Study of Viral infections associated with Male infertility in

Hillah city-Iraq

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Abstract

Objectives: To investigate the association of viral infections present in the lower genital tract of males and relationship with male infertility.

Methods: ELISA technique of 175 semen and serum specimens, collected over 12 months from males investigated for infertility, were retrospectively assessed.

Results: One hundred and seventy five seminal fluid, blood and serum specimens from men investigated for infertility over a period of 1 year from February 2011 to February 2012 were analyzed. The seminal fluids and serum of patients mentioned to the laboratory from the fertility clinics of Babylon maternity and children Hospital and outer clinics. The results had shown that from 284 viral infection there were, rubella consist 20.6%, Herpes simplex I 22.9%, Herpes simplex II 55.4%, Cytomegalovirus 33.1%, Human papilloma virus 15.4%, Adeno-associated virus 10.8%, Epstein-Barr virus 4%.

Also the infection with viruses revealed that it is higher in azoospermic+oligospermic patients than normospermic group (control) as shown in table 2 and figure2. We revealed in this study that azoospermic and oligospermic patients formed the highest ratio of the viral infections. there is 88.9% rubella infection, 90% Herpes simplex I, 83.5% Herpes simplex II, 76.2% Cytomegalovirus, 77.7% Human papilloma virus, 100% Adeno-associated virus, 85.7% Epstein-Barr virus in azoospermic and oligospermic compare with normospermic patients which are 11.1%, 10%, 16.5, 13.8%, 22.3%, 0%, 14.3% respectively.

Conclusions: using sensitive procedures for assays, we detected an unexpectedly high prevalence of sexually transmitted pathogens in semen from asymptomatic infertility patients.

Introduction

Male infertility accounts for 20-50% infertile couples and is often associated with genital infections **[1,2]**. Some effects on reproductive function have been proposed for such viruses as the human immunodeficiency virus, human papillomavirus, herpes simplex virus, Epstein-Barr virus and Cytomegalovirus**[3]**. Herpes virus and adeno-associated viruses have been linked to OAT without significant leukospermia **[4,5]**.

Serologic surveys indicate that the prevalence of infection with herpes simplex virus (HSV) type 2 (HSV-2) among adults approaches 25 percent in the United States and ranges from 4 to 18 percent in western Europe.[6] In most studies, only 10 to 25 percent of subjects with HSV-2 infection report a history of genital lesions. [7] Historically, it has been assumed that persons with asymptomatic HSV infections have less frequent and less severe reactivations than those with symptomatic disease. However, two lines of evidence suggest this may not be true. First, many HSV-2–seropositive subjects who initially report having no history of genital lesions do, after an educational session with a clinician, subsequently report having such lesions. Such subjects most likely have unrecognized symptomatic infection. Second, most HSV-2 infections are acquired from a person with no history of genital herpes infection. These data suggest that viral shedding in seropositive subjects may be frequent, regardless of the presence or absence of a reported history of genital herpes. The viruses present in human semen and their consequences are HIV, CMV, HBV, HTLV-1, HIV, HBV, HSV, Papillomavirus Adenovirus [6].

Human T-lymphotropic virus type I. HTLV-I, like HIV, is a retrovirus that infects T cells. It is epidemiologically linked to adult T-cell leukemia-lymphoma. HTLV-I is sexually transmitted by semen **[8,9]**, most probably via contaminated lymphocytes in the semen. Human herpesvirus 8. Kaposi's sarcoma (KS) is frequently associated with AIDS and occurs mainly in homosexual men. The epidemiology of KS in HIV-infected patients suggests that it may be caused by a sexually transmitted infectious agent **[10]**.

This agent has recently been identified as a new human herpesvirus called human herpesvirus 8 (HHV-8) or KS-associated herpesvirus. The nature of the infected cells in semen and the origin of the virus also remain to be determined. **Cytomegalovirus**. CMV also belongs to the Herpesviridae family. It is very common, with 50% of the otherwise healthy population being infected. Infection is spread by intimate contact with infected body fluids including semen, and 40% of the semen from healthy donors is infected. This virus was previously erroneously reported to be cold labile, whereas it in fact survives in frozen and thawed semen. CMV is thought to be a possible causative agent of hematospermia **[9]**. The virus generally remains in a

latent form and causes a lifelong infection, but it may be activated either by a primary infection, for example after organ transplantation, or by the impairment of cellular immunity. Prospective studies in the United States have demonstrated that CMV is responsible for more prenatal and perinatal virus infections than is any other transmissible agent identified to date **[11]**.

Sexually transmitted infections (STIs) are of major concern to clinicians and researchers in the field of reproductive medicine. Many STI pathogens cause incurable, often fatal diseases, and have been transmitted through insemination procedures **[12]**. Furthermore, several of these pathogens can be transmitted from infected mothers to the fetus or newborn **[13]**. Men can harbor subclinical infections in the genital tract over extended periods of time. Several viruses, including cytomegalovirus (CMV), Epstein-Barr virus (EBV), human papillomavirus (HPV), hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex virus type 2 (HSV-2), human herpes virus type 6 (HHV-6), and human immunodeficiency virus type 1 (HIV-1), as well as the intracellular bacterium, Chlamydia trachomatis (CT), have been detected in semen from asymptomatic men **[14]**.

Patients and Methods:

One hundred and seventy five seminal fluid and serum specimens from men investigated for infertility over a period of 1 year from February 2011 to February 2012 were analyzed. These seminal fluids of patients submitted to the laboratory from the fertility clinics of Babylon maternity and children Hospital and private clinics. The specimen was collected by patients themselves into sterile cubs. The subjects were instructed on how to collect the specimens and submit to the laboratory within one hour of production. The semen was collected after the patient had abstained from coitus for at least three days for the detection of Azoospermia and Oligospermia.

Serum specimens was used in ELISA technique for the detection of Rubella, Herpes simplex I, Herpes simplex II, Cytomegalovirus, Human papilloma virus, Adeno-associated virus, Epstein-Barr virus.

The sperm density, volume, viscosity (liquefaction), the percentage of actively motile sperms, the percentage of abnormal forms, the presence or absence of pus cells were assessed. The semen was analyzed on two different occasions at eight weeks interval for those semen specimens which gave abnormal results. The average of the two readings was calculated. Analysis was carried out immediately they were received.

Results and Discussion:

Sexually transmitted infections (STIs) are of major concern to clinicians and researchers in the field of reproductive medicine. Many STI pathogens cause

incurable, often fatal diseases, and have been transmitted through insemination procedures **[15].** Furthermore, several of these pathogens can be transmitted from infected mothers to the fetus or newborn **[16].** Men can harbor subclinical infections in the genital tract over extended periods of time. Several viruses, including cytomegalovirus (CMV), Epstein-Barr virus (EBV), human papillomavirus (HPV), hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex virus type 2 (HSV-2), human herpes virus type 6 (HHV-6), and human immunodeficiency virus type 1 (HIV-1), as well as the intracellular bacterium, Chlamydia trachomatis (CT), have been detected in semen from asymptomatic men **[17].** The importance of genital tract microorganisms as an etiologic factor in male infertility is still a controversial topic. The purpose of this study was to determine the prevalence of several common sexually transmitted pathogens in healthy, male, infertility patients **[18]**

As shown in table 1 and figure 1, rubella consist 20.6%, Herpes simplex I 22.9%, Herpes simplex II 55.4%, Cytomegalovirus 33.1%, Human papilloma virus 15.4%, Adeno-associated virus 10.8%, Epstein-Barr virus 4%. The results in our study was higher than Guntram Bezold et al study (CMV 8.7%, HPV 4.5%, HHV-6 3.7%, HSV 3.7%, CT 2.5%, EBV 0.4%, and HBV 0%) **[16,18].** This difference may belong to the type of procedure that used in detection process. In this study we used ELISA technique while in Bezold et al study PCR was used.

Type of Causes	No.	%
Rubella	36	12.67606
Herpes simplex I	40	14.08451
Herpes simplex II	97	34.15493
Cytomegalovirus	58	20.42254
Human papilloma virus	27	9.507042
Adeno-associated virus	19	6.690141
Epstein-Barr virus	7	2.464789
Total	2	84

Table 1- Types of Viral infection associated with Y chromosome microdeletions patients and control.

For this study, we chose 7 common STI viruses that can chronically infect the male genital tract, often without causing symptoms. Two other common sexually transmitted pathogens were not included in the study: HIV-1 was not studied because it is a retrovirus and requires a different processing and assay system; Neiserria gonorrhea was not studied because its infection is usually symptomatic in men.

In table 2 and figure2, the results of viral infection compare between azoospermia, oligospermia and normospermia. We revealed in this study that azoospermic and oligospermic patients formed the highest ratio of the viral infections. there is 88.9% rubella infection, 90% Herpes simplex I, 83.5% Herpes simplex II, 76.2% Cytomegalovirus, 77.7% Human papilloma virus, 100% Adeno-associated virus, 85.7% Epstein-Barr virus in azoospermic and oligospermic compare with normospermic patients which are 11.1%, 10%, 16.5, 13.8%, 22.3%, 0%, 14.3% respectively.

Causes	Azoos	Azoospermia		Oligospermia		Normospermia		Total	
	No.	%	No.	%	No.	%	No.	%	
Rubella	15	41.7	17	47.2	4	11.1	36	100	
Herpes simplex I	22	55	14	35	4	10	40	100	
Herpes simplex II	42	43.3	39	40.2	16	16.5	97	100	
Cytomegalovirus	22	37.9	28	48.3	8	13.8	58	100	
Human papilloma virus	13	48.1	8	29.6	6	22.3	27	100	
Adeno-associated virus	1	5.3	18	94.7	0	0	19	100	
Epstein-Barr virus	5	71.4	1	14.3	1	14.3	7	100	
Total	12	20	1	25	3	9	284	100	

Table 2- Types of Viral infection associated with azoospermia and oligospermiapatients.

CMV is a member of the herpesvirus family and can cause a variety of teratogenic effects in newborns as well as a clinical illness in adults resembling infectious mononucleosis **[19]**. Its presence and persistence in semen has been reported previously **[20]**. In the current study, CMV was the most frequently detected pathogen in semen of infertility patients (8.7%) with copy numbers ranging from 110 to 12 million. Detection of a low level of CMV in serum from one of the CMV-seronegative commercial serum donors could reflect a recent infection in this individual. We did not observe a significant association of CMV DNA with semen parameters in this study, although there was a trend for lower motile sperm and α -glucosidase concentrations in the CMV-infected group, indicating that CMV infection could have a modest effect on semen quality, perhaps by affecting epididymal function. Previous studies have not shown an association of CMV infection with a reduction in semen parameters **[21]**.

Herpes simplex virus mainly affects epithelial surfaces and is a major cause of genital ulcers. Whereas primary infection usually occurs through direct or indirect contact with herpetic lesions, HSV-1 and -2 have been detected in semen and on sperm, and transmission of HSV-2 has occurred through donor insemination [20].

However, HSV was associated with the strongest effect of any of the pathogens on semen parameters. HSV positive samples had significantly reduced sperm

concentration, sperm motility, motile sperm concentration, total motile sperm count, neutral α -glucosidase and citrate concentrations. This is consistent with earlier studies that found associations between HSV-1 or -2 in semen and low sperm count and poor motility, as well as infertility **[19]**.

Thus, HSV-infection of the male genital tract could explain some cases of male infertility, due to its association with decreased semen quality. Our study showed decreased concentrations of neutral α -glucosidase and citrate in HSV-positive samples, suggesting that the effect on fertility may be due to impaired epididymal and prostate function. Human papillomaviruses represent a group of small DNA viruses that induce epithelial cell proliferation. More than 35 types of HPV infect the genital tract. DNA from HPV 16, 18, 31 and 45 have been associated with invasive squamous cell cancers of the genital tract and anus **[20,22]**.

HPV is primarily transmitted through direct epithelial contact , but high risk HPV types have been detected in both semen and spermatozoa , as well as in the vas deferens . In the current report, 27 out of the 175 cases that tested positive for HPV were.

HPV was associated with a significant decrease in total sperm count, and a statistically non-significant trend for lower total motile sperm count and neutral α - glucosidase concentrations. A previous study reported an association between HPV



and reduced sperm motility **[23]**, while another study did not find any effects on sperm quality. Thus, additional research is needed to determine whether HPV infection contributes to male infertility. CT infects the genital tract and eye, and has been associated with conjunctivitis and pneumonia in infants infected via the birth canal **[24]**. Our study found CT in serum from 175 cases there are 58 cases and 50 cases positive of infertility patients, with copy numbers ranging from 8,300 to 590,000. This prevalence rate is consistent with earlier publications **[23,20]**.

CT DNA was associated with a significant decrease in neutral α -glucosidase concentration (and a trend towards lower total sperm and total motile sperm counts). Other studies have shown that men with CT in semen have reduced sperm concentration, motility, velocity, viability, morphology, acrosome reaction and citrate **[25]**. Spermatozoa incubated with elementary bodies of CT had decreased motility and increased cytotoxicity (81); these effects have been attributed to lipopolysaccharide **[26]**. Thus, CT infection of the male genital tract may contribute to male infertility. EBV, a member of the herpesvirus family, causes infectious mononucleosis and has been associated with Burkitt's Lymphoma **[27]**.

EBV is found in semen and is thought to be sexually transmitted **[28].** In the current study, EBV was detected in only seven samples. The low detection rate probably reflects the low prevalence of EBV infection in this country, and the fact that the virus' primary host cell, the B-lymphocyte, represents only a small minority of all WBC in semen. Previous studies have reported a higher prevalence of EBV in infertility patients in Europe, although no association of seminal EBV with reduced semen quality has been found **[25,28]**.



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دراسة الاصابة الفيروسيه المشتركه مع الذكور العقيمين في مدينه الحله- العراق

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الخلاصه

الهدف: للتحري عن الاصابات الفيروسيه المشتركه في الجهاز التناسلي الذكري للذكور العقيمين وعلاقتها مع حالات العقم في الذكور.

طرق العمل: تقنية الاليزا استخدمت لتشخيص ١٧٥ عينه مصل وسائل منوي ، جمعت على طول فترة ١٢ شهرا من الذكور المشخصين والمصابين بالعقم.

النتائج: ١٧٥ عينه سائل منوي ومصل استحصلت من الذكور المصابين بحالات عقم بالاضافه الى مجموعة السيطره خلال فترة سنه من شباط ٢٠١١ الى شباط ٢٠١٢. عينات السائل المنوي والمصل اخذت وشخصت في مختبرات مستشفى الولاده والاطفال-وحدة العقم ومن العيادات الخارجيه. اظهرت النتائج ان Herpes simplex I فيروسيه فان اصابات فيروس rubella مكلت ٢٠٠٦% ، المعادات الخارجيه. Human ، ٢٢٠٩ ، ٢٠٩٦ عند العابات فيروس المعادات العام من العيادات الخارجيه. اظهرت النتائج ان ويت بين ٢٨٤ اصابة فيروسيه فان اصابات فيروس المعادات العقم ومن العيادات الخارجيه. ويت بين ٢٠٢٩ اصابة فيروسيه فان اصابات فيروس عليه العقم ومن العيادات العارجيه. ويت بين ٢٠٩٢ اصابة فيروسيه فان اصابات فيروس المعاد العقم ومن العيادات العادي العاد من بين ٢٠٩٢ اصابة فيروسيه فان اصابات فيروس المعاد العقم ومن العيادات العادي العادي العادي ويت العادي معند العاد العادي العادي العادي العادي العادي العادي العادي العادي المعادي المادي العادي ويت العادي ال

اضافة الى ذلك فان الاصابات الفيروسيه اظهرت بان اعلى الاصابات كانت بين الذكور المصابين بقلة النطف وانعدام النطف اكثر من مجموعة السيطره اي الذكور الغير عقيمين كما هو موضح في جدول٢ وشكل ٢. اظهرت نتائجنا في هذا البحث ان الاصابات تالقيروسيه كانت اكبر في الذكور الغير عقيمين حيث شكلت هذه النسبه ٨٨.٩% في حالات الاصابه ب ٩٩، rubella ، ٩٩% في حالات ا ٢٨٨% في حالات الاصابه ب ٥.٣٠% في حالات الاصابه ب ٩٠٠٢ ، ٢٠٢٣ ، ٩٠٠% في حالات ٢ ٥.٣٠% في حالات الاصابه ب ٩٠٠٢ ، ٢٠٠% في حالات ٢ ٥.٣٠% في حالات الاصابي بقلة النطف وانعدام المطف مقارنة بذكور السيطره ٢٠٠%، ١٠. ، ٥.٣٠% ، ٢٢.٣% ، ٣٠.٠% ، ١٤.٠% بالتتابع.

الاستنتاجات: باستخدام تقنيات حساسه للتشخيص ، تمكنا من تحديد نسب عاليه لانتشار الاصابات الفيروسيه المساهمه الامراض المنتقله جنسيا في السائل المنوي للذكور العقيمين.