Sero-Epidemiological Studies of Toxoplasmosis among Pregnant Women in Hail region Saudi Arabia

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Abstract: Toxoplasmosis is a zoonotic (cat born) disease caused by the *Toxoplasma gondii* parasite. This disease pass from animal to human by eating undercooked meat or food, drink contaminated water with oocysts, management of contaminated soil or eating vegetables and fruits directly without proper cleaning. Research has established that toxoplasmosis can be passed through the placenta during pregnancy causing a hazard of abortion or even death of the fetus inside the uterus. If infection takes place during the last trimester of pregnancy, the baby will be born with congenital toxoplasmosis. This study was regulated to verify the percentage of pregnant women affected with toxoplasmosis in Hail region. Serum samples of 318 pregnant women ranging in age from 20 to more than 31 years old were collected from patients at the women's hospital in Hail. Serum samples were separated and examined to evaluate toxoplasmosis antibodies by using four serological tests: (LAT- IHA – IFAT – ELISA IgM, IgG). The study results were obtained by applying serological tests (LAT, IHAT, IFAT, ELISA IgG, ELISA IgM) indicate that infection has reached (73.0%, 60.7%, 44.3%, 28.9%, 2.8%) correspondingly. In addition, it was set that the infection rate among women between 20 -30 years old has increased. The study indicated there was no Significance different among age groups for all the serological tests except for the tests (LAT, IHAT, ELISA IGM) and its value is (0.909, 0.483, 0.036) respectively. These results show that there is a significant percentage of pregnant women in Hail Region infected by the toxoplasmosis parasite, which expresses that women in the Kingdom are predisposed to this infection. So, raise awareness programs for pregnant women have to be drive everywhere in the Kingdom to inhibit primary infection that may lead to injurious complications for the unborn child.

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1. Introduction

Toxoplasmosis is a cat born disease caused by an obligate intracellular coccidium protozoan *Toxoplasma gondii* (Torda,2001), proved for the first time in 1908 in an African rodent(the gondii) ,and in 1909 the disease was distinguished from Leishmania and named *Toxoplasma gondii* (Ryan and Ray, 2003 and Sukthana,2006). The disease passed to human by eating of under cooked meat and oocysts passed in the feces of the infected felines (John, 1984 and Bailey and Scott`s,2007), or passed from mother to fetus (Torda,2001, Ryan and Ray,2003 and Sukthana,2006).greater than 500 million people allover the world are infected with *T. gondii*. In human, symptoms are variable. Most of cases are completely asymptomatic, when they appear differ from host to another (Ryan and Ray, 2003 and Rosemary Drisdelle,2007).

Acute form occurs during the first days of infection and makes flu like symptoms, headache, fever, lymphadenopathy, muscle pain, and lasts for month or more .So far, it gets more significant in pregnant woman mainly in first and second trimester and causes abortion, still birth (dead fetus in uterus), and congenital abnormalities (Garcia and David,1997 Torda, 2001, Ryan and Ray,2003 , Rosemary Drisdelle, 2007)

Chr12onic form occurs in people infected with *T. gondii* that encysted in nervous and muscular tissue regulated by host immune system, most people are asymptomatic (Roberts John, 2000&Torda, 2001)

Serological tests are available despite that they are not routinely offered by clinical laboratories due to high fee, lack of qualified technician, low number of test orders and problems with sensitivity and specificity and analysis slandered techniques that have been used include complement fixation CF, indirect hemagglutination IHA, indirect fluorescent antibody IFA, soluble antigen fluorescent antibody, latex agglutination LA, counter electrophoresis, enzyme-linked immunofluorescence assay ELISA, radioimmunoassay and intradermal tests (Roberts John,2000).

2. Materials and Methods

This study was applied in Hail region, Saudi Arabia from Feb 2011 to June 2011. The clinical diagnosis was carried out at obstetric and gynecological hospital in Hail region. The total number of women included in this study was 318 pregnant women, age ranged between (20 –30 years). These women were registered and had a feedback form to inform individual and epidemiological data as the name ,age, address and abortion parity and times.

Serum samples were taken from each woman, the serological tests were done with

LAT (Toxocell, Biokit),IHA (Cellognost-Toxoplasmosis H, SIEMENS) , IFAT(ToxoIgG IFA, VIRGO) – IFAT-ELISA IgM,IgG(ToxoIgG and IgM,UDI)

3. Results

This study was carried out from February2011 to June 2011. It includes 318 pregnant women, attending the women's hospital in Hail region. Out of 318 pregnant women (42.58%) women had antibodies against Toxoplasmosis.

The Toxoplasma latex agglutination test in 318 women showed the result of pregnant women with toxoplasmosis in this study was 232(72.96%) women table(1). The majority of women were 90(28.30%) women between (20 –25) years,80(28.30%) women between(26-30) years and 28(8.81) women over(31) year figure (1). By statistical analysis p >0.05 and its value was x^2=0.825 .f=.0181 (Table 6).

Regarding IHAT test in 318 women, 193(60.69%) women had antibodies against Toxoplasmosis figure (2) and table(2) .The majority of women 69(21.70%) werebetween (20–25) years, 68(21.38%) women were between(26-30)years and 25(7.86) women were over(31) year figure (3). By statistical analysis p>0.05 and its value was x^2=0.2.66 .f=0.821 table(6).

Regarding Toxoplasma antibody titer of IgM in 318 pregnant women, titer was9 (2.83%) case figure(3) table(3). The majority of women were (0.94%) women between (26 –30) years, and (0.94%) women over(31) year. By statistical analysis p>0.05 and its value was x^2=03.96 .f=782.8 table(6), while IgG titer was 48(15.09%)women between(20-25) years and 24(7.55%)women between(26-30)years and only 2(0.63%)women less than 20 years figure(4) table(4). P<0.05 and its value was x^2=60.32.

Regarding IFAT test in 318 women, 141(44.34%) women had antibodies against Toxoplasmosis figure(5)and table(5).The majority of women were 59(18.55%) women between (26-30) years, 45(14.15%) women between(20-25)years and 12(3.77%) women less than 20 years table (4). By statistical analysis p<0.05 and its value was x^2=22.18.f=9.56 table(6).

Table(1) Seropositivity of *Toxoplasmosis* by latex in relation to age.

TEST	RESULT	AGE GROUPS BY YEARS				SUM	X^2	ARRY
		<20 %	20 – 25 %	26 – 30 %	>31 %			
LATEX	-VE	14(4.40%)	35 (11%)	25 (7.86%)	12 (3.77%)	86 (27.04%)	0.901	0.825 P<0.05
	+VE	34(10.69%)	90(28.30%)	80(25.16%)	28 (8.81%)	232(72.96%)		
		48(15.09%)	125(39.30%)	105(33.02%)	40(12.58%)	318(100.0%)		

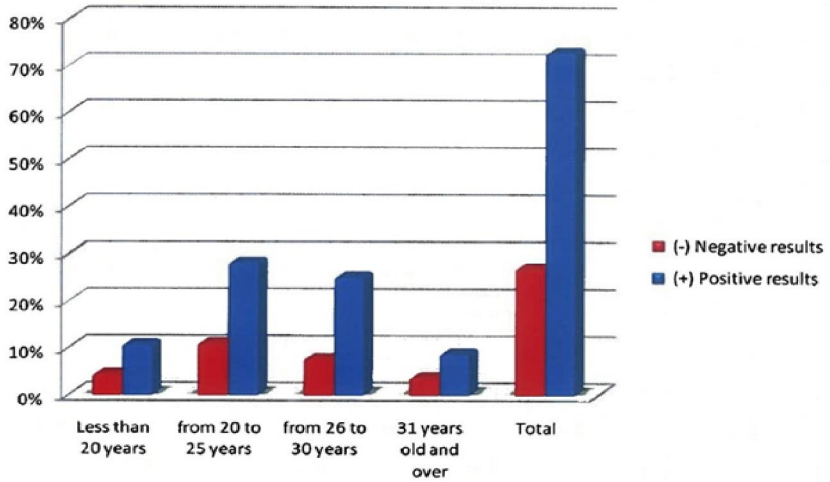
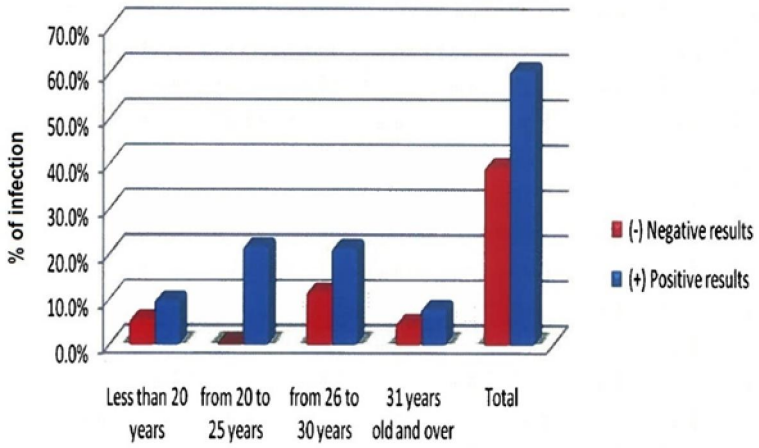


Figure (1): Seropositivity of Toxoplasmosis by latex in relation to age

Table(2) Seropositivity of *Toxoplasmosis* by IHAT in relation to age.

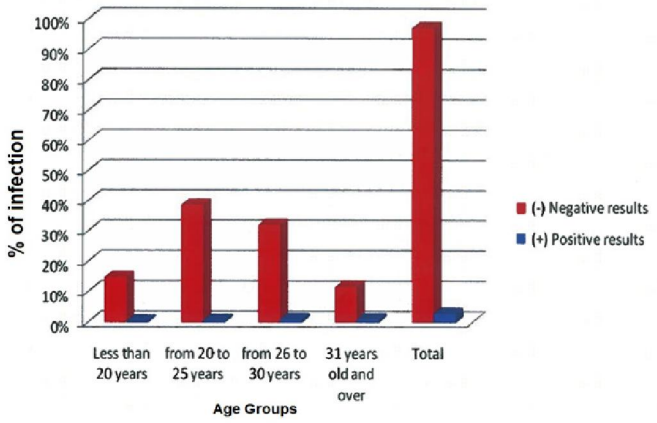
TEST	RESULT	AGE GROUPS BY YEARS				SUM	X^2	ARRY
		<20 %	20 – 25 %	26 – 30 %	>31 %			
IHAT	-VE	17(5.35%)	56(17.61%)	37(11.64%)	15(7.72%)	125(39.31%)	2.677	0.825 P>0.05
	+VE	31(9.75%)	69(21.70%)	68(21.38%)	25(7.86%)	193(60.69%)		
		48(15.09%)	125(39.31%)	105(33.02%)	40(12.58%)	318(100.0%)		



Fig(2) Seropositivity of Toxoplasmosis by IHAT in relation to age

Table(3)Seropositivity of Anti-Toxoplasma IgM in relation to age.

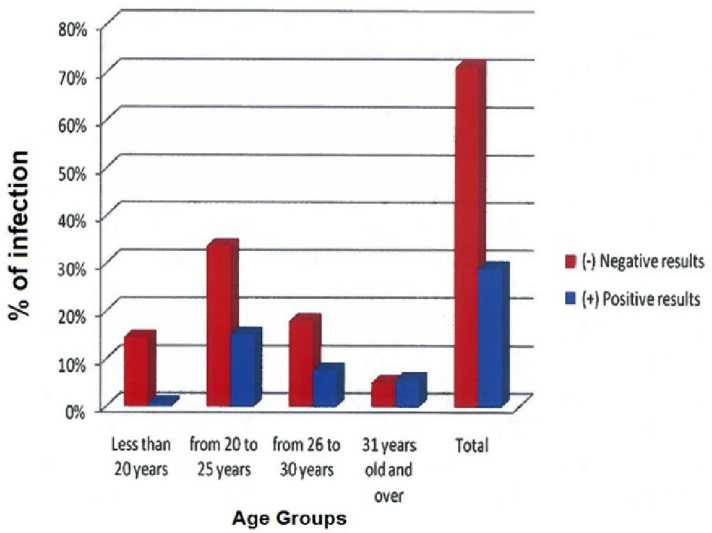
TEST	RESULT	AGE GROUPS BY YEARS				SUM	X^2	ARRY
		<20 %	20 – 25 %	26 – 30 %	>31 %			
ELISA IgM	-VE	47(14.78%)	123(38.68%)	102(32.08%)	37(11.64%)	309(97.19%)	3.96	0.27 P>0.05
	+VE	1(0.31%)	2(0.63%)	3(25.16%)	3(0.94%)	9(2.83%)		
		48(15.09%)	125(39.30%)	105(33.02%)	40(12.58%)	318(100.0%)		



Fig(3) Seropositivity of Anti-*Toxoplasma* IgM in relation to age.

Table(4) Seropositivity of Anti-*Toxoplasma* IgG in relation to age.

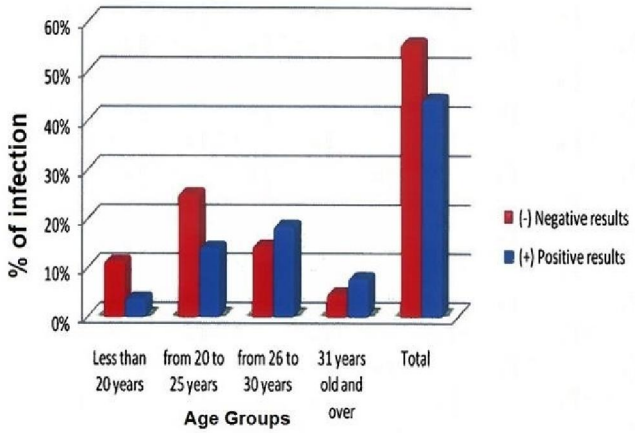
TEST	RESULT	AGE GROUPS BY YEARS				SUM	X^2	ARRY
		<20 %	20 – 25 %	26 – 30 %	>31 %			
ELISA IgG	-VE	46(14.47%)	107(33.65%)	57(17.92%)	16(5.03%)	226(71.07%)	60,32	0.0 P<0.05
	+VE	2(0.63%)	48(15.09%)	24(7.55%)	18(5.66%)	92(28.93%)		
		48(15.09%)	125(39.30%)	105(33.02%)	40(12.58%)	318(100.0%)		



Fig(4) Seropositivity of Anti-*Toxoplasma* IgG in relation to age.

Table(5)Seropositivity of *Toxoplasmosis* by IFAT in relation to age.

TEST	RESULT	AGE GROUPS BY YEARS				SUM	X^2	ARRY
		<20 %	20 – 25 %	26 – 30 %	>31 %			
IFAT	-VE	36(11.32%)	80(25,16%)	46(14.47%)	15(4.72%)	177(55.66%)	22.18	0.00 P<0.05
	+VE	12(3.77%)	45(14.25%)	59(18.55%)	25(7.86%)	141 44.34%)		
		48(15.09%)	125(39.30%)	105(33.02%)	40(12.58%)	318(100.0%)		



Fig(5) Seropositivity of *Toxoplasmosis by* IFAT in relation to age

Table(6) Analysis of variance results between the serological tests

Test	Source	Square sum	DF	Mean Square	F	Sig
LATEX	Between	0.107	3	0.36	0.181	0.909
	Within	61.7	314	0.197		
	Total	61.81	317	0.233		
IHAT	Between	0.589	3	0.196	0.821	0.483
	Within	75.06	314	0.239		
	Total	75.65	317	0.435		
IFAT	Between	6.596	3	2.199	9.625	0.00
	Within	72.2	314	0.23		
	Total	78.79	317	2.429		
ELISA IgG	Between	12.93	3	4.31	52.02	0.00
	Within	54.089	314	0.172		
	Total	67.019	317	4.842		
ELISA IgM	Between	0.158	3	0.053	2.878	0.036
	Within	5.729	314	0.018		
	Total	5.887	317	0.071		

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4. Discussion

Nearly about one third of world`s population are infected with Toxoplasmosis. Acute infection during pregnancy causes agrand maternal risks concerning to congenital toxoplasmosis (Jones. 2001 and Sensini, 2006). Serological tests are used for detecting *Toxoplasma gondii* antibodies, most cases with positive IgG titer can point to chronic infection with *Toxoplasma gondii*, while positive IgM indicate acute infection. Negative IgM titer may indicate too early infection that antibody response has not yet developed or isunnoticeable (Liesenfeld *et al.,* 1997) and Suzuki *et al.*(2001).

This study was approved in Hail region, Saudi Arabia to evaluate the prevalence of *T. gondii* infection. Most earlier studies in Saudi Arabia have focused on the prevalence of *Toxoplasma* infection among general population in the kingdom, likewise the present study .The seroprevalence of *Toxoplasmosis* obtained in this study among pregnant women in Hail was 42.58% which is comparable to results previously reported in Abha 31.6% (El Hady, 1991),39% (Hafeez,1992)in Al Madinh Al Monawarh and in Makkah 35.6% (Ghazi *et al*., 2002). A comparable seroprevalence result was obtained from healthy people in two rural areas in the Eastern Region 25% to 26.36% (Al-Qurash i*et al*., 2001; Al-Qurashi,2004), in Trinidad and Tobago 39.3% (Ramsewak *et al.,*2008) and in Qater 35.1% (Abu-madi *et al.,*2010).A higher seroprevalence 52.1% was reported in Asir (Al-Amari, 1994), 55% were reported in France (Ancelle *et al*., 1996). Elevated prevalence rates were recorded in other Arab countries as Kuwait 58.2% (Al-Nakib *et al*., 1983) and Jordan 37% (Morsey& Michael, 1980).The average prevalence rate of *T. gondii*in most areas of the world is 20-30% (Wilson &McAuley, 1991). Low prevalence rates of 10% were recorded in the United Kingdom (Allain *et al*., 1998) and Norway (Jenum *et al*., 1998. Regional variations in the incidence of *Toxoplasma* infection rates from one country to another or even within the same country, has been well documented. This difference had been related to weather, cultural variation regarding food and hygiene habits (Jenum *et al*., 1998; Remington *et al*., 2001; Dupouy-Camet *et al*., 2003). The presence of stray cats in a humid rainy climate increasing the survival of oocysts resulting in elevated *Toxoplasma* prevalence in Central America (Remington *et al*., 2001). Stray cats are widely spread in Makkah city, however, the hot and dry conditions are not the best for oocyst survival, compared to cooler and Rainey environmental conditions in the Eastern and Southern regions of the kingdom, which support a higher prevalence. Farming and animal breeding are common too .The big relation showed in the existing study between *Toxoplasma* prevalence rate and the woman's age confirms the fact that seroprevalence of *Toxoplasmosis* is increased with age; the higher prevalence, the earlier rise (Remington *et al*., 2001; Dupouy-Camet *et al*., 2003) This relationship does not mean that older age predisposes to infection but might be explained by the older the age the longer time being exposed to the infection and may maintain a fixed level of anti-*Toxoplasma* IgG in serum for years. Opposite result was reported in the Eastern Region where seropositivity turned down with age (Al-Qurashi *et al*., 2001). The present study showed highest level of seroconversion was (33%) among 26-30 years age group then was (39.30%) in 20-25 years age group this result fall in with the common result that supports most recurrent seroconversion in

15-35 years age groups (Jackson & Hutchison, 1989). The obtained outcome fit with (Alvarado-Esquivel *et al.,*2007) in Mexico and (Boia *et al.,*2008) in Brazil while it differ with that of (Doehring *et al.,*1995) in Tanzanya who established highest level of seroconversion among 12-25 years age group and (Al-Qurashi*et al*., 2001) in the Eastern Region.

In this study,sera from 318 pregnant women were tested for toxoplasma antibodies by four serological methods, i.e. latex agglutination test (LAT), indirect hemagglutination tests (IHAT), enzyme linked immune sorbent assay (ELISA, IgM&IgG) and indirect fluorescent antibody test (IFAT). The percentage of toxoplasmosis was (32.70%,46.23%,0.94%,71.7%&60.38%).
Correspondingly This result is similar to that of (Al-Mashari *et al.,*1980) who used five serological methods For inspection of toxoplasmosis (LAT,IHAT,EIA(IgM,IgG) &IFAT)and he found prevalence of toxoplasmosis in pregnant women varied between 25.4% and 36.3% .Also (Sarwat, 1993) used three serological tests (IHAT,ELISA&IFAT) and he found the incidence of toxoplasmosis was 24%,46% and 49.3% respectively. In this study LAT has been proved to be the most sensitive and suitable test as it is cost effective and simple to do .Al-Mashari *et al.* (1980), Rye *et al.(*1996) and Al-Obeidi. (2004) proved LAT as the best test for screening of toxoplasmosis else . In this study of the three tests (IHAT, ELISA IgM&IgG&IFAT) , the IHAT test proved to be the most suitable because of its 99% specificity the same as proved by (Al-Mashari *et al.,*1980).

This study lends a hand to the public health community as most of pregnant women aren't aware of being tested for *Toxoplasma.* It also suggests that there is a require to make an awareness program for not only the pregnant women but even the unmarried ones and support more studies to improve the facts of the population about the risks of contact of pregnant women in Saudi Arabia to *Toxoplasma gondii*.

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