

HERPES SIMPLEX VIRUS INFECTION (TYPE I AND TYPE II) AS AN ETIOLOGIC FACTOR IN SPONTANEOUS ABORTIONS (AN IMMUNOHISTOCHEMICAL STUDY)

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ABSTRACT: *A reliable and simple method of immunoperoxidase, Avidin-Biotin staining method was used to identify HSV-Type I and II antigens in paraffin embedded placental tissues. 20 clinically diagnosed spontaneous abortion cases (group 1) and 10 normal delivery cases (group 2) were chosen for this study. Placental HSV positivity was detected in 8 cases of group 1 and 3 cases of group 2. There was a significant concordance between our findings and the literature. Namely, there were five positive cases in group 1 where both the decidual cells and the chorionic villi reacted positively with Anti-HSV-Type II monoclonal antibody; as compared to three positive cases of group 2 with the same positive reaction in chorionic membranes only. This data supports that HSV-Type II has an etiologic role in spontaneous abortions and if the placental tissue is somehow protected from infection, healthy deliveries can occur.*

INTRODUCTION

Genital Herpes Simplex Virus (HSV) infection, which is

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probably responsible for some of the spontaneous abortions of unknown etiology, has experienced an increasing incidence in the recent years (9,10,11,12). Some studies indicate that Herpes Simplex infection during pregnancy causes not only spontaneous abortions, but congenital and perinatal infections, maternal systemic infections and exitus of the mother as well (5,9,12).

Following the genital infection, through a transneural route; HSV can establish a latent endometrial epithelial infec-

Table-I: Evaluation of HSV Type I and Type II in placentar tissues of 20 spontaneous abortion cases (Group I) by monoclonal antibodies.

	No. of cases	
	Negative	Positive
HSV Type I	20 (% 100)	-
HSV Type II	12 (% 60)	8 (% 40)
$\chi^2 = 10.0, DF = 1, p < 0.01.$		

Table-II: Evaluation of HSV Type I and Type II in placentar tissues of 10 Normal Delivery cases (Group 2) by monoclonal antibodies.

	No. of cases	
	Negative	Positive
HSV Type I	10 (% 100)	-
HSV Type II	7 (% 70)	3 (% 30)
Fisher's exact test, $p = 0.105.$		

tion. The fact that this infection can not be clinically detected unless fertilization occurs (2,9), is the main indication for early diagnosis.

The most recently utilized detection procedures of HSV include; enzyme-linked immunosorbent assay (ELISA); isolation in cell culture by the use of smear samples from the genital areas, lips, nose and perianal regions; and immunofluorescent and immunoperoxidase techniques in tissue samples (1,6,8,12).

In this study endometrial curettage materials clinically diagnosed as spontaneous abortions and placentar tissues from normal deliveries were examined for HSV-Type I and Type II Ag to find out the rate of HSV infection and tissue localization of the virus in normal and abnormal pregnancies.

The Streptavidin-biotin immunoperoxidase technique (with monoclonal antibodies) was used in the paraffin embedded tissues. The study was carried out in the Pathology Department of the Medical School of Cukurova University.

MATERIALS AND METHODS

We examined placentar tissues of 30 cases of gynecology department of the Medical School of Cukurova University.

The first group consisted of 20 cases clinically diagnosed as spontaneous abortions (Group 1) and the second group of 10 normal delivery cases (group 2). None of the patients in either groups had an history of genital HSV infection.

After reviewing the Hematoxylen-Eosin stained slides of the cases, 5 μ -thick slides were obtained from paraffin embedded tissues for Avidin-Biotin Immunoperoxidase technique (13) and mouse monoclonal antibodies HSV Type I (50 % Dilution) (Serotec Cath. No: MCA 405) and HSV Type II (50 % Dilution) (Serotec Cath. No: MCA 405) were used. For HSV-Type I, orolabial smear samples of herpetic ulcer were used as positive controls. At least two slides were prepared for each specimen; one to serve as the test slide, the other as a negative control slide. The corresponding negative control reagents were added to the negative control slides. The slides were viewed on a brightfield microscope and photographed under an Olympus microscope.

RESULTS

The mean age of clinically diagnosed spontaneous abortion cases was 31, with a range of 19 through 43. In group 1; HSV-Type I antigen was negative in all cases, in spite of

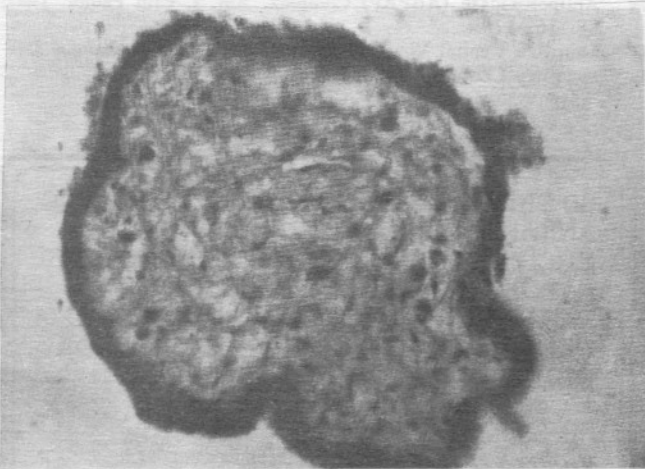


Fig. I: Immunoperoxidase staining of a chorionic villus in a case of spontaneous abortion, with HSV-Type II monoclonal antibody. Positivity in Hofbauer cells and trophoblastic cells is seen (x 120).



Fig. II: Immunoperoxidase staining of the chorionic membrane in a case of Group 2, with HSV-Type II monoclonal antibody. Positivity, limited to some parts of the membrane is seen (x 100).

the HSV-Type II positivity in 8 of them (Table I). Placental tissues from normal deliveries stained negatively in all the 10 cases of group 2 by monoclonal HSV-Type I Ab but 3 cases were positive for HSV-Type II (Table II).

Localization of Monoclonal Type II Ab in positive cases:

a) In five cases of group 1, both decidua and Hofbauer cells and trophoblastic cells of the chorionic villi were stained positively (Fig. 1). There was one case with positive staining only in the decidua cells, and two cases with positive staining only in the chorionic villi.

b) In group 2, the staining was limited to the chorionic membranes in all cases (Fig. 2). No reaction was established in decidua cells and chorionic villi.

DISCUSSION

There exist prevalence studies relating to genital HSV infections in normal populations (7,11,12) and also a study indicating the inverse relation of asymptomatic shedding of the virus by increasing age (4). Such an inverse relation was not detected in this present study, a finding which may be due to the two characteristics of the Turkish Society; mainly, relatively higher age, especially in the big cities at the initial sexual relation, and lower number of sexual partners.

In the diagnosis of HSV infection, virus culture, in spite of its high cost, is an important and specific technique (12). The enzyme-linked immunosorbent assay (ELISA) for the detection of HSV Antigen has been evaluated in the recent years, and some sensitive results have been obtained. By the use of this diagnostic assay, however, the typing of HSV may somewhat be difficult, as HSV-Type I and Type II Antigens can cross-react with each other (1). Viral antigen can be shown in tissue sections and diagnosed specifically by the help of Immunoperoxidase techniques and monoclonal antibodies (8,9,12). These techniques can be used in cytologic smears as well.

HSV, has the ability of producing latent infection in nervous tissue and many factors are accused of the reactivation and systemic shedding of the virus. Hormonal changes in delivery, systemic or local rise in prostaglandin levels and physiologic immunosuppression are some of the situations which reactivate HSV and are associated with asymptomatic viral shedding (2,9). As a complication of this shedding, virus can produce infection in placental and fetal tissues and can cause spontaneous abortions. In our cases of HSV positive spontaneous abortions, the antigen was localized both in decidua tissues and chorionic villi, which is in concordance with the literature (4,5,6,9,12).

In some reports, it is established that HSV does not produce infection in placental and fetal tissues without causing endometritis and it can only be detected clinically if the fertilization does occur (3,9). In most of the recurrent infections, anti-HSV antibodies in amniotic fluid protect the fetus and the chorionic villi and these pregnancies are ended as normal deliveries (3,9). This fact is demonstrated in our study as well; since in three of the cases of group 2 (the normal delivery group) the antigen was localized in chorionic membranes only, sparing the chorionic villi.

In the eleven positive cases belonging to both groups, we could not detect any HSV-type I antigen by our technique. As a result; in spontaneous abortion cases with unknown etiology, without a history of genital HSV infection, detection of HSV antigens by the help of monoclonal antibodies can be helpful to identify the etiologic agent. We

plan to continue our study on larger series and correlate our findings with clinical signs and virus cultures.

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