IMMUNOCYTOCHEMICAL STUDIES FOR THE LOCALISATION OF HERPES SIMPLEX VIRUS TYPE II ANTIGENS IN CERVICAL INTRAEPITHELIAL NEOPLASIA AND CARCINOMA CERVIX

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SUMMARY

1544 cervical biopsies and 6869 cervical smears were studied.Out of 1544 cervical biopsies, immunocytochemical studies were performed in 417 cases for the localisation of herpes simplex virus II antigens (HSV II). It was found that the positivity for HSV II antigen increased as the grades of cervical intra-epithelial neoplasia (CIN) increased and it was much more in cases of carcinoma cervix thereby explaining a case relationship of HSV II antigen with CIN and carcinoma cervix.

INTRODUCTION

Carcinoma of cervix is the most common malignancy among Indian women. The incidence of carcinoma cervix in Nagpur city agglomeration is 18.3 per lac population (Jussawela et al 1987). Carcinoma cervix does not arise de novo, but it is preceded by lesions such as dysplasia, precancerous changes and most widely by cervical intraepithelial neoplasia (CIN) which has been graded as CIN I CIN II and CIN III. Viruses are known to produce tumors in animals, but their roles in the etiology of cancer in humans is a matter of great importance and research. Various seroepidemiological and histological studies have revealed that the two classes of viruses namely Herpes Simplex Virus (HSV II) and a human papilloma virus (HPV 16) are directly or indirectly related with precancerous and cancerous lesions of cervix. Women with

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Herpetic cervicitis have 4 to 16 fold greater risk of developing carcinoma cervix than those without this lesion (Shariff et al 1989).

The study of carcinoma cervix has covered a long pathway from epidemiological studies as back as middle of 19th century to the present era of immunocytochemical studies such as immunoperoxidase (IP) and immunofluroscence (IF) increased levels of antibodies to HSV II antigens in the sera and other body fluids have been demonstrated by techniques such as microneutralization, radiolimmuno assay (RIA) and Elisa in the past but direct demonstration of HSV II antigen in the cervical epithelium by immunocytochemical techniques have further strengthened the fact that there is a close relationship between HSV II antigen and cancerous lesions of carvix.

In view of these facts a study was conducted in Governmemt Medical College, Nagpur for a period of two years with the following aims.

1. To identify and localize HSV II antigen in CIN and carcinoma cervix lesions by immunocytochemical techniques.
2. To know the association of HSV II lesions with risk factors.

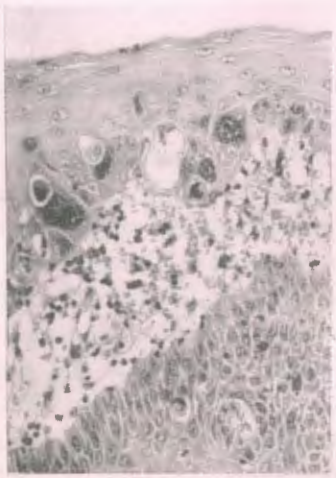

Fig. 1

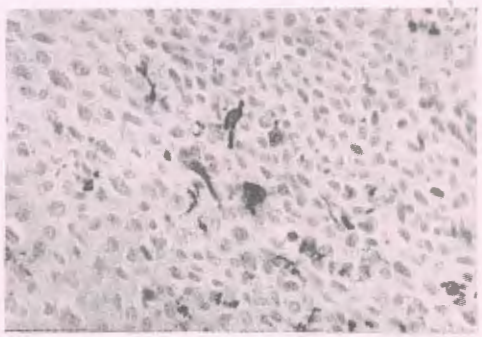

Fig. 2

MATERIAL AND METHODS

The present study comprised of 1544 cervical biopsies and 6869 cervical smears received in the department of pathology, Govt. Medical Colloge, Nagpur for a period of Two years (Table I and II). These cases were further classified into groups such as inflammatory, metaplastic, CIN and its grades and squamous cell carcinoma (as shown in table I & II)

Out of 1544 cervical biopsies, 221 cases of CIN, 146 cases of well differentiated squamous cell carcinoma and 30 cases of chronic cervicitis were subjected to immunocytochemical techniques for the localization of HSV II antigen. For the identification of HSV II antigen immunoperoxidase (IP PAP Peroxidase Antiperoxidase) technique and direct immunofluorescent (IF) were used. Negative and positive controls were always kept while doing immunocytochemical tecani-

ques. Antibody reaction sites were seen in the form of apple green (greenish yellow) flourescence, while positive immunoperoxidase staining was in the form of golden brown colour in the cytoplasm of cells.

RESULT

In the present study 1566 cervical biopsies were further classified into different groups as shown in Table I. There were 820 (53.11%) cases of squamous cell carcinoma, 126 (8.14%) of cervical intraepithelial neoplasia and 560 (36.30%) of chronic cervicitis.

Out of 6869 cervical smears 104 (1.52%) belonged to squamous cell carcinoma, 35 (0.51%) to squamous cell carcinoma, 35 (0.51%) to CIN while 5654 (82.18%) were inflammatory as shown in Table II.

In 417 cervical biopsies which were

Table I

Showing Distribution of Cervical Biopsies

No.	Group	No. of cases	Percentage
1.	Normal	29	1.85
2.	Inflammation	560	36.30
3.	Metaplasia	09	0.60
4.	C. I. N.	126	8.14
5.	Squamous cell Carcinoma	820	53.11
	Total	1544	100.00

Table II

Showing Distribution of Cervical SMEAR

No.	Group	No. of cases	Percentage
1.	Normal	1030	14.99
2.	Inflammation	5654	82.18
3.	C. I. N.	35	0.51
4.	Metaplasia	46	0.70
5.	Squamous cell Carcinoma	104	1.52
	Total	6869	100%

Table III

Showing Distribution of Immunoperoxidase and Immunofluorescence in Cervical Biopsies

No.	Diagnosis	Total Cases	No. of Cases I.P. Positivity	%	No. of Cases with I. F.	%
1.	Normal	10	0	-	0	-
2.	Chronic cervicitis	30	0	-	0	-
3.	Metaplasia	10	0	-	0	-
4.	C.I.N. I	70	0	-	4	5.71
5.	C.I.N. II	87	4	4.59	7	8.04
6.	C.I.N. III	64	13	20.31	13	20.31
7.	Squamous cell carcinoma	146	30	20.54	32	21.91
	Total	417	47	45.44	56	55.97

Table IV

Showing HSV II Positivity with Risk Factors in CIN and Carcinoma Cervix

No.	Risk factors	% of HSV II positivity in CIN and Carcinoma Cervix
1.	Early Menarche	62.5
2.	Early Marriages	53.57
3.	Multiparity	44.64
4.	Low Socioeconomic status	66.07
5.	Oral contraceptives	50.00
6.	Age group	20.40 year

subjected to IP and IF studies, it was seen that the imunoperoxidase positivity and immunoflurescence increased with the increasing grades of CIN and it was much more in carcinoma cervix. Table III shows the percentage of immunoperoxidase pos-

itivity and immunofluorescence.

During this study various risk factors were also taken into consideration so as to find the association of HSV II with CIN and carcinoma cervix. Table IV shows the percentage of HSV positivity in CIN and carcinoma cervix with the risk factors.

DISCUSSION

In the present study the incidence of CIN was found to be 0.51% as shown in Table II. Luthra et al (1983) observed the incidence of CIN in Indian women to be as high as 1.7%. The incidence observed by Briggs (1979) was 0.54%. The incidence of CIN reported by other authors varies from 0.54% to 1.33 % (Patten S.F. 1978, Bibbo 1971). These differences may be due to size and type of samples, different populations surveyed and subjective interpretation implicated in diagnosing dysplasia or CIN.

In the present study the percentage of carcinoma cervix was found to be 1.52 % (Table II) on cytological examination. While the frequancy of CIN on histopathological examination is 8.14% (Table I). GUIN (1953) has stated it to be 3.1%. These differences could be due to the fact that only symptomatic patients biopsies were taken in this study, different populations surveyed and different criteria used for the histopathological diagnosis.

It was observed that the severity of CIN increased with the number of risk factors.Such association of CIN, carcinoma cervix an high risk factors was also stated by various workers (Schneider et al 1983, Reeves et al 1987) It was observed in the present study that (5.71%) and of 70 cases of CIN I were positive on immunoflurescent staining and no case was found to be positive on immunoperoxidase staining. In CIN II, HSV II positivity was found to be 4.5% on immunoperoxidase and 8.04% on immunofluorescent staining, while it was positive in 20.3% both on immunofluorescent and immunoperoxidase staining in CIN III. In well differentiated squamous cell carcinoma, HSV II positivity was 20.54% on immunoperoxidase staining and 21.91% on immunofluoresence staining. It was seen in the present study that HSV II antigen positivity increased as the grades of CIN increased. Our findings were well correlated with those of Dressman et al (1980) Cabral et al (1983)

In the present study, it was observed that risk factors like early onset of menarche, early marriage, multiparity and low socioeconomic condition were significantly associated with HSV II positivity in CIN and carcinoma cervix (Table IV) such association is also observed by other authors (Schneider et al 1983, Reeves et al 1987).

Thus it can be concluded that an increased positivity for immunoperoxidase and increased immunofluroescence was seen in the increasing grades of CIN and squamous cell carcinoma, there by explaining a strong relationship between HSV and CIN and carcinoma cervix. Therefore HSV II should be considered as one of the important factors in the multifactorial etiopathogenesis of cervical intraepithelial neoplasia and carcinoma cervix.

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