giemsa).

not significantly different between FLT and LGL. High cellularity, relatively monomorphic population and frequent occurrence of lymphoglandular bodies were common in LGL compared to FLT. However, these findings were not statistically significant.

DISCUSSION

PTL usually presents with rapidly enlarging, painless thyroid mass [17]. The early diagnosis of thyroid lymphoma can be established by



[Table/Fig-7]: a) Oncocytic cells, polymorphous population of reactive lymphoid cells in FLT (200X, May-Grunwald-Giemsa); b) Lymphoepithelial lesion, plasma cells and macrophages in FLT (400X, May-Grunwald-Giemsa).

Ultrasound, Fine Needle Aspiration Cytology (FNAC), IHC and flow cytometry [17]. Diagnosis of thyroid lymphoma is important as it can be treated by chemotherapy and surgery may not be required which is an option with better outcome [17]. FNAC is an important tool in early diagnosis of PTL. Early diagnosis reduces morbidity of major surgery [18]. In present study, the diagnostic accuracy of FNAC for thyroid lymphoproliferative lesions was 78.1%. This is comparable and slightly higher as compared to studies by Morgen EK et al., while Sangalli G et al., and Seningan JL et al., showed a higher sensitivity of 82.3% [14,19,20]. Also, studies by Dustin SM et al., and Gupta N et al., demonstrated >90% sensitivity [13,15]. In the study by Khanna S et al., 12 histology proven lymphoma cases were studied out of which, only three patients (25%) were correctly diagnosed as lymphoma on FNAC, five were diagnosed as chronic lymphocytic thyroiditis and four as follicular neoplasms [21].

The diagnosis of HGL has very high sensitivity and specificity which is uniform across various studies [13,14]. The presence of monomorphic large cells which are >3 times the size of small lymphocyte with coarse chromatin along with presence of lymphoglandular bodies are highly suggestive of HGL. Most of the studies, including the present study had >90% accuracy for diagnosis of HGL with least interobserver variation. A single case of HGL was misinterpreted as FLT on FNA due to coexisting lymphocytic thyroiditis which resulted in polymorphous appearance of the cellular infiltrate along with presence of plasma cells.

The distinction between LGL and FLT is less obvious on FNA with high interobserver variation due to overlapping cytomorphological features such as polymorphous population of lymphoid cells, plasma cells, macrophages. These may be the reasons for misdiagnosis of lymphoma and in cases where, florid thyroiditis co-exists with lymphoma, the utility of FNAC may be limited [22]. Though high cellularity, lymphoglandular bodies and paucity of plasma cells favour LGL, these features do not always help and no single cytological feature is diagnostic of LGL in a background of FLT. Lerma E et al., compared the cytological features of 25 cases of lymphocytic thyroiditis and 12 cases of thyroid lymphoma (Sensitivity for lymphoma diagnosis-92.3%) found that heterogeneous population of small and large lymphocytes was the most frequent pattern in both the diseases [23]. While Abraham LK et al., noted the abundance of lymphoid cells and high proportions of intermediate centrocyte like cells in LGL as compared to FLT with macrophages and polymorphous mixed population in thyroiditis [12]. Gupta N et al., studied 10 cases of lymphoma in which one case of lymphoma was misdiagnosed as lymphocytic thyroiditis due to polymorphous

population of lymphoid cells [15]. Similarly, in the present study four LGL cases by observer 1, two LGL cases by observer 2 and three LGL cases by observer 3 were diagnosed as FLT, as all the cases lacked monomorphism, no significant lymphoglandular bodies and had plasma cell infiltration. Also, a single case of FLT was cytologically diagnosed as LGL due to increased cellularity, absent colloid and presence of lymphoglandular bodies. Usually MALT lymphomas/LGLs are easily missed or remain under-diagnosed on FNAC [18]. Despite of its difficulty in diagnosing LGLs, few studies reported sensitivity and positive predictive (90-100%) values of FNAC. Dustin SM et al., reported sensitivity and positive predictive value of FNAC to be 100% [13]. Ancillary studies including flow cytometry, immunocytochemistry increases the sensitivity, though Morgen EK et al., noted the difficulty of flow cytometry in cytology specimens due to fragile cytoplasm of lymphoma cells [14]. Marginal zone B cell lymphoma usually presents with lower stage disease and initiating early therapy is crucial for achieving complete recovery and better long term outcome. Therefore, differentiation between the two entities is important. Hence core biopsy, though not the initial diagnostic investigation significantly improved the diagnostic yield [12,24]. Therapeutic strategies are distinct from the other thyroid cancers and include local therapy or most commonly combined multimodality treatment, mainly chemoradiation [17]. The percentage of cases of thyroid lymphoma was high for present study because selected type of cases were included only and it does not refer to true incidence of thyroid lymphoma. The main aim was to evaluate cytomorphological pointers for thyroid lymphoma. Present study showed that sensitivity and specificity of FNA was high for HGL and centres which do not have flow cytometry and immunophenotyping facility, cytolomorphological features such as high cellularity, absent of colloid, lymphoglandular bodies and paucity of plasma cells favour LGL. These features do not always help and no single cytological feature is diagnostic of LGL in a background of FLT, but it is helpful in putting a one of the differential diagnosis of LGL on cytology and histological confirmation is important. Main features of interobservation variations are lymphoepithelial lesions and cell size. It is challenging to diagnose PTLs on FNA. For this reason, it should be kept in mind that patients with chronic lymphocytic thyroiditis should be evaluated carefully. The role of FNAC was limited but it is simple safe and cost-effective for initial workup.

Limitation(s)

Lacking flow cytometry and immunocytochemistry is the limiting factors for the present study.

Future perspective: FNA cytology in combination with flow cytometric immunophenotyping is an accurate method of diagnosing and classifying thyroid lymphoma. Ancillary tools and general knowledge of haematopathology are needed in addition to cytological diagnosis.

CONCLUSION(S)

The diagnosis of HGL on FNA is usually straight forward and interobserver variation is least. Cytomorphological features such as high cellularity, absent of colloid, lymphoglandular bodies and paucity of plasma cells favour LGL. All cases of FLT or suspected LGL should undergo histological confirmation with IHC or light chain restriction on cell block and flowcytometry, if feasible. The role of FNAC in diagnosing thyroid lymphoma is limited but it is still useful in the initial work-up.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Pathology and Cancer Genetics, Superspeciality Cancer Institute and Hospital, Lucknow, Uttar Pradesh, India.
2. Senior Resident, Department of Pathology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India.
3. Professor, Department of Pathology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India.
4. Professor, Department of Pathology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Niraj Kumari,
Professor, Department of Pathology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow-226014, Uttar Pradesh, India.
E-mail: nirajpath@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: May 21, 2020
- Manual Googling: May 30, 2020
- iThenticate Software: Jun 11, 2020 (11%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? No
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **May 21, 2020**
Date of Peer Review: **May 30,2020**
Date of Acceptance: **Jun 02, 2020**
Date of Publishing: **Jul 01, 2020**