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Molecular Detection of Human Herpes Virus types 1 and 2 Among pregnant women in Khartoum State, Sudan

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ABSTRACT

Background : Infection with herpes simplex is one of the most common sexually transmitted infections. Because the infection is common in women of reproductive age it can be contracted and transmitted to the fetus during pregnancy and to the newborn. Herpes simplex virus is considered as an important cause of neonatal infection, which can lead to death or long-term disabilities. The greatest risk of transmission to the fetus and the newborn occurs in case of an initial maternal infection contracted in the second half of pregnancy. The risk of transmission of maternal-fetal-neonatal herpes simplex can be decreased by performing a treatment with antiviral drugs or resorting to a caesarean section in some specific cases. The purpose of this paper is to determine the prevalenceof HSV-1 and HSV-2 infections among pregnant women in Khartoum State using ELISA and Real-time PCR

Methods: Pregnant females suspected to have herpes simplex infection, were enrolled in the study. Ninghty serum samples were collected from such patients in different hospitals in Khartoum State.The sera were Tested for Herpes simplex virus types 1 and2 specific immunoglobin (IgM) antibodies using enzyme-linked immunosorbent assay(ELISA), and Real-time PCR was used for detection of HSV1&2 DNA

Results : Out of the 90 sera tested, 4 patient's (4.4%) showed HSV1 IgM, and 4 (4.4%) HSV1 DNA and 7 patients (7.7%) showed HSV2 IgM with 6 samples (6.6%)showing HSV-2 DNA .

Conclusion: The prevalence of HSV1 and HSV2 in pregnant females in Khartoum State, was documented through the molecular detection of HSV1 and HSV2 antibodies and detection of HSV1DNA and HSV 2 DNA. Further study using various diagnostic methods should be considered to determine the prevalence of HSV1 and HSV2 diseases at the national level.

Keywords : ELISA, Real-time PCR, Sudan, Herpes simplex, Pregnancy.

I. INTRODUCTION

Herpes simplex virus (HSV) infection is one of the most common sexually transmitted viral diseases (STD) [1,2]. Herpes simplex virus type 1 (HSV-1) is usually transmitted during childhood via non-sexual contacts. However, HSV-1 has emerged as a principle

causative agent of genital herpes in some developed countries [1,3,4].

HSV-2 is almost exclusively sexually transmitted, causing infection in the genital or anal area (genital herpes).It can be transmitted from skin in the genital or anal area that looks normal and is often transmitted in the absence of symptoms. In rare circumstances, HSV-2 infection can be transmitted from a mother to her infant during delivery (WHO,2017).

The greatest incidence of HSV infections occurs in women of reproductive age, the risk of maternal transmission of the virus to the foetus or neonate has become a major health concern [2,5-6].

Recent findings reveal that first-time infection of the mother is the most important factor for the transmission of genital herpes from mother to foetus/newborn. In fact, the pregnant woman who acquires genital herpes as a primary infection in the latter half of pregnancy, rather than prior to pregnancy, is at greatest risk of transmitting these viruses to her newborn. Additional risk factors for neonatal HSV infection include the use of a fetal-scalp electrode and the age of the mother less than 21 years. Interventions based on these findings led to new management of the pregnant patient with genital herpes prior to pregnancy and to prevention measures to avoid the acquisition of herpes during pregnancy [7].

II. MATERIAL AND METHODS

STUDY AREA:

The study were conducted in Khartoum Hospitals (Basher Hospital, Ibrahim Malek Hospital , Soba Teaching Hospital and Omdurman Delivery Hospital) during period November 2015 to June 2016.

Patients Inclusion Criteria and Sample:

Target patients were pregnant females with age ranging between 15 and46years old and weight 55-104 kg.

Ninety pregnant females with or without abortion history and congenital babies were recruited. All participating patients were given written informed consent.

Blood Sample Collection:

Blood (2-5 ml) was collected in plain containers and transported on wet ice to the laboratory for immediate processing. Sera were separated from blood samples by centrifugation for 5 mints at 3000 RPM. Obtained sera were used for antbodies detection and DNA extraction .

Capture ELISA IgM: Serum samples were tested for the presence of HSV-1 and HSV-2 IgM antibodies using 3rd generation commercially available ELISA kits (GENESIS Diagnostic, Omega Diagnostic Group PLC, Cambridge Shine, UK), according to the manufacturer's Protocols.

DNA extraction: Commercial DNA extraction kits (analytikjena, Germany) were used to extractDNA of HSV according to the procedure described by the manufacturer instructions.

Real-time PCR

Optimized Real-time PCR reaction for HSV1and HSV2 DNA was performed using the commercial kit (innuMIX Q PCR MasterMi`x probe_analytic jena_Germany). The real-time HSV-typing PCR is a multiplex PCR which can detect both HSV-1 and HSV-2 DNA in the same reaction. The optimized gB primer pairs (GbTyF:5-CGCATCAAGACC ACC TCC TC-3, GbTypR: 5-GCT CGC ACC ACG CGA-3) amplify both HSV-1 and HSV-2 with the same efficiency) [12].

Probe:

Type specific probes are labeled with different fluorescent dyes. HSV-1 typing probe (GbTyp1: 5-TGG CAACGCGGCCCAAC-3) is labeled at the 5 end with VIC and at the 3 end with TAMRA. HSV-2

typing probe (GbTyp2: 5-CGG CGA TGC GCC CCA G-3 is labeled at the 5 end with FAM and at the 3 end with TAMRA.

Master Mix:

The MasterMix was innuMIX qMasterMix Probe (analytikajena_German)

The reaction mixture content was 10μ of master mix 0.5μl of probe (0. 5μl for each specific type of HSV) 2μl of primer، 2.5μl of H2O and 5μl of DNA (Total volume of Mix is 20μl) .

The PCR reaction was carried out as described by 12].with slight modification.

After 2 min of incubation at 50C°, and 5sec of denaturation at 95C°, the PCR mixture was subjected to 45 cycles of 95C° for 20 sec and 60C for 1 min. The intensities of the fluorescent dyes in each reaction were read automatically during PCR cycling in T.optical thermo_cycler(analytikajena, Germany).

III. RESULTS AND DISCUSSION

Four (4.4%) out of 90 sera were found to be HSV-1 IgM antibodies positive and 7 (7.7%) were HSV-2 IgM positive(table 1).

In addition, 4 samples were positive for HSV-1 DNA and 6 were positive for HSV-2 DNA. All HSV-1 and HSV-2 positive DNA samples were about 11%(table 2).No significant differences in the prevance of IgM antibodies or incidence of DNA detection were observed anmong different age groups were observed,

Table 1 : Incidence of HSV-1 and HSV-2 DNA among different age groups as detected by Real Time PCR

Age group	HSV1			HSV2		
	No of positive	Percent of positive	**P- value**	No of positive	Percent of positive	**P- value**
15- 25	1	1.1	0.776	1	1.1	0.600
25- 35	2	2.2		3	3.4	
35- 45	1	1.1		2	2.3	
Total	4	1.5		6	2.3	

Table 2 : Prevalence of HSV-1 and HSV-2 IgM in different age groups .

Age group	HSV1			HSV2		
	No of positive	Percent of positive	**P- value**	No of positive	Percent of positive	**P- value**
15- 25	0	0	0.364	2	2.2	0.358
25- 35	2	2.2		4	4.4	
35- 45	2	2.2		1	1.1	
Total	4	4.4%		7	7.7%	

Table 3 : Incidence of HSV1 and HSV2 DNA detected by real-time PCR according to stage of pregnancy

	HSV1			HSV2		
	No of positive	Percent of positive	**P-value**	No of positive	Percent of positive	**P-value**
First	2	2.2	0.776	1	1.1	0.600
Second	1	1.1		3	3.3	
Third	1	1.1		2	2.2	
Total	4	4.4%		6	6.7%	

DISCUSSION

The main aim of this study was to determine the prevalenceof HSV-1 and HSV-2 infections among pregnant women in Khartoum State using ELISA and Real-time PCR. In the present study, HSV1 IgM antibodies were found in 4(4.4%),HSV2 IgM 7(7.7%) and virus DNA for HSV1was found in 4 (4.4%) and HSV2 DNA in 6 (6.7%) in the tested patients. No significant differences were detected among various groups in the prevalence of IgM antibodies or regarding the incidence of DNA detection for both viruses. Also, no significant differences were discerned regarding the prevalence of IgM antibodies or incidence of DNA detection among the two viruses. An earlier study, [8] reported that 45 out of 130(34.6%) of pregnant women who undergone lower segment caesarean section were positive for HSV IgG but none were positive for IgM. The authors stated that their results indicated immunity in their study subjects and no active cases of HSV infection were detected. The same authors [8] also indicated that there were no significant differences in the seroprevalence of infection among various age groups. The discrepancy noted between the results of our study and that of [8] regarding the prevalence of IgM Antibodies may be related to different diagnostic tests used in the two studies.

Detection of HSV1/2 DNA is most likely to be useful in this way because it has high sensitivity and more specificity than serological methods (ELISA). Real-time PCR offers numerous advantages over conventional methods used to detect HSV DNA. It requires less manual involvement; therefore, it is faster, and more importantly, there is a reduced possibility of cross-contamination. Also, real-time PCR has consistently been reported to be significantly more sensitive than viral culture and antigen detection.[9,10].

Positive IgM ELISA and negative DNA results might be to due to the persistence of the IgM antibodies for a longer time after infection in some individuals, where virus load may be too low to be detected by Real_time PCR.

IV.CONCLUSION

In conclusion, pregnant females in Khartoum state showed low prevalence of HSV-1 and HSV-2 IgM antibodies and low incidence of DNA detection. This study should open the way for more research on various areas of HSV-1 and HSV-2 infection including different sources of virus, the association between HSV-1 and HSV-2 acquisition and various risk factors such occupation, ethnicity geographical location, hospitalization, antibiotic usage, surgery and disinfection. Also other causes of pregnant females viral infection such as rubella virus, CMV, VZV should deserve further investigationto decrease children congenital infections.

Ethical Approval

Ethical approval for this study was obtained from the Ministry of Health, Khartom State, AL- Neelain University ethical committee board. Only patients who agreed to participate were enrolled in this study and informed consents were obtained regarding the data and collection of blood samples.

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