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A study some biomarkers associated with rubella virus infection in women

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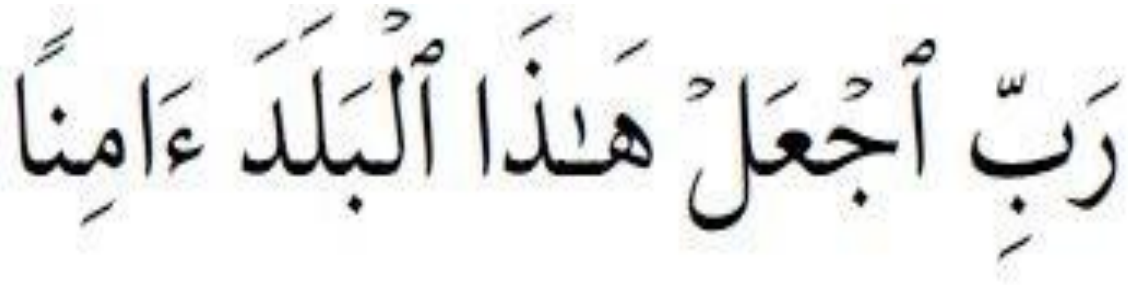
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صدق الله العظيم

سورة البقرة (126)

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Thank you very much, for any one who has helped me

Dedication

Peace to those who are in my life…….

To them I bend a letter of love and gratitude...

Here is my mother and father ... and peace be upon you…….

To my helpers and joy in life my brothers, my wife, and my children…

Hashim

Supervisor Certification

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Summary

This study aims to assess the link among biochemical, hematological and immunological parameters with rubella virus and to determine code diagnosis for rubella virus. It also aims to study of the effect of Rubella on body functions (liver, kidney, immune system) as well as anemia, white blood cell count, rheumatism among women in Baghdad governorate. In this study, 180 women with a positive result of the rubella virus tested ELISA. The control group was also selected with 50 healthy women with a negative result of the rubella virus by testing the ELISA, collecting venous blood from control individuals and patients for the serum. The function of the liver was evaluated by TSB. The function of the kidney was evaluated by measuring the concentrations of urea and creatinine.

The study group was divided into married women (50) 21% and married (non-pregnant) women 45 (19%). This work likewise revealed a quantity of unmarried women (85) percentage (36.7 %) and number of control 50 (21%). The prevalence of IgG was 82.78%, IgM (2.22%), IgG + IgM. (15.00%) described several vital signs that are associated with the rubella virus. These are Interleukin-10 (IL-10), which accounted for 96.11%, Interleukin-6 (IL-16), which was 65% low, and RF for positive and negative cases was 10.56% and 89.44%. The result of rubella infection with CD8 that showed a significant in the level ($P<0.01$), high in CD8 (98.33%) compared with normal value (1.67%) and low values (0%) and in CD4 that showed the normal level of (80.56%) compared to the low value of (19.44%) and the high value was (0%). Creatinine which accounted for the normal ratio and its decrease (98.33%, 1.67%) respectively, the normal ratio and the rise in urea (67.778% 31.11%), respectively, The normal ratio, the decrease and the rise in the White blood cells (WBC) (65%, 16.667% and 18.333%), respectively, was the normal ratio, the decrease and the rise in the Hb (47.778%, 48.333% and 3.889%),

respectively, The normal ratio, the decrease and the rise in the TSB (18.333%, 53.889% and 27.778%), respectively.

The results pointed out a substantial growth ($p < 0.01$) in Rubella virus activity rates, parameters, WBC, Hb, TSB, B. Urea, S.creatnine, IL6, IL10, CD4, and CD8. Rubella virus affects kidney and liver function in addition to increasing or decreasing in the morbidity of the levels of some vital indicators in the patient group, which is likely to affect some members of a body because of serious emergence diseases such as inflammations of liver and lung, immune system disorders, anemia and rheumatism.

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List of abbreviations

Interleukin-6	IL-6
Interleukin-10	IL-10
Human Cluster of differentiation 4	CD4
Human Cluster of differentiation 8	CD8
Arthritis	RA
Rheumatoid Factor	RF
Rubella virus	RV
Enzyme-linked immune sorbent assay	ELISA
White blood cells	WBC
Hemoglobin	Hb
Red blood cell	RBC
creatinine clearance	Ccr
Total serum bilirubin	TSB
Measles virus	MV
Congenital RV syndrome	CRS
Radio immune assay	RIA
complement fixation	CF
measles , mumps and rubella vaccine	MMR
major histocompatibility complex class I	MHCI
major histocompatibility complex class II	MHCII

1. Introduction

The *rubella* virus belongs to the Togviridae family. It is the only virus of the genus Rubi. It is an enveloped virus between 60 and 70 nanometers in diameter and it contains a single strand of RNA and it encompasses three major polypeptides including - E1, E2, and C (Vynnycky *et al*., 2016). Rubella virus spreads via the respiratory route from one human to another. The virus primarily duplicates in local lymph nodes and nasopharyngeal mucosa. It is transmitted either by direct contact or by droplet contact from nasopharyngeal secretions with an incubation period extending from twelve to twenty-three days, averaging in 14 days (Fauci, *et al* 2012; Lanzieri, *et al*., 2017). It is the principal cause of global birth flaws (Kancherla, *et al* 2014).

The virus infects the placenta in pregnant women. It reaches the fetus and ultimately alters various functions of fetal systems by hindering organ formation and triggering systemic inflammation (Moss and Griffin, 2015). Rubella is simply temperately contagious. It is highly contagious only once the rash first detected, nonetheless, it might be shed from seven days before to five to seven days or more after rash onset (Atkinson, *et al*., 2011).

It is a vital virus that causes many diseases, a contagious virus that is often call the measles, this virus affects both sexes and different ages, and the incidence of children and adults is mild. This type of postnatal infection is called postnatal infection (Hay-JR et al., 2007). Pregnant women who do not have the IgG antibodies to the rubella virus are at risk of contracting the virus. If infection happens at pregnancy start, specifically throughout the first trimester, it can lead to serious complications, most notably abortion, stillbirth or the birth of children with congenital rubella syndrome (Levinson, 2006).

Rubella infection could outcome a fetal death, miscarriage premature delivery and constellation of severe birth defects known as Congenital Rubella Syndrome (CRS) when it happens just before conception and throughout the first trimester of pregnancy (Lanzieri, *et al* 2017).

The specific antibodies of the virus are detected by ELISA technique and other diagnostic methods to identify the complete gene structure in sera prevalence in different regions of the world which has been shown to have a prevalence of immunoglobulin M (IgM) and immunoglobulin G (IgG) titers increase at first, with IgG persisting though IgM antibody titers wane properly quickly, and by eight weeks the latter are typically untraceable, a rubella-specific T-cell response starts a week after the humoral response, and the cell mediated immunity seems to continue forever. Nevertheless, rarely, serologically documented re-infections have happened, and re-infection throughout pregnancy causing CRS was stated. The presence of rubella IgG antibodies ≥10 IU/ml is normally well-thought-out to deliver evidence of protection, though there are few alterations among countries in terms of the concentration of IgG antibodies well thought-out protective (Geneva, 2011).The prevalence of IgG infection among the sample of pregnant women who are not vaccinated with rubella virus in China ranged from 5.4 to 14.1% (Sa *et al*., 2006). As show in non-pregnant women, sera positivity rates were between 90.9% and 96.8% (Doroudchi *et al.,* 2001; Jarour *et al.,* 2007).

Some biomarkers are used to determine associated with rubella virus infection in women. Immune biomarkers consists: cytokines (IL6, IL10) cytokines are regulatory proteins originating from immune system cells, they act as mediators of several functions including differentiation of lymphocytes, stimulating growth, stimulating the development of

hematopoietic cells, and activating effector cells (Garrote *et al*., 2008; Kleiner *et al*., 2013). Human cluster of differentiation 4 (CD4) Receptor CD4, is adifferentiation marker that is mainly articulated on the surface of T lymphocytes. It is a 55 kDa type I integral membrane glycoprotein that is essential throughout adaptive immune responses. As well as T-cells, CD4 is expressed by monocyte-derived macrophages, monocytes, mast cells, Langerhans cells, megakaryocytes, B-lymphocytes, dendritic cells, and eosinophils (Badoual *et al*., 2004). Human cluster of differentiation 8 (CD8) CD8+ T-cells are a subset of lymphocytes with cytotoxic functions prompting Tcell receptors (TCR) which recognize peptides offered by MHC class I molecules existing on each nucleated cell (Sallusto *et al.*, 1999).

Serology biomarker: Arthritis (RA), Rheumatoid Factor (RF) Symptoms are related to inflammation of joints; examples, stiffness, temperature, pain, and aching one or more of the joints, which result in a joint mobility restraint and disability , the serological studies showed that there are still people who are ready to accept the virus, these are among the most dangerous groups (Kurtin, 2000). Hematology biomarkers: white blood cells (WBC) are of boundless prominence for human immune system and are essential for their aptitude to fight viral and bacterial infections, WBC are divided due to their differs in structure and cell division lineage into five key forms monocytes, neutrophils, lymphocytes, eosinophils, and basophils (Hematological Atlas, 2013). Hemoglobin (Hb) A hemoglobin test quantity blood hemoglobin amount, It is the main component of red blood cells (RBC) (erythrocytes), RBC are formed in the bone marrow, Hemoglobin encompasses iron allowing oxygen binding, RBC move oxygen from lungs to other tissues and organs,

they also transport CO_2 to lungs therefore it may be removed from the body (Jensen, 2009). Biochemical biomarkers: urea, creatinine, and bilirubin are waste product. Bilirubin, the main pigment in bile, comes from hemoglobin breakdown, after some degradation steps, the free bilirubin will be bounded by albumin and it transmitted by blood to the liver. (Bosma, 2003)

Aims of the study

1 – Detecting some biomarkers associated with rubella virus infection in women through immunological, biochemical and hematological tests and facilitating the diagnosis of the doctor

2 - Finding the relationship between biochemical and hematological tests on the one hand and viral infections on the other and their impact on the immune system and some organs of the body, such as liver and kidney

2-1: Rubella virus (RV)

Rubella virus is categorized as the single associate of the genus Rubivirus inside Togaviridae family.The term "togaviurs" is derived from Latin word "toga", which means shroud or cloak. Genus Alphavirus was lone extra genus inside its family, which encompasses no less than 26 members. Human is the single natural host of rubella virus. It can be spread via the respiratory route from one human to another. It initially copies in local lymph nodes and nasopharyngeal mucosa. It infects the placenta in pregnant women and spreads to the fetus and modifies the function of several fetal systems by interfering with organ formation and triggering systemic inflammation (Moss and Griffin, 2015). Infants with congenital rubella syndrome (CRS) shed huge virus amount from body secretions in a 1-year period and might consequently spread to persons carrying them who are liable to the disease. Rubella can be spread by humans with subclinical or asymptomatic cases (reaching half infections of rubella virus) (Atkinson *et al*., 2011). It may exist in nasopharyngeal samples from one week before rash onset to two weeks after, with utmost molting happening one to five days after rash onset (Wkly, 2011). It is highly contagious only once it primarily shows, nonetheless it might be hut starting seven days before to five to 7 days or further after rash onset (Atkinson *et al*., 2011).

Rubella disease or sense as German measles or three-day measles is an acute viral infection triggered using *Rubivirus*. It is accompanied by some symptoms as fever, discrete erythematous maculopapular skin rash in all part of body and auricular, cervical and occipital lymphadenopathy in which usually is mild in children and adults, even though in most cases are asymptomatic and resolve spontaneously, While developing

fetus infection occurs followed by transmission from mother to fetus, especially during the first trimester of pregnancy, creates severe side effects that cause congenital rubella syndromes recognized as congenital rubella syndrome (CRS) (Hatami, *et al*. 2013). (Plans, 2014).

CRS frequency differs globally, contingent upon naturally acquired immunity levels, overcrowding and immunization policies and practices (Castillo *et al*., 2011); (WHO. 2013). The incidence of congenital anomalies that follows the infection in the first trimester of pregnancy is 80 to 85% of cases (Zimmerman and Reef, 2012). Side effects could contain abortion, premature birth, fetal death, or live birth defect including congenital cataracts, hearing impairment, cardiac abnormalities, central nervous system involvement, and low weight at birth, the temporary effects of the adenopathy, bone lesions, enlarged liver and spleen, hepatitis, meningoencephalitis and late effects such as diarrhea (Arya, and Agarawal, 2014).

It is usually a trivial febrile viral illness containing a diffuse punctate and maculopapular rash occasionally approximating that of measles or scarlet fever (Chin, 2000). Nevertheless, throughout the early stage of pregnancy of the mother, infection encourages severe congenital disorders of the infected fetus; this condition is recognized as congenital rubella syndrome (Ushida *et al.*, 2003).

The diagram below shows the core section of Rubella virus (RV) with genomic detail, while on the right is the surface structure. Figure (2-1)

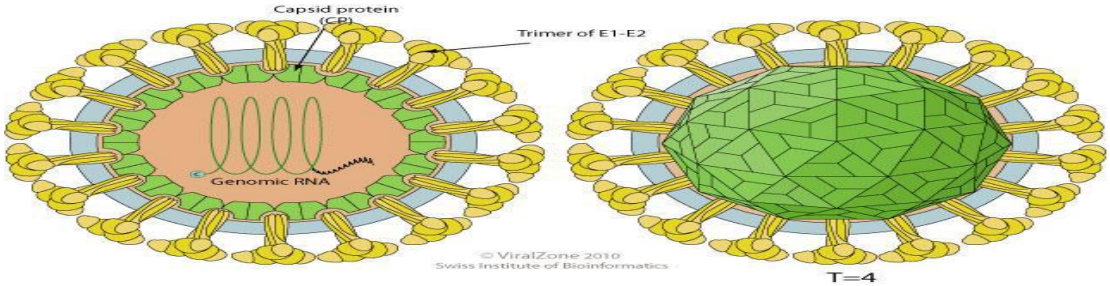


Figure (2-1) Rubella virion (ViralZone, 2010)

The danger of emerging CRS in the fetus is up to 90% once the pregnant mother is diseased with RV through pregnancy first trimester (Best, 2004). The major cause for rubella vaccination programs is the prevention of CRS (Reef *et al.,* 2006). Rubella virus has relatively high sensitivity to ultraviolet light, heat, pH extremes of less than 6.8 and more than 8.1, and to a variety of chemical agents like ether, acetone, and formalin; however at 4c^{o} the virus titer is relatively stable for 24 hours (Gershon *et al*., 2004).

2.1.1 Structure of rubella virus

The RV is spherical-shaped with a width ranging between 60 to 70 nm. RV encompasses three main polypeptides including -E1, E2, and C (Wolnisky, 1996). E1 (MW 58000) and E2 (MW 42000 to 47000) are glycosylated and are positioned on the viral surface membrane, E1 is the viral hemagglutinin that is found on five to six nm surface projections. Nucleocapsid has a diameter of 30 to 40 nm and comprises of polypeptide (C protein) and the genomic RNA (DuBois *et al*, 2013).

The E1 glycoprotein is 481 amino acids long and it is further exposed on the virus surface than E2. Accordingly, E1 protein is the major antigenic determinant on the virus, and it is responsible for receptor recognition (Cong *et al*., 2011) and membrane virus fusion with the endosomal membranes at low-pH (Yang *et al*., 1998). The E1 glycoprotein binds to the recently identified receptor, myelin oligodendrocyte glycoprotein (MOG). Rubella virus has single-stranded RNA nucleic acid with a molecular weight of 3.2 to 3.8 × 10^{6}, the outer coat of the virus (envelope) is lipoprotein in nature with host-cell lipid and virus-specified polypeptides (Cong *et al*., 2011, Feigin and Cherry, 1992).

The E2 glycoprotein is 282 amino acid residues in length, it is essential for proper folding and maturation of the E1 glycoprotein (Garbutt *et al*., 1999; DuBois *et al*, 2013). Capsid protein has created along with glycoproteins like poly protein precursor amino terminus of the E2 glycoprotein by a signal peptide, this signal peptide targets both these proteins to the endoplasmic reticulum (ER), the E2 glycoprotein gets transported to the ER lumen and gets cleaved off by a host cell signal peptidase, the signal peptide stays linked to capsid protein anchoring it to the ER membrane Figure (2-2) (Suomalainen *et al*., 1990).

Rubella virus RNA genome is infectious, and complementary DNA copies facilitate study of its records (Wang *et al.,* 1994). Rubella virus is immunologically distinct from the other togaviruses. A single serotype of rubella virus exists. The analysis of sequence changing among several separates reveal highly amino acid structure conservation (Bosma *et al.*, 1996).

The capsid protein forms the nucleocapsid, which contains and interacts with the viral genome. The capsid protein existing as a disulphide linked dimer in the virus. The capsid monomer comprises of 277 amino acids with a molecular weight of about 31 kDa, and a high pI (iso-electric point) value of 10.6 and its amino acid sequence is rich in arginine and proline residues. The amino terminal region of approximately 100 amino acids is highly basic and interacts with the viral genomic RNA to form the nucleocapsid (Baron and Forsell, 1991; Frey, 1994). The amino acid region of residues 28 to 56 of the capsid protein binds specifically to nucleotides 347-375 of the viral RNA (Liu *et al*., 2006). Phosphorylation of capsid protein, especially Serine-46, restricts binding of RNA to the capsid protein. Thus, phosphorylation

and de-phosphorylation of the capsid protein is regulated in infected cells to coincide with budding of the virus (Law *et al*., 2003).

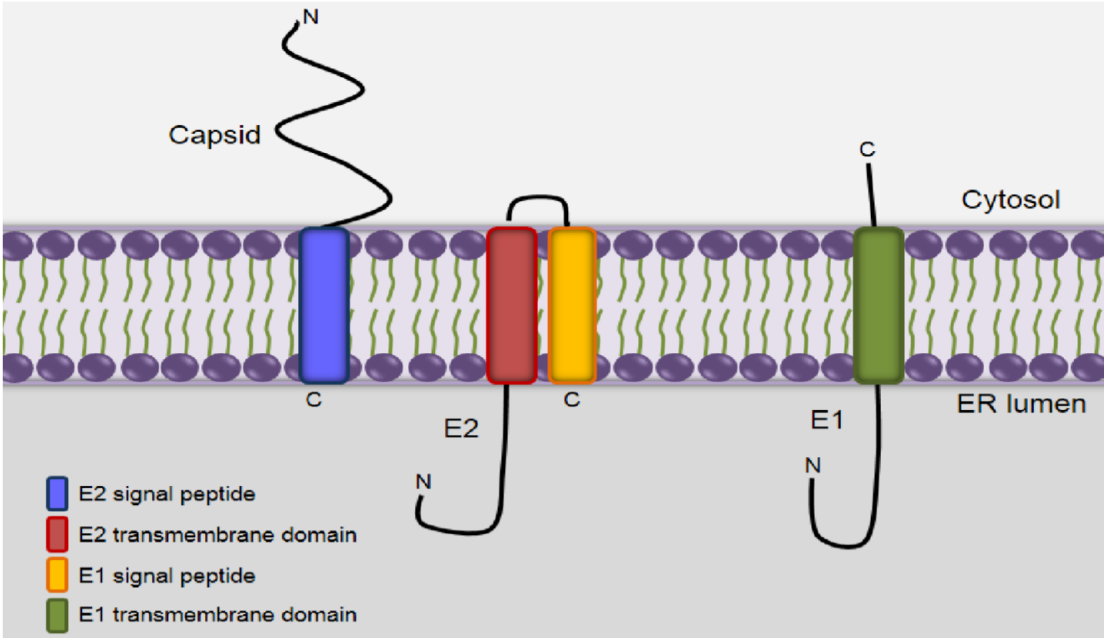


Figure (2-2) Position of RV structural proteins inside cell. (Suomalainen *et al*., 1990)

The different proteins are shown after post-translational cleavage, along with the signal peptides and transmembrane domains. The letters N and C denote the amino and carboxyl terminus of the proteins respectively. The figure is reproduced from (Mangala Prasad, *et al.*, 2013).

Apart from forming the nucleocapsid, interaction of the capsid protein with the E1 and E2 glycoproteins is required for assembly and budding of the virion (Qiu *et al*., 1994). The capsid protein also has other non-structural functions in the infected host cell. The capsid protein was reported to control the viral RNA transcription and replication (Chen *et al*., 2004). It inhibits protein translation of host-cell mRNAs by binding to the initiation factor PABP (Ilkow *et al*., 2008). The capsid protein causes dramatic changes in the morphology and

distribution of mitochondria in infected cells.It also inhibits mitochondrial import in infected cells (Beatch *et al*., 2005, Ilkow *et al*., 2010). It has been revealed toward binding proteins such as mitochondrial matrix protein (p32), prostate apoptosis response (Par-4) protein and Bax, which are involved in apoptotic pathways in host cells, thereby influencing apoptosis mechanisms in diseased cells (Beatch and Hobman, 2000; Beatch *et al*., 2005; Ilkow *et al*., 2011). This function may form the basis for the observed delay in lytic release of virus during rubella infection as compared to other RNA viruses (Ilkow *et al*., 2011). Multiple functions of capsid protein are depicted in (Figure 2-3) (Ilkow *et al*., 2010)

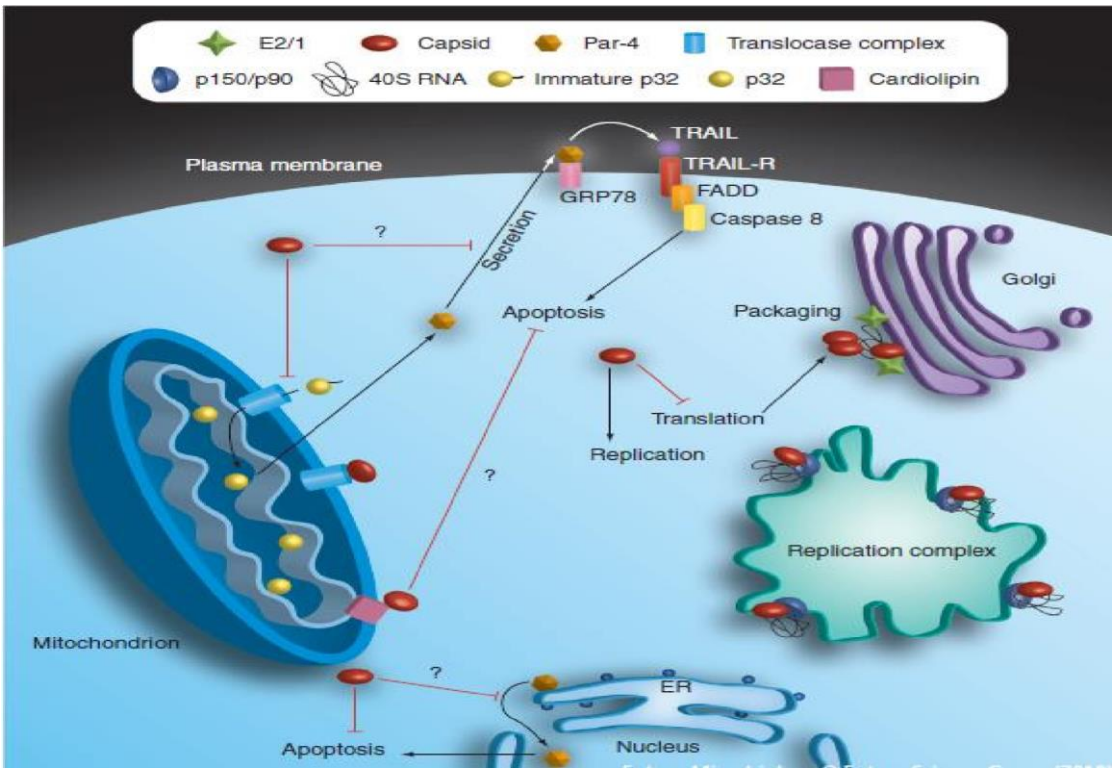


Figure (2-3) Different functions of rubella capsid protein in an infected cell. (Ilkow *et al*., 2010)

2.1.2 Non-structural proteins

They are interpreted as a polyprotein precursor after viral genomic RNA. The polyprotein is later sliced into two products, and p90 and p150, through the proteolytic activity of p150's carboxyl terminal region. These two proteins together form the viral replicas (Hobman and Chandler, 2007).

2.1.3 Virus replication and assembly

The rubella virus replication has the virus genomic positive, single-stranded RNA act as a template for the translation of the non-structural proteins, the genomic RNA serves as a messenger RNA for non-structural proteins and as a template for the synthesis of negative single stranded RNA (Fauquet *et al*., 2005; David *et al*., 2001). The viral RNA replicate the sequences of the structural proteins through a complimentary negative single stranded RNA which acts as a templates and translated as a short mRNA. This short sub genomic RNA is additionally packed in a virion (Atreya *et al*., 2004; Lee *et al*., 2000; Ono *et al*., 2001). The polyprotein precursor p110 is translocated into the endoplasmic reticulum by two separate signal peptides 23 and 20 amino acids in length located at the amino termini of E2 and E1. In the ER the rubella virus capsid protein is cleaved from E2 and E2 is cleaved from E1 (Zheng *et al.*, 2003).

The virus coating takes place in the acidic environment contained by endosome leading to viral genomic RNA releasing inside cytoplasm. Viral genome replication and subsequent manufacturing of viral proteins in the infected cells causes big changes in the intracellular organelle distribution and organization (Lee *et al*. 1996). Formation of cytopathic vacuoles occurs within infected cells. This is a characteristic of toga

viruses (Hobman and Chantler, 2007). These cytopathic vacuoles are derived from the lysosomes in cells and are sites for the viral replication complexes (Lee *et al*., 1994; Magliano *et al*., 1998). Cytopathic vacuoles are frequently found in association with mitochondria, the ER and Golgi stacks (Risco *et al*., 2003). On translation, the structural polyprotein of rubella virus is transported to the ER, the glycoproteins E1 and E2 are transported into the ER lumen while the capsid protein remains linked with cytoplasmic side of ER membrane (Suomalainen *et al*., 1990). The structural polyprotein is cleaved into its corresponding proteins by the host cell signals (Oker-Blom *et al*., 1990). Glycosylation and other posttranslational modifications of the glycoproteins occur within the ER. The structural proteins subsequently accumulate in the Golgi complex, where the assembly of virion particles takes place, the transmembrane regions of the E2 glycoprotein contain a Golgi retention signal and is predicted to determine the site of virion assembly (Garbutt *et al*., 1999). Interactions between the cytoplasmic domains of the glycoproteins and the capsid protein possibly form the trigger for virus budding (Garbutt *et al*., 1999; Qiu *et al*., 1999). The physical association observed between the Golgi stacks and the viral replication complexes form the basis for passage of the newly synthesized viral genome RNA to the sites of virus assembly (Fontana *et al*., 2010).

2.1.4 Viral genome

It is a single stranded RNA of positive polarity capable of interpreted instantaneously using host ribosomes. RV genome contains numerous elements, which are supposed to take part in replication, packing. Interaction with host cell factors, there are three untranslated regions (UTRs): a 5' 40bp UTR, a 59bp 3' UTR, and a 123 nucleotide UTR between the non-structural

and structural ORFs (Hobman, 2013). Two stem loop structures, one in the 5' untranslated region and one in the 3' untranslated region, were found to stimulate translation (Pogue *et al.*, 1993)

In the Figure (2-4) below, that show Rubella virus genomic structure (Lee and Bowden, 2000)

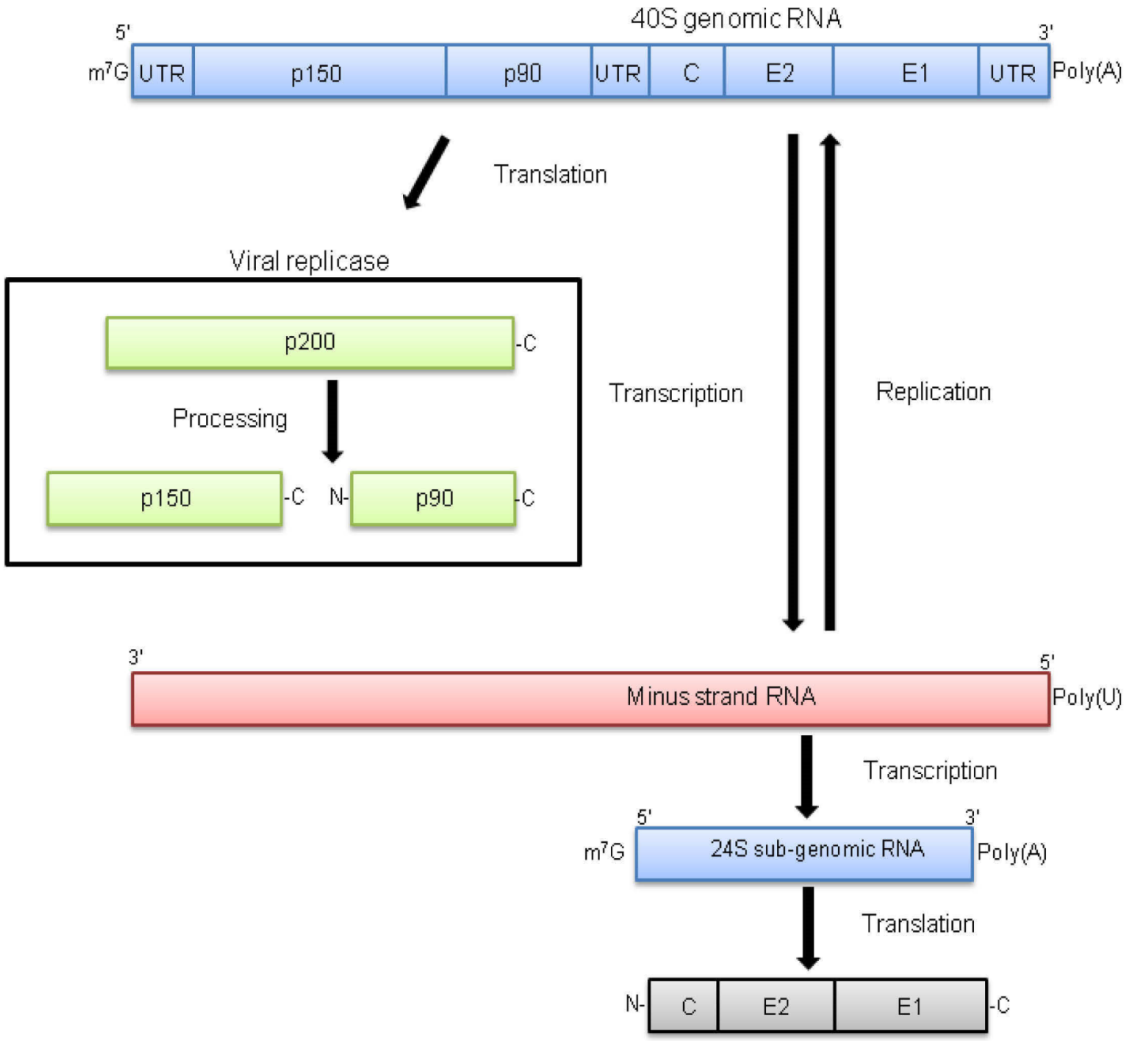


Figure (2-4) Rubella virus genomic structure. (Lee and Bowden, 2000)

The Rubella virus genome contains five genes and three untranslated regions (UTRs), the 5' ORF of the positive sense, 40S, genomic RNA is translated to produce the viral replicas polyprotein p200 that is later cleaved by a protease domain in the p150 protein, the replicas proteins, p150 and p90 transcribe the positive strand RNA into a minus strand that is used as a template to produce plus sense genomic and sub genomic RNAs, the sub genomic RNA is translated in association with ER membranes into the structural proteins capsid (C), E2 and E1(Ilkow *et al* 2010).

2.1.5 Pathology

Generally Rubella virus is establish a chronic non lytic infection in the fetus and has the potential to infect any organ (Wolnisky, 1996). 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 cataracts 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 stria vascularis (Webster, 1999).

2.1.6 Pathogenesis

Infection main place is the respiratory epithelium of nasopharynx. Initial infection of respiratory epithelium apparently is minor; a more important event is the early transmit of the virus to the regional lymphatics. Rubella virus multiplies at the respiratory site, and initiates a viremia of approximately 7 days duration (Fields, 1996).

Viremia peaks before the onset of exanthem and vanishes soon afterward. Alternatively, virus endures to be consistently contemporary in the nasopharynx for a 6-day period after the onset of rash and occasionally for an extra week thereafter, in addition to the blood and nasopharynx, rubella virus has been improved from the succeeding sites: lymph node, lung, synovial fluid, breast milk, conjunctival sac, cerebrospinal fluid, and urine, rubella virus was recovered from the skin of rubella patients at positions where rash was both absent and present, 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 50% 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 regularly may 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 Figure (2-5) (Feigin and Cherry, 1992).

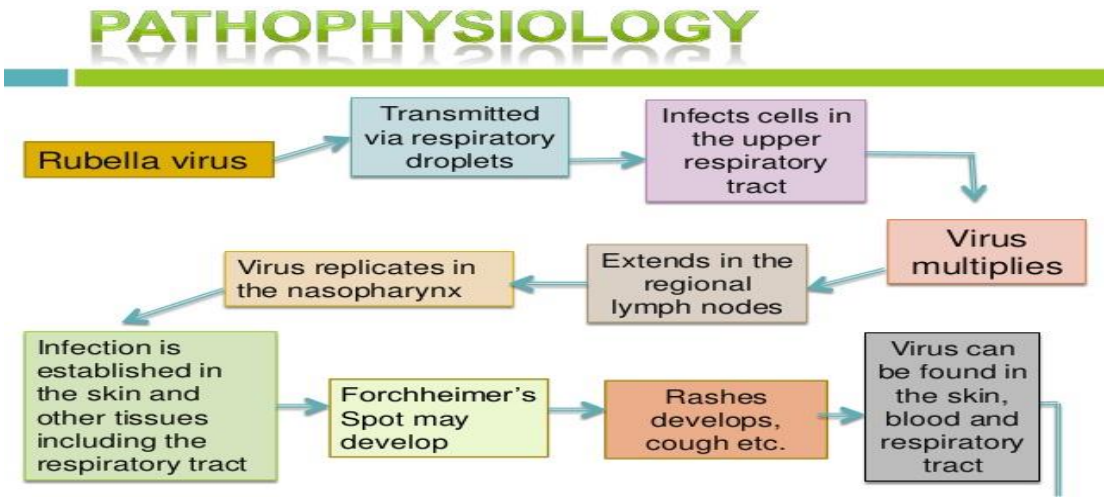


Figure (2-5) Proposed pathogenesis of congenital rubella (Feigin and Cherry, 1992).

2.1.7 Postnatally acquired rubella

2.1.7.1 Clinical manifestations

Clinical symptoms of rubella virus infection assimilated postnatally are typically mild, and twenty to fifty percent of infected patients were asymptomatic, incubation period of rubella is 12-21days with an average of 16-17 days (Green-wood *et al.*, 2002).

In the child, the first signal of disease is the attendance of the rash. it performs first on the face and then transmits downward fast to the neck, arms, trunk and extremities, eruption performs, spreads, and disappears extra rapidly than does of rash of measles. The duration and amount of the rash may be variable, which as a rule lasts for 3 days, may persist for 5 days or may be so evanescent that it disappears in less than a day. "In adolescents and adults, there is frequently a one - five day prodrom with low grade fever, lymphadenopathy, malaise, and upper respiratory symptoms preceding the rash" (Gershon *et al.*, 2004).

Lymphadenopathy can start a week previous to the rash lasting for numerous weeks. Post auricular, posterior cervical, and suboccipital nods are frequently involved (Lee and Bowden, 2000). At times, splenomegaly also may be noted, during the acute stages of disease. Other rubella symptoms including conjunctivitis, testalgia, and orchitis. Forschheimer spots are reddish, pinpoint or larger in size, and can be renowned on the soft palate, however aren't diagnostic for RV. WBC count is low but it may be normal. Occasionally, there may be an increased percentage of abnormal lymphocytes or a decrease in platelets Figure (2-6) (Gershon *et al.*, 2004).

Natural infection is accompanied with larger order of protection from reinfection. Nevertheless, reinfection evidence, that commonly, symptomatic, can be achieved by representing an important rise in antibody concentration subsequent natural an investigational contact with RV. It is hard to differentiate primary infection and reinfection, especially in the lack of blood soon later contact or if sera occupied prior to contact (for screening purposes) are not obtainable. Rubella reinfection is not linked to the de-neutralizing antibodies or a explicit defect in rubella specific lymphoproliferative responses, nonetheless a catastrophe in harvesting epitope-specific antibodies is likely (Zuckerman *et al.*, 2000).

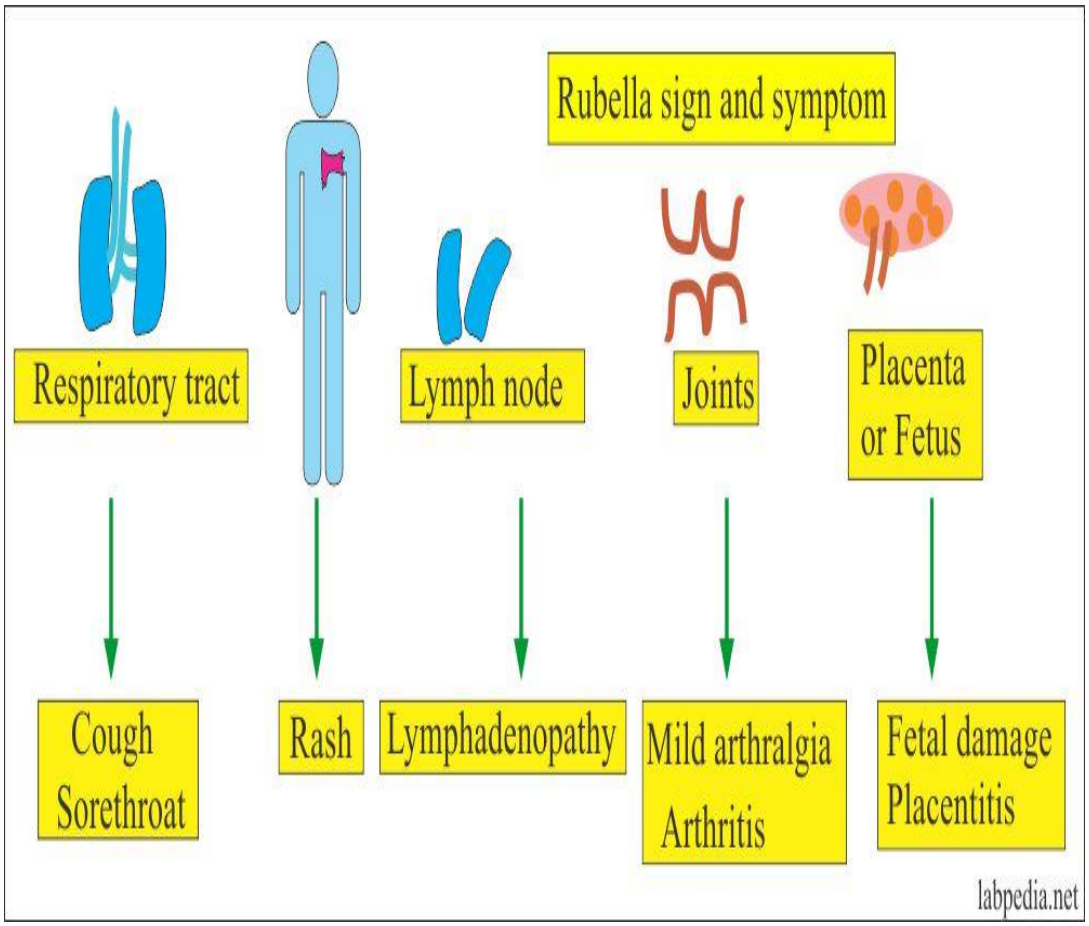


Figure (2-6) Clinical symptoms of rubella virus (Gershon *et al.*, 2004).

B- 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.*, 2004). Arthralgia and arthritis can happen in up to seventy percent of adult women who have RV, nonetheless it is infrequent in children and adult males. Shared symptoms happen around equal time or soon after the entrance of the rash, and they can persist for up to one month (Frey, 1994). 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.*, 2004).

At a ratio of 1/6,000 cases, central nervous system complications (i.e., encephalitis) happen which probably affect adults. On the other hand, at a ratio of 1/3,000 cases thrombocytopenia happens which probably affects children (Frey, 1994).

2.1.8 Congenital Rubella Syndrome (CRS)

Congenital rubella was identified as a clinical entity more than a century after the disease was first recognized. In 1941 Gregg stated congenital cascades incidence for about 78 infants after maternal incidence learned throughout the 1940 epidemic in Australia (Gershon *et al.*, 2004).

CRS is an assembly of physical irregularities which were established in infants due to maternal infection and following fetal infection with rubella virus (Robert-Gnansia, 2004). Congenital infection frequency is crucially relying upon the time of virus exposure. Roughly 50% of infants within 4 weeks of conception exposing to the virus will have signs of congenital infection. Once maternal infection happens in weeks 5 to 8 after conception, nearly 25% of fetuses will be infected. Once it grows in weeks 9 to 12, about 10% of fetuses will be infected. Once it happens beyond this point in time, less than 1% of fetuses are affected (Creasy *et al.*, 2004).

The utmost regularly designated anomalies linked to congenital rubella syndrome are, cardiac (peripheral pulmonary artery stenosis, patent ductus arteriosus), ophthalmologic (retinopathy, cataracts, and congenital glaucoma), neurologic (meningoencephalitis, behavioral disorders, and mental retardation), and auditory (sensorineural hearing impairment). Furthermore, infants having congenital rubella syndrome frequently were development retarded and could have radiolucent bone

disease, thrombocytopenia, hepatosplenomegaly, and purpuric skin lesions (showing a "blueberry muffin" attendance), initial reports of maternal reinfection advocated asymptomatic reinfection in primary pregnancy were doubtful to be linked to fetal infection, even once certain IgM response were noticed, reinfection can deliver danger to the fetus once it is linked to viremia; this has infrequently been documented (Morgan, *et al.,* 1991). Intended danger of fetal disease is nearly (8.0%) of following reinfection in the first sixteen weeks of pregnancy, however, fetal malformations are infrequent (Zuckerman *et al.,* 2000).

2.1.9 Epidemiology

Rubella virus is spread (congenital) to a fetus using a diseased mother (John, 2005). Acquired RV (non-congenital) is spread through airborne droplet emission from the upper respiratory region of active cases; may be approved by RV infected persons breath. Similarly, can be transmitted by a sneeze or a cough. The virus can be contemporary on the skin, carrier state is absent, reservoir happens completely in active human cases. The disease has an incubation period of 2 - 3 weeks (Anon, 2004)

Rubella virus is transmitted through interaction with nasopharyngeal secretions of infected people. Infected people shed great 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 virus for mite-hand respiratory or hand-hand respiratory routes (Feigin and Cherry, 1992).

The age at infection varies from area to area. Infection was maximum mutual in the 5 to 9 years old group before vaccine development. By initiation of childhood vaccination in the US, there were major shifts in virus incidences to young adult, it is evident to note that there were much advanced announcement rates in males, in countries accepting the selective vaccination policy (Lee and Bowden, 2000).

2.1.10 Immune Responses

A- Rubella virus with autoimmunity

Viral infections can promote autoimmunity using numerous dissimilar and occasionally joint mechanisms. The link of diseases and autoimmunity development were well labelled in the medical literature (Pordeus, *et al.,* 2008 and Shoenfeld, 2011). Viral infections may promote autoimmunity via several different and sometimes combined mechanisms; these mechanisms include direct cytolysis of virus-infected cells; induction of autoimmune responses to "altered self-antigens"; molecular mimicry resulting in the activation of autoreactive T and/or B cells by viral antigens; and bystander activation of autoreactive T cells that may be driven by viral super antigens. In addition, viruses may induce disturbances of the finely tuned balance between T regulatory cells (Tregs) and autoreactive T cells in favor of the auto reactivity, either by specific infection or destruction of T regulatory cells or by expansion of autoreactive T cells, and viruses may lead to the activation of innate immunity by triggering toll-like receptors. It could well be that multiple mechanisms need to act in concert to result in disease onset (Pordeus, *et al.,* 2008 and Christen, *et al.,* 2010). Numerous viruses were concerned as potential triggers or advocates of autoimmune responses, viral infections usually harvest transient auto immune responses, usually

aimed alongside blood cells, escorted with transient elevation in autoantibodies titers. Cytomegalovirus (CMV), hepatitis viruses, Epstein-Barr virus (EBV), and Parvovirus B19 are disreputably linked to arthritis induction, thrombocytopenia, and autoimmune hemolytic anemia (Pordeus, *et al.*, 2008 and Christen, *et al.*, 2010).

"The clinical symptoms and antibody titers are transient, and decline over numerous weeks for mainstream diseased patients", humeral and cell-mediated immunity improve resulting RV infection. IgG and IgM antibodies are perceived approximately fourteen-eighteen days after RV infection, in approximately time once rash seems to show **(Figure 2-7)**. RV IgM antibodies wane rapidly, are typically untraceable after two months. "Although RV IgG antibodies persevere, a rubella-specific cell-mediated lymphocyte response starts one week after the humoral response and remains forever" (Wkly, 2000)

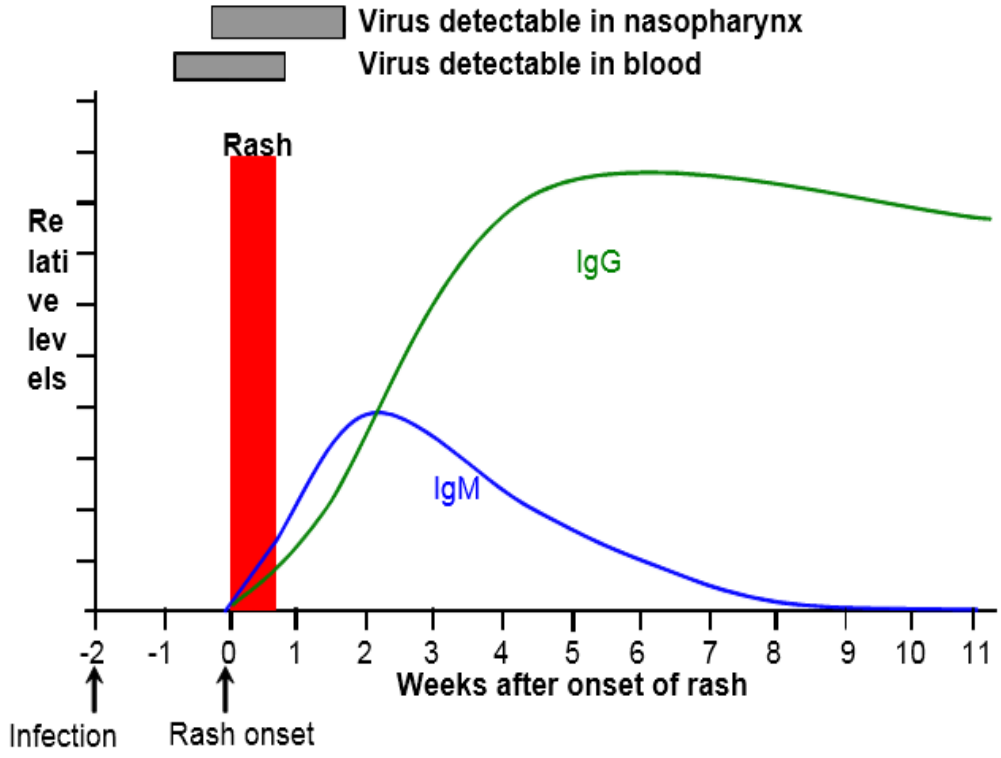


Figure 2-7 Immune response in typical rubella infection (Wkly, 2000.)

B- Naturally acquired immunity

Antibodies are initially noticeable approximately fourteen – eighteen days after acquired rubella infection, coinciding approximately with the appearance of the maculopapular rash. Immunoglobulin M (IgM) and immunoglobulin G (IgG) titers rise initially, with IgG persisting while IgM antibody titers wane rapidly, and by 8 weeks, the latter are usually undetectable. A rubella-specific T-cell response begins a week after the humeral response, and the cell-mediated immunity appears to persist throughout life. However, on rare occasions, serologically documented re-infections have occurred, and re-infection during pregnancy resulting in CRS has been reported. (Geneva, 2011). Although there is some variation among countries in terms of the concentration of IgG antibodies considered protective, the presence of rubella IgG antibodies ≥10 IU/ml is commonly considered to provide evidence of protection. (Skendzel, 1996 and Geneva, 2011).

C- Cellular Immune Response

Some degree of lymphocyte suppression might occur in the first weeks afterward natural rubella infection, but this phenomenon is only transient. Normally, cell mediated immune responses go before the presence of humoral immunity by 1 week, reach a peak value simultaneously as antibody response, and then persevere for several months or years as measured by migration inhibitory factor, lymphokine release, interferon secretion or lymphocyte transformation (Skendzel, 1996).Rubella specific cell mediated immune responses were considered in children with congenital RV by 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, 1992).

D- Passive Immunization

Before the development of vaccine, immune globulin (IG) which contains rubella antibody has frequently been presented to pregnant women exposed to rubella hoping that it could prevent fetal infection. Investigational studies have established the effectiveness of passive antibody in precluding clinical rubella; nonetheless, several failures of gamma globulin existed for precluding congenital fetal abnormality in real practice (Plotkin and Orenstien, 1999).

2-1-11 Diagnosis of rubella

Serological testing is the favored technique in monotonous laboratory diagnosis of RV, attendance of RV IgM or protest of an important increase in "RV IgG from paired acute and convalescent serum samples delivers evidence of ongoing or current RV infection. Rarely, false-positive IgM outcomes can happen once IgM antibody detection kits were employed, like ELISA". Where RV was exceptional, false-positive serological outcomes developed moderately more mutual, thus raising confirmatory tests necessity (Best, 2007). IgM antibody existence can continually be deduced with caution once strong clinical context lacks (if testing is regularly done throughout pregnancy). "Congenital RV infection is utmost usually diagnosed by detection of RV IgM in serum or oral fluid sampled throughout the early months of life" (Skendzel, 1996).

"RV infection may be serologically confirmed by a noteworthy increase in RV antibody titer between acute and convalescent phase serum specimen or using serum RV IgM attendance". Sera can be collected as early as possible (in seven to ten days) after onset of illness, and again as a minimum seven-fourteen days (if possible two-three weeks) later (Chin, 2000). The standard serological test is the hem agglutination inhibition (HAI) test which has high sensitivity and early availability of results (Plotkin and Orenstein, 1999). The most usual used test nowadays is ELISA, which may be employed in measuring IgG and IgM antibodies to RV (Gershon *et al.,* 2004). ELISA also had advantage over the comparable sensitive radio – immune assay (RIA), "using detection of antibodies deprived of usage of hazardous radioactive isotopes, the expense of reagents with short shelf lives and without costly equipment requirement" (Yaseen, 1992). Other assays that are used include latex agglutination, complement fixation (CF), and indirect immunofluorescence (Gershon *et al.,* 2004). "False-positive serum RV IgM tests have happened in persons with parvovirus infections, with a positive heterophile test for infectious mononucleosis, or with a positive rheumatoid factor" (Mahoney and Chernesky, 1997).

"RV virus might be isolated from nasal, blood, urine, throat and cerebrospinal fluid specimens from RV cases. It can be insulated from pharynx one week before and up to two weeks after onset of rash" (Chin, 2000). "Though isolation of the virus is diagnostic of RV infection, viral cultures are labor intensive and consequently, unperformed in numerous laboratories; they are not commonly employed in routine diagnosis of RV" (Chin, 2000).

The PCR was revised to the detection of RV RNA using opposite transcription and amplification (Ho-Terry and Lonesborough, 1990). The technique seems to be sensitive and specific, and is chiefly beneficial for prenatal detection of RV infection of the fetus (Tanemura *et al.*, 1996). RV reinfection can be diagnosed using noteworthy rise in RV IgG titers, occasionally to extreme levels, or specific IgM detection in a patient having antibodies. It is frequently likely to differentiate reinfection from main infection by investigating the antigen-binding avidity of certain IgG. Sera taken from samples of RV reinfection having low IgG avidity, though sera taken from persons having distant infection, counting cases of RV reinfection, having greater avidity. The RV IgM response is typically lower and more transient than subsequent main infection (Zuckerman *et al.*, 2000).

2-1-12 Vaccine Recommendations

RV vaccine is endorsed to be taken in combination with measles and mumps vaccine (MMR) "if a child is twelve to fifteen months of age, with a second dose at four – six years", in line with endorsements for routine measles immunization. People who do not receive the dose at early ages have to get their second dose once available nonetheless no later than eleven to twelve years (AAP, 2003).

Vaccine is endorsed for all susceptible non-pregnant females deprived of contraindication. Susceptible young adults who have interaction with young children or congregate at colleges, military recruits and other types of institutions should be immunized. All medical personals must be immunized to RV, in particular those who are in interaction with patients in prenatal clinics (Chin, 2000). People who were born in 1957 or after and did not receive one dose of vaccine at

least or who did not have any serologic evidence of immunity to RV are regarded susceptible and must be immunized with MMR vaccine (AAP, 2003).

2-2 Some Biomarkers to Determine Diagnostic Code for RV Viral

2-2-1 Immunological markers

2-2-1-1 Cytokines

"Cytokines are regulatory proteins originating from immune system cells and act like intermediaries of numerous functions extending from stimulating growth and differentiation of lymphocytes, activating effector cells and stimulating the progress of hematopoietic cells" (Garrote *et al*., 2008; Kleiner *et al*., 2013).

"Their main effect is exerted locally or systemically, these regulating proteins mediate processes together inside the innate and adaptive immune response categorized by activation of T cells in addition to being signature cytokines of diverse subsets of Th-cells e.g. Th1 and Th2" (Monteleone *et al*., 2004; Abbas *et al*., 2015). "They were conventionally categorized into families depending on origin immune cell and immunological effects that they carry. CD4+ T-helper cells are the main immune cells related to cytokine production, and may be categorized into functional subsets based on their cytokine production, T- helper 1 (Th1) cells harvest interferon gamma (IFNg), IL-2 and tumor necrosis factor beta (TNFb) are key players of cell intermediated immune response , T-helper 2 (Th2) cells produce IL-4, IL-5, IL-6 and IL-10, that are key players of antibody-mediated humoral responses" (Wumy, *et al*.,2001).

Cytokines have synergistic, antagonistic, redundant and pleiotropic biological effects and categorized to pro-inflammatory "(IL-18, IL-12,

IL-8, IL-6, IL2, IL-1, TNF-α, and IFN-γ) and anti-inflammatory (IL-4, IL-10 and TGF-β), pro-inflammatory and anti-inflammatory cytokines balance thus seems to dictate the fate of T cell polarization" during the immune response (paramalingam *et al*., 2007; Romero-Adrian *et al*., 2009).

2-2-1-1 1 Interleukin-6 (IL-6)

The molecule that we now call IL-6 was originally given various designations by different groups, designations including interferon-β2 (IFN-β2), B cell stimulatory factor 2 (BSF-2), hybridoma growth factor (HGF) and hepatocyte-stimulating factor (HSF), each reflecting the different characteristics of the protein. IL-6 promotes specific differentiation of naïve $CD4^+$ T cells, thus performing an important function in the linking of innate to acquired immune response. It has been shown that IL-6, in combination with transforming growth factor (TGF)-β, is indispensable for Th17 differentiation from naïve $CD4^+$ T cells nonetheless that IL-6 also inhibits TGF-β-induced Treg differentiation Up-regulation of the Th17/Treg balance is considered to be responsible for the disruption of immunological tolerance, and is therefore pathologically relate the growth of autoimmune and chronic inflammatory illnesses (Korn *et al.* 2009; Bettelli et al. 2006; Kimura and Kishimoto 2010). "Additionally, IL-6 promotes T-follicular helper-cell differentiation in addition to production of IL-21, that controls immunoglobulin (Ig) synthesis and IgG4 production in particular. IL-6 similarly encourages the differentiation of $CD8^+$ T cells into cytotoxic T cells" (Ma *et al*. 2012; Nakahara *et al.,* 2003). "BSF-2, IL-6 was found capable of inducing the differentiation of activated B cells into Ab-

producing plasma cells, so that continuous over synthesis of IL-6 causes hyper gamma globulinemia and autoantibody production".

2-2-1-1-2 Interleukin-10 (IL-10)

An important feature of the effector T Regulatory (Treg) cells is their production of IL-10, which is an anti-inflammatory cytokine that was first called human cytokine synthesis inhibitory factor, and it is associated with inhibition of viral infection after a stimulation of NK cell activity. It also functions as and inhibitor of Th1cells and its major immune-biological effect is the regulation of Th1:Th2 balance (Pestkoi *et al*., 2004). It is also a potent inhibitor of IL-1, IL-6, IL-12, IL-18, and TNF-α, and IL-10 not just inhibits production of pro-inflammatory mediators but also enhances the production of anti-inflammatory factors; such as IL-1RA, and associated with a down-regulation of MHC class II expression (Murray, 2006).

2-2-1-2 Human Cluster of differentiation 4 (CD4)

Receptor CD4, a differentiation marker mainly articulated on the surface of T lymphocytes, "is a 55 kDa type I integral membrane glycoprotein a key contributor throughout adaptive immune responses. Besides T-cells, CD4 was revealed to be articulated on monocytes, monocyte-derived macrophages, Langerhans cells, B lymphocytes, dendritic cells, eosinophils, megakaryocytes and mast cells" (Badoual *et al*., 2004). "In T-cells, CD4 alleviates TCR interactions with peptide-major histocompatibility complex class II (MHCII) complexes on antigen awarding cells and mediates intracellular T-cell signaling" (Bos and Sherman, 2010). "TCR and CD4 together are necessary for making contact with the peptide MHCII complex with a purpose of transducing

suitable proliferative signal to the antigen specific T-cell. This signal will be capable of coordinating numerous effector activities critical for the maturation of a robust, antigen-specific immune response. On the other hand, binding of TCR to MHCII deprived of a CD4-MHCII co-stimulatory signal may cause T-cell energy and apoptosis" (Shevach, 2002). There is an overall consensus that the key function of CD4 is to employ the Src tyrosine kinase Lck to the TCR-MHCII complex upon co-receptor binding to MHC that causes the formation of a TCR-MHCII-CD4 complex (Sakaguchi, 2004). Lck recruitment, that happens through its linking to the cytoplasmic tail of CD4, promotes phosphorylation of the immune receptor tyrosine activation motifs (ITAMs) situated inside the cytoplasmic tails of the TCR. "Therefore, it results in a signal amplification. By destabilizing serious immunological function of CD4, a probable contribution of gp120-mediated anergic effects on $CD4^+$ T cells to the pathogenesis of AIDS was established, (Carapetis, *et al*., 2005). Right after the discovery of CD4 as the receptor for HIV" (Maloy and Powrie, 2001).

Immunoprecipitation of these two molecules as an intricate from infected cells establishes a specific interaction between the external subunit of the viral envelope glycoprotein (gp120) and CD4. These findings propose the initiation of virus infection by main interaction between gpl20 and CD4 (Bos and Sherman, 2010).

2-2-1-3 Human Cluster of differentiation 8 (CD8)

"CD8+ T-cells are a subset of lymphocytes with cytotoxic functions that prompt Tcell receptors (TCR) which recognize peptides presented by MHC class I molecules present on all nucleated cells. Their cytolytic (CTL) activity is important in anti-viral, anti-tumor, and certain anti-

bacterial responses, during an infection, CD8+ T-cells are triggered by binding of their TCR to peptide-MHCI complexes on antigen presenting cells (APC). They are co-stimulated with B7.1, B7.2 (aka CD80/86) receptors and the cytokine microenvironment, activating cytokines derived from such APC initially include, but are not limited to, type 1 IFNs (α and β), IL-2, IL-12, and IL-6, Upon activation, CD8+ naïve T (TN) cells undergo clonal expansion and differentiation into short-lived effector cells (TE), TE cells will migrate to the site of infection to specifically target affected cells. CD8+ T-cells release perforin which forms pores in target cells to allow entry of other cytotoxic particles such as granzymes to disrupt the mitochondria and fragment DNA, and granulizing which has antimicrobial properties, Expression of Fast ligand can also trigger apoptosis of target cells, In addition, CD8+ T-cells produce IFN-γ (blocks viral replication and enhances host anti-viral function), LT-α (kills tumor cells), TNF-α (activates microvascular endothelium) to help clear infection, and IL-2 to augment their other functions. TE cells then undergo massive contraction whereby 95% of cells die by apoptosis. The remaining T-cells become memory precursors and then central (TCM) and effector memory (TEM) cells, which can quickly expand into TE cells upon re-stimulation with the same antigen (Cox et al., 2013). Another subset of CD8+ T-cells known as terminally differentiated effector memory (TEMRA) cells proliferate less than other memory cells, produce high concentrations of IFN-γ and have increased susceptibility to apoptosis. CD8+ T-cells express different surface proteins and exhibit different gene expression patterns and functions at each stage of development. Cell markers to distinguish between stages of human CD8+ T-cell differentiation have classically included CD45RA (long isoform of tyrosine phosphatase) and CCR7 (chemokine receptor

that allows for homing to the lymph nodes), but also include many others (Sallusto et al., 1999).

2-2-2 Serology marker

2-2-2-1 Detection of RV virus by ELISA tests

The standard serologic test for RV is HI test; nevertheless, serum should be pretreated to eliminate general inhibitors before testing. Serum pretreatment isn't obligatory and "they may be altered to identify definite IgM. IgG detection is an indication of immunity since there is only one serotype of RV virus, to exactly settle the latest RV infection this is why ELISA tests are preferred (critically vital in the case of a pregnant woman), either a rise in antibody titer should be established between 2 serum samples occupied ten days apart at least or RV-specific IgM should be perceived in a single specimen". (Huang, 2012).

2-2-2-2 Arthritis (RA) and Rheumatoid Factor (RF)

"Arthritis" was derived from the Greek words *Arthron* (joint) and *itis* (inflammation) it is related to symptoms linked to inflammation of joints. For example, pain, stiffness, temperature, aching one or more of the joints that consequence is a limitation of joint mobility and disability. Over hundreds of conditions linked to arthritis that range from severe to mild forms. They are specific forms of inflammatory arthritis linked to abnormal functions of the immune system alongside joints. Consequently, RA is delineated as autoimmune disorders, nevertheless pathological mechanisms are not well defined that lead to such abnormality even though genetic and environmental factors are taken into consideration and result in autoimmune responses wherein cytokines are the key mediators. Rheumatoid factor (RF) results in a painful

swelling in wrists, neck, ankles, toes, hips, fingers, knees, and jaws" (Helmick *et al*., 2008; Kurtin, 2000; Prahalad and Glass, 2008; Batthish *et al*., 2011; Albers, 2015).

It is recommended that RA to be intervened by triggered pro-inflammatory Th1 cells categorized mostly by secretion of IL-2, IFN-γ and TNF-α promoting cellular immune response. Cytokines of Th2 cells (IL-13, IL-6, IL-5 and IL-4) that enhance humoral antibody mediated immune response are likewise related in pathogenesis of RA. have emphasized Th17 (IL-17A), Th22 (IL-22) and Treg (IL-10) cytokines role in RA progression. RA Iraqi patients, verified that evaluation of IL-2, IL-6, IFN-γ and TNF-α serum levels can endorse a disease activity. (Walshe *et al*., 2008; Mohammed *et al*., 2010; Burska *et al*., 2014; Manhal, 2009)

2-2-3 Hematology markers

2-2-3-1 White blood cells (WBC)

WBC are of significant status for the immune system and important for the aptitude in fighting viral and bacterial infections. They are allocated into five-key categories because of their structural differences and likewise cell division lineage; basophils, neutrophils, monocytes, lymphocytes, and eosinophils. Both the morphology and the function of these cells are unlike nonetheless, they are all related to protect our body alongside unknown, although cells possess their own method of protection *(Hematological Atlas*, 2013).

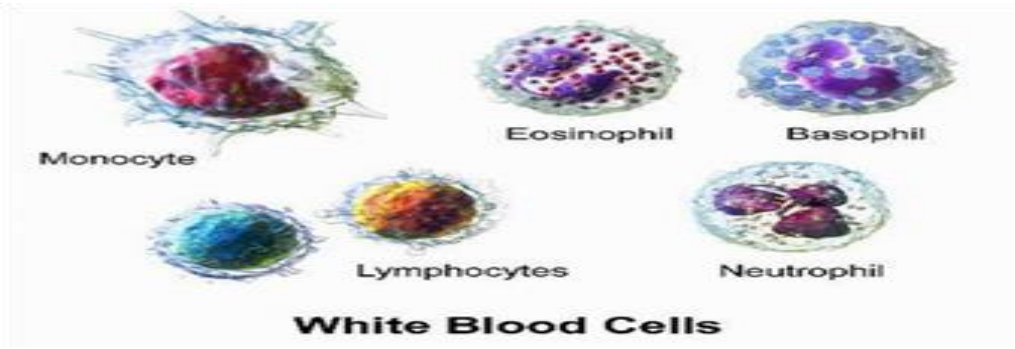


Figure 2-8 blood cells present in human blood [The different kinds of white]. (Kaplan, 2013)

In the case of autoimmune diseases, this protection mechanism alongside unknown may well rebound and initiate an attack to original healthy cells. The body will begin to produce defected WBC, as realized by infectious mononucleosis. The activation of WBC itself will result in variations of the cells morphology *(Hematological Atlas*, 2013). This fact was recognized for a time and was under research topics. WBC have changes in their morphology besides cells number presented in the blood because of e.g. bacterial or viral infection in addition to different diseases, including leukemia and various autoimmune diseases (Kaplan, 2013). WBC morphology changes might be because of a disease and might also be because of vitamin deficiency, i.e. hyper segmented neutrophils might be noticed if patients have either folic acid or vitamin B12 deficiency. To enable deficiencies detection an equipment like the microscope and extremely qualified healthcare workforces is obligatory (Abbas *et al*., 2014) .

Neutrophils activation varies according to pathogenic or autoimmunity diseases. This is because in autoimmunity diseases neutrophils have revealed both phenotyping and functional abnormalities in various autoimmunity diseases. On the other hand, in pathogenic infections neutrophils have their complete functions (Kaplan, 2013) Changes in the neutrophil function and activation of various cells

depending on the reason for infections. i.e. in the course of a parasitic infections the main immune cells being activated are eosinophils. This can enable differentiation among dissimilar reasons for infections (Abbas *et al.*, 2014) .

2-2-3-2 Hemoglobin (Hb)

The amount of Hb in the blood is measured by Hb tests. It is a protein that is the main component of RBC (erythrocytes), which are created in the bone marrow. It comprises of iron, allowing oxygen binding. RBCs move O_2 from lungs to further tissues and organs and transfer CO_2 back to the lungs so that the body will eliminate it (Jensen, 2009).

Anemia occurs when hemoglobin levels are lower than normal. It signifies that body organs might lack O_2 supply. This will lead to a deficiency in cells energy, causing fatigue and additional problems (Hall, 2010). When the bone marrow is not creating sufficient RBCs, or the destroyed cells are more than newly generated cells, it will be noticed by a low Hb level. Chronic blood loss can lead to a drop in Hb levels and it is one of the utmost joint reasons for anemia, An Hb test can be done alongside with a routine physical examination to evaluate Hb amount in the blood. This test is part of CBC test that tries to find RBC, WBC, and platelets numbers in blood samples, A CBC may similarly be performed if there is a symptom of low Hb level, including dizziness, weakness and fatigue (Forget, and Bunn, 2013). Hb test can be employed in anemia screening and display the development throughout treatment. It may only create whether anemia is present and its degree of severity. The test is not able to check the reason for Hb abnormal level. Extra tests are obligatory (Nelson, 2000).

2-2-4: Biochemical markers

2-2-4-1: Urea

It is a pioneering marker to evaluate GFR, however presently it is not used in clinical practice due to numerous reasons. Urea production is erratic and changes with protein intake and readily it is reabsorbed by tubules and again amount of reabsorption is erratic. Hydration status of the individual also affects urea clearance clearly; in patients with depleted intravascular volume, high plasma levels accompany decreased urine flow. In addition, many substances may interfere with its estimation (Delgado, and Straseski, 2015).

2-2-4-2 Creatinine

It is produced through no enzymatic dehydration of creatinine and phosphocreatine, muscle mass therefore is the main determinant (98%) of the creatinine pool, dietary consumption of meat is another source of creatinine, endogenous creatinine clearance which gives as an estimate of GFR was first proposed by Popper and Mandal in 1937 (Popper and Mandel, 1937) and is still highly patronized in clinical practice. However, its performance and interpretation present alarming difficulties such as: Changes in the rate of production of creatinine, accurate measurement of plasma creatinine, some level of secretion by the renal tubules and the struggle of getting comprehensive, precise urine specimens (Delgado, and Straseski, 2015).

Creatinine is generally measured by Jaffa colorimetric reaction using over the past century, employing alkaline picrate that makes a red orange complex, numerous substances, such as uric acid, ascorbic acid, ketones and ketoacidosis, plasma proteins, bilirubin, fatty acids, urea, cephalosporin's and glucose interfere with Jaffa's colorimetric assay for

estimation of plasma creatinine resulting in erroneously great values (Horowitz, 2014)

Furthermore, tubular secretion and induction of true assessment of plasma creatinine are inhibited using drugs such as triamterene, spironolactone, amyloid, trimethoprim, cimetidine, probenecid, and high dose salicylates or pyrimethamine , Enzyme based assays have enhanced precision comparable to high performance liquid chromatographic techniques because they lack this interference (Salazar, 2014). As a result of tubular secretion creatinine clearance (Ccr) usually overestimates GFR. This represents 10-40% of GFR in normal renal function with clear interindividual variability. In patients with decreased kidney function, tubular secretion can increase to above 100% especially among those with glomerulopathy and proteinuria (Dugdale, 2013).

2-2-4-3 Total serum bilirubin (TSB)

Bilirubin is the chief pigment in bile (waste product) derived from the breakdown of Hb. Free bilirubin will be bound by albumin after numerous degradation steps, and then move by blood to liver (Bosma, 2003). "It is insoluble in water; indirect, or unconjugated, bilirubin is rendered soluble in the liver by conjugation with glucuronide. Bilirubin water-soluble referred to as direct or conjugated, is moved alongside with other bile constituents into the bile ducts, then to the intestines where bacterial enzyme action transforms bilirubin to numerous associated compounds, together known by urobilinogen" (Bosma, 2003) In normal plasma a low concentration of bilirubin is found, virtually all of it is indirect. Total bilirubin is the summation of direct and indirect bilirubin. Repetitive analytical procedures occur for the measuring total bilirubin and direct bilirubin. Indirect fraction is acquired by subtraction.

Direct determination in addition to total bilirubin is employed to differentiate some kinds of jaundice (Takeuchi *et al*, .2004).

2-2-6 Relationship between rheumatoid Arthritis (RA), Interleukin 6(IL-6) and Human Cluster of differentiation 4(CD4)

RA is a chronic disease disturbing 0.5-1% of adults. It is described by persistent synovitis, systemic inflammation and immunological abnormalities. Unrestrained active RA leads to combined damage, disability, reduced life quality, cardiovascular and additional co-morbidities (McInnes and Schett, 2011), (Smolen, *et al 2007)*. IL-6, a usual cytokine including redundancy and pleiotropic activity, is the main contributor to RA development (Hashizume and Mihara, 2011). It endorses the imbalance between T17 cells and Treg and the production of autoantibodies including RF and ACPA. It encourages synovial inflammation and cartilage and bone destruction in addition to systemic features such as cardiovascular, psychological and skeletal disorders (Tanaka, *et al 2013*). It is consequently encouraging Th17 predominance over Treg in the effector CD4+Tcell subsets that is the main contributor to RA development and numerous additional immune-mediated diseases (Dienz, *et al* 2009).

2-2 Measles virus

It is genus Morbillivirus member of the Paramyxoviridae family, it seems antigenically stable. Although viral antigens significant changes over time evidence is lacking, viral genes arrangement analysis have revealed distinct lineages (genotypes) of wild-type measles viruses (Washington, 2005). WHO distinguishes 24 genotypes of wild-type measles virus (H2, H1, G3, G2, G1, F, E, D11, D10, D9, D8, D7, D6, D5,

D4, D3, D2, D1, C2, C1, B3, B2, B1, and A) (Strebel, *et al*.2013). Recognition of a definite virus genotype alongside with epidemiological information may propose geographic origin of an eruption (Washington, 2005).

Human is the only natural host of measles virus. It may be upheld in human inhabitants by an unbroken chain of acute infections. People with measles are infectious for numerous days before and after the onset of a rash, once the symptoms of cough, coryza and sneezing are most severe. These symptoms enable virus spread, being contagious before the onset of identifiable illness delays the effectiveness of isolation measures (Moss and Griffin, 2012).

It is spread mainly among people by large respiratory droplets, nonetheless it as well can be transmitted by the airborne route as aerosolized droplet nuclei (Moss and Griffin, 2012). It is one of the greatest contagious human pathogens and it has the capacity to seek out a small number of residual measles-susceptible persons. Subordinate attack rates amongst vulnerable domestic associates was 75–90%. Because of high spread efficiency of measles, outbreaks was in populations only 3–7% of the individuals were vulnerable (Washington, 2005). There were neither latent nor persistent infection with measles virus causing prolonged contagiousness (Moss and Griffin, 2012).

In developing countries, its infection runs a devastating course in children and mortality rates may range from 2% to 15%. RV is typically a mild viral disease in children; nonetheless, infection in a pregnant woman may have serious consequences on the developing fetus. Congenital RV syndrome (CRS) happens in 90% of cases of RV infection early in pregnancy. Miscarriage or stillbirth may happen, and babies born with CRS may grieve from problems including ophthalmic,

auditory, cardiologic and craniofacial defects, in addition to mental retardation (Strebel, *et al.*2013). Table2.1 shows the difference and similarity between RV and measles virus

Table 2-1 the similarities and differences between the RV and Measles virus

Disease		
Causative agent	**Measles virus**	**Rubella virus**
Incubation period	7-18 day	12-14 day
Fever	Yes	Yes
Rash	Yes	Yes
Conjunctivitis	Yes	Yes
Coryza	Yes	Yes
Joint symptoms	Yes	Yes and severe in adult women
Lymphadenopathy	No found	Yes
Diagnostic test	IgM	IgM
Infection during pregnancy	No found	Yes
Stillbirth	Yes	Yes

(Friden and Resnick, 1991)

3-1: Subjects

This study was conducted from March to October 2018, including an overall of 230 women in childbearing age (15-40 and above) years were classified into three groups: the group of unmarried girls, the group of married women (pregnant and non-pregnant women) and control group, showed that, the married women (pregnant) 50 (21.8%), married women (non-pregnant) were 45 (19.7%). The study also showed that the number of unmarried women (85) percentage (36.7%) and number of control 50 (21.8%) were selected randomly from urban and rural region of Baghdad Governorate. Blood samples (5 ml) were drawn; the specimen was transported to the Virology Laboratory within 48hours and stored at -30°C.Maintaining cold chain transport to the Virology laboratory where extraction was done. Extraction buffer was added to the sponge and agitated, the fluid squeezed out and inverted into the tube. The sample was centrifuged at 10000 rpm for 5 minutes. Samples are collected from women attending Baghdad Maternity and Children Hospital (Al-Kadhimiyah Pediatric Hospital, Alnomaan Hospital, dhari alfad General Hospital, medicine city General Hospital, Al- yarmook General Hospital, Fatima Al- Zahra Hospital and Ibn-Albaldy Hospital)

By directly interviewing each woman a questionnaire form was filled. The data demanded comprise residence, age, occupation, level of education, pregnancy (pregnant and non-pregnant), trimester, and parity. We omitted recent disease history women with rash, or contact with a known case of rubella.

3.2: Materials

3.2.1: Equipment and instruments

Equipment and common laboratory instruments that were employed in the study and their manufactures are briefed in table 3-1.

Table 3-1: The equipment and instruments and their manufactures.

APPARATUS	**COMPANY**	**ORIGIN**
Autoclave	Portable	England
Caps	AFCO	China
Centrifuge	Eppendorf	Germany
Cotton	Kardelen	Turkey
Dagon	C cell	Spain
Deep freezer	Sanyo	Japan
Disposable tips (100µl, 1000µl)	Promega	USA
ELISA system (reader, washer and printer)	Human	Germany
Ethylene-diamine tetra acetate (EDTA) Vacuum Blood Collection Tubes	AFCO	China
Filter paper	AFCO	Lebanon
Gloves	AFCO	China
Incubators	Memmerts	Germanys
Lights microscopes	Olympuss	Japans
Multi pipettes (10-100µl)	Slamed	Germany
Micropipettes (size 1-10µl,10-100µl and 100-1000µl)	Dragon	China
Oven	Memmert	Germany
Plain tube	AFCO	China
Refrigerator	Beko	Turkey
Shaking water bath	Jeio tech	South Korea
Slides	AFCO	China
Spectrophotometer	Apple	Japan
Test tube rack	AFCO	China
Timer	AFCO	China

3.2.2: Kits

Kits that were employed to achieve the study were listed in table 3-3 with their manufacturers.

Table 3-2: A list of kits and their manufactures.

Kits	Company	Originy
Human Cluster of differentiation 4 (CD4)	Bio Elisa	USA
Human Cluster of differentiation 8 (CD8)	Bio Elisa	USA
Total serum bilirubin (TSB)	Liner	Aspin
IL-10 Enzyme Immune Assay Kit	Bio Elisa	USA
IL-6 Enzyme Immune Assay Kit	Bio Elisa	USA
Rheumatoid Factor (RF)	Randox	England
White blood cells (WBC)	Randox	England
Urea	Liner	Aspin
Creatinine	Liner	Aspin

3-3: Immunology examination

3-3-1: ELISA of rubella IgG and IgM kit

3-3-1-1- A: Principle assay of rubella IgG and IgM

Rubella IgG and IgM kit assay levels in samples employed distilled rubella IgG and IgM antibody for coating micro titer plate wells, making solid-phase antibody, subsequently adding rubella IgG and IgM to the wells. Joint antibody with HRP labeled, resulting in antibody - antigen -enzyme -antibody complex, after a complete wash, adding TMB substrate solution , TMB substrate turn into bluish color at HRP enzyme-catalyzed. The reaction ended using sulphuric acid solution addition and a color change noticed by spectrophotometer at 450 nm.

Rubella IgG and IgM concentration in samples are formerly concluded by connecting samples O.D. (Zfferey et al., 1995)

3-3-1-2: Components of bio Elisa rubella IgG and IgM kit

Bio Elisa rubella IgG and IgM kit produced by bio-kit company-Barcelona- Spain, have been used to diagnose anti RV IgG and IgM antibodies in human serum using ELISA technique. It consists of the following components:

1. Micro plate 12x8 well strips coated with deactivated RV antigen.
2. High positive calibrator; 0.6 ml of human gamma globulin dilution having 200 IU/ml of anti-rubella IgG and IgM. Containing stabilizers protein, green dye and < 0.1 percentage sodium azide.
3. Low positive calibrators; 0.6 ml of human gammaglobulin dilution containing 10 IU/ml of anti-rubella IgG and IgM have stabilizers protein, green dye and sodium azide .
4. Negative control; 0.6 ml of human gammaglobulin dilution negative for anti- rubella IgG and IgM having stabilizers protein, yellow dye and < 0.1% sodium azide.
5. Concentrate conjugate; 0.35 ml of rabbit anti-human IgG and IgM anti bodies conjugated with peroxidase having red dye, stabilisers protein, 0.02% thimerosal and 0.001% gentamicin sulphate .
6. Conjugate diluent; 15 ml of Tris buffer having yellow dye, additives, 0.02% thimerosal and 0.001% gentamicin sulphate .
7. Sample diluent; 2x50 ml of phosphate buffer containing detergent, stabilizers protein, green dye and < 0.1% sodium azide.
8. Washing solution; 2x50 ml of concentrate phosphate buffer (10 x) having 1% tween 20 and 0.01% thimerosal .

9. Substrate buffer; 14 ml of citrate -acetate buffer having hydrogen peroxide and 0.002 % gentamicin sulphate .
10. Chromogenic; 1.5 ml of 3, 3', five, 5' Tetramethylbenzidine (TMB) dissolved in Dimethylsulfoxide (DMSO).
11. Stopping solution; 12 ml of 1N sulphuric acid.
12. Adhesive seals ; they are used for containing the micro plate throughout incubations. The method was done according to the manufacturer's instructions

3-3-1-3: Procedures of rubella IgG and IgM kit

The diagnostic kit was used by bio Elisa, ELISA to investigate IgG and IgM antibodies against the Rubella virus as instructed by the manufacturer (Gravell et al., 1977).

1. We reserved six wells for blank, calibrators and control. The calibrators and negative control should be run in duplicate, 100 µl of every diluted sample, control the calibrators added into wells, with a well-left empty for the blank.
2. Microplate was enclosed by an adhesive seal and incubated for 1 hour at 37 C°.
3. After incubation the adhesive seal was removed, and the wells washed 5 times with a washing solution by filling the wells with washing solution, shake gently and discard the solution after each shake.
4. 100µl of diluted conjugate was added to each well, except blank one, an adhesive seal and incubated for 30 minutes at 37 C° cover the plate.
5. After incubation, adhesive seal was removed and the well washed 5 times with a washing solution as in (5) above.

6. In each well a 100µl solution of substrate-chromogen was added including the blank, and incubated uncovered for 30 min.
7. The reaction ended using addition 100µl of ending solution (1N H_2SO_4 solution).
8. After the reaction had been stopped, the absorbance of each well had been read by using ELISA reader at 450 nm.

3-3-1-4: Calculation of specimen OD ratio

Calculate an OD ratio for each specimen by dividing its OD value by the cut –off value

Set up the cut –off value

The cut –off value = N+ 0.15

N: Mean OD of the negative control.

3-3-2 Serum level of cytokine IL-6 and IL-10 ELISA Kit instruction:

3-3-2 -1 Principle of the assay of IL-6 and IL-10

The ELISA kit is proposed for research and analysis only therefore, diagnostic or therapeutic procedures are not applicable. Stopping solution modifies color from blue to yellow and color intensity was measured at 450 nm. With the intention of measuring IL-6 concentration in the sample , this Kit contains a set of standard calibrations. Standard calibrations are assay simultaneously as the samples and permit lab operator to harvest a typical curve of OD with IL-6 and IL-10 concentration. (Zfferey *et al., 1995*)

3-3-2 -2: Procedures of IL-6 and IL-10 ELISA Kit instruction:

1. Preparing all reagents beforehand initiation assay procedure. It is recommended that the entire Standards and Samples beaded induplicate to the Micro Elisa Strip plate.

2. Adding standard: Set Standard wells, tasting sample wells. Adding standard 50µl to standard well.
3. Adding Sample: Adding testing sample Ten µl at that time adding Sample Diluent 40µl to test sample well; Blank well left alone.
4. Adding 100µl of HRP-conjugate reagent to all wells, covering with an adhesive strip and incubate for 60 minutes at 37°C.
5. Aspirating all wells and washing, repeating the process 4 times for 5 washes. Washing by filling all wells with Wash Solution (400µl) by a squirt bottle, manifold dispenser or auto washer. Removing liquid at all steps is vital to worthy qualification. After wards, delete any remaining wash Solution by aspirating or decanting. Inverting the plate and blot it against clean paper towels.
6. Adding chromogen solution A50µl and chromo gen solution B 50µl to all wells. Gently mix and incubate for15minutes at37°C.Protect from light.
7. Adding 50µl Stopping Solution to all wells. Wells color must alter from blue to yellow. If it was green or the color modification was uneven, moderately hit the plate to make sure of in-depth mixing.
8. Reading OD at 450 nm by a micro titer plate reader within fifteen minutes. Materials that were used in the study and their companies are illustrated in table 3-4. The working method is prepared for the manufacturer (Bio Elisa)

Table 3-3: Materials provided with the kit of IL-6 and IL-10 ELISA Kit instruction:

Materials provided with the kit	48determinations	96 determinations	Storage
User manual	1	1	
Closure plate membrane	2	2	
Sealed bags	1	1	
Micro Elisa strip plate	1	1	2-8℃
Standard	0.3ml×6 bottle	0.3ml×6 bottle	2-8℃
HRP-Conjugate reagent	5ml×1 bottle	10ml×1 bottle	2-8℃
Sample diluent	3ml×1 bottle	6ml×1 bottle	2-8℃
Chromogen Solution A	3ml×1 bottle	6ml×1 bottle	2-8℃
Chromogen Solution B	3ml×1 bottle	6ml×1 bottle	2-8℃
Stop Solution	3ml×1 bottle	6ml×1 bottle	2-8℃
20×Wash solution	15ml×1 bottle	25ml×1 bottle	2-8℃

3-3-2 -3: Calculation of specimen OD ratio

Calculate an OD ratio for each specimen by dividing its OD value by the cut –off value

Set up the cut –off value

The cut – off value = N × 0.27

N: Mean OD of the negative control.

3-3-3: Serum level of CD4 and CD8 ELISA Kit instruction:

3-3-3-1: Principle assay of CD4 and CD8

CD4 and CD8 level in the sample, distled CD4 and CD8 antibody is employ for coating micro titer plate wells, making solid-phase antibody, after adding CD4 and CD8 to the wells, Combining antibody with HRP labeled, becoming antibody-antigen-enzyme-antibody complex, after a total wash, adding TMB substrate solution, TMB substrate will be bluish color at HRP enzyme-catalyzed, reaction is ended by sulphuric acid solution additives and a color

change was noticed by spectrophotometer at 450 nm. CD4 and CD8 concentration in the samples is at that time found by associating samples O.D. with normal curve.

3-3-3-2: Procedures of CD4 and CD8 ELISA Kit instruction:

1. Adding standard: Setting Standard wells, test sample wells. Adding standard fifteen μl to standard well.
2. Adding sample：Setting blank wells distinctly (blank comparison wells prevent adding sample and HRP-Conjugate reagent, other steps are identical). Test sample well. Adding Sample dilution forty μl to testing sample well, after adding testing sample ten μl (sample final dilution is five-fold), adding sample to wells, do not touch the well wall to the extent that it can be, and smoothly mix.
3. Enzyme addition： HRP-Conjugate reagent of 100μl addition to every well, excluding blanks.
4. Incubating: After closing plate with Closure plate membrane, incubate for 60 minutes at 37℃.
5. Liquid configuration: 20-fold wash solution diluted 20-fold with distilled water and reserve.
6. Washing：Uncovering Closure plate membrane, discarding Liquid, dry by swing, adding washing buffer to all wells, stilling for thirty seconds then draining, repeating five times, dry by pat.
7. Coloring：Adding Chromogen Solution fifty μl and Chromogen Solution B collectively, avoid light preservation for fifteen minutes at 37℃
8. Stopping reaction：Adding Stopping Solution fifty μl to every well, stopping reaction (blue color will be yellow).

9. Assay：taking blank well as zero，Reading absorbance at 450nm after Adding Stop Solution and in fifteen minutes. Materials that were used in the study and their companies are illustrated in table 3-5.preparing the working method for the manufacturer. (Bio Elisa)

3-3-3-3 Calculation of specimen OD ratio

Calculate an OD ratio for each specimen by dividing its OD value by the cut –off value

Set up the cut –off value

The cut – off value = 0.08+N

N: Mean OD of the negative control.

Materials provided with the kit	48determinations	96 determinations	Storage
User manual	1	1	
Closure plate membrane	2	2	
Sealed bags	1	1	
Micro Elisa strip plate	1	1	2-8℃
Standard	0.3ml×6 bottle	0.3ml×6 bottle	2-8℃
HRP-Conjugate reagent	5ml×1 bottle	10ml×1 bottle	2-8℃
Sample diluent	3ml×1 bottle	6ml×1 bottle	2-8℃
Chromogen Solution A	3ml×1 bottle	6ml×1 bottle	2-8℃
Chromogen Solution B	3ml×1 bottle	6ml×1 bottle	2-8℃
Stop Solution	3ml×1 bottle	6ml×1 bottle	2-8℃
20×Wash solution	15ml×1 bottle	25ml×1 bottle	2-8℃

Note: Standard concentration was follow by:80、40、20、10、5、0 ng/mL.

3-4: Hematology examination

3-4-1-Procedure of hematology

A phlebotomist gathers samples by venipuncture, drawing blood into the test tube having an anticoagulant (EDTA, citrate) to end clotting. Transporting samples to laboratories. Occasionally samples are drawn off a finger prick by means of a Pasteur pipette to directly process using an automated counter. CBCs were done by hand before the age of automated hematology analyzers, counting cells in a diluted sample of blood on a hematology cytometer, and by inspecting slides prepared with samples of patient's blood under a microscope. Nevertheless, manual blood cell counts are being not as much of use, as an alternative, the process was commonly done using the automated analyzer.

Classically, the analysis begins once a good-mixture of whole blood sample is placed on an analyzer rack. Devices use flow cells, photometers, and apertures with the intention of analyzing diverse components in blood, cell-counting component counts, numbers and sorts of diverse cells inside the blood. An Hb meter quantify the amount of Hb. Diluent additions lyse RBCs that are at that moment pumped into a spectro-photometric computing cuvette. Color alteration of lysate equates to blood Hb content.

Due to a computerized hematology cell counter samples and counts a large number of cells, results were accurate. Nevertheless, specific abnormal blood cells are unable to get recognized appropriately, require a physical assessment of the device's outcomes and recognition by microscopy of each abnormal cell the device might not be classified. Complicated modern analyzers may offer extended WBC differential

counts that has hematopoietic progenitor cells, erythroblasts, and immature granulocyte. (Buttarello, 2008)

By flow cytometry blood cell counting happens if a tiny specimen magnitude was aspirated, thinned and flows by an aperture and a laser flow cell. Sensors compute and recognize cells flowing by the aperture. Laser light detectors and electrical impedance are most commonly used sensors. By examining size data and features of light as they cross the cells the device specifies blood cell kind. Some devices measure altered cells features with the purpose of categorizing them.

3-4-2: Principles of hematology

Electrical resistance to count PLT, RBC, WBC and cyanide-free technique for Hb.

3-4-3: Sample Volume

- Pre diluted 20µL
- Whole Blood 17µL.

3-5: Biochemistry examination

3-5-1-1: Principle of urea

Urea was hydrolyzed by urease (Chanery and Marbach, 1962) resulting in NH_3 and CO_2. NH_3 produced responds to alkaline hypochlorite and sodium Salicylate when sodium nitroprusside is found like a coupling agent yielding a green chromophore. Color intensity form is directly proportional to urea concentration.

3-5-1-2: Content of kit Urea

Table 3-6: Content of kit Urea

R1: Enzyme reagent		
R2: Color reagent	Phosphate 6.7	20mmol\l
	EDTA	2mmol\l
	Sodium salicylate	60mmol\l
	Sodium nitroprusside	3.4mmol\l
R3: Standard urea		50mg\dl
R4: Alkaline reagent	Sodium hypochlorite NaCLO	10mmol\l
	Sodium hypochlorite NaOH	150mmol\l

3-5-1-3: Reagent preparation

Working reagent: mix 1 volumes of R1 + 24 volumes of R2. Stable for 4 weeks at 2-8c and for 7 days at 15-25c

3-5-1-4: Procedure for urea:

1-Preincubate working reagent, sample and standard to reaction temperature (37c)

2-set the photometer to 0 absorbance with distilled water.

3-Pipette into cuvette, 1ml of working reagent (R2) and added 10ul of sample or standard

4-Mix and incubate for 5min at 37c or 10 min at room temperature.

5- After incubate added 1ml of working reagent (R3).

6-Mix thoroughly and incubate the tubes for 5min at 37c or for 10 min at room temperature

7-Read the absorbance of samples and standard at 600 nm against the reagent blank. Prepare the working method for the manufacturer. (Liner)

3-5-1-5: Calculation:

Urea concentration = **(A** sample**A** standard**)** x concentration. Standard

3-5-2: Principle of Creatinine

This procedure was created by an alteration of original picrate reaction. Creatinine under alkaline condition responds to picrate ion forming a reddish complex. Formation rate of the complex restrained over the rise of absorbance in a prefixed interval of time is proportional to creatinine concentration in the sample (Jaffe, 1886; Bartels, *et al* 1971).

3-5-2-1: Content of kit Creatinine

- **R1:** picric acid 25mmol/L
- **R2:** Alkaline buffer phosphate buffer 300mmol/L
- **CAL:** creatinine standard. Creatinine 2mg/dL

3-5-2-2: Procedure of kit Creatinine

1-Preincubate working reagent, sample and standard to reaction temperature (37c)

2-Set the photomrter to 0 absorbance with distilled water.

3-Pipette into cuvette, 1ml of working reagent and add 100ul of sample or standard

4-Mix smoothly. Inserting cuvette into the temperature control instrument and initiate a stopwatch.

5-Record absorbance at 510 nm after thirty sec. (A1) and after ninety sec. (A2) of sample or standard addition. Prepare the working method for the manufacturer. (Liner)

3-5-2-3: Calculation:

{(A2−A1) sample/(A2−A1) standard}× Standard = mg/dL creatinine

3-5-3: Principle of Bilirubin

Bilirubin was transformed to colored azo bilirubin by diazotized sulfanilic acid and it was computed photo metrically, of the two-bilirubin fractions in serum –bilirubin-glucuronide and free bilirubin that is bound to albumin– just former straight reaction, though free albumin reacts after being exiled from protein by an accelerator, the dissimilarity of two quantities total and direct bilirubin permits indirect calculation bilirubin. The terms «direct» and «indirect» bilirubin refer exclusively to the reaction characteristics in the attendance or non attendance of an accelerator or solubilizer and may merely estimate equivalents of the two-bilirubin fractions (Walters, and Gerarde, 1970), (Pearlman, and Lee, 1974).

3-5-3-1: Reagent composition

RT: Sulfanilic acid 29 mmol/L, hydrochloric acid 0.24 mol/L, Duposol® 3% (w/v).

RD: Sulfanilic acid 29 mmol/L, hydrochloric acid 0.24 mol/L.

RN: Sodium nitrite 11.6 mmol/L.

CAL: Bilirubin Calibrator

3-5-3-2: Reagent preparation

Working reagents. Mix 1 mL RN + 4 mL RT (Total) or 1 mL RN + 4 mL RD (Direct). Stable for 8 days at 2-8ºC.

Calibrator. Reconstitute the vial by adding exactly 1.0 mL of distilled water. Mix carefully and let stand for 5-10 minutes before use. The stability of bilirubin in the dark is 8 hours 16-25ºC, 2 days 2-8ºC and 28 days –20ºC frozen once.

3-5-3-3: Procedure of total bilirubin

1-Preincubate working reagent, sample and blank to reaction temperature (37c)

2-Pipette into cuvette, 1ml of working reagent and added 100ul of sample or standard.

3-Pipette into other cuvette, 1ml of working reagent and added 100ul of distilled water as blank

4-Mix thoroughly and letting the tubes stands for two min. at room temperature.

5-Read the absorbance (A) of the sample blanks at 540 nm against purified water.

6-Read the absorbance (A) of the samples at 540 nm against the reagent blank. Prepare working method for the manufacturer. (Liner)

3-5-3-4: Calculations

{(A Sample – A blank)/standard} x F = mg/dL total or direct bilirubin.

3-6: Serological examination

3-6-1: Principle of Rheumatoid Factor (RF)

A number of techniques are presented for testing of RF. The utmost used serological technique was based on latex agglutination test. The RF-Latex is a slide agglutination test for the qualitative and semi-quantitative detection of RF in human serum latex particles coated with human gamma globulin. Figure **3-1** shows Principle of Rheumatoid Factor (RF) (Frederick *et al*, 1987)

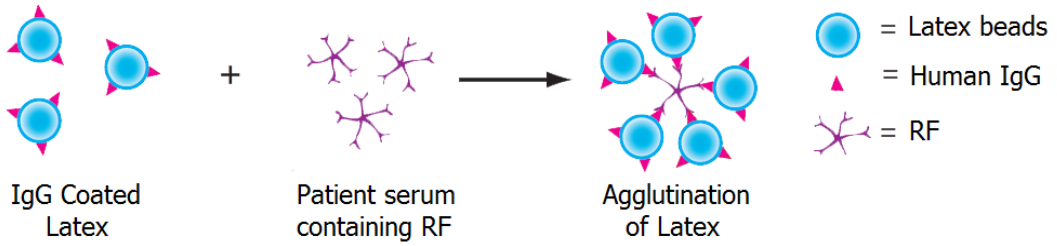


Figure 3-2 Principle of Rheumatoid Factor (RF) (Frederick *et al*, 1987)

3-6-2: Procedure of Rheumatoid Factor (RF)

1. Carry all reagents and specimens in room temperature.
2. Place one drop of the positive control and 40ul of the patient serum into separate circles on the slide.
3. Gently add one drop of RF latex reagent on each circle of sample to be tested and control.
4. Use separate Applicator sticks/stir sticks to spread reaction mixture over the entire area of the particular field.
5. Tilt the slide back and forth for 2 minutes in a rotary shaker so that the mixture rotates slowly.
6. Observe for agglutination after 2 minutes under bright artificial light.

3-6-3: Content kit of Rheumatoid Factor (RF)

Agglutination of latex particles was a positive reaction, indicating the attendance of RF at a noteworthy and noticeable level.

Positive result: An agglutination of the latex particles suspension will occur within 2 min., indicating an RF level higher than 18 IU/ml. **Negative result**: No agglutination of the latex particles suspension within 2 min.

3-7: Statistical Analysis

The Statistical Analysis System- SAS (2012) program was employed to show the effect of different factors in study parameters. Chi-square test was employed to compare the percentages and T-Test was employed to substantially associate means in this study. Chi-square was used to test the statistical significance of the association between the categorized variables. A p-value not more than 0.05 level of significance was well thought-out to be statistically substantial.

4. Results and Discussion

The results in this chapter are based on the statistical data analysis.

4-1 Study groups:

The current 230 women were classifyed into three groups: the group of unmarried girls, the group of married women (pregnant and non-pregnant women) and control group as depicted in the Table (4-1), as shown in appendix 1, results showed that the married girls (50) pregnant (21.8%), married (non-pregnant) women were 45 (19.7%). The study also showed that the number of unmarried women (85) percentage (36.7%) and number of control 50 (21.8%).This increase in the incidence of rubella among married and unmarried women can be attributed to a number of reasons, including the absence of vaccination programs that take into account the vaccination of women who are ready to accept infection at the age of childbearing, lack of health awareness and low attention to primary health care for pregnant women (Turbadkar *et al*., 2003).

A few studies have examined the presence of pregnancy as a factor in IgG antibodies prevalence among pregnant and non-pregnant women. Our findings were consistent with Punjab, India, which documented non significant effect of pregnancy on IgG antibodies prevalence for pregnant and non-pregnant women (Singla *et al.,* 2004). The few of vaccination agendas for teenage girls and women of childbearing age who are ready for accepting the rubella virus have made consistent with the previous study, which showed that 10-20% of women reach reproductive age without acquiring normal immunoglobulin or Infected with the Rubella vaccine (Turbadkar *et al.*, 2003).

Table 4-1. Study group according to rubella infection

Specimens		Number	Percentage(%)
Married	Pregnancy	50	21.8
	No pregnancy	45	19.7
No married		85	36.7
Control		50	21.8
Total		230	100%

The results in **Table 4-2** show the demographical distribution patients such as age, living, and educational level of females involved. Table 4-2 shows rubella virus prevalence in the totals of women, according to age, living (rural and urban) and educational level (illiteracy, primary education, middle school, secondary school and higher education), the number of women infected with the age of 15-19 is 40, (20%), 21-29, 81, (47.2%), 30-39, 48, (26.7%), 40 and above years is 11 and (6.1%). Rubella infection is more prevalent in the ages of 20-29 than in married women because this age where more review of appetizers marriage at than in the other ages. The results of urban and rural living in the numbers (54 and 126) in percentages (30% and 70%), respectively, show that rural infestation is much higher than urban areas due to rural women's health neglect and lack of health awareness, as opposed to urban areas (Sasaki *et al.,* 2007). As for the level educational that was (illiteracy, primary education, middle school, secondary school and higher education) in the numbers (81,34,31,18 and16) and in percentages (18.4%,18.9%, 17.2%,10% and 8, 9%), respectively, shows that the highest incidence is in women who have the lowest level of education in the illiterate women. We also note lower rubella virus incidences for higher levels of education, the greater the health awareness and care for health care than the uneducated women. In this study, the level of education was

uneven among women that educational level (illiteracy, primary education, middle school, secondary school and higher education) with rubella virus low awareness level. It is necessary to enable women to know about the infection and prevention of rubella, and pregnant women, whenever possible, avoid contact with people with rash in the skin (Sallam et al., 2003). As shown in appendix 1

Table 4-2 demographical distribution patients of patients group among rubella infection.

patients group	**Number**	**Percentage**
Age (years)		
15-19	36	20
20-29	85	47.2
30 -39	48	26.7
40 and above	11	6.1
Total	180	100
Living		
Urban	54	30
Rural	126	70
Total	180	100
Educational level		
Illiteracy	81	45
Primary education	34	18.9
Middle school	31	17.2
secondary school	18	10
Higher Education	16	8.9
Total	180	100

4-2 Immunological parameters:

Serum level of immunological parameters which belong to interleukins IL-10, IL-6, CD4 and CD8) was determined in rubella patients and control samples.

4-2-1: IgG and IgM antibodies prevalence for rubella among women

The results showed that IgG antibodies prevalence among women was higher by a statistical difference (P <0.01) compared with IgM, because infection time of IgM was low, again, IgG was found to be 149 and 82.78% and IgM 4 and 2.22% respectively. IgG and IgM antibodies prevalence for rubella among women were 27 and 15% .These results indicated significant difference in (P <0.01). As Table (4-3)

Rubella antibodies (IgG) absence could indicate unexposed people to Rubella virus or vaccine they shielded against it. On the other hand, IgG antibodies presence signifies a history of past exposure to the virus or vaccination while IgM antibodies presence signifies recent or active infection (Sallam *et al.*, 2003; Goh *et al.*, 2007 ; Pennap *et al*., 2009). Our findings present correlate with previous studies which showed that specific antibodies prevalence to the rubella virus among female samples, as this non-significant increase in IgM antibodies prevalence to the rubella virus among women may be caused by the fact that these women have never experienced the rubella virus Consequently, IgG antibodies level is insufficient to prevent infection, the absence of recent infections or recent exposure to the virus can be attributed to the fact that this group has high levels of protection represented by the emergence of IgG antibodies due to previous exposure (natural injury or previous vaccination). The present study is consistent with a similar study of young girls in Yemen (Sallam et al., 2003). Our study has comparable results with two previous studies that showed that IgG antibodies prevalence to the rubella virus among samples of pregnant women In Baghdad (88%) and Germany (87%) (Abbas, 2015

and Reiche *et al*., 2000), as confirmed by previous studies, they do not have previous abortions that are more likely to accept the rubella virus (Singla *et al*., 2004; Levinson, 2006).

While less than the prevalence of these antibodies in two separate Saudi studies (94.2%, 93.3%), Kuwait (95%), Turkey (95.0%). As well as two separate studies in Iran (94.6%, 91.1%), India (91.37%) and Spain (99.95%), (EL-Makki and Zaki, 1998; Gazi, 1999; Ganjooie and Mohammadi, 2003; Gutierrez-Zufiaurre *et al*., 2004; Kaushal and Baxi, 2007; Ocak *et al*., 2007 and Majlessi *et al*., 2008).

This increase in specific antibodies prevalence to the rubella virus among women has been caused by the absence of vaccination programs that take into account the vaccination of women who are ready to accept infection at the reproductive age and the low interest in primary health care for women. Results of this study were in agreement with a former research that showed that IgM prevalence of the rubella virus with the study in the city of Kashmir, which indicated that the highest incidence of the rubella virus represented by the emergence of IgM antibodies was among women, our results are consistent also with three previous studies which indicated IgG antibodies prevalence among a sample of non-pregnant women in Mexico (87%), Peru (87.2%) and India (88.93%) (Sagarnaga-Durante *et al*., 2004; Kaushal and Baxi, 2007; Suàrez-Ognio *et al*., 2007).

Results of this study showed a high prevalence of these infections which might be the result of previous exposure to the rubella virus. Argentina (91.2%) and Switzerland (94.3%) (Dayan *et al.*, 2005*;* Jarour *et al.*, 2007). Lack of vaccination programs that take into account the vaccination of women who are ready to accept infection at the age of childbearing, lack of health awareness and low attention to primary health care for pregnant women, the results revealed a higher IgG antibodies prevalence among pregnant women than in two separate studies in Baghdad; (2.8%)

(AL-Zubaidy, 1993) and 2% Turkey (0.54%), Brazil (1.2%) and China (7.4%) (Reiche *et al*., 2000 ; Zheng *et al*., 2002 ; Ocak *et al*., 2007).

Table 4-3. Distribution of patients according to Rubella infection

Rubella infection	Number	Percentage (%)
Rubella IgG	149	82.78
Rubella IgM	4	2.22
Rubella IgG + IgM	27	15.00
Total	180	100%
Chi-Square	---	13.738 **
P-value	---	0.0001
** (P<0.01).		

4-2-2 Interleukins IL-10 and IL-6

- **Comparison between women with rubella infection and control in IL-10 and IL-6**

IL-10 serum level was considerably increased ($p \leq 0.01$) in IL-10 patients in comparison with control IL-10 (214.63 ± 12.70. 9.67 ± 0.64 pg/ml), though insignificant differences were perceived between IL-6 patients and their controls (8.89 ± 1.63. 9.27 ± 0.40 pg. /ml). As Table (4-4) It displays the mean value of concentration (pg/ml) of IL-10 and IL-6 in sera of studied group that reveal extremely considerable increase ($p<0.01$) IL-10 expression with rubella women in comparison to control, due to the immune system's preoccupation with the immune

response to the virus, Several virals merchandises improve DNA-binding activity of NF-κB and NF-IL-6, leading to a rise in IL-6 mRNA transcription, as example, interaction with NF-κB of derived from the human T lymph tropic virus improves IL-6 production (Lee et al, 2012).

Table 4-4. Comparison between women with rubella infection and control in IL-10 and IL-6

Group	Mean ± SE	
	IL-10 (Pg/ml)	IL-6 (Pg/ml)
Rubella infection	214.63 ± 12.70	8.89 ± 1.63
Control	9.67 ± 0.64	9.27 ± 0.40
T-Test	47.123 **	6.059 NS
P-value	0.0001	0.898
** (P<0.01).		

4-2-2-A: Relationship between Rubella infection IgG and IgM of patients with IL-10 and IL-6

show that there was no significant difference in rubella virus (IgG + IgM) associated with (IL-6, IL-10), (17.43 ± 7.64, 225.89 ± 32.29) respectively, no significant differe in rubella virus (IgG) associated with (IL-6, IL-10), (211.28 ± 14.04, 7.37 ± 1.38). There is no significant difference in rubella virus (IgM) associated with (IL-6, IL-10), (263.50 ± 94.62, 7.45 ± 0.42), and these results are significantly higher than controls. Table 4-5 and Figure (4-1) Antiviral effects of T-cells can be mediated through cytokines excretion which overturns virus replication also diseased cells cytotoxic elimination. Since T-cells will not block infection

straight for worldly. However, they respond to controller or remove viral diseased cells when disease have happened. T cells contribution to protection is commonly thought to be negligible as compared with neutralizing antibodies. Moreover, T cell immunity alone is not able to shield from rubella virus disease, nonetheless it enables RNA authorization and creation of a vigorous T cell and antibody reaction afterward encountering disease. (Lin, *et al.*, 2015).

Level of IL6 (65%) of people infected with the rubella virus was lower than normal values (27.7%) and compared to the very low level of IL6 (7.22%) due to the immune system's preoccupation with the immune response to the virus, Several viral merchandises improve DNA-binding activity of NF-κB and NF-IL-6, leading to a rise in IL-6 mRNA transcription, as example, interaction with NF-κB of the Tax derived from the human T lymphotropic virus improves IL-6 production (Lee *et al*, 2012).

Table 4-5. Relationship between Rubella infections of patients with IL-10 and IL-6

Rubella infection	Mean ± SE	
	IL-10 (Pg/ml)	IL-6 (Pg/ml)
Rubella IgG	211.28 ± 14.04	7.37 ± 1.38
Rubella IgM	263.50 ± 94.62	7.45 ± 0.42
IgG + IgM	225.89 ± 32.29	17.43 ± 7.64
LSD value	149.22 NS	14.943 NS
P-value	0.778	0.088
NS: Non-Significant.		

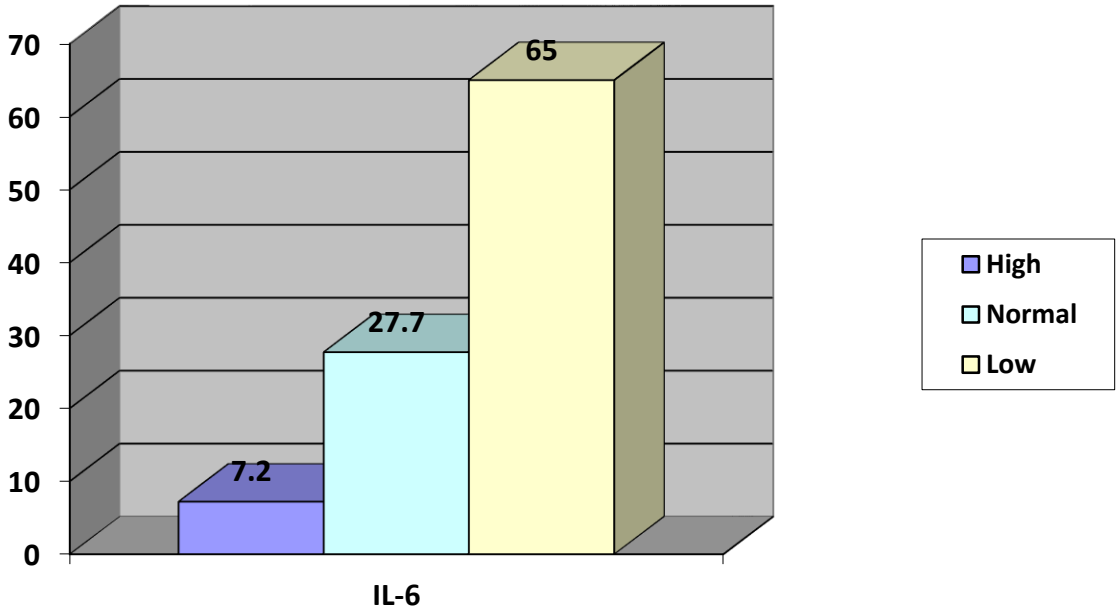


Figure 4-1 Rubella infection with Interleukin-6 (IL6)

4-2-2-B: Relationship between women with rubella infections with interleukin-10 (IL-10)

In figure 4-2, the level of IL10 for women infected with the rubella virus (96.11%) was higher than normal values (3.89%) because the height of IL10 was because of immune system stimulation and IL10 releasing significantly IL10 is produce by T-cells, which is an anti-inflammatory cellular and tolerance by inhibiting the release of anti-inflammatory cellular motifs (Cheng *et al.*, 2005). This is in line with the observed significant increase in IL10, which may contribute to the maintenance of women. Conversely, women with rubella showed the lowest sera positivity level of this cellular motor, which may confirm that its low level may be associated with the induction of Automatic abortion; the studies have shown that Treg cells are dominant in immune regulation and fetal loading. These cells inhibit the proliferation and production of cellular motions produced from CD4 + and CD8

+ T cells, toxicity of natural killer cells, and regenerative cell maturity, Of the mother, so the functions of Th1 and Th2 cells are affected by cellular motions produced from CD4 + CD25 + Treg cells. These cells are therefore conservative cells (Sakaguchi, 2005; Akbar *et al*., 2007). IL-10 as well inhibits Th1 cells function and phagocytic cells and inhibits rejection of fetal-allograft rejection (Saini *et al*., 2011). Treg cells are dominant in maintaining pregnancy and few in these cells lead to abortion, as many researchers have shown (Zenclussen *et al*., 2005; Sasaki *et al*., 2007; Darmochwal-Kolarz *et al*., 2007).

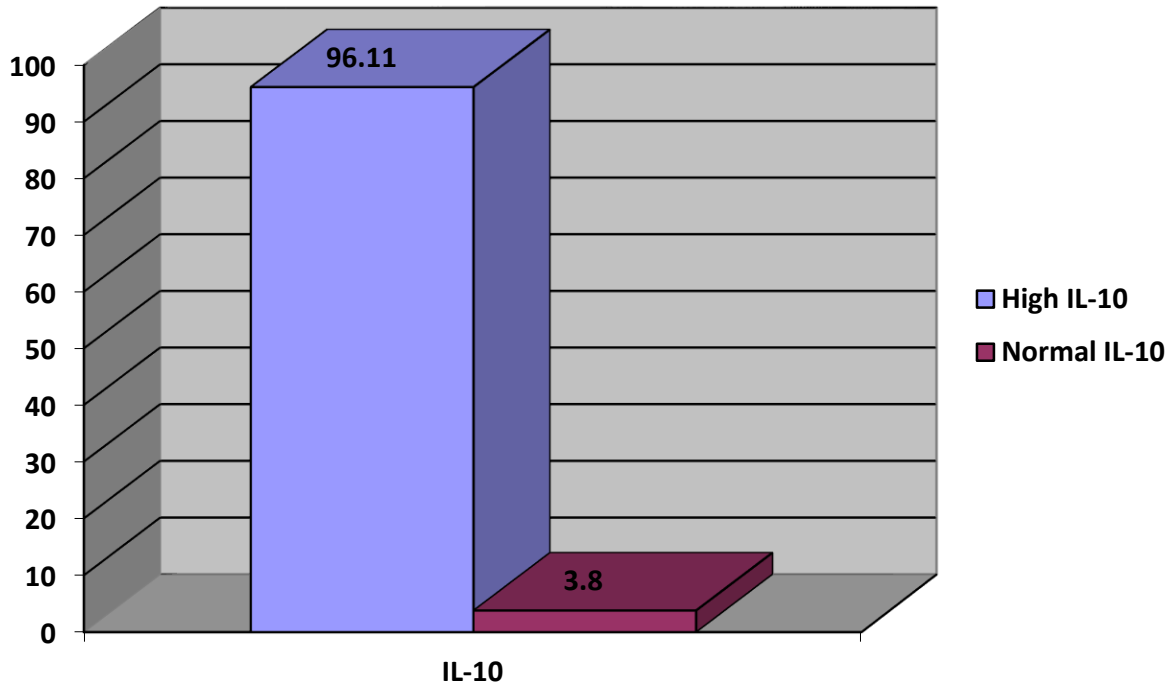


Figure 4-2 Rubella infection with Interleukin-10 (IL-10)

4.2.3 CD8 and CD4

4.2.3.1 Comparison between women with rubella infection and control in CD8 and CD4

Table 4-6 shows that CD8 serum level was considerably ($p \leq 0.01$) improved in CD8 in comparison to control (21.57 ± 1.47. 129.41 ± 14.22 pg/ml), though this difference was noticed between CD4 patients and their controls (14.07 ± 0.98. 20.33 ± 1.56 pg/ml), it indicates the mean value of concentration (pg/ml) of CD4 and CD8 in sera of studied group that reveal extremely momentous ($p<0.01$) increase CD8 expression in with rubella women in comparison to control that because CD8 level increase in viral infection but CD4 level is decrease, because the CD4 is high in secondary infections such as bacteria and decreases when viral infections and due to the immune response of the virus (De Vries, et al., 2012).

Table 4-6. Comparison between rubella infection and control in CD8 and CD4

Group	Mean ± SE	
	CD4 (pg/mL)	CD8 (pg/mL)
rubella infection	14.07 ± 0.98	21.57 ± 1.47
Control	20.33 ± 1.56	129.41 ± 14.22
T-Test	3.992 **	15.818 **
P-value	0.0022	0.0001
** (P<0.01).		

Table 4-7 shows that serum level of CD4 and CD8 were significantly different ($p \leq 0.05$), it shows that the mean of anti-rubella antibodies titer in IgG by CD4 and CD8 test were (14.04 ± 1.13 b and 21.51 ± 1.70 b), Sequentially, but IgM with CD4 and CD8 test were (27.67 ± 5.68 a and 42.97 ± 12.59 a) . In mean of anti-rubella antibodies titer (IgG, IgM) with CD4 and CD8 test shows that they were significant in ($P<0.05$). (12.22 ± 1.57 b and 18.73 ± 1.73 b) sequentially.

It demonstrates the mean value of concentration (pg/ml) of CD4 and CD8 in sera of studied group that reveal extremely considerable ($p<0.05$) increase of CD8 with rubella women in comparison to control. Diseased immune cells (monocytes, CD8+ memory T cells, CD4+, and B cells) at this point go into the flow and transmit virus to various lymphoid organs (thymus, Lymph nods, spleen) and non-lymphoid organs (liver, lung, kidney, skin) in which replications happen in epithelial and endothelial cells, Lymphocytes and macrophages. (De Vries, *et al.*, 2012). Since CD4+ T cell assistance was obligatory to both maturations namely affinity and isotype of concealing antibody cells, memories of maturation of CD8+ T cell B cell. Response of immune cells is vital for the initiation of defensive immunity; the immune response controls manifold phases of disease. Preliminary inborn resistant reaction are constrained because of interferon (IFN) response, constrained because of interferon (IFN) response, permits extensive virus replication, transmit throughout silent latent period of 10–14 days (Mina, *et al.*, 2015).

Table 4-7. Comparison between Rubella infections of patients with CD8 and CD4

Rubella infection	Mean ± SE	
	CD4 (pg/mL)	CD8 (pg/mL)
Rubella IgG	14.04 ± 1.13	21.51 ± 1.70
Rubella IgM	27.67 ± 5.68	42.97 ± 12.59
IgG + IgM	12.22 ± 1.57	18.73 ± 1.73
LSD value	11.45 *	17.18 *
P-value	0.042	0.043
* (P<0.05).		

In figure 4-3 revealed an important result in the level (P<0.01), high in CD8 (98.33%) compared with normal value (1.67%) and low values (0%) due to stimulation of the immune system, Diseased immune cells (monocytes, $CD8^+$, $CD4^+$ T cells, B cells) at this point go into flow then transmit the disease to manifold lymphoid organs (thymus, LNs, spleen) and non-lymphoid organs (liver, lung, kidney, skin) in which replications happen in epithelial and endothelial cells, Lymphocytes and macrophages, as well as because CD8 is high in viral infections and decreases in secondary infections such as bacteria (De Vries *et al*, 2012).

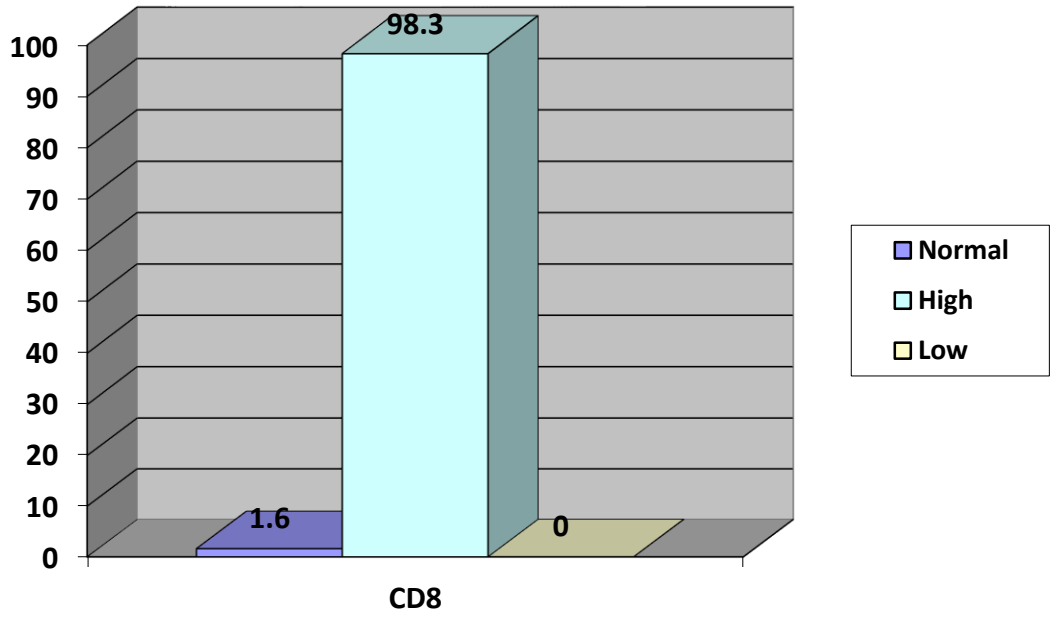


Figure 4-3 Rubella infection with human Cluster of differentiation 8 (CD8)

Figure 4-4, CD4 did not affect people who were infect with the rubella virus. The natural value was not affected at the normal level of 80.56% compared to the low value of 19.44% and the high value was 0% , because the CD4 is high in secondary infections such as bacteria and decreases when viral infections and due to the immune response of the virus, which did not significantly affect it. (De Vries, et al., 2012). The immune response controls manifold phases of disease, first inborn resistant reaction was constrained because of IFN inhibition reaction and permits wide virus repetition and transmits throughout a clinically hushed dormant age of ten–fourteen day. (Mina *et al*, 2015)

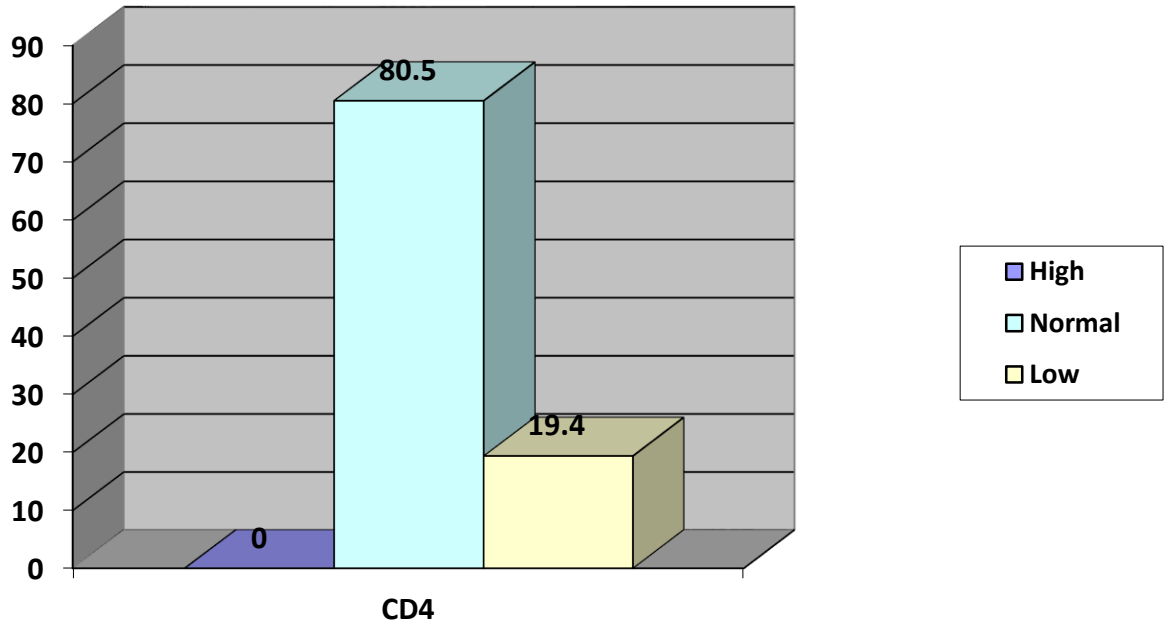


Figure 4-4 Rubella infection with human Cluster of differentiation 4 (CD4)

4-3: Serological examination

4-3-1 Relationship between rubella infections with RF

Table 4-8 show that there is no effect at this level (P<0.01) on rubella virus, because RF is a genetic factor, where the number of women positive for RF is 19 and (10.56%), while the number of women with negative result 161 and (89.44%). The result of this study are different from the results of London study that presented antibodies presence to the rheumatoid factor (65%) of patients (Veerapen *e t al.*, 1993). These antibodies from rheumatism in the mother's blood can cause abortion in women through their transmission to the fetus's blood by causing a increase in the heartbeat in fetus. (Goeldner *e t al.*, 2010)

Table 4-8 Relationship between women with rubella infections and levels of RF in study groups

RF	Number	Percentage (%)
Positive	19	10.56
Negative	161	89.44
Total	180	100%
Chi-Square	---	15.069 **
P-value	---	0.0001
** (P<0.01).		

4-4-Biochemstry examination

4-4-1 Comparison between rubella infection and control in Creatinine, Urea and TSB

Table 4-9 shows the mean value of concentration (mg/dl) of creatinine, urea and TSB in sera of studied group that reveal extremely momentous ($p<0.01$) increase creatinine expression, urea and TSB with rubella women in comparison to control. Furthermore, it reveals a substantial correlation ($P>0.05$) among creatinine, urea and TSB in women with rubella and control groups.

Serum level of creatinine, urea and TSB were considerably ($p \leq 0.01$) decreased in creatinine patients in comparison with control (0.902 ± 0.02, 0.817 ± 0.02 mg/dl), despite the fact that these variations were between urea patients and their controls (36.99 ± 0.78, 33.06 ± 0.83 mg/dl). Whereas these differences were lacking between urea patients and their controls (0.911 ± 0.04. 0.700 ± 0.03 mg/dl). Women who had

a normal urea ratio this difference in results since the virus reached multiple lymphoid (LNs, thymus, spleen) and non-lymphoid (liver, lung, Kidney) organs in which they duplicated in epithelial, endothelial cells, macrophages, and lymphocytes (De Vries et al, 2012). This condition is initiated either using impairment in the metabolic pathway or by an overproduction of bilirubin, the increase in bilirubin production is typically originated using fast destruction of erythrocytes, causing blood diseases like haemolilytic anemia. (Young, 2000).

Table 4-9 Comparison between rubella infection and control according to level of Creatinine, Urea and TSB

study groups	Mean ± SE		
	Creatinine (mg/dl)	Urea (mg/dl)	TSB (mg/dl)
rubella patients	0.902 ± 0.02	36.99 ± 0.78	0.911 ± 0.04
Control	0.817 ± 0.02	33.06 ± 0.83	0.700 ± 0.03
T-Test	0.0827 *	3.049 **	0.148 **
P-value	0.0438	0.0019	0.0053
* (P<0.05), ** (P<0.01).			

Table 4-10 shows the mean value of concentration (mg/dl) of creatinine, urea and TSB in sera that reveal non-significant ($p<0.01$) rise in creatinine, urea and TSB with rubella women in comparison to control. Moreover, it shows non-significant correlation ($P>0.05$) among creatinine, urea and TSB in women with rubella and control groups.It shows that the mean of anti-rubella antibodies titer in IgG by

Creatinine, Urea and TSB test were (0.897 ± 0.02, 37.11 ± 0.83 and 0.921 ± 0.04), but in IgM (0.825 ± 0.16, 40.40 ± 5.88 and 0.875 ± 0.25). In mean of anti-rubella antibodies titer (IgG, IgM) with Creatinine, Urea and TSB test shows no significant (0.944 ± 0.06, 35.83 ± 2.40 and 0.859 ± 0.07). Women who had a normal urea ratio this difference in results since the virus reached multiple lymphoid (LNs, thymus, spleen) and non-lymphoid (liver, lung, Kidney) organs in which they duplicated in epithelial, endothelial cells, macrophages, and lymphocytes (De Vries et al, 2012). An impairment in the bilirubin metabolism is because either using an enzyme deficiency or using a physical obstruction in bilirubin flow like biliary (bile duct) obstruction, the hyperbilirubinemia leads to jaundice (discoloration of mucus membranes, sclera and skin caused by the deposition of bilirubin pigment). (Neerga, 2007)

Table 4-10. Relationship between women with Rubella infections with Creatinine, Urea and TSB

Rubella infection	Mean ± SE		
	Creatinine (mg/dl)	Urea (mg/dl)	TSB (mg/dl)
Rubella IgG	0.897 ± 0.02	37.11 ± 0.83	0.921 ± 0.04
Rubella IgM	0.825 ± 0.16	40.40 ± 5.88	0.875 ± 0.25
IgG + IgM	0.944 ± 0.06	35.83 ± 2.40	0.859 ± 0.07
LSD value	0.251 NS	9.261 NS	0.453 NS
P-value	0.635	0.686	0.838
Non-Significant.			

4-4-2: Comparison between women with rubella infections and creatinine

The results showed a high level of creatinine that was (1.67%) correlated with normal values which were (98.33%) since the virus reached multiple lymphoid (LNs, thymus, spleen) and non-lymphoid (liver, lung, Kidney) organs in which they duplicated in epithelial, endothelial cells, macrophages, and lymphocytes (De Vries *et al*, 2012).

4-4-3: Comparison between women with rubella infections and urea

Table 4-11 Show a low in urea (1.111%), incidence of cases of viral infections and urea high of 56 (31.11%), although there were 122 (67.778%), women who had a normal urea ratio this difference in results since the virus reached multiple lymphoid (LNs, thymus, spleen) and non-lymphoid (liver, lung, Kidney) organs in which they duplicated in epithelial, endothelial cells, macrophages, and lymphocytes (De Vries *et al*, 2012).

Table 4-11. Comparison between women with rubella infections and urea

UREA	Number	Percentage (%)
High	56	31.11
Low	2	1.111
Normal	122	67.778
Total	180	100%

4-4-4 Rubella virus with total serum bilirubin (TSB)

In Figure 4-5 showed variation in some cases of viral infections and TSB rise of 27.778%, low in TSB 53.889%, although 18.333% of the people with TSB were normal, the liver is the controlling organ in hematopoiesis instead of bone marrow, high temperature of the symptoms of the virus of the rubella and this high in temperature due to high sweating and decomposition and this leads to anemia in liver from infected woman, necrotizing and inflammatory variations signifying viral hepatitis were existing. Nonetheless, obvious inflammatory variations triggered by virus infection were slight in further organs from infected woman. *Hyperbilirubinemia* (an abnormal elevation of bilirubin, conjugated or unconjugated) in plasma is an indication of a disturbance in bilirubin metabolism. This condition is initiated either using impairment in the metabolic pathway or by an overproduction of bilirubin, the increase in bilirubin production is typically originated using fast destruction of erythrocytes, causing blood diseases like haemolilytic anemia. (Young, 2000). An impairment in the bilirubin metabolism is because either using an enzyme deficiency or using a physical obstruction in bilirubin flow like biliary (bile duct) obstruction, the hyperbilirubinemia leads to jaundice (discoloration of mucus membranes, sclera and skin caused by the deposition of bilirubin pigment). This result agree with (Neerga, 2007) that showed bilirubin high with rubella virus (18.33 %).

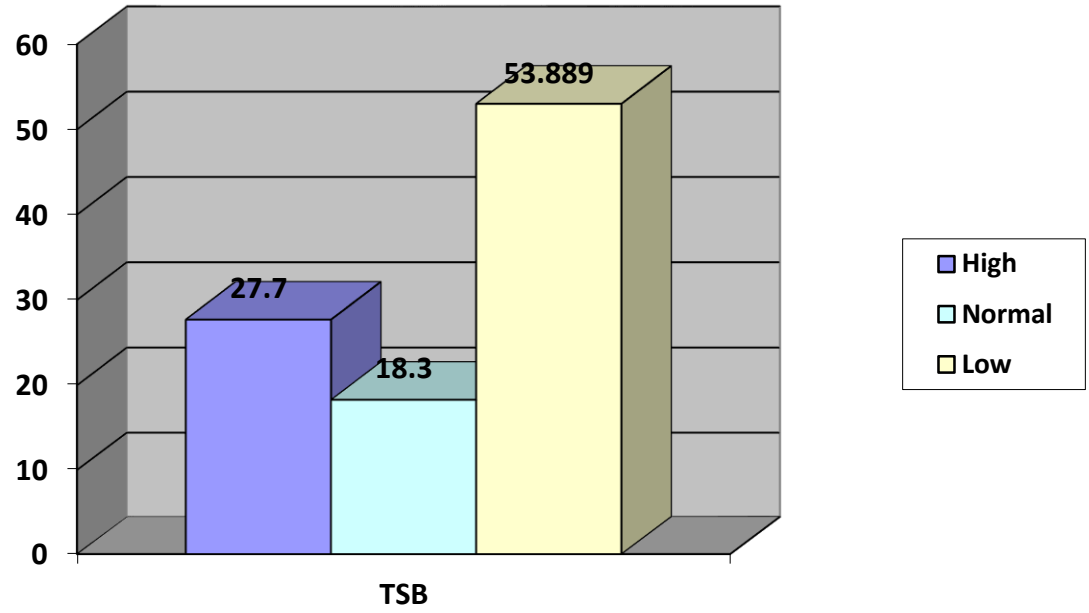


Figure 4-5 Rubella infection with total serum bilirubin (TSB)

4-5: Hematology examination

4-5-1: Comparison between rubella infection and control in WBC and Hb

Table 4-12 shows that blood level of white blood cell count (WBC) and Hemoglobin (Hb) were considerably ($p \leq 0.01$) reduced in WBC count patients in comparison with control (7571.67 ± 269.67. 7062.75 ± 297.63 c/m), while the difference was between Hemoglobin (Hb) patients and their controls (11.75 ± 0.14. 13.33 ± 0.12 mg/dl) increased.

It shows the mean value of concentration (c/m and mg/dl) of WBC and Hb in blood of studied group which shows extremely substantial ($p<0.01$) improved expression of in WBC and Hb with rubella women compared with control. Through the above results, the main reason for the decrease and increase in Hb and WBC levels is shown by comparison with control, because anemia is considered a

symptom of rubella and an increase in WBC due to what this virus does from stimulating the immune system. (Fine, *et al*., 2007).

Table 4-12 Compare between rubella infection and control in WBC and Hb

Group	Mean ± SE	
	WBC	**Hemoglobin(Hb) (mg/dl)**
rubella infection	7571.67 ± 269.67	11.75 ± 0.14
Control	7062.75 ± 297.63	13.33 ± 0.12
T-Test	1046.90	0.551 **
P-value	0.339	0.0001
** (P<0.01).		

Table 4-13 Shows that the mean of anti-rubella antibodies titer in IgG by WBC and Hb were (7564.43 ± 284.88 and 11.82 ± 0.15), but in IgM (8225.00 ± 3148.38 and 13.05 ± 1.17). In mean of anti-rubella antibodies titer (IgG, IgM) with WBC and Hb test shows that there were no significant difference (7514.81 ± 785.11 and 11.17 ± 0.35). Decreased production of erythrocytes is associated most commonly with iron deficiency, although other factors, such as bone marrow disorders, TORCH infections (rubella), congenital leukemia, and nutritional deficiencies other than iron, also can be responsible. This agree with (Neerga, 2007) that show Hb low with rubella virus (3.889gm %).

Table 4-13 Relationship between rubella infections of patients with WBC and Hb

Rubella infection	Mean ± SE	
	WBC (c/m)	Hb (mg/dl)
Rubella IgG	7564.43 ± 284.88	11.82 ± 0.15
Rubella IgM	8225.00 ± 3148.38	13.05 ± 1.17
Rubella IgG + IgM	7514.81 ± 785.11	11.17 ± 0.35
LSD value	3180.50 NS	1.968 NS
P-value	0.934	0.112
Non-Significant.		

In figure 4-6 show that the effect of white blood cell count was higher (18.333%), compared to normal (65%) and the low of (16.667%). In acute infection of the rubella virus, there will be an increase in white blood cells while in chronic infection, white blood cells will be less than normal, this increase or decrease in WBC due to virus's replication nature and its potential activity, in some cases do not affect (normal) number of white blood cells where this virus is latent, in latent infections , overt illness is not found, nonetheless the virus was uneradicated, the virus can occur in an actually covert noninfectious occult form, probably as an integrated genome or an episomal agent , or as an infectious and uninterruptedly replicating agent , termed a persistent viral infection (Mina, *et al.*, 2015).

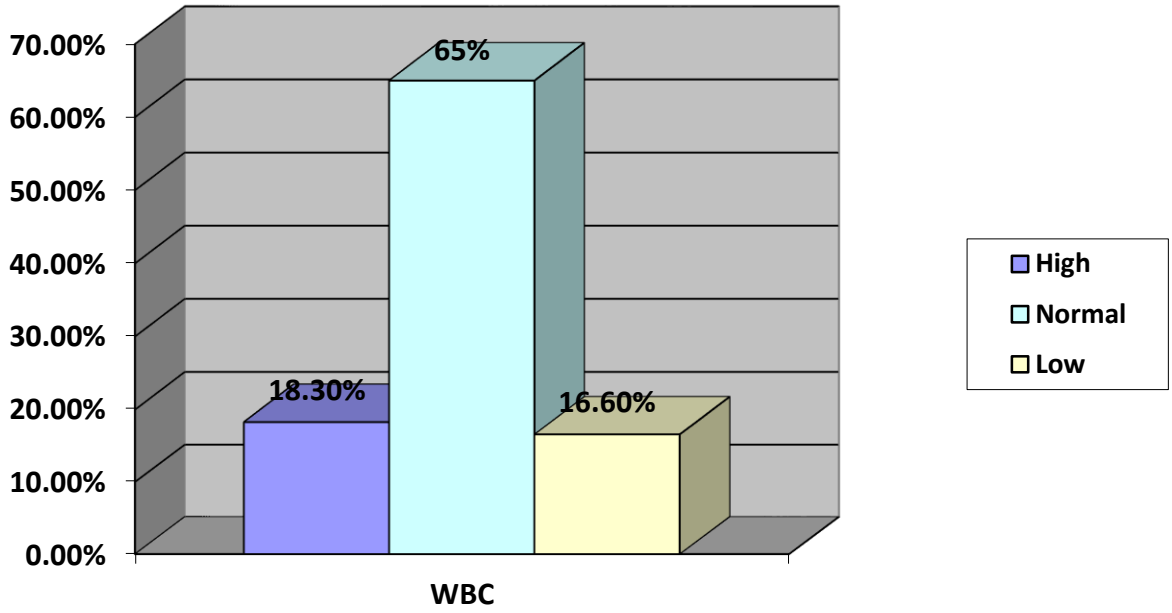


Figure 4-6 Rubella infection with white blood cell

In figure 4-7 show a decrease in the level of hemoglobin, which was (48.333%) compared to normal values, which was (47.778%) because of the symptoms that accompany the rubella virus and High in hemoglobin (3.889%). Decreased production of erythrocytes is associated most commonly with iron deficiency, although other factors, such as bone marrow disorders, TORCH infections (rubella), congenital leukemia, and nutritional deficiencies other than iron, also can be responsible. This result agree with (Neerga, 2007) that showed high Hb with rubella virus (3.88gm %).

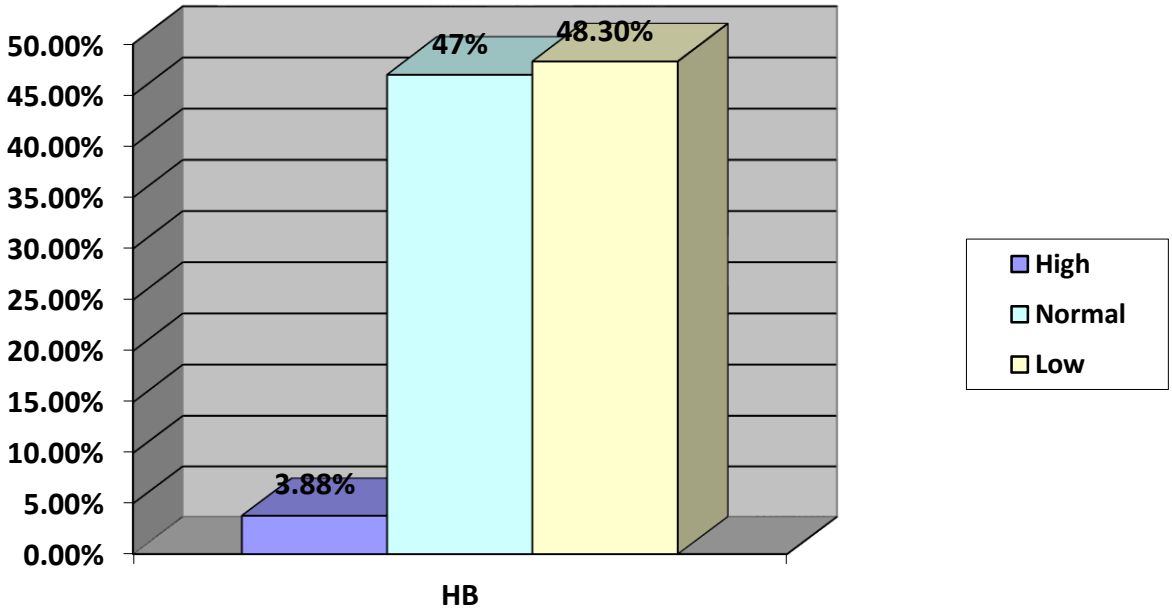


Figure 4-7 Rubella infection with hemoglobin (Hb)

The results of the present study to determine the diagnostic code of the rubella virus in women according to the **Figure4-8** below.

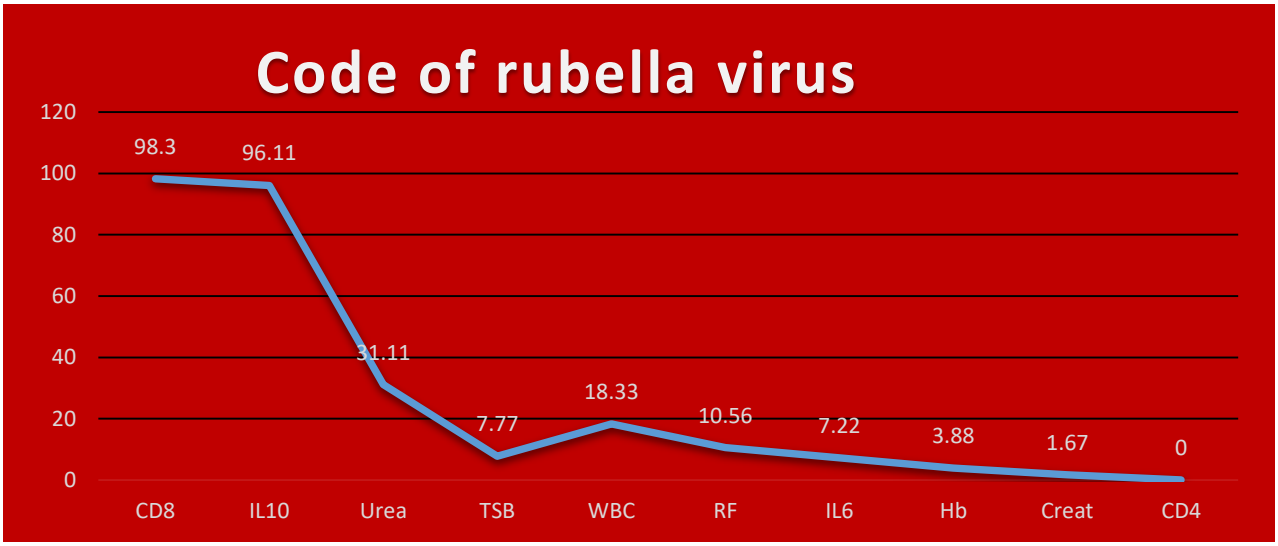


Figure 4-8 diagnostic code for the rubella virus with some biomarkers

Conclusions

1. Using the biological code include chemical, immunological, and hematology, has a role in the diagnosis of rubella infections

2. High in results for immunologic assays CD8, IL10

3-The study shows high in the Urea examination among chemical tests taken with patients of rubella virus

4-High in the IgG test result for the rubella virus and decrease in IgM

5-High in screening (WBC) among hematological tests taken with patients of rubella virus

6. Provide materials for screening serological tests for IgM, IgG antibodies of rubella virus in viral testing of health institutions.

7. The rubella virus vaccine is important given the serious health and social complications caused by the disease.

Recommendations

1 - Studying the genetic variation of cytokines (IL6, IL10) in women infected with the rubella virus

2 - Investigating of viral load in women infected with the rubella virus and its relationship with the immune indicators

3 - Comparing of diagnostic techniques used in the diagnosis of the rubella virus

4 - Working on the study of other vital indicators such as hormones, enzymes and immunological markers

5 - Disseminating of the results of this study to health institutions to take benefit through the preparation of health awareness programs for citizens.

References

Abood, A. S. A.; Ahmed, H. T. and Hamid, A. N. (1995). Is estimation of the rubella serum antibody level of the women before marriage justified.*J. Islamic Acad. Sci.* **8 (3):** 127 – 130

Abbas, A. K., Lichtman A. H., Pillai, S. (2014). *Cellular and molecular immunology*. 8th ed. Philadelphia, USA: Elsevier Sanders. Pages: **13-29**

Abbas, A.K.; Lichtman, A.H.; Pillai, A. and Shiv, N. (2015). Cellular and Molecular Immunology. Eight Edition Elsevier Saunders, USA, pp**190-87.**

Abbas, A. K; Lichtman A. H.and Pillai, S. (2014). *Cellular and molecular immunology*. 8th ed. Philadelphia, USA: Elsevier Sanders. Pages: **13-29**

Akbar, A. N., Vukmanovic-Stejic, M., Taams, L. S. and Macallan, D. C. (2007). The dynamic co-evolution of memory and regulatory CD4+ T cells in the periphery. *Nature Reviews Immunology*, **7: 231-237**.

AL – Zubaidy, A. H. N. (1993). Rubella and CMV infection in Iraqi pregnant and aborted females and those congenitally malformed children, a retrospective study. M. Sc. Thesis. *Al nahreen College of Medicine.*

Albers, H. M. (2015). Genetics in juvenile idiopathic arthritis. Leiden University. Rotterdam, the Netherlands. A dissertation, p: **218**.

Arya, S.C., Agarwal, N. and Agarawal, S. (2014). Apropos "Congenital rubella syndrome and immunity status of immigrant Women living in southern Italy: A cross-sectional, seroepidemiological investigation".

Badoual C, Hans S, Rodriguez J, Peyrard S, Klein C, Agueznay Nel H, Mosseri V, Laccourreye O, Bruneval P, Fridman WH, Brasnu

DFTartour E. (2004) Prognostic value of tumor-infiltrating CD4+ T-cell subpopulations in head and neck cancers. Clin Cancer Res; **12:465–72**.

Beatch, M., and Hobman, T. (2000). Rubella virus capsid associates with host cell protein p32 and localizes to mitochondria. J. Virol. **74**, 5569-5576.

Beatch, M.D., Everitt, J.C., Law, L.J., and Hobman, T. C. (2005). Interactions between rubella virus capsid and host protein p32 are important for virus replication. J. Virol. **79**, 10807–10820.

Best and K. Corbett. (1996). Nucleotide sequence analysis of a major antigenic domain of the E1 glycoprotein of 22-rubella virus isolates. J. Gen. Virol**. 77**:2523-2540

Best JM. Rubella. Seminars in Fetal & Neonatal Medicine, (2007), **12**:182–192.

Banatvala JE, and Brown D.W.G. (2004) Rubella. Lancet; **363**(9415):1127-37.

Baron, E. J.; Peterson, L. R. and Finegold, S. M. (1994). Bailey and Scott's diagnostic microbiology 9th ed. Mosby St. Louis Baltimore Boston Chicago London Madrid Philadelphia Torento **408**.

Bartels, h, and bohner, M, Clin. Chim. Acta. **32**:81 (1971)

Batthish, M.; Feldman, B.M.; Babyn, P.S.; Tyrrell, P.N.and Schneider R. (2011). Predictors of hip disease in the systemic arthritis subtype of juvenile idiopathic arthritis. J Rheumatol. **38**(5):954-958.

Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK (2006). Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature**441**: 235–238

Bos R, Sherman L A. (2010). CD4+ T-cell help in the tumor milieu is required for recruitment and cytolytic function of CD8+ T lymphocytes. Cancer Research; **70**(21):8368–77.

Bosma PJ, Chowdhury JR, Bakker C, *et al*. (1995). The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. *N Engl J Med*; **333:** 1171–5. 43.

Bosma TJ, Best JM, Corbett KM, Banatvala JE, Starkey W.G. (1996). Nucleotide sequence analysis of a major antigenic domain of the E1 glycoprotein of 22-rubella virus isolates. *J Gen Virol*. **77:**2523–30.

Bosma PJ. Inherited disorders of bilirubin metabolism. (2003). J Hepatol; **38**: 107–17. 41.

Brooks, G. F.; Butel, J. S. and Morse, S. A. (2001). Rubella. In: Medical Microbiology. Brooks, G. F.; Butel, J. S. and Morse, S. A. 22nd Ed. McGraw – Hill. P: **484 – 487**.

Burska, A.; Boissinot, M.and Ponchel, F. (2014). Cytokines as biomarkers in rheumatoid arthritis. *Mediators Inflamm*. 2014:**545493**-545517

Buttarello, M; Plebani, M (2008). "Automated blood cell counts: state of the art". American Journal of Clinical Pathology. 130 (1): 104–16. doi:10.1309/EK3C7CTDKNVPXVTN. PMID 18550479.Bosma, T.

Carapetis, J.R., McDonald, M., and Wilson N.J. (2005). Acute rheumatic fever. Lancet; **366:155-68**.

Castillo-Solórzano, C., Marsigli, C., Bravo-Alcántara, P. Flannery, B., Ruiz, C., *et al*. (2011). Elimination of rubella and Congenital

Rubella Syndrome in the Americas. J Infect Dis **204** Suppl 2: S571-578.

Chaney,A.L., and Marbach,E.P. Clin. (1962). Chem. **8: 132**.

Chen Q, Wang WC, Bruce R, Li H, Schleider DM, Mulbury MJ, Bain MD, Wallace PK, Baumann H, Evans SS (2004) Central role of IL-6 receptor signal-transducing chain gp130 in activation of L-selectin adhesion by fever-range thermal stress. Immunity **20:59-70**.

Cheng, X., Liao, Y. H., Ge, H. Li, B., Zhang, J. and Yuan, J. (2005). TH1/TH2 functional imbalance after acute myocardial infarction: coronary arterial inflammation or myocardial inflammation. *Journal of Clinical Immunology*, **25**: 246-253 .

Christen U, Hintermann E, Holdener M, von Herrath MG. (2010) viral triggers for autoimmunity: Is the "*glass of molecular mimicry*" half-full or half empty. J Autoimmun; **34**(1):38-44.

Cong, H., Y. Jiang, and P. Tien, (2011). Identification of the Myelin Oligodendrocyte Glycoprotein as a Cellular Receptor for Rubella Virus. Journal of Virology, **85** (21): p. 11038-11047.

Connie Mahon; Donald Lehman; George Manuselis. (2014)Textbook of Diagnostic Microbiology.

Cox, M.A., Kahan, S.M., and Zajac, A.J. (2013). Anti-viral CD8 T cells and the cytokines that they love. *Virology* 435, 157-169.

Creasy, R.K., R. Resnik and J.D. Iams. (2004). Maternal Fetal Medicine: principles and practices. 5 th ed. Philadelphia, Pennsylvania. Saunders an Imprint of Elsevier. p. **760-762**.

Cutts, F.T., S.E. Robertson. J.L. Diaz-Ortega and R. Samuel. (1997). Control of rubella and congenital rubella syndrome in developing countries, part 1: burden of disease from CRS. Bull. WHO. **75**: 55-68.

Darmochwal-Kolarz, D., Saito, S., Rolinski, J., Tabarkiewicz, J., Kolarz, B., Lezczynska-Gorzelak, B. and Oleszczuk, J. (2007). Activated T lymphocytes in pre-eclampsia. American Journal of Reproductive Immunology, **58**: 39-45.

Davidson, J. (2000). Juvenile idiopathic arthritis: a clinical overview. Eur J Radiol., **33(2)**:128-134.

Dayan, G. H.; Panero, M. S.; Urquiza, A.; Molina, M.; Prieto, S.; *et al.* (2005). Rubella and measles seroprevalence among women of childbearing age, Argentina, 2002. *Epidemiol. Infect.* **133 (5)**: 861 – 9.

De Vries, R.D.; McQuaid, S.; van Amerongen, G.; Yüksel, S.; Verburgh, R.J.; Osterhaus, A.D.; Duprex,W.P.; de Swart, R.L. (2012) Measles immune suppression: Lessons from the macaque model., **8**, e1002885.

Dienz O, Eaton SM, Bond J.P, *et al*. (2009).The induction of antibody production by IL 6 is indirectly mediated by IL-21 produced by CD4+Tcells. *J Exp Med*. **206**:69-78.

Doroudchi, M.; Dehaghani, A. S.; Emad, K. and Ghaderi, A. A. (2001). Seroepidemiological survey of rubella immunity among three populations in Shiras, Islamic Republic of Iran. East. Medit. Health. J. **7(1-2)**: 128 – 138.

DuBois, R.M., Vaney, M.C., Tortorici, M.A., Kurdi, R.A., Barba-Spaeth, G., Krey, T., and Rey, F.A. (2013). Functional and

evolutionary insight from the crystal structure of rubella virus protein E1. Nature **493**, 552-556.

EL – Mekki, A. A. and Zaki, Z. M. (1998). Screening for rubella antibodies among Saudi women of childbearing age. *Saudi Med. J.* **19 (5)**: 575 – 577.

Fauci S, Kasper L, Hauser L, Harrison R. (2012) .Principles of Internal Medicine: Rubella. 18th ed. USA: The McGraw-Hill Companies, Inc.

Feigin, R.D and J.D. Cherry. (1992). Textbook of Pediatrics Infectious Diseases.3 th ed. W.B. Saunders Co. Philadelphia, Pennsylvania. P.**1792-1817**.

Forget, B. G.; Bunn, H. F. (2013). "Classification of the Disorders of Hemoglobin". Cold Spring Harbor Perspectives in Medicine. Cold Spring Harbor Laboratory. **3 (2)**: a011684–a011684. doi:10.1101/cshperspect.a011684. ISSN 2157-1422. PMC 3552344. PMID 23378597.

Fine, A. M., Nigrovic, L. E., Reis, B. Y., Cook, E. F., Mandl, K. D. (2007). Linking Surveillance to Action: Incorporation of Real-time Regional Data into a Medical Decision Rule. J. Am. Med. Inform. Assoc. **14**: 206-211

Frederick Wolfe *et al* .Clinica Chimica Acta (1987); **167**:1-21.

Frey TK, Abernathy ES, Bosma TJ, Starkey WG, Corbett KM, Best JM. (1998). Molecular analysis of rubella virus epidemiology across three continents, North America, Europe, and Asia, 1961–1977. J Infect Dis.**178**:642–50.

Frey, T.K. (1994). Molecular biology of rubella virus. Adv. Virus Res. **44**, 69-160.

Frieden, I. J. and Resnick, S. D. (1991). Childhood exanthema old and new. Pediatric Clinical of North American. **38**: 859 – 889.

Gabbe, S.G., J.R. Niebly and J.L. Simpson. (2002). Obstetrics Normal and Problem pregnancies. 14th ed. Churchill Livingstone. p. **627-630**.

Garrote, J.; Gómez-González, E.; Bernardo, D.; Arranz, E. and Chirdo, F. (2008). Celiac Disease Pathogenesis: The Proinflammatory Cytokine Network. *Journal of Pediatric Gastroenterology and Nutrition,* **47(1)**:27-32.

Goeldner I, Skare TL, de Messias Reason LT, et al. (2010). Anti-cyclic citrullinated peptide antibodies and rheumatoid factor in rheumatoid arthritis patients and relatives from Brazil. Rheumatology.

Greenwood, D., R.C.B. Slack and J.F. Peutherer. (2002). Medical Microbiology. 16 th ed. Churchill Livingstone. Nottingham and Edinburgh. U.K. p. **501-504**.

Hall, John E. (2010). Guyton and Hall textbook of medical physiology (12th end). Philadelphia, Pa.: Saunders/Elsevier. p. 502. ISBN 9781416045748.

Hashizume M, MiharaM. (2011). the roles of interleukin-6 in the pathogenesis of rheumatoid arthritis. Arthritis: **765624**.

Hatami, H., Mansouri, F., Mehrabi, Y. and *et al*. (2013). Int J Prev Med. **4(8)**:917-21.

Haukenes, G. (2002). Rubella virus. In: Clinical Virology. Haaheim, L. R.; Pattison, J. R. and Whitley, J. R. 2nd Ed. John Wiley and Sons, LTD. P: **105 -110**.

Hay – JR, W. W. ; Levin, J. M.; Sondheimer, J. M.; Deterding, R. R. (2007). Rubella. In: Current Diagnosis and Treatment in Pediatrics. Sydor, A. M.; Lebowitz, H. and Carr, P. 19th Ed.*The McGraw – Hill Companies, Inc*. P: **1132 – 1133**.

Helmick, C.G.; Felson, D.T.; Lawrence, R.C.; Gabriel, S.; Hirsch, R.; Kwoh, C.K.; Liang, M.H.; Kremers, H.M.; Mayes, M.D.; Merkel, P.A.; Pillemer, S.R.; Reveille, J.D.; Stone, J.H.and National Arthritis Data Workgroup.(2008). Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part I. Arthritis Rheum. **58(1)**:15-25.

Hobman, T.C., and Chantler, J. (2007). Rubella virus. In Fields Virology, D.M. Knipe, and P.M. Howley, eds. (Philadelphia, Lippincott Williams & Wilkins), pp. **1069-1100**.

Hobman, T. (2003). Phosphorylation of rubella virus capsid regulated its RNA binding activity and virus replication. J. *Virol*. **77**: 1764-1771.

Horowitz, G. (2014 December 10, Updated). Creatinine. Medscape Drugs & Diseases. Available online at emedicine.medscape. /article/2054342- through emedicine.medscape.com. Accessed 07/18/15.

Huang, J.L. (2012). New advances in juvenile idiopathic arthritis. *Chang Gung Med. J*., **35**: 1-14.

Ilkow, C., Goping, I., and Hobman, T. (2011). The rubella virus capsid is an anti-apoptotic protein that attenuates the pore-forming ability of Bax. PLOS Pathogens **7**.

Ilkow, C., Weckbecker, D., Cho, W., Meier, S., Beatch, M., Goping, I., Herrmann, J., and Hobman T. (2010). The rubella virus capsid protein inhibits mitochondrial import. J.Virol. **84**, 119–130.

Ilkow, C.S., Mancinelli, V., Beatch, M.D., and Hobman, T.C. (2008). Rubella virus capsid protein interacts with poly (A)-binding protein and inhibits translation. J. Virol. **82**, 4284-4294.

Ilkow, C.S., Willows, S.D., and Hobman, T.C. (2010). Rubella virus capsid protein: a small protein with big functions. Future Microbiology **5**: 571-584.

Jaffe, M.Z.Physiol.Chem.**10:391** (1886)

Jarour, N.; Hayaineb, W. A.; Balbeesi, A.; *et al*. (2007). Seroprevalence of rubella among Jordanian women of childbearing age. *Vaccine*, **25 (18)**: 3615 – 8.

Jarour, N.; Hayaineb, W. A.; Balbeesi, A.; et al. (2007). Seroprevalence of rubella among Jordanian women of childbearing age. Vaccine, **25 (18)**: 3615 – eight.

Jensen, Frank B (2009). "The dual roles of red blood cells in tissue oxygen delivery: oxygen carriers and regulators of local blood flow". *Journal of Experimental Biology*. **212 (Pt 21)**: 3387–93. Doi: 10.1242/jeb.023697. PMID 19837879

Kancherla V, Oakley GP, Brent RL. (2014) Urgent global opportunities to prevent birth defects. Semin Fetal Neonatal Med. **19(3)**:153–60.

Kaplan, M. J. (2013). Role of neutrophils in systemic autoimmune diseases. Arthritis Research & Therapy, **15(5)**, 219. Available at: doi.org/10.1186/ar4325 [Accessed 5 November 2016]

Kaushal, M. and Baxi, A. (2007). Rubella immune status of pregnant and nonpregnant women in Indian population. *Internet. J. Gynecol. Obstetr*. **6 (2)**: 1528 - 8439.

Kenneth Todar. (2013) Staphylococcus aureus and Staphylococcal Disease". Textbook of Bacteriology: Retrieved Dec **7**,

Kleiner, G.; Marcuzzi, A.; Zanin, V.; Monasta, L. and Zauli, G. (2013). Cytokine Levels in the Serum of Healthy Subjects. Mediators of Inflammation, 2013: **1-6**.

Korn T, Bettelli E, Oukka M, Kuchroo V.K (2009). IL-17 and Th17 cells. *Annu Rev Immunol* **27**: 485–517

Kurtin, P.S. (2000). Outcomes assessment: meeting the challenge of accountability in pediatric rheumatology. J Rheumatol Suppl., **58**:8-10.

Lanzieri T, Redd S, Abernathy E, Icenogle J. (2017) Surveillance Manual. Rubella: Chapter. **14:1**.

Lateef, I. I. (1996). Rubella and congenital rubella infection in Baghdad. M. Sc. Thesis. Saddam College of Medicine.

Law, L.M.J., Everitt, J.C., Beatch, M.D., Holmes, C.F.B., and Lawrence A.Kaplan, Amadeo J., Steven C.Kazmierczak. Clinical Chemistry, Theroy, Analysis, Correlation. 2003; fourth edition, volume **1&2**.

Lee YH, Bae SC, Choi SJ, Ji JD, Song G.G. (2012). The association between interleukin-6 polymorphisms and rheumatoid arthritis: A meta-analysis. Inflamm Res: **665–671**

Lee, J.Y and D.S. Bowden. (2000). Rubella virus replication and link to teratogenicity. Clin. Microbiol. Rev. **13 (4)**: 571-587.

Levinson, w. (2006). Rubella. In: Review of Medical Microbiology and Immunology. Malley, J.; Pancotti, R. and Davis, K. 9th Ed. The McGraw - Hill Companies, Inc. P: **277 – 278**.

Liebhaber, H. (1970). Measurement of rubella antibody by hemagglutination inhibition. I. *Variables affecting rubella hemagglutination. J. Immunol*. **104**: 818-825.

Lin, W.H.; Pan, C.H.; Adams, R.J.; Laube, B.L.; Griffin, D.E. (2014). Vaccine-induced measles virus-specific T cells do not prevent infection or disease but facilitate subsequent clearance of viral RNA. Bio **5**: 1047.

Liu, C. H., Fan, Y. T., Dias, A., Esper, L., Corn, R. A., Bafica, A., Machado, F. S. and Aliberti, J. (2006). Cutting edge: dendritic cells are essential for in vivo IL-12 production and development of resistance against Toxoplasma gondii infection in mice. Journal of Immumology, **177**: 31-35.

Ma CS, Deenick EK, Batten M, Tangye S.G (2012). The origins, function, and regulation of T follicular helper cells. J Exp Med **209**: 1241–1253

Maloy, K.J., and Powrie, F. (2001). Regulatory Tcells in the control of immune pathology. Nat Immunol. **2**: 22-816. 4.

Mangala Prasad, V., Willows, S., Fokine, A., Battisti, A.J., Plevka, P., Sun, S., Hobman, T., and Rossmann, M.G. (2013). Rubella virus capsid protein structure and its role in virus assembly and infection. Proc Natl Acad Sci U.S.A (*in press*).

Manhal, F.S. (2009).Cytokine Profile in Patients with Rheumatoid Arthritis. Fac Med Baghdad. **51 (4)**:433-436.

McInnes IB, Schett G The pathogenesis of rheumatoid arthritis. N Engl J Med (2011); **365**:2205-19.

Measles elimination field guide. Washington, DC: WHO Regional Office for the Americas; (2005).

Mina, M.J.; Metcalf, C.J.; de Swart, R.L.; Osterhaus, A.D.; Grenfell, B.T. (2015) Long-term measles-induced immunomodulation increases overall childhood infectious disease mortality. Science. **348**: 694–699.

Mina, M.J.; Metcalf, C.J.; de Swart, R.L.; Osterhaus, A.D.; Grenfell, B.T. (2015). Long-term measles-induced immunomodulation increases overall childhood infectious disease mortality. Science .**348**: 694–699.

Monteleone, I.; Monteleone, G.; Del Vecchio-Blanco, G.; Vavassori, P.; Cucchiara, S.; MacDonald, T.T. and Pallone, F. (2004). Regulation of the T helper cell type 1 transcription factor T-bet in coeliac disease mucosa. Gut, **53(8)**: 1090-1095.

Moss WJ, Griffin DE. Measles. Lancet. (2012); **379**:153–164.

Moss WJ, Griffin DE. Rubella. Lancet. (2015); **385**:2297–2307.

Murphy, F.A., C.M. Fauqet, D.H.L. Bishop, S.A. Ghabrial, A.W. Jarvis, G.P. Martelli, T.M. Mago and M.D. Summers. (1995). Virus taxonomy. 6th report of the International Committee on Taxonomy of Virus (ICTV). Arch. Virol. **10**: 1 – 586.

Murray, P.J. (2006). Understanding and exploiting the endogenous interleukin-10/STAT3-mediated anti-inflammatory response. *Curr Opin Pharmacol*. **6(4)**:379-386.

Neal R. Chamberlain, July. (2013) "Genitourinary Diseases" Medical Microbiology.

Nelson, D. L.; Cox, M. M. (2000). *Lehninger Principles of Biochemistry*, 3rd Ed. New York, Worth Publishers. p. **217**, ISBN 1572599316.

Ocak, S.; Zeteroglu, S.; Ozer, C.; *et al*. (2007). Seroprevalence of Toxoplasma gondii, Rubella, Cytomegalovirus among pregnant women in Southern, Turkey. *Scand. J. Infect. Dis*. **39 (3)**: 231 – 234.

Paramalingam, S.; Thumboo, J.; Vasoo, S.; Thio, S.; Tse, C. and Fong, K. (2007). Invivo pro- and Anti-inflammatory cytokines in normal and patients with rheumatoid arthritis. Annals Academy of Medicine, **36**: 96-100.

Parkman, P.D. 1999. Making vaccination policy: the experience with rubella. Clin. Infect. Dis. **28**: 140 – 146.69.

Pearlman, F.C. and Lee, R.T.Y. Clin. Chem. 20/4, 447 (1974).

Pennap G, Amauche G, Ajoge H, Gabadi S, Agwale S, Forbi J. (2009).Serologic survey of specific rubella virus IgM in the Sera of pregnant women in Makurdi, Benue state, Nigeria. African Journal of Reproductive Health. **13 (2)**: 69-73.

Persico M, Persico E, Bakker CT, et al. (2001). Hepatic uptake of organic anions affects the plasma bilirubin level in subjects with Gilbert's syndrome mutations in UGT1A1. Hematology; **33**: 627–32.

Pestkoi, S.; Krause, C.D.; Sarkar, D .and Walter, M.R. 2004. Interleukin-10 and related cytokines and receptors. *Ann Rev Immunol*. **22**:929–979.

Plans-Rubio, P. (2014). Is the current prevention strategy based on vaccination coverage and epidemiological surveillance sufficient to achieve measles and rubella elimination in Europe, Expert Rev Anti Infect there.

Plotkin, S.A and W.A. Orenstein. (1999). Vaccines. 3rd Ed. Philadelphia, PA: W.B. Saunders Co. p.**409-439**.

Pordeus V, Szyper-Kravitz M, Levy RA, Vaz NM, Shoenfeld Y. (2008). Infections and autoimmunity: a panorama. Clin Rev Allergy Immunol; **34**:283-99.

Prahalad, S.and Glass, D.N. (2008). A comprehensive review of the genetics of juvenile idiopathic arthritis. Pediatr Rheumatol J. **6**:11-27.

Qiu, Z., Ou, D., Wu, H., Hobman, T.C., and Gillam, S. (1994). Expression and characterization of virus-like particles containing rubella virus structural proteins. J. Virol. **68**, 4086-4091. 88

Qiu, Z.; Tufaro, F. and Gillam, S. (1999). The influence of N-linked glycosylation on the antigenicity and immunogenicity of rubella virus E1 glycoprotein .*Virology*, **190**: 876 – 881.

Raijmakers MT, Jansen PL, Steegers EA, Peters WH. (2000). Association of human liver bilirubin UDP-glucuronyltransferase activity with a polymorphism in the promoter region of the UGT1A1 gene. J Hepatol; **33**: 348–51. 44.

Reef SE, et al. Rubella vaccines. In: Plotkin SA, Orenstein WA, Offit PA, eds., (2013). Vaccines, 6th ed. Philadelphia, PA: Elsevier Saunders; **689–717**.

Reiche, E. M. ; Morimoto, H. K. ; Farias, G. N. ; Hisatsugu, K. R.; Geller, L.; *et al.* (2000). Prevalence of American Trypanosmiasis, Syphilis, Toxoplasmosis, Rubella, Hepatitis B, Hepatitis C and Human Immunodeficiency Virus infection, assayed through serological tests among pregnant patients from 1996 to 1998 at The Regional University Hospital Note do Parana. *Rev. Soc. Bras. Med. Trop.* **33 (6)**: 519 – 27.

Rhodes, Carl E.; Varacallo, Matthew (2019). "Physiology, Oxygen Transport". NCBI Bookshelf. PMID 30855920. Retrieved 2019-05-04. It is important to note that in the setting of carboxyhemoglobinemia, it is not a reduction in oxygen-carrying capacity that causes pathology, but an impaired delivery of bound oxygen to target tissues.

Risco, C., Carrascosa, J.L., and Frey, T.K. (2003). Structural maruation of rubella virus in the Golgi complex. Virology **312**, 261-269.

Robertson, S. E.; Featherstine, D. A.; Gacic – Dobo, M. and Hersh, B. S. (2003). Rubella and congenital rubella syndrome: global update. Rev. Panam. Salud. Publica. **14 (5)**: 306 – five.

Rubella Vaccines. WHO position paper? Wkly Epidemiol Rec (2000). **75 (20)**: p. 161-169.

Rubella vaccines: WHO position paper? Wkly Epidemiol Rec. (2011); **86**:301–316.

Sagarnaga – Durante, D.; Delgado – Monge, C.; Seanz – Flores, G.; *et al.* (2004). Frequency of antibodies against rubella virus in puerperium women. *Ginecol. Obstet. Mex.* **72**: 445 – 9.

Saini, V., Arora, S., Yadav, A. and Bhattacharjee, J. (2011). Cytokines in recurrent pregnancy loss. International Journal of Clinical Chemistry, **412**: 702-708.

Sakaguchi, S. (2005). Naturally arising Foxp3-expressing CD25+ CD4+ regulatory T cells in immunological tolerance to self and non-self. Nature Immunology, **6**: 345-352.

Sakaguchi, S. (2004). Naturally arising CD4+ regulatory T-cells for immunologic selftolerance and negative control of immune responses. Annu. Rev. Immunol. **22**:62-531. 3.

Sallam TA, Raja'a YA, Benbrake MS, Alshaibani KS, Al-Habani AA. (2003). Prevalence of Rubella Antibodies among school girls in Sana'a, Republic of Yemen. Eastern Mediterranean Health Journal. **1–4**.

Sallam, T. A.; Raja,a, Y. A. ; Benbrake, M. S. ; *et al*. (2003). Prevalence of rubella antibodies among schoolgirls in Sana'a, Republic of Yemen. *East. Medit. Health J*. **9 (1-2)**: 148 – 151.

Sallusto, F., Lenig, D., Forster, R., Lipp, M., and Lanzavecchia, A. (1999). Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature **401**, 708-712.

SAS. (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

Sasaki, Y., Darmochwal-Kolarz, D., Suzuki, D., Sakai, M., Ito, M., Shima, T., Shiozaki, A., Rolinski, J. and Saito, S. (2007). Proportion of peripheral blood and decidual CD4+CD25+ bright regulatory T cells in pre-eclampsia. *Clinical Experimental Immunology*, **149**: 139-145.

Shevach, E.M. (2002). CD4+CD25+ suppressor Tcells: more questions than answers. Nat. Rev. Immunol. **2**:389-400. 2.

Shoenfeld Y, Agmon-Levin N. (2011)"ASIA" - Autoimmune/inflammatory syndrome induced by adjuvants. J Autoimmun . **36(1)**:4-8.

Signore, C. (2001). Rubella. J. Prim. Care. Update. Ob. Gyns. **8(4)**: 133-137.

Singla, N.; Jindal, N. and Aggarwal, A. (2004). The seroepidemiology of rubella in Amritsar (Punjab). *Indian. J. Med. Microbiol.* **22 (1)**: 61 – 63.

Skendzel LP. (1996). Rubella immunity: defining the level of protective antibody. American Journal of Clinical Pathology, **106**:170–174.

Smolen JS, Aletaha D, Koeller M, Weisman MH, Emery P New therapies for treatment of rheumatoid arthritis. Lancet Strebel PM, Papania MJ, Dayan GH, et al. Measles vaccines. In: Plotkin SA, Orenstein WA, Offit PA, eds. Vaccines, 6th ed. Philadelphia, PA: Elsevier Saunders; (2013);352–387.

Suárez-Ognio, L.; Adrianzen, A.; Ortiz, A.; Martínez, C.; Whittembury, A.; *et al*. (2007). A rubella serosurvey in postpartum women in the three regions of peru. Rev. *Panam. Salud. Publica*. **22 (2)**: 1020 – 4989.

Suomalainen, M., Garoff, H., and Baron, M. (1990). The E2 signal sequence of rubella virus remains part of the capsid protein and confers membrane association in vitro. J. Virol. **64**, 5500-5509.

Takeuchi K, Kobayashi Y, Tamaki S, et al. (2004). Genetic polymorphisms of bilirubin uridine diphosphate-glucuronosyltransferase gene in Japanese patients with Crigler–Najjar syndrome or Gilbert's syndrome as well as in healthy Japanese subjects. J Gastroenterol Hepatol; **19**: 1023–8. 42.

Tanaka T, Ogata A, and Kishimoto T. (2013). Targeting of Interleukin-6 for the Treatment of Rheumatoid Arthritis: A Review and Update. Rheumatol Curr Res; **S4**:1-14

Tong S.Y, Davis J.S, Eichenberger E, Holland T.L, Fowler V.G (2015). "Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management". Clinical Microbiology Reviews. **28 (3)**: 603–61. doi:10.1128/CMR.00134-14. PMC 4451395. PMID 26016486

Turbadkar, A.; Mathur, M.; Rele, M. (2003). Seroprevalence of TORCH infection in bad obstetric history. Indian. *J. Med. Microbiol.* **21 (2)**: 108 – 110.

United States Centers for Disease Control and Prevention. Rubella. In: Atkinson W et al., eds. Epidemiology and prevention of vaccine-preventable diseases, 12th ed. Washington, DC: Public Health Foundation; (2011).

Universiteit Stellenbosch University Medicine and Health Sciences. 2013. *Division of Haematological Pathology Haematological Atlas White blood cell morphology*.

Ushida, M., S. Katow and S. Furukawa. (2003). Congenital rubella syndrome due to infection after maternal antibody conversion with vaccine. Jpn. J. Infect. Dis. **56**:68 – 69.

Veerapen, K.; Mangat, G.; Watt, I. and Dieppe, P. (1993). The expression of rheumatoid arthritis in Malaysian and British patients: A comparative study. Br J. Rheumatol., **32**:541–545.

Vijayalakshmi, P.; Anurdha,R. ; Parkash, K. ; Narendran,K. ; Ravindran, M. ; et al. (2004). Rubella serosurvey at three Aravind Eye Hospital in Tamil Nadu, India. Bulletin of the World Health Organization. **82 (4)**: 259 – 64.

Walshe, C.A.; Beers, S.A.; French, R.R.; Chan, C.H.; Johnson, P.W.; Packham, G.K.; Glennie, M.J.and Cragg, M.S. (2008). Induction of Cytosolic Calcium Flux by CD20 Is Dependent upon B cell Antigen Receptor Signaling. Journal of Biological Chemistry, **283(25)**: 16971-16984.

Walters, M.I. and Gerarde, H.W. (1970). Microchemical Journal. **15**, **231**

Wang, C., G. Domingues and T. Frey. (1994). Constriction of rubella virus genom-length cDNA clones and synthesis of infectious RNA transcripts. *J.Virol*.**68**: 3550-3557.

Wang, W.Y. Lee, S.Y. Chiueh, T.S. and Lu, J.J. (2009). Molecular And Phenotypic Characteristics of Methicillin-Resistant andancomycin-Intermediate Staphylococcus aureus Isolates from Patients with Septic Arthritis. J. Clin. Microbiol

Webester WS. (1998).Teratogen update: congenital rubella. Teratology; **58(1)**:13-23.

Webster, W.S. (1999). Teratogen update: congenital rubella. Teratology. **58**: 13-23.

Wolinsky, J. S. (1996). Rubella. In B. N. Fields, D. M. Knipe, P. M. Howley, R. M. Chanock, J. L. Melnick and B. Roizman (ed.). Virology. 3rd ed. Lippincott – Raven Publishers. Philadelphia. p. **889-929**.

WHO, Western Pacific Regional Office. (2012). Rubella and Congenital Rubella Syndrome (CRS). Manila, Philippines: World Health Organization.

WHO. (2013). Rubella and Congenital Rubella Syndrome (CRS). In: Immunization surveillance, assessment and monitoring.

Wolinsky, J. S. (1996). Rubella. In B. N. Fields, D. M. Knipe, P. M. Howley, R. M. Chanock, J. L. Melnick and B. Roizman (ed.). Virology. 3rd ed. Lippincott – Raven Publishers. Philadelphia. p. **889-929**.

Wu MY, Chen HF, Chen SU, Chao KH, Yang YS and Ho HN. (2001). Increase in the production of interleukin-10 early after implantation is related to the success of pregnancy. Am J Reprod Immunol **46**:386–392.

Yang, D., Hwang, D., Qiu, Z., and Gillam, S. (1998). Effects of mutations in the rubella virus E1 glycoprotein on E1-E2 interaction and membrane fusion activity. *J. Virol*. **72**, 8747-8755.

Young D.S. (2000). Effects of drugs on clinical laboratory tests, 5th ed. AACC Press.

Zenclussen, A. C., Gerlof, K., Zenclussn, M. L., Sollwedel, A., Bertoja, A. Z., Ritter, T., Kotsch, K., Leber, J. and Volk, H. D. (2005). Abnormal T-cell reactivity against paternal antigens in spontaneous abortion: adoptive transfer of pregnancy induced CD4+CD25+ T regulatory cells prevent fetal rejection in a murine abortion model. American Journal of Pathology, **166**: 811-822.

Zheng DP, Zhu H, Revello MG, Gerona G, Frey TK. (2003). Phylogenetic analysis of rubella virus isolated during a period of epidemic transmission in Italy, 1991–1997. J Infect Dis. **187**:1587–97.

Zheng, F.; Du, J. and Hu, Y. (2002). Astudy of rubella virus infection during pregnancy. Zhonghua Fu Chan Ke Za Zhi. **37 (7)**: 391 – four.

Zimmerman, L.A. and Reef. S.A. (2012). Rubella (German measles). In: Fauci, A.S., *et al*. Harrison's Principles of internal medicine, Infectious Diseases. 18th Ed. New York: 2012. pp: **1605-1607**.

Zufferey, J.; Jacquier, P.; Chappuis, S.; *et al.* (1995). Seroprevalence of rubella among women of childbearing age in Switzerland. *Eurp. J. Clin. Microbiol. Infect. Dis*. **14 (8)**: 691 – 696.

Zuckerman, A.J., J.E. Banatvala and J.R. Pattison. (2000). Principles and Practice of Clinical Virology. 24th ed. John Wiley and Sons Ldt. UK. p. **387-418**.

Appendix 1: Question list of women of patient study

No:

Patient name:

Age:

Marital status: Married: single:

Place of living:

Equation:

Type of infection: chronic: Acute:

History of patient (after disease):

No. of abortion:

الخلاصة

الهدف من هذه الدراسة هو ايجاد العلاقة بين معلمات الكيمو حيوية والدم مع فيروس الحصبة الالمانية وتحديد الشفرة التشخيصية للحصبة الألمانية, دراسة تأثير الإصابة بفايروس الحصبة الألمانية على وظائف الجسم (الكبد، الكلى، الجهاز المناعي) وكذلك مع فقر الدم، نسبة كريات الدم البيضاء، الروماتزم بين النساء في محافظة بغداد. شاركت في هذه الدراسة (180) أمرأه ذات نتيجة موجبة لفيروس الحصبة الألمانية باختبار مقايسة امتصاصية مناعية للإنزيم المرتبط واختيرت كذلك مجموعة السيطرة ب عدد (50) امرأة بصحة جيدة وذات نتيجة سالبة لفيروس الحصبة الألمانية باختبار مقايسة امتصاصية مناعية للإنزيم المرتبط ، جمع الدم الوريدي من افراد السيطرة والمرضى للحصول على المصل. تم تقيم وظيفة الكبد بقياس Total serum bilirubin (TSB) (تحليل أبو صفار العام)، اما وظيفة الكلى فقد تم تقيمها بقياس تراكيز اليوريا والكرياتين

تم تقسيم مجموعة الدراسة الى نساء متزوجات تمثلت بالنساء الحوامل التي شكلت (50) وبنسبة (21٪)، ونساء المتزوجات (غير الحوامل) 45 بنسبة (19 ٪). كما أظهرت الدراسة أن عدد النساء غير المتزوجات (85) بنسبة (36.7٪) وعدد العينات للنساء الغير مصابات بفايروس الحصبة الألمانية كانت 50 وبنسبة (21٪) حيث كانت نسبة الإصابة فايروس الحصبة الألمانية IgG, (2.22%) IgM (82.78%)IgG + IgM (15.00%) .

أظهرت النتائج بان هنالك زيادة معنوية ($p<0.01$) في معدلات فعاليات Rubella virus، WBC، Hb TSB، B. Urea، S.creatnine، IL6, IL10, CD4, CD8. في النساء المصابات بفايروس الحصبة الألمانية مقارنة بمجموعة السيطرة.

وصفت العديد من الدلائل الحيوية التي ترتبط مع فايروس الحصبة الألمانية ومن هذه الدلائل Interleukin-10 (IL-10) التي شكل نسبة مرتفعة 96.11% ، Interleukin-6 (IL-6) التي شكل نسبة منخفضة 65% وكانت نسبة RF للحالات الموجبة والسالبة (89.44%،10.56%) على التوالي وشكلت نسبة ارتفاع Human Cluster of differentiation 4 (CD4) (0%) ،النسبة الطبيعية(80.56%) والنسبة المنخفضة كانت(19.44%) ، كانت النسبة الطبيعية والمنخفضة والارتفاع في Human Cluster of differentiation 8 (CD8) (0% ، 1.67% و 98.3%) على التوالي ، Creatinine شكلت نسبته الطبيعية ,انخفاضه (98.33%، 1.67%) على التوالي ، وكانت النسبة الطبيعية والارتفاع

في urea (67.778%، 31.11%) على التوالي، وكانت النسبة الطبيعية ، الانخفاض والارتفاع في TSB (18.333%، 53.889%, 27.778%) على التوالي ، وكانت النسبة الطبيعية ، الانخفاض والارتفاع في White blood cells (WBC) (65%، 16.667% , 18.333%) على التوالي ، وكانت النسبة الطبيعية ، الانخفاض والارتفاع في Hemoglobin (Hb) (47.778%، 48.333% , 3.889%) على التوالي .

يستنتج من هذه الدراسة بأن فايروس الحصبة الألمانية يؤثر على وظائف الكلى والكبد وكذلك الزيادة او النقصان المعنوية في مستويات بعض المؤشرات الحيوية في مجموعة المرضى والذي من المحتمل ان يؤثر على بعض أعضاء الجسم فيسبب بظهور امراض خطيرة كالالتهاب في الكبد والرئة واضطرابات في الجهاز المناعي وفقر الدم والروماتزم.

وزارة التعليم العالي والبحث العلمي
جامعة ديالى
كلية التربية للعلوم الصرفة
قسم علوم الحياة



دراسة بعض المعلمات الحيوية المرتبطة مع أصابة فايروس الحصبة الألمانية في النساء

رسالة مقدمة إلى مجلس كلية التربية للعلوم الصرفة – جامعة ديالى وهي جزء من متطلبات نيل درجة دكتوراه فلسفة في علوم الحياة

من قبل

هـاشـم عـبد عبـاس الـعنبكـي

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