

Argyrophilic Nuclear Organizer Regions (AgNORs) in Lymphocytic Leukaemia

T TASNEEM A RASHID S AKHTAR S AHMED

Department of Pathology, Postgraduate Medical Institute, Lahore

Correspondence: Dr. Tahira Tasneem, Associate Professor

Nuclear organizer regions code for ribosomal RNA and are associated with non-histone nucleoproteins that can be identified by silver staining (AgNORs). AgNORs have been correlated to proliferative activity of tumours. The present study was carried out to observe the AgNOR pattern in lymphocytic leukaemias. A significant increase in AgNOR counts ($p < 0.001$) was observed in acute lymphoblastic leukaemia (ALL) patients as compared to normal controls. The proliferative index was found to be significantly higher in ALL. In chronic lymphocytic leukaemia and reactive lymphocytes, AgNOR pattern was found similar to the normal lymphocytes. These results suggest that AgNORs counts are significantly higher in acute lymphoblastic leukaemia.

Key words: AgNOR, (Argyrophilic, nuclear organizer regions), nucleus, acute lymphoblastic leukaemia

Nuclear Organizer Regions (NORs) are loops of DNA present in the nucleoli of cells. They contain genes that code for ribosomal RNA (rRNA). These are associated with non-histone nucleoproteins which can be identified by silver staining²¹. The abbreviation 'NOR' is generally applied to silver stained nuclear dots observed in the interphase nuclei. AgNOR means silver stained NOR proteins⁶. These are of vital significance for synthesis of proteins¹⁴. These are located on acrocentric chromosomes 13, 14, 15, 21, 22²³.

The amount of AgNOR is related to the cell cycle phases, low for G1 phase and high for S-G2 phase. Thus it is a measure of the relative proportion of cells in each phase. The high values indicate that the major part of the cell are S-G2 phase and relatively a few are in G1 phase; this characterizes rapid cell cycle²⁰. Since AgNOR area of interphase nuclei correlate well with the proliferative activity of the cell^{7,8} the AgNOR number is significantly higher in malignant cells than that of normal, reactive or benign neoplastic cells²².

Leukemias are neoplastic disorders in which there is unregulated clonal proliferation of haemopoietic stem cell. This commonly result in an immature morphological appearance of cells⁹. Leukemias are divided into myeloid and lymphatic varieties and into acute and chronic forms¹⁰. Acute leukemias are diagnosed by the presence of Blast count >30% of nucleated marrow cells². Since AgNORs are indicative of proliferative activity of the cells, increased counts are found in acute leukaemia²¹.

Three types of AgNOR configurations are described⁶.

1. NORs are fully aggregated to form a solitary round argyrophil structure corresponding to the nucleolus often seen in resting lymphocytes.
2. Multiple dots seen in nucleolar region as observed in proliferating cells.
3. Distribution of small true multiple AgNORs throughout the nucleoplasm as seen in highly malignant cells.

The number and size of AgNORs associated proteins correlates with the level of rDNA transcription, the degree of cell proliferation and the tumour growth fraction. This method allows a reliable identification of cell type and their maturation¹⁶. The purpose of this study was to characterize the AgNOR pattern in lymphocytic leukaemia.

Materials and methods

Twelve (12) patients of acute lymphoblastic leukaemia, 6 patients of chronic lymphocytic leukaemia, 6 patients with absolute increase in lymphocytes counts with reactive changes in lymphocytes were included in the study. Ten (10) normal smears were stained for studying AgNOR pattern of normal lymphocytes. In leukaemic patients, both peripheral and bone marrow smears were stained.

The diagnosis of lymphocytic leukaemia was established by microscopic examination of Giemsa stained smears. ALL was confirmed by Sudan Black stain.

The prepared smear were stained by the AgNOR staining method of Nickicicz and Norback¹⁶. The smears were examined with an optical microscope using x100 oil immersion lens with total magnification of 1000.

Each AgNOR was counted as a unit and AgNOR dots in 100 cells were counted and average number of dots per cell were calculated. AgNOR proliferative index (p AgNOR) was calculated as percentage of cells with 5 or more AgNOR dots as described by Mourad et al¹⁵.

Results

Acute Lymphoblastic Leukaemia (ALL)

It included twelve patients 6 had L1 morphology based on FAB and 6 had L2 morphology. The AgNOR count of acute lymphoblastic leukaemia was $3.5 \pm 0.3SD$ (Table 1, Fig 1 and Fig 2a). The proliferative index was 19.8 ± 4.4 . The AgNOR count and proliferative index in acute lymphoblastic leukaemia was found to be significantly higher as compared with normal, CLL and reactive lymphocytes ($p < 0.001$).

Chronic Lymphoblastic Leukaemia (CLL)

AgNOR count in CLL was 1.02 ± 0.01 the proliferative index was 0 (Table 1, Fig 1, and Fig 2b). There was no significant difference between the AgNOR count and proliferative index in chronic lymphocytic leukaemia as compared to normal lymphocytes.

Reactive Lymphocytes

The AgNOR count of reactive lymphocytes was 1.11 ± 0.13 and proliferative index was 0. No significant difference was found when compared with normal lymphocytes (Table 1, Fig. 1 and Fig. 2c).

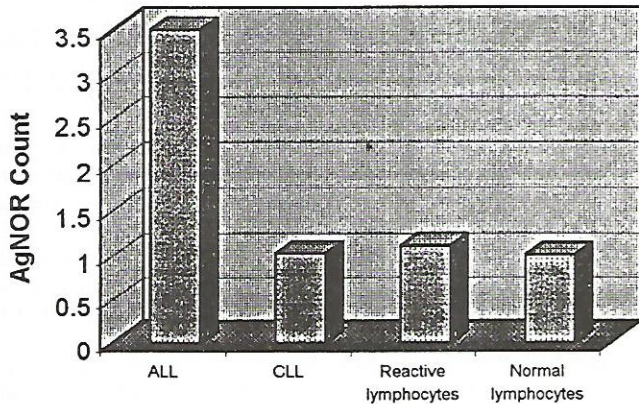


Fig. 1. Comparison of AgNOR count in ALL, CLL, Reactive Lymphocytes and Normal Lymphocytes

Table 1. Results of silver staining in ALL, CLL, reactive lymphocytes and normal lymphocytes

Samples	No.	AgNOR Count	Proliferative Index (%)
ALL	12	$3.5 \pm 0.27^*$	19.8 ± 4.4
CLL	6	1.02 ± 0.01	0.0
Reactive lymphocytes	6	1.11 ± 0.13	0.0
Normal lymphocytes	12	1.00	0.0

* $P < 0.001$ as compared with CLL, normal and reactive lymphocytes

Discussion

AgNOR proteins are a set of argyrophilic nucleolar proteins that accumulate in highly proliferating cells. Whereas their expression is very low in non-proliferating cells. Number of AgNOR regions and proliferative index, both depict the proliferative activity of the cells.

Silver staining had been employed in paraffin embedded sections. Thus AgNOR could render an indication of the status of the cells with regard to activation, induction or malignancy. When applied to lymph node sections, it was observed that reactive lymphocytes possessed approximately one AgNOR per nucleus while in low grade lymphoma the regions were fewer than those of high grade lymphoma⁴. Silver staining had also been employed in benign as well as malignant effusions¹⁴. It was found very useful in discriminating benign from malignant effusions¹¹.

Silver staining has also been used on bone marrow and peripheral blood films to study the AgNOR pattern in haematological malignancies and haemopoietic cells¹. Busch et al³ observed the AgNOR count of normal lymphocytes and reactive lymphocytes. He demonstrated a single dot in these cells.

We employed silver staining on peripheral blood smears and bone marrow smears. The result of our study indicated that a single silver staining dot is observed in mature lymphocytes. Majority of the reactive lymphocytes show single dot with occasional lymphocytes showing two dots. However, the difference was not statistically significant when compared with normal lymphocytes.

Proliferative index in mature as well as reactive lymphocytes was zero. Lymphocytes from patients with chronic lymphocytic leukaemia showed a single dot and the proliferative index was zero. AgNOR count of the lymphoblasts in acute lymphoblastic leukaemia was significantly increased as compared to normal lymphocytes, reactive lymphocytes and chronic lymphocytic leukaemia. The proliferative index of these lymphoblasts was also very high.

Klobusicka et al^{12,13} showed a significantly high AgNOR count in acute leukaemias as compared to normal. The results of our study are in accordance with other studies, whereas enumeration of AgNOR is concerned, since proliferative index indicates the number of cells with more than five dots per 100 cells. Assessment of AgNOR count and proliferative index reflects the proliferation capacity of the leukaemic cells. These parameters could be helpful in detecting the neoplastic behaviour of the cells. Razumovic¹⁹ has described that 2.9 NORs per nuclei is cut off point for separating low grade and high grade lymphoma. Hence NORs number has a prognostic significance.

AgNOR number was shown to have a prognostic significance in multiple myeloma^{17,18}. In the light of this study it is suggested that further study should be carried out to evaluate the prognostic significance of AgNOR in acute leukaemias.

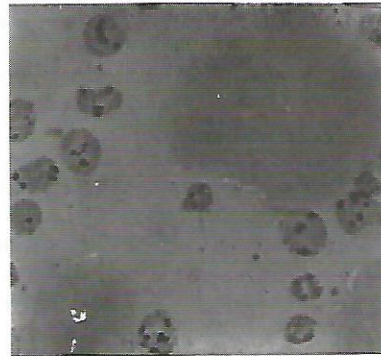


Fig. 2(a) AgNOR staining in Acute Lymphoblastic Leukaemia

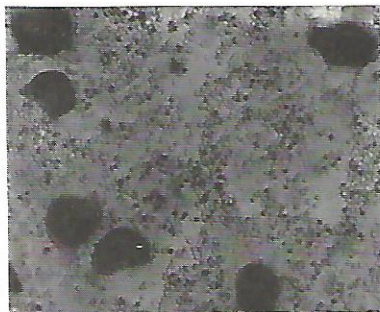


Fig.2(b) AgNOR staining in Chronic Lymphoblastic Leukaemia



Fig.2(c) AgNOR staining in Reactive Lymphocytes.



Fig.2(d) AgNOR staining in Normal Lymphocytes.

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