EVALUATION OF ANTI-RUBELLA ANTIBODIES AMONG CHLIDBEARING AGE WOMEN IN BABYLON GOVERNORATE

A THESIS

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By

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تقييم لأضداد الحصبة الألمانية بين النساء في سن الحمل

في محافظة بابل

رسالة مقدمة إلى

مجلس كلية الطب, جامعة بابل

كجزء من متطلبات نيل شهادة

الماجستير في الأحياء المجهرية الطبية

من قبل

وسام حمزة حمد

٢٠٠٦ م ١٤٢٧ هـ

بسم الله الرحمن الرحيم

وَهوَ الَذي أَنشَأَكُمْ مِنْ نَفْسٍ واحِدَةٍ فَمُستقرٌّ

ومسْتَودعٌ قَدْ فَصّلنا الآيات لقومٍ يَفْقَهُونْ

صدق الله العلي العظيم

(سورة الأنعام، الآية ٩٨)

الخلاصة

أجريت دراسة للفترة من الأول حزيران عام ٢٠٠٥ إلى الثلاثين من تموز عام ٢٠٠٦, فحصت خلالها عينة متكونة من ٢٥٠ امرأة في سن الحمل (١٥–٤٥) سنة, وقد اختيرت هذه العينة عشوائيا من المناطق الحضرية والريفية من محافظة بابل. وقد أجريت هذه الدراسة لتحديد الحالة المناعية للنساء في سن الحمل ضد مرض الحصبة الألمانية.

فحصت النساء بواسطة فحص المقايسة المناعية الماصة والرابطة للأنزيم للكشف عن الأجسام المناعية المضادة نوع (IgG) وكانت نسبة الانتشار الإجمالية (٧٧.٦%) . و فحص اثباط التلازن الدموي للكشف عن الأجسام المناعية المضادة (IgG and IgM) وكانت نسبة الانتشار الإجمالية (٨٠.٠%) .

وبالنسبة للعوامل الديموغرافية, فقد أظهرت الدراسة أن أعلى نسبة للايجابية بواسطة فحصي ELISA و HAI (٨٥.٩٦% و ٨٧.٧% على التوالي) كانت في المراحل العمرية (٢٥–٢٩) سنة بينما أوطأ نسبة (٦٦.٧٪)كانت في النساء اللواتي أعمارهن أكبر أو يساوي أربعين سنة. كما أظهرت الدراسة أن أعلى نسبة الايجابية كانت بين مجاميع النساء اللواتي يسكن المناطق الحضرية (٨٢.٥٦٪), عاليات التعليم (٨٨.٩٪), و النساء العاملات (٩٤.٤٤٪) . وكذلك أن أعلى نسبة للايجابية كانت بين النساء الحوامل عنها دون غير الحوامل (٧٨.٣٣٪), مع ذلك فالنساء الحوامل في الثلاثة اشهر الأولى من الحمل اظهرن أعلى نسبة ايجابية (٧٩.٦٪) من النساء الحوامل في الثلاثة اشهر الثانية والثالثة. وفيما يخص الإنجابية فان الدراسة أظهرت أن أعلى نسبة للايجابية كانت بين النساء اللواتي لهن ٢–٣ أطفال (٨٥.٠٪).

وقد أشارت الدراسة أن وسط عيار الأجسام المضادة للحصبة الألمانية بواسطة فحص اثباط التلازن الدموي كان عاليا (١٩٥٢) في النساء اللواتي يتراوح أعمارهن (٢٥–٢٩) سنة وواطئاً (٢٨٠) في النساء اللواتي أعمارهن اكبر أو يساوي ٤٠ سنة. أن وسط العيار كان أعلى في النساء اللواتي يسكن المناطق الحضرية (١٢٦٢.٤), اللواتي لديهن تعليم ثانوي (١٣٠٧.٩) و النساء العاملات (١٤٩٦.٥). بينما لم يكن هنالك فرق مهم في وسط العيار بين النساء الحوامل وغير الحوامل. على الرغم من ذلك, فان النساء الحوامل في الثلاثة اشهر الثانية من الحمل أظهرت وسطا أعلى من غيرهن (١٠١٤.٤). كما أن النساء اللواتي لديهن طفلين اظهرن وسطا أعلى من النساء الأخريات (١١٢٤.٥).

Summary

A study was conducted from ١st June ٢٠٠٥ to ٣٠th July ٢٠٠٦, in which a sample of ٢٥٠ women in childbearing age (١٥-٤٥) years, were selected randomly from urban and rural regions of Babylon Governorate. These women were selected from those attending Babylon Maternity and Children Hospital, and Public Health Laboratory. This study has been carried out to determine anti-rubella antibodies among women in childbearing age.

The women included in this study were tested by enzyme-linked immunosorbent assay for IgG antibodies giving an overall prevalence of (٧٧.٦٪), and hemagglutination-inhibition test for rubella IgG and IgM antibodies giving an overall prevalence of (٨٠.٠٪).

Regarding the sociodemographic variables, the study revealed that the highest rate of seropositivity was in age group (٢٥-٢٩) years by ELISA and HAI tests (٨٥.٩٦% and ٨٧.٧١% respectively), while the lowest rate was in older age group ≥٤٠ years (٦٦.٧%) for both tests. Also the study revealed that the rate of seropositivity was higher among women who lives in urban areas by ELISA and HAI tests (٨٢.٥٦٪ and ٨٣.٤٨٪ respectively), women with high educational level (٨٨.٩%) for both tests, and employees women (٩٤.٤٤٪) for both tests. Also, pregnant women had higher rate of seropositivity (٧٨.٣٣٪) than non-pregnant ones. However, pregnant women in the first trimester had highest rate of seropositivity by ELISA and HAI tests (٧٩.٦% and ٨١.٦٣٪ respectively) than women in the second and third trimester. Regarding the parity, the study revealed that the multipara women with three children had the highest rate of seropositivity (٨٥.٠٪) for both tests than others.

The present study showed that the mean of the titer of anti-rubella antibodies by hemagglutination-inhibition test, was high (١٩٥٢ ± ١٦٤١.٩) in women with age group (٢٥-٢٩) years, and low (٢٨٠ ± ٨٠) in the older age women ≥٤٠ years. Also, the mean of the titer was higher among women who live in urban areas (١٢٦٢.٤ ± ١٣٧٥.٤), women with secondary educational level (١٣٠٧.٩ ± ١٣٥٠.٢), and employees women (١٤٩٦.٥ ± ١٦٨٣.٣). While in pregnant women and non-pregnant ones, there is no significant difference in the mean of the titer between them. However, the pregnant women in the second trimester show high mean of the titer (١٠١٤.٤ ± ١٣٥٢.٩) than others, and multipara women with two children had high mean of the titer (١١٢٤.٥ ± ١٣٨٣) than others.

Introduction

Rubella is an acute febrile illness, which is caused by rubella virus, from Togavirus family genus Rubivirus. The disease is characterized by a rash and lymphadenopathy that affects children and young adults. It is the mildest of common viral exanthems (Brooks *et al.,* ٢٠٠٤). However, infection during early pregnancy may result in serious abnormalities of the fetus, including congenital malformations and mental retardation. The consequences of rubella in utero are referred to as the congenital rubella syndrome (CRS) (Creasy *et al.,* ٢٠٠٤). With a rare exception, the mother is the source of infection for the fetus or neonates; thus, virtual infection is said to be vertically transmitted (Hanshaw and Dudgeon, ١٩٧٨).

Rubella was known as German measles, because it was first described by two German physicians in the mid-eighteenth century. For many years German measles was confused with other diseases causing a rash (such as measles and scarlet fever). It was eventually recognized as a distinct disease by an International Congress of Medicine in London in ١٨٨١, and the name rubella was accepted at about that time (Zuckerman *et al.,* ٢٠٠٠).

Although maternal viral illness is a common event during pregnancy, many viral infections are limited to a localized site, such as the respiratory epithelium, gastrointestinal epithelium, or skin. Only viruses that produce a maternal viremia are capable of infecting the placentofetal unit (Modlin, ١٩٨٦). However, the list of viruses that may cause congenital infection is growing. In addition to rubella viruses, cytomegalovirus, varicella zoster virus,

the human immunodeficiency viruses, and human parvovirus B١٩ may infect the developing fetus. Transplacental infections with Japanese encephalitis and Lassa fever viruses have also been reported, as has occasionally been the case with hepatitis B virus and Herpes simplex virus (Best and Banatvala, ١٩٩٠).

Before use of rubella vaccine, rubella epidemics involved about ٥٪ of the population, although only nearly ١٠٪ of these cases were reported to public health authorities (Horstmann, ١٩٧١). Since the licensure of rubella vaccine in ١٩٦٩, the number of CRS cases has declined (CDC, ١٩٩٧). The goal of the rubella vaccination program is to prevent the consequences of infection during pregnancy. Many countries do not have rubella vaccination programs or have only recently implemented such programs, and many adults throughout the world remain susceptible (CDC, ٢٠٠١). In ١٩٩٦, the World Health Organization (WHO) estimated that ٣٦٪ of member countries offered routine rubella vaccination (Robertson *et al.,* ١٩٩٧). In ١٩٩٩, WHO estimated that ٥٢٪ of countries offered routine rubella vaccination, in the region of the Americas, ٨٩٪ of countries used rubella vaccine (WHO, ٢٠٠٠).

In Iraq, some aspects of the rubella antibodies have been studied by researchers (Al-Moslih *et al.,* (١٩٨٨); Yaseen (١٩٩٢); and Al-Heety (٢٠٠٠).

In Babylon, no study was conducted on rubella antibodies, therefor; this study was conducted with the following aims:

١. Determination of anti-rubella antibodies among women in childbearing age.
٢. Study of sociodemographic variables: age, residency, level of education, occupation, pregnancy (pregnant or non-pregnant), trimester, and parity.

٣. Study the titer of anti-rubella antibodies in relation to sociodemographic variables.

Review of Literature

٢-١: History

Feigin and Cherry (١٩٩٢), had reported that In ancient history, rubella as a disease is lost among the other prominent exanthematous diseases (i.e., scarlet fever, measles, and smallpox). Also they mentioned that In an extensive review, Griffith suggested that rubella was known to the early Arabian physicians under the name al-hamikah; they considered rubella a form of measles (Feigin and Cherry, ١٩٩٢). Forbes (١٩٦٩), had reported that two German physicians, de Bergan in ١٧٥٢ and Orlow in ١٧٥٨ are generally credited with the first clinical description of rubella as a specific entity (Forbes, ١٩٦٩). In early writings, rubella was generally referred to as Rötheln. However, because of the great interest of German physicians in the disease during the period from the mid-١٨th to the mid-١٩th centuries, the name German measles was frequently employed in other countries (Feigin and Cherry, ١٩٩٢).

Zuckerman *et al*.,(٢٠٠٠), had reported that in ١٨٦٦, a Scottish physician named Veale described ٣٠ cases of German measles. In his paper, he gave the illness its present name, rubella. It was his opinion that the German name, Rötheln, was too harsh and foreign, and that other possible names- rubeola notha and rosalia idiopathica- were too long for general use and could be confused with measles. Also he had reported that in ١٨٨١ at the International Congress of Medicine in London, a consensus was reached that rubella was a

distinct disease. By the beginning of the ٢٠th century, the clinical description of rubella was quite complete (Zuckerman *et al.,* ٢٠٠٠). Lee and Bowden (٢٠٠٠), had reported that in ١٩٣٨, Hiro and Tasaka demonstrated that rubella was a disease of viral etiology by transmission of disease in humans by the subcutaneous injection of filtered nasal washing (Lee and Bowden, ٢٠٠٠).

The notion that rubella was only a mild illness of children was dispelled in ١٩٤١, when Norman Gregg, an Australian ophthalmic surgeon, reported the occurrence of congenital cataract among ٧٨ infants born following maternal infection in early pregnancy. This report was soon followed by others by Australian, Swedish, American and British epidemiologists and teratologists, who confirmed the role of rubella virus in congenital cataracts, and also noticed the presence of heart disease and deafness in infant. Thus, the characteristic congenital rubella triad was established (Plotkin and Orenstein, ١٩٩٩).

In ١٩٦٢, the isolation in cell culture of the etiological agent of rubella was reported by two independent groups, Parkman *et al.,* infected African green monkey kidney cells with throat washing from patients with rubella and indirectly demonstrated the presence of rubella virus by resistance to challenge with an echovirus. While Weller and Neva detected cytopathic effects in human amnion cells infected with rubella virus from blood and urine specimens. It had now been shown that rubella virus could grown in a wide variety of cell culture systems, and this had been the corner stone of vaccine development (Lee and Bowden, ٢٠٠٠).

٢-٢: Rubella Virus

Rubella virus is classified as the only member of the genus Rubivirus within the family Togaviridae; the name "togaviurs" was derived from Latin "toga", meaning cloak or shroud, a reference to the virus envelope. The genus Alphavirus was the only other genus within this family and comprises at least ٢٦ members (Murphy *et al.,* ١٩٩٥).

While humans are the only known natural hosts for rubella virus, vertebrates and arthropods, such as mosquitoes, were recognized hosts for alphaviruses. Rubella virus and the alphaviruses possess similar characteristics in terms of replication strategy and genomic organization (Lee and Bowden, ٢٠٠٠).

` The rubella virion is spherical with a diameter of ٦٠ to ٧٠ nm, and it contains three major polypeptides-E١, E٢, and C (Wolnisky, ١٩٩٦). E١ (MW ٥٨٠٠٠) and E٢ (MW ٤٢٠٠٠ to ٤٧٠٠٠) are glycoselated and are located on the viral surface membrane. E١ is the viral hemagglutinin that is found on ٥ to ٦ nm surface projections (Petterson *et al.,* ١٩٨٥). The nucleocapsid has a diameter of ٣٠ to ٤٠ nm and is composed of polypeptide (C protein) and the genomic RNA. The nucleic acid of rubella virus is single-stranded RNA with a molecular weight of ٣.٢ to ٣.٨ × ١٠^{٦}. The outer coat of the virus (envelope) is lipoprotein in nature with host-cell lipid and virus-specified polypeptides (Feigin and Cherry, ١٩٩٢).

The RNA genome of rubella virus is infectious, and complementary DNA copies facilitate study of its transcripts (Wang *et al.,* ١٩٩٤). Rubella virus is immunologically distinct from the other togaviruses; there is only one serotype

of rubella virus, and analysis of sequence variation among several isolates reveal high conservation of amino acid structure (Bosma *et al.,* ١٩٩٦).

Rubella virus will grow in many different tissue cultures including cell strains, cell lines and primary cells (Cunningham and Fraser, ١٩٨٥). In tissue culture, rubella virus growth can be identified by either cytopathic effect (CPE) or the ability to produce interference of the growth of another tissue culture-susceptible virus. For primary isolation of rubella virus from clinical material, the most commonly used method is the interference technique employing primary African green monkey kidney (AGMK) cells. Infection is demonstrated in the AGMK tissue culture by the failure of typical enterovirus CPE to occur after challenge of the culture with echovirus ١١ or another suitable enterovirus. A common alternative to the AGMK- echovirus ١١ interference system for primary rubella virus isolation is the use of the RK-١٣ rabbit kidney cell line in which infection can be identified by CPE (Feigin and Cherry, ١٩٩٢). BHK-٢١ and Vero cells cultures are useful for virus isolation because they do not produce interferon, and virus can therefor replicate more rapidly to high titer (Zuckerman *et al.,* ٢٠٠٠). Rubella virus is relatively high sensitive to heat, ultraviolet light, pH extremes of less than ٦.٨ and more than ٨.١, and to a variety of chemical agents like ether, acetone, and formalin; however at ٤cº the virus titer is relatively stable for ٢٤ hours (Gershon *et al.,* ٢٠٠٤).

Rubella virus infection of tissue culture cells result in the production of infectious virus that can be neutralized by specific antiserum. Specific viral antigens can be identified by hemagglutination, complement fixation, precipitation, platelet aggregation, and immunoflourescence (Feigin and Cherry, ١٩٩٢). Techniques employing careful control of test system diluents have revealed that red cells from many different animals (like pigeon, goose,

and lamb) are agglutinated by rubella virus. Viral hemagglutinin is stable at -٢٠ Cº for many months and at ٤Cº for several weeks but is rapidly destroyed by heat (Liebhaber, ١٩٧٠). There are two distinct rubella component fixing antigens. One of the antigens is similar in size and weight to both hemagglutinin and infectious virus, the other is soluble antigen, smaller, non-infectious, and does not contain nucleic acid. Two major small-particle antigens have been identified in the medium of tissue culture-infected cells by immunodiffusion. These two soluble antigens are structural components of the virion, and natural infection with rubella virus results in the formation of serum precipitating antibodies, these antigens have been designated theta and iota (Feigin and Cherry, ١٩٩٢). Their importance lies in the fact that antibody to the iota antigen is rarely noted in the serum of recipients of some rubella vaccine; therefore, they may be of value in studying vaccine-induced immunity (Cappel *et al.,* ١٩٧٥)

٢-٣: Definition of disease

Rubella is usually a mild febrile viral disease with a diffuse punctate and maculopapular rash sometimes resembling that of measles or scarlet fever (Chin, ٢٠٠٠). However, infection during the early stage of pregnancy of the mother induces serious congenital disorders of the infected fetus; this condition is called congenital rubella syndrome (Ushida *et al.,* ٢٠٠٣).

٢-٤: Pathology

Almost no data relating to the histologic finding in uncomplicated rubella are available, but occasionally postmortem tissue has been studied from patients with encephalitis. Guiliani and associates studied lymph nodes from rubella infected patients and noted edema, reticulum cell hyperplasia, and loss of the usual follicular morphologic features. Sherman and associates reported six cases of rubella encephalitis and noted the autopsy finding in three cases. Mild, nonspecific, follicular hyperplasia in the spleen and nodes was only seen. Histologic examination of the brain of a ٧-year –old girl who died of encephalitis revealed diffuse swelling; nonspecific degeneration; and a sparse, mononuclear, perivascular and meningeal exudate. The synovial biopsy specimens in a woman with rubella arthritis revealed scattered areas of fibrinopurulent exudate and synovial cell hyperplasia; there was inflammatory cell infiltration composed mainly of lymphocytic cells, and vascularity was increased (Feigin and Cherry, ١٩٩٢).

Rubella virus generally establishes a chronic nonlytic infection in the fetus and has the potential to infect any organ (Wolnisky, ١٩٩٦). Microscopic analyses of aborted infected fetuses revealed cellular damage in multiple sites, with inflammatory necrosis being common in the structures of the eyes, heart, brain, and ears of aborted infected fetuses. Examination of rubella-induced cataractous eye lenses from first-trimester fetuses revealed pyknotic nuclei, cytoplasmic vacuoles, and inclusion bodies in primary lens cells; lens development was found to be retarded. Necrosis is also detected in the endothelial cells within the blood vessels lining the heart and can cause thrombosis of small vessels and necrosis of surrounding tissue; cell destruction

of the myocardial is common. Vascular necrosis lesions within the walls of the cerebral blood vessels may contribute to ischemic brain damage. As with rubella virus-induced deafness in CRS infants, examination of rubella virus-infected fetuses revealed cellular damage to the epithelium of the cochlear duct and/or stria vascularis (Webster, ١٩٩٩).

٢-٥: Pathogenesis

The primary site of infection is the respiratory epithelium of the nasopharynx. Initial infection of the respiratory epithelium apparently is minor; a more important event is the early spread of the virus to the regional lymphatics. Rubella virus multiplies at the respiratory site, and initiates a viremia of approximately ٧ days duration (Fields, ١٩٩٦). Respiratory tract shedding of virus, viremia peaks just before the onset of exanthem and disappears shortly thereafter. In contrast, virus continues to be consistently present in the nasopharynx for a ٦-day period after the onset of rash and occasionally for an additional week thereafter. In addition to the blood and nasopharynx, rubella virus has been recovered from the following sites: lymph node, urine, cerebrospinal fluid, conjunctival sac, breast milk, synovial fluid, and lung. Rubella virus was recovered from the skin of rubella patients at sites where rash was both present and absent (Feigin and Cherry, ١٩٩٢).

With maternal infection during the first trimester, placental infection regularly occurs and often persists throughout the reminder of pregnancy. In therapeutic abortion studies of Alford and associates, fetal infection occurred in about ٥٠٪ of placental infection. Persistence infection is the usual occurrence of first trimester fetal infection. This fetal infection usually involves

multiple organs, and at birth, virus can regularly be isolated from the throat, rectum, and urine. Little is known about events in second and third trimester maternal rubella infection. It is most probable that placental infection is a regular occurrence, and transmission of virus to the infant *in utero* may also occur regularly (Feigin and Cherry, ١٩٩٢). With maternal rubella infection, the cervix is also involved, so that fetal infection could occur by the ascending rout as well as by primary placental infection (Seppala and Vaheri, ١٩٧٤).

Rubella virus can be recovered regularly from infants with congenital rubella after their birth. The percentage of infants with persistence infection decreases over the first year of life; by the first birth day, between ١٠ to ٢٠ % of children will still be shedding virus in nasopharyngeal secretions (Feigin and Cherry, ١٩٩٢).

٢-٦: Postnatally Acquired Rubella

٢-٦-١: Clinical Manifestations

The clinical symptoms of rubella virus infection acquired postnatally are usually mild, and ٢٠٪--٥٠٪ of infected persons are asymptomatic (CDC, ٢٠٠١). The incubation period of rubella is ١٢-٢١days with an average of ١٦-١٧ days (Green-wood *et al.,* ٢٠٠٢).

In the child the first sign of illness is the appearance of the rash. It appears first on the face and then spreads downward rapidly to the neck, arms, trunk and extremities. The eruption appears, spreads, disappears more quickly than does of rash of measles. The duration and extent of the rash may variable, which as a rule lasts for ٣ days, may persist for ٥ days or may be so evanescent that it disappears in less than a day. In adolescents and adults, there is often a ١-٥ day prodrom with low grade fever, malaise, lymphadenopathy, and upper respiratory symptoms preceding the rash (Gershon *et al.,* ٢٠٠٤).

Lymphadenopathy may begin a week before the rash and last for several weeks. Post auricular, posterior cervical, and suboccipital nods are commonly involved (Lee and Bowden, ٢٠٠٠). At times, splenomegaly also may be noted, during the acute stages of disease. Other symptoms of rubella include conjunctivitis, testalgia, and orchitis. Forschheimer spots are reddish, pinpoint or larger in size, and may be noted on the soft palate, but are not diagnostic for rubella. The white blood cell count is low but it may be normal. Occasionally, there may be an increased percentage of abnormal lymphocytes or a decrease in platelets (Gershon *et al.,* ٢٠٠٤).

Natural infection is followed by a very high order of protection from reinfection. However, evidence of reinfection, which is generally, a symptomatic, may be obtained by demonstrating a significant increase in antibody concentration following natural an experimental exposure to rubella. It may be difficult to distinguish between primary infection and reinfection, particularly if blood was not obtained shortly after contact or if sera taken prior to contact (e.g. for screening purposes) are not available. Rubella reinfection is not associated with a lack of neutralizing antibodies or a specific

defect in rubella specific lymphoproliferative responses, but a failure to produce epitope-specific antibodies is possible (Zuckerman *et al.,* ٢٠٠٠).

٢-٦-٢: Complications

Rubella in childhood is rarely followed by complications. Secondary bacterial infections, which are so common in measles, are not encountered in rubella (Gershon *et al.,* ٢٠٠٤). Arthralgia and arthritis may occur in up to ٧٠٪ of adult women who contract rubella, but is rare in children and adult males. Joint symptoms tend to occur about the same time or shortly after the appearance of the rash, and may persist for up to one month (Tingle *et al.,* ١٩٨٦). Rubella arthritis with involvement of the knees, ankles, or elbows may simulate the poly arthritis of rheumatic fever. When there is fusiform swelling of the fingers, it may resemble rheumatoid arthritis (Gershon *et al.,* ٢٠٠٤).

Central nervous system complications (i.e., encephalitis) occur at a ratio of ١ per ٦,٠٠٠ cases and are more likely to affect adults. Thrombocytopenia occurs at a ratio of ١ per ٣,٠٠٠ cases and is more likely to affect children (CDC, ١٩٩٨; Frey, ١٩٩٤).

Guillian-Barre syndrome after rubella has also been reported (Yaginuma *et al.,* ١٩٩٦). Additional complications include orchitis, neuritis and a rarely fatal neurodegenerative disorder termed progressive rubella panencephalitis, which considered as a late complication of childhood rubella (Abe *et al.,* ١٩٨٣).

٢-٧: Congenital Rubella Syndrome (CRS)

Congenital rubella was identified as a clinical entity more than a century after the disease was first recognized. In ١٩٤١ Gregg reported the occurrence

of congenital cataracts among ٢٨ infants born after maternal rubella infection acquired during the ١٩٤٠ epidemic in Australia (Gershon *et al.,* ٢٠٠٤).

Congenital rubella syndrome is a group of physical abnormalities that have developed in an infant as a result of maternal infection and subsequent fetal infection with rubella virus (Robert-Gnansia, ٢٠٠٤). The frequency of congenital infection is critically dependent on the time of exposure to the virus. Approximately ٥٠٪ of infants exposed to the virus within ٤ weeks of conception will manifest signs of congenital infection. When maternal infection occurs in weeks ٥ to ٨ after conception, approximately ٢٥٪ of fetuses will be infected. When it develops in weeks ٩ to ١٢, about ١٠٪ of fetuses will be infected. When it occurs beyond this point in time, less than ١٪ of fetuses are affected (Creasy *et al.,* ٢٠٠٤; Gilbert, ١٩٩٧).

The most commonly described anomalies associated with congenital rubella syndrome are ophthalmologic (cataracts, retinopathy, and congenital glaucoma), cardiac (patent ductus arteriosus, peripheral pulmonary artery stenosis), auditory (sensorineural hearing impairment), and neurologic (behavioral disorders, meningoencephalitis, and mental retardation). In addition, infants with congenital rubella syndrome often are growth retarded and may have radiolucent bone disease, hepatosplenomegaly, thrombocytopenia, and purpuric skin lesions (giving a "blueberry muffin" appearance). Mild forms of the disease can be associated with few or no obvious clinical manifestations at birth (CDC, ٢٠٠١).

Initial reports of maternal reinfection suggested that asymptomatic reinfection in early pregnancy was unlikely to be associated with fetal infection, even when a specific IgM response was detected. Reinfection would provide a hazard to the fetus if it is associated with viremia; this has only rarely

been documented. Morgan-Capner *et al.,* (١٩٩١) have calculated that the risk of fetal infection is nearly (٨.٠%) of following reinfection in the first ١٦ weeks of pregnancy, but fetal malformations are rare (Zuckerman *et al.,* ٢٠٠٠).

٢-٨: Epidemiology

Rubella has a worldwide distribution (Green-wood *et al.,* ٢٠٠٢). Rubella is a winter and spring disease, with the largest number of cases in March, April, and May in the United States. This seasonal pattern occurs in years of both high and low rubella incidence. Presumably, some transmission and sporadic illness occur throughout the year in large urban areas (Wolinsky, ١٩٩٦).

Before wide spread use of rubella vaccine, rubella was an epidemic disease, occurred every ٦ to ٩ years in the United States with the last major United States epidemic occurring in ١٩٦٤-١٩٦٥ (Parkman, ١٩٩٩). Epidemic rubella has been documented in Czechoslovakia in ١٩٧٢; in Australia in ١٩٦٩-١٩٧٠ and ١٩٧٥-١٩٧٦; in Japan in ١٩٧٥ to ١٩٧٧; in Brazil in ١٩٨١; and in the United Kingdom in ١٩٧١ to ١٩٧٣, ١٩٧٨, and ١٩٨٣ (Feigin and Cherry, ١٩٩٢). The epidemiology of rubella in developing countries can be deduced from the seroprevalence of rubella antibodies. The large variation seen in seroprevalence suggests that rubella occur in sporadic epidemics except where population density is high. In the metropolis of Sao Paulo, Brazil, nearly everyone is seropositive by ٢٠ years of age, whereas in rural Mexico, seropositivity varies from ٢٩.٠٪ to ٧٦.٠٪ (Plotkein and Orenstein, ١٩٩٩). Cutts and colleagues reviewed rubella susceptibility data from ٤٥ developing countries and found remarkable differences among them not correlated with geography. For example, Malaysia, Peru, and Nigeria were among the

countries where more than (٢٥.٠٪) of women were found to be seronegative (Cutts *et al.,* ١٩٩٧). With licensure of an effective rubella vaccine in ١٩٦٩, the frequency of rubella has declined by almost ٩٩٪ (CDC, ١٩٩٠). However, a small number of cases still occur each year. Persistence of this infection appears to be due to failure to vaccinate susceptible individuals rather than to lake of immunogenicity of the vaccine (Gabbe *et al.,* ٢٠٠٢).

The age at infection varies from area to area. In developed countries before vaccine development, infection was most common in the ٥ to ٩ years old group, corresponding to the early school years. With the advent of childhood vaccination in the United States, there was a shift in disease incidence to young adult. In countries practicing the selective vaccination policy, there were much higher notification rates in males, as expected (Lee and Bowden, ٢٠٠٠).

Rubella is a human disease, there is no known animal reservoir (Alford, ١٩٨٤). Rubella is transmitted by contact with nasopharyngeal secretions of infected people. Infected persons shed large concentrations of virus in the nose and throat, and droplets of secretions are released into the environment, which allows respiratory to respiratory transmission. It is also quit possible that the initial host may contaminate his or her own hands and then transmit the infectious agent to environmental surfaces or directly to contacts. Under this circumstance, the new host can acquire infection via the formite-hand respiratory or hand-hand respiratory routes (Feigin and Cherry, ١٩٩٢).

Rubella is a highly contagious (Davis *et al.,* ١٩٩٠). The period of communicability is about ١ week before and at least ٤ days after onset of rash; highly communicable. Infants with congenital rubella shed virus from the nose

and throat for many months and have been responsible for spread of virus to susceptible contacts (Chin, ٢٠٠٠).

The incidence of clinical rubella is similar in boys and girls. In adults, more cases of rubella are reported in women. This finding is quite possibly the result of interest and concern relating to congenital rubella rather than a true difference on the basis of sex. It is of interest that in rubella vaccine trials, girls have been noted to have higher geometric mean convalescent-phase antibody titers than do boys (Feigin and Cherry, ١٩٩٢).

No ethnic differences in incidence have been clearly shown, although the characteristic rash is more difficult to diagnose in person with dark skin (Horstmann, ١٩٨٢). There is a trend toward higher rates in lower than in upper socioeconomic groups, this may be due to increased exposure in crowded homes (Cohen *et al.,* ١٩٨٥).

٢-٩: Immune Responses

٢-٩-: Humoral Immune Response

Since viral replication is wide spread throughout the body at least a week before illness begins, specific antibodies can be detected in the serum during the disease or occasionally even before (Alford, ١٩٨٤). Serum antibodies to different rubella viral antigens can be measured by hemagglutination–inhibition (HAI), complement fixation (CF), neutralization, immunoflurescence (IFA), precipitation, radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), single radial hemolysis (SRH), passive

hemagglutination (PHA), and latex agglutination (LA) (Millians and Wegman, ١٩٧٢).

In natural postnatal rubella infection, HAI and neutralization antibodies appear ١٤ to ١٨ days after exposure, at the time of the rash. HAI antibody titers usually peak about ٢ weeks after the onset of clinical illness, stay at a high level for several weeks, decrease about fourfold over a year's time, and then generally persist for life. Antibody detected by PHA does not appear until ٣ to ٤ weeks after rash onset, and that detected by SRH is delayed until ١ to ٢ weeks after rash onset. CF antibody first appears about a week after HAI antibody, peaks about ١ month after illness, and in general does not persist as long as HAI. Occasionally the CF antibody response is delayed, appearing ١ month after exanthem with peak titers ٢ to ٥ months' later (Feigin and Cherry, ١٩٩٢). IFA, ELISA, RIA, LA, and neutralization techniques reveal antibody patterns similar to those determined by the HAI method (Herrmann, ١٩٨٥).

Primary rubella virus infection, either naturally acquired or vaccine induced, is characterized by the initial appearance of antibody in the IgM and IgG serum components. In general, the IgM-specific response is short lived and not detectable more than ٨ weeks after the onset of infection. Occasionally, it has been detected in the serum for extended periods of time. While the IgG antibody probably persist for life, therefor, detection of IgG antibody is useful indicating immunity. IgA nasal HAI and neutralizing antibody also regularly occurs after viral infection. After immunization, the IgA antibody response varies with type of vaccine and rout of administration (Feigin and Cherry, ١٩٩٢).

Humoral antibody in the congenitally infected fetus is both transplacentally acquired from the mother and actively produced by the fetus.

In the normal maternal-fetus relationship, the transport of antibody to the fetus is minimal until the midpoint of the second trimester (١٦ to ٢٠ weeks). The fetal immune system becomes functional during the second trimester, and small amounts of rubella fetal IgM antibody can be detected. From the midpoint of pregnancy, antibody levels in the developing fetus rise so that at birth the maternal and infant values are similar. Maternal antibody at the time of delivery is usually all composed of IgG. In contrast, the infant titer is composed of fetal IgM, presumably fetal IgG, and occasionally fetal IgA, and transplacentally derived maternal IgG (Feigin and Cherry, ١٩٩٢).

٢-٩-٢: Cellular Immune Response

In the first weeks after natural rubella infection, some degree of lymphocyte suppression may occur, but this phenomenon is only transient. Generally, cell mediated immune responses precede the appearance of humoral immunity by ١ week, reach a peak value at the same time as antibody response, and subsequently persist for many months or years as measured by migration inhibitory factor, lymphokine release, interferon secretion or lymphocyte transformation (Alford, ١٩٨٤).

Rubella specific cell mediated immune responses have been studded in children with congenital rubella by the following assays: lymphocyte-mediated cytotoxicity, lymphocyte transformation, lymphocyte interferon production, and leukocyte migration-inhibition factor production. By all methods of study, infants with congenital rubella have decreased rubella specific cell-mediated responses, compared with persons with previous postnatally acquired rubella (Feigin and Cherry, ١٩٩٢).

Buimovici-Klein and associates noted that the degree of suppression correlated with the time of in utero infection: the earlier in pregnancy the maternal infection, the greater the depression of specific cell-mediated immune responses (Buimovici-Klein *et al.,* ١٩٧٩). In the study of an infant with late-onset congenital rubella syndrome, Verder and associates noted decrease activity of killer and natural killer cells and alloreactive direct cytotoxic cells. Their data indicated that defective cytotoxic effector cell function was the primary cause for the failure to eliminate virus in the illness (Feigin and Cherry, ١٩٩٢).

٢-١٠: Diagnosis

Clinical diagnosis of rubella is often inaccurate. Laboratory con-firmation is the only reliable evidence of acute infection (Plotkin and Orenstein, ١٩٩٩).

Serological diagnosis is the most common method of confirming the diagnosis of rubella. Rubella infection can be serologically confirmed by a significant rise in rubella antibody titer between acute and convalescent phase serum specimen or by the presence of serum rubella IgM. Sera should be collected as early as possible (within ٧ to ١٠ days) after onset of illness, and again at least ٧-١٤ days (preferably ٢-٣ weeks) later (Chin, ٢٠٠٠).

The standard serological test is the hemagglutination inhibition (HAI) test which has the advantages of high sensitivity and early availability of results (Plotkin and Orenstein, ١٩٩٩). HAI test is the most widely accepted method for the diagnosis of rubella virus infection and for evaluation of immune status (Zartarian *et al.,* ١٩٨١). However, the HAI test is time-consuming, labor

intensive, difficult to standardize between laboratories (Meegan *et al.,* ١٩٨٢). The demonstration of a fourfold rise or greater in HAI antibody titer is reliable evidence of rubella infection (CDC, ٢٠٠١). The most commonly used test nowadays is an enzyme-linked immunosorbent assay (ELISA), which can be used to measure IgG and IgM antibodies to rubella (Gershon *et al.*, ٢٠٠٤). The ELISA is more sensitive and specific than many other serological teachings (Bidwell *et al.*, ١٩٨٠). ELISA also had advantage over the comparable sensitive radio – immuno assay (RIA), by detection of antibodies without use of hazardous radio active isotopes, the expense of reagents with short shelf lives and without the requirement of costly equipment (Yaseen, ١٩٩٢). Other assays that are used include latex agglutination, complement fixation (CF), and indirect immunofluorescence (Gershon *et al.*, ٢٠٠٤). False-positive serum rubella IgM tests have occurred in persons with parvovirus infections, with a positive heterophile test for infectious mononucleosis, or with a positive rheumatoid factor (Mahoney and Chernesky, ١٩٩٧).

Rubella virus can be isolated from nasal, blood, urine, throat and cerebrospinal fluid specimens from rubella cases. Virus may be isolated from pharynx ١ week before and up to ٢ weeks after onset of rash (Chin, ٢٠٠٠). Although isolation of the virus is diagnostic of rubella infection, viral cultures are labor intensive and therefore, not done in many laboratories; they are generally not used for routine diagnosis of rubella (CDC, ٢٠٠١).

Congenital rubella infection can be diagnosed in the infant by the isolation of virus, by the demonstration of IgM antibodies, or by the detection of antibodies persisting beyond the predicted decay of passively transmitted maternal antibodies. Virus can often be isolated from tissues obtained at biopsy, autopsy, or surgical procedures such as cataract extraction, but more

often nasopharyngeal swabs, urine specimens, or cerebrospinal fluid serve as the sources. IgM antibodies are present for as long as a tear after birth. Persistence of IgG antibodies beyond ٦ months of age can be detected in ٩٥٪ of infant with congenital rubella syndrome (Plotkin and Orenstein, ١٩٩٩).

The polymerase chain reaction (PCR) has been adapted to the detection of rubella RNA by reverse transcription and amplification (Ho-Terry and Lonesborough, ١٩٩٠). The method appears to be sensitive and specific, and is particularly useful for prenatal detection of rubella infection of the fetus (Tanemura *et al.,* ١٩٩٦).

Rubella reinfection may be diagnosed by a significant rise in rubella IgG titers, sometimes to very high levels, or detection of specific IgM in a patient with pre-existing antibodies. It is often possible to distinguish reinfection from primary infection by examining the antigen-binding avidity of specific IgG. Sera taken from cases of rubella reinfection have low IgG avidity, while sera taken from persons with distant infection, including cases of rubella reinfection, have higher avidity. The rubella IgM response is usually lower and more transient than following primary infection (Zuckerman *et al.,* ٢٠٠٠).

٢-١١: Differential Diagnosis

Because similar symptoms and rash can occur with many other viral infections, rubella is a difficult disease to diagnose clinically except when the patient is seen during an epidemic. Particularly in its more severe forms, rubella may be confused with the mild types of scarlet fever and measles (Behrman *et al.,* ٢٠٠٤). Distinction from measles may be made on the basis of a fainter, non-staining rash, the milder course, and the minimal or absent

systemic complaints. Sore throat is a more prominent compliant in scarlet fever (Goldman and Bennett, ٢٠٠٠). Roseola infantum (exanthem subitum) is distinguished by a higher fever and the appearance of rash at the end of the febrile episode rather than at the height of the signs and symptoms. Infectious mononucleosis may have a rash but is associated with generalized lymphadenopathy and characteristic a typical lymphocytosis. Enteroviral infections accompanied by a rash can be differentiated in some instances by accompanying respiratory or gastrointestinal manifestations and the absence of retroauricular lymphadenopathy. Drug rashes may be extremely difficult to differentiate from the rash of rubella but the characteristic enlargement of the lymph nodes supports a diagnosis of rubella (Behrman *et al.,* ٢٠٠٤).

Differential diagnosis of congenital rubella from cytomegalovirus infection, congenital toxoplasmosis, and congenital syphilis may also be characterized by the following manifestations of congenital rubella: thrombocytopenic purpura, jaundice, hepatosplenomegaly, and bone lesions. Herpes simplex virus infection may show the same manifestations, with the exception of bone lesions and a vesicular skin rash. The diagnosis may be clarified by the presence of other findings more compatible with congenital rubella, like cataract, glaucoma, patent ductus arteriosus, or maternal history of rubella (Gershon *et al.,* ٢٠٠٤).

٢-١٢: Treatment

No specific therapy is necessary or indicated in uncomplicated rubella. Starch baths may be useful for the adult with troublesome pruritis. It is important that the affected patient understand that he or she is contagious

and that transmission of infection to a pregnant woman could have serious consequences. Occasionally, in adults, arthritis can be quite severe. When weight-bearing joints are affected, rest is encouraged. Symptoms readily respond to aspirin therapy; corticosteroids are not indicated. In rubella encephalitis, care is supportive, with adequate maintenance of fluids and electrolytes. Thrombocytopenia is usually self-limited; on occasion, sever bleeding has occurred. Corticosteriods therapy is often employed (Feigin and Cherry, ١٩٩٢).

٢-١٣: Rubella Immunization

٢-١٣-١: Passive Immunization

Before the development of vaccine, immune globulin (IG) which contains rubella antibody has frequently been offered to pregnant women exposed to rubella in the hope that it would prevent fetal infection. Experimental studies have confirmed the efficacy of passive antibody in preventing clinical rubella, but there were numerous failures of gammaglobulin to prevent congenital fetal abnormality in actual practice (Plotkin and Orenstien, ١٩٩٩). Therefore, IG is not recommended for routine post exposure prophylaxis of rubella in early pregnancy or any other circumstances. Infants with congenital rubella have been born to women who received IG shortly after exposure. Administration of IG should considered only if a pregnant woman who has been exposed to rubella will not consider termination of pregnancy under any circumstances. In such cases, intramuscular administration of ٢٠ ml of IG within ٧٢ hours of rubella exposure might reduce, but will not eliminate the risk for rubella (CDC, ٢٠٠١).

٢-١٣-٢: Active Immunization

Three rubella vaccines were licensed in the United States from ١٩٦٩ to ١٩٧٠ as follows:

HPV-٧٧ (duck embryo), HPV-٧٧ (dog kidney), and Cendehill (rabbit kidney) strains. Soon thereafter, the RA ٢٧/٣ human diploid fibroblast vaccine was licensed in the Europe. In January ١٩٧٩, the RA ٢٧/٣ human diploid fibroblast strain in the United States was licensed and all other strains were discontinued. The RA ٢٧/٣ strain is also the most widely used throughout the world with the exception of Japan (Plotkin and Orenstein, ١٩٩٩).

A-Characteristics

The RA٢٧/٣ rubella vaccine is a live attenuated virus. It was first isolated in ١٩٦٥ at the Wistar Institute from a rubella-infected aborted fetus. The virus was attenuated by ٢٥ – ٣٠ passages in tissue culture, using human diploid fibroblasts (Plotkin, ١٩٩٦).

Vaccine virus is not communicable, except in the setting of breast-feeding, even though virus may be cultured from the nasopharynx of vaccinees (Plotkin and Orenstein, ١٩٩٩). Rubella vaccine is available as a single antigen preparation, combined with mumps vaccine, or combined with measles and mumps vaccines (AAP, ٢٠٠٣).

B- Immunogenicity and Vaccine Efficacy

RA٢٧/٣ rubella vaccine is safe and more immunogenic than the previously used rubella vaccines. In clinical trials, ٩٥٪ or more of vaccinees

aged ١٢ months and older have developed serological evidence of rubella immunity after a single dose. More than ٩٠٪ of vaccinated persons have protection against both clinical rubella and viremia for at least ١٥ years. Follow-up studies indicate that one dose of vaccine confers long lasting, and will usually provide lifelong protection (AAP, ٢٠٠٣). Failure to respond may result from not complying with the manufacturer's instructions during storage or following reconstitution (with consequent inactivation of virus or loss of potency), or pre-existing low levels of antibody (Zuckerman *et al.,* ٢٠٠٠).

C- Vaccine Recommendations

Rubella vaccine is recommended to be administered in combination with measles and mumps vaccine (MMR) when a child is ١٢ to ١٥ months of age, with a second dose at school entry at ٤ – ٦ years, according to recommendations for routine measles immunization. People who do not receive the dose at school entry should receive their second dose as soon as possible but no later than ١١ to ١٢ years of age (AAP, ٢٠٠٣).

Vaccine is recommended for all susceptible non-pregnant females without contrandication. Susceptible young adults who have contact with young children or congregate at colleges, military recruits and other types of institutions should be immunized. All medical personals should be immunized to rubella, in particular those who are in contact with patients in prenatal clinics (Chin, ٢٠٠٠).

People who were born in ١٩٥٧ or after and have not received at least ١ dose of vaccine or who have no serologic evidence of immunity to rubella are

considered susceptible and should be immunized with MMR vaccine (AAP, ٢٠٠٣).

D-Dosage and Rout of Administration

The rubella vaccine is administered subcutaneously. Efforts at administering the vaccine intranasally have proved ineffective with dose below ١٠.٠٠٠ plaque-forming units (PFU) of virus (Plotkin and Orenstein, ١٩٩٩).

Meanwhile, subcutaneously immunization only requires ١٠٠٠ PFUs and shows similar humoral antibody and slightly reduced secretory antibody production as the intranasal rout (Davidkin and Martti, ١٩٩٨).

٣-١-A: Materials

This study was conducted from ١st June ٢٠٠٥ to ٣٠th July ٢٠٠٦, a total of ٢٥٠ women in childbearing age (١٥-٤٥) years, were selected randomly from urban and rural region of Babylon Governorate. Blood samples (٥ ml) were drawn from women attending Babylon Maternity and Children Hospital, and Public Health Laboratory seeking premarital checking.

The representative number was estimated by using the following equation (Moser and Calton, ١٩٧٩; Schsselman, ١٩٨٢):

$N = ١.٩٦^{٢} \times P\ (١-P) / SE^{٢}$

Where:

$١.٩٦^{٢}$ = level of confidence at ٠.٠٥

P = prevalence expected from previous studying. It was (٧٩.٠%) from the study of Yaseen (١٩٩٢) in Basrah.

SE = the error tolerated at ٠.٠٥ level

Based on the above equation, the minimal estimated sample size would be ٢٤٥ women, anyhow a total of ٢٥٠ women in childbearing age were selected from the total ٣٤٧٢٧٧ women in childbearing age in Babylon Governorate (Public Health Directorate, personal communication).

A questionnaire form was filled for each woman by direct interview. The data requested include age, residence, level of education, occupation, pregnancy (pregnant and non-pregnant), trimester, parity. We excluded the women who have had history of recent illness with rash, or contact with a known case of rubella.

٣-١-B: Instruments and tools

Equipment and tools used in this study include the followings:

Device	Company	Origin

Centrifuge	Heraeus	Germany
ELISA reader	Bechman coulter	U.S.A
ELISA washer	Beckman coulter	U.S.A
Freezer	Liebher	Austria
Incubator	Fisher scientific	U.S.A
Micropipette	Slamed	U.S.A

٣-٢: Methods

The women included in this study were tested by enzyme-linked immunosorbent assay (ELISA) technique for IgG antibodies, and hemagglutination inhibition test (HAI) for IgG and IgM antibodies.

٣-٢-١: Collection of blood samples

Blood (٥ ml) were collected by venipuncture, allowed to clot at room temperature and sera were separated by centrifugation at ٥٠٠-١٠٠٠ rpm for ٥ minutes, then stored at -٢٠ C°.

٣-٢-٢: Serological examination

٣-٢-٢-١: Enzyme-linked immunosorbent assay (ELISA)

Bioelisa rubella IgG kit, produced by bio-kit company- Barcelona- Spain, has been used to diagnose anti rubella IgG antibodies in human serum by

enzyme-linked immunosorbent assay (ELISA) technique. The kit consist of the following components:

١- Microplate; ١٢x٨ well strips coated with inactivated rubella virus antigen.

٢- High positive calibrator; ٠.٦ ml of diluted human gamma globulin containing ٢٠٠ IU/ml of anti-rubella IgG. Contains stabilisers protein, green dye and < ٠.١ % sodium azide.

٣- Low positive calibrators; ٠.٦ ml of diluted human gammaglobulin containing ١٠ IU/ml of anti-rubella IgG contains stabilisers protein, green dye and sodium azide.

٤- Negative control; ٠.٦ ml of diluted human gammaglobulin negative for anti-rubella IgG contains stabilisers protein, yellow dye and < ٠.١٪ sodium azide.

٥- Concentrate conjugate; ٠.٣٥ ml of rabbit anti-human IgG anti bodies conjugated with peroxidase contains red dye, stabilisers protein, ٠.٠٢٪ thimerosal and ٠.٠٠١٪ gentamicin sulphate.

٦- Conjugate diluent; ١٥ ml of Tris buffer containing yellow dye, additives, ٠.٠٢٪ thimerosal and ٠.٠٠١٪ gentamicin sulphate.

٧- Sample diluent; ٢x٥٠ ml of phosphate buffer containing detergent, stabilizers protein, green dye and < ٠.١٪ sodium azide.

٨- Washing solution; ٢x٥٠ ml of concentrate phosphate buffer (١٠ x) containing ١٪ tween ٢٠ and ٠.٠١٪ thimerosal.

٩- Substrate buffer; ١٤ ml of citrate-acetate buffer containing hydrogen peroxide and ٠.٠٠٢ % gentamicin sulphate.

١٠- Chromogin; ١.٥ ml of ٣, ٣', ٥, ٥' Tetramethylbenzidine (TMB) dissolved in Dimethylsulfoxide (DMSO).

١١- Stopping solution; ١٢ ml of ١N sulphuric acid.

١٢- Adhesive seals; They are used to cover the microplate during incubations.

٣-٢-٢-١-١: Preparation of samples and reagents

١- Calibrators, negative control and serum samples were diluted ١/١٠١ with sample diluent. For example, ١٠ µl of serum samples to ١ml of sample diluent.

٢- Washing solution was prepared by diluting the concentrated washing solution ١/١٠ with distilled water

٣- The conjugate was diluted ١/٥١ with the conjugate diluent to make working diluted conjugate according to the following criteria:

Strips required	١	٢	٤	٦	٨	١٠	١٢
Conjugate diluent ml	١.٠	٢.٠	٤.٠	٦.٠	٨.٠	١٠.٠	١٢.٠
Concentrated conjugate µl	٢٠	٤٠	٨٠	١٢٠	١٦٠	٢٠٠	٢٤٠

٤- The substrate chromogen solution consisted of substrate buffer and chromogen (TMB). The solution is prepared as in the following example:

Strips required	١	٢	٤	٦	٨	١٠	١٢
Substrate buffer ml	١.٠	٢.٠	٤.٠	٦.٠	٨.٠	١٠.٠	١٢.٠
Chromogen µl	٢٠	٤٠	٨٠	١٢٠	١٦٠	٢٠٠	٢٤٠

٣-٢-٢-٢-٢: Procedure

We depended upon bioelisa rubella IgG kit instructions to perform ELISA test.

١. We reserved ٧ wells for blank, calibrators and control. The calibrators and negative control should be run in duplicate, ١٠٠ µl of each diluted sample, control the calibrators added into the wells, with a well left empty for the blank.
٢. The micro plate was covered with an adhesive seal and incubated for ١ hour at ٣٧ C°.
٣. After incubation the adhesive seal was removed, and the wells washed ٣ times with a washing solution by filling the wells with washing solution, shake gently and discard the solution after each shake.
٤. ١٠٠µl of diluted conjugate was added to each well, except the one for the blank. The plate was covered with an adhesive seal and incubated for ٣٠ minutes at ٣٧ C°.
٥. After incubation the adhesive seal was removed and the well washed ٣ times with a washing solution as in (٣) above.
٦. ١٠٠µl of substrate-chromogen solution were added to each well including the blank, and incubated uncovered for ٣٠ min. at room temperature in a dark place.
٧. The reaction was stopped by adding ١٠٠µl of stopping solution (١N $H_٢SO_٤$ solution).
٨. After the reaction had been stopped; the absorbance of each well had been read by using ELISA reader at ٤٥٠ nm.

٣-٢-٢-٢-٣: Calculation the results

The mean absorbance of the low positive calibrator represents the cut-off value.

Cut- off = LPCx

The sample absorbance is divided by the cut-off value.

Positive (immune) : ratio absorbance / cut- off ≥ ١.٠.

Negative (non- immune) : ratio absorbance / cut- off < ٠.٩.

Equivocal : ratio absorbance /cut- off ≥ ٠.٩ < ١.٠.

٣-٢-٢-٢: Hemagglutination-Inhibition Test (HAI)

The method described previously by Collee *et al.,* (١٩٩٦) was depended in this study.

٣-٢-٢-٢-١: Reagents

١- Phosphate buffered saline (PBS).

٢- Rubella virus antigen (Hemagglutinin) (was prepared in the Vaccine and Sera Institute). Available in vials of ٣ ml each. All required ٢٠ vials were stored in the lab under -٧٠ C° until the end of test.

٣- Avian red cell suspension (pigeon blood was transferred to a sterile tube containing heparin, and red cells was washed three times by adding of PBS on the side of tube, then the mixture was centrifuged at ١٠٠٠ rpm for ٣ min.; the buffy coat was removed each time, the washing red cells were stored in the refrigerator under ٤ C° until used. The suspension was prepared by adding ٠.١ ml of packed red cells to ١.٠ ml of PBS).

٤- The serum was diluted ١:١٠ in PBS, heated at ٥٦ C° for ٣٠ minutes, to remove non-specific inhibitors that might give false-negative results.

٣-٢-٢-٢-٢: Procedure

١. The first row of the microtiter plate was marked off for each serum to be tested.
٢. ٠.٠٢٥ml of PBS was added to the second, and each of the remaining wells of the microtiter plate.
٣. ٠.٠٥ml of ١:١٠ serum dilution was added to first well in each row of the microtiter plate.
٤. Serial dilutions of the serum were prepared (١:٢٠, ١:٤٠, ١:٨٠, ١:١٦٠, ١:٣٢٠, ١:٦٤٠, ١:١٢٨٠, ١:٢٥٦٠, ١:٥١٢٠, ١:١٠٢٤٠). ٠.٠٢٥ ml is discarded from the final well through transfer of ٠.٠٢٥ ml from first well to the second well and from this to the third and so on to the final well .
٥. ٠.٠٢٥ml of the rubella antigen (hemagglutinin) was added to each of the mentioned wells and mixed thoroughly.
٦. The microtiter plate was incubated for ١hour at room temperature to allow serum and virus to interact.
٧. ٠.٠٥ml of red cell suspension were added to all wells.

٨. The microtiter plate was shaked, sealed with tape, and incubated for ١hour at room temperature.

٩. The result was read by naked eye. The titer of the serum is the highest dilution that causes complete inhibition of hemagglutination. Titers of ١:٨ or higher were considered as positive.

٣-٢-٣: Statistical Analysis

Data were translated into a computerized database structure. Statistical analysis was computer assisted using SPSS ver ١٣ (statistical package for social sciences). Frequency distribution from selected variables was done first. The titer for hemagglutination inhibition test was a discrete variable (ordered), the median was therefor used to describe the central tendency.

Chi-square was used to test the statistical significance of association between the categorized variables. P value less than ٠.٠٥ level of significance was considered statistically significant.

Results

The number of women included in this study was ٢٥٠, their ages ranged from ١٥ to ٤٥ years (Mean ± SD= ٢٤.٠٨ ± ١٠.٠٧). The Distribution of the studied sample are presented in figures (١, ٢, ٣ and ٤). In figure (١) we can see that (٢٣.٢٪) of the studied women were (١٥-١٩) years of age group. The next group which may represent the most common age of childbearing that is ٢٠-٢٤

years represent (٣٢.٠٪) of our studied sample. The random selection had resulted that (٢٢.٨٪) fall in the third age group of ٢٥-٢٩ years. The three groups collectively with the fourth age group of ٣٠-٣٤ years may represent (≥٩٢.٠٪) of our studied sample and may represent the most selected age for marriage. The other two groups ٣٥-٣٩ and ≥ ٤٠ years were only (٧.٦٪) of our sample and possibly enough to give an idea about the anti-rubella antibody titer in the population at that age group.

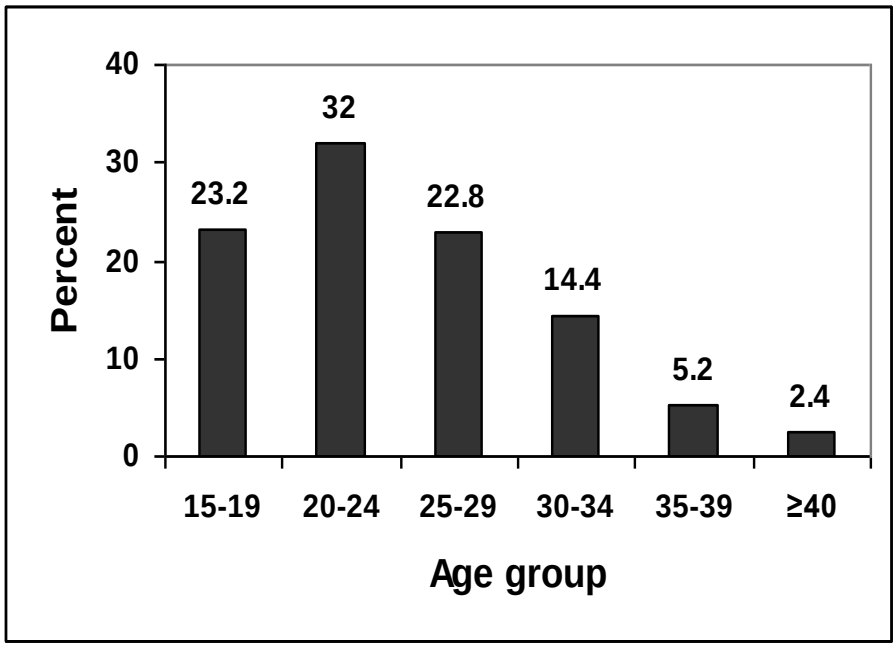
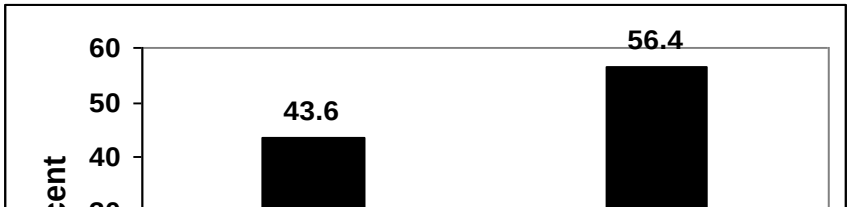


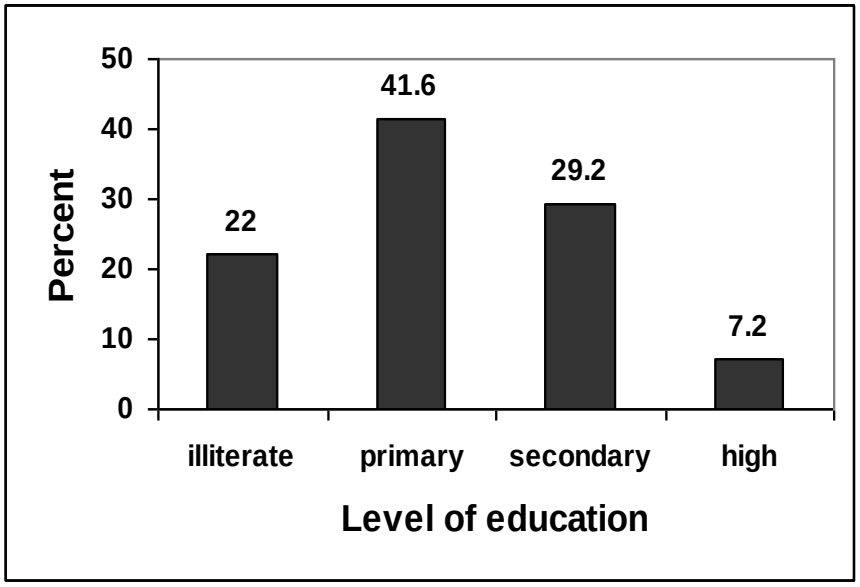
Figure (١): distribution of the sampled women according to age.

Figure (٢-A) shows that ١٠٩ (٤٣.٦٪) women were from urban areas and ١٤١ (٥٦.٤٪) were from rural areas. Regarding the level of education, ٥٥ (٢٢.٠٪) women were illiterate, ١٠٤ (٤١.٦٪) had primary school qualification, ٧٣ (٢٩.٢٪) were secondary school graduate, and ١٨ (٧.٢٪) with high education (figure ٢-B).



(B)

(A)

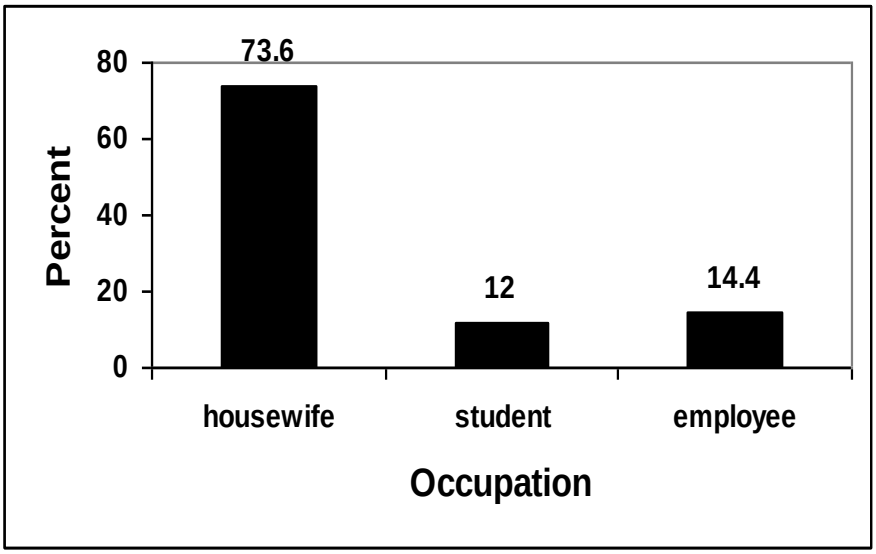


(B)

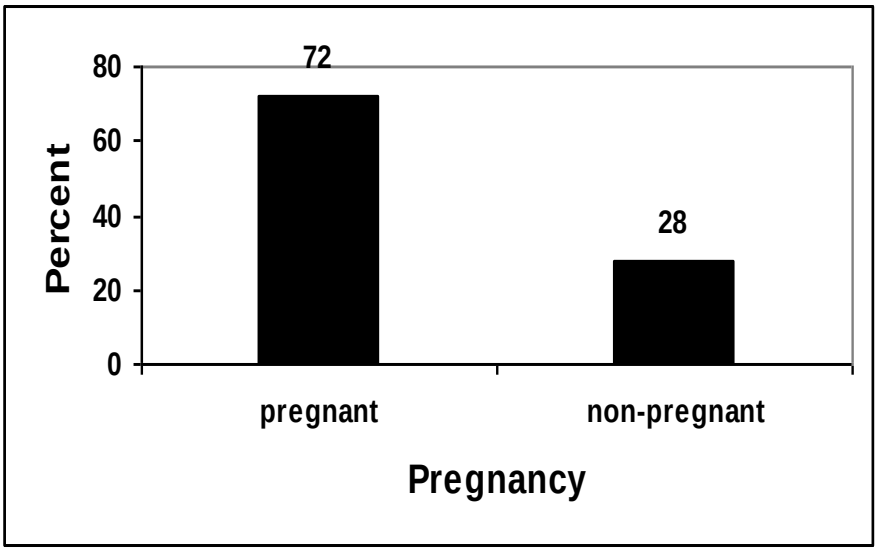
Figure(٢): Distribution of the sampled women according to:

A) Residency. B) Level of education.

In respect to occupation, one hundred eighty four (٧٣.٦٪) of the women were housewives, ٣٠ (١٢.٠٪) were students and ٣٦ (١٤.٤٪) were employees (figure ٣-A). One hundred and eighty (٧٢.٠٪) of studied women were pregnant and ٧٠ (٢٨.٠٪) were not (figure ٣-B).



(A)

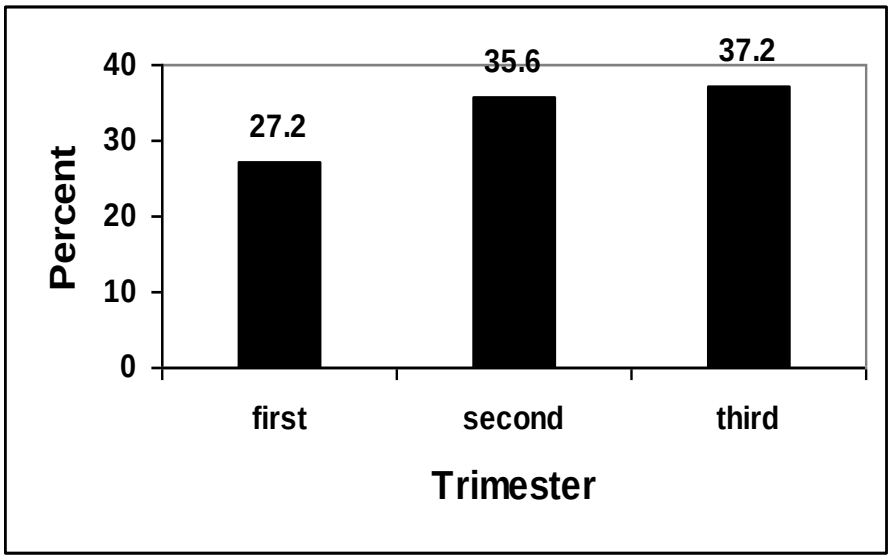


(B)

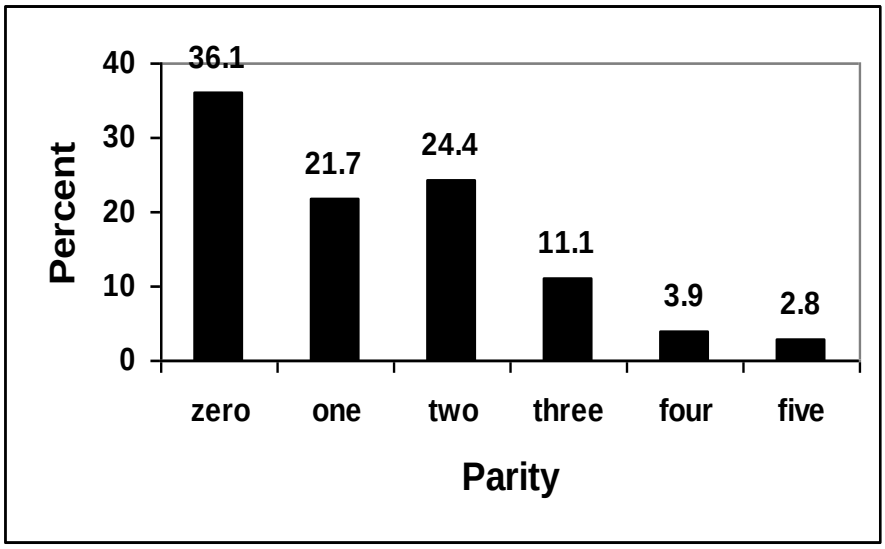
Figure (٣): Distribution of the sampled women according to:

A) Occupation. B) Pregnancy.

Regarding pregnant women, ٤٩ (٢٧.٢٢٪) of them were in first trimester, ٦٤ (٣٥.٥٪) were in second trimester and the rest ٦٧ (٢٦.٨٪) were in third trimester (figure ٤-A). In respect to parity, ٦٥ (٣٦.١١٪) have no children, ٣٩ (٢١.٦٦٪) were have one child, ٤٤ (١٤.٠٪) have two children, ٢٠ (١١.١٪) have three children, ٧ (٣.٨٪) have four children and ٥ (٢.٧٪) have five children (figure ٤-B).



(A)



(B)

Figure (٤): Distribution of the sampled women according to:

A) Trimester. B) Parity.

Figure (٥) shows that ١٩٤ out of ٢٥٠ women had positive anti-rubella antibodies by ELISA (IgG) test giving an overall prevalence of (٧٧.٦٪), whereas ٢٠٠ out of ٢٥٠ women had anti-rubella antibodies by HAI test (IgG and IgM) giving an overall prevalence of (٨٠.٠٪).

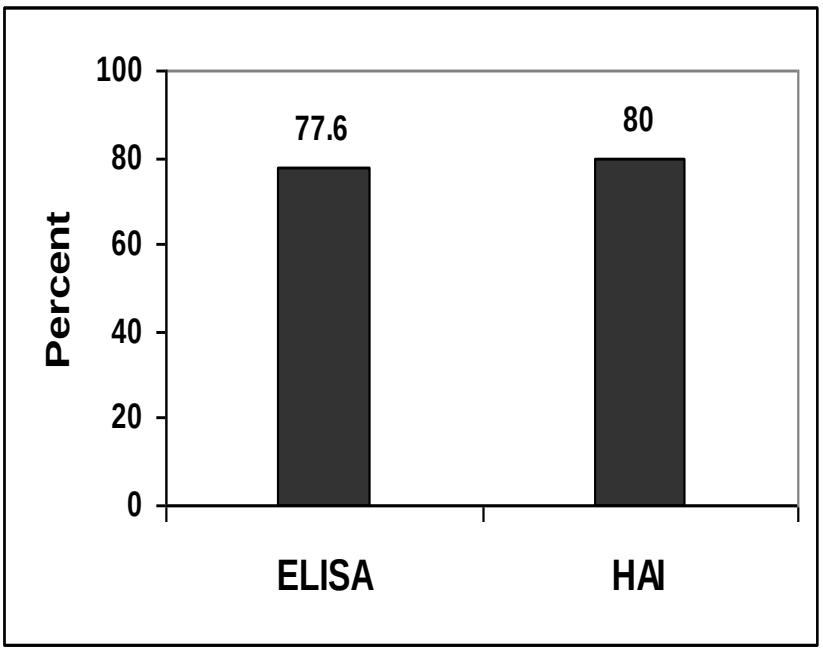
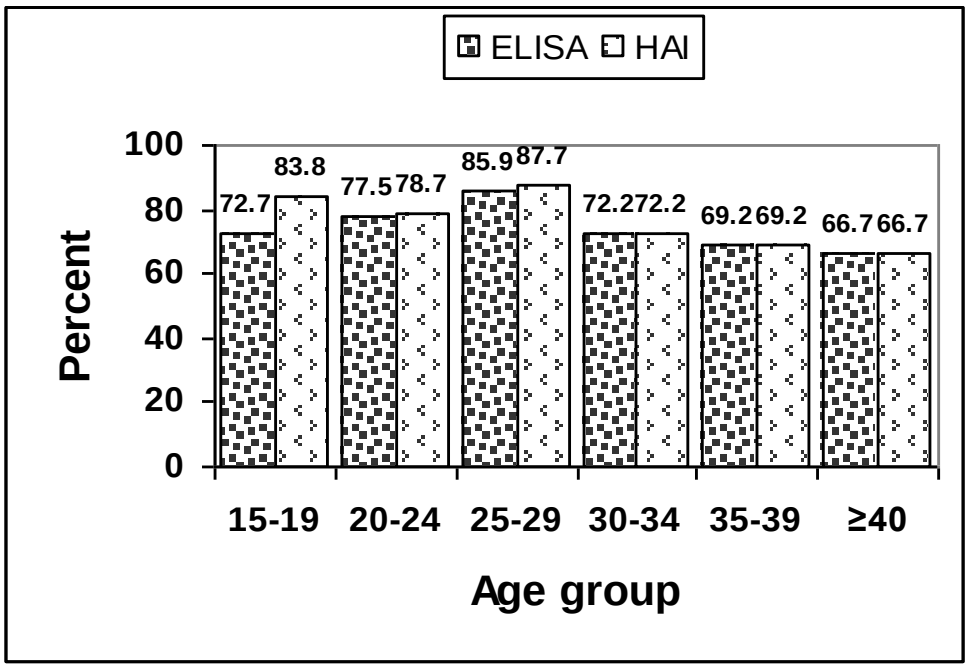
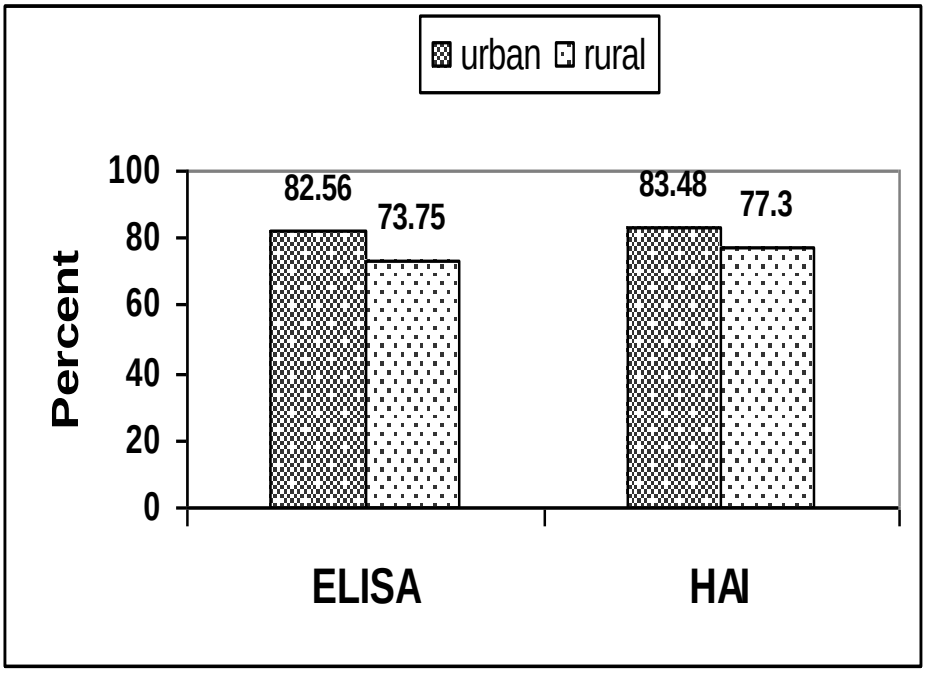


Figure (٥): The rate of anti-rubella antibodies by ELISA and HAI tests.

Figure (٦-A) shows that the highest rate of seropositivity of anti-rubella antibodies was among women at age group ٢٥-٢٩ years by ELISA and HAI tests (٨٥.٩٦٪ and ٨٧.٧١٪ respectively), whereas the lowest rate among women aged ≥ ٤٠ years by both tests (٦٦.٧%) for both tests. Regarding the residency, the rate of seropositivity was higher among women who live in urban areas than those who were live in rural areas by ELISA and HAI tests (٨٢.٥٦٪ and ٨٣.٤٨٪ respectively) (figure ٦-B).


ELISA
HAI
100
80
60
40
20
0
Percent
72.7
83.8
77.5
78.7
85.9
87.7
72.2
72.2
69.2
69.2
66.7
66.7
15-19
20-24
25-29
30-34
35-39
≥40
Age group

(A)

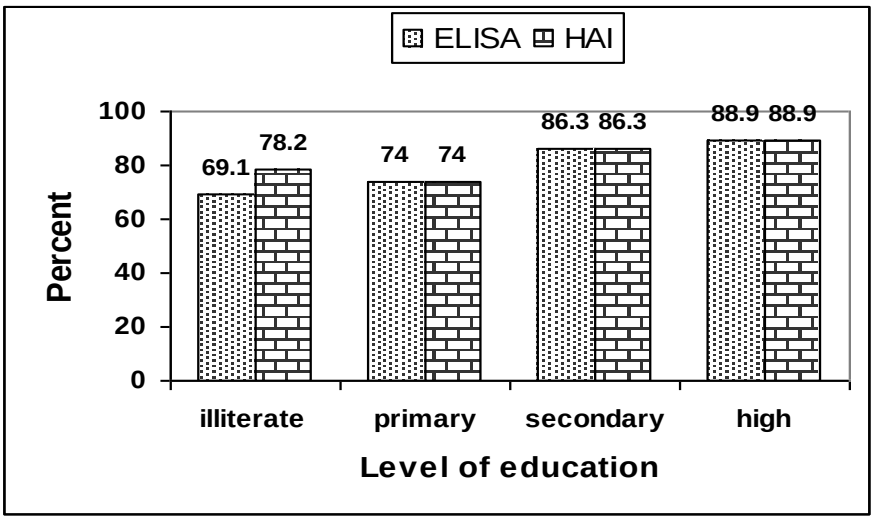

urban
rural
100
80
60
40
20
0
Percent
82.56
73.75
83.48
77.3
ELISA
HAI

(B)

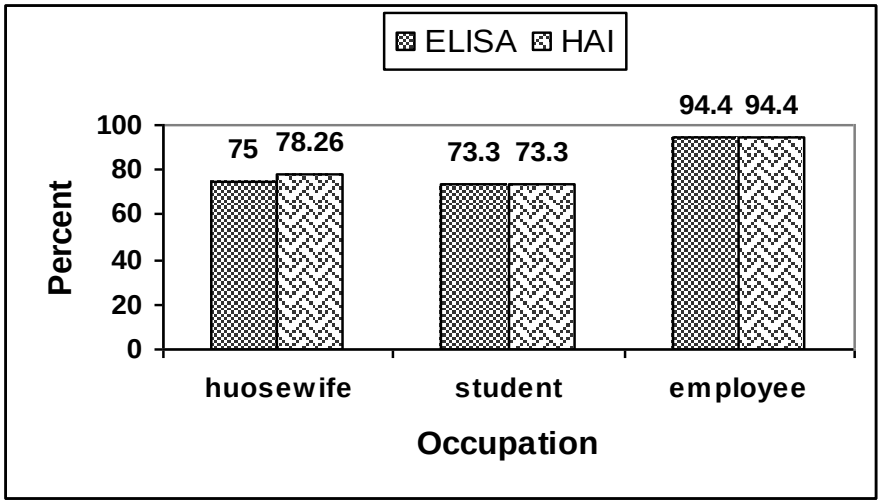
Figure (٦): The rate of seropositivity of anti-rubella antibodies by ELISA and HAI tests according to: A) Age.

B) Residency.

The rate of seropositivity was highest among women who were highly educated by ELISA and HAI tests than others (٨٨.٩%) for both tests. The lowest rate of seropositivity was among illiterate women (٦٩.١٪) by ELISA, whereas among primary educated women (٧٥.٠٪) by HAI test (figure ٧-A). In respect to occupation, the employees women have the highest rate of positivity (٩٤.٤٤٪ by both tests) than students and housewives (figure ٧-B).



(A)

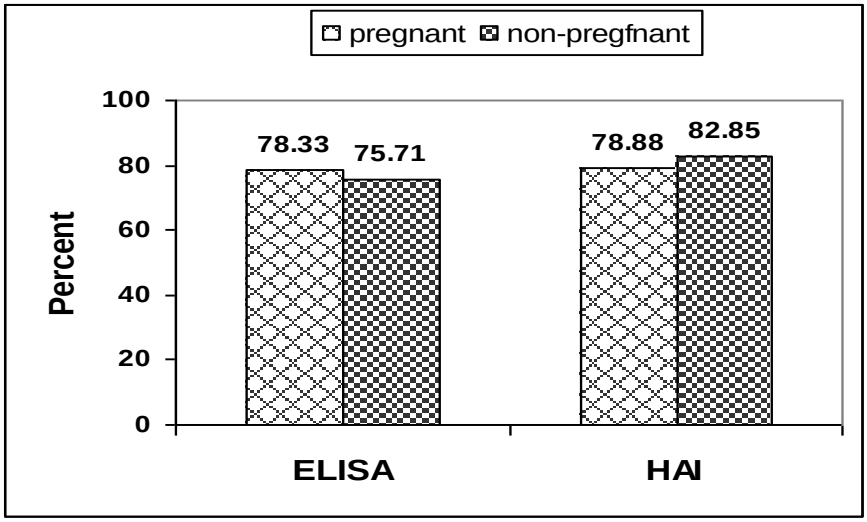


(B)

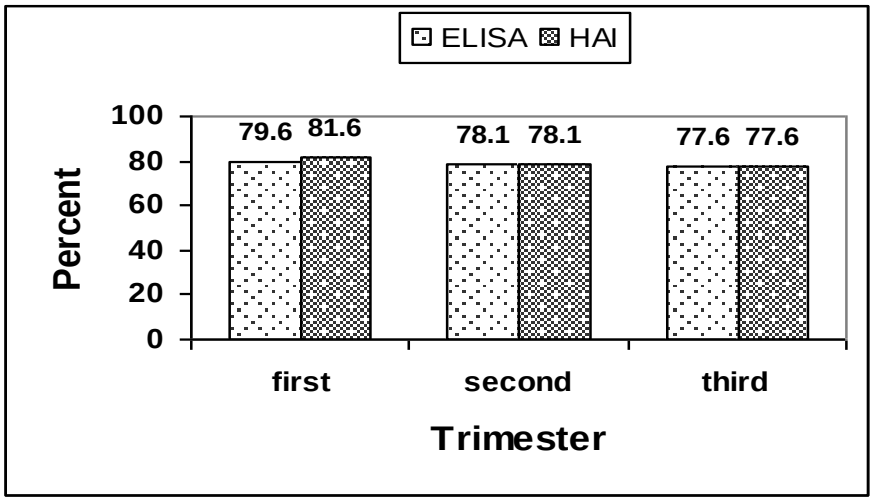
Figure (٧): The rate of seropositivity of anti-rubella antibodies by ELISA and HAI tests according to: A) Level of education.

B) Occupation.

Pregnant women were have higher rate of positivity (٧٨.٣٣٪) than non-pregnant ones by ELISA test, whereas non-pregnant women have higher rate of positivity than pregnant ones (٨٢.٨٥٪) by HAI test (figure ٨-A). Pregnant women who were in first trimester have the highest rate of seropositivity by ELISA and HAI tests (٧٩.٦% and ٨١.٦٣٪ respectively) than the women in second and third trimester (figure ٨-B).



(A)



(B)

Figure (٨): The rate of seropositivity of anti-rubella antibodies by ELISA and HAI tests according to: A) Pregnancy. B) Trimester.

The highest rate of seropositivity by ELISA and HAI tests was among multipara women with three children (٨٥.٠٪) for both tests than others, and the lowest rate among women who have four children (٥٧.١٤٪) for both tests (figure ٩).

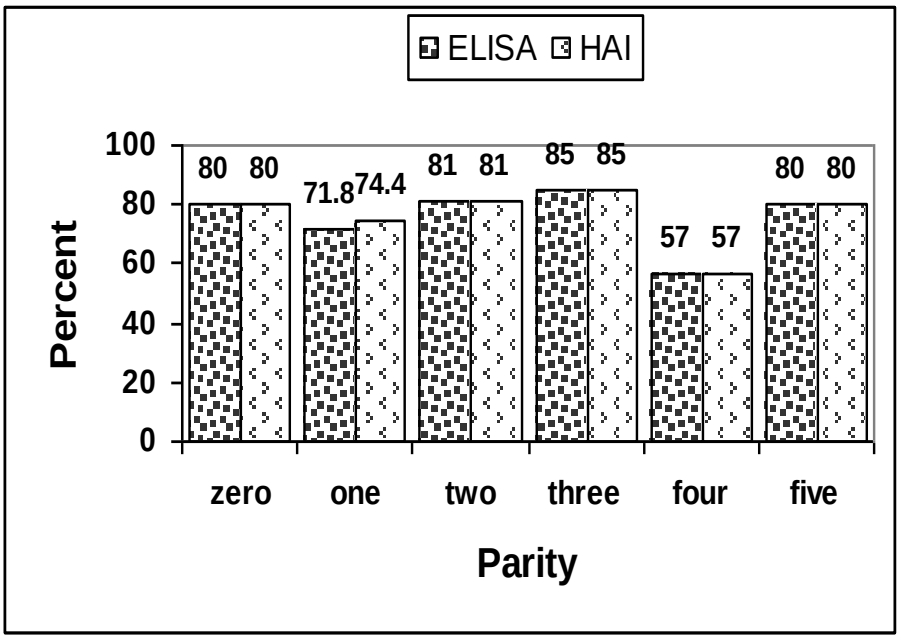


Figure (٩): The rate of seropositivity of anti-rubella antibodies by ELISA and HAI tests according to Parity.

The rate of positive anti-rubella antibodies by ELISA (IgG) was significantly associated with (age and residency) (P=٠.٠٢ and ٠.٠٢٣ respectively). The rate of positive anti-rubella antibodies by HAI was also significantly associated with (age and residency) (P = ٠.٠٢ and ٠.٠٢٥ respectively).

Table no. (١) shows that the highest mean of anti-rubella antibodies titer by HAI test (١٩٥٢ ± ١٦٤١.٩) was among women in age group ٢٥-٢٩ years, and the highest percentage (٣٦.٠٪) of them were presented with a titer of (١٢٨٠). Whereas the lowest mean titer (٢٨٠ ± ٨٠) among age group ≥ ٤٠ years and (٢٥.٠٪) of them were presented with a titer of (٣٢٠). Women at age group ١٥-

١٩ years, show the mean titer of (٤٣١.٧ ± ٤٣٥) and (٣٣.٣٪) of them were presented with a titer of (١٦٠). Women at age group ٢٠-٢٤ years, show the mean titer of (٦٠٩.٢ ± ٤١٧.٤) and (٤٤.٤٪) of them were presented with a titer of (٦٤٠). Women at age group ٣٠-٣٤ years, show the mean titer of (٤١٢.٣ ± ٤٧٨.٨) and (٣٤.٦٪) of them were presented with a titer of (٣٢٠). Women at age group ٣٥-٣٩ years, show the mean titer of (٣٠٢.٢ ± ١٤٨.٥) and (٥٥.٥٪) of them were presented with a titer of (٣٢٠).

Table No. (١): Anti-rubella antibodies titer by HAI test according to age.

Age No.(%)	Titer								P value
	٨٠	١٦٠	٣٢٠	٦٤٠	١٢٨٠	٢٥٦٠	٥١٢٠	Mean ± SD	
	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)		
١٥_١٩ ٤٨ (٢٤.٠)	٣ (٦.٢)	١٦ (٣٣.٣)	١٤ (٢٩.١)	١١ (٢٢.٩)	٣ (٦.٢)	١ (٢.٠)	٠	٤٣١.٦ ± ٤٣٥	<٠.٠٥
٢٠_٢٤ ٦٣ (٣١.٥)	٠	٧ (١١.١)	١٨ (٢٨.٥)	٢٨ (٤٤.٤)	٩ (١٤.٢)	١ (١.٥)	٠	٦٠٩.٢ ± ٤١٧.٤	
٢٥_٢٩ ٥٠ (٢٥.٠)	٠	٢ (٤.٠)	٢ (٤.٠)	١١ (٢٢.٠)	١٨ (٣٦.٠)	٨ (١٦.٠)	٩ (١٨.٠)	١٩٥٢ ± ١٦٤١.٩	
٣٠_٣٤ ٢٦ (١٣.٠)	٢ (٧.٦)	٨ (٣٠.٧)	٩ (٣٤.٦)	٦ (٢٣.٠)	٠	١ (٣.٨)	٠	٤١٢.٣ ± ٤٧٨.٨	
٣٥_٣٩ ٩ (٤.٥)	٠	٣ (٣٣.٣)	٥ (٥٥.٥)	١ (١١.١)	٠	٠	٠	٣٠٢.٢ ± ١٤٨.٥	
≥ ٤٠ ٤ (٢.٠)	٠	١ (٢٥.٠)	٣ (٧٥.٠)	٠	٠	٠	٠	٢٨٠ ± ٨٠	

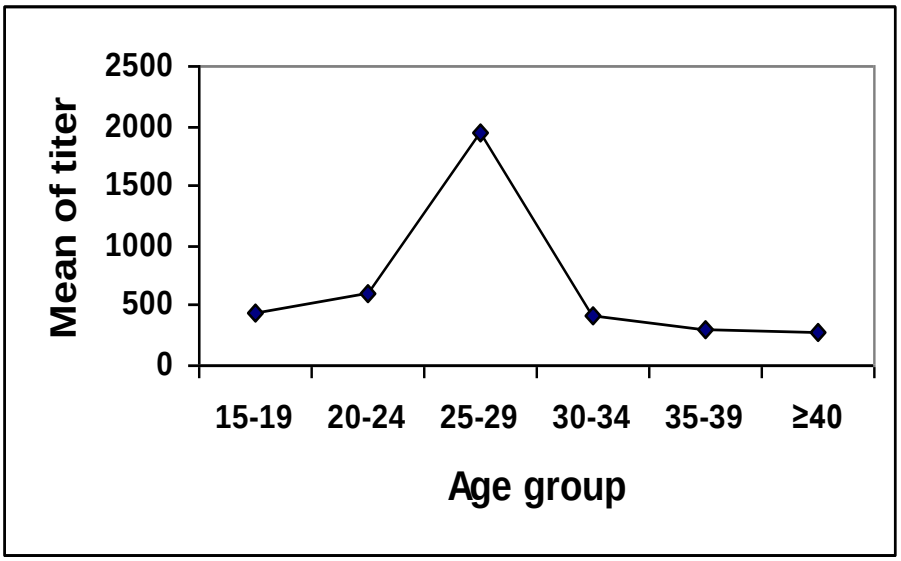


Figure (١٠): The mean of titer of anti-rubella antibodies of different age groups.

Table no. (٢) shows that the mean of anti-rubella antibodies titer by HAI test was higher among women who live in urban areas (١٢٦٢.٤ ± ١٣٧٥.٤), and (٣١.٨%) of them were presented with a titer of (٦٤٠). While the women who live in rural areas showed a lower mean of titer (٥٢١.٨ ± ٦٢١.٧) and (٣١.٢٪) of them were presented with a titer of (٣٢٠).

Table No. (٢):Anti-rubella antibodies titer by HAI test according to residency.

Residency No. (%)	Titer								P value
	٨٠	١٦٠	٣٢٠	٦٤٠	١٢٨٠	٢٥٦٠	٥١٢٠	Mean ± SD	
	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)		
Urban ٩١ (٤٥.٥)	٠	٨ (٨.٧)	١٧ (١٨.٦)	٢٩ (٣١.٨)	٢٠ (٢١.٩)	٩ (٩.٨)	٨ (٨.٧)	١٢٦٢.٤ ± ١٣٧٥.٤	

Rural ١٠٩ (٥٤.٥)	٥ (٤.٥)	٢٩ (٢٦.٦)	٣٤ (٣١.٢)	٢٨ (٢٥.٦)	١٠ (٩.١)	٢ (١.٨)	١ (٠.٩)	٥٢١.٨ ± ٦٢١.٧	<٠.٠٥

Regarding the level of education, table no. (٣) shows that the highest mean titer of anti-rubella antibodies by HAI test (١٣٠٧.٩ ± ١٣٥٠) was among women who had secondary level of education and (٣١.٧٪) of them were presented with a titer of (٦٤٠). While the women who were illiterate have the lowest mean of titer (٤٦٥.١ ± ٣٨٨.٣) and (٣٢.٥٪) of them were presented with a titer of (١٦٠). The women with primary and high level of education shows that the same mean titer (٦٢٤.٦ ± ٨٣٩.٧ and ١٢٩٠ ± ١٥٥٠.٧ respectively). (٣٠.٧٪) of women with primary education level presented with a titer of (٦٤٠) and (٣١.٢٪) of women with high education level presented with a titer of (١٢٨٠).

Table No. (٣): Anti-rubella antibodies titer by HAI test according to level of education.

Level of education No. (%)	**Titer**								**P value**
	٨٠	١٦٠	٣٢٠	٦٤٠	١٢٨٠	٢٥٦٠	٥١٢٠	**Mean ± SD**	
	N.(%	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)		
Illiterate ٤٣ (٢١.٥)	٢ (٤.٦)	١٤ (٣٢.٥)	١٣ (٣٠.٢)	٩ (٢٠.٩)	٤ (٩.٣)	١ (٢.٣)	٠	٤٥٦.١ ± ٣٨٨.٣	>٠.٠٥
Primary ٧٨ (٣٩.٠)	٣ (٣.٨)	١٧ (٢١.٧)	٢٣ (٢٩.٤)	٢٤ (٣٠.٧)	٨ (١٠.٢)	١ (١.٢)	٢ (٢.٥)	٦٢٤.٦ ± ٨٣٩.٧	
Secondary ٦٣ (٣١.٥)	٠	٥ (٧.٩)	١١ (١٧.٤)	٢٠ (٣١.٧)	١٣ (٢٠.٦)	٩ (١٤.٢)	٥ (٧.٩)	١٣٠٧.٩ ± ١٣٥٠	
High	٠	١ (٦.٢)	٤ (٢٥.٠)	٤ (٢٥.٠)	٥ (٣١.٢)	٠	٢ (١٢.٥)	١٢٩٠ ± ١٥٥٠.٧	

١٦ (٨.٠)									

Table no. (٤) shows that the employees women had the highest mean titer of anti-rubella antibodies by HAI test (١٤٩٦.٥ ± ١٦٨٣.٣) and (٢٩.٤٪) of them were presented with a titer of (٣٢٠). The students and housewives had a mean titer of (٨١٤.٥ ± ٥٤٥.٢ and ٧١٥ ± ٩٢٥.٣ respectively). (٤٥.٤٪) of students and (٢٧.٠٪) of housewives presented with a titer of (٦٤٠).

Table No. (٤): Anti-rubella antibodies titer by HAI test according to occupation.

Occupation No. (%)	Titer								P value
	٨٠	١٦٠	٣٢٠	٦٤٠	١٢٨٠	٢٥٦٠	٥١٢٠	Mean ± SD	
	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)		
Employee ٣٤ (١٧.٠)	٠	٢ (٥.٨)	١٠ (٢٩.٤)	٨ (٢٣.٥)	٥ (١٤.٧)	٤ (١١.٧)	٥ (١٤.٧)	١٤٩٦.٤ ± ١٦٨٣.٣	>٠.٠٥
Student ٢٢ (١١.٠)	٠	٢ (٩.٠)	٣ (١٣.٦)	١٠ (٤٥.٤)	٦ (٢٧.٧)	١ (٤.٥)	٠	٨١٤.٥ ± ٥٤٥.٢	
Housewife ١٤٤ (٧٢.٠)	٥ (١١.٣)	٣٣ (٢٢.٩)	٣٨ (٢٦.٣)	٣٩ (٢٧.٠)	١٩ (١٣.١)	٦ (٤.١)	٤ (٢.٧)	٧١٥ ± ٩٢٥. ٣	

Table no. (٥) shows that the mean of anti-rubella antibodies titer by HAI test was slightly high in pregnant women than non-pregnant ones (٩٠٧.٦ ± ١١٣٦ and ٧١٧.٢ ± ٩٩١.٧ respectively), but (٣٠.٩٪) of pregnant women were presented with a titer of (٦٤٠) and (٢٩.٣٪) of non-pregnant ones presented with a titer of (٣٢٠).

Table No. (٥): Anti-rubella antibodies titer by HAI test according to pregnancy.

Pregnancy No. (%)	Titer								P value
	٨٠	١٦٠	٣٢٠	٦٤٠	١٢٨٠	٢٥٦٠	٥١٢٠	Mean ± SD	
	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)		
Pregnant ١٤٢ (٧١.٠)	٣ (٢.١)	٢٤ (١٦.٩)	٣٤ (٢٣.٩)	٤٤ (٣٠.٩)	٢١ (١٤.٧)	٩ (٦.٣)	٧ (٤.٩)	٩٠٧.٦ ± ١١٣٦	>٠.٠٥
Non-pregnant ٥٨ (٢٩.٠)	٢ (٣.٤)	١٣ (٢٢.٤)	١٧ (٢٩.٣)	١٣ (٢٢.٤)	٩ (١٥.٥)	٢ (٣.٤)	٢ (٣.٤)	٧١٧.٢ ± ٩٩١.٧	

Table no. (٦) shows that the mean titer of anti-rubella antibodies by HAI test of pregnant women in first and third trimester was (٨٣٠ ± ٩٤٥.٨ and ٨٦٤.٦

± ١٢٢٦.٣ respectively). (٣٥.٠٪) of pregnant women in first trimester presented with a titer of (٦٤٠) and (٢٦.٩٪) of pregnant women in third trimester presented with a titer of (٣٢٠). While the pregnant women in second trimester had the mean titer of (١٠١٤.٤ ± ١٣٥٢.٩), and the highest percent (٣٤٪) of them presented with a titer of (٦٤٠).

Table No. (٦): Anti-rubella antibodies titer by HAI test according to trimester.

Trimester No. (%)	Titer								P value
	٨٠	١٦٠	٣٢٠	٦٤٠	١٢٨٠	٢٥٦٠	٥١٢٠	Mean ± SD	
	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)		

First ٤٠ (٢٨.٢)	١ (٢.٥)	٧ (١٧.٥)	٨ (٢٠.٠)	١٤ (٣٥.٠)	٦ (١٥.٠)	٣ (٧.٥)	١ (٢.٥)	٨٣٠ ± ٩٤٥.٨	>٠.٠٥
Second ٥٠ (٣٥.٢)	٠	٩ (١٨.٠)	١٢ (٢٤.٠)	١٧ (٣٤.٠)	٥ (١٠.٠)	٣ (٦.٠)	٤ (٨.٠)	١٠١٤ ± ١٣٥٢.٩	
Third ٥٢ (٣٦.٦)	٢ (٣.٨)	٨ (١٥.٣)	١٤ (٢٦.٩)	١٣ (٢٥.٠)	١٠ (١٩.٢)	٣ (٥.٧)	٢ (٣.٨)	٨٦٤.٦ ± ١٢٢٦.٣	

Table no. (٧) shows that the mean titer of anti-rubella antibodies by HAI test of nullipara women was (٨١٥.٤ ± ١٠٠.٩) and the highest percent of them (٣٦.٥٪) were presented with a titer of (٦٤٠). The women with a parity of ١, ٢ and ٣ had a mean titer of (١٠٥٦.٥ ± ١٠٨٥.٧, ١١٢٤.٥ ± ١٣٨٣, and ٨٥٦.٤ ± ١٠٠٨.٩ respectively), and the highest percent of them (٢٧.٥٪, ٢٥.٠٪, and ٤٧.٠٪ respectively) had a titer of (٦٤٠). The women high parity (٤ and ٥) had a mean titer of (٢٠٠ ± ٨٠ and ٢٤٠ ± ٩٢.٤ respectively), and (٧٥.٠٪) multipara women (٤) had a titer of (١٦٠) and other multipara women (٥) were that equally (٥٠.٠٪) had (١٦٠) and (٣٢٠) titer.

Table No. (٧): Anti-rubella antibodies titer by HAI test according to Parity.

Parity No.(%)	Titer								P value
	٨٠	١٦٠	٣٢٠	٦٤٠	١٢٨٠	٢٥٦٠	٥١٢٠	Mean ± SD	
	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)		
٠ ٥٢ (٣٦.٩)	٠	٧ (١٣.٤)	١٥ (٢٨.٨)	١٩ (٣٦.٥)	٧ (١٣.٤)	٢ (٣.٨)	٢ (٣.٨)	٨١٥.٤ ± ١٠٠٩.١	> ٠.٠٥
١ ٢٩ (٢٠.٦)	١ (٣.٤)	٣ (١٠.٣)	٦ (٢٠.٦)	٨ (٢٧.٥)	٦ (٢٠.٦)	٤ (١٣.٧)	١ (٣.٤)	١٠٥٦.٥ ± ١٠٨٥.٧	
٢ ٣٥ (٢٤.٨)	٢ (٥.٥)	٥ (١٣.٨)	٦ (١٦.٦)	٩ (٢٥.٠)	٨ (٢٢.٢)	٢ (٥.٥)	٣ (٨.٣)	١١٢٤.٥ ± ١٣٨٣	
٣ ١٧ (١٢.١)	٠	٣ (١٧.٦)	٤ (٢٣.٥)	٨ (٤٧.٠)	٠	١ (٥.٨)	١ (٥.٨)	٨٥٦.٤ ± ١٠٠٨.٩	
٤ ٤ (٢.٨)	٠	٣ (٧٥.٠)	١ (٢٥.٠)	٠	٠	٠	٠	٢٠٠ ± ٨٠	
٥ ٤ (٢.٨)	٠	٢ (٥٠.٠)	٢ (٥٠.٠)	٠	٠	٠	٠	٢٤٠ ± ٩٢.٤	

Discussion

The evaluation of anti-rubella antibody profiles among females in childbearing age and in various geographical areas is essential for effective administration of rubella vaccine to lessen congenital rubella syndrome in non-immunized women during pregnancy.

The study revealed that the overall prevalence rate of anti-rubella antibodies by ELISA technique was (٧٧.٦٪), whereas the study revealed that the overall prevalence by HAI test was (٨٠.٠٪), this might be due to that ELISA test is more sensitive and specific than HAI test (Best *et al.,* ١٩٨٠). Similar results were reported in Pakistan (٧٧.٠٪) (Azmi *et al.,* ١٩٨٧); in Thailand (٧٥.٠٪) (Boonruang and Buppasiri, ٢٠٠٥); and in the southern of Iraq (Basrah) (٧٩.٠٪) (Yaseen, ١٩٩٢). Higher result were reported in Al-Doora, Baghdad (٩٤.٨٪) (Al-Heety, ٢٠٠٠).

Higher results were reported in other Arab countries, in Kuwait (٩٢.٣٪) (Makhseed *et al.,* ٢٠٠١); in Jeddah, Saudi Arabia (٩٣.٠٪) by HAI test (Basalamah and Serebour, ١٩٨٢); also in Saudi Arabia (٩٣.٣٪) by ELISA (Hani, ٢٠٠١); in Sana'a, Yemen (٨٥.٤٪) (Sallam *et al.,* ٢٠٠٦); and in other parts of the world, in Tehran (٩٨.١٪) (Soleimanjahi *et al.,* ٢٠٠٥); in Catalonia, Spain (٩٨.١٪) (Dominques *et al.,* ٢٠٠٥); in Switzerland (٩٤.٣٪) (Zufferey *et al.,* ١٩٩٥); in Dakar, Senegal (٩٠.١٪) (Dromiqny *et al.,* ٢٠٠٣); in Malaysia (٩٢.٣٪) (Sekawi *et al.,* ٢٠٠٥); in Maputo, Mozambique (٩٥.٣٪) (Barreto *et al.,* ٢٠٠٦); and in

Taiwan (٨٥.١٪) (Tsenq *et al.,* ٢٠٠٦); in Cameron (٨٣.٩٪) (bioELISA rubella IgG kit literature). This explained by an efficient vaccination programs or there is high rate of clinical or subclinical infection. While lower results of seropositivity were reported in Leon, Guanjuato, Mexico (٧١.٠٪) (Macias-Hernandes *et al.,* ١٩٩٣); in Korea (٧٣.١٪) (Park and Kim, ١٩٩٦); in Amritsar, Punjab (٦٨.٨٪) (Singla *et al.,* ٢٠٠٤); in Thrace, Greece (٦٧.٠٪) (Mela, ٢٠٠٤); and in Mersin, Turkey (٥٥.٠٪) (Sasmaz *et al.,* ٢٠٠٦). In India the rate of anti-rubella antibodies increasing from (٤٩.٠٪) in ١٩٨٨ to (٨٧.٠٪) in ٢٠٠٢ (Gandhoke *et al.,* ٢٠٠٥).

Regarding the age, the present study revealed that the highest rate of seropositivity (٨٥.٩٦٪) was in age group (٢٥-٢٩) years, while the lowest rate (٦٦.٧%) was in age group (≥٤٠) years (figure ٦-A). Similar results were reported by others (Boonruang and Buppasiri, ٢٠٠٥); (Singla *et al.,* ٢٠٠٤); (Al-Heety, ٢٠٠٠); and (Yaseen, ١٩٩٢). This could be explained by that increase chance of exposure to the virus which could be either in form of vaccine or infection, or due to that the antibody response declines, overtime, to below the protective level. Prospective serological surveillance has shown that vaccine induced antibodies would persist in the majority of persons over a period of ٧-١٠ years (Horstmann, ١٩٨٢), and might persist for as long as ١٨ years after vaccination (Kudesia *et al.,* ١٩٨٥).

Regarding the residency, the present study revealed that the rate of seropositivity was higher among women who live in urban areas than those who live in rural areas (figure ٦-B). This could be explained by that the knowledge about the rubella as a preventable disease in urban areas is more likely, and more contact with information regarding it than in rural areas. Similar results were reported by (Barreto *et al.,* ٢٠٠٦); (Singla *et al.,* ٢٠٠٤); and (Macias-Hernandes, ١٩٩٣).

Although education should have an effect on knowledge about rubella and its prevention by vaccination program. The present study revealed that the level of education had no-significant effect on the prevalence of anti-rubella antibodies (figure ٧-A). Similar results were reported by (Boonruang and Buppasiri, ٢٠٠٥); and (Yaseen, ١٩٩٢).

Regarding the occupation, the present study revealed that the highest rate of seropositivity was in the employees women than the students and housewives with no significant difference (figure ٧-B, table-٤). This could be explained by that the employees women had more contact with people, so more likely susceptible to get infection whether clinical or subclinical to be seropositive more. Similar results were reported by (Boonruang and Buppasiri, ٢٠٠٥); and (Yaseen, ١٩٩٢).

In respect to pregnancy, the study revealed that the rate of seropositivity was higher in pregnant women, than non-pregnant ones (figure ٨-A). But the difference between them was not significant (table-٥). Similar results were reported by (Al-Heety, ٢٠٠٠); and (Barreto *et al .,* ٢٠٠٦). While Singla *et al.,* (٢٠٠٤) reported that the prevalence of rubella antibodies in pregnant women was less than that observed in non-pregnant ones. Also the highest rate of seropositivity was among women in the first trimester of pregnancy than other period of pregnancy (figure ٨-B), but there is no significant difference had seen (table-٦). Similar result was reported by (Boonruang and Buppasiri, ٢٠٠٥). While Al-Heety (٢٠٠٠), reported that the prevalence of rubella antibodies was high among women in the second trimester than those in the first and third trimester. The reason for this is not clear, but we are in need for further

studies stressing on non-pregnant women, then to follow them in pregnancy through the three trimesters to give an exact explanation for this finding.

The study, also, revealed that the highest rate of seropositivity was among multipara women with two and three children than other women (figure ٩), but there is no significant difference (table-٧), this might be related to the age factor. Similar result was reported by (Boonruang and Buppasiri, ٢٠٠٥). But Yaseen (١٩٩٢) was reported that the majority of seronegative women were multipara with two children. Miller *et al.,* (١٩٨٢), was mentioned that the attack rate in para-٢ women was about ٢.٥ times that in para-٠ of the same age.

The present study revealed that the mean of the titer of anti-rubella antibodies by HAI test was elevated with age as the mean of the titer was (٤٣١.٧) in age group (١٥-١٩) years, then (٦٠٩.٢) in age group (٢٠-٢٤) years, until reach its maximum level (١٩٥٢) in age group (٢٥-٢٩) years, after that, it started to decrease gradually with increasing age (table-١). This could be explained by that the immunoglobulin level had been maximum in the first ten years after vaccination or exposure, and persist for life in lower levels.

The study, also, revealed that the mean of the titer was higher among women who live in urban areas (١٢٦٢.٤ ± ١٣٧٥.٤), than those residing in rural areas (table-٢). This might be related to fact that the women who live in urban areas had more chance to expose to infection because of more likely contact with infected persons. The mean of the titer was higher among women with secondary education level (١٣٠٧.٩ ± ١٣٥٠) than others (table-٣), which is related to the age factor. Also the mean of the titer was higher among employees women (١٤٩٦.٤ ± ١٦٨٣) than students and housewives (table-٤), this could be explained by that the employees women had more contact with

individuals, so higher chance of exposure to infection, while the mean of the titer shows no significant difference between pregnant and non-pregnant ones (table-٥). However, the pregnant women in the second trimester shows high mean of the titer (١٣٥٢.٩ ± ١٠١٤) (table-٦) (similar result was reported by Al-Heety, ٢٠٠٠). The study revealed that the mean of the titer was high in multipara women with two children (١١٢٤.٥ ± ١٣٨٣), which is commonly young than women with a multipara until it reach its lowest level at older women who were with a parity of four and five children (table-٧), this mean that this effect of parity on the titer is related to age factor.

According to figure (١٠), the least protective level of anti-rubella antibody titer were assessed to be ١:٣٢٠, and in this category all women have had ≤٣٢٠ titer of antibody should be revaccinated to maintain a protective level of immunity against rubella. About ٦٨.٦٪ of women ١٥-١٩ years of age, ٣٩.٦٪ of women ٢٠-٢٤ years, ٨.٠٪ of women ٢٥-٢٩ years, ٧٢.٩٪ of women ٣٠-٣٤ years, ٨٨.٨٪ of women ٣٥-٣٩ years, and all women ≥٤٠ years had a titer of ≤٣٢٠ have to be revaccinated. According to table (٢), about ٢٧.٠٪ of the urban and ٦٢.٠٪ of the rural women have to be revaccinated against rubella to reach a protective level of antibodies. In the same sequence, ≥٦٧.٠٪ of the illiterate, about ٥٥.٠٪ of the primary educated. ٢٥.٠٪ of the secondary educated and ٣٠.٠٪ of high educated have to be revaccinated. About ٣٥.٠٪ of the employees women, ٢٠.٠٪ of the students and ٦٠.٠٪ of the housewives have to be revaccinated to have a protective level of immunity. In respect to pregnancy, about ٤٣.٠٪ of pregnant women, and ٥٥.٠٪ of non-pregnant ones have to be revaccinated. Those who were pregnant, ٤٠.٠٪ of them in first trimester, ٤٢.٠٪ in second trimester, and ٤٦.٠٪ in third trimester have to be revaccinated to reach a protective level of immunity. About ٤٩.٠٪ of nullipara women, ٣٤.٠٪ of women with one child, ٣٧.٠٪ with two child, ٤١.٠٪ with

three child, and all multipara women with four and five child have to be revaccinated against rubella.

Conclusions

١. There were a fair number of women in childbearing age still at high risk for acquiring rubella virus infection.
٢. Women who lives in urban areas show high possibility to develop seropositivity, which may be due to exposure or vaccination.
٣. The titer of anti-rubella antibodies mainly affected by age and residency than other factors.

Recommendations

١. Examining of women in premarital stage and pregnancy for anti-rubella antibodies is recommended.

٢. Rubella immunization program in secondary schools should be continued to ensure that all girls are immunized to rubella before they reach childbearing age.

٣. Encouragement the health education for the public about the hazard of rubella, the importance of vaccination for prevention this disease and other information regarding rubella.

٤. Health worker should be educated to the importance of proper handling and storage of rubella vaccine to avoid the failure of vaccination.

٥. For non immune women, vaccination at premarital visits, post abortion, post partum, or during any contact with the health care system with warning to avoid pregnancy for three month following vaccination will be very useful.

٦. A continuous serosurveillance is needed to monitor vaccine efficacy in the field and to ensure that a protective level of antibody is maintained throughout the female reproductive period.

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