TEMIDAYO OLADIRAN ADENIYI

SCREENING OF TORCH PANEL TESTS IN PREGNANT WOMEN

NEU 2020

T.R.N.C

TURKISH REPUBLIC OF NORTH CYPRUS
NEAR EAST UNIVERSITY
GRADUATE INSTITUTE OF HEALTH SCIENCES

SCREENING OF TORCH PANEL TESTS IN PREGNANT WOMEN

A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF HEALTH SCIENCES
OF
NEAR EAST UNIVERSITY

By
TEMIDAYO OLADIRAN ADENIYI

In Partial Fulfillment of the Requirements for
The Degree of Master of Science
In
Medical and Clinical Microbiology

ADVISOR
Assist. Prof. Dr. Ayse SARIOGLU

Nicosia, 2020

T.R.N.C

TURKISH REPUBLIC OF NORTH CYPRUS

NEAR EAST UNIVERSITY

GRADUATE INSTITUTE OF HEALTH SCIENCES

SCREENING OF TORCH PANEL TESTS IN PREGNANT WOMEN

TEMIDAYO OLADIRAN ADENIYI

Master of Science
In
Medical and Clinical Microbiology

ADVISOR

Assist. Prof. Dr. Ayse SARIOGLU

Nicosia, 2020

The Directorate of Health Sciences institute

This study has been accepted by the Thesis Committee in Medical Microbiology Program as a Master of Science Thesis.

Thesis committee

Chairman of the committee: Assoc. Prof. Dr. Meryem Guvenir

Supervisor: Assist. Prof. Dr. Ayse Sarioglu

Members: Assist. Prof. Dr. Ozel Yuruker

Approval:

According to the relevant articles of the Near East University Postgraduate study-Education and Examination Regulations, the members of the thesis committee and the decision of the Board of Directors of the Institute have approved this thesis.

Prof. Dr. K. Husnu Can Baser

Director of Health Sciences Institute

DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Temidayo Oladiran Adeniyi

ACKNOWLEDGEMENT

I want to thank my lecturer and advisor, Assist. Prof. Ayşe Arikan-Sarioğlu, whose gudiance was unrelentless during the course of this thesis**.** I would also like to thank myfamily for their great support throughout my education, Adeola for her love and patience throughout this course.I extend my thanks and appreciation to all those who contributed with me tocomplete this study, without them, I would not have been able to complete mymaster's degree.

I also want to say thank you to Prof. Dr. Nedim Çakır, our Head of Department for having this project best interest at heart, always.

I will also like to acknowledge Mr. Emrah Guler for his correction and guidance during the course of this project.

DEDICATION

I dedicate my thesis to my beloved parents

Screening of Torch Panel Tests in Pregnant Women

Temidayo Oladiran Adeniyi

Assist. Prof. Dr. Ayse Sarioglu

Near East University, Medical Microbiology and Clinical Microbiology Department

ABSTRACT

Objective: It is important to diagnose maternal infections that may cause various congenital anomalies in the newborn in the early period of pregnancy. In this study, it was aimed to investigate the seroprevalence of antibodies developed against maternal infections in pregnant women who applied to the Near East University Hospital Gynecology and Obstetrics Polyclinic and to determine some factors affecting these infections.

Materials and Methods: Samples from 1286 pregnant women sent to Near East University Microbiology Laboratory between January 2016 and December 2018 were included in the study. IgG and IgM antibodies developed against Toxoplasma, Rubella and Cytomegalovirus infections in serum samples taken from pregnant women were investigated in Abbott architect i1000SR device by ELISA method. In the study, the age ranges of pregnant women were categorized and evaluated as 17-25, 26-35 and above 35 years.

Results: A total of 1286 pregnant women between the ages of 17-46 (mean: 29 ± 4.9) were included in the study. Seroprevalence respectively: Toxoplasma IgM 1.3%, IgG 17.9%; Rubella IgM 2.0%, IgG 90.2%; Cytomegalovirus (CMV) IgM 1.0%, IgG 94.5%. While the Toxoplasma Avidity test was low in 17% of the avidity tests, the Rubella Avidity and Cytomegalovirus Avidity tests were high in all pregnant women. In the study, while there was no statistical relationship between Rubella IgG / IgM, CMV IgG / IgM and Toxoplasma IgM positivity and age groups (respectively: $p = 0.318 / 0.309$; $p = 0.719 / 0.710$; $p = 0.954$), 35 Toxoplasma IgG seropositivity was found to be significantly higher in pregnant women of age and older than those younger than 35 years ($p = 0.002$). In addition, it was found that Toxoplasma IgG positivity increased as the age progressed.

Conclusion: It has been determined that it is appropriate to perform maternal tests in terms of laboratory routines during pregnancy follow-up, routine screening of IgG and IgM antibodies against infections, and the necessity of checking avidity tests when necessary.

Keywords: TORCH. Pregnant women. Avidity, IgM, IgG

Gebelerde TORCH Panel Testlerinin Araştırılması

Temidayo Oladiren Adeniyi

Yrd. Doç. Dr. Ayşe Sarıoğlu

Yakın Doğu Üniversitesi, Tıbbi Mikrobiyoloji ve Klinik Mikrobiyoloji Ad.

ÖZET

Amaç: Yenidoğanda çeşitli doğumsal anomalilere neden olabilen maternal enfeksiyonların gebeliğin erken döneminde teşhis edilmesi önemlidir. Bu çalışmada,Yakın Doğu Üniversitesi Hastanesi Kadın Hastalıkları ve Doğum Polikliniği'ne başvuran gebelerde maternal enfeksiyonlarına karşı gelişen antikorların seroprevalansını araştırmak ve bu enfeksiyonlara etki eden bazı faktörleri belirlemek amaçlanmıştır.

Gereç ve Yöntem: Ocak 2016- Aralık 2018 tarihleri arasında Yakın Doğu Üniversitesi Mikrobiyoloji Laboratuvarı'na 1286 gebeden gönderilen örnekler çalışmaya dahil edilmiştir. Gebelerden alınan serum örneklerinde, Toksoplazma, Rubella ve Sitomegalovirüsenfeksiyonlarına karşı gelişen Ig G ve Ig M antikorları ELISA yöntemiyle Abbott architect i1000SR cihazında araştırılmıştır. Çalışmada gebelerin yaş aralıkları 17-25, 26-35 ve 35 yaş üstü olarak kategorize edilmiş ve değerlendirilmiştir.

Bulgular: Çalışmaya yaşları 17-46 (ort: 29±4.9) arasında toplam 1286 gebe kadın alınmıştır. Seroprevalans sırasıyla: Toksoplasma IgM %1.3, IgG %17.9; Rubella IgM %2.0, IgG %90.2; Sitomegalovirus (CMV) IgM %1.0, IgG %94.5'dir. Avidite testlerinden Toksoplazma Avidite testi %17 kişide düşük saptanırken, Rubella Avidite ve Sitomegalovirüs Avidite testleri tüm gebelerde yüksek idi. Çalışmada,Rubella Ig G/Ig M, CMV Ig G/ Ig M ve Toksoplasma Ig M pozitiflikleri ile yaş grupları arasında istatistiksel bir ilişki saptanmaz iken (sırasıyla: p=0.318/0.309; p=0.719/0.710; p=0.954), 35 yaş ve üzerindeki gebelerde, 35 yaşından küçük gebelere göre toksoplasma IgG seropozitifliğinin anlamlı oranda yüksek olduğu tespit edilmiştir (p=0.002). Ayrıca yaş ilerledikçe Toksoplazma Ig G pozitifliğinin de arttığı saptanmıştır.

Sonuç: Gebelik takibinde laboratuvar rutinleri açısından maternal testlerininyapılmasının uygun olduğu, enfeksiyonlara karşı gelişen Ig ve Ig M antikorlarının rutin taramada bakılması, gerek duyulan hallerde avidite testlerinin de bakılması gerekliliği belirlenmiştir.

Anahtar kelimeler: TORCH. Hamile kadın. Avidity, IgM, IgG11'

.

TABLE OF CONTENTS

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

TORCH	Toxoplasmosis, Rubella, Cytomegalovirus, and Herpes simplex,
CMV	Cytomegalovirus
VZV	Varicella Zoster Virus
IgM	Immunoglobulin M
IgG	Immunoglobulin G
TRNC	Turkish Republic of Northern Cyprus
CDC	Center for Disease Control
AIDS	Acquired immunodeficiency syndrome
WHO	World Health Organization
SPSS	Statistical Package for the Social Sciences
PCR	Polymerase chain reaction
ELISA	Enzyme-linked Immunosorbent assay
BOH	Bad Obstetric History
DNA	Deoxyribonucleic acid,
HAART	Highly active antiretroviral therapy
SNHL	Sensorineural hearing loss
TORC	Toxoplasmosis, Rubella, and Cytomegalovirus,

CHAPTER ONE

INTRODUCTION

1.0 Introduction

According to El-Tantawy & Taman (2014)TORCH stands for Toxoplasmosis, Rubella virus also called German measles, Cytomegalovirus and Herpes Simplexvirus. This group oforganisms has been implicated in causingsevere complications in pregnant women and the growing fetus. These infections enter into fetal circulation through trans-placenta. Transmission may also be possible during the developmental stages of pregnancy or sometime during the time of birth. Primary infection has high mortality than recurrent infection and may cause congenital abnormality, fatal miscarriage, Intra-uterine fetal demise, development hindrance, preterm labor and new born babies with signs of the infection (El-Tantawy & Taman, 2014).

The placenta acts as a boundaryconnecting mother and developing fetus during the first three months'gestation period, the fetus is protectedfrom the cell mediated and humoral immunological response. The developing baby is seriously affected by these viruses because of absence of immunity after the first trimester of pregnancy, despite that the fetus gets immunity from mother. Different infections have their own causative agent and they most times spread through water, soil, poor hygienic conditions, contaminated blood, and airborne respiratory droplet. According to Pizzo (2011)in respect to the infections caused secondary or reactivated infection causes less complications as compared to primary infection which causes more severe damages. Each microbial agent has specific manifestations or symptoms but some are commonly seen. Complications are confirmed if a fetus presents withmicrocephaly, rash, intracranial calcifications, jaundice, intrauterine growth restriction, hepatosplenomegaly, elevated trans-aminase concentrations and thrombocytopenia(Pizzo, 2011).

Specific symptoms of these infections are shown in Table 1.1.

Table 1.1: Specific clinical symptoms of TORCH Disease

Symptoms	Organism
Intracranial Calcification	CMV, Toxoplasmosis
Vesicles	HSV, VZV, Syphilis
Chorioretinitis	Toxoplasmosis, CMV
Bone lesions	Syphilis, Rubella
Blueberry muffin lesions	Rubella
Cataracts	Rubella, HSV
Microcephaly	CMV
Hydrocephalus	Toxoplasmosis

*Toxoplasma gondii*is the causative agent of Toxoplasmosis and it is an obligate intracellular parasite, which causes infection in mammals. On the basis of serological studies, *T. gondii* is one of those TORCH infections which are most prevalent.Countries like Austria, France and Belgium, it is mandatory that pregnant females should be screen for TORCH infection during pregnancy. The high prevalence of these infections during pregnancy had been reported in different region of the world like Latin America, Africa, parts of Eastern, the Middle East, andparts of Southeast Asia (El-Tantawy & Taman, 2014).Whenever a woman has infected with these infections, the immune system produces IgM antibodies against these infections. TORCH IgM antibodies usually present for about 3 months. The presence of IgM indicates recent infection or recurrent infection(Sadik, Fatima, Jamil, & Patil, 2012).The cause of contamination of this disease has also been shown in Figure 1.

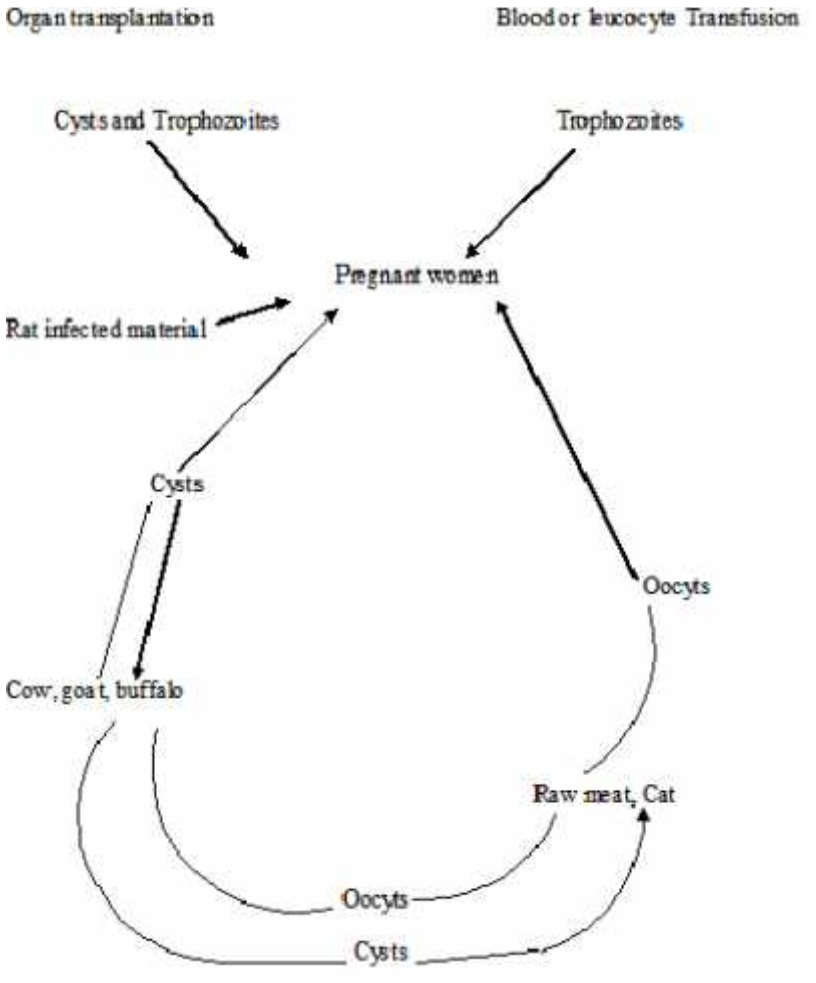

Figure 1**:** Causes and occurrence of TORCH disease

According to Sharma *et al*(2012), the TORCH panel tests comprise of antibody investigation to test for the microorganisms that causes congenital diseases transferred from mother to baby. However, the four diseases are usually mild in exposed and treated adults, patients affected during the pregnancy period are at risk for fetal death, still birth, or different severe birth entanglements or potentially disease. Thusly, this test is done when pregnancy is analyzed to decide clinical history of the mother or presentation to the TORCH micro-organisms. TORCHtest is conducted on fetal serum when infant show manifestations steady with an inherently gained contamination by one of the living beings above. Sharma*et al.*(2012)expressed that poor obstetric result suggests past complication fetal result regarding at least two sequential unconstrained premature birth, history of intrauterine fetal loss, intrauterine development impediment, despite everything births, early neonatal demise as well as inborn abnormalities.

Reasons for Poor obstetric result might be hereditary, hormonal, unusual maternal immune reaction and maternal disease.

Repetitive pregnancy lossbecause of maternal diseases is transferred in utero during different phase in the incubation can be brought about by diverse number of microorganisms which incorporate the TORCH panel complex not limited to different operators like Treponema pallidum, Chlamydia trachomatis, N. gonorrhea, HIV and so on. Toxoplasmosis gained before the birth of the baby may harm the baby (Colak, 2007).Sero-epidemiological examinations have indicated that about 20% of fertile womenwithin the childbearing age in India are vulnerable to Rubella disease. Inherent abnormality in 10-54% of cases is been attributed to rubella during pregnancy.

According to Stagno *et al* (2010), the disease brought about by cytomegalovirus (CMV) in grown-ups is typically asymptomatic however its consequences are commonly more fatal during pregnancy period. Notwithstanding, the consequence of primary CMV disease is usually more severe in pregnant ladies from regions of low standards of living. The birth mother is the main mode of infection of Herpes simplex infection to the baby or infant. Essential Herpes simplex infection in the first trimester of pregnancy is related with higher recurrence of unconstrained fetus loss, poor fetal development, and inherent deformity. These maternal diseases with antagonistic result are at first in clear or asymptomatic and are in this manner hard to analyze on clinical grounds. Along these lines, conclusion of intense TORCH contamination in pregnant ladies is generally settled by showing of seroconversion in matched sera or by exhibit of explicit IgM antibodies(Stagno, Pass, & Cloud, 2010).

The screening of TORCH infections can in some cases give false negative and false positive outcomes. False negative IgM tests can occur when IgG antibodies attach to the antigen utilized in the test or from immunodeficiency conditions that decrease the antibody reaction to these organisms. False positive test outcomes can be brought about by rheumatoid, immune system or heterophile antibodies in the mother's serum. Newborn children probed for IgG immune response may be positive as a result of a past disease or a progressing maternal contamination, and this doesn't mean the infant is tainted. Maternal antibodies to HSV and CMV may not satisfactorily defend the neonate (Stagno, Pass, & Cloud, 2010).This study gives a

report on the screening forboth IgG and IgM antibodies and also avidity testing of TORCH panelinfection in pregnant women.

1.1 Aim and Objectives of the Study

The aim of the study is to determine the seroprevalence of TORCH infections through antenatal screening in Near East University, North Cyprus.

CHAPTER TWO

LITERATURE REVIEW

2.0 Pregnancy

Most pregnant women experience less severe complexities of pregnancy, in any case, for a minority increasingly significant issues occur. In severe cases, these can bring about the loss of the child and, once in a while in advanced nations, that of the mother. Issues run from disappointment of the embryo to be embed in the womb of the mother, prompting a miscarriage, rupture of the placenta and pre-eclampsia (pregnancy induced hypertension). Developing fetus can encounter problems in the womb such as genetic disorders (Arvin & Whitley, 2011).

Pregnancy is a period, where a woman should take for her own as well as the fetus, considering the reviews ofJamieson et al (2016)“the emerging infectious disease threats a keen attention should be given to the special populations, especially women who are pregnant”. During pregnancy a few concerns are applicable to infectious disease risks. To start with, pregnant women are vulnerable to infectious diseases due to immunological changes during pregnancy. Secondly the impact of irresistible illnesses on the newborn child might be hard to recognize, and identification of fetal infections are difficult to diagnose. Lastly, treatment and prophylaxis administered generally may not be suitable for pregnant women.

According to Gilbert (2012)couples ought to be counsel their primary care physicians when arranging pregnancy. Health testing before pregnancy ought to incorporate intermittent antenatal screening tests; Screening ought to for the most part be done to every single pregnant woman. Specific screening dependent on risk factors is not dependable in showing characteristic risk factors for every single significant infection is tedious and improbable to recognize each one of the risks.

2.2 Pregnancy Related Complications

Pregnancy in which the mother, fetus, or newborn will be atan increased risk of morbidity or mortality before or after delivery is termeda high-risk pregnancy. Normally pregnancies proceed normally and result in a healthy baby. The most significant step in ensuring a safe and healthy pregnancy is by identifying women that are at a higher risk of birthcomplications and the

best time to identify them is before they get pregnant. Factorssuch as family health history, lifestyle and the mother's overall health offer significantdata about potential risks. For instance, women below the age of 35 are considered at lower risk than older women for pregnancy-related complications. Severe health problems such as asthma, diabetes, heart problems, lupus and Rh disease also require particular care during pregnancy. Some factors, such as the older age range age, anemia and bleeding in pregnancy are considered as low-risk

Consistently, women worldwidedie from a complication related to pregnancy or childbirth with a total death toll of almost 6 lakh women a year (Sivanandhan, 2015). Furthermore, in the year 2015 the "lives of eight million women are threatened, and more than 500,000 women were at a high risk as a result of causes related to pregnancy and childbirth. Ninety-nine percent of these deaths occur in developing countries (Population Reference Bureau, 2012),Of these, India accounts for 1.36 lakh maternal deaths, that are in India, due to pregnancy related causes a woman to die in every five minutes.

According to Nielson,(2015)in 2000 Uttar Pradesh a state in the northern India, Madhya Pradesh and Bihar accounted for half of neonatal deaths. This was accounts for about15 percent of the worldwide neonatal mortality. The absolute number of fetalmortalities was significantly lowin 1,000 births 10 fatalities was recorded in Kerala, however, it was recorded to be 60 in Madhya Pradesh and Orissa province. Neonatal mortality are noted to be caused by fetal infections, birth asphyxia, and preterm birth". A study donein the Tamil Nadu district showed a rate of 1.35% stillbirth, 3.53% neonatal mortality and 4.2% perinatal mortality (Nielson, 2015).In the underdeveloped region of Tamil Nadu, women had a controlled reproductive pattern. The high rate of neonatal mortality among young women establishes around 33% of the perinatal death rate as a result of their preference to sons.

In a study by Rao, Zamir, Rilkis, & Ben-David (2005) in the southern part of India, the mortality rate was still higher (1.67%). A report from Pondicherry shows that the septic abortions were the cause of 30.2% of the maternal mortality (Rajaram, Agrawal, & Swain, 1995)Situations where the fertilized egg becomes attached outside the womb (Ectopic pregnancy)has been increasing significantly in the UK in moderntimes. The causes for ectopic pregnancy are unascertained, but research shows infections causing damage to fallopian tubes, such as STDs, could be a factor(Anne & Elizabeth, 2011) opined that severe infectious affecting the pregnant

mother can have nonspecific fetalconsequences orobstetric effects and lead to fetal loss, premature labor or miscarriage. These infections must be treated as any other serious illness. Infections acquired in utero or during the birth process are a significant cause of fetal and neonatal mortality and an important contributor to early and later childhood morbidity.

Normally the fetus is infected by trans-placental spread after maternal disease, in which the microorganism courses in the mother's blood. These diseases, procured in utero, can be sufficiently extreme to cause fetal miscarriage or can bring about intrauterine development limitation, premature birth, or constant postnatal infection. Most times the maternal ailment is less severe yet the effect on the embryo is increasingly extreme. The level of seriousness is subject to the gestational age of the baby when tainted, the destructiveness of the life form, the harm to the placenta, and the seriousness of maternal infection. For instance, an essential maternal contamination, for example, Herpes simplex is bound to be vertically transmitted and cause a more serious ailment than repeat of same disease in the mother (Boyer & Boyer, 2004).Wilcox *et al.*(1988)observed the difficulty to decide the level of fetal miscarriages because of disease during early pregnancy.

2.3 TORCH Infections

From a cross-sectional study, sero-epidemiological overview on the predominance of TORCH infections during different phases of development, a general rate of 13-15% seropositivity to Toxoplasma gondii and, rubella, HSV, and CMV were in the proportions of85-87 %, 79-81 %, and 100%, were recorded individually. A statistically significant increment in antibodies titer and specific IgM immune response was found with the progress in the gestation period to CMV infection was reported. The results suggested a rise in CMV disease or reactivation during pregnancy while an increment in the other TORCH contaminations was not self-evident (Taechowisan, Sutthent, Louisirirochanakul, Puthavathana, & Wasi, 1997).

Kaur, Gupta, Nair, & Kakkar (1999)conducted the study on the seroprevalence of IgM antibodies to Toxoplasma gondii, rubella virus and cytomegalovirus and IgG antibodies to Herpes simplex virus type 1 and 2. Out of the 120 women included for the study, 112 (93.4%) had found to be positive for one or more infections. Prevalence of IgG antibodies to HSV was 70%. Seropositivity for toxoplasmosis, rubella and CMV respectively were 11.6, 8.3 and 20.8%.

The result demonstrated a high frequency of primary infections during pregnancy which may lead to any of the congenital anomalies and abnormalities.

Cao, Qui, & Zhang(1999)studied the prevalence of TORCH infection in pregnant women with histories of abnormal pregnancies and normal pregnant women that acted as the controls by using Enzyme-Linked immunosorbent assay (ELISA) combined with polymerase chain reaction (PCR) technique. In the study group, the rates of previous TORCH infection were reported as 16.67%, 16.29%, 46.29%and 29.63%respectively. The rates of active infection were 38.89%, Rubella 59.26%, CMV (57.40%) and HSV- 2 (46.29%). The rates of recurrent infection were as Toxoplasma (11.11%), Rubella (38.89%), CMV (38.89%) and HSV-2 (22.22%). These three sorts of infection rates in study group were essentially elevatedcompared to the rates in the control group. The frequency of maternal-fetal vertical transmission in study group was 73.08%. The study suggested the absolute necessity to screen TORCH infection for ladies who had the narratives of irregular pregnancies so as to forestall birth absconds and perinatal inconveniences. They presumed that ELISA joined with PCR procedure is a significant technique for the analysis of TORCH disease.

Reiche (2000)made a researchat Londrina State University, Parana and the rates of seropositivity to determine the status of various infections. The prevalence rate was recorded for various infections as: American (Ghazi, Telmeseno, & Mahomed, 2002). Trypanosomiasis 0.9%, Syphilis 1.6%, Toxoplasmosis 67% (IgG) and 1.8% (IgM), Rubella 89% (IgG) and 1.2% (IgM), Hepatitis B surface antigen 0.8%, hepatitis C virus 0.8% and HIV infection 0.6%.Odland(2001)surveyed the pervasiveness of various viral diseases corresponding to late premature births, stillbirths, and inherent distortions in sera from Russian pregnant ladies and intermittent aborters. There was little distinction in all out antibodies to cytomegalovirus or parvo B19 between the groups, while the ordinary pregnant women had a fundamentally higher commonness of rubella antibodies. These outcomes show that less women were vulnerable to primary CMV infection in pregnancy in Russia. Characteristic vaccination against rubella infection was lower than in other, unvaccinated female populaces.Based on these observations, vaccination strategies for rubella are now initiated in the Russian Federation.

A seroprevalence report for TORCH agents in pregnant Saudi women were demonstrated by Ghazi *et al.*(2002)using indirect ELISA. 926 samples were screened for antibodies to

TORCH agents, Toxoplasma IgG antibodies were detected in 35.6%, 92.1% for CMV total IgG, 93.3% for rubella -IgG, 90.9% were detected in HSV-1 IgG, 27.1% for HSV-2, and VZV IgG antibodies were found in 74.4%. A 0% seroprevalence rate for HIV-1 and 2was reported from the study. In a prevalence study among the 300 pregnant females attending the antenatal clinic in Amritsar, the seropositivity of Toxoplasma, Rubella and Cytomegalovirus infections were demonstrated. Out of the 300 samples 200 were with BOH and 100 serum samples werewithout any obstetric complications. From 200 samples with BOH 42.5% were with toxoplasmosis, 17.5% were found to be positive for rubella and 29.5% were positive for CMV. The higher seropositivity were reported among the abortion cases with 71.8%, 59.9% and 61% rate of positivity among toxoplasma, rubella and CMV infections.

Alanen *et al*. (2009)recorded the seroprevalences in Finland as 96.2% for Varicella-zoster virus (VZV), 56.3% for Cytomegalovirus (CMV), 54.3% forherpes simplex virusHSV, 46.8% for HSV-1, 9.3% for HSV-2 and 58.6% for parvovirus B19. No infants with anti-CMV IgM antibodies were born to CMV IgG positive women. Surpam, Kamlakar, Khadse, & Qazi(2006)conducted a serological survey of this group of infections in women with BOH. Their study comprised a total of 225 women that includes 150 with bad obstetric history and 75 clinically normal ones with previous full-term deliveries. They recorded the seropositivity for toxoplasma as14.66%, rubella 4.66%, cytomegalovirus 5.33% and herpes simplex virus as 8.66%. Maximum number positivity cases (50%) belonged to 26 – 30 years of age group. They have demonstrated that among the 150 BOH cases 50 (33.33%) were with any one of the TORCH infections, similarly 6 (8%) out of 75 healthy controls were positive for TORCH infections. Seropositivity rate among the women with BOH were significantly higher than the normal healthy women ($p>0.00$).

The predominance of serum antibodies to TORCH infectionsamong women pregnancy or in the early time of pregnancy in Beijing were estimated by (Li, 2009)According to their investigation in different seasons, the prevalence was demonstrated for TORCH in the order as 3%,1.6%, 0.7% and 10.8% in spring, 4.6%, 1.3%, 2.55 and 12.0% during the summer, 4.6%, 3.8%, 2.1% and 13.1% in fall and 3.5%, 3.1%, 1.4% and 7.7% in winter separately. Al-Taie (2010)made a serological study for TORCH infections in women with high delivery risk factors in Mosul. Toxoplasmosis was observed among 43% of women of child bearing age, 12% of

them were positive for rubella, 16% were withCMV and11% were positive for herpes simplex virus.

A prevalence of 19.4%for toxoplasmosis,rubella infection30.4%, 34.7%CMV infection andherpes infection 33.5% were demonstrated**,** among the 380 serum samples collected in and around Varanasi, North India. The seropositivity's for each infection are in the age range of 19-25 years. Mixed infections were reported among 74 positive cases as 37.83% (28/74). It was concluded that the seroprevalence of anti-TORCH complex IgM were more prevalent amongst the pregnant women and the antenatal cases with BOH to be routinely tested for the TORCH to avoid fetal outcomes (Sen, Shukla, & Banerjee, 2012).

2.2.1 *Toxoplasma gondii*

Toxoplasma gondii is one of the well-studied parasites as it is gaining its importance both in medical and veterinary science and it is considered as a suitable model unicellular organism for the cell biology and molecular studies. The discovery of the parasite was first discovered by Nicolle & Manceaux (1908)and its nomenclature was accordingly to its morphology and the host. Toxoplasma gondii is considered as an obligate intracellular parasite infecting a wide range of living organisms, serving as an intermediatehost. It undergoes a complete life cycle with three significant stages: 1) tachyzoite is the infective stage that occurs during the acute stage of the infection and it multiplies in most cell types, 2) bradyzoites is a stage found in latent infection, this form is present in tissue cysts and 3) sporozoite is the resistant form of the parasite and it is found in oocysts(Jones, Dundar, & Caliskan, 2011).

Humans are infected through three principal routes, initially by consuming raw or meat poorly cooked containing spores. According to a study by Beazley & Egerman (1998)bradyzoites were seen in 8% of beef, 20% of lamb and 20% of pork, whereas Dubey(1996)demonstrates that cooking these meats to an internal temperature of 67ºC or freezing the meat below -12ºC kills bradyzoites, thus minimizing the risk of transmission through this route. Secondly, people can consume oocystsdeposited by felines in their defecation,oocysts present in the feline waste box or in an earth. Thirdly, by the transfusion of blood from the carrier to a recipient who isnot immuneor possibly from pregnant mother to the child(also known as congenital toxoplasmosis).

Perinatal infection can be caused by primary infection during pregnancy.In uncommon cases, congenital transmission happens in incessantly infected women, where the reactivation of the parasite occurred due to the immuno-compromised state of the parasite. Jones, Dundar, & Caliskan(2011)demonstrated that in spite of the fact that these diseases are typically either asymptomatic or symptomatic indicative with self-limiting side effects in adults, infection when transmitted from pregnant women to fetus can cause severe health complications and leads to the chronic sequelae in infants not limited to mental retardation, loss of sight and epilepsy. Avelino, Sandhya, Senthamarai, Sivasankari, & Anitha(2009)reasoned out that alternations in mother's immunity during pregnancy were analyzed as a risk factor for toxoplasmosis sera conversion. Among the 3,564 women between 12 and 49 years of age, in the state if Goiana, Goias in Central Brazil, the rate of seroconversion among the pregnant women were 2.2 times higher than the women who were not pregnant. This risk increased to 7.7 in adolescents (12 to 20 years old).

Remington, Mcleod, Thulliez, & and Desmonts(2006)reported that most of the women that are pregnant during acute infection do not give rise to clear manifestations or signsand a few can have general feeling of discomfort, low-grade fever anddisease of the lymph nodes (lymphadenopathy). Whereas Garweg, Gowri, Malini, Bl, & Bv(2015)explained thatvisual changes caused by Toxoplasmic chorioretinitis occurs in rare casescauses reactivation of a chronic infection or recent infection. Lopes, Gonclaves, Mitsuka-Bregano, Freire, & Navarro (2007)demonstrated that chorioretinitis whichis an inflammation of the choroid (thin pigmented vascular coat of the eye) and retina of the eyeare most timesimplicatedwith congenital toxoplasmosis. Less than 60% of uveitis is caused byT. *gondii*. Twolesionscaused by acute retinitis are usually accompanied with progressive visual impairment,serious inflammation and chronic retinitis which could results in the loss of sight.

2.2.2 Rubella Virus

Rubella, also known as German measles, is a disease that affects childhood it has uniquely declined in frequency in North America since the adaptation of the rubella immunization. Without pregnancy, it is normally clinically showed as a gentle self-constrained disease. During the period of pregnancy, be that as it may, the infection can have possibly destroying impacts on the fetus. It has been implicated for countless wastage and for extreme congenital abnormalities (Centers for Disease Control, 2001)

Rubella happens around the world. In the mild zones, top frequency is in pre-spring and late-winter. Prior to the broad utilization of rubella vaccination, which was authorized in 1969, pinnacles of rubella occurrence happened in the United States (U.S.) each six to nine years, and most cases happened in young children presently, in the United States less than 25 cases have been accounted for yearly. In 2004, just ten instances of rubella were accounted for in the United States. In 2005, no affirmed instances of rubella were accounted for in New Jersey. Late serologic studies demonstrate that about 10% of youthful grown-ups are prone to contacting rubellaas of late in the United States, due to the lack of rubella immunization programs in the country of origin of immigrants there has been outbreaks in the immigrant population. In work environments and in the community outbreaks of rubella is now common. CRS presently lopsidedly influences newborn children born to foreign women. Distinguishing and overseeing vulnerable pregnant women who may have been exposed to rubella has been a challenge, particularly in community related outbreaks. In 2004, no instances of CRS were accounted for in the United States. There has not been an announced instance of CRS in New Jersey for about 20 years(Services, 2010)**.**

2.2.3 Cytomegalovirus

Human cytomegalovirus belonged to the Herpesviridae family; they are double stranded linear DNA encircled by an icosahedral viral coat protein encased by a twofold layer of cell a viral glycoproteins and phospholipids. Humans are the only reservoir of CMV. Cytomegalovirus is an ubiquitous organism that can cause infection at any time during the course of life. In various parts of the world, the prevalence of CMV ranges from 40-100% and the rate of seropositivity in developed countries is 50%. About less than15% of new born with congenital CMV display clinical symptoms during childbirth, albeit even kids who seem asymptomatic during childbirth are in danger for neurodevelopmental sequelae(Bopana, 2008)**.**CMV congenital infection causes the syndrome of cytomegalic disease such as hepatosplenomegaly, jaundice, petechia, purpura and microcephaly.

CMV is notably the mostfrequently seen viral opportunistic disease affecting people with Acquired Immune Deficiency Syndrome (AIDS) and it has been recognized in up to 25% of patients with Acquired Immune Deficiency Syndrome (Murray, Rosenthal, Kobayashi, & Pfaller, 2000)**.** Furthermore, it was evidenced by Vandkova & Dvorak(2015)in their study on CMV

Infection in immuno-compromisedpatients recorded the preceding the appearance of Highly Active Antiretroviral Therapy (HAART) used to treat HIV**,** CMV retinitis which is an inflammation of the retina of the eye that can lead to blindness, was the most widely recognized reason for visual deficiency in grown-ups with AIDS. (Emery, 2012) During his investigation among the CMV infected immuno-suppressed patients, CMV is found to be an important cause of morbidity and mortality. In his study he further explained that the primary infection is significant during childhood, at the point when the resistant framework is undermined reactivations happen, by means of through disease, for example, HIV immunity(infant) or through organ transplantation, the infection can display its full pathogenic potential.

Adler, Palmer, & Pearson (2014)demonstrated that the seronegative child-bearingmothers (less than 44 years of age) who have an acute primary infection have the highest riskof acquiring transplacentaltransmission of CMV. They added that for child-bearing mothers,in contact with the infected children even her own children who had acquired the infection in group day care, was a common primary infectionroute. In addition, about70% of young children get infected with CMV infection in a private residence or other structure in which day care is provided and keep on shedding the infection for 6 to four years (a year and half) after initial infection. This is due to the severityof the virusespecially in young children serving as a major reservoir for the virus.

Hodinka & Friedman (2009)called CMV as a lifelong silent killer or a potential killer. Cannon & Davis (2005)during their investigation on the congenital infection reported that in the congenital CMV infection has been majorly implicated in causing birth malformation and childhood disorders. Approximately 8,000 children were indicated to be affected each year with some neurological sequelae identified with in utero CMV infection with childhood disorders for example, Down syndrome in 4,000 births yearly, fetal alcohol syndrome 5,000 births per year, or spinal bifida 3,500 births per year, Congenital CMV disease tops the most well-known reason for birth, malformations and childhood disorders in the United States. Consideringall these, CMV-related long-term neurological disabilities, more attention should be paid to understand the neuropathogenesis of congenital CMV infection.

CMV diseases are present in nature and for the most part show no manifestations however clinically significant infections are seen often among the pregnant women, neonates and

also the immunocompromised patients (Brooks, Caroll, & Butel, 2007)It was evaluated that worldwide 40,000 kids (about 2% of deliveries) are born with CMV and it causes around 400 severe cases each year. For up to 90% of childrenwho are symptomatic, and around 15%asymptomatic to the infection, create at least one lifelong neurological sequelae, for example, mental impediment, psychomotor hindrance, SNHL, and ophthalmologic abnormality(Cheeran, Lokensgard, & Schleiss, 2009)

2.2.4 Herpes Simplex Virus

Herpes simplex viruses are divided into two major groups, HSV type 1 and HSV type 2. They both can be transmitted through epithelial mucosal cells and skin interruptions, and then move to nerve tissues,where they linger in a latent stage. HSV 1 prevails in orofacial lesions and found in the trigeminal ganglia,while HSV 2 is most normally found in the lumbosacral ganglia. Both of these infections can contaminate any district of the body, with rates of HSV 1 at present expanding in the genital tract (Kriebs , 2008)**.**HSV 1 or HSV 2 incubation period ranges from 2 to 12 days. Most people infected with HSV are mostly asymptomatic to the virus,pregnant women who have the virus are also asymptomatic**.**

Gupta, Warren, & Wald (2007)explained that a primary disease creates when a vulnerable individual (lacking of previous HSV 1 and 2 antibodies) is presented to HSV. For sure, a latent disease happens when an individual with previous HSV antibodies encounters a first episode with the other HSV type. Recurrent infections of HSV occurs when an individual with previous antibodies against the equivalent HSV type. Contaminations during pregnancy might be transferred to infants: both HSV types may causemeningoencephalitis, eye or skin injuries, disseminated diseases, or fetal deformities.

Gardella & Brown (2011)reported that if the infections occur during the first trimester of pregnancy, an expansion in spontaneous premature births and instances of intrauterine fetal development limitation. Just in uncommon cases there is the transmission of the infection through the placenta, bringing about an extreme intrinsicinfection that occurs withhepatosplenomegaly, microcephaly, intrauterine fetal loss, and intra uterine growth retardation. The antivirals are usedduring the first three months of pregnancy if the mother's lesions are especially severe**.**There is a higher risk of infection during the third trimester of

pregnancy**.** In this situation there is a short period for case IgG development**,** their passage to the fetus and the risk of fetal is as high in 50% of cases(Corey & Wald, 2009).

Kapranos & Kotronia (2009)while reviewing the herpes infections demonstrated that the mechanism of the role of HSV in the loss of pregnancy is still under speculation. Reactivated endometrial HSV disease might be related with a resulting increment in killer cell action which has been seen as identified with pregnancy mortality, or it might cause dysregulation of the cytokine shift mechanism. HSV may likewise be connected with improved apoptosis in decidual and trophoblastic tissues, which has been proposed as a potential component of spontaneous pregnancy misfortune. Besides, a thrombogenic activity brought about by HSV on the uteroplacental vessels prompting circulatory unsettling disruption can't be precluded as an instrument of spontaneous pregnancy loss, given the way that HSV just as cytomegalovirus (CMV) can cause production of thrombin and endothelial impairment**.**

2.3 TORCH infections diagnosis

Scheduled examination for this group of infection in new borns with just rashness or intrauterine development impediment is probably not going to yield positive outcomes and is, along these lines, not suggested (Greenough, et al., 1994).To distinguish those newborn children in whom further clinical assessment (i.e., cranial computed tomography scan, ophthalmology assessment) or research facility examinations might be advantageous.Recognizable proof of a congenital infection in the fetal stage of pregnancy in both symptomatic and therapeutic interest. it is only during the developmental stage of pregnancy testing and follow-up's permit affirmation of a congenital infection. along these lines, inherent illness must be accepted in light of the fact that postnatal acquisition can't by and large be blocked. For example, it is only possible to recognize inherent CMV disease by the presence of CMV in urinal specimens procured in the primary month of life. After that time, perinatal or postnatal obtainment can't be restricted. Antimicrobial treatment is effective in upset or restricting the outcomes of sequelae in children with syphilis and toxoplasmosis at whatever point it begins not long after birth.

The identification of primary TORCH infection is investigated in terms of seroconversions of these infections are measured. Be that as it may, since reports of seroconversion is uncommon, as pregnant women are not routinely screened for TORCH antibodies before pregnancy, the identification of IgM antibodies has been utilized as a pointer to

the stage of infection. Various units can be utilized; understanding changes from 56% to 75% with affectability somewhere in the range of 30% and 88%(Lazzarotto, Brojanac, Maine, & Landini, 1997). At the point when against IgM antibodies are recognized in the patient, the identification stays open, since they can't generally be corresponded to primary infection. pregnant women during pregnancy can create IgM during infection reactivations as explained byLazzarotto *et al*.(1997). Moreover, anti-CMV IgM antibodies have been recognized during pregnancy from six to nine months after the resolution of the acute period of primary infection.

2.4 Treatment of TORCH infections

Most healthy people recover from toxoplasmosis without treatment. Persons who are ill may be treated with a combination of drugs such as pyrimethamine and sulfadiazine, plus folinic acid. Pyrimethamine is conceivably teratogenic and ought not be utilized in the early trimester of pregnancy (Frenkel, 2013).The medication produces reversible, typically slow, portion related paralysis of the bone marrow. All patients who get pyrimethamine ought to have total platelet which are observed closely. Folic acid is utilized for decrease and counteraction of the hematological toxicity levels of the medication. Administered Pregnant women, newborns, and infants can be treated, although the parasite is not eliminated completely. The parasites can remain within tissue cells in a less active phase; their location makes it difficult for the medication to completely eliminate them.

In the management of CMV infection, distinctmethods are used in antiviral treatment, prophylaxis precautionary and suppressive treatment.Ganciclovir, a powerful inhibitor in vitro and a nucleoside simple of guanine. Valganciclovir, is effective in CMV treatment, is a prodrug of ganciclovir. The use of the two doses has been related with myelosuppression whichdepends on doses used. Foscarnet, a pyrophosphate simple with in vitro movement against all HSV infections just as human immunodeficiency viruses has likewise been valuable in treatment of CMV retinitis in AIDS patients. Nephrotoxicity and electrolyte irregularity are the most well-known harmfulness related with foscarnet**.** Foscarnet could be used as an alternative drug of choice in cases of ganciclovir resistance**.** Oral Ganciclovir can be administered orally to patients with more severe AIDS.

Routine prophylaxis has not been used as a standard in most HIV treatment centers, for the most part because of the significant expense of prophylaxis, possible toxicity and the

inconvenience of taking 12 tabs daily. Pregnant women with a first clinicaloccurrence or a repeat might be treated using acyclovir or valacyclovir at the suggested doses. The use of Acyclovir and valacyclovir are not formally affirmed for treatment**,** patients ought to be educated to give assent before the organization. In any case, no expansion of fetal variations from the norm was attributed to these medicines, albeit long haul results were not assessed (Centers for Disease and control, 2006).Drug administration is usually by the 36 week of pregnancy to reduce the recurrence of, vertical transmission,clinical signs**,**and reduction of the viral load during birth of the baby and also eliminating the need for caesarean,acyclovir and valacyclovir are the drugs of choice(Anzivino, Fioriti, & Mischitelli, 2009)

CHAPTER THREE

METHODOLOGY

3. MATERIALS AND METHODS

3.1. Research Design

A cross-sectional research design which is ideally suited to assess the prevalence of TORCH infections among pregnant women was done based on the investigation made at one specific point of time fromJanuary 2016 to December 2018 over a period of three years. For the evaluation of association between the frequency of infection and the TORCH infections, the samples from women attending the hospitals were collected.

3.2. Study Area and Population

The samples were collected from Near East University laboratory Cyprus, established in 1988, is located in Nicosia, capital of North Cyprus, is the capital and largest city of the de facto state of Northern Cyprus. A census conducted in 2011 puts the city population 61,378, inhabitants.

3.3. Sample Size and Study Procedure

A total of 1286 serum samples from pregnant women.The age ranges of the study population were between 17 years to 46 years. Socio demographic data were obtained at the sampling site from the study subjects using a pre-designed structured questionnaire. The patients were well informed and a written consent was obtained at the time of sample collection, preceding blood testing an exceptional survey was completed by every patient, which contains all necessary data.

3.4. Specimen Collection and Preparation for Analysis

For each woman; 5ml of blood samples were collected using a sterile disposable needle and syringe. Blood samples gotten from the patient were taken to the laboratory within 30 minutes of sampling and centrifuged to separate the serum. The Serum was stored at -20ºC.with and were labelled accordingly for further study. Screening of Torch IgG and IgM testing was performed using IgM and IgG Abbott architect i1000SR kits.

3.4.1 ELISA lgG/1gM

In the laboratory, the evaluation of specific TORC IgG and IgM antibodies was carried out using the commercial testing kit "ELISA" (Architect i1000 SR ABBOTT, USA). The levels of anti-TORC IgG and IgM were measured in the beginning of pregnancy in accordance with the manufacturer's instructions (Architect i1000 SR ABBOTT, USA). The measurement of TORC-IgG was quantitative and it is expressed in international units per milliliter (IU/mL); but the TORC -IgM measurement was done using an index report by the computation of the cut-off point

Eight ml blood sample was collected in aseptic condition in plain tube without anticoagulant. Serum was separated and store at -20°C. These samples were tested for the presence of Anti-TORC IgG and IgM antibodies by using ELISA technique according to the manufacture's instruction. Both positive and negative controls were proceeds with tests samples. In this study ELISA method was chosen for the detection of IgM antibodies as ELISA method is a sensitive technique for the detection of IgM antibodies. The test value more than 1.2 was considered as a positive sample while the value less than 1.0 was considered as negative. The values between 1.0 and1.2 were considered equivocal. The test was repeated after 1-2 weeks for those patients whose sample results were inthe range of equivocal. In the present study, we included those samples from pregnant females that were suspected of TORCH infection. Those females were excluded who had chronic infection of TORCH. Inform consent was taken from all the participants. The present study was approved by the ethical committee of the Near East University. North Cyprus.TORC Avidity test was performed by ELISA method in order to make differential diagnosis of acute or chronic disease in cases with TORC IgG and IgM positivity.

3.4.2 Preparationof Analysis

The study was carried out following the manufacturer's instructions. Samples with serum separation completed were vortexed at low speed before starting the study.

Serum samples were examined visually and homogenous samples were studied**.**

Non-homogeneous sera were vortexed until homogeneous.

Serums separated from blood samples can be stored at room temperature if they are to be taken into operation within 3 hours. Serums separated from blood samples can be stored at 2-8 degrees if they are to be taken into the study within 14 days. Serums separated from blood samples can be stored at -20 ° C if the study is to be performed for more than 14 days.

3.4.3 Assay Procedure

The microparticle bottle was resuspended before loading the reagent kit into the instrument. . If microparticles are still confined to bottle, continue to invert the bottle until the microparticle have been completely resuspended. Calibration of the device is done with control sera. The Architect TORC IgM or IgG control value must be within the acceptable ranges as specified in the control package insert. If a control out of its specific range the associated test result are invalid and sample must be retested recalibration may be indicated. Abbott Architect i1000 automated system prepared for the study of pregnant women's serum is loaded into the device. Minimum sample cup volume is calculated by system and printed on the order list report to minimize the effect of evaporation, verify adequate sample cup volume is present prior to running.

Specimen cannot be diluted for the ARCHITECT TOXO IgM assay. If the Toxo IgG result is> 2000 IU / ML, an automatic dilution protocol is applied. The system performs 1:10 dilution of specimen and automatically calculates concentration of specimen before dilution and reports the result. When test is conduct using TOXO IgG assay file specimen flagged as > 2000IU/ML will be automatically retested in 1 :10 dilutions.

3.5 Interpretation of the result

TORCH agents, the results are provided in the form of ratios which are a relative measure for the concentration of antibodies

Negative Result or non-reactive- Any sample ratio less than 0.9should be considered as negative.

Borderline result or equivocal- Any sample ratio between 0.9-1.1). These samples should be tested again and if the same result is obtained, another assay should be performed after 1-2

weeks. Possible causes of these borderline results could be contaminated samples, non-specific reactions.

Positive Results or reactive- Any sample ratio greater than1.1 should be considered as reactive.

TORC IgG Avidity test method

The IgG avidity ELISA test consists of an immunoenzymaticallyassay in which a destabilizing agentof hydrogen bridges, such as urea and thiocyanate, is used to dissociate the binding between thespecific IgG and the antigen, in such a way that in recent infections, low avidity IgGs are almost totally dissociated from the antigen-antibody complex, while high-avidity chronic infections they remain mostly bound to T. gondii antigens

Procedure of avidity

The methodology is explained in more detail as follows: the microtiter plates coated with toxoplasma antigens and then blocked are washed 3 times with PBS plus 0.05% Tween 20 (PBST). The serum samples were diluted 1/200 and added (100 µl / well) in 2 rows of a plate (row A and row B), after incubation for 45 min at 37°C; row B is washed 3 times with PBST, and row A is washed 3 times with the modified PBST buffer containing 6 M urea and a fourth time with PBST.Anti-human IgG conjugated with horseradish peroxidase (HRP) is added at the dilution of 1/1000 in PBST. After incubation and washing, the chromogenic substrate, o-Phenylenediamine (OPD) is added. The reaction was stopped by addition of sulfuric acid 20%. The absorbance (Abs) was read with an automated ELISA reader at 492 nm. Avidity index (AI) expressed in percentage was calculated as the result of Abs of wells washed with PBS-urea (U +), divided by the Abs of wells washed with PBST (U-), and multiplied by 100, based on the formula; AI = Abs (U +) / Abs (U-) × 100.

3.6 Statistical Analysis

The information was processed by utilizing Statistical Package of Social Science (SPSS) version16. Information depiction was introduced in percentages with their meanswere determined to show the size and precision of the assessed qualities calculated.

CHAPTER FOUR

RESULT

In this study, for the TORCH screening, ELISA technique was used. The samples collected from 1286 pregnant women were tested for IgM and IgG antibodies at Near East University Hospital. This chapter contains the result of the findings after conducting the test.

Table 4.1:Table showing the year, frequency and percentages of each year studied

		Frequency	Percent
Valid	2016	503	39,1
	2017	452	35,1
	2018	331	25,7
	Total	1286	100,0

Table 4.1 shows the years studied were between 2016 to 2018 and the total number of samples for TORCH were 1286, the frequency of occurrence from highest to lowest are in the order of 2016(506), 2017 (452), and year 2018(321).

Table 4.2: Different age groups and frequency

		Frequency	Percent
Valid	17-25	339	26,4
	26-35	812	63,1
	>35	135	10,5
	Total	1286	100.0

Table 4.2 above illustrates the age specific distribution and clinical presentation of TORCH cases. These females were categorized into three groups,17-25, 26-35 and ages above 35 and the mean age of the females was 28.95 ± 4.97. Majority of TORCH cases were in the age group of

26-35(63,1%) this age gap is the most productive stage of motherhood, followed by ages 17-25 (26,4%)] and the least were ages greater than 35 (10,5%).

Table 4.3: Patients descriptions enrolled in the study

Information	Number
Total number examined	1286
Age ranges 17 up to 46 years	28.95 ± 4.97

Table 4.4: Result of Rubella IgM, IgG and Avidity

Rubella IgM	1249 (%97.1) Nonreactive (<0.9) 25 (%2.0) Reactive (>1.1) 12 (%0.9) Grayzone (0.9-1.1) 1286 patients
Rubella IgG	73 (%7.0) Nonreactive (<5) 939 (%90.2) Reactive (>10) 29 (%2.8) Grayzone (5-10) 1041 patients
Rubella Avidity	0 (%0) Low avidity (<40) 1 (%9.1) Borderline (40-60) 10 (%90.9) High avidity (>60) 11 patients

Table 4.5: Result of Toxo IgM, IgG and Avidity

Toxo IgG	746 (%81.1) Nonreactive (<1.6) 165 (%17.9) Reactive (>3) 9 (%1.0) Grayzone (1.6-3) 920 patients
Toxo IgM	1249 (%98.7) Nonreactive (<0.5) 17 (%1.3) Reactive (>0.6) 0 (%0) Grayzone (0.5-0.6) 1266 patients
Toxo Avidity	1 (%16.7) Low avidity (<0.2) 2 (%33.3) Borderline (0.2-0.3) 3 (%50) High avidity (>0.3) 6 patients

Table 4.6: Result of CMV IgM, IgG and Avidity

CMV IgG	47 (%5.5) Nonreactive (<6) 806 (%94.5) Reactive (>6) 853 patients
CMV IgM	1227 (%98.8) Nonreactive (<0.85) 13 (%1.0) Reactive (>1) 2 (%0.2) Grayzone (0.85-1) 1242 patients
CMV Avidity	0 (%0) Low avidity (<40) 0 (%0) Borderline (40-65) 6 (%100) High avidity (>65) 6 patients

.

Table 4.7: Table showing Rubella IgG Cross tabulation

			RubellaIgG			
			Nonreactive (<5)	Reactive (>10)	Grayzone (5-10)	Total
Age	17-25	Count	15	251	5	271
		Expected Count	19,0	244,4	7,5	271,0
		% within age2	5,5%	92,6%	1,8%	100,0%
		% within RubellaIgG2	20,5%	26,7%	17,2%	26,0%
	26-35	Count	49	594	18	661
		Expected Count	46,4	596,2	18,4	661,0
		% within age2	7,4%	89,9%	2,7%	100,0%
		% within RubellaIgG2	67,1%	63,3%	62,1%	63,5%
	>35	Count	9	94	6	109
		Expected Count	7,6	98,3	3,0	109,0
		% within age2	8,3%	86,2%	5,5%	100,0%
		% within RubellaIgG2	12,3%	10,0%	20,7%	10,5%
Total		Count	73	939	29	1041
		Expected Count	73,0	939,0	29,0	1041,0
		% within age2	7,0%	90,2%	2,8%	100,0%
		% within RubellaIgG2	100,0%	100,0%	100,0%	100,0%

Table 4.7 shows a consistent reduction in % within age and a reduction in % within RubellaIgG2at the age range of above 35 years (10,0%). Statistically there was no significant relationship between age groups and Rubella IgG positivity. (p=0.318).

Table 4.8: Tableshowing Rubella IgM Cross tabulation

			RubellaIgM			
			Nonreactive (<0.9)	Reactive (>1.1)	Grayzone (0.9-1.1)	Total
Age	17-25	Count	264	6	1	271
		Expected Count	262,4	5,5	3,1	271,0
		% within age2	97,4%	2,2%	,4%	100,0%
		% within RubellaIgM2	26,2%	28,6%	8,3%	26,0%
	26-35	Count	636	15	10	661
		Expected Count	640,0	13,3	7,6	661,0
		% within age2	96,2%	2,3%	1,5%	100,0%
		% within RubellaIgM2	63,1%	71,4%	83,3%	63,5%
	>35	Count	108	0	1	109
		Expected Count	105,5	2,2	1,3	109,0
		% within age2	99,1%	,0%	,9%	100,0%
		% within RubellaIgM2	10,7%	,0%	8,3%	10,5%
Total		Count	1008	21	12	1041
		Expected Count	1008,0	21,0	12,0	1041,0
		% within age2	96,8%	2,0%	1,2%	100,0%
		% within RubellaIgM2	100,0%	100,0%	100,0%	100,0%

Table 4.8 above depicts the nonreactive and the reactive result of the Rubella IgM tests. For the reactive tests, the % within RubellaIgM2reduced drastically with increase in age at above 35 years with, 0%. Statistically there was no significant relationship between age groups and Rubella IgM positivity. (p=0.309).

Table 4.9: Table showing Toxo IgM Cross tabulation

			ToxoIgM		
			Nonreactive (<0.5)	Reactive (>0.6)	Total
Age	17-25	Count	332	4	336
		Expected Count	331,5	4,5	336,0
		% within age2	98,8%	1,2%	100,0%
		% within ToxoIgM2	26,6%	23,5%	26,5%
	26-35	Count	786	11	797
		Expected Count	786,3	10,7	797,0
		% within age2	98,6%	1,4%	100,0%
		% within ToxoIgM2	62,9%	64,7%	63,0%
	>35	Count	131	2	133
		Expected Count	131,2	1,8	133,0
		% within age2	98,5%	1,5%	100,0%
		% within ToxoIgM2	10,5%	11,8%	10,5%
Total		Count	1249	17	1266
		Expected Count	1249,0	17,0	1266,0
		% within age2	98,7%	1,3%	100,0%
		% within ToxoIgM2	100,0%	100,0%	100,0%

Table 4.9 shows the % increase within ToxoIgM2 increase for the age range of 17 -25 and 26-35 (23,5%; 64,7% respectively) years and drops drastically with age increase of above 35 years (10,5%). Statistically there was no significant relationship between age groups and Toxo IgM positivity. (p=0.954).

Table 4.10:Table showing Toxo IgG Cross tabulation

			ToxoIgM			
			Nonreactive (<1.6)	Reactive (>3)	Grayzone (1.6-3)	Total
Age	17-25	Count	212	36	0	248
		Expected Count	201,1	44,5	2,4	248,0
		% within age2	85,5%	14,5%	,0%	100,0%
		% within ToxoIgG2	28,4%	21,8%	,0%	27,0%
	26-35	Count	465	107	5	577
		Expected Count	467,9	103,5	5,6	577,0
		% within age2	80,6%	18,5%	,9%	100,0%
		% within ToxoIgG2	62,3%	64,8%	55,6%	62,7%
	>35	Count	69	22	4	95
		Expected Count	77,0	17,0	,9	95,0
		% within age2	72,6%	23,2%	4,2%	100,0%
		% within ToxoIgG2	9,2%	13,3%	44,4%	10,3%
Total		Count	746	165	9	920
		Expected Count	746,0	165,0	9,0	920,0
		% within age2	81,1%	17,9%	1,0%	100,0%
		% within ToxoIgG2	100,0%	100,0%	100,0%	100,0%

A significant relationship existsin Toxo IgG positivity and age groups (p = 0.002). Accordingly, women over 35 years of age were significantly higher (23.2%) of Toxo IgG positive. In addition, it has been determined that Toxo IgG positivity increases with age.

Table 4.11:Table showing CMV IgM Cross tabulation

			CMVIgM			Total
			Nonreactive (<0.85)	Reactive (>1)	Grayzone (0.85-1)	
Age	17-25	Count	326	5	0	331
		Expected Count	327,0	3,5	,5	331,0
		% within age2	98,5%	1,5%	,0%	100,0%
		% within CMVIgM2	26,6%	38,5%	,0%	26,7%
	26-35	Count	771	7	2	780
		Expected Count	770,6	8,2	1,3	780,0
		% within age2	98,8%	,9%	,3%	100,0%
		% within CMVIgM2	62,8%	53,8%	100,0%	62,8%
	>35	Count	130	1	0	131
		Expected Count	129,4	1,4	,2	131,0
		% within age2	99,2%	,8%	,0%	100,0%
		% within CMVIgM2	10,6%	7,7%	,0%	10,5%
Total		Count	1227	13	2	1242
		Expected Count	1227,0	13,0	2,0	1242,0
		% within age2	98,8%	1,0%	,2%	100,0%
		% within CMVIgM2	100,0%	100,0%	100,0%	100,0%

Table 4.11 above shows the reduction in % within CMVIgM2 as the age increased from 35 years. The % drop occurs after 26-35 (53,8%), when the age is > 35 (7, 7%).Statistically there was no significant relationship between age groups and CMV IgM positivity. (p=0.710).

Table 4.12:Table showing CMV IgG Cross tabulation

			CMVIgG		Total
			Nonreactive (<6)	Reactive (>6)	
Age	17-25	Count	10	214	224
		Expected Count	12,3	211,7	224,0
		% within age2	4,5%	95,5%	100,0%
		% within CMVIgG2	21,3%	26,6%	26,3%
	26-35	Count	32	507	539
		Expected Count	29,7	509,3	539,0
		% within age2	5,9%	94,1%	100,0%
		% within CMVIgG2	68,1%	62,9%	63,2%
	>35	Count	5	85	90
		Expected Count	5,0	85,0	90,0
		% within age2	5,6%	94,4%	100,0%
		% within CMVIgG2	10,6%	10,5%	10,6%
Total		Count	47	806	853
		Expected Count	47,0	806,0	853,0
		% within age2	5,5%	94,5%	100,0%
		% within CMVIgG2	100,0%	100,0%	100,0%

Table 4.12: This table depicts the drop in % within CMVIgG2 with increase in age at 35 years (10,5%). The drop occurred from age range of 26-35(62,9%) to >35 (10,5%). Statistically there was no significant relationship between age groups and CMV IgG positivity. (p=0.719).

Table 4.13:Comparative results of TORCH

TORCHinfections	IgG No(%)		IgM No (%)	
	Positive	Negative	Positive	Negative
Toxoplasma	165(17,9)	746(81,1)	17(1,3)	1249(98.7)
Rubella	939(90,2)	73(7,0)	21(2,0)	1008(96,8)
CMV	806(94,5)	47(5,5)	13(1)	1227(98.8)

Out of total 1286 cases with the TORCH antibody (IgG and IgM) titers showed the following results: In Toxoplasma, 17,9% IgG positive, 1,3% IgM positive, 81,1% IgG negative, and 98,7% IgM negative. In Rubella, 90,2% IgG positive, 2,0% IgM positive, 7,0% IgG negative, and 96,8% IgM negative. In CMV, 94,5% IgG positive, 1,0% IgM positive, 5,5% IgG negative, and 98,8% IgM negative.

CHAPTER FIVE

DISCUSSION

5.1 Discussion

T. gondii, CMV and rubella cause just asymptomatic or mild disease in the mother yet can have substantially more extreme ramifications for the unborn baby. Congenital, intra-uterine contaminations are regularly the reason for inborn variations from the norm, intra-uterine development lacks and fetal death, bringing about both financial and social concerns. A significant part of pre-birth care is the acknowledgment of these infections in the mother and the fetus. At present, routine pre-birth screening for some TORCH diseases is usually during the first trimester of pregnancy since patients who are seronegative develops primary infections, which increases risk of vertical transmission to the growing baby.

Antibody screening for antibody results is deciphered as (I) IgG-positive result, IgM-negative outcome during an early gestational period affirms previous infection, with no need for additional activity; in any case, IgG avidity testing might be suggested. (ii)IgG-positive result, IgM-positive outcome recommends a current infection and is be followed by IgG avidity testing. High levelsof avidity demonstrate lesser chance of congenital infection; though lower levels of avidity show a higher risk. In situations of lower avidity levels, the patient ought to be directed that vertical transmission is certainly not inevitable, and counter measures can be utilized to recognize fetal infections. (iii) IgG-negative result, IgM-positive outcome recommends an ongoing infection and a high chance of vertical transmission. The patient ought to be followed to report seroconversion and advised equivalent to the patient with an IgG-positive, IgM-positive outcome. (iv) IgG-negative result, IgM-negative outcome shows a possibility of primary infection, and the patient ought to be guided to take precautionary measures to lessen the risk of infection, for example, evasion of direct contact with bodily materials from others (especially young children) and frequent hand washing (Coll, et al., 2009);(Guerra, et al., 2007); (Adler, Finney, Manganello, & Best, 2004)

In this study 1286 samples were collected from pregnant women. These samples were tested for the presence of IgM and IgG antibodies by using Abbott architect i1000SR technique. Mean age of the females was 28.95 ± 4.97. These females were categorized from 17-25, 26-35 and ages

above 35 (Table 2). Out of 1286 patients, 1249 (97.1%) were nonreactive for Rubella IgM TORCH infection, 25 (2.0%) were reactive while 12 (0.9%) were within grayzone . Out of 1041 patients 73 (7.0%) were nonreactive for Rubella IgG, 939 (90.2%) were reactive while 29 (%2.8) were within grayzone. Out of 920 patients, 746 (81.1%) were nonreactive for Toxo IgG TORCH infection, 165 (17.9%) were reactive while 9 (%1.0) were within grayzone also, a relationship between Toxo IgG and age groups ($p = 0.002$). Accordingly, women over 35 years of age were significantly higher (23.2 %) of Toxo IgG positive. In addition, it has been determined that Toxo IgG positivity increases with age. Out of 1266 patients, 1249 (98.7%) were nonreactive for Toxo IgM TORCH infection, 17 (1.3%) were reactive, while 0 (0%) were within grayzone. Out of 853 patients, 47 (5.5%) were nonreactive for CMV IgG TORCH infection while 806 (94.5%) were reactive. Out of 1242 patients, 1227 (98.8%) were nonreactive for CMV IgM TORCH infection, 13 (1.0%) were reactive while 2 (%0.2) were within grayzone in this study.

As of late, IgGavidity have been proposed to be a reasonable technique in distinguishing between past, recurrent infections or acute infections. These are used to investigate for Toxoplasma gondii, CMV, and for some different agents. Low avidityfor a pathogenic infection shows an ongoing disease, however high rate of avidity rules out infection in the last 3-4 months(Curdt, et al., 2009); (Murat, et al., 2012). In this study, the avidity detected in all patients screened for anti-CMV IgG avidity was considered favorable in terms of reduced risk of congenital infection. The avidity results in this study is shown to be significant across the organism's in order of the highest to the lowest 100% in cytomegalovirus, (6/6), Rubella 90.9% (10/11), and Toxoplasma 50%(3/6), significantly.High or intermediate level of avidity indicates a low risk of congenital infection; low avidity indicates ahigher risk of congenital infection. In cases of low avidity, the patient should be counseled that vertical transmission is still possible therefore shouldn't be ruled out, and additional methods can be utilized to distinguish fetal disease.

These results indicates a low T.*gondii* seroprevalence, which is in accordance with several studies conducted in other countries such as United States, United Kingdom, Sweden, Norway and Spain.((Nash, Chissel, & Jones, 2005); (Allain, Palmer, & Pearson, 1998); (Jenum, Stray-Pedersen, & Malby, 1998);(Gutierrez-Zufiaurre, Sanchez-Hernandez, & Munoz, 2004); (Peteerson, Strat-Pedersen, Malm, Forsgren, & Evengard, 2000)and(Jones, Kruszon-Moran,

Sanders-Lewis, & Wilson, 2001).The climatic, topographic and socioeconomical characteristics of these countries may be attributed to the lower T. gondii seroprevalence present in the regions. The frequency of inherent Toxoplasma infection likewise varies from nation to nation and is evaluated to influence 1-10 for each 10,000 infants in Europe(Gilbert R. , 1999). In a study in Turkey, the general pace of seropositivity for T. gondii antibodies is between 43-85%.(Harma, Gungen, & Demir, 2004)**.** Seroprevalence of Toxoplasma was additionally quite lower in the western part of Turkey than in the eastern and mid-anatolian parts.(Harma, Gungen, & Demir, 2004),The high seroprevalence of T. gondii in Turkey is credited to the nearness of an incredible number of stray animals such as cats in both rustic and urban territories of the nation. The Cypriot diet, which consists of large amounts of raw, wild vegetables, salads and undercooked meats that could easily be contaminated with parasite. Primary prevention of toxoplasmosis in the seronegative pregnant mother can be accomplished through training to rehearse prudent steps, which incorporate washing the hands often, washing all vegetables and foods grown from the ground, critically, avoiding the consumption of raw meat. Besides, as detailed in this investigation, there is a mild difference in the seropositivity of toxoplasma antibodies among age group studied, which requires further examination to evaluate whether, is there any critical affiliation exists among toxoplasmosis and age.

The seropositivities of the pregnant women for anti-rubella out of 1286 patients, 1249 (97.1%) were nonreactive for Rubella IgM TORCH infection which confirms a current or recent infection, 25 (2.0%) were reactive while 12 (0.9%) were within grayzone. Out of 1041 patients 73 (7.0%) were nonreactive for Rubella IgG, 939 (90.2%) were reactive while 29 (%2.8) were within grayzone. The presence of IgM rubella antibodies in the blood demonstrates an ongoing disease Seropositivities of rubella were reported to be 87 % in USA(Danovaro-Holliday;, LeBraron, & Allensworth, 2000).93.3-94% in Saudi Arabia. (Ghazi *et al.*,2000; (El-Mekki & Zaki, 1998),98% in Spain (Pedranti, Adamo, Macedo, & Zapata, 2007),95.3% in Mozambique (Barreto, Sacramento, & Robertson, 2006), and 95-96.2% in Turkey (Ocak, Zeteroglu, Ozer, Dolapcioglu, & Gungoren, 2007),(Kanbur, Derman, Kutluk, & Kinik, 2003), and(Aksakal, Maral, Cirak, & Aygun, 2007) in pregnant ladies. The study of disease transmission of rubella contamination has been altered since the time the presentation of the rubella vaccination. Danovaro-Holliday *et al.*(2000) revealed that childhood immunization strategies alone may not be sufficient, and that work environment immunization of high-risk adults should be thought of.

Despite the fact that rubella vaccine introduced in the national childhood immunization program for quite a long while in Turkey, there are as yet unvaccinated women in childbearing age.

Cytomegalovirus is the most frequently implicated in congenital infection among children causing long term neurodevelopment sequlae (Idris, Adamu, & Mohammad, 2016).Infections caused by CMV canoccur in any period of pregnancy. congenital CMV infection rate is between 0.2 and 2.2% of all births in the world(Tekay & Özbek, 2007). In this study, the seropositivities of the pregnant women for anti-CMV IgG was 94.5% (806) Seropositivities of CMV were reported in pregnant women as 39%-94.7% in USA(Colugnati, Staras, Dollard, & Cannon, 2007); (Staras, Dollard, & Radforf, 2006)), 56.8% in Australia (Munro, Hall, & Whybin, 2005),30.4% was also reported in Ireland(Knowles, Grundy, Cahill, Cafferkey, & Geary, 2005),84% in Spain (Estripcaut, Moreno, Ahumada, & et al, 2007)and 100% in Thailand. (Wong, Tarik, Tee, & Yeo, 2000). Several studies have reported between 84.5% and 95% prevalence of anti-CMV IgG among pregnant women in Turkey (Yilmazer, et al., 2004); (Ocak, Zeteroglu, Ozer, Dolapcioglu, & Gungoren, 2007)

This study shows seropositivity for CMV IgM to be 1.0%. The findings were in agreement with studies by Turbadkar *et al.*. and Padmavathy *et al*., where seropositivity rates were also low at 8.42% and 9.2 % respectively. (Turbadkar, Mathur, & Rele, 2003); (Padmavathy, et al., 2007),However studies conducted by Yasodhara *et al.*, Rajendra Surpam *et al*., and Gumber *et al.*, showedsignificantly lower seropositivities of 5.8%, 5.33%, 4.67% respectively**.**(Yasodhara & et al., 2001)(Rajendra, Surpam, & et al., 2006)(Grumber & et al, 2008)). Such low seropostivitycan be attributed to betterliving conditions. CMV IgG avidity testing has been asignificant research center apparatus for investigating essential CMV contamination during pregnancy. Low avidity shows primary infection inside the first trimester of pregnancy, with an elevated risk of intrauterine transmission to the growing baby. High avidity during the first trimester of pregnancy rules out post conception primary infection and demonstrates significant lesser chance of intrauterine transmission. Anti-CMV IgG avidity was high at 100% in all serum samples (n=6) analyzed. Periodic antenatal screening alongside with behavioral and educational intervention is important to control CMV transmission.

CHAPTER SIX

6.1 CONCLUSION AND RECOMMENDATIONS

In Conclusion, the Toxoplasma and Rubella and CMV IgM antibody positivity rate was discovered low in this study, on the other hand the antibodies of CMV IgG and Rubellapositivity was high among TORCH infections and have also has a high rate of avidity than Toxoplasma. It is possible that the prevalence of these infections may be increased in Cyprus, but due to lack of awareness, eating or dietary habits, life style, climate conditions, cultural limitation, and mostly people are reluctant to visit to doctors during pregnancy. We also concluded that TORCH has an adverse effect on child birth during pregnancy. We therefore, make an effort to find out the prevalence and the importance of these infections during pregnancy to reduce the risk of-morbidity and mortality in the region. Serologic screening before pregnancy is important to diminish or possibly reduce the morbidity and mortality caused by T. *gondii*, rubella and CMV. Antibody test for TORCH organisms among women during the gestation period, and follow-up assessments until delivery are standard procedures during pregnancy. Widespread screening may contribute largely to the prevention of congenital infections caused by TORCH agents

Subsequently, all the prenatal, cases ought to be frequently evaluated for Rubella antibodies, as ahead of schedule as identification and mediation will help in legitimate administration of these cases. Likewise, this examination accentuates the need to plan a viable rubella vaccination program to support the declining immunity. Perinatal infections are a major source of serious congenital anomalies, and can lead to significant fetal morbidity and mortality. By recognizing these infections, the physician is better ready to screen and treat both the mother and baby, and prompt guardians on conceivable fetal results.

REFERENCES

Adler, J. P., Palmer, C. R., & Pearson, G. (2014). Epidemiological study of latent and recent infection by Toxoplasma gondii in pregnant women from a regional population in the UK. *Journal of infection, 36*, 189-196.

Adler, S. P., Finney, J. W., Manganello, A. M., & Best, A. M. (2004). Prevention of child-to-mother transmission of cytomegalovirus among pregnant women. *The Journal of Pediatrics, 145*, 485–491.

Aksakal, F. N., Maral, I., Cirak, M. Y., & Aygun, R. (2007). Rubella seroprevalence among women of childbearing age residing in a rural region: Is there a need for rubella vaccination in Turkey? *Japanese Journal of Infectious Diseases, 60*, 157-160.

Alanen, E., Fioriti, D., Mischitelli, M., & Bellizzi, A. (2009). Herpes simplex virus infection in pregnancy and in neonate: Status of art of epidemiology, diagnosis, therapy and prevention. *Virology journal, 112*, 1-11.

Allain, J. P., Palmer, C. R., & Pearson, G. (1998). Epidemiological study of latent and recent infection by Toxoplasma gondii in pregnant women from a regional population in the UK. *Journal of infection*, 189-196.

Al-Taie, A. (2010). Serological Study For TORCH Infections In Women With High Delivery Risk Factors In Mosul. *Tikrit Journal of Pure Science, 15*(1), 193-197.

Anne, M. F., & Elizabeth, C. (2011). Stewart Diagnostics, Etiology and outcome of fetal ascites in a South African hospital. *Internationall journal of gynecology and obstetrics*, 148-152.

Anzivino, E., Fioriti, D., & Mischitelli, M. (2009). Herpes simplex virus infection in pregnancy and in neonate: status of art of epidemiology, diagnosis, therapy and prevention. *Virology of Journal*(6), 40.

Arvin, S., & Whitley, U. (2011). Prevalence of IgM antibodies to toxoplasma, rubella and cytomegalovirus infections during pregnancy. *JK Science*, 190-192.

Avelino, S., Sandhya, B., Senthamarai, S., Sivasankari, S., & Anitha, C. (2013). Serological evaluation of herpes simplex virus Type-1/Type-2 infections in pregnant women with bad obstetric history in a tertiary care hospital,. *International journal of advanced research, I*, 123-128.

Barreto, J., Sacramento, I., & Robertson, S. E. (2006). Antenatal rubella serosurvey in Maputo, Mozambique. *Tropical Medicine & International Health, 11*, 559-564.

Beazley, D., & Egerman, R. (1998). Toxoplasmosis . *Seminars in Perinatology, 22*, 332- 338.

Bopana, K. D. (2008). Seroprevalence of TORCH in women with still birth in RIMS hospital. *The Journal of the Medical Society*(22), 2-4.

Boyer, S., & Boyer, K. M. (2004). Update on TORCH infections in the newborn infant. *Newborn and Infant Nursing Reviews, 4*, 70-80.

Brooks, G., Caroll, K., & Butel, J. (2007). Herpes viruses In: . In M. &. Jawetz.

Cannon, M. J., & Davis, K. (2005). Washing our hands of the congenital cytomegalovirus disease epidemic. *BMC Public Health*(5), 70.

Cao, Y., Qui, L., & Zhang, Q. (1999). Study on the relationship between the history of abnormal pregnancy and TORCH infection in pregnant woman. *Zhonghua fu chan ke za zhi34:*, 517-520.

Centers for Disease and control. (2006, August 4). Sexually Transmitted Diseases Treatment Guidelines / 55(RR11);. *MMWR Recommendations and Report*, 55(RR11);1-94.

Centers for Disease Control. (2001). Control and prevention of rubella: evaluation and management of suspected outbreaks, rubella in pregnant women, and surveillance for congenital rubella syndrome. *MMWR Recommendations and Reports , 50*, 1–23.

Cheeran, M. C., Lokensgard, J. R., & Schleiss, M. (2009). Neuropathogenesis of congenital cytomegalovirus infection: disease mechanisms and prospects for intervention. *Clinical microbiology reviews, 22*(1), 99–126.

Colak. (2007). Control of the risk of human toxoplasmosis transmitted by meat. *international journal of parasitology*, 1359-1370.

Coll, O., Benoist, G., Ville, Y., Weisman, L. E., Botet, F., Maurizio M. Anceschi, t., . . . Carbonell-Estrany (coordinator), X. (2009). Guidelines on CMV congenital infection. *Journal of Perinatal Medicine, 37*(5), 433-445. doi:https://doi.org/10.1515/JPM.2009.127

Colugnati, F. A., Staras, S. A., Dollard, S. C., & Cannon, M. J. (2007). Incidence of cytomegalovirus infection among the general population and pregnant women in the United States. *BMC Infectious diseases, 7*, 71.

Corey, L., & Wald, A. (2009). Maternal and neonatal herpes simplex virus infections. *The New England Journal of Medicine, 361*(14), 1376–1385.

Curdt, I., Praast, G., Sickinger, E., Schultess, J., Herold, I., & Braun, H. B. (2009). Development of fully automated determination of markerspecific immunoglobulin G (IgG) avidity based on the avidity competition assay format: application for Abbott Architect Cytomegalovirus and Toxo IgG Avidity assays. *Journal of clinical Microbiology*, 603-613.

Danovaro-Holliday;, M. C., LeBraron, C. W., & Allensworth, C. (2000). A large rubella outbreak with spread from the workplace to the community. *JAMA, 284*, 2733-2739.

Diagnosis of and screening for cytomegalovirus infection in pregnant women, . (n.d.). *Journa;*.

Dubey, J. (1996). Strategies to reduce transmission of Toxoplasma gondii to animals and humans. *Veterinary Parasitology, 64*, 65-70.

El-Mekki, A. A., & Zaki, Z. M. (1998). Screening for rubella antibodies among Saudi women of child bearing age. *Saudi Medical Journal, 19*, 575-577.

El-Tantawy, N., & Taman, A. a. (2014). Toxoplasmosis and female infertility: is there a coorelation? *American journal of epidemiology and infectious and diseases, 2*, 29-32.

Emery, V. C. (2012). Cytomegalovirus: recent progress in understanding pathogenesis and control. *QJM, 105*(5), 401-405.

Estripcaut, D., Moreno, Y., Ahumada, R. S., & et al. (2007). Seroprevalence of cytomegalovirus infection in puerperal women and its impact on their newborns. *Anales de Pediatria*, 66:135-139.

Frenkel, C. (2013). When should the "TORCH" study be requested? *Paediatrics & Child Health, 23*, 226-228.

Gardella, A., & Brown, Z. (2011). Prevention of neonatal herpes. *Journal of Obstetrics and Gynaecology, 118*(2), 187-192.

Garweg, M., Gowri, M., Malini, J., Bl, U., & Bv, N. (2015). Seroprevalence of TORCH infections and adverse reproductive outcome in current pregnancy with bad obstetric history. *Journal ofclinical and boimedical sciences*(3), 62-71.

Ghazi, O. H., Telmeseno, A. M., & Mahomed, M. F. (2002). TORCH agents in pregnant saudi women. *Medic\al principles and practice*(11), 180-182.

Gilbert, R. (1999). *Epidemiology of infection in pregnant women. Congenital toxoplasmosis: scientific background, clinical management and control.* (P. P. Ambroise-Thomas, Ed.) Paris, France: springer verlag.

Gilbert, R. E. (2012). Mother-to-child transmission and diagnosis of Toxoplasma gondii infection during pregnancy. *Indian journal of medical microbiology*, 69-76.

Greenough, W., Armstrong, K., Comery, T., Hawrylak, N., Humphreys, A., Kleim, J., . . . Wang, X. (1994). *Plasticity related changes in synapse morphology. In: Cellular and molecular mechanisms underlying higher neural functions.* (A. P. (Selverston AI, Ed.) Chichester: Wiley.

Grumber, S., & et al. (2008). Occurance of Cytomegalovirus and Herpes simplex virus infections in Pregnancy. *International Journal of Medical Microbiology*, 204-205.

Guerra, B., Simonazzi, G., Banfi, A., Lazzarotto, T., Farina, A., Lanari, M., & Rizzo, N. (2007). Impact of diagnostic and confirmatory tests and prenatal counseling on the rate of pregnancy termination among women with seropositive cytomegalovirus immunoglobulin M antibody titers. *American Journal of Obstetrics and Gynecology, 221*, e1–e6. doi:10.1016/j.ajog.2006.08.039

Gupta, R., Warren, T., & Wald, A. (2007). Genital herpes. *The Lancet, 370*, 2127–2137.

Gutierrez-Zufiaurre, N., Sanchez-Hernandez, J., & Munoz, S. (2004). Seroprevalence of antibodies against Treponema pallidum, Toxoplasma gondii, rubella virus, hepatitis B and C virus, and HIV in pregnant women. *Clinical Microbiology and Infection*, 512-516.

Harma, M., Gungen, N., & Demir, N. (2004). Toxoplasmosis in pregnant woman in Sanliurfa, South-eastern Anatolia city in Turkey. *Journal of the Egyptian Society of Parasitology, 34*, 519-525.

Hodinka, J., & Friedman, M. E. (2009). Utility of newborn screening cards for detecting CMV infection in cases of stillbirth. *Journal of clinical virology*(44), 215-218.

Idris, A. N., Adamu, B., & Mohammad, S. S. (2016). Clinical Significance of IgG Avidity Testing and other considerations in the diagnosis of congenital Cytomegalovirus infection: A Review Update. *Medical science (Basel), 4*(1), 5.

Jamieson, D. J., Theiler, R. N., & Rasmussen, S. A. (2006). Emerging Infections and Pregnancy. Emerging Infectious Diseases, 12(11),. (2016). *12*(11), 1638-1643.

Jamieson, D. J., Theiler, R. N., & Rasmussen, S. A. (2016). Emerging Infections and Pregnancy. *Emerging Infectious Diseases, 12*(11), 1638-1643.

Jenum, P. A., Stray-Pedersen, B., & Malby, B. (1998). Incidence of Toxoplasma gondii infection in 35 940 pregnant women in Norway and pregnancy outcome for infected women. *Journal of clinical microbiology*, 2900-2903.

Jones, G., Dundar, D., & Caliskan, E. (2011). Seroprevalence of Toxoplasma gondii, rubella and cytomegalovirus among pregnant women in western region of Turkey. *Clinical and investigative medicine, 32*, E43-47.

Jones, J. L., Kruszon-Moran, D., Sanders-Lewis, K., & Wilson, M. (2001). Toxoplasma gondii infection in the United states: Seroprvalence and risk factors. *American journal of epidemiology*, 357-365.

Kanbur, N., Derman, O., Kutluk, T., & Kinik, E. (2003). Age-specific rubella seroprevalance of an unvaccinated populatıon of adolescents in Ankara Turkey. *Japanese journal of infectious diseases, 56*, 23-25.

Kapranos, N., & Kotronia, D. (2009). *Detection of Herpes Simplex Virus in First Trimester Pregnancy Loss Using Molecular Techniques. in vivo, 23*(5), 839-842.

Kaur, R., Gupta, N., Nair, D., & Kakkar, M. M. (1999). Screening for TORCH infections in pregnant women: a report from Delhi. *Southeast Asian J. Trop. Med. Public Health, 30*, 284-286.

Knowles, S. J., Grundy, K., Cahill, I., Cafferkey, M. T., & Geary, M. (2005). Low cytomegalovirus sero-prevalence in Irish pregnant women. *Irish medical journal*, 98:210-212.

Kriebs , J. M. (2008). Journal of Midwifery & Women's Health,. *Breaking the cycle of infection: TORCH and other infections in women's health, 53*(3), 173-174.

Lazzarotto, T., Brojanac, S., Maine, G. .., & Landini, M. (1997). Seroprevalence of HSV1 and HSV2 infections in family planning clinic attenders. *Journal of communicable diseases*(4), 307-309.

Li, Z. Y. (2009). The prevelance of the serum antibodies to TORCH among women before pregnancy or in the early period of pregnancy in Beijing. *Chinica Chimica Acta, 404*, 212-215.

Lopes, F., Gonclaves, D., Mitsuka-Bregano, R., Freire, R., & Navarro, I. (2007). Toxoplasma gondii infection in pregnancy. *Brazilian Journal of Infectious Diseases, 11*(5), 496-506.

Munro, S. C., Hall, B., & Whybin, L. R. (2005). Diagnosis of and screening for cytomegalovirus infection in pregnant women,. *Journal of medical microbiology*, 43:4713-4718.

Murat, J. B., L'Ollivier, C., Fricker, H. H., Franck, J., Pelloux, H., & Piarroux, R. (2012). Evaluation of the new Elecsys Toxo IgG avidity assay for toxoplasmosis and new insights into the interpretation of avidity results. *Clinical and Vaccine Immunology*, 1828-1843.

Murray, P., Rosenthal, K., Kobayashi, & Pfaller, M. (2000). *Human Herpesviruses. Medical Microbiology*. Mosby Inc.

Nash, J. Q., Chissel, S., & Jones, J. (2005). Risk factors for toxoplasmosis in pregnant women in Kent, United Kingdom. *Epidemioligy of infection*, 475-483.

Nicolle, C., & Manceaux, L. (1908). Sur une infection a corpos de Leishman (ou organisms voisins) du gondi. *Comptes rendus de l'Académie des Sciences, 147*, 763-766.

Nielson, A. (2015). Control of the risk of human toxoplasmosis transmitted by meat. *International journal of parasitology*, 1359-1370.

Ocak, S., Zeteroglu, S., Ozer, C., Dolapcioglu, K., & Gungoren, A. (2007). Seroprevalence of Toxoplasma gondii, rubellaand cytomegalovirus among pregnant women in southern Turkey. *Scandanavian journal of infectious diseases*, 231-234.

Odland, J. S.-P. (2001). Seropositivity of cytomegalovirus, parvovirus and rubella in pregnant women. *Acta Obstetricia et Gynecologica Scandinavica*, 1025-1029.

Padmavathy, M., Mangala, G., Malini, J., Umapathy, B. L., Naveneeth, B. V., Mohit, B., & Shruthi, H. (2007). Seroprevalence of TORCH Infections and Adverse Reproductive Outcome in Current Pregnancy with Bad Obstetric History. *Journal of clinical and medical science*, 39:.

Pedranti, M. S., Adamo, M. P., Macedo, R., & Zapata, M. T. (2007). Prevalence of anti-rubella and anti-parvovirus B19 antibodies in pregnant women in the city of Córdoba, and in women of fertile age in the city of Villa Mercedes, province of San Luis. *Revista Argentina de Microbiología, 79*, 47-50.

Peteerson, K., Strat-Pedersen, B., Malm, G., Forsgren, M., & Evengard, B. (2000). Seroprevalance of Toxoplasma gondii among pregnant women in Sweden. *Acta Obstetricia et Gynecologica Scandinavica*, 824-828.

Pizzo, A. (2011). Management f neonatal herpes simplex virus. *paediatric drugs*, 81-90.

Population Reference Bureau. (2012, July 25). *World Data Sheet.* Retrieved from http://www.prb.org/pdf12/2012-population-data-sheet_eng.pdf

Rajaram, P., Agrawal, A., & Swain, S. (1995). Determinants of maternal mortality: a hospital based study from south India. *Indian Journal of Maternal & Child Health, 6*, 7-10.

Rajendra, B., Surpam, & et al. (2006). Department of Microbiology, Indira Gandhi Govt. Medical College, Nagpur. Serological study for TORCH infections in women with BOH. *Journal of Obstetrics and Gynaecology* , 947-1091.

Rao, E., Zamir, C. S., Rilkis, I., & Ben-David, H. (2005). Congenital toxoplasmosis--prenatal aspects of Toxoplasma gondii infection. *Reproductive toxicology*, 458-472.

Reiche, E. e. (2000). Prevalence of American trypanosomiasis, syphilis, toxoplasmosis, rubella, hepatitis B, hepatitis C, human immunodeficiency virus infection, assayed through serological tests among pregnant patients, from 1996 to 1998, at the Regional University Hospital N. *Revista da Sociedade Brasileira de Medicina Tropical, 33*, 519- 527.

Remington, J., Mcleod, R., Thulliez, P., & and Desmonts, G. (2006). Toxopladsmosis In:. In K. J. Remington JS, *Infectious disease of the fetus and newborn infant* (6TH ed., pp. 947-1091.). Philadelpphia: Elsevier Saunders.

Sadik, M. S., Fatima, H., Jamil, K., & Patil, C. (2012). Study of TORCH profile in patients with bad obstetric history. *Biology and Medicine, 4*, 95-101.

Sen, M., Shukla, B., & Banerjee, T. (2012). Prevalence of serum antibodies to TORCH infection in and around Varanasi, North India. *Journal of Clinical and Diagnostic Research, 6*(9), 1483-1485.

Services, N. J. (2010). Rubella and Congenital Rubella Syndrome. *Communicable Disease Service Manual*, 1-43.

Sharma, M. S., Grupta, H., Ganguly, k., Mahajan, C., & Malla, G. (2012). study of TORCH profile in patients with bad obstetric history. *Biology and medicine*, 95-101.

Sivanandhan, E. (2015). Maternal serologic screening to prevent congenital toxoplasmosis: a decision-analytic economic model. *PLOS Neglected Tropical Disease*, e1333.

Stagno, S., Pass, B., & Cloud, E. (2010). Infectious diseases of the fetus and newborn infant. In *Infectious diseases of the fetus and newborn infant* (pp. 740-781). Philadelphia: Elsevier.

Staras, S. A., Dollard, S. C., & Radforf, K. W. (2006). Seroprevalence of cytomegalovirus infection in the United States, 1988-1994. *Clinical infectious diseases, 43*, 1152-1153.

Surpam, R. B., Kamlakar, U., Khadse, R. K., & Qazi, M. (2006). , Serological study for TORCH infections in women with bad obstetric history. *The Journal of Obstetrics and Gynecology of India, 56*(1), 41-43.

Taechowisan, T., Sutthent, R., Louisirirochanakul, S., Puthavathana, P., & Wasi, C. (1997). Immune status in congenital infections by TORCH agents in pregnant Thais. *Asian Pacific Journal of Allergy and Immunology,, 15*, 93-97.

Tekay, F., & Özbek, E. (2007). The seroprevalence of Toxoplasma gondii in women from Sanlıurfa a province with a high raw meatball consumption. *31*, 176=179.

Turbadkar, D., Mathur, M., & Rele, M. (2003). Seroprevalence of torch infection in bad obstetric history. *Indian journal of medical microbiology*, 108-110.

Vandkova, D., & Dvorak, A. (2015). Seroprevalence of torch infections in bad obstetrics history in HIV and non-HIV women in Solapur district of Maharashtra. *Journal of Human Virology & Retrovirology*.

Wilcox, A., Weinberg, C., & O'Connor, J. (1988). Incidence of early loss of pregnancy. *The New England Journal of Medicine: Research & Review*(319), 189-194.

Wong, A., Tarik, K. H., Tee, C. S., & Yeo, G. S. (2000). Seroprevalence of cytomegalovirus, Toxoplasma and parvovirus in pregnancy. *Singapore medical journal, 41*, 151-155.

Yasodhara, P., & et al. (2001). Prevalence of specific IgM due to Toxoplasma, Rubella, Cytomegalo virus and Chlamydia trachomatis infections during pregnancy. *International Journal of Medical Microbiology, 19*(2), 52-56.

Yilmazer, M., Altindis , M., Cevrioglu, S., Fenkc, V., Aktepe, O., & Sirthan, E. (2004). Toxoplasma, Cytomegalovirus, Rubella, Hepatitis B and Hepatitis C Seropositivity Rates in Pregnant Women Who Live in Afyon Region. *Kocatepe medical journal*, 2:49-53.

YAKIN DOĞU ÜNİVERSİTESİ
BİLİMSEL ARAŞTIRMALAR ETİK KURULU

ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi : 24.12.2020

Toplantı No **:** 2020/86

Proje No **:**1218

Yakın Doğu Üniversitesi Tıp Fakültesi öğretim üyelerinden Yrd. Doç. Dr. Ayşe Arıkan Sarıoğlu'nun sorumlu araştırmacısı olduğu, YDU/2020/86-1218 proje numaralı ve **"Frequency of TORCH infections in pregnant women in Northern Cyprus"** başlıklı proje önerisi kurulumuzca online toplantıda değerlendirilmiş olup, etik olarak uygun bulunmuştur.

Prof. Dr. Rüştü Onur

Yakın Doğu Üniversitesi

Bilimsel Araştırmalar Etik Kurulu Başkanı