

Positive Serological markers associated with Infertility. Across sectional study on 50 couples

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Abstract

Background

The current prevalence of medical contact infertility varies from (3.6%-17%) of other infertility causes.(Shmidt et al: 1995).Natural infection by a variety of organisms have been linked to pregnancy loss and infertility, including Toxoplasma gondii, Viruses such as Rubella, Cytomegalo virus and Herpes simplex virus(William et al: 1998).

Patients and Methods: A cross sectional study was carried out during the first 6 months of 2009 for 50 infertile couples, the sample members were assessed clinically and investigated for their infertility state. A blood sample was collected for serological detection of specific Toxoplasma, Rubella, Cytomegalo virus and Herpes simplex virus IgG and IgM anti bodies .The results were statistically analyzed against the age (in years),the type, the cause, the duration of infertility and the history of Hepatitis in each.

Results: Toxoplasmosis found among 3 wives (3.6%) and one (2%) husbands, but was shared in one couple only out of 50 couples of the sample (2%).Rubella virus was found in 18 (36%) of wives and 19 (38%) husbands also shared in 16 couples (32%) of the sample. Cytomegalo virus found to be positive in18 wives (36%) and in 16(32%) husbands, shared in 14 couples (28%) of the sample while Herpes simplex virus was positive in 6 wives (12%) and 5(10%) husbands but shared in4 couples(8%). The eldest wives and the youngest husbands had higher association with the positive (TORCH) serological markers (100%), (66.7%) respectively, also the primary infertile wives and the secondary infertile husbands shared high association with positive serological markers, (59.1%). and both wives and husbands with duration of infertility (2-5) years had higher association with positive markers (76%) and (57.7%) respectively .The history of previous Hepatitis in both wives and husbands had similar association (100%) with positive serological markers, and all the results were statistically non significant.

Conclusion: The positive serological findings in infertile couples are important factors to assess, that can high light to the fact that infertility has a multiple correlating factors including serological markers and Medical causes, enhancing to arrange more researches in future with increasing sample size and variables.

Key words

Infertility, TORCH test for Toxoplasma gondii, Rubella virus, Cytomegalo virus, and Herpes simplex virus.

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Introduction

The current prevalence of infertility among women in the fertility age group varies from 3.6% to 14.35, the life time prevalence varies from 12% to 32%. The prevalence of voluntary infecundity from 2.6% to 5.9% and medical contact infertility from 3.6% to 17% (Shmidt et al:1995). Natural infections by a variety of organisms have been linked to pregnancy loss including Mycoplasma (M.hominis), Ureaplasma (Urealyticum), group B. Beta-hemolytic streptococci, Chlamydia, Treponema pallidum, Toxoplasmosis and viruses such as Rubella, Cytomegalo virus, Herpes simplex and Coxsacki virus (William et al:1998). So serological investigations for the commonest microbiological organisms related to infertility are recommended for assessment of infertile couples and of obstetric and gynecology (RCOG) 2004 (National Collaborating center-2004)., such as Toxoplasma gondii, Rubella, Cytomegalo virus and Herpes simplex type 2 virus (TORCH) test (Home rapid diagnostic tests-2009). Toxoplasma gondii is a protozoan parasite cause toxoplasmosis which is a zoonotic disease that can infect all mammals and birds species throughout the world approximately one-third of humans has been exposed to parasite worldwide (Sukthana : 2006)., it is an intra cellular parasite that may cause abortion or congenital disease in its intermediate hosts ,but infection in human is generally asymptomatic except in congenitally infected children and immunosuppressed individuals (Konstantinos et al:2009). Toxoplasmosis infection in human may occur vertically by tachyzoites that are passed to the fetus via the placenta ,or horizontal transmission which may involve 3 life cycle stages ,i.e. ingesting sporulated oocytes from cats or ingesting tissue cysts in raw or undercooked meat or tachyzoites in blood products or viscera of many different animals ,tissue transplants or unpasteurized milk (Innes : 1998). Serious disease can be found in immunocompromised patients, the fetus is only at risk of congenital disease when acute infection occurs during pregnancy , although congenital infection has also been reported from a chronically infected immunocompromised mother with a reactivation of toxoplasmosis leading to abortion and fetal abnormalities (Nahed et al:2009). In males as it was concluded by many previous researches Toxoplasmosis can affect men's infertility and cause sterility, a pathological changes found in testes, epididymus, vasdeference, prostate and thalamus with a significant decrease in sperm motility and concentration and increase in sperm abnormalities of male mice that was documented by Konstantin

L,etal,during 2009(Nahed et al:2009). Toxoplasmosis can induce encephalopathies and meningoencephalitis in central nervous system infection giving rise to more severe damage especially in immuonosupressed although not rare in others with primary infection resulting in infertility due to dysfunction of Hypothalamus-Pituitary – Gonadal axis in both genders(Shibata : 1990),(Zhang et al: 2002).Toxoplasmosis can cause severe damage in congenitally acquired infected fetus due to primary infection of pregnant women so it is important to diagnose the timing of infection that require a highly sensitive and quantitative assay for IgG and IgM antibodies so presence of toxoplasma specific IgM antibodies in the serum is an indicators of recent infection and a high avidity of IgG excludes recent infection within the last 4 months , if it is found during 1st trimester of pregnancy (the risk time for fetus) it will exclude an acute infection during pregnancy(Home rapid diagnostic tests: 2009).Rubella is endemic in countries without Universal Vaccination Policies can affect 80%-85 and of young adults as having past infection but as a subclinical manifestations found in most cases of child hood and Sir Norman Gregg recognized in 1941 the strong association between Rubella infection in early pregnancy and the congenital anomalies of the fetus (W.T.A.Toood et al:2006).Rubella characterized by mild self – limiting disease associated with characteristic rash ,when occur in 1st trimester of pregnancy fetal infection rates are near 80% dropping to 25% in the late 2nd trimester and increasing again in the 3rd trimester from 35% at(27-30)weeks of gestation to nearly 100% beyond 36 weeks gestation(Gabbe et al: 2002)There transplacental vertical infection by the rubella virus can have catastrophic effects on the developing fetus resulting in spontaneous abortion ,fetal infection,still birth or fetal growth restriction (Reef et al : 2002) therefore women who are concerned about their fertility should be offered Rubella secreening and Vaccination and advice her to not become pregnant for at least one month following vaccination (National Collaborating center: 2004), also IgG and IgMtests may be ordered for a person or pregnant who has symptoms of rubella infection (Home rapid diagnostic tests: 2009). therefore positive serological finding of rubella specific IgM anti bodies is indicative of acute infection and a four fold rise in rubella IgG antibody titer between acute and convalescent serum specimen ; best performed within 7-10 days after the onset of the rash and should be repeated 2-3 weeks later, also the virus can be found in cultures drawn from nasal , blood,throat, urine or cerebrospinal fluid(Lorraine Dontigny et al: 2008).

Cytomegalo virus (CMV) ; is found throughout all geographic locations and socioeconomic groups and infects between 50% and 80% of adults in United States by 40 years of age .This virus is more widespread in developing countries and in area of lower socioeconomic conditions with symptoms of prolonged fever and mild hepatitis , once the person becomes infected , the virus remains alive but usually dormant within that person's body for life .The virus infection is higher among fetus in utero and people who work with children or in close contact with a person excreting the virus in their saliva, urine, breast milk or other body fluids ,the transmission rate to the fetus is between 24% -75% according to the organization of Tetrology Information Service(OTIS) with an average transmission rate of 40% from women who have been infected at least 6 months prior to conception (Guidelines: 2000). The rate of new born infection is 1% (American pregnancy association:2010). Human (CMV) is often detected at the uterine-placental interface impairing cytotrophoblast differentiation and invasion , infected cytotrophoblast express CMV IL-10 which up regulate human IL-10 and decreases matrix metalloproteinase 9 activity an enzyme which degrades the extra cellular matrix and increased the depth

of invasion(Yammamoto et al:2004) the diminished degradation of extra cellular matrix could contribute to the shallow invasion of the uterus and restriction of fetal growth in pregnancies affected by CMV (Fisher et al:2000), moreover it is found By several studies that single intra peritoneal immunization with recombinant immune CMV induce permanent infertility with no systemic illness in female BALB/c mice due to induction of ovarian auto immune pathology leading to progressive oocyte depletion and eventual ovulation failure associated with recruitment of inflammatory leukocytes predominantly CD 4 and CD 8 T cells evident from the immunization (Senn et al: 2008).But CMV presence in semen was not significantly related to semen quality including sperm functional capacity,local Anti Sperm Anti Body or seminal WBC also CMV in endo cervical material was not associated with reduced quality of the cervical mucus or with other female infertility factors (Waltraud Eggert et al:2008).Laboratory techniques are the sole means of diagnosing acute infections, however the established of primary infection as opposed to reactivation, chronic infection diagnosed by sensitive and qualitative CMV IgM, with highly specific and quantitative IgG,so positive IgM and low avidity IgG are suggestive of primary infection wheres high IgG indicates IgM presence due to persistency or reactivation ,in addition low IgM responses in primary infection, the IgM test will point to the need of IgG avidity determination to overrule primary infection (Home rapid diagnostic tests:2009).

Herpes simplex virus (HSV):types 1 and 2 of this virus affects human ,Type 1 HSVproduces mucocutaneous lesions ,predominantly of the head and neck, while type 2 is a sexually transmitted anogenital infection .By puberty 30%-100% of United Kingdom adults will have antibodies to HSV depending on socio economic status(W.T.A.Toood et al: 2006).The source of infection is a case of primary active recurrent disease, primary infection normally occurs as a gingivo stomatitis in infancy and may be subclinical or mistaken for teething , it may present as keratitis (dendritic ulcers),Viral paronychia,Valvovaginitis,Cervicitis, and rarely encephalitis .Recurrent disease involving reactivation of HSV from latency in dorsal root ganglia produces the classical (cold sore) or herpes labials, ,prodromal hyperesthesia is followed by rapid vesciculation postulation and crusting.Type 2(genital disease)is a common cause of recurrent painful genital ulcerations .Neonatal HSV disease (from birth canal) is disseminated and is potentially fatal .Active HSV in preterm mother is an indication for elective cessarian section (Gabbe et al:2002).HSVwas significantly related to low sperm count and poor motility in contrast to CMV(Nikiferos Kapranos et al:2003) also the HSV positive infertile patients was two time more frequent oligozoospermia than HSV negative one in addition to a damage of the spermatozoa structures as microhead(consequence of the defect in acrosome or reduced genome) and cytoplasm drops on the neck(an immature forms) occurred more often in HSV infection patients than others (Abdulhamed Zhidova et al:2007).

A highly specific and sensitive screening tool for determination of IgG antibodies to HSV 1 and HSV 2 as well as a complementary test for detection of IgG and IgM have been developed to decrease viral transmission and to avoid life threatening consequences in new bourns were Herpes simplex viral infection is 10 times greater for women with primary infection In the last trimester of pregnancy (Home rapid diagnostic tests: 2009).

This study was done:

1. To assess the positive serological markers among infertile wives and husbands .
2. To find the association of positive serological results with other infertility variables

Patients and Methods

This cross sectional study was carried out during first 6 months of 2009 to assess 50 infertile couples sera for presence of specific *Toxoplasma gondii*, Rubella, Cytomegalo virus and Herpes simplex type 2 anti bodies or (TORCH) TEST. and their association with other infertility variables in the sample.

The age distribution was from < 30 years to >40 years

The evaluation criteria was inability to conceive for one year or more during unprotected intercourse ,all wives and husbands were evaluated according to standard protocols that included thorough history and physical examination , hormonal assessment for follicle stimulating hormone(FSH), Leutinizing hormone(LH),Prolactin and Progesterone hormones,Testosterone hormone and Antiphospholipid Antibody test for wives with hysterosalpingography followed by laparoscopy , hysteroscopy if indicated to exclude anatomical abnormalities in wives .For the husbands , seminal fluid analysis was done to evaluate the fertility state.

The unexplained cause of infertility was diagnosed according to negative results of all the above investigations in both husbands and wives.

A blood sample equal to 5cc was collected by vein puncture from each member without anti coagulant ,after agglutination ,centrifugation by 2500 round/min.m for 10 min. separation of the serum , every 50ml mixed with tip wait kit for half hour by using Enzyme linked immuno sorbent (ELIZA) technique , the results appear automatically for specific IgM and IgG anti bodies (European Biochek kit for *Toxoplasma* ,German human kit for CMV, A European Biochek kit for Rubella and American Cal Biochek for HSV.)

The results were statically analyzed by computer aids using SPSS Ver 13 frequency distribution for selective variables , the statistical significance of differences in mean of a qualitative normal distribution variables between two groups was assessed by Chi-square , the prevalence ratio was used to assess the magnitude of association between two categorical variables, the 95%CI(confidence interval) of prevalence rates was calculated by logarithm method ,the 95% CI of the sample properties was calculated by using the exact

Binominal distribution method ,P value< 0.05 was considered statistically significant .(Abdulhamed Zhidova et al: 2007).The selective infertility variables were the age in years, the type of infertility,(primary or secondary),the cause of infertility(known or unknown),the duration of infertility and the positive history of Hepatitis.

Results

In table 1: The youngest wives and husband constituted 46% , 24% of the sample respectively . The husbands between (30-39) years were 44%. While the eldest wives were only 12%, the secondary infertile wives were more 56% than those with primary infertility 44% the same was found in secondary infertile husbands 58% and those with primary infertility 42%. Table 2 revealed that the duration of infertility (1-20) years mean 4.7 found to be more among wives than husbands (1-17) years mean 4.3. In table 3: Toxoplasmosis was found among 6% of wives and 2% of husbands and shared by both members of the couple in 2%. Rubella positive serological markers found in 36% of the wives and in 38% of husbands but shared in 32% of the same couple members. Cytomegalo virus infection was positive in 36% of the infertile wives and 32% husbands but 28% of the studied couples had the

same infection in both members. Herpes simplex type 2 was positive in 12% of wives and 10% of husbands and shared by both members of the same couple in 8% of the sample. The positive serological (TORCH) test markers as a whole found in 64% of the wives, 54% husbands and shared by both members in 52% of the couples. The association between positive TORCH test results and other infertility variables in wives were shown by

Table 4. The eldest wives (>40 years) had highest association with positive serological (TORCH) markers (100%) than other age groups studied in the sample (<30 years), (30-39 years) who had 60.9% and 57.1% respectively. The primary infertile wives showed higher association (68.2%) than those with secondary infertility (60.7%). The unexplained infertility in wives had higher association (64.3%) than the explained type (63.9%). The duration of infertility from 2-5 years had highest association (76%) than one year duration (76%) and the duration from 6 and more (50%). History of Hepatitis infection had higher association (100%) in infertile wives than others (61.7%). Although all the results were statistically not significant. Table 5 described the association of positive serological TORCH test markers in husbands studied in the sample with their infertility variables. The youngest age group (<30 years) had highest association (66.7%) than the eldest group (40 years and more) (56.3%) and than the group of (30-39 years) (45.5%).

The secondary infertile husbands were highly associated (58.6%) and those with primary infertility had (47.6%). The husbands with a diagnosed infertility cause showed higher association (59.15%) than the unexplained type (50%). The duration of infertility (2-5) years was highly associated (57.7%), the one year duration (57.1%) and the longest duration had lowest association (47.1%). The history of positive Hepatitis infection had higher association (100%) than others (53.1%). But all the results were statistically not significant.

Table 1 : The distribution of the sample according to the age groups and type of infertility

	Wife		Husband	
(n=50)	N	%	N	%
Age group of (years)				•
<30	23	46	12	24
30.39	21	42	22	44
40+	6	12	16	32
Type of infertility				
Primary	22	44	21	42
Secondary	28	56	29	58

Table 2 : The duration of infertility of wives and husbands in the studied sample

	Range'	Mean	SD
Duration of infertility (years) - wife	(1 to 20)	4.7	3.9
Duration of infertility (years) - Husband	(1 to 17)	4.3	24

Table 3 : The proportions of positive serological markers in the studied sample

Positive serological markers	Wife			Husband				<i>Both members of couple positive</i>		
	N	%	95% confidence interval for proportion	N	%	95% confidence interval for proportion	McNemar Test	N	%	95% confidence interval for proportion
Toxoplasmosis	3	6	(1.6-17.5)	1	2	(0.1-12.0)	0.5(NS)	1	2	(0.1-12) .
Rubella	18	36	(23.3-50.9)	19	38	(25.0-52.8)	1(NS)	16	32	(19.9-46.8)
Cytomegalovirus	18	36	(23.3-50.9)	16	32	(19.9-46.8)	0.69(NS)	14	28	(16.7-42.7)
Herpes simplex virus	6	12	(5.9-25.0)	5	10	(3.6-22.6)	KNS)	4	8	(2.6-20.1)
TORCH	32	64	(49.1-76.7)	27	54	(39.5-67.9)	0.13(NS)	26	52	(37.6-66.1)

Table 5 : The association of positive TORCH screening test with the infertility variables of husbands in the studied sample

Husband	Total N	Positive N	TORCH Screening %	P(Chi-square)
Age group (years)				0.48(NS)
<30	12	8	66.7	
30-39	22	10	45.5	
40-	16	9	56.3	
Type of infertility				0.44(NS)
Primary	21	10	47.6	
Secondary	29	17	58.6	
Infertility attributed to a possible medical Condition				0.52(NS)
unexplained	28	14	50	
Explained	22	13	59.1	
Duration of infertility (years)				0.78(NS)
First year	7	4	57.1	
(2-5)	26	15	57.7	
6+	17	8	47.1	
Hepatitis				0.35(NS)
Negative	49	26	53.1	

Discussion

Toxoplasma gondii, Rubella, Cytomegalovirus and Herpes simplex virus (TORCH) test is a routine test for the infertile couples especially those with unobvious cause of infertility. Toxoplasma infection can cause severe damage in cases of congenitally acquired infection, serology is the only method to determine if the female has been infected by Toxoplasma gondii (recent or chronic infection). Rubella test is used to confirm the presence of adequate protection against the virus and to detect recent or past infection it can also be used for identification of those not exposed to viral infection or vaccinated (Home rapid diagnostic tests: 2009). Cytomegalovirus is the most common cause of viral intra uterine infection as infections are asymptomatic or accompanied by which are not specific for Cytomegalovirus, laboratory techniques are the sole mean of diagnosing acute infections (American pregnancy association: 2010). Herpes simplex virus type 2 is the main cause of genital herpes, since most individuals infected are asymptomatic, the serological evaluation of this infection can reduce viral transmission. (Abdulhamed Zhidova et al: 2007). By this study 3(6%) out of 50 infertile wives and only one infertile husband (2%) had Toxoplasmosis that was shared by both members in one couple (2%). These results are lower than what was found by Zhou Y H et al at 2002, (34.8%) Toxoplasmosis among infertile couples studied to explore

the relationship of this infection and infertility (Zhou et al:2002).Our result was also lower than the prevalence (20.4%) of Toxoplasma infection found among infertile wives involved in Nahed H.Choneim et al in their research during 2009,to detect Toxoplasma infection among high risk women(Nahed et al: 2009).These differences could be due to developing laboratory means and good health education in our society and or because of small sample size. But Rubella infection was positive in 18 infertile wives(36%) and in 19(38%) husbands and shared in 16 (32%) couples ,as Rubella is endemic in countries without universal vaccination policies, in non immunized countries 80%-85% of young adults have evidence of past infection (W.T.A.Toood et al: 2006).So it must be lower incidence in our country than others because of the Universal vaccination policies applied for last 35 years as documented by several global studies to assess the incidence of rubella infection that revealed a marked decline in after 1970 at a mean endemic rate of 4/100000 population /year ,this is an average 1000 cases reported /year range 237 to2450(Lorraine Dontigny et al:2008).Cytomegalo virus infection found among 18(36%) of infertile wives and in 16(32%) husbands and the infection was shared in 14(28%) of the infertile couples , that was lower than the percentage found among American infected adults (50% and 85%) in 2000 (Guidelines: 2000).Since this virus is mainly transmitted sexually and by body fluids , it seems to have less opportunity in our Islamic eastern society than in United states although it can be caused by transplanted organs or blood transfusion and among workers in contact with infected children in day care centers (Guidelines: 2000).Herpes simplex type 2 infection found among 6(12%) out of 50 examined wives and in 5(10%) husbands but was shared in 4(8%) coupled complained of infertility ,that was lower than the percentage found in 2007 (25%) among infertile males by Abdulhameed Zhedan A G, research for detection of Herpes simplex virus infection and the negative male infertility (Abdulhamed Zhidova et al: 2007), also lower than that was detected by Mikiferas Kapranos,M.D , et al (49%) in 2003 in their study for investigation of Herpes simplex virus in infertile males (Nikiferos Kapranos et al: 2003), also this sexually transmitted contagious virus can affect 30%-100% in U K adults (as anti Herpes simplex virus anti body found) depending on socio economic status (W.T.A.Toood et al: 2006). I think this is not applied in our country because of religious habits in general population and the hygienic care with health education and it could be lower in our society due to decreased registration process because of wrong diagnosis or low clinic attendance due to patients shameful feelings .Positive serological (TORCH) test markers found among 32(64%) of the infertile wives and in 27(54%) husbands and was shared in 26(52%) out of 50 couples ,although all the above results were statistically non significant , it was documented by many studies before that (TORCH) test was significantly higher in infertile couples than in others (Zhou et al:2002).The eldest infertile wives (40 years and more) had highest association with positive (TORCH) test as a whole 6(100%) while that found in youngest husbands (<30) years 8(66.7 and) and those between (30-39)years had lowest association 10(45.5) the same as in youngest wives 12(57.15%).These results were statistically non significant but was near to the age association found with Cytomegalo virus infection as 50% and 80% percent among adults in the United states by 40 years age (American pregnancy association: 2010),another study documented that highest association of Toxoplasma infection found among infertile wives in age (35-45) years(66.7%-62.5%) by Nahed H.Ghoein et al in 2009 (Nahed et al : 2009).The primary infertile wives had higher association with positive serological TORCH markers 15(68.2%) than those with secondary infertility 17(60.7%) while the secondary infertile husbands had higher

17(58.6%) association than the primary infertile members(47.6%) while other study in 2009 arranged by Nahed et al showed that there was high prevalence of Toxoplasma infections among the pregnant women (30.5% and 20.4%) than non pregnant or primary infertile women.(13.6% and 7.95%) (Nahed et al: 2009). This difference could be due to more social and familial care given to the primary infertile couples to achieve conception therefore increasing infertility attendance and managed more than others. The wives with unexplained infertility had higher association with positive TORCH results (64.3%) than others while husbands with well defined cause of infertility had higher association also(59.1%). That could be due to the strong association of Toxoplasmosis , Cytomegalo, and Herpes simplex viral infections with low sperm count and poor motility(well defined cause of infertility) (Konstantinos et al: 2009). (Nikiferos Kapranos et al: 2003), but Cytomegalo virus was highly associated with unexplained repeated miscarriage revealed by Wafaa M et al in 2006(Waafa M.et al-2006). The duration of infertility between (2-5) years had highest association(76%) in wives while the longer (6 years and more)had lowest association (50%), the same was found in husbands. This result could be due to a relation of long duration of infertility with more chance of exposure to infectious factors in spite of more time for investigations and or treatments. Both infertile wives and husbands showed a higher association between positive serological TORCH test markers and the history of Hepatitis infection(100%) than others, that may be due to the fact that a causative agents of Hepatitis were included in the test like (Cytomegalo virus, Rubella) in addition to other causative agents of hepatitis like Epstein Barr virus and Mumps that affect large number of population as one of the major type of diseases in the world , although all the results were statistically not significant,

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