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Significance of AgNOR Counts in Thyroid Cytology.

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ABSTRACT

To determine the value of argyrophilic nucleolar organizer regions (AgNORs) in fine needle aspiration smears of thyroid lesions. Fine needle aspiration smears of 33 histologically confirmed thyroid lesions comprising11 cases of colloid goitre,8 cases of follicular adenoma,5 cases of follicular carcinoma, 5 cases of papillary carcinoma, 2 cases of medullary carcinoma and 2 cases of anaplastic carcinoma were stained for AgNORs. Mean AgNOR counts were compared to see if they could help in distinguishing the different lesions from each other especially follicular adenoma from follicular carcinoma. Mean AgNOR counts in colloid goitre were significantly higher than those in neoplastic lesions. Among the neoplastic lesions mean AgNOR counts were not statistically significantly different except for those in anaplastic carcinoma. AgNOR counts were of no use in differentiating among the various neoplastic thyroid lesions on cytology smears because of considerable overlap.

INTRODUCTION

Thyroid nodules are acommon clinical problem in India, but only a small proportion of them are malignant. The prevalence of thyroid nodules in the general population is about 5% by palpation and increases to 10–55% with the use of ultrasonography [1]. To avoid unnecessary surgery, a thyroidscan, ultrasonography, and fine needle aspiration cytology (FNAC) are used as diagnostic tools to differentiate malignantnodules from a benign lesion. FNAC is widely accepted and has becomecornerstone in evaluation of thyroid nodules because it is asimple and accurate screening test with high sensitivity and specificity in the preoperative evaluation of thyroid lesions [2]. However, even FNAC haslimitations because of low yield of cells, loss of histologicalarchitecture and inability to distinguish follicular adenomaand well differentiated follicular carcinoma [2, 3].

Various attempts have been made to improve the diagnostic accuracy of FNAC, including morphometric studies, DNA measurement, immunohistochemical, and enzyme techniques for thyroid cancer with varying degrees of success. It is well known that the silver staining technique for nucleolar organizer regions (AgNORs) has been successfully applied to a wide variety of neoplastic lesions on pathological materials in order to distinguish benign from malignant lesions [4]. The nucleolar organizer regions (NORs) are genomic DNA segments encoding for ribosomal RNA. They appear on the short arms of the five acrocentric chromosomes 13,14,15,21 and 22[5] NORs are associated with argyrophilic proteins that can be localized through silver staining. The result of staining is dots of silver (AgNOR dots or AgNORs).It was found that the mean number of AgNORS was related to the proliferation rate of tumor cell populationsince it is it is proportional to the rapidity of cell duplication [6]. It has been suggested that the number of AgNOR s may have some diagnostic and prognostic value in different neoplasms [7, 8].

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In recent times AgNOR analysis is carried out through standardised morphometry. However such techniques are not widely available especially in developing countries. The manual evaluation of AgNOR scores is a cost-effective alternative to automated methods of evaluation. The staining technique is relatively simple and rapid and can be applied to both aspiration smears and tissue sections. The reliability of this method in cancer evaluation has been frequently demonstrated even by a simple visual assessment. Thus, counting of AgNOR dots appears to be very useful and simple way of obtaining data on the proliferative index of cancerous as well as benign lesions [9]. Hence, the aim of this study was to determine the usefulness of AgNOR counts in differentiating various thyroid lesions on fine needle aspiration cytology especially follicular adenoma from follicular carcinoma.

MATERIALS AND METHODS

This study was based on AgNOR staining of FNAC smears of 33 patients with histologically confirmed thyroid swellings. The thyroid lesions were aspirated with a 23 gauge needle attached to a 20 ml disposable syringe. From the aspirated material 4- 6 smears were made, out of which 2 -4 were immediately fixed in 95% ethyl alcohol for Papanicolau stain and 2 smears were air dried and subsequently fixed in 95% alcohol for AgNOR stain.

AgNOR staining was done as per the silver colloid reaction method described by Crocker and Nar [10]. AgNORs were visualised as black or brown dots within the yellowish background of the nucleus. The number of individually discernible and separate black dots was counted in 100 nuclei under an oil immersion lens and the mean number of AgNORs per nucleus was calculated. Where 2 or more dots were so closely aggregated that the precise number within the aggregate could not be counted they were taken as one. The data was statistically analyzed by student's t test.

RESULTS

The present study included FNAC smears of 33 cases of histologically confirmed thyroid lesions. There were 19 benign lesions (11 cases of colloid goitre and 8 cases of follicular adenoma) and 14 malignant lesions (5 cases of follicular carcinoma, 5 cases of papillary carcinoma, 2 cases of medullary carcinoma and 2 cases of anaplastic carcinoma). Mean AgNOR counts for all benign lesions combined were 2.12 ± 0.45 and for all malignant lesions combined were3.04 ± 0.79 (Table 1) and the difference was statistically significant.

Mean AgNOR counts per cell in the different thyroid lesions are shown in Table 2 and Figure 1- 6.

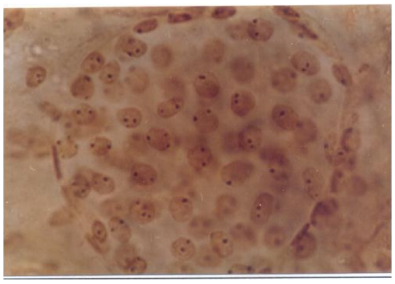

Figure 1: AgNOR staining in FNA smear of colloid goitre

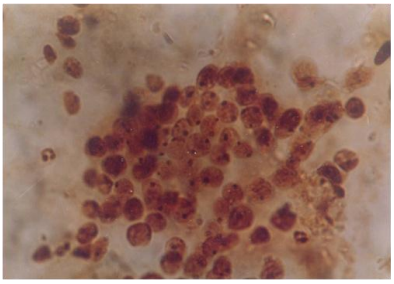

Figure 2: AgNOR staining in FNA smear of follicular adenoma

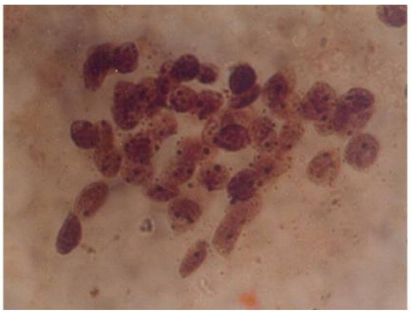

Figure 3: AgNOR staining in FNA smear of follicular carcinoma

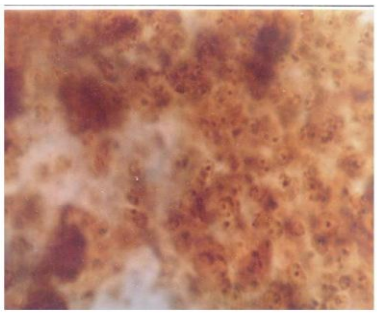

Figure 4: AgNOR staining in FNA smear of papillary carcinoma

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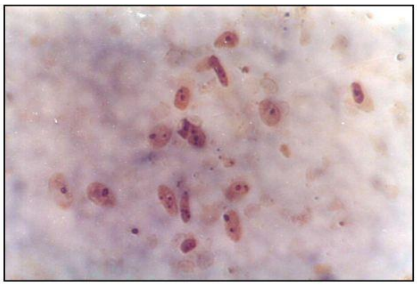

Figure 5: AgNOR staining in FNA smear of medullar carcinoma

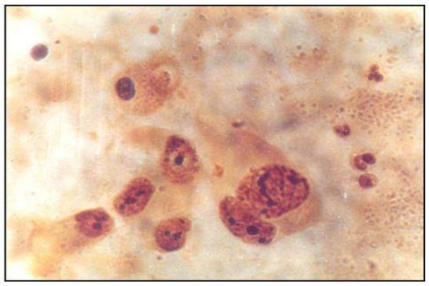

Figure 6: AgNOR staining in FNA smear of anaplastic carcinoma

Table 3 shows that mean AgNOR counts were significantly higher in neoplastic groups compared to colloid goitre. Among the neoplastic lesions the counts in anaplastic carcinoma were significantly higher than those in other neoplastic groups. The counts were not significantly different among other neoplastic groups except for medullary carcinomavs papillary carcinoma.

Table 1: AgNOR count in benign and malignant thyroid lesions

Group	AgNOR count per cell (Mean ± SD)
Benign (n=19)	2.12 ± 0.45
Malignant (n=14)	3.04 ± 0.79

t=4.26; p<0.001

Table 2: Correlation of AgNOR count with the type of lesions

Group	AgNOR count per cell (Mean ± SD)
Benign: Colloid Goitre (n=11) Follicular adenoma (n=8)	1.82 ± 0.20 2.45 ± 0.45
Malignant: Follicular carcinoma (n=5) Papillary carcinoma (n=5) Medullary carcinoma (n=2) Anaplastic carcinoma (n=2)	2.63 ± 0.64 2.33 ± 0.32 3.11 ± 0.05 4.15 ± 0.23

Table 3: Statistical analysis of Mean AgNOR count on cytology

Follicular adenoma vs Colloid goitre	t =4.146; p < 0.001
Follicular carcinoma vs Colloid goitre	t =3.936; p < 0.01
Papillary carcinoma vs Colloid goitre	t =3.932; p < 0.01
Medullary carcinoma vs Colloid goitre	t =8.776; p < 0.001
Anaplastic carcinoma vs Colloid goitre	t =14.938; p < 0.001
Follicular carcinoma vs Follicular adenoma	t =0.599; p = NS
Papillary carcinoma vs Follicular adenoma	t =0.516; p = NS
Medullary carcinoma vs Follicular adenoma	t =1.981; p = NS
Anaplastic carcinoma vs Follicular adenoma	t =5.016; p < 0.01
Papillary carcinoma vs Follicular carcinoma	t =0.938; p = NS
Medullary carcinoma vs Follicular carcinoma	t =1.002; p = NS
Anaplastic carcinoma vs Follicular carcinoma	t =3.124; p < 0.05
Medullary carcinoma vs Papillary carcinoma	t =3.247; p < 0.05
Anaplastic carcinoma vs Papillary carcinoma	t =6.249; p < 0.05
Anaplastic carcinoma vs Medullary carcinoma	t =7.152; p < 0.001

DISCUSSION

Nodular thyroid disease is a common clinical problem. Although most thyroid nodules can be diagnosed easily based on cytological features some cases still impose diagnostic difficulty. Therefore various proliferation markers such as Ki 67 [11], proliferating cell nuclear antigen (PCNA) [12] and argyrophilic nucleolar organizer regions (AgNORs) have been examined in thyroid nodules [13]. In the present study, AgNOR staining was undertaken in FNAC smears of 33 histologically confirmed thyroid

lesions to see if the technique could differentiate between benign and malignant lesions particularly follicular neoplasms.

The results show that mean AgNOR counts in malignant thyroid lesions significantly exceeded those in benign thyroid lesions (Table 1). Similar findings were reported by Shechtman et al who in a study of AgNOR counts in smears of 70 thyroid lesions found mean counts were statistically significantly higher in malignant lesions as compared to benign lesions [14]. Mean AgNOR counts in colloid goitre were significantly higher than those in neoplastic lesions.

Mehrotra *et al.*,[15] studied cytomorphological features and the value of the silver colloidal staining method in distinguishing nonneoplastic, benign and malignant neoplasms in 140 FNAC smears of thyroid nodules and concluded that AgNOR study could be used as an additional diagnostic method with cytomorphological features to differentiate benign and malignant follicular lesions. In their study lower AgNOR counts were recorded in cases of thyroiditis (1.375 ± 0.414) whereas follicular carcinoma had higher number of AgNOR counts (5.04 ± 0.52). In our study, the mean AgNOR count in colloid goiter was 1.82 ± 0.2, in follicular adenoma it was 2.45 ± 0.45 and in follicular carcinoma it was 2.63 ± 0.64. In a study by Aiad HA et al mean AgNOR number =2.91 and marginal AgNORs = 2.67 were useful cut-off values above which follicular carcinoma can be diagnosed with 100% sensitivity, 79% specificity, 76% positive predictive value, 100% negative predictive value and 85% diagnostic accuracy for both parameters [16].

In the present study there was considerable overlap among the mean AgNOR counts in follicular adenoma and carcinoma hence AgNORs could not be used as a diagnostic criterion to differentiate between them (Table 3). Similar findings were reported by Khan EM and Pandey R who studied AgNOR counts in FNAC smears of 60 thyroid nodules and found that there was considerable overlap among the various groups and thus AgNOR counts had no significant diagnosticutility [17]. Slowinska –Klencka D et al also found evaluation of mean number of AgNORs per nucleus did not improve the diagnosis of malignancy in follicular lesions of thyroid [12]. However Mehrotra A et al, 2002 found mean AgNOR count to be sensitive, simple and cost effective method for differentiating between benign and malignant thyroid follicular neoplasms [13]. Aiad et al also suggested that quantification of AgNORs and Ki67 L1 can be used as helpful ancillary method in differentiation between different thyroid lesions [16].

The mean AgNOR counts in anaplastic carcinoma were significantly higher than those in other lesions but since the cytomorphology in an aplastic carcinoma is diagnostic hence AgNORs have little role as a diagnostic criterion. The counts were also not significantly different among other neoplastic groups except for medullary carcinoma vs papillary carcinoma where again cytomorphology alone is fairly distinctive.

CONCLUSION

Mean AgNOR counts in colloid goitre were significantly higher than those in neoplastic lesions. AgNOR counts showed considerable overlap among the various neoplastic lesions and could not be used reliably to differentiate amongst them except for anaplastic carcinoma. AgNOR counts were not useful in differentiating follicular neoplasm i.e. follicular adenoma from follicular carcinoma which is the main problem in thyroid cytopathology. In these cases excision and histological evaluation may be necessary for a definitive diagnosis.

REFERENCES

1. Young JK, Lumapas OG, Mirasol R. Sonography guided fine-needle aspiration biopsy of thyroid nodules:correlation between cytologic and histopathologic findings. Philippine J Int Med. 2011;49(1): 8–14.
2. Handa U, Garg S, Mohan H, Nagarkar N. Role of fine needle aspiration cytology in diagnosis and management of thyroid lesions: A study on 434 patients. J Cytol. 2008;25 (1):13-17
3. Borget I, Vielh P, Leboulleux S, Allyn M, Iacobelli S, Schlumberger M, de Pouvourville G. Assessment of the cost of FNAC as a diagnostictool in patients with thyroid nodules. Am J Clin Pathol. 2008 ;129(5):763-71
4. S Asotra and J Sharma. Role of AgNORs in thyroid lesions on fine needle aspiration cytology smears. J Cytol. 2008; 25 (1): 18-22
5. Howell WM. Selective staining of nucleolus organizer regions. In: Busch H., Rothblum L (Eds).The cell nucleus. Academic Press. New York:p 89-143; 1982

6. Ofner D, Hittmair A, Marth C, ofner C, Tötsch M, Daxenbichler G, Mikuz G, Margreiter R, Schmid KW. Relationship between quantities of silver stained nucleolar organizer regions associated proteins (Ag-NORs) and population doubling time in ten breast cancer cell lines. Pathol Res Pract. 1992; 188(6):742-6.
7. Bankfalvi A, Schmitz K, Mock T, Kemper M, Cubick C, Bocker W. Relatioship between AgNOR proteins, Ki 67 antigen, p 53 immunopheno type and differentiation markers in archival breast carcinomas. Anal Cell Pathol. 1998; 17(4):231-42.
8. Pich A, Chiusa L, Margaria E. Prognostic relevance of AgNORs in tumor pathology Micron 31 133-141 2000
9. Hossain M I, Hassan M Q, Bhattacharjee P, Ahamad M S, and Rahman Z. Role of Multiparameter Analysis of AgNORs in FNA Smears of Thyroid Swellings in Differentiating Benign and Malignant Lesions. Pathology Research International, vol. 2012, Article ID 908106, 7 pages, 2012.
10. Crocker J, Nar P. Nucleolar organiser regions in lymphomas. J Pathol. 1987; 151: 111-118
11. Sofiadis A, Tani E, Foukakis T, Kjellman P, Skoog L, Hoog A, Wallin G, Zedenius J, Larsson C. Diagnostic and prognostic potential of MIB-1 proliferation index in thyroid fine needle aspiration biopsy. Int J Oncol. 2009;35(2):369-74
12. Slowińska-Klencka D, Klencki M, Popowicz B, Sporny S, Lewiński A. Multiparameter analysis of AgNOR in thyroid lesions: comparison with PCNA expression. Histol Histopathol. 2004;19(3):785-92.
13. Mehrotra A, Goel MM, Singh K. Ki-67 and AgNOR proliferative markers as diagnostic adjuncts to fine needle aspiration cytology of thyroid follicular lesions. Anal Quant Cytol Histol. 2002;24(4):205-11
14. Shechtman L, Koren R, Horowitz A, Shechtman I, Halpern M, Gal R. Diagnostic value of AgNOR staining in thyroid cytology. Anal Quant Cytol Histol. 1998; 20(3):187-91.
15. Mehrotra A, Agarwal PK, Chandra T. Cytopathology and AgNOR counts in fine-needle aspiration cytology smears of thyroid lesions. Diagn Cytopathol. 1998; 19(4):238-43.
16. Aiad HA, Bashandy MA, Abdou AG, Zahran AA. Significance of AgNORs and ki-67 proliferative markers in differential diagnosis of thyroid lesions. Pathol Oncol Res. 2013; 19(2):167-75.
17. Khan EM, Pandey R. Differential diagnosis of fine needle aspiration smears of thyroid nodules. Cytologic features and AgNORs. Acta Cytol. 1996;40(5):959-62.