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Prevalence of *Toxoplasma* infection in pregnant women and their infants in Makkah Hospitals

Naser A. ElSawy[1,2], Abdullah G.Alkushi[3] .Amal Almattry[4] and Hataba A.A[5]

[1]Department of Laboratory Medicine, Faculty of Applied Medical Sciences, Umm Al-Qura University, Saudi Arabia

[2]Department of Anatomy & Embryology, Faculty of Medicine, Zagazig University, Egypt

[3]Department of Anatomy, Faculty of Medicine, Umm Al-Qura University, Saudi Arabia

[4]Department of Anatomy & Embryology, Faculty of Medicine, Zagazig University, Egypt

[5]Department of Chemistry, Faculty of Science, Zagazig University, Egypt.

Address for correspondence: Abdullah G. Alkushi, Department of Anatomy, Faculty of Medicine, Umm Al-Qura University, Makkah, K.S.A, P.o. Box: 57039, Postal code: 21955

Mobile: 00966532570960, Email: dr.alkushi@gmail.com

Abstract:

Toxoplasma gondii (*T. gondii*) is found worldwide in many species, including carnivorous and herbivorous mammals and birds. The definitive host of this parasite has been shown to be cats, which have been associated with the transmission of the parasite in every population investigated.

Serum samples were examined for antibodies to *T. gondii*, and the analysis for toxoplasma specific antibodies IgG, and IgM were completed within one day in the lab. The prevalence of *T. gondii* was 47.7% in female patients and 41.6% in male patients. From 225 patients with positive antibodies IgG Abs was detected in 113 women and 88 male and IgM was detected in 13 women and 11 male. There was a highly significant correlation between the (18>40years age group and Toxoplasma infection in this population. Most of the study group were nulliparous with seropositive for IgG and IgM Abs to *T.gondii*. The high rate of abortion (30.2%) was determined with seropositive IgG and seropositive IgM., IgG Abs were observed in cases with a history of recurrent pregnancy loss while IgM detected with women who had a history of previous abortion. *T. gondii* is one of the widest spread protozoal parasites infecting abroad spectrum of vertebrates.

Toxoplasmosis can cause detrimental effects on anyone who contracts the disease. Thus, it is very important to practice the prevention of the disease so that treatment does not need to be employed.

Key words: *Toxoplasma gondii*, stillbirth, prenatal mortality, pregnancy, congenital anomalies

Introduction

Toxoplasmosis is a zoonotic disease that occurs worldwide and is caused by the protozoan parasite *Toxoplasma gondii* (*T. gondii),* (Liesenfeld and Janitschke 2005; Hill et al. 2002). *T. gondii* requires both a definitive host and an intermediate host to complete its life cycle. Although felines are the only definitive host of *T. gondii*, any warm-blooded animal, including humans, can be infected by the parasite (Dubey 2010). Up to one-third of the world's human population is estimated to be carrying a Toxoplasma infection (Montoya and Liesenfeld 2004). Moreover, it is estimated that one-quarter of the population (over 12 years of age) in the United States is positive for *T. gondii* infection (Center for Disease Control 2012).The prevalence in some areas can be as high as 95% in the older populations. Latent, chronic infection, which is characterized by parasite encystment in the host muscle and brain cells (particularly neurons and glial cells), persists following the resolution of acute infection and continues with seropositivity throughout the host's lifetime (Dubey 2010).

T. gondii infection can be acquired by ingestion of viable tissue cysts in meat or oocysts excreted by cats that contaminate food or water (Montoya and Liesenfeld 2004). However, *T. gondii* infection in pregnant women represents a risk for congenital disease, i.e., congenital transmission may occur when the mother acquires a primary infection during pregnancy (Remington et al. 2006). Although pregnant women are often asymptomatic or have only mild symptoms, *T. gondii i*nfection may cause spontaneous abortion, stillbirth, or serious fetal damage. The gestational age at which the infection is contracted is a key variable affecting the clinical fetal outcome (Ades and Nokes 1993; Lopez et al. 2000; Martin 2001). While the prevalence rates of *T. gondii i*nfection were up to 50–80% in Central and South American as well as some European populations, the primary infection with *T. gondii i*n pregnant women occurs all over the world with frequencies between 0.1–1% (Stray-Pedersen 1993). However, there is scarce information on the epidemiology of *T. gondii* infection in pregnant women.

When a pregnant woman (even one who has no symptoms) catches toxoplasmosis during pregnancy and remains untreated, there is a chance that the mother could pass the infection on to her developing fetus. Babies who were infected during their mother's first trimester tend to have the most severe symptoms. It is rare for a woman who got infected with toxoplasmosis before getting pregnant to pass the infection on to her unborn baby because she (and her baby) will have built up immunity to the infection. However, it can occur if a pregnant woman who had a previous infection becomes immune-compromised and her infection is reactivated. Generally, it is probably a good idea to wait to try to get pregnant until at least 6 months after a toxoplasmosis infection.

Therefore, the aims of the study include the following:

1- Prevalence of toxoplasmosis in Makkah hospital from the year 1427 H till 1432 H

2- Determination of the percentage of stillbirth from the year 1427H to 1432 H

3- Prevalence of acute and chronic cases of toxoplasmosis according to gender and age of patients in 1432 H

4- Incidence of prenatal mortality to total number of pregnancy

5- Percentage of congenital anomalies cases

MATERIALS AND METHODS

Sample Collection

After medical history taking, the participants were subjected to a physical and obstetric examination followed by a prenatal ultrasound evaluation. A venous blood sample was taken (median cubital vein) from mothers and after separation; the sera were kept at -20 ℃ until analyzed. Every serum sample was tested for toxoplasma antibodies using commercially available ELISA kits (Pishtaz–Iran) in which microplate wells are coated with anti-human IgM or IgG antibody to detect IgM or IgG respectively and were completed within one day in the laboratory.

Sample collection and storage

Peripheral blood leukocytes were collected in sterile tubes containing ethylenediaminetetracetic acid (EDTA). The temperature during transportation was kept at around 0–10 ℃ by using isolated boxes with ice. The samples were stored at −20°C in the Molecular Genetics Laboratory in the Department of Medical Genetics, Faculty of Medicine. After the DNA extraction, the samples were kept at −80°C for long-term storage.

DNA Isolation

DNA isolation from whole blood

Genomic DNA was isolated from EDTA-peripheral leukocytes using Mini/Spin-Column protocol (QIAGEN, USA). Proteins K enzyme (20 µl) was pipette into the bottom of a 1.5-ml-microcentrifuge tube. Then 200 µl of whole blood was mixed with 200 µl of phosphate-buffered saline (PBS). We used up to 200 µl of the mixture and pipette the mixture into the micro centrifuge tube. Then, Llysis buffer (AL) (200 µl) was added to the sample and incubated at 56°C for 10 min for complete haemolysis of the RBCs, lysis of the cell pellet and digestion of the proteins.

Absolute ethanol (200 µl) was mixed with the sample to precipitate the DNA. The sample-ethanol mixture was carefully applied to the QIAamp spin-column (QIAGEN, USA), and then the mixture was centrifuged at 8,000 rpm for 1 min. The filtrate was discarded. The column was carefully washed with the washing buffer (AW1) (500 µl). The tube was centrifuged at 8,000 rpm for 1 min. Another 500 µl of the washing buffer (AW2) was added and again centrifuged at full

speed for 2 min. The column was opened, and 200 µl buffer AE (elution buffer) was added to the filter, incubated at room temperature for 1 min and then centrifuged at full-speed for 1 min. The highly pure DNA sample was refrigerated at 4 ℃ until further use, –20 ℃ for longer time. Finally, the DNA quantity was measured by UV absorption at A260 (1.0 OD unit is equivalent of 50 µg), and the genomic DNA quality was assessed by an analytical 0.7% agarose gel.

RESULTS

Gender and age of patients

A total of 1617 patients with toxoplasmosis were hospitalized from the year 1427 H till 1432 H (Table 1). Of them, only 502 patients were included in this study for eligibility to participate in the study. Some patients were refused to participate and others were lost and could not be followed up (Table 2).

With respect to gender, 238 (47.4%) of the patients were male while 264 patients (52.6%) were females. Considering the age of patients, 30 patients were above 60 years of age,65 patients (%) were in the age group 40–60 years, 218patients (%) were aged 18–40 years, and 70 patients (%) were aged between12–18 years. Sixty-nine patients were aged less than 12 years while 50 patients were newborns (Tables 3 and 4).

Clinical manifestations of illness

The severity of the disease ranged from mild illness to sever painful manifestations. The most commonly presenting symptoms were fever (34 patients), myalgia (29 patients) and headache (28 patients). In addition, seven patients presented with arthralgia either in one or more joints; the most frequently affected joints were the hip, knee, shoulder, wrist and ankle joints. Manifestations of anemia such as general weakness and easy fatigability together with pallor were observed in 15 patients (Tables 5 and 6).

Incidence of *T. gondii* in pregnant women in Makkah

With respect to the incidence of prenatal mortality to the total number of pregnancy, it was found that the total number of prenatal mortality was 223 (105 of them with positive antibodies and 118 with negative one) (Table 7). Congenital anomalies, such as oculopathy, hepatomegaly,

pulmonary/respiratory distress, meningoencephalitis, hyper-extended knee (Genu recurnatum), were observed in 18 patients (Table 8). Moreover, there were 95 cases of abortion out of the total 328 pregnancy cases (Table 9 and Table 10).

Other laboratory investigations

Erythrocyte sedimentation rate (ESR)

In cases presenting with high titers (1:640–1:2560), the ESR ranged from 32 to 74.However, after the treatment for 3 weeks, the ESR value returned to normal (less than 20).In cases presenting with lower titers (1:40–1:640), there was a slight increase in ESR, i.e., above the normal value up to 25.

Hemoglobin level

In cases presenting with high titers (1:640-1:2560), the laboratory findings proved lower hemoglobin levels (8 mg/dl) when the normal range was 12.5–17 mg/dl. This suggests normocytic anemia with general weakness, pallor, and easy fatigability. However, in cases presenting with lower titers, there was no decrease in the hemoglobin level although there were manifestations of anemia such as general weakness and easy fatigability.

Haematocrit value

The laboratory findings showed lower levels (26.4) of hematocrit value (the normal range was 36-47), suggesting normocytic anemia with general weakness, pallor, and easy fatigability.

Prothrombin time

The laboratory findings revealed lower levels (11.2) of prothrombin time (normal from 12–16), suggesting prolonged bleeding in patients.

White blood cell (WBC) count

In cases presenting with high titers (1:640–1:2560), there was an elevation in the WBC count (leukocytosis), suggesting acute bacterial infection. In cases presenting with lower titers (1:40–1:640), three patients (2.91%) presented with a slight elevation of the WBC count suggesting previous old infection.

DISCUSSION

T. gondi is one of the most widespread protozoa parasites infecting abroad spectrum of vertebrates. In general, the infections in healthy adults are usually asymptomatic. However, the infections in neonates and immuno-compromised individuals can be devastating. *T .gondii* is indeed the causative agent of congenital toxoplasmosis, the leading cause of neurological birth defects, and toxoplasmic encephalitis is the most common cause of focal brain lesions in AIDS patients (Ananvoranich and Perreault, 2000).The link between the immunological profile of newborns and the time of fetal infection has been pointed out previously by many author (Bessieres et al. 1992).

The immune system development needs the interaction of cellular and humoral components. The IgM antibodies which the first antibody to be produced in intrauterine life and may disappeared at birth, followed later by IgG and IgA antibodies, while the IgG antibodies produced later on (Decoster et al. 1988).

The prevalence of anti -Toxoplasma IgM and IgG among pregnant women was 30.5% and 20.45%, respectively and that 21.5 % of them were PCR positive (Ghoneim et al. 2009) that is similar with our results.

We examined the samples for *Toxoplasma* antibodies to study the prevalence and possible transmission route(s). The prevalence of antibodies gradually increased with age. The percentage of toxoplasmosis in children was less than that in adults. This may be attributed to the fact that children are frequently forbidden from getting contact with the soil for fear of helminthes infections.

The prevalence rate among the female patients (47.7%) was insignificantly different from that in the male patients (41.6%). This result is similar to the findings of Rorman et al. 1986 and that confirmed by Remington et al. 2006 that refer the main problem of toxoplasmosis to primary infection during pregnancy.

In addition, although the rate of transmission of infection is very high among pregnant women, the percentage of congenital anomalies is low because most of these infections are probably not recognized. Thus, it can be said that maternal infection as well as the infection in newborn infants may be asymptomatic. The symptoms in infants may develop insidiously and can be non-specific

in nature. It may be difficult to demonstrate *T. gondii* as the etiological agent when the symptoms gradually emerge. The immunity transmitted from primary maternal infection protects the fetuses so the rate of congenital toxoplasmosis is low at about 1 per 1000 cases.

Thus, it is important to adopt strategies for prevention of *T. gondii* infection. Some prevention measures that can be taken include wearing gloves or washing hands after and during gardening or yard work, avoiding consumption of undercooked or raw meat, washing kitchen utensils thoroughly after use, especially the cutting boards used to cut vegetables, washing and/or peeling fruits and vegetables before consumption, covering children's sandboxes and keeping outdoor play areas free from cat access, wearing gloves/washing hands after changing litter boxes, etc.

We recommend that a nationwide prospective study should be conducted with the aim to prevent congenital toxoplasmosis. It should include the screening of pregnant women for toxoplasma-specific antibodies so as to collect information on the risk factors for infection. Moreover, the incidence of primary *T. gondii* infection in pregnant women should be further studied. The rate of transmission of infection to the foetus should also be determined. Further studies should shed light on the feasibility of a serological screening program.

Toxoplasmosis can have extremely detrimental effects on anyone who contracts the disease. Although most immune-competent patients are able to combat the parasite, many individuals suffering from a disease that renders them immune-compromised, pregnant women, or individuals whose medication weakens their immunity might find that toxoplasmosis has more of a negative impact on their bodies than expected. Thus, it is very important to practice the prevention of the disease so that treatment does not need to be employed.

TABLES

Table 1. The number of hospitalized patients with toxoplasmosis from the year 1427 H till 1432 H was found to be 1617 distributed as follows:

Year	1427	1428	1429	1430	1431	1432
Number	77	112	264	230	456	478

Table 2. The annual distribution of hospitalizes still birth from the year 1427 to 1432

Year	1427	1428	1429	1430	1431	1432
Number	8	11	16	8	31	76

Table 3. Prevalence of acute and chronic cases of toxoplasmosis according to gender and age of patients in 1432 H by IFAT toxoplasma antibody from 502 patient with clinical manifestation and 225 positive antibodies .The titer from1024 to 4096 only in 25 cases from 225 (11.11) in percentage and mild titer from 16 to 64 in 157 cases which equal (69.77) in percentage.

Age group	Number	Number of IgM	%	Number of IgG	%
0>12	119	2	1.7	11	9.2
12>18	70	2	2.82	22	31.4
18>40	218	19	8.7	109	50
40>60	65	1	1.5	39	60
<60	30			20	66.7
Total	502	24(11male-13female)		201(88 male-113 female)	

Table 4. Prevalence ratio of mean toxoplasma titer according to gender and age of patients in 1432 H by IFAT toxoplasma antibody from 502 patients with clinical manifestation.

Age group	Mean age	Mean titer
0>12	10.9	194.5

Age group	Mean age	Mean titer
12>18	14.3	304.7
18>40	28.7	427.6
40>60	46.5	93.6
<60	66.7	44

Table 5. Manifestations of the illness and its percentage.

Manifestation	Number of positive 225	%	Number of negative 277	%	P
Fever	34	15.1	20	7.2	<0.01
Headache	28	12.4	20	7.2	<0.05
Myalgia	29	12.9	16	5.8	<0.01
Sore throat	16	7.1	15	5.4	<0.05
Back pain & Arthralgia	7	3.1	10	3.6	<0.05
Maculopapular rash	9	4	6	2.2	<0.05
Respiratory infection	3	1.3	2	0.7	<0.05
Jaundice	1	0.4	3	1.1	<0.05
Asymptomatic	174	77.3	244	88.1	<0.001
Other symptoms like anemia etc	15	6.7	33	11.9	<0.05

Table 6. Manifestations of the illness and its percentage in relation to titer.

Manifestation	N.of high titer 25	%	N.of med titer43	%	N.of low titer 157	%	Sero–ve	%	P
Fever	20	80	3	7	11	7	20	7.2	<0.01
Headache	15	60	3	7	10	6.4	18	6.5	<0.01
Myalgia	18	72	2	4.7	9	5.7	16	5.8	<0.01
Sore throat	6	24	2	4.7	9	5.7	16	5.8	<0.01

Arthralgia	1	4	1	2.3	5	3.2	10	3.6	<0.01
Rash	6	24	0	0	3	1.9	6	2.2	<0.01
Respiratory	2	8	0	0	1	0.6	20	7.2	<0.01
Jaundice	1	4	0	0	0	0	3	1.1	<0.01
Asymptomatic	1	4	34	79	139	88	244	88	<0.01
Other symp.	24	96	9	21	18	11	33	12	<0.01

Table 7. Incidence of prenatal mortality to total number of pregnancy

Spontaneous vaginal delivery	Lower seg C S	Still birth	Congenital anomalies	Intra uterine fetal death	Total
4142	1463	76	18	129	5828

Table 8. Percentage of congenital anomalies cases to the total number 18

Patient	Number	%
Oculopathy	6	33.3
Hepatomegally	4	22.2
Pulmonary(Respiratory distress)	2	11.1
Meningoencephalitis	3	16.6
Hyper extended Knee (Genu Recurvatum)	3	16.6

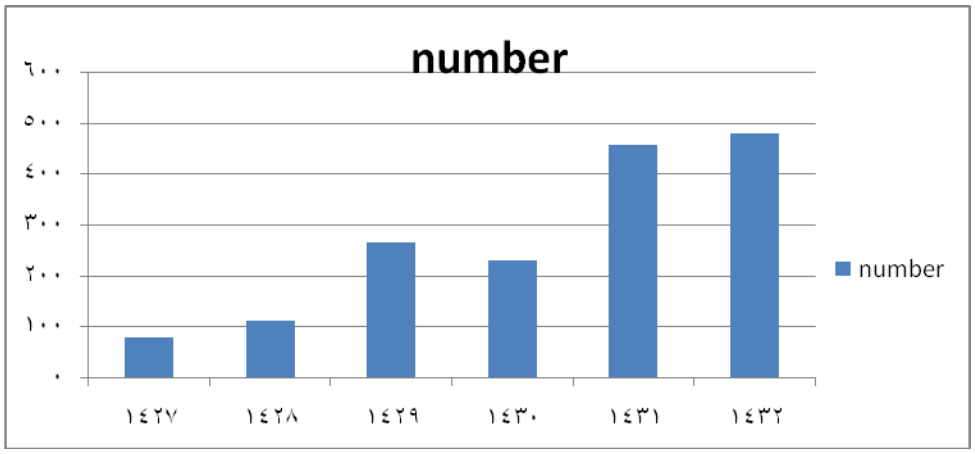
Table 9. Incidence of abortion in pregnant women in Makkah with previous adverse pregnancy outcome.

Toxoplasma antibody	Total of pregnant	Abortion 1st	Abortion 2nd	Abortion 3rd	%of abortion to pregnant	P
Ab positive	159	4	14	30	30.2	<0.01
Ab negative	169	27	13	7	27.8	<0.01
Total	328	95			29	<0.01

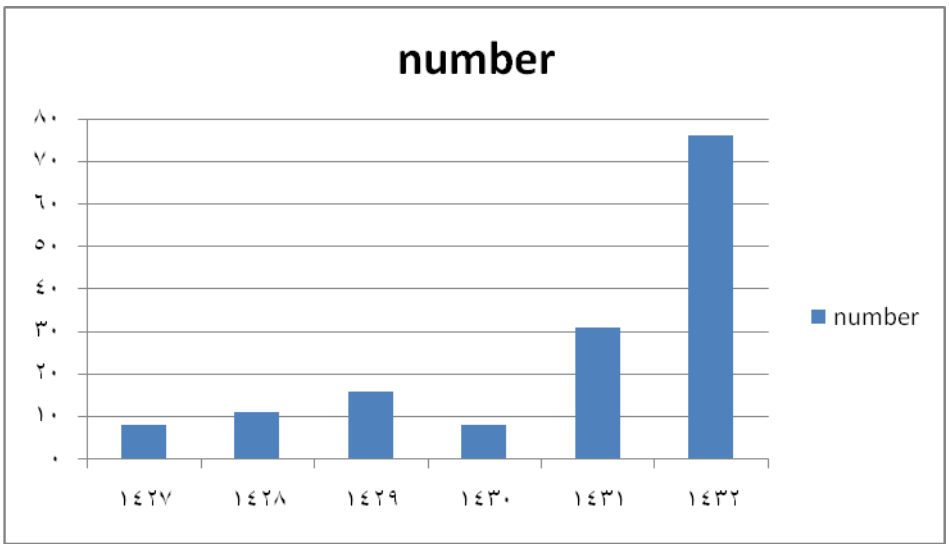
Table 10. Evaluation of a rapid ELISA technique for detection of circulating antigens of toxoplasma gondii in 70 cases and 20 controls in the last 6 months.

Manifestation	Number of positive	%	Number of negative	%	P
Immuno comprised 10	8	80	2	20	<0.001
Control 20	0	0	20	100	<0.001
Chronic Toxoplasmosis 25	1	4	24	96	<0.001
Acute Toxoplasmosis 35	24	68.6	11	31.4	<0.001

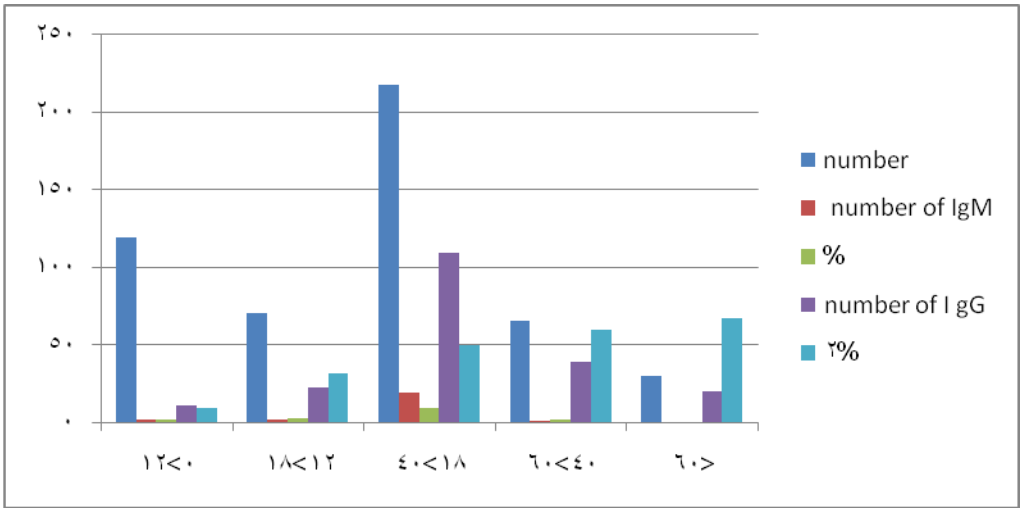
CURVES



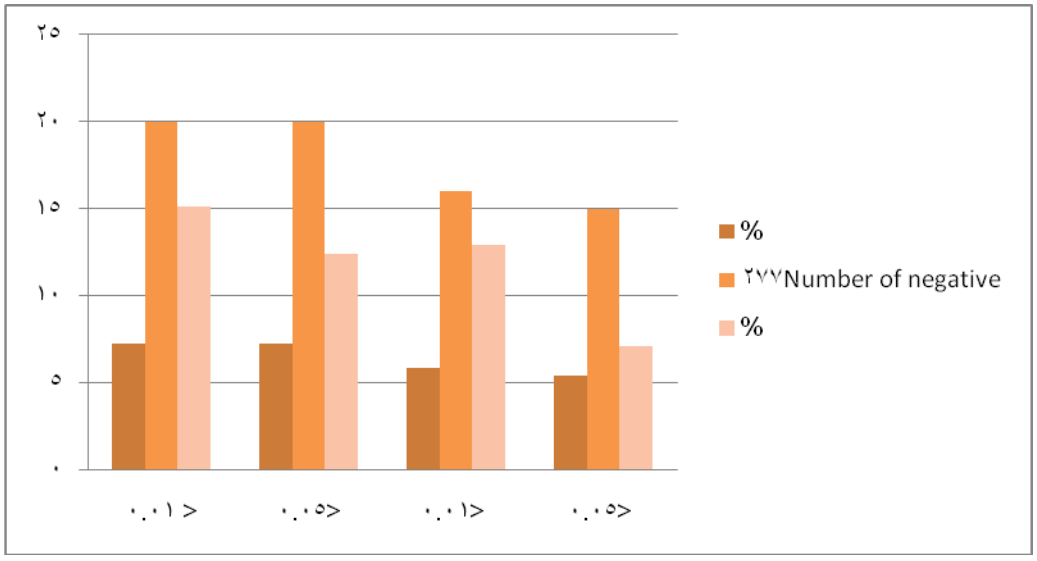
Curve 1. The number of hospitalized patients with toxoplasmosis from the year 1427 H till 1432 H was found to be 1617 distributed as follows



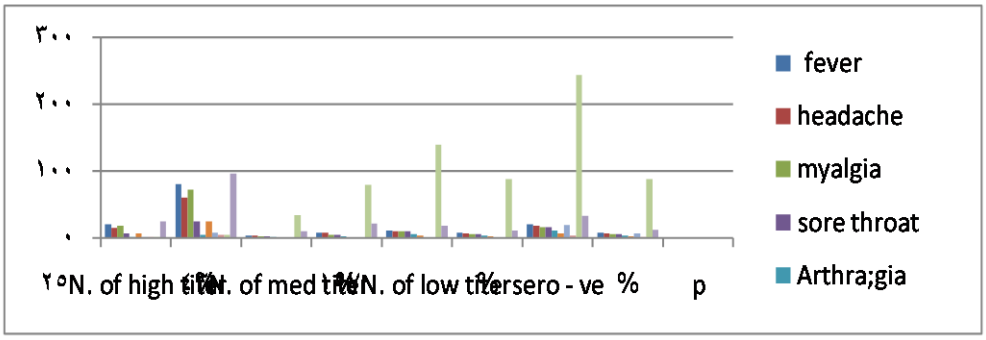
Curve 2. The annual distribution of hospitalizes still birth from the year 1427 to 1432



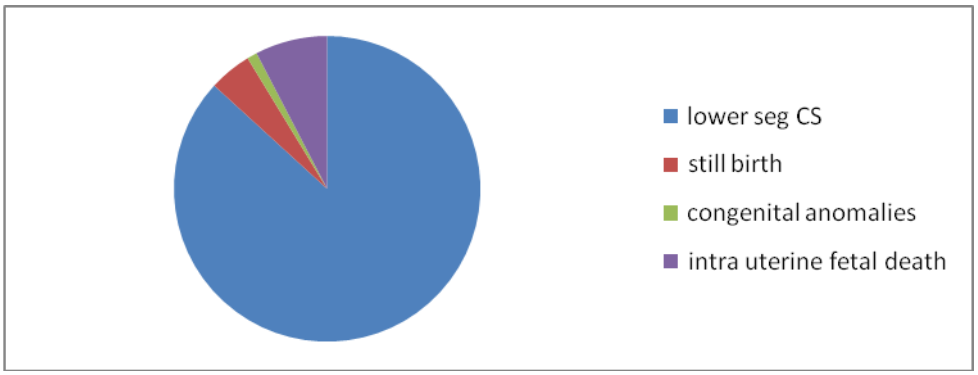
Curve 3. .Prevalence ratio of mean toxoplasma titer according to gender and age of patients in 1432 H by IFAT toxoplasma antibody from 502 patients with clinical manifestation.



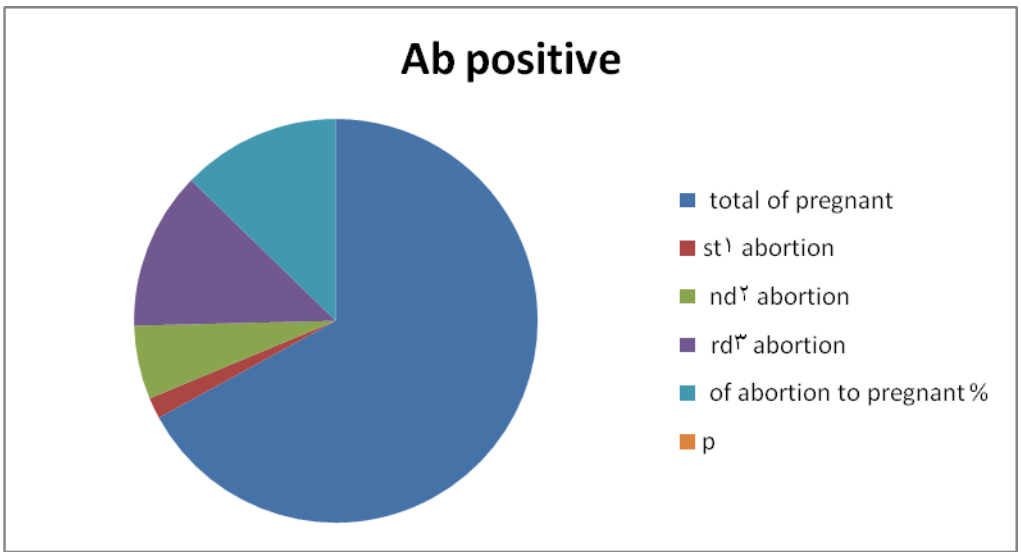
Curve 4. Manifestations of the illness and its percentage.



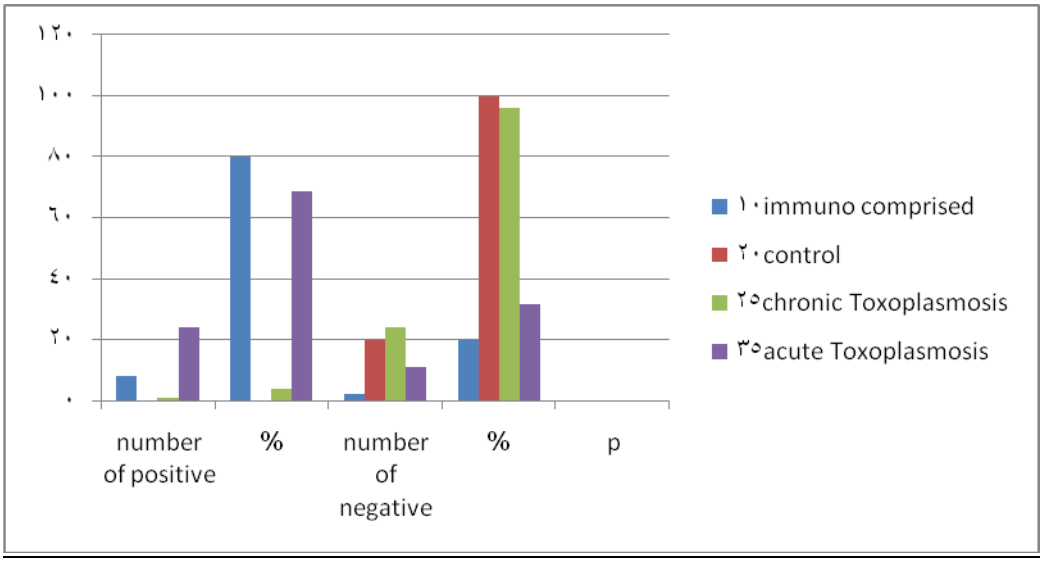
Curve 5. Manifestations of the illness and its percentage in relation to titer.



Curve 6. Percentage of congenital anomalies cases to the total number 18



Curve 7. Incidence of abortion in pregnant women in Makkah with previous adverse pregnancy outcome.



Curve 8. Evaluation of a rapid ELISA technique for detection of circulating antigens of toxoplasma gondii in 70 cases and 20 controls in the last 6 months.

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