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Research Article

AGNORS –The Way to Diagnose Proliferative Rate of Cells

Dr. Humaira Nazir[1*], Dr. Imran Nazir Salroo[2], Dr. Fouzia Nazir[2], Dr. Waseem Shah[3]
[1]PG Student,Dept. Of Oral Pathology and Microbiology,Sri Siddhartha Dental College,Tumkur, India
[2]Senior Resident,Dept. of Radiodiagnosis and Imaging,Govt.Medical College, Srinagar.
[2]Senior Resident, Dept. of Preventive & Community Dentistry,Govt.Medical College, Srinagar.
[3]Senior Resident, Dept. of ENT, SKIMS Medical College, Srinagar.

***Corresponding author**
Dr. HumairaNazir
Email:humairanazir15@gmail.com

Abstract: The objective is to assess the significance of argyrophilic nuclear organizer region (AgNOR) counts in diagnosis of squamous cell carcinoma of buccal mucosa treated by radiotherapy and evaluation of different grades of sqamous cell carcinoma by AgNORcounting. Study was conducted on 40 histologically proven, previously untreated cases of various grades of squamous cell carcinoma of the buccal and compared with controls. AgNOR staining was performed. The patients were subjected to Cobalt-60 radiotherapy and sections obtained 2 and 4 weeks after therapy were assessed for AgNOR score. In squamous cells of the control group, the AgNORs were fine, tightly packed and centrally placed in the nucleoli. AgNORs in carcinoma of the buccalmucosa prior to radiotherapy were large and variable in size and shape. The cases studied for AgNOR count 2 weeks after radiotherapy showed multiple AgNOR dots which were fewer in number and less coarse as compared to the pre-radiation group. After 4 weeks of radiation therapy, the AgNOR count further declined in number, and the dots were usually single and fine. In patients who showed persistence of malignancy, the AgNOR dots were found to be coarse and present in large clumps. AgNOR is an effective tool reflecting the proliferation rate of the tumor.
Keywords:squamous cell carcinoma of buccal mucosa, radiation therapy, AgNOR counts.

INTRODUCTION

Nucleolar organizer regions (NORs) are loops of ribosomal DNA (rDNA) that is transcribe into ribosomal RNA (rRNA)[1].Consequently, they are of diagnostic value for in characterization of the invasiveness in carcinomas NORs are located on the stalks of the acrocentric chromosomes[1,2].Silver stained nucleolar organizer regions (AgNORs) are argyrophilic non-histone proteins associated with NORs and include RNA polymerase I, C23 proteins and B23 proteins[3].The number of AgNORs in the cell is believed to reflect nucleolar activity and to be related to cellular proliferative activity[3,4]. They are intimately related to the cell cycle and may be related to proliferation and ploidy. AgNORs are argyrophilic proteins. Binding of silver and protein occurs in carboxyl and sulphydryl groups by colloidal precipitation of ionic silver. The carboxyl groups on the protein reduce the silver solution forming micronuclei of silver. The large aggregates of silver get deposited at disulphide and sulphydryl group sites. These are seen by light microscopy as black intranucleargranules . The AgNOR count is an important index for assessment of proliferating cells. In normal cells, the AgNORs are tightly packed in the nucleoli and are indiscernible[5]. They also play a role in the estimation of the cellular activity that is applied to a variety of neoplastic or hyperplastic lesions[6]. Since some of the NOR associated proteins are argyrophilic and can be demonstrated as black dots by a silver staining technique, such demonstrated structures are known as AgNORs[7].

MATERIALS AND METHOD:

This study was conducted in the squamous cell carcinoma of buccal mucosa from 40 patients and 10 control cases.All specimen were collected by surgical excision or by as a biopsy who were treated with Cobalt-60 (Co-60) radiation. 40 untreated cases of different grades of squamous cell carcinoma of the buccal mucosa diagnosed by histopathology. Control group were chosen from normal oral mucosal epithelium. A detailed clinical history was recorded in all cases. The tissue section were cut into slices 4um thick from control and each patient prior to radiation therapy, and were subjected to single step AgNOR staining.

Technique

Single step AgNOR staining technique was employed for the demonstration of AgNORs. The freshly prepared solution was poured on to the sections

which were then left in the dark at 370 C for 30 minutes. Slides were dehydrated in 3 changes of acetone, cleared in xylene and mounted in DPX.
The patients were given 40-50 Gray of Co-60 teletherapy divided in 25 fractions over a period of 5 weeks, followed by intracavitary radiation. The same procedure was carried out on buccal mucosa sections collected at intervals of 2 and 8 weeks after completion of radiotherapy. AgNOR counting was carried.

RESULTS

In squamous cells of the control group, the AgNORs were fine, tightly packed and centrally placed in the nucleoli. AgNORs in carcinoma of the buccal mucosa prior to radiotherapy were large and exhibited great variation in size and shape and they were mostly irregular and in large clusters (Figure1). The patients studied for AgNOR count 2 weeks after radiotherapy showed multiple AgNOR dots which were fewer in number and less coarse as compared to the pre-radiation group (Figure 2). After 4 weeks of radiation therapy, the AgNOR counts further declined in number, and the dots were usually single and fine (Figure 3) whereas in patients who showed persistence of malignancy (poor response to radiotherapy), the AgNOR dots were found to be coarse and present in large clumps. ONE WAY ANOVA was used to test the statistical significance.

AgNOR counts in control group ranged from 2.83 to 2.98 with a mean of 2.91 ± 0.01. In all the 15 cases of carcinoma cervix prior to radiation therapy the AgNOR counts ranged from 3.30 to 4.10 with a mean of 3.75 ± 0.05, which was significantly higher than that in the control group ($P<0.001$). After irradiation a significant decline was noted in AgNOR counts which varied from 2.40 to 2.90 with a mean of 2.85 ± 0.01 after 2 weeks of irradiation and from 2.10 to 2.18 with a mean of 2.0 ± 0.05 after 4 weeks of irradiation.

Although conventional clinic pathological staging or histological grading or both, may be useful in clinical assessment of squamous cell carcinoma of buccal mucosa, these methods do not determine the growth rate of the tumor in individual patients. The tumor growth rate has been estimated by the cellular proliferative activity of the tumor. In the present study of evaluation of AgNOR count in SCCs of buccal mucosa, mean value was compared with study of other workers and thus it was suggested that this method can be used to differentiate different grades of carcinoma.

Table-1: Shows mean value of AgNORs with and without radiation theraphy.

Group	No. of cases	AgNOR count	
		Range	Mean
Control	15	2.83-2.98	2.91±0.01
Prior to radiation	15	3.30- 4.10	3.75±0.05
Post radiation			
2 weeks	10	2.40-2.38	2.85±0.01
4 weeks	10	2.10 – 2.18	2.0±0.05

Table-2: Shows increasing mean value of Agnors in different grades of squamous cell carcinoma

Grades	Mean AgNOR count
I	1.26
II	2.90
III	3.73
IV	5.83

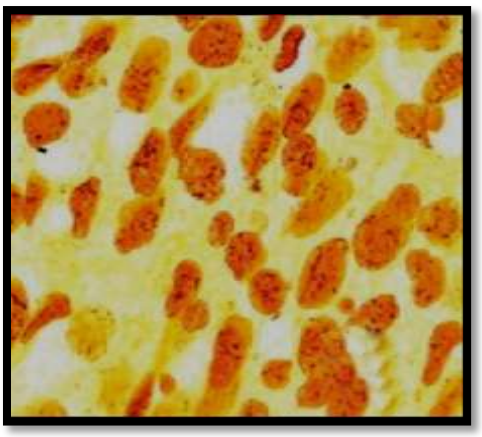

Fig-1: Showing large variation in size and shape and AgNOR are irregular and in clusters.

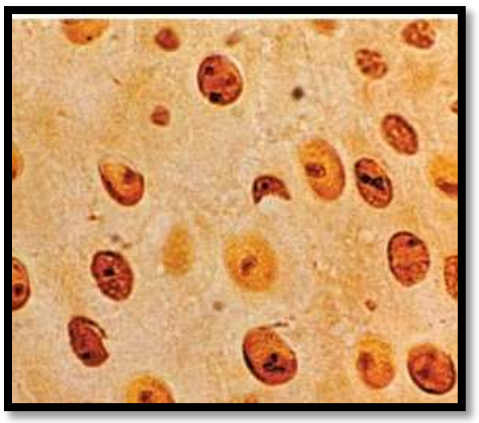

Fig-2: Showing AgNORdots singly and fine.

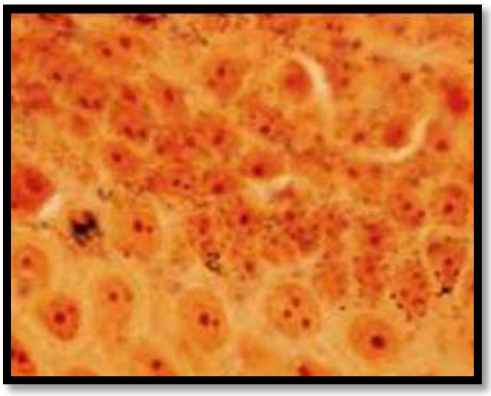

Fig-3: Showing AgNOR dots fewer and less coarse.

DISCUSSION

Millions of new cases of invasive cancer are diagnosed each year of which oral cancer has a considerably high incidence. This high incidence of oral cancer and its attendant morbidity and mortality has instigated workers to devise methods for its early diagnosis. Nucleolar organizer regions (NORs) are segments of chromosomes encrypted for ribosomal RNA (rRNA) which are present on specific loops of DNA. NORs have received a great deal of attention recently because of the observations that their frequency within the nuclei is significantly higher in malignant cells than in normal, reactive or benign neoplastic cells[8,9]. Accordingly, an increase in the nucleolar structures (AgNORs), where rRNA synthesis takes place, is expected in rapidly diving cells. AgNOR parameter is thus a convenient marker for studying the rate of proliferation of cells in tissues being studied histologically. AgNOR counts in squamous cell carcinoma were more than others. The same results have been obtained between aggressive and nonaggressive lesions14 and also other benign and malignant lesions[10]. Silva *et al* showed a direct association between the number of NORs in OSCC and histologic tumor grade[11].

Our study shows significantly lower AgNOR scores after radiation when compared with the control group and with the patients who responded to radiation. Similar results were obtained by Kinoshita Y *et al*[12].

We also noted a significant difference (P <0.001) in AgNORs between grade I and grade IV of squamous cell carcinoma (Table 2). These findings strongly support the view that proliferative activity and malignant potential of neoplastic lesions of the buccal mucosa increase progressively as the grade of the lesion becomes higher. Similar AgNOR scores were obtained by da Silva SO *et al* who recorded a mean score in cases of well differentiated squamous cell carcinoma of the cervix and in poorly differentiated cases[13].

Even though the reason for the greater number of AgNORs in the nuclei of malignant lesions is uncertain, it may represent an escalation in ploidy19, increased gene amplification or a rise in chromosomal segregation with more cells being in the S phase of their cycle.

CONCLUSION

In the present study this technique was followed for its convenience and feasibility. The AgNORtechnique whichwas earlier used extensively in cytogenetics has now gained importance as an indicator of cell proliferation. AgNOR scores differentiate between different grades of malignancy, high counts indicating a higher grade, while significant decline in AgNOR counts after radiotherapy denotes a good prognosis. Hence further AgNOR studies in a large number of patients, in conjunction with other

parameters, could help better establish the status of AgNOR as a prognostic indicator.

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