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Original Article

Assessment of monoclonal antibody MIB-1 labeling indices in cervical intraepithelial lesions of the uterine cervix in paraffin section

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Abstract

Objectives: To analyze the role of MIB-1 immunostaining for grading of cervical intraepithelial lesions (CIN) and microinvasive carcinoma as an index of cellular proliferation of dysplastic lesions and neoplastic progression. *Methods:* One hundred and fifty three cases of paraffin sections were stained by streptavidin - biotin method after antigen retrieval. Statistical analysis was done by using SPSS 10.0 package and comparisons were done by ANOVA method and independent sample 't' test. *Results:* MIB-1 labeling index (LI) increased from dysplasia to carcinoma group. Statistical analysis showed that MIBLI was significantly higher in diseased group as compared to normal group ($P<0.0001$ for all the groups) but few cases of CINI lesion showed high proliferative index. The mean values present linear progression from normal to metaplastic to dysplastic to cancerous lesion. A significant positive correlation was present between intensity of marker and labeling index of MIB-1 in all the groups ($P=0.05$) except nonSCC group. Statistically no important correlation was found with age and menopausal status. *Conclusion:* This marker may be useful in grading CIN lesions and identifying low-grade CIN cases with high proliferative index.

Key words: CIN, MIB-1, Immunostaining

Introduction

Cervical cancer continues to be the leading cause of cancer deaths for women in developing countries. Incidence and death rates are particularly high in Latin America, Africa, India and eastern Europe[1]. India accounts for one fifth of the worlds' burden of cervical cancer.

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Cervical intraepithelial neoplasia is a precursor of invasive squamous cell carcinoma of the uterine cervix[2]. Invasive carcinoma can also develop from CIN I, while CIN II and CIN III cases do not always progress into cervical cancer[3]. Often pathologists rely on standard histomorphologic criteria such as nuclear pleomorphism, loss of polarity, absence of maturation, and mitoses to identify and subclassify the squamous lesions of the uterine cervix. The presence of atypical mitotic figures and the localization of mitoses are used for the grading of the CIN lesions. In CIN lesions the mitotic figures occur more frequently in suprabasal layer of epithelium[4]. However, grading of CIN lesion on histological basis is subjective and difficult. Therefore,

additional methods are required to improve grading and perhaps also for the identification of biologically unfavorable CIN lesions.

Ki-67 antigen, a tumor growth marker is present throughout the cell cycle (G, S, G2 and M phase) of proliferating cells but is absent in quiescent (G0) cells. It can be detected by monoclonal antibody MIB-1 (standing for molecular immunology Borstel) in immunohistochemical assay. This antibody works satisfactorily on formalin fixed tissue sections[5]. Therefore it is interesting to study the immunohistochemical expression of MIB-1 in different grades of CIN lesions especially in low-grade CIN lesion developing into invasive carcinoma.

One hundred & fifty three cases were selected where clinical data was available. These cases were divided into four groups normal (n=35), CIN (n=60), SCC (Squamous cell carcinoma) (n=44) and nonSCC (n=14) group. Multiple sections of 3-4 μ thickness were cut from each paraffin block. One section was stained with hematoxylin eosin staining for histological typing and rest of the sections were kept for MIB-1 immunostaining. Primary antibody MIB-1 (Code No-N1633) of Dako Cytomatin Ltd. and B sap universal kit (Code No - 37101) of Span Diagnostics Ltd. were used.

Immunostaining method for MIB -1

The method described by Key et al[6] was employed. Immunostaining was done by streptavidin - biotin method. Paraffin sections were rehydrated and kept in citrate buffer (pH 6.0) for antigen retrieval in microwave oven. Sections were kept in 3% H_2O_2 followed by protein blocking antibody (25 minutes). After washing with TBS (tris buffer saline) sections were incubated overnight into primary antibody (MIB:1) at 4^0C. On the next day sections were put into biotinylated secondary antibody (30 minutes). After washing with TBS, sections were kept in streptavidin - peroxidase reagent (45 minutes) followed by DAB (Diaminobenzidine) solution for 45 minutes and counterstained with hematoxylin and mounted in DPX (Distyrene plasticizer xylene).

Positive control for MIB-1: A histological section of gall bladder adenocarcinoma was used as positive control with each batch of staining.

Negative control for MIB:1: For negative control 1% nonimmune serum was used in place of primary antibody, with rest of the steps being the same as far the positive control.

Calculation of MIB-1 labeling index

MIB-1 labeling index was calculated by the number of positive cells per 100 cervical epithelial cells in different areas under X400 magnification in triplicate and the mean was calculated. Positive nuclei were expressed as the percentage of total nuclei counted. MIB-1 labeling index was calculated as follows

$$\text{Labeling index} = \frac{\text{No. of cells showing positive staining}}{\text{Total no of cells}} \times 100$$

Statistical analysis

Statistical analysis was done by using SPSS 10.0 package. Means were calculated for each of the quantitative values. The comparisons were made using ANOVA and independent 't' test. Correlations were obtained by using bivariate correlation and Pearson's correlation coefficient®. In order to correlate intensity of MIB-1 values, the intensities were graded on a scale of 0-3 as 0 - negative, 1 - weak, 2 - moderate, and 3 - intense.

In order to ascertain significance, probabilities were also taken into account.

Results

MIB-1 immunostaining was positive in 112/153 (73.2%) cases. Labeling index of MIB-1 increased as we move from dysplasia to carcinoma group (Figure 1).

Mean labeling index of carcinoma group was higher than that of CIN group (37.692±11.5426 vs 8.233±6.1709, Table 1). In case of dysplasia CIN-III cases present maximum labeling index as compared to other CIN lesions but 5 cases of CIN-I lesion showed high proliferative index of MIB-1 than high-grade dysplastic lesion. These cases are important and should be kept in higher grade for timely and appropriate intervention. This marker may be useful in low grade CIN lesion with high labeling index, which could not be diagnosed in histopathological sections.

In order to compare the difference among different groups, analysis of variance was performed. 'F' values of 83.50703 was found to be statistically significant at $P<0.005$ ($P=9.52\times10^{-32}$, Table II).

MIB-1 labeling index was correlated with age, menopausal status and intensity of marker. Statistical

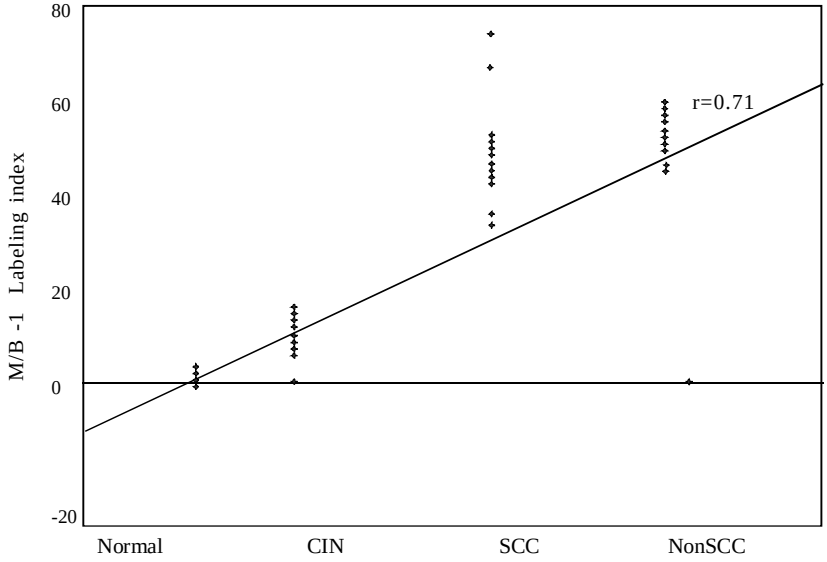

Figure 1. MIB -1 Labeling index.

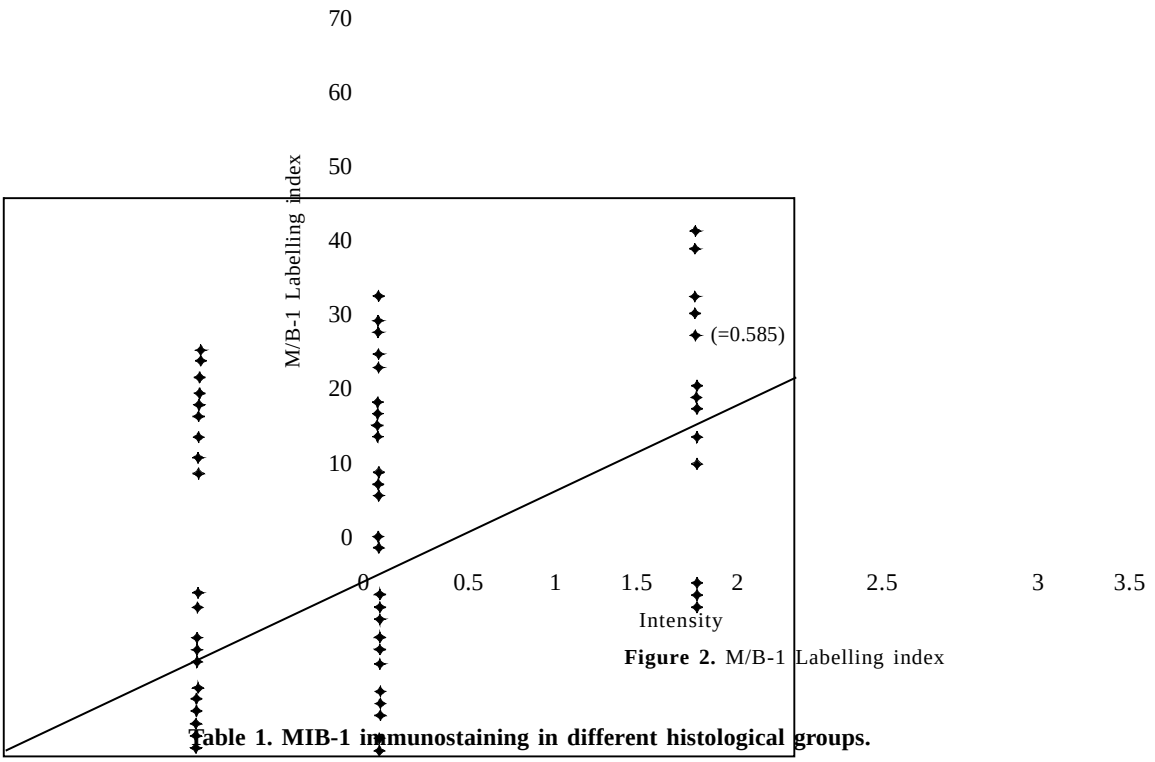

Figure 2. M/B-1 Labelling index

Table 1. MIB-1 immunostaining in different histological groups.

Lesion	Number	MIB-1 Mean±SD	SE	t (P)
Normal	35	1.448±1.0684	0.1806	—
CIN	60	8.233±6.1709	0.7967	6.435(<0.0001)
SCC	44	31.409±15.6804	2.3639	11.268(<0.0001)
NonSCC	14	37.692±11.5426	3.2013	17.702(<0.001)

Table 2. Analysis of variance for cervical biopsies for different histological groups.

Groups	Count	MIB-1 Sum	Average	Variance
Normal	35	50.7	1.448571	1.141395
CIN	60	494	8.23333	38.08023
SCC	44	1382	31.40909	245.8753
NonSCC	14	490	35.00000	224.4615

Source of variation	SS	df	MS	F	P value
Between groups	26525.27	3	8841.757	83.50703	952E-3
Within groups	15776.18	149	105.8804		
Total	42301.45	152			

Table 3. Intensity of MIB - 1 immunostaining in different cervical lesions.

Lesion	Number	Staining intensity		
		Weak	Moderate	Intense
Normal	35	31	4	-
CIN	60	35	15	10
SCC	44	8	29	7
NonSCC	14	3	3	8

Table 4. Correlation between MIB-1 immunostaining with intensity.

Group	N	MIB-1 Mean (SD)	Intensity of MIB Mean (SD)	MIB-1 vs intensity of MIB-1
Normal	35	1.4486(1.0684)	0.9429(0.6391)	0.482*
CIN	60	8.23333(6.1709)	1.1833(0.9476)	0.7775*
SCC	44	31.4091(15.6804)	1.5455(0.9010)	0.685*
NonSCC	14	35.0000(14.9820)	2.1429(0.7703)	0.233

Significant at $P<0.05$

analysis showed no significant correlation between age and menopausal status of the patient with MIB-1 labeling index (data not shown). Table 3 presents MIB-1 intensity in different cervical lesions. Intense positive staining was seen in CIN III grade of dysplasia. In carcinoma group seven cases of SCC showed positive staining. Statistical analysis present (Table IV) a significant positive correlation in all the groups (P=0.05) except for the nonSCC group where the correlation is statistically not significant. This could be attributed to the fewer number of the samples included in the group and thus making it prone to chance error. Scatter diagram

also reflects a direct relationship between the MIB labeling index and intensity of the marker (Figure 2).

Discussion

In normal cervical epithelium Ki-67 antigen is exclusively found in parabasal and basal cells. Parabasal cells are the main source for cells renewal in the exocervical epithelium and basal cells serve as reserve cells[7]. Gibbons et al [8] reported a change in the expression of MIB-1 from parabasal cells (normal and metaplastic epithelium) to intermediate (low grade SIL) and superficial layers high grade SIL). In their opinion invasive carcinoma had high labeling index than high-grade dysplasia.

Maeda et al [9] observed that Ki-67 positive cells increased with increasing grades of cervical lesions. McCluggage et al [10] also found that the number and distribution of Ki-67 positive cells increased with the grade of CIN lesion. Our findings are also consistent with these studies. Our results showed that MIB-1 staining levels increased with the progression of lesion from normal through increasing grades of dysplasia to invasive carcinoma.

MIB-1 staining might be useful in selected cases in the grading of CIN and especially in low lesion showing high proliferative index. Our findings are in agreement with those of Ter Harmsel et al[11] who reported that a few cases of low grade CIN showed higher proliferative index. Equally some cases of high-grade CIN lesions present small number of Ki-67 positive nuclei than observed in most CIN lesion. Our findings are in agreement with these observations that MIB-1 labeling index will be specifically useful in seemingly low-grade lesion i.e. CIN I with high proliferative index. Kruse et al[12] also reported that Ki-67 may be a sensitive biological indicator of progression of seemingly low grade CIN lesion. al-Saleh et al[13] reported no overlapping between low grade and high grade SIL groups but a partial overlap between the densities of Ki-67 positive cells in low grade SILs and squamous metaplasia. In contrast our findings suggest a clear distinction between squamous metaplasia and CIN grade of cervical intraepithelial lesion. Gargetti et al[14] reported a significant difference between MIB-1 proliferative indices in paraffin sections of cervical carcinomas in young and older patients suggesting a biologic aggressiveness of age related cervical carcinoma. In our study MIB-1 was found to be an independent marker irrespective of age and menopausal status.

The assessment of cell proliferation with MIB-1 is useful and less expensive in comparison to other technics like thymidine and bromodeoxyuridine labeling quantitation of cellular DNA, which are more expensive and cannot be used in routine diagnostic practice. MIB-1 can be used as an independent discriminant of progression and biological behavior of CIN lesion irrespective of age and menopausal status. This could be useful a developing country where HPVDNA testing as screening is still out of reach because of high cost.

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