



## Molecular Detection of HSV type 2 Infection among Infertile Males in Khartoum State, Sudan

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### Abstract

**Background:** Herpes simplex virus (HSV) infections of humans were first documented in ancient Greece. HSVs belong to family Herpesviridae, and are characterized by being enveloped, double-stranded DNA viruses with relatively large complex genomes. Herpes simplex virus type 2 (HSV-2) DNA seems to play a significant role in male infertility, it is significantly related to low sperm count as well as poor motility.

**Aim:** This study aimed to determine the incidence of HSV-2 DNA among infertile males in Khartoum State, Sudan by using molecular methods.

**Methods:** The study was carried out in Khartoum State, Sudan, during May-August 2018. A total of 50 semen samples from infertile males were included. HSV-2 was detected using real-time polymerase chain reaction (RT-PCR).

**Result:** Among 50 semen samples from infertile males, 6(12%) samples were found positive for HSV-2 DNA.

**Conclusion:** Our findings showed no association between HSV-2 DNA and low sperm count in infertile Sudanese men. Our study also revealed the need for further investigations in different parts of the country to highlight the extent of the viral related men infertility problem in Sudan.

**Keywords:** HSV-2, RT-PCR, infertility, men, Sudan

### Introduction

Herpes simplex virus (HSV) infections of humans were first documented in ancient

Greece. Greek scholars, particularly Hippocrates, used the word “herpes,” meaning

to creep or crawl, to describe spreading lesions. The classification now in use came into being in the late eighteenth century, and although the vesicular nature of lesions associated with herpetic infections was previously well characterized, it was not until 1893 that Vidal specifically recognized person-to-person transmission of HSV infections <sup>(1)</sup>.

Herpesviruses belong to family Herpesviridae, that are enveloped, double-stranded DNA viruses with relatively large complex genomes. They replicate in the nucleus of a wide range of vertebrate hosts cells <sup>(2)</sup>.

Human herpesviruses infections are endemic and sexual contact is a significant mode of transmission for several including both HSV-1, HSV-2, also human cytomegalovirus (HHV-5) and likely Karposi's sarcoma herpesvirus (HHV-8). The increasing prevalence of genital herpes and corresponding rise of neonatal infection and the suggestion of Epstein-Barr virus (HHV-4) and Karposi's sarcoma herpesvirus as cofactors in human cancers created an urgent need for a better understanding of this complex, and highly successful virus family <sup>(3)</sup>.

HSV-2 continues to be a common infection, affecting approximately 22% of adults ages 12 and older, representing 45 million adults in the United States alone <sup>(4)</sup>. HSV-2 is more commonly the prime suspect when patients present with genital lesions. Despite this, most outbreaks of the infection will present with nonspecific symptoms such as genital itching, irritation, and excoriations <sup>(4)</sup>. HSV-2 is mainly transmitted through sexual coitus, contributing to its predominant rise starting at puberty <sup>(5)</sup>.

HSV-2 DNA seems to play a significant role in male infertility, it is significantly related to low sperm count as well as poor motility <sup>(6)</sup> and has been detected by polymerase chain reaction (PCR) technique in the sperm of men with genital HSV-2 infection <sup>(7,8)</sup>.

## Material and Methodology

### Study design

This is a Cross sectional study carried out in Khartoum State's hospitals.

### Clinical samples

A total of 50 semen samples were collected from patient groups at Khartoum State, between May and August 2018. Patients included in this study were infertile men and were given a written informed consent to participate.

Semen samples (3ml) in cryovials tubes, were collected from males after ejaculation and stored immediately at -20°C for DNA extraction for RT-PCR. Morphology was done by strict Kruger method and total number count of sperm was done using routine counting procedure (improved Neubauer haemocytometer).

### DNA extraction kits

Commercial kit QIAamp viral DNA mini kits (Qiagen, Germany) were used to extract DNA of HSV-2 according to procedure described by manufacture. The viral DNA was finally eluted in 60µl of elution buffer and stored at -20°C

### RT-PCR

Bio-Rad (USA) commercial kits were used according to manufactures instructions.

### Results

Semen samples had a homogeneous, grey-opalescent appearance and were less opaque due to low sperm concentration. Sperms had low motility, less than (40%). Morphology done by strict Kruger method, showed normal sperm morphology (1-3%) and abnormal (defect sperms) morphology (97-99%). Total number count of sperm done by routine counting procedure (improved Neubauer haemocytometer), revealed that all samples have low sperm count. In samples numbered from 1 to 16 the counts were less than  $10 \times 10^6$  sperm per millimeter, indicating very low count (cryptozoospermia), and for samples numbered 17 to 50 the count were from  $10-20 \times 10^6$  sperm per millimeter.

6 of the 50 semen samples from infertile males were found positive for HSV-2 DNA as shown in table 1. More positive HSV-2 DNA was

detected in 4 low count (less than  $10 \times 10^6$ ) samples while only 2 samples were positive in the higher count ( $10-20 \times 10^6$ ) samples,

**Table 1: HSV2 DNA in infertile males in Khartoum State detected by PCR (2018).**

Sperm count	Positive (%)	Negative (%)	Total (%)
Less than $10 \times 10^6$	4(8)	12(24)	16(32)
From $10-20 \times 10^6$	2(4)	32(64)	34(68)
Total	6(12)	44(88)	50(100)

## Discussion

About 50% of infertility cases are due to male factors. In the majority of male infertility cases, the cause of infertility remains unknown<sup>(9)</sup> The major causes of male infertility include varicocele, hormonal disturbances, immunological conditions, genital duct obstruction, cryptorchidism, medications, gonadotoxins, infectious diseases, sexual dysfunction, and ejaculatory failure<sup>(10)</sup>. Infections may deteriorate fertility by damaging spermatogenesis, impairment of sperm function, and occlusion of the seminal tract<sup>(11)</sup>. A mounting body of evidence now indicate that viral infections play a role in the pathogenesis of male infertility. Viral infections may reduce male fertility, either directly, or indirectly by invading the male genital tract cells or by causing local inflammatory or immunological responses that might deteriorate reproductive functions by proinflammatory cytokines and reactive oxygen species<sup>(12, 13)</sup>.

The role of some viral infections in male infertility has been investigated, and many previous studies revealed that HSV infections were associated with abnormal sperm parameters<sup>(14-16)</sup>. HSV-2 is mainly transmitted through sexual intercourse, contributing to its predominance starting at puberty and seems to play important role in male infertility.

Kotronias et al.<sup>(17)</sup> detected HSV-1 and HSV-2 infections in the semen of 21% and 20% of infertile men, respectively. Moreover, HSV infection was related with reduced sperm count and reduced motility. In another study by Monavari et al, HSV-1 and HSV-2

DNA was detected in 16 (22.9%) and 10 (14.3%) of 70 semen samples, respectively. All HSV-positive samples had abnormal semen parameters<sup>(18)</sup>. In yet another study by Kapranos et al.<sup>(19)</sup>, HSV DNA was detected in 49.5% of semen samples and HSV infection was significantly related to low sperm count as well as poor motility. In the present study, lower incidence rate (12%) was detected in Sudanese patients. This relatively lower positivity might be rooted in the conservative nature of the Sudanese society which prohibits extramarital relationships. Similar to other studies<sup>(17,19)</sup>, however, our study showed that HSV-2 may be implicated in lower sperm counts in the infected patients as most of the positive patients (n=4, 67%) were cryptozoospermia patients..

Due to logistical reasons we were not able to test our patients for HSV 1 and other herpesviruses, that would have given us a more complete depiction of the implications of these viruses infections in men infertility cases in

Sudan. Thus more detailed investigations are required in this domain of study.

### Conclusion

Detection of HSV-2 DNA in cases of men infertility by using RT-PCR is fast, accurate, sensitive, simple and more practical method for diagnosis.

Our findings show no association between HSV2 DNA and low sperm count. Our study also reveals the need for further investigations in different parts of the country to highlight the extent of the viral related men infertility problem in Sudan.

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