بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Seroprevalence of Varicella-Zoster Virus (Chickenpox) among Pregnant Women in Prenatal Clinics at Al-Turkey Teaching Hospital in Khartoum State

الانتشار المصلي لفيروس جدري الماء لدى النساء الحوامل اللائي يترددن على عيادات الولادة في المستشفى التركي التعليمي بولاية الخرطوم

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الآية

قال تعالي :

(اللَّهُ لاَ إِلَهَ إِلاَّ هُوَ الْحَيُّ الْقَيُّومُ لاَ تَأْخُذُهُ سِنَةٌ وَلاَ نَوْمٌ لَّهُ مَا فِي السَّمَاوَاتِ وَمَا فِي

الأَرْضِ مَن ذَا الَّذِي يَشْفَعُ عِندَهُ إِلاَّ بِإِذْنِهِ يَعْلَمُ مَا بَيْنَ أَيْدِيهِمْ وَمَا خَلْفَهُمْ وَلاَ

يُحِيطُونَ بِشَيْءٍ مِّنْ عِلْمِهِ إِلاَّ بِمَا شَاء وَسِعَ كُرْسِيُّهُ السَّمَاوَاتِ وَالأَرْضَ وَلاَ يَؤُودُهُ

حِفْظُهُمَا وَهُوَ الْعَلِيُّ الْعَظِيمُ)

صدق الله العظيم

سورة البقرة الآية (255)

DEDICATION

TO my dear parents,

Sisters, Family,

Teachers, Colleagues

And Friends

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Firstly, I thank ALMIGHTY ALLAH for giving me strength, health, patience, and knowledge to complete this work.

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ABSTRACT

Varicella zoster virus (VZV), the causative agent of chickenpox and shingles, can cause severe systemic infections of the central nervous system (CNS) and the respiratory tract in immunocompetent individuals as well as in immunocompromized patients.

This is a cross-sectional study has been conducted on 90 pregnant women, attending prenatal care clinics at Al-Turkey Teaching Hospital, Khartoum in Sudan during the period from May to June, 2016. The aim of the study was to identify and evaluate their IgG response to VZV infection, and to compare the history of chickenpox infection with the presence of Varicella IgG antibodies. Serologic testing for Varicella was performed for the 90 pregnant women with different trimesters and different ages. Enzyme Linked Immunosorbent Assay was used to detect IgG antibodies against Varicella-zoster virus, and the obtained results were analyzed statistically. A total of 90 pregnant women aged 19-45 years were examined. The study demonstrated that 44 of the 90 pregnant women (48.9%) were serologically positive for VZV IgG antibodies. The positive case in pregnant women aged 28-36 years was 21 (61.8%) followed by 20 (38.5%) in pregnant women between 19-27 years of age. However, the pregnant women between 37-45 years of age were only 3 (75%) which was the lowest percentage among the group tested. VZV IgG seropositivity was 12 (54.5%) among the pregnant women with a history of abortion, and this percentage was statistically insignificant. Most pregnant women have VZV IgG antibodies, were within the second trimester 19 (55.9%), followed by the first trimester 18 (64.3%), and the third trimester 7(25%). The seropositivity of VZV IgG antibodies among pregnant women that had a child with congenital abnormality was 1(50%). Sensitivity, specificity, positive and negative predictive values of pregnant women

with self-reported history was (80%), (63.1%), (45.5%) and (89.1%) respectively. Serologic screening of pregnant women is essential to determine their immunity to VZV (chickenpox) regardless of the age and history of the infection. As VZV vaccination is not part of routine national wide immunization, women of reproductive age were recommended as a target group for a future immunization program in Sudan.

ملخص الاطروحة

الفيروس النطاقي الحماقي / جدري الماء (فارسيلا زوستر) هو العامل المسبب لجدري الماء والقوباء المنطقية ويمكن أن يسبب التهابات موضعية شديدة للجهاز العصبي المركزي والجهاز التنفسي في الأفراد ذوي المناعة وكذلك في المرضى الذين يعانون من نقص المناعة.

أجريت هذه الدراسة المقطعية على 90 من النساء الحوامل اللائي يترددن على عيادات الولادة في المستشفى التعليمي التركي في الخرطوم خلال الفترة من مايو إلى يونيو 2016. والهدف من هذه الدراسة هو تحديد وتقييم الحالة المناعية لدى النساء الحوامل والكشف المصلي عن الأجسام المضادة لفيروس فارسيلا زوستر. تم جمع عينات دم من 90 امرأة حامل تراوحت أعمارهن بين 19-45 سنة، في فترات حمل وأعمار مختلفة. تم اختبار جميع العينات باستخدام تقنية الاليزا للكشف عن وجود الأجسام المضادة لفيروس فارسيلا زوستر، وتم اخضاع النتائج التي تم الحصول عليها لتحليل إحصائي. ونتائج التحليل المصلي للعينات للكشف عن الأجسام المضادة لفيروس فارسيلا زوستر كانت إيجابية المصل في 44 امرأة من مجموعة النساء الحوامل بنسبة (48.9٪). وكانت النتائج الإيجابية المصل لدى 21 من مجموعة النساء الحوامل أعمارهم بين 28-36 سنة بنسبة (61.8٪)، يليها نتائج إيجابية المصل لدى 20 من مجموعة النساء الحوامل أعمارهم بين 19-27 سنة بنسبة (38.5٪). بينما أدنى نتائج إيجابية للمصل كانت لدى 3 فقط من مجموعة النساء الحوامل أعمارهم بين 37-45 سنة بنسبة (75٪). ونتائج الكشف عن الأجسام المضادة لفيروس فارسيلا زوستر كانت إيجابية للمصل لدى 12 من النساء الحوامل بتاريخ تعرض لإجهاض بنسبة (54.5٪)، وهذه النسبة لم تصل إلى أهمية إحصائية. ومعظم النساء الحوامل اللائي لديهن أجسام مضادة لفيروس فارسيلا زوستر 19 منهن في الأشهر الثلاثة الأولى من الحمل بنسبة (55.9٪)، تلي تلك 18 نساء حوامل في الأشهر الثلاثة الأولى من الحمل بنسبة (64,3)، وتليها 7 نساء حوامل في الأشهر الثلاثة الأخيرة من الحمل بنسبة (25٪). أما إيجابية المصل للأجسام المضادة لفيروس فارسيلا زوستر بين النساء الحوامل اللائي ولدن اطفال بتشوهات خلقية فقد كانت النتيجة 1 امرأة حامل بنسبة (50٪). وحسب المقاييس الإحصائية للحساسية والنوعية والقيم التنبؤية الإيجابية والسلبية للنساء الحوامل اللاتي أصبن بمرض معين في تاريخ سابق كانت النتائج (80٪)، (63.1٪)، 45.5٪) و (89.1٪) على التوالي. إن إجراء الاختبارات المصلية لدم النساء الحوامل يعتبر أمرا ضرورياً لتحديد المناعة لجدري الماء بغض النظر عن العمر وتاريخ الإصابة، ونوصي بأن تكون النساء في سن الإنجاب مجموعة مستهدفة في برنامج التحصين المستقبلي في السودان.

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List of abbreviations:

Abrrevation	Complete word
A549	Adenocarcinomic human alveolar basal epithelial cells 49
ANS	Autonomic nervous system.
AP1	Activator protein1
CPE	Cytopathic effect
CMI	Cell mediated immunity
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
g	Glycoprotein
G-C	Glycine - Cytosine
HIV	Human immunodeficiency virus
HZ	Herpes zoster
Ig	Immunoglobulin
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LATs	Latency-associated transcripts
MAPK	Mitogen-activated protein kinase

MRC-5	Medical Research Council cell strain 5.
mRNA	messenger ribonucleic acid.
PCR	Polymerase chain reaction
PKR	Protein kinase R
RNA	Ribonucleic acid
RNase L	Ribonuclease L
rER	rough Endoplasmic Reticulum.
SP1	Specificity Protein 1(transcription factor).
SP2	Specificity Protein 2
SPSS	Statistical Package for the Social Science
T-A	Thyamine - Adenine
VCA	Viral Capsid Antigen
VZV	Varicella Zoster Virus.

CHAPTER ONE

INTRODUCTION

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INTRODUCTION

1.1 Introduction

Varicella-zoster virus (VZV) is an air-borne, extremely contagious a human alpha herpes virus found worldwide. It causes Varicella (chickenpox**)**, which usually is a benign illness in childhood. Adults, pregnant women and immunocompromised patients often get a severe disease with complications. After infection the virus stays latent in sensory nerve ganglia. It can be reactivated and cause zoster (shingles) if the cell mediated immunity (CMI) deteriorates (Svahn, 2008).

In tropical countries, for reasons that are not yet entirely clear, Varicella occurs mainly among young adults. The population of women who are susceptible to VZV during their pregnancy period is higher in these regions, due to late seroconversion, who in turn, pass on the virus to their fetus. This may cause a congenital Varicella syndrome in the child (Mamani *et al*., 2012).

Varicella is more severe in adults than in children and associated with a greater risk of complications and death. Varicella occurring during pregnancy is a growing clinical and public health concern. The fetuses and neonates of pregnant women who are affected by Varicella may develop congenital or neonatal disease, with high case-fatality rates. Furthermore, the primary infection with Varicella –zoster virus during early stages of pregnancy could lead to further infectious complications such as pneumonia and encephalitis (Mamani *et al*., 2012).

Differences in immunity against VZV, among pregnant women from tropical and temperate countries have been reported. Most people who live in countries with

temperate climate are infected with VZV before they reach adolescence, so chickenpox occurs only rarely during pregnancy. A study in England showed that VZV antibody prevalence was 93.1% in white pregnant British women while 86.8% in Bangladeshi women. The difference may imply an increased risk of Varicella infection during pregnancy for women who live in tropical areas (Talebi-Taher *et al.*, 2014).

A live attenuated vaccine was developed in the 1970s based on the Oka strain and has been incorporated in the routine pediatric immunization schedule in the United States of America since 1995. In a recent survey of 23 countries in the European region, the inclusion of VZV vaccine in the routine pediatric immunization programs was reported in Germany, in the Italian region of Sicily and by some Health Maintenance Organizations (HMOs) in Israel (Nardone *et al*., 2007).

In Sudan, no research has been published until now to determine the seroprevalence of anti-Varicella-zoster virus IgG in pregnant women and VZV vaccination is not part of routine national wide immunization.

1.2 Rationale

Varicella (chickenpox) is a highly contagious disease caused by VZV, which is human alpha herpesvirus. Varicella is a mild disease in most children, but in adults, pregnant women and immunocompromised patients are life threatening disease.

Primary maternal Varicella-zoster infection during pregnancy can have devastating effects on the mother (maternal pneumonitits), the fetus and the newborn (neonatal Varicella, and congenital Varicella syndrome respectively) (McIntosh and Isaacs, 1993).

No research has been published in Sudan to determine the seroprevalence of anti-Varicella-zoster virus IgG in pregnant women and VZV vaccination is not part of routine national wide immunization so this study will provide the health policy makers with primarily information about the vaccination status against Varicella (chickenpox) among pregnant women to ensure that the screening of Varicella (chickenpox) will become a part of antenatal care program and developing preventive measures.

1.3 Objectives

1.3.1 General objectives

To study the seroprevalence of anti-VZV IgG in a group of pregnant women attending prenatal care clinics located in Al-Turkey Teaching Hospital, Khartoum, Sudan.

1.3.2 Specific objectives

- To identify the VZV IgG antibodies in a group of pregnant women attending prenatal care clinics located in Al-Turkey Teaching Hospital, Khartoum, Sudan.
- To compare the history of Varicella infection (chickenpox) with the presence of Varicella IgG antibodies in mentioned group of pregnant women.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

LITERATURE REVIEW

2.1 History

Descriptions of vesicular rashes characteristic of Varicella (chickenpox) date back to the ninth century. In 1875, Steiner showed that chickenpox was an infectious agent by transmitting the disease from chickenpox vesicle fluid to previously uninfected people (steiner,1875). Zoster (shingles) has been recognized since ancient times. In 1892, Von Bokey suggested that chickenpox and shingles were related infections, an idea that was confirmed experimentally in the 1920s and 1930s, when children inoculated with fluid from zoster vesicles were shown to contact chickenpox (Von Bokey, 1909) . In 1943, Garland suggested that zoster was due to reactivation of Varicella virus that remains latent in sensory nerve ganglia (Garland,1943), and in 1954, Hope-Simpson reaffirmed this hypothesis (Hope-Simpson, 1954).

The viral agents of Varicella and zoster were first cultivated by Weller in 1952 and shown on morphological, cytopathic, and serologic criteria to be identical (Weller *et al*.,1958). In 1983, Gilden and colleagues showed that Varicella-zoster virus (VZV) DNA is latent in human sensory ganglia (Gilden *et al*., 1983) and, Hyman and colleagues showed that VZV RNA is present in human trigeminal ganglia (Hyman *et al*., 1983). In 1984, Straus and colleagues showed that viruses isolated during sequential episodes of chickenpox and zoster from the same patient had identical restriction endonuclease patterns, proving the concept of prolonged latent carriage of the virus (Straus *et al*., 1984). In 1974, Takahashi and colleagues developed a live attenuated VZV vaccine for prevention of Varicella (Takahashi *et al*., 1974), in 2005, Oxman and colleagues showed that a high potency formulation of this vaccine is effective in reducing rates of zoster and post herpetic neuralgia (Oxman *et al*., 2005).

2.2 Virus classification and genome

Varicella-zoster virus (Chickenpox), also known as human herpes virus 3 (HHV3) and is DNA virus which belongs to herpes virus family (Herpesviridae). This classification is based on morphological characteristics of the virus and its physical and chemical properties. The Herpes virus study group of the International Committee on the Taxonomy of Viruses (ICTV) divided the members of this family into three subfamilies: Alphaherpesvirinae, Betaherpesvirinae and Gammaherpesvirinae. Based on host spectrum, the length of the replicative cycle, the cytopathic effect invitro and the particularities in the establishment of latency, VZV together with herpes simplex virus type 1 (HSV1;HHV1) and type 2(HSV2;HHV2) were grouped into the subfamily of Alphaherpesvirinae. Moreover, by its genome organization, VZV was classified into the genus varicellovirus, where as HSV was classified into the class simplex virus (Rahaus *et al*., 2006).

Though symptoms of an infection with one of these herpesviruses differ strongly from each other, the morphology of the particles and the biological properties are very similar. VZV is characterized by a strongly limited spectrum of infectable host cells, which are, in fact, exclusive cells of human or simian origin.

An important characteristic of herpesviruses is the architecture of the virion. Its size varies between 120 and 300 nm and is described to have a polygonal or round shape with clearly visible central dot. Until now, it is not exactly known, how many polypeptides are involved in the assembly of the virion, but an average of 30-35 is reported. The virion is structured by for distinct components: envelope, tegument, capsid and core with the genome (fig- 1a).

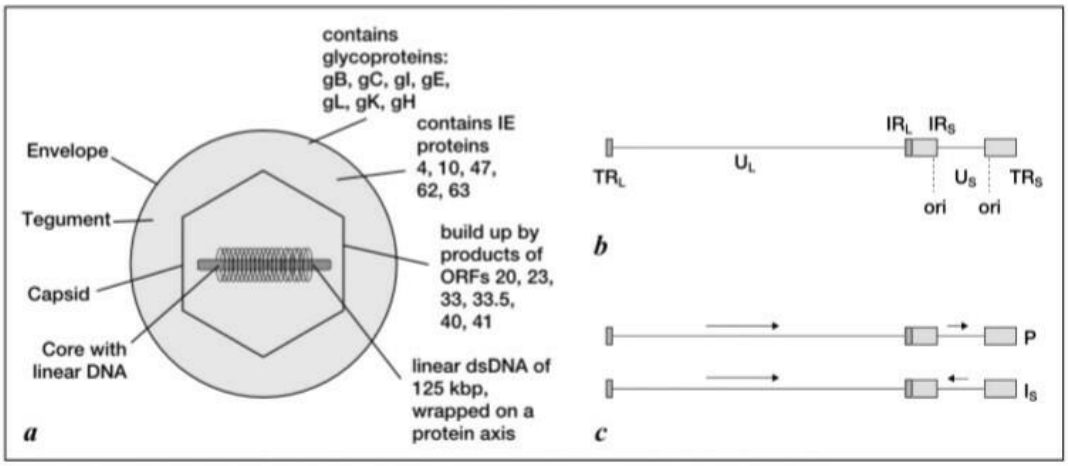


Fig. 2.1 schematic drawing of the VZV: **(a**) overview of its morphology : the important elements-envelope, tegument, capsid and core are indicated on the left-hand side, important components of each element are given on the right-hand side (**b**) General structure of the VZV genome. The genome (124,884bp) can be divided in to the unique long (U_L) and unique short(U_S) region, which are flanked by terminal repeats long and short (TR_L, TR_S) and internal repeat long and short (IR_L, IR_S). The origins of DNA replication are located in $IR_S$and TR_S. (**c**) Isomeric forms of VZV DNA: the P and I_S isomers makeup more than 95% of the packaged VZV DNA (Rahaus *et al*., 2006).

The outer covering envelope has a typical trilaminar appearance. It consists of different membrane network,Gologi apparatus, rough endoplasmic reticulum, cytoplasmic vesicles and cell surface elements. The envelope is interspersed but spikes made up by of viral glycoproteins. The VZV genome encodes glycoproteins gB, gC, gE, gH, gI, and gL as well as the putative glycoproteins gM and gN. The enveloped particles have a final diameter of 180-200 nm and a pleomorphic to spherical shape (Rahaus *et al*., 2006).

The next inside layer, located directly underneath the envelope, is the tegument. It does not have any distinct properties, but its thickness seems to be variable: virions

located in cytoplasmic vacuoles obviously have a thicker tegument than those located in the perinuclear space. The proteins encoded by the open reading frame (ORF) 4, 10, 47, 62 and 63 are found inside the tegument. The tegument surrounds the nucleocapsid.

The nucleocapsid has an icosahedric shape of 100-110 nm in diameter. It is composed of exactly 162 capsomers. Due to the morphology of this capsid structure, it is not possible to distinguish between members of the Herpesviridae. All capsomers occur in a 5:3:2 axial symmetry in which pentameric proteins form the vertices of an 80-120 nm icosahedrons. The facets are comprised by hexameric elements. The capsid is built up by the proteins encoded by ORFs 20, 23, 33, 33.5, 40 and 41 (fig. 2.1a) (Rahaus *et al*., 2006).

The VZV genome is located inside the nucleocapsid. The DNA is coiled upon a protein axis. This combination of linear DNA and proteins is called core. The genome is a linear double stranded DNA molecule of approximately 125 kbp in length and an average G-C content of 46%. This smallest genome known in the family of herpesviruses. During transition of the DNA from the capsid into the nucleus of an infected cell, it changes from a linear state into a circular one. It contains at least 69 unique ORFs and three duplicated genes (ORFs 62-71, 63-70 and 64-69). The VZV genome consists of two covalently linked segments, U_L and U_s (long, L and short, S), which are composed of unique sequences. Both of these unique segments are flanked by inverted repeat sequences: U_L by IR_L (internal repeat long)and TR_L (terminal repeat long), U_S by IR_S (internal repeat short) and TR_S (terminal repeat short)(fig 2.1b). In the genome of the VZV strain Dumas, which is completely sequenced, the U_L element has a length of 104,836bp flanked by 88 bp inverted repeats and the U_S

region, which is 5,232 bp in length, is surrounded by inverted repeats of 7,319 bp (Rahaus *et al*., 2006).

VZV DNA isolated from purified virions can be found in two predominant isomeric forms designated as P (protype) and I_s (showing an inverted U_s region) (fig.1c). Other isomeric forms can only be found at very low levels representing 2-5% of the virions DNA. DNA purified from VZV nucleocapsids is infectious as it was first demonstrated by Dumas et al. The VZV genome contains two origins of replication (ori). These elements, consisting of a 46 bp palindromic sequence which centers are composed of 16 TA dinucleotide repeats, are located within the inverted repeats flanking the U_s region (fig. 1b). Three internal elements inside these ori-sequences, designated A, B, and C, are recognized by the viral origin binding protein encoded by ORF 51(Rahaus *et al*., 2006)

2.3 The replication cycle

The replication cycle of VZV is divided into three different phases according to Rahaus *et al*., (2006): 1.Virus adsorption and entry, uncoating, transportation of the capsid to the nucleus and release of the viral DNA into it, 2. Viral gene transcription and translation as well as synthesis of viral DNA and 3. Assembly of new virions, enveloping and egress.

The replication cycle begins when the virus adsorbs to its specific receptors on the surface of the target cell. The adsorption is mediated by viral glycoproteins; the receptors have not yet been precisely identified. However, recent data indicate that the mannose 6-phosphate receptor plays a major role during attachment since at least four VZV envelope glycoproteins contain mannose 6-phosphate. After fusion of the viral envelope and the cellular membrane, capsid and tegument proteins are released into

the cytoplasm. The capsid is transported to the nuclear pores and releases its nucleic acids by an unknown mechanism. With regard to this process, it is not worthy that the cytoskeletal architecture of the host cell was found to be altered after infection. Microfilaments and microtubules were subject to reorganization, while intermediate filaments remained unaffected. These data support the thesis that cellular filament systems play an important role in the transport of virions or nucleocapsids as it is known from HSV. The following expression of viral genes runs according to a very precise cascade. Immediate-early genes (IE, ORFs 4, 61, 62, and 63) are transcribed first within a few hours of infection in the absence of de-novo protein synthesis. The IE proteins have regularity functions on the subsequent gene transcription. Next to the virus-encoded trans-activator proteins, cellular transcription factors are also involved in the regulation of VZV gene expression. Most VZV promoters contain cis-acting elements which are recognized by ubiquitously expressed cellular factors. The bi-directional promoter of the ORFs 28 and 29 is activated by cooperation of cellular upstream stimulatory factor and the major trans-activator protein encoded by ORF 62(IE62). Other cellular factors of importance are Sp1 and Ap1. Sp1 is one essential factor for the trans-regulation of the activating upstream sequence-element inside the viral glycoprotein 1 promoter as well as it is implicated in the regulation of the viral glycoprotein E expression by substituting the TATA-box binding protein to initiate transcription. The expression and activation of AP1 increased significantly after infection of cells with VZV and a knockout of this factor leads to a significant decrease of virus replication. To achieve AP1 activation, VZV takes advantage of pre –existing cellular signaling pathways such as the MAPK cascades. The ORF61 protein has been demonstrated to be involved in the regulation of this pathway. The induction of transcription of a secondary class of genes, named early-(E) genes, which

can be translated in to early proteins before the onset of viral DNA replication is dependent on the cooperation of viral IE proteins and cellular transcription factors. Almost all E genes encode proteins with enzymatic properties involved in the replication of viral DNA, like the DNA polymerase (ORF 28), the polymerase processivity factor (ORF16), the helicase (ORF55), the primase (ORF6), the heliase/primase accessory factor (ORF 52), the single-strand DNA binding factor (ORF 29) and origin binding protein (ORF 51).the VZV DNA replication process itself can be divided into different steps. At first, the linear viral DNA circularizes followed by the start of the replication process, which involves the rolling cycle-mechanism leading to the formation of head-to-tail cocatemers. Isomerization may occur by homologous recombination between the inverted repeats. Finally, the concatemers are cleaved to generate linear DNA, which is packaged into virions. After DNA replication has begun, late (L) genes are transcribed. Proteins belonging into the group of L products are the glycoproteins as well as those proteins that build up the virus particles. Due to the aim to achieve a strict and efficient expression of all classes of genes and to repress an up-come of host defense mechanisms, VZV mediates a process known as host shut-off which result in degradation of cellular mRNA. In contrast to HSV-1, the VZV mediated shut-off is not an immediate early process but a delayed one. The ORF 17 protein, which is the homologue to the HSV virion host shut-off (vhs) factor U_L41, is not the main actor to gain the shut-off. Due to trans-repressing properties, recent reports indicate a role of IE 63 in putting on the shut-off effect. The degradation of mRNA includes also transcripts of VZV IE genes what is thought to be a part the switching process from the IE to the E and L gene transcription during the replication cascade. Viral E and L transcripts are also degraded as a consequence of the shut-off. However, evidence is increasing that broad

range of cellular genes are not influenced by the shut-off. In addition to the host shut-off as a mechanism against host defense, VZV is also capable to prevent the induction of interferon-stimulated anti-viral systems such as PKR and RNase L.

After the expression of all three classes of genes has occurred, the newly replicated genomes are wrapped on the protein core, packed inside the newly synthesized capsids and transported outside the host cell. It is still not definitely clear how and in which form nucleocapsids are transported out of the nucleus and towards the egress. Different hypothesis are still proposed. A widely accepted model is that the capsids get a temporary envelope gained from inner nuclear membranes while entering the perinuclear space. These newly formed particles reach the lumen of the rough endoplasmic reticulum (rER). The envelope fuses the rER membrane. The processes resulting in this temporary enveloping at the inner nuclear membrane and the fusion with the rER membrane are not understood yet. Further hypothesis give unknown functions of some glycoproteins in these events. Following this dis-envelopment, naked particles bud into large cytoplasmic vesicles. The viral glycoproteins are released from the trans-Golgi network in additional vesicles that fuse with the cytoplasmic vesicles prior to virion formation. The assembly of fully enveloped virions with functional glycoproteins occurs in these vesicles while they forwarded to the cell surface. The viral particles are released by exocytosis. According to another scenario, cytosolic capsids are wrapped by cisternae of the trans-Golgi network which already contain the glycoproteins. The tegument is thought to bind to those glycoprotein's (Rahaus *et al*, 2006).

2.4 Transmission

VZV is transmitted primarily from person to person by airborne respiratory secretions and by direct inoculation onto mucus membranes or the conjunctivae. The incubation period is classically 14 days (range 10 to 21 days) with the initial presentation being fever with mild respiratory symptoms, followed within 1 to 2 days by a pruritic vesicular rash that exists in different stages of development and, in uncomplicated cases, lasts for 5 to 7 days. Varicella occurs most commonly in late winter and spring.

Patients are contagious for a variable period ranging up to 72 h before onset of rash. For practical purposes, particularly for exposure management, the pre-rash infectious period taken as a maximum of 48h. This period of infectivity is challenging because a large number of individuals, including patients, staff and visitors in a healthcare setting, may be exposed before diagnosis occurs, thus perpetuating the outbreak. Moreover, patients remain contagious until the vesicles have crusted over, usually taking a further five days after rash onset. The incubation period may be prolonged to 28 days if Varicella zoster immunoglobulin (VZIG) has been administered. This poses important strategic problems in relation to patient placement inwards, as well as staff deployment issues.

Transplacental transmission from mother to fetus occurs during maternal viraemia and the incubation period for neonatal infection is 11 days (range 9-15 days) from onset of maternal disease. Attack rates among susceptible contacts range from 60 to 90%; therefore efficient outbreak or exposure management is required, particularly in the hospital setting (Daley *et al.*, 2008).

2.5 Pathogenesis

Recently, the pathogenesis of Varicella (chickenpox) was thoroughly reviewed by, and a probable schema is illustrated in Figure (2.2). Based on epidemiologic evidence, VZV is assumed to spread by droplet nuclei or airborne droplets. Airborne transmission of chickenpox in hospitals has been demonstrated. The initial site of infection may be the conjunctiva, the upper respiratory tract, or both. Conjunctival and respiratory infection was demonstrated in animal model experiments using guinea pigs. The virus then replicates at a local site, probably the regional cervical lymph tissue. Large multinuclear giant cells have been found in adenoidal and tonsillar tissue 3 days before the onset of chickenpox. The duration of viral replication at the local site may be estimated from observations on direct injection into human subjects of vesicle fluid from patients with Varicella or zoster. In these studies, the incubation period in the 33 cases that developed symptoms of Varicella was usually 8 to 10 days, with a range of 7 to 12 days. As the incubation period of natural VZV infection is usually 14 to 15 days, the duration of viral replication at the local site may be estimated to be 4 to 6 days.

Primary viremia, which may occur after the release of a virus that has replicated at the local site of infection, may be explained by the observation of a neonatal chickenpox on day 1 or 2 after birth following maternal varicella in the last 2 weeks of gestation. The major sites of viral replication are not yet clear, but may be deduced from the results of autopsies of neonates who died of chickenpox. Although data on fluorescent-antibody staining were not available, pathologic changes, including formation of intranuclear inclusions were found in the liver, spleen, lungs, and other organs, but not the thyroid gland, heart, bladder, and ureter.

Secondary viremia may occur after viral replication in some or all of the above sites. The virus will quickly invade cutaneous tissue, where viral replication results in vesicle formation.

Recently, secondary viremia was detected by a sensitive culture technique for viral isolation in immunocompetent children after close exposure to wild-type VZV. Naturally infected children who developed clinical Varicella had viremia between 5 days before and 1 day after development of rash (Takahashi, 1988).

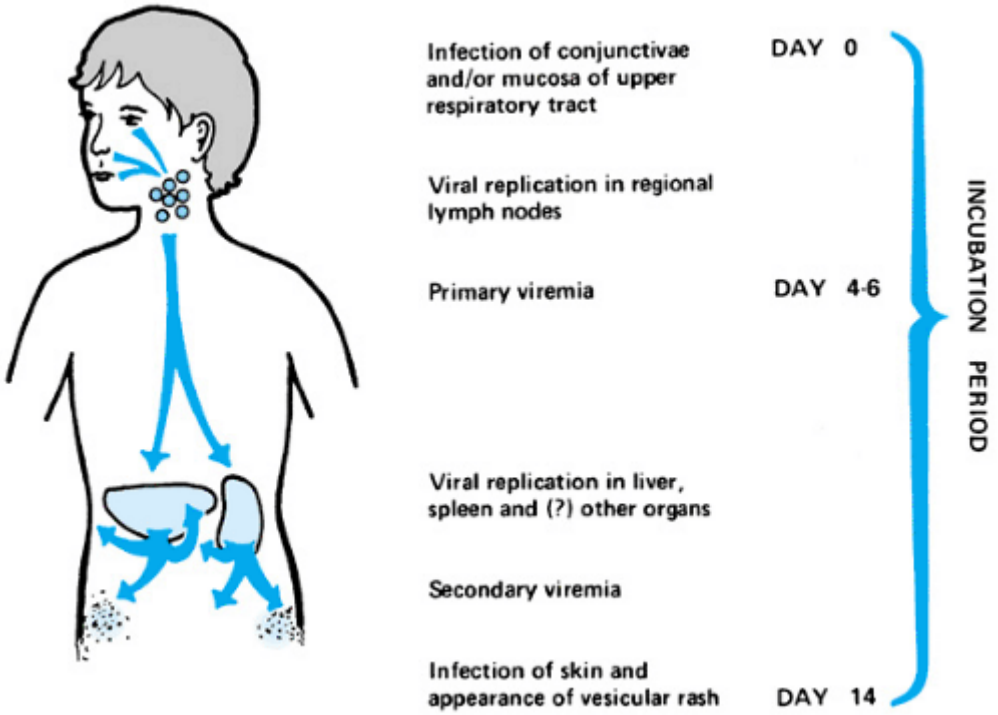


Fig.2.2 The pathogenesis of chickenpox. Primary infection with varicella-zoster virus occurs when virus-laden water droplets contact the respiratory mucosa or conjunctivae of a susceptible host. The pathogenesis most likely includes a biphasic course with primary and secondary viremia followed by the typical vesicular exanthem of chickenpox **(**Grose, 1981).

2.6 Latency and reactivation

The hallmark property of VZV replication in vivo is the establishment of a permanent latent state in several types of sensory neuronal cells, including trigeminal, dorsal root, and cranial ganglia. Although non-neuronal cells proximate to neuronal cells have been reported to harbor latent VZV, viral DNA was found only in neuronal cells of 34 of over 2,000 human ganglia and none in the satellite, nonneuronal cells. Guinea pigs and rats, in particular, the cotton rat, can be latently infected with VZV, mimicking a limited degree of latency in humans. Most of the VZV transcripts expressed during latency in human neurons also are expressed in the latently infected cotton rat. Notably, however, VZV cannot be reactivated from latency in rat cells, either in vivo or in vitro.The latent state of VZV is unique among herpesviruses in that it expresses different RNA transcripts and is present in unusual anatomic and cellular sites compared to other herpesviruses, particularly HSV. Although most of the VZV ORFs studied to date have no role in latency, establishment of latency is controlled in part by ORF63, an IE transcriptional regulator of VZV, as shown in human neural cells in vitro and in the cotton rat model. Indeed, ORF63 has been the most frequently detected and abundantly transcribed VZV gene in human ganglia. ORF63 encodes a tegument protein that has a significant function in both lytic and latent VZV infection by regulating the transcription of several other VZV proteins involved in VZV replication. Other ORFs that are transcribed during latency are ORF4, ORF21, ORF29, ORF62, and ORF66. An average of 4% of neuronal cells in the trigeminal ganglia harbors latent VZV. The key to VZV establishing and maintaining VZV latency in neuronal cells is localization of the transcribed regulatory proteins in the cytoplasm of latently infected neurons rather than their usual location in the nucleus during lytic replication. In addition, it has been suggested that host CTL

reactivity to latency proteins such as ORF63 inhibits reactivation of latent virus. The mechanisms of reactivation of latent VZV have not been elucidated but presumably involve modulation of these regulatory proteins. Reactivation of latent VZV probably occurs throughout the life of the host after primary infection but is controlled by immune mechanisms and normally remains subclinical. Indeed, VZV can be detected by PCR amplification of viral DNA in the blood of older, asymptomatic individuals as well as immunocompromised, asymptomatic patients. Reactivation of latent VZV in dorsal root ganglia occurs primarily in elderly persons, presumably due to a progressively lower T-cell immune response to the virus during older age. This results in either a subclinical outcome, or clinical syndrome, including a chronically painful zoster without rash (zoster sine herpete) or overt zoster with a classic, thoracic dermatoform vesicular rash (shingles). These clinical conditions can be much more severe and life threatening in immunocompromised hosts, such as organ and tissue transplant recipients, cancer patients, and persons infected with HIV-1(Specter *et al*., 2009).

2.7 Epidemiology

Varicella represents primary infection with VZV, where as shingles represents reactivation of latent VZV. Varicella is highly communicable with secondary attack rates in susceptible household contacts of approximately 90%. Baba et al studied three institutional outbreaks in normal infants and found a 100% attack rate in susceptible children.

It has been estimated that approximately 3.5 million cases occur annually; 95% of Varicella infections are clinically apparent. 90% of cases occur in children aged 1 to

14 years and approximately 2% of cases occur in adults 20 years of age or older. Children aged 5 to 9 account for 30 to 60% of cases.

Data on the epidemiology of Varicella in pregnant women are less clear, but the incidence of Varicella has been estimated to be 1 to 5 cases per 10,000 pregnancies. Gershon et al used fluorescent antibody to VZV membrane antigen to measure antibody status in pregnant women who had no history of Varicella and found that only 5% of childbearing women were susceptible to Varicella. Childbearing women from subtropical and tropical areas were more susceptible (~16%). This may in part due to the " geographical" spread of this virus (Riley, 1994).

2.8 Clinical manifestations

In typical Varicella there is a generalized rash, with a concentration of skin vesicles on the head, including the scalp and trunk (Fig.2.3 A). There are fewer skin lesions on the extremities; the distribution of the rash is a diagnostic clue and may be used to differentiate Varicella from smallpox. The rash evolves over a few days from maculopapular lesions to vesicles, pustules, and scabs. In contrast to the case for smallpox, in any one area of skin, vesicles, pustules, and scabs may be present at the same time. Contagion is highest during the phase when the rash is vesicular; most of the transmissible VZV comes from the skin lesions it is difficult to rule out the possibility of respiratory spread of VZV in the pre-eruptive phase; it is usually assumed that the illness is transmissible 48 h before rash onset, but the evidence for this is limited. After the pustular stage is reached, transmissibility is no longer occurring. Other symptoms associated with Varicella are malaise and fever. Varicella usually lasts 5 to 7 days in immunologically normal hosts and is represented by a spectrum of illness, ranging from mild symptoms and scant rash (which may be

overlooked or forgotten) to severe illness with over 1,000 vesicles. The occurrence of Varicella without an accompanying rash is rare. Varicella is 25 times more likely to be severe (and may even be fatal) in adults than in children. Adults with Varicella are especially at risk to develop primary Varicella pneumonia, a dreaded complication but one that usually responds to prompt antiviral therapy.

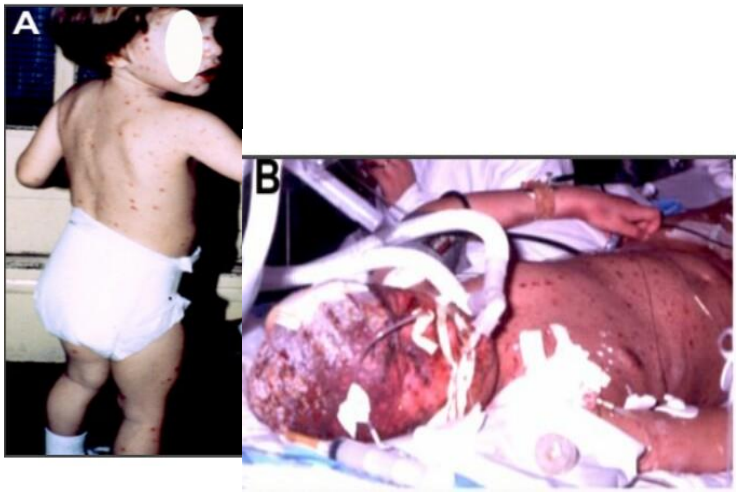


Fig. 2.3 The clinical spectra of VZV infection. (A) An otherwise healthy 2-year-old child with typical Varicella (primary infection). (B) A child with underlying malignant disease receiving chemotherapy who died from disseminated Varicella with pneumonia (Gershon and Gershon, 2013).

The cutaneous vesicles that arise during varicella are usually pruritic; scratching is common and may contribute to aerosolizing VZV with subsequent spread to others. Shedding of squames, however, is sufficiently common and profuse that even if scratching is prevented, VZV may still aerosolize from the skin and transmit disease. Scratching, however, may promote super infection with bacteria such as streptococci and staphylococci. As a result, bacterial cellulities, pneumonia, and/or sepsis, requiring hospitalization and intravenous antimicrobial therapy, are complications of Varicella two forms of central nervous system (CNS) complications may occur in

varicella. One is the usually self-limited cerebellar ataxia (1 in 4,000 cases); the other is the more serious encephalitis (1 in 10,000 cases), which can be severe or fatal Immunocompromised patients who contract varicella may experience severe or fatal illness with a prolonged course, including high fever, extensive rash that may become hemorrhagic, pneumonia, hepatitis, and encephalitis. Usually one attack of varicella provides lifelong immunity, but reinfections have been reported (Gershon and Gershon, 2013).

The clinical features of Varicella vary with form of infection. For example, primary infection in childhood (chickenpox) is typically a self –limiting illness with a rash. Adolescent, adults, and the immunocompromised are more likely to have poor outcomes, with complication including secondary bacterial infection of rash lesions, pneumonitis, hepatitis, meningoencephalitis, cerebellar ataxia, and Reye`s syndrome.Four forms can affect pregnancy or the newborn: Varicella pneumonitis, congenital varicella, neonatal varicella, and infant herpes zoster (Robert *et al*., 2015).

2.8.1 Varicella pneumonitis

Maternal infection during pregnancy is associated with an increased risk of pneumonitis**.** Clinically, respiratory symptoms develop about 4-5 days after the onset of the Varicella rash and include tachypnea, dyspnea, cough and fever. Radiologically there are bilateral, diffuse, peribronchial nodular infiltrates. Radiological and clinical findings may be disparate, with abnormal radiology in the absence of abnormal clinical findings. Varicella pneumonitis can be life threatening when it occurs in the second or third trimester of pregnancy (Robert *et al*., 2015).

2.8.2 Congenital Varicella

Congenital Varicella can affect the skin, limbs, eyes, and central and autonomic nervous systems (table2.1).

Table 2.1 Stigmata of congenital Varicella

System	Characteristic
Skin	Cicatricial lesions that may be depressed, pigmented or hypopigmented and follow a dermatomaldistrbusion
Limb	Hypoplasia of bones or muscles, absent or malformed digits.
Eyes	Cataract, chorioretinitis, horner`s syndrome, microphthalmia, nystagmus, ptosis.
CNS	Cortical atrophy, mental retardiation, microcephaly, seizures.
ANS	Esophageal dilation and reflux, hydroureter, neurogenic bladder

The highest risk (2%) was observed between weeks 13 and 20 (Robert *et al*., 2015).

2.8.3 Neonatal Varicella

Maternal infection rash developing from 5 days before delivary to 2 days after delivery has an attack rate in the newborn of approximately 20% with a mortality rate of about 30%. Overwhelming neonatal infection ensues as there is insufficient time for maternal VZV IgG to develop and cross the placenta. Clinical feature may include a typical Varicella rash, respiratory distress and pneumonia, hepatitis, and wide spread focal necrosis (Robert *et al*., 2015).

2.8.4 Infant Herpes Zoster

Infants born to mothers who suffered VZV infection between 14 and 33 can demonstrate infant zoster in the first 2 years of life. Disease is typically benign,

presenting with a vesicular rash in dermatomal distribution. Infants may be irritable and febrile and demonstrate lymphoadenopathy (Robert *et al*., 2015).

2.9 Laboratory diagnosis

The importance of laboratory determination of VZV infection is highly dependent on the nature of the clinical illness. Thus the laboratory diagnosis of VZV is not recommended for conventional, uncomplicated Varicella or zoster syndromes in otherwise healthy children and adults. This is based on recognition of the classic signs and symptoms of Varicella, the backdrop of endemic Varicella in the geographic location of known exposure to someone with Varicella, and the lack of indicators of an underlying immune deficiency. This is also prudent for the management of health care costs.

Nevertheless, there are bonafide circumstances where laboratory-based diagnosis of Varicella is appropriate. These are, first, the presence of immunosuppressive conditions in the patient, such as cancer or HIV-1 infection; second, the occurrence of Varicella in a neonate, a presumed immune person, or a person who has been vaccinated; third, zoster in young person; fourth, occurrence of significant clinical complications of VZV infection; fifth, when a typical lesions occur that, for example, could be due to HSV infection; sixth, to distinguish VZV lesions from smallpox lesions in the event of a bioterrorism threat. In these cases, a laboratory diagnosis of VZV infection can be essential to determining the clinical prognosis and making therapeutic decisions, i.e., when to start, stop, or change antiviral therapy (Specter *et al*., 2009).

2.9.1 Specimen collection

In collecting specimens for viral isolation, the labile infectivity of VZV should be taken into consideration. To minimize loss of infectivity, it is important to choose suitable media for collecting vesicular fluid or pharyngeal secretions. SPGA and PSGA media may be suitable for preserving the infectivity of VZV. When these media are used, 15 to 50% of VZV survive for 24h at 4°C. furthermore, comparison of viral recovery from sucrose phosphate (0.2 M SP) and from 70% sorbitol at -70, 4 and 20°C showed 0.2 M SP to be superior in preserving the stability of VZV. From these results, media containing 0.2 M sucrose and fetal calf serum or albumin are recommended. Virus from vesicle fluid is reasonably stable even without added diluents at -65 to70°C, but rapidly loses infectivity at -20°C.

Some preservation of infectivity was obtained by simply freezing the infected cells as monolayers with this medium. Rapid freezing of infected cells with 5 to 10% glycerol preserved infectivity for long periods. No particular caution is needed for biosafty in collecting specimen from suspected cases of Varicella-zoster (Takahashi, 1988).

2.9.2 Direct Identification of CPE:

A traditional method for determining Varicella(chickenpox) infection is to identify IN inclusions and giant cells in scrapings from the base of fresh vesicular lesion that have been stained with either Tzanck, Giemsa or hemotoxylin and eosin preparations. Such histological diagnosis is complicated, however, by the identical inclusion bodies formed in HSV- infected cells. This becomes of major significance in determining disease prognosis and proper antiviral drug treatment regimens. Staining of cell smears with colloidal gold complexed to anti-VZV IgG also has been used to identify VZV directly in cell smears by electron microscopy (Specter *et al*., 2009).

2.9.3 Culture of infectious virus:

A conventional method for diagnosing VZV infection has been identified of characteristic CPE consisting of small clusters of ovoid cells in monolayers of cultures of human fibroblast. The cell lines of choice for isolation of VZV are human diploid cell lines HF, MRC-5 and A549. This procedure, however, is fraught with problems, including the need of fresh vesicle fluid the relatively long time to positivity (usually 7 to 10 days), and the loss of the cell cultures to microbial contamination. An improvement in the culture technique that is now the standard method in most diagnostic laboratories is the inoculation of specimens into fibroblast cultures (e.g., MRC-5) that have been grown on glass cover slips in shell vials. The cover slips are fixed with acetone after 3 and 6 days of culture and stained with fluorescein isothiocyanate-conjugated monoclonal IgG antibody specific for VZV glycoprotein. Positive cells have cytoplasmic, apple-green fluorescence. This procedure is more sensitive and usually more rapid for identification of VZV than standard tube cultures (Specter *et al*., 2009).

2.9.4 Direct Identification of Viral Antigens

A method for more timely diagnosis of VZV infection is direct staining of vesicular epithelial cell smears with fluorescent dye-conjugated, monoclonal antibodies to VZV IE and glycoprotein antigens. This procedure can be done within hours of obtaining the specimen. The method is highly dependent on the quality of the specimen, as too few epithelial cells are common problems. The procedure is more sensitive than conventional tube cell culture (97.5% versus 49.4%) and is less costly, particularly for labor (Specter *et al*., 2009).

2.9.5 Molecular Diagnosis:

Molecular detection of VZV DNA by in situ hybridization and PCR is sensitive and specific for diagnosis of Varicella (chickenpox) infection in vesicle fluids and skin biopsy specimens. These molecular procedures are gradually replacing antigen detection method for diagnosis of Varicella (chickenpox) infection as they are becoming more cost effective for the routine laboratory. Rapid, differential diagnosis of zoster and HSV lesions, which is important for prognosis and treatment, can be done using PCR. Detection of Varicella (chickenpox) infection in CSF directly by PCR and hybridization, and indirectly by measure of anti-VZV antibodies, is of value in the diagnosis and treatment of Varicella and zoster encephalitis. A problem is that PCR also detects VZV DNA in the CSF of persons with subclinical virus reactivation.

Detection of VZV DNA in oropharyngeal swabs of patients with zoster sine herpete and in the aqueous and vitreous humor of patients with retinitis is used for diagnosis of these syndromes. Additionally, PCR has been used to detect VZV in amniotic fluids of mothers with incident Varicella in early pregnancy. This may be of benefit in diagnosing and possibly treating congenital Varicella.

The technical ease and specificity of detection of VZV by PCR has been improved by the advent of real-time PCR. Using primers for different targets within VZV ORFs, detection 0f VZV DNA by real-time PCR has been shown to be more sensitive and specific and technically superior to culture or immunofluorescence for dermal and CNS specimens. Addition of phocine (seal) herpes virus to the clinical samples as an internal control prior to extraction allows monitoring for accurate control of the whole process. An extension of this technology has been the detection of multiple human herpesviruses in the same clinical specimen, termed "multiplex PCR." Thus, DNA

from VZV, HSV-1 and HSV-2, and in some cases, also CMV, EBV AND HHV6, as well as an internal control, can be simultaneously detected by real-time PCR in a single sample extracted from a clinical specimen. This may be of value in the care of patient who are susceptible to multiple herpes virus infections due to immunosuppressive conditions. Finally, an automated, DNA microarray PCR method has been developed for high-through put detection of multiple herpes viruses, including VZV, in clinical samples. Blood several days before the onset of clinical symptoms of VZV infection, thus allowing for early initiation of therapy to preempt the onset of disease. Real-time PCR has been used to quantitate levels of VZV DNA in blood. This should be useful in monitoring patients at high risk for severe VZV infection, such as stem cell transplant recipients. In some cases, VZV DNA can be detected in blood several days before the onset of clinical symptoms of VZV infection, thus allowing for early initiation of therapy to preempt the onset of the disease (Specter *et al*., 2009).

2.9.6 Serum Antibody

Serologic assays for detection of anti-VZV IgG and IgM antibodies have been in use for many years. They are of limited value in diagnosis due to the need for matching acute and convalescent-phase or chronic samples, long turn-around time, indirect measure of infection, and false positives for IgM. The determination of VZV antibody is important, however, when there is a question regarding the immune status of a person, particularly in immunosuppressed patients, and for determining the immunogenicity of VZV vaccination. They are also of critical value in the epidemiologic studies of VZV, where they have been adapted to use with oral fluid specimens. The accepted standard in the field for assessing immunity to VZV in the fluorescent- antibody to-membrane antigen (FAMA) test. This assay detects

antibodies specific for viral envelope glycoproteins using human fibroblasts infected for several days with a defined infectious dose of VZV oka strain that produces sufficient surface glycoproteins. The infected cells are fixed with glutaraldehyde in microtiter wells and mixed with serial dilutions of the patient's serum. These cells are then stained with fluorescein isothiocyanate-labelled rabbit antihuman IgG and Evans blue dye. The percentage of infected cells at each dilution of serum is determined by fluorescence microscopy. Titers of ≥2 are considered positive. VZV antibody standards are available to validate the assay. Unfortunately, this assay is best done using in house procedures, as commercial FAMA kits have been unreliable. Improved, indirect immunofluorescence assays have been reported to be more sensitive and less labor-intensive to perform than FAMA. Commercial ELISAs are also available for detecting and measuring VZV antibodies (Specter *et al*., 2009).

2.10 Prevention of Varicella (chickenpox) in pregnancy

2.10.1 Vaccination

Varicella vaccine contains live attenuated virus derived from the Oka strain of VZV and has been licensed for use in the USA since March 1995. Following its introduction, the incidence of primary infection (chickenpox) in the general population has fallen by over 80% and the mortality related to the condition has decreased by two-thirds. Immunity from the vaccine persists for up to 20 years. Two Varicella vaccines are licensed for use in the UK for the prevention of chickenpox: Varivax® (Oka/Merck; Sanofi Pasteur MSD Limited, Maidenhead, Berkshire, UK) and Varilrix® (Oka–RIT; GlaxoSmithKline UK, Uxbridge, Middlesex, UK). Both are live attenuated vaccines administered in two separate doses 4–8 weeks apart.

The Varicella immune status of women planning a pregnancy or receiving treatment for infertility can be determined by obtaining a past history of chickenpox and by testing the serum for Varicella antibodies in those who have no history or an uncertain history of previous infection. In 2009, the UK National Screening Committee reviewed the evidence for antenatal screening for susceptibility to Varicella-zoster infection. The committee concluded that there was insufficient evidence to support antenatal screening because of a lack of reliable information on the true incidence of VZV infection in pregnancy and on the outcomes following treatment.

If a woman of reproductive age is vaccinated, she should be advised to avoid pregnancy for 4 weeks after completing the two-dose vaccine schedule and to avoid contact with susceptible pregnant women should a post-vaccination rash occur. Transmission of vaccine virus is rare, despite it being a live attenuated virus. Inadvertent exposures to the vaccine in pregnancy have been reported to a register. There have been no cases of FVS (Fetal Varicella Syndrome) and no increase in the risk of fetal abnormality above the background risk. Small studies have not detected the Varicella vaccine in the breast milk of women who have been vaccinated postpartum (Royal College of Obstetricians and Gynecologists, 2015)

2.10.2 Management of exposure incidents

An essential part of the prevention strategy to avoid or reduce the incidence of Varicella infection in pregnancy and the cost of managing an outbreak requires an organized approach to management of exposure incidents. Screening should be carried out pre-pregnancy if there is an opportunity to do so (family planning/infertility clinics). Screening should also be carried out in early pregnancy so that those who are uncertain can be tested, and those who are susceptible can be

counseled about the risks, instructed on procedures should contact occur, and co-opted into a protocol for management of exposure incidents. All healthcare workers who deal with pregnant women should be screened and vaccinated or identified as susceptible, to permit the redeployment to non-patients areas. An evaluation of the economic and clinical outcomes of a program of routine antenatal screening and post-partum vaccination of seronegative women found that a selective sero-testing strategy prevented nearly half of VZV cases in their cohort. This will be particularly true in those areas with high immigrant populations from tropical climates where immunity is much less likely and immune status is much less likely to be known. However, this evaluation was based upon an analytical cost-effective model following a hypothetical cohort of over 4 million women over a 20 year provided (Lamont *et al*., 2011).

2. 11 Management of Varicella infections in pregnancy

2.11.1 Antivirals used in VZV infections

Acyclovir is a synthetic nucleoside analogue of guanine which is highly specific for cells infected by VZV or HSV. When phosphorylated by viral thymidine kinase in the cells infected by VZV, there is inhibition of viral DNA polymerase which stops replication of human herpes viruses. Oral acyclovir has low bioavailability and must be given in frequent doses to achieve therapeutic levels. Further bioavailability data suggests that the physiological changes of pregnancy do not alter maternal pharmacokinetics from non-pregnant women. Valacyclovir and famciclovir are pro-drugs of acyclovir and penciclovir respectively. As pro-drugs they have a longer half-life and better oral absorption and bioavailability, so due to less frequency of administration are a better choice of oral therapy with improved compliance.

Antiviral therapy either alone or in combination with VZIG has been recommended in the management of Varicella (chickenpox) in pregnancy. Antiviral prophylaxis is best given on the 7th day post exposure. All pregnant women with established chickenpox should receive oral acyclovir 800 mg five times daily for seven days or valacyclovir 1 g three times daily. Compared with placebo, this reduces the duration of fever and symptoms of Varicella (chickenpox) in immunocompetent adults if commenced within 24 hours of rash development. If given within 24 hours and upto 72 hours of the development of rash, acyclovir is effective in the reducing the feto-maternal mortality and morbidity associated with VZV infection, particularly if used IV. Intravenous acyclovir in severe pregnancy complications such as pneumonia is preferred to oral treatment because of bioavailability, especially in the second half of pregnancy. The dose is usually 10-15mg/kg of body weight IV every 8 hours for 5-10 days for VZV pneumonia and should be started within 24-72 hours of rash. There is no evidence of fetal benefits with respect to CVS or Varicella (chickenpox) but acyclovir crosses the placenta and can be found in AF(Amniotic Fluid), umbilical cord blood and other fetal tissues, though it doesn't appear to accumulate in the fetus. Acyclovir may inhibit viral replication during maternal viremia which May inhibit transplacental transmission of VZV. Neonates showing signs of Varicella (chickenpox) or those with chickenpox showing evidence of neurological or ophthalmic complications have been reported to benefit from the use of acyclovir intravenously.

Registries of neonates exposed to acyclovir in utero have found no significant risk of teratogenesis from the use of acyclovir in pregnancy but theoretical risks exist with use in the first trimester. Though there is a potential for complications of in utero exposure, small studies of valacyclovir use in late pregnancy have found no clinical or

laboratory evidence of toxicity in infants followed up to one month or six months of age (Lamont *et al*., 2011).

2.11.2 Varicella Zoster Immune Globulin (VIZG)

As prevention is the most effective strategy for the reduction of maternal complications associated with Varicella infections, immunoglobulin prophylaxis is an important objective for susceptible, exposed pregnant women.

VZIG has been shown to lower Varicella infection rates if administered within 72 to 96 hours after exposure. The effectiveness of VZIG when given beyond the 96 hours after initial exposure has not been evaluated. Protection is estimated to extend through 3 weeks, which corresponds with the half-life of the immunoglobulin. The principal indication for the use of VZIG in pregnant women is reduction of the maternal risks of Varicella infection-related complications associated with adult disease. If the mother does not acquire Varicella infection, this eliminates risk for the neonate, but this has not been studied as an end point because of the low frequency of cases. The dose is 125 units per 10 kg given intramuscularly, with a maximum dose of 625 units. VZIG is recommended for all susceptible pregnant women. To determine if an exposed pregnant woman is susceptible, a history of Varicella infection should be taken, and, if this is positive, the woman can be assumed to be immune. If the history is negative and no Varicella antibody testing was done in early pregnancy, antibody testing with Enzyme Linked Immunosorbent Assay or Fluorescent Antibody to Membrane Antigen should, if possible, precede use of VZIG. However, in settings where pregnant women might be tested too late and/or results may not be available quickly, using VZIG before antibody testing results are available might be practical.

The value of VZIG in averting fetal Varicella is primarily in its ability to prevent maternal infection, but it may have some effect in decreasing the risk of fetal infection even in those women who go on to develop Varicella. In a study of 1373 women who had Varicella during pregnancy, 9 cases of Congenital Varicella Syndrome were identified, all occurring after maternal Varicella during the first 20 weeks of gestation. However, no cases of Congenital Varicella Syndrome were reported in any of the 97 women in whom Varicella occurred after post-exposure prophylaxis with anti VZIG.

The most frequent adverse reaction following VZIG administration is local discomfort at the injection site, with pain, redness, and swelling occurring in approximately 1% of people.32 Less frequent adverse events include gastrointestinal symptoms, malaise, headache, rash, and respiratory symptoms, which occur in approximately 0.2% of recipients. Severe events, such as angioneurotic edema and anaphylactic shock, are rare (occurring in < 0.1% of recipients). Obstetrical care providers need to be aware of the availability of testing and therapy in their local environment. As both testing and therapy are time sensitive, it is important to know the turnover time for the test in local laboratories, and how to arrange VZIG administration. As VZIG is a blood product, patient consent is required (Shrim *et al*., 2012).

2.12 Management of perinatal infections

Primary maternal infection with VZV around the time of delivery poses important problems**.** Following maternal chickenpox around term, elective delivery may be delayed by 5-7 days to facilitate passive immunity of the neonate but experience with this practice is limited. Theoretically, epidural rather than spinal anesthesia may be safer because the dura mater is not penetrated and a site which is free of cutaneous lesions should be chosen for needle placement. A neonatal ophthalmic examination

should be performed together with serological testing of the neonate for IgM at birth and IgG at seven months of age.

VZIG is recommended for neonates whose mothers develop VZV rash from five days before delivery up to two days after delivery. Neonates born before or after this time probably do not need passive immunization because they are not at risk of severe neonatal chickenpox. While VZIG may not prevent infection, it may reduce the severity of neonatal infection, but is of no benefit once signs of chickenpox become evident. monitoring of the neonate should be prolonged to 28 days because VZIG may prolong the incubation period. VZIG is also recommended for the non-immune neonate who is exposed to VZV or HZ from an index subject other than the mother in the first seven days of life.

If signs of neonatal infection develop despite VZIG, the neonate should be treated with acyclovir, and there are anecdotal reports of benefit of a combination of VZIG and acyclovir in maternal VZV exposure near term or in exposed neonates to prevent neonatal Varicella. Maternal HZ peripartum does not require any action because the neonate will have passive immunity. This does not apply to babies born before 28 weeks or those less than 1000g birth weight because they may not have developed passive immunity (Lamont *et al*., 2011)**.**

CHAPTER THREE

Materials and Methods

CHAPTER THREE

Materials and Methods

3.1 Study approach

Semi-quantitative approach.

3.2 Study design

The study was prospective cross-sectional study.

3.3 Study period

This work was carried out in the period between April to June 2016.

3.4 Study area

This study was carried out in Al-Turkey Teaching Hospital-Khartoum.

3.5 Study population

Pregnant women in different ages and trimesters attending to Al-Turkey Teaching Hospital

3.6 Inclusion criteria

Pregnant women in different ages and different trimesters were included in this study.

3.7 Exclusion criteria

Non pregnant women were excluded.

3.8 Sample size

A total of 90 subjects of pregnant women

3.9 Data collection

The data were collected by using a direct interviewing questionnaire (appendix 3)

3.10 Ethical consideration

An approval for the work was taken from Ministry of Health. Consent was taken from the pregnant women after inform them with the objective of this study.

3.11 Laboratory method

The preserved sera from pregnant women was tested for the presence of IgG antibodies against the Varicella-zoster virus using ELISA (appendix 2), see micro titer ELISA (appendix 2).

3.11.1 Specimens collection and preservation

Under sterile condition 3 ml of venous blood were collected by using venous puncture technique. The collected blood was drawn in to a plain container, allowed to clot and then centrifuged at 3000 rpm for 5 minutes. The sera were separated in to an eppendorf tubes and preserved at -20°C until used.

3.11.2 Principle

The test kit contains micro titer strips each with 8 break-off reagent wells coated with Varicella –zoster virus antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect bound anti-bodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalyzing a colour reaction.

3.11.3 Procedure

According to manufacturer guidelines (EUROIMMUN, Germany) the following steps were followed (see appendix 1).

All reagents were brought to room temperature (18°C to 25°C) approximately 30 minutes before use.

Washing buffer was prepared by adding 30ml of buffer to 270 ml of distilled water in flask.

Samples were diluted by adding 10µl to 1ml of samples diluents.

Step 1: 100µl of calibrators, controls, and diluted sera were dispended into appropriate wells incubated for 30 minutes at room temperature (18°C to 25°C), and then washed three times using 300µl diluted washing buffer.

Step 2 : Then 100µl of enzyme conjugate (peroxide-labeled anti-human IgG) were dispensed in to all wells, incubated for 30 minutes at room temperature (18°C to 25°C), and then washed three times using 300µl diluted washing buffer.

Step 3: 100µl of chromogen /substrate (TMB-$H_2O_{2)}$ solution were dispensed in to all wells incubated for 15 minutes at room temperature.

Step 4: 100µl of stock solution (0.5M sulphuric acid) was added to the wells containing chromogen /substrate solution.

The color intensity was measured at wave length of 450 nm within 30 minutes of adding stop solution. Reading of results (see appendix 2).

3.11.4 Calculation and interpretation of the result

Photometric measurement of the color intensity should be made at wavelength of 450 nm, within 30 minutes of adding the stop solution. The result evaluated by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator 3.

Interpretation of the results as follows:

Ratio <0.8 negative.

Ratio >0.8 to <1.1 borderline.

Ratio >1.1 positive.

Sensitivity refers to the percentage of persons with a history of Varicella among seropositive pregnant women.

Sensitivity = True positive/ (True positive+ false negative)*100

Specificity refers to the percentage of persons without a history of varicella among

seronegative pregnant women.

Specificity = True negative/ (True negative+ false positive)*100

PPV refers to the percentage of seropositive among pregnant women with a positive history of Varicella.

PPV = True positive (diseased)/total seropositive*100

NPV refers to the percentage of seronegative among pregnant women with a negative history of Varicella

NPV = True negative (not diseased) /total seronegative*100.

3.12 Data analysis

Data were analyzed by using SPSS software version 22.0. Significance of differences was determined using chi-square test and statistical significance was set at $p<0.05$.

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

A total of 90 pregnant women aged 19-45 years were enrolled in this study. Among these 44 individuals (48.9%) were found to be seropositive and 46 (51.1%) were seronegative as illustrated in Table (4.1) and Figure (4.1). Twenty one (61.8%) of positive cases were in pregnant women aged between 28-36 years, followed by 20 (38.5%) in pregnant women between 19-27 years of age, while, those positive between 37-45 years were 3(75%). There was no significant difference between the presence of VZV antibodies and age as illustrated in Table (4.2) and Figure (4.2), (p =0.061). Varicella IgG seropostivity was 12 (54.5%) among the pregnant women with a history of abortion as illustrated in Table (4.3) and Figure (4.3), (P = 0.541) and this percentage did not reach statistical significance. Most of pregnant women who have VZV IgG antibodies were within the second trimester 19 (55.9%), followed by the first trimester 18 (64.3%), and the third trimester 7 (25%) (P =0.008). The results indicated that there was a significant relation between the presence of VZV antibodies and the trimester as illustrated in Table (4.4) and Figure (4.4). The seropostivity of VZV IgG antibodies among pregnant women that had a child with congenital abnormality was 1(50%) (p value 0.975), so the results indicated that there was no significant association between seropositivity and child with congenital abnormality as illustrated in Table (4.5) and Figure (4.5). Distribution of VZV IgG antibodies in pregnant women, according to a history of Varicella indicated that there was a significant relation between the presence of VZV antibodies and history of chickenpox in this study as illustrated in Table (4.6) and Figure (4.6), (P = 0.000).

Sensitivity, specificity and positive and negative predictive values of pregnant women with self-reported history were 80%, 63.1%, 45.5%, and 89.1% respectively.

Table 4.1 of VZV IgG antibodies among pregnant women

Result	Frequency n	Percent %
Positive	44	48.9
Negative	46	51.1
Total	90	100

Fig. 4.1 The Seroprevlance of VZV IgG among pregnant women (n=90)

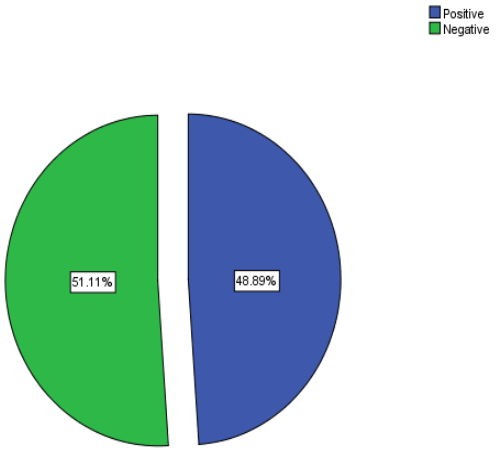


Table 4.2: The seroprevalence of VZV IgG antibodies among pregnant women according to their age:

Age group(years)	VZV IgG		Total n(%)
	Positive n(%)	Negative n(%)	
19-27	20 (38.5)	32 (61.5)	52(100)
28-36	21 (61.8)	13 (38.2)	34 (100)
37-45	3 (75)	1 (25)	4 (100)
Total	44 (48.9)	46 (51.1)	90 (100)

P=0.061

Fig. 4.2: The seroprevalence of VZV IgG among pregnant women according to their age:

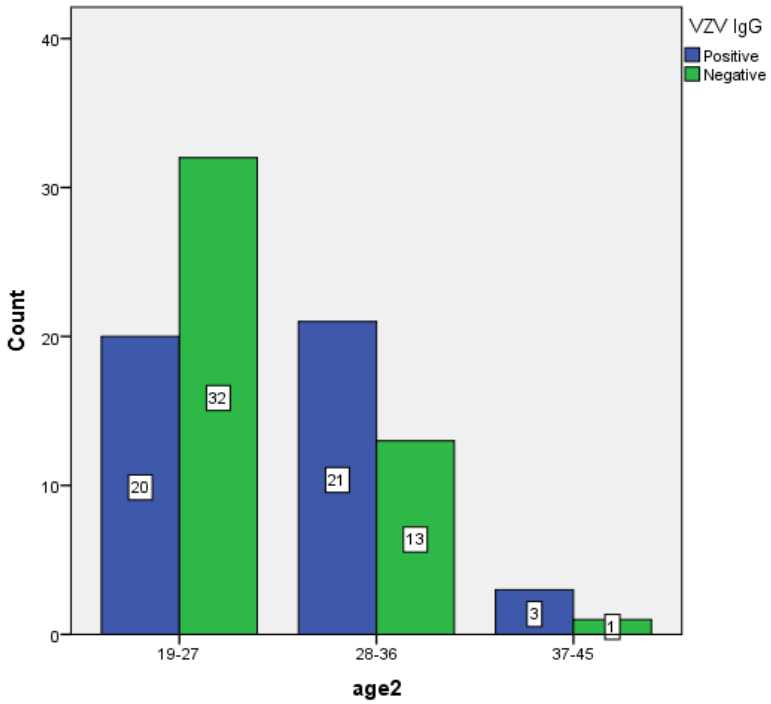
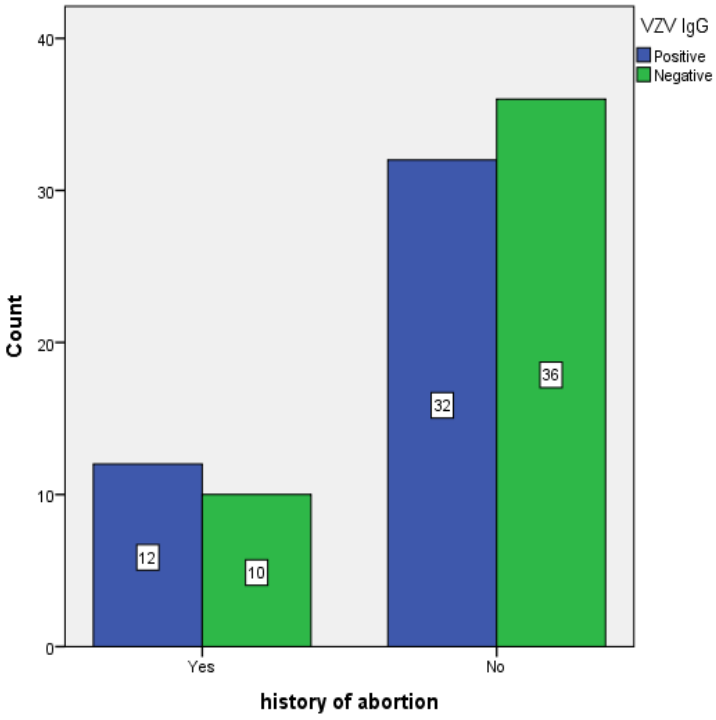


Table 4.3: The seroprevalence of antibodies to VZV in pregnant women according to a history of abortion:

History of abortion	VZV IgG		Total n(%)
	Positive n(%)	Negative n(%)	
Yes	12(54.5)	10 (45.5)	22(100)
No	32(47.1)	36(52.9)	68(100)
Total	44(48.9)	46(51.1)	90(100)

P=0.541

Fig. 4.3: The seroprevalence of antibodies to VZV in pregnant women according to history of abortion:



The seroprevalence of antibodies to VZV in pregnant women according to trimester:

Trimester	VZV IgG		Total n(%)
	Positive n(%)	Negative n(%)	
1st	18 (64.3)	10 (35.7)	28 (100)
2nd	19 (55.9)	15 (44.1)	34 (100)
3rd	7 (25)	21 (75)	28 (100)
Total	44 (48.9)	46 (51.1)	90 (100)

P=0.008

Fig. 4.4: The seroprevlance of VZV IgG among pregnant women according to their trimester:

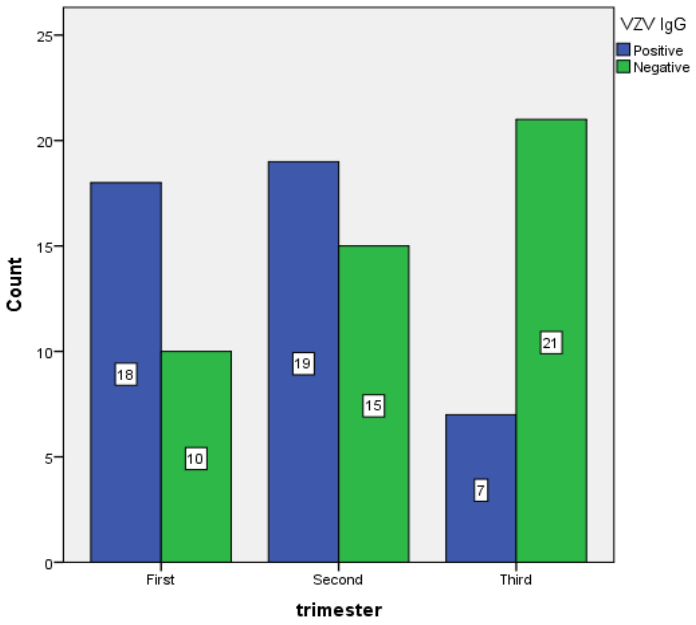


Table 4.5: The seroprevalence of VZV IgG in pregnant women that had child with congenital abnormality.

Child with congenital abnormality	VZV IgG		Total n(%)
	Positive n(%)	Negative n(%)	
Yes	1 (50)	1 (50)	2 (100)
No	43(48.9)	45(51.1)	88 (100)
Total	44(48.9)	46(51.1)	90 (100)

P=0.975

Fig. 4.5 The seroprevalence of VZV IgG in pregnant women that had child with congenital abnormality:

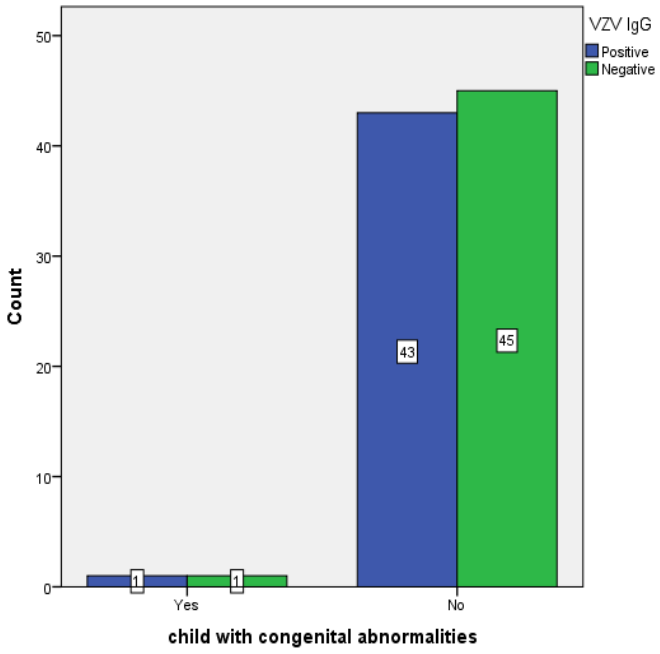
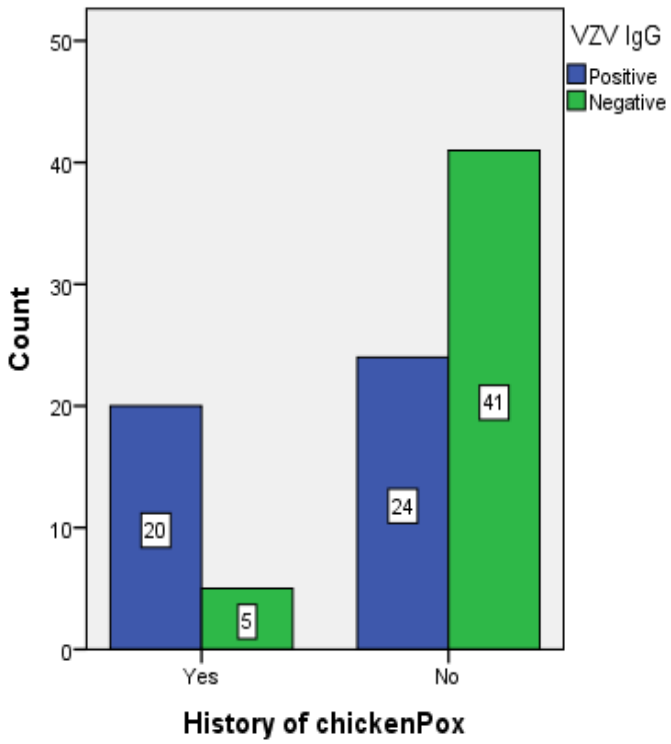


Table 4.6: The seroprevlance of VZV IgG among pregnant women according to the history of chickenpox:

History of chickenpox	VZV IgG		Total n(%)
	Positive n(%)	Negative n(%)	
Yes	20 (80)	5 (20)	25 (100)
No	24 (36.9)	41 (63.1)	65 (100)
Total	44 (48.9)	46 (51.1)	90 (100)

P=0.000

Fig. 4.6: The seroprevlance of VZV IgG among pregnant women according to the history of chickenpox:



CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

DISCUSSION

5.1 Discussion

The prevalence of Varicella (chickenpox) infection varies substantially in different geographic regions, including those within the same country. Varicella infection contracted during pregnancy may be associated with high risk for VZV transmission to the fetus or newborn and can have serious adverse effects and serious complications on both maternal and infant health such as spontaneous abortion, premature labor, Varicella pneumonitis, premature delivery and CVS (Guido *et al*., 2012).

This is the first cross-sectional descriptive study conducted to detect seroprevalence of Varicella (chickenpox) infection among pregnant women in Khartoum, Sudan. As the aim of this study was to identify anti VZV IgG of the pregnant women and to compare the history of Varicella infection (chickenpox) with the presence of Varicella antibodies, the study indicated that approximately 51.1% of them were susceptible to Varicella infection and 48.9% were immune against it. This result is lower than those obtained in other countries. Studies from European countries and other parts of developed countries showed that the seroprevalence level of VZV IgG among pregnant women varies as follows : UK, Bradford, the seroprevalence of VZV among pregnant women reported by 95% (Pembrey *et al*., 2013), in Lyon, France the seroprevalence of VZV IgG antibodies in pregnant women was 98.8% (Saadation-Elahi *et al*., 2007), 96.1% were immune in Catalonia, Spain (Plans *et al*., 2007), in

central Italy the overall seropostivity to VZV IgG antibodies was found to be 80.9% (Alfonsi *et al*., 2007), similar study from Italy, province of leece the seropostivity among pregnant women was 89.4% (Guido *et al*., 2012). The IgG seroprevalence was 84.3 % among Croatian women during their fertility period (Hannachi *et al*., 2011). In Hong Kong, it has been reported by 95.4% seroprevalence among pregnant women (Fung *et al*., 2011). Similar studies were carried out in different regions of Iran: in Babol, Northern Iran, 90.2% of pregnant women were VZV seropositive and 8.7% were susceptible to Varicella infection. Another study from Hamadan, west of Iran showed that 78.4% of pregnant women were immune to Varicella. Another study from Jahrom, Southern Iran revealed 72.7% seroprevalence among young women prior to their marriage (Bayani *et al*., 2013).

As this study was conducted in a limited area on a small sample size of pregnant women, seroprevalence of VZV IgG antibodies among young, mid-age and among elder pregnant women seem to be had no clear association between age and the prevalence of the antibodies that affect pregnant women irrespective of their age. As well age had no significant association among pregnant with a history of abortion and with those who had a child with congenital abnormality. But the results showed high prevalence of seropositivity among pregnant women in the first trimester and the second trimester, but very low in the third trimester, so there was a significant relation between the seroprevalence of VZV antibodies among pregnant women and the history of chickenpox. A positive history of a chickenpox showed a sensitivity of 80% and specificity of 63.1% and that compared to other studies in other countries was very high percentage and that may be also due the small size of the sample and the limited in which the study conducted.

This study showed PPV of 45.5%, which is a low percentage in comparison to other studies in the populations of Iran, Singapore, and Brazil the PPV of a clinical history of VZV ranged from 85% to 98% (Talebi-Taher *et al*., 2010; Almuneef *et al*., 2004; Dashraath *et al*., 2007; Lafer *et al*., 2005). Thus, the absence of the chickenpox infection in the patient's past medical history is an unreliable predictor of susceptibility to infection in pregnant women.

In comparison with other studies our results showed that self-reported positive history of Varicella continues to be low predictors of Varicella immunity, whereas a negative history was high predicted with the negative predictive value of 89.1%.

5.2 Conclusion

In this study the seroprevalence percentage of VZV IgG among pregnant women attending prenatal clinics at Al-Turkey Teaching Hospital was 44(48.9%). There was a significant relation between the presence of VZV antibodies and the history of chickenpox and the trimesters, and there was no significant relation between the presence of VZV antibodies and age, the history of abortion and child with congenital abnormality.

5.3 Recommendations

1. More studies with larger sample size are needed to identify and evaluate the immune status of all pregnant women in other regions in Sudan.

2. Serologic screening of pregnant women is essential to determine their immunity to VZV (chickenpox) regardless of the age and history of the infection.

3. Vaccination program should be introduced, national wide in Sudan, to all women of reproductive age to prevent and to reduce risk factors of Varicella zoster virus among pregnant women.

4. Pregnant women who are not immune to Varicella should be educated to avoid exposure to persons who have Varicella zoster, as Varicella vaccine is contraindicated during pregnancy.

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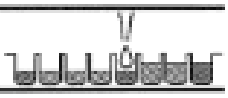
Appendices

Appendix 1

EUROIMMUN Medizinische Labordiagnostika AG

[EV_0310_F_DEUK_A04] 1335100D-0092_EV_0310_F_DEUK_A04.doc

ELISA-Inkubation
ELISA Incubation

		Antigen-beschichtete Reagenzgefäße *antigen-coated wells*	
	Pipettieren: 100 µl je Reagenzgefäß ***Pipette:*** *100 µl per well*	Kalibratoren, Kontrollen, verdünnte Proben *calibrators, controls, samples*	
1.	**Inkubieren:** 30 min bei Raumtemperatur (18°C bis 25°C) ***Incubate:*** *30 min at room temperature (18°C to 25°C)*		
	Waschen: 300 µl (man.)/450 µl (aut.) je Reagenzgefäß Einwirkzeit: 30-60 s je Waschzyklus ***Wash:*** *300 µl (man.)/450 µl (aut.) per well* *residence time: 30-60 s per washing cycle*	3x	
	Pipettieren: 100 µl je Reagenzgefäß **Pipette:** 100 µl per well	Enzymkonjugat *enzyme conjugate*	
2.	**Inkubieren:** 30 min bei Raumtemperatur (18°C bis 25°C) ***Incubate:*** *30 min at room temperature (18°C to 25°C)*		
	Waschen: 300 µl (man.)/450 µl (aut.) je Reagenzgefäß Einwirkzeit: 30-60 s je Waschzyklus ***Wash:*** *300 µl (man.)/450 µl (aut.) per well* *residence time: 30-60 s per washing cycle*	3x	
	Pipettieren: 100 µl je Reagenzgefäß **Pipette:** *100 µl per well*	Chromogen/Substrat *chromogen/substrate*	
3.	**Inkubieren:** 15 min bei Raumtemperatur (18°C- 25°C) ***Incubate:*** *15 min at room temperature (18°C- 25°C)*		
	Pipettieren: 100 µl je Reagenzgefäß **Pipette:** *100 µl per well*	Stopplösung *stop solution*	
	Auswerten: Photometrische Messung (450 nm) ***Evaluate:*** *photometric measurement (450 nm)*		

EUROIMMUN · D-23560 Lübeck · Seekamp 31 · Tel.: 0451-5855-0 · Fax: 0451-5855591 · euroimmun@euroimmun.de · www.euroimmun.de

EUROIMMUN Medizinische Labordiagnostika AG

Qualitätskontrollzertifikat
Quality Control Certificate

Produkt: Anti-VZV ELISA (IgG) — **Best.-Nr.:** EI 2650-9601 G
Product: — ***Order No:***

Ch.-B.: E160413AW — **Verw. bis:** 12-Apr-2017
Lot: — ***Exp. Date:***

		Referenzwert *Reference value*		Valider Bereich *Valid range*	
Kalibrator 1 *Calibrator 1*	5000 IU/l	1,883	O.D.	> 0,700	O.D.
Kalibrator 2 *Calibrator 2*	500 IU/l	0,979	O.D.		
Kalibrator 3 *Calibrator 3*	100 IU/l	0,287	O.D.	> 0,140	O.D.
Kalibrator 4 *Calibrator 4*	10 IU/l	0,054	O.D.		
Pos. Kontrolle 1 *Pos. Control 1*	quantitativ *quantitative*	320	IU/l	152 - 479	IU/l
Pos. Kontrolle 1 *Pos. Control 1*	semiquantitativ *semiquantitative*	2,4	Ratio	1,3 - 3,6	Ratio
Neg. Kontrolle *Neg. Control*	quantitativ *quantitative*	10	IU/l	0 - 20	IU/l
Neg. Kontrolle *Neg. Control*	semiquantitativ *semiquantitative*	0,2	Ratio	0 - 0,7	Ratio

O.D. Kalibrator 1 > O.D. Kalibrator 2 > O.D. Kalibrator 3 > O.D. Kalibrator 4
O.D. *Calibrator 1* > O.D. *Calibrator 2* > O.D. *Calibrator 3* > O.D. *Calibrator 4*

Die Charge wurde von der Qualitätskontrolle getestet und erfüllt alle Spezifikationen.
The lot has been tested by the quality control laboratory and meets the specifications.

Anti-VZV ELISA (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2650-9601 G	Varicella zoster virus (VZV)	IgG	Ag-coated microplate wells	96 x 01 (96)

Indication: The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human antibodies of the IgG class against varicella zoster virus (VZV) in serum or plasma for the diagnosis of Varicella zoster virus infections.

Application: The determination of specific antibodies is the method of choice for confirmation of suspected infections (Varicella) or reactivations (Zoster) with corresponding clinical symptoms. The determination of IgG and IgM also indicates an acute infection; in the case of reactivations, specific IgA antibodies have an important diagnostic value. In both cases however, antibodies of both class IgA and IgM may be present, so that the determination of avidity for antibodies of class IgG can be of great importance. The determination of the immune status in early pregnancy and the investigation of successful immunisation is possible by means of VZV-IgG antibodies.

Principles of the test: The test kit contains microliter strips each with 8 break-off reagent wells coated with varicella zoster virus antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound anti-bodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. **Microplate wells coated with antigens:** 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	—	12 x 8	STRIPS
2. **Calibrator 1** 5000 IU/l (IgG, human), ready for use	red coloured in decreasing intensity	1 x 2.0 ml	CAL 1
3. **Calibrator 2** 500 IU/l (IgG, human), ready for use		1 x 2.0 ml	CAL 2
4. **Calibrator 3** 100 IU/l (IgG, human), ready for use		1 x 2.0 ml	CAL 3
5. **Calibrator 4** 10 IU/l (IgG, human), ready for use		1 x 2.0 ml	CAL 4
6. **Positive control** (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
7. **Negative control** (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
8. **Enzyme conjugate** peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
9. **Sample buffer**, ready for use	light blue	1 x 100 ml	SAMPLE BUFFER
10. **Wash buffer**, 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
11. **Chromogen/substrate solution** TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
12. **Stop solution** 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
13. **Test instruction**	—	1 booklet	
14. **Quality control certificate**	—	1 protocol	

LOT Lot description — CE — Storage temperature
IVD In vitro diagnostic medical device — Unopened usable until

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Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.
- **Calibrators and controls:** Ready for use. The reagents must be mixed thoroughly before use.
- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.
- **Sample buffer:** Ready for use.
- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to 37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.
- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.
- **Stop solution:** Ready for use.

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: The controls and calibrators used have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2 using enzyme immunoassays and indirect immunofluorescence methods. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted **1:101** in sample buffer.
Example: Add 10 µl of sample to 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: Calibrators and controls are prediluted and ready for use, do not dilute them.

Incubation

For **semiquantative analysis** incubate **calibrator 3** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1 to 4** along with the positive and negative controls and patient samples.

(Partly) manual test performance

Sample incubation: (1st step)
Transfer 100 µl of the calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol.
Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Wash reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Modus").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction values.
Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells.
Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells.
Incubate for **15 minutes** at room temperature (+18°C to +25°C), protect from direct sunlight.

Stopping the reaction:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution.** Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on enquiry.
Automated test performance using other fully automated, open system analysis devices is possible. However, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	C 1	P 6	P 14	P 22			C 1	P 3	P 11	P 19		
B	pos.	P 7	P 15	P 23			C 2	P 4	P 12	P 20		
C	neg.	P 8	P 16	P 24			C 3	P 5	P 13	P 21		
D	P 1	P 9	P 17				C 4	P 6	P 14	P 22		
E	P 2	P 10	P 18				pos.	P 7	P 15	P 23		
F	P 3	P 11	P 19				neg.	P 8	P 16	P 24		
G	P 4	P 12	P 20				P 1	P 9	P 17			
H	P 5	P 13	P 21				P 2	P 10	P 18			

The pipetting protocol for microtiter strips 1-4 is an example for the **semiquantitative analysis** of 24 patient samples (P 1 to P 24).
The pipetting protocol for microtiter strips 7-10 is an example for the **quantitative analysis** of 24 patient samples (P 1 to P 24).

The calibrators (C 1 to C 4), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.
The wells can be broken off individually from the strips. Therefore, the number of tests performed can be matched to the number of samples, minimising reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

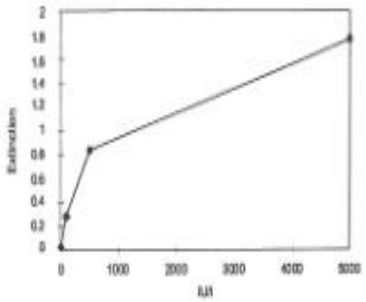
Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator 3. Calculate the ratio according to the following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 3}} = \text{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8:	**negative**
Ratio ≥0.8 to <1.1:	**borderline**
Ratio ≥1.1:	**positive**

Quantitative: The standard curve from which the concentration of antibodies in the patient samples can be taken is obtained by point-to-point plotting of the extinction values measured for the 4 calibrators against the corresponding units (linear/linear). Use "point-to-point" plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.



If the extinction for a patient sample lies above the value of calibrator 1 (5000 IU/l), the result should be reported as ">5000 IU/l". It is recommended that the sample be retested at a dilution of e.g. 1:400. The result in IU/l read from the calibration curve for this sample must then be multiplied by a factor of 4.

The upper limit of the reference range of non-infected persons (**cut-off value**) recommended by EUROIMMUN is **100 International Units (IU/l)**. EUROIMMUN recommends interpreting results as follows:

<80 IU/l:	**negative**
≥80 to <110 IU/l:	**borderline**
≥110 IU/l:	**positive**

Evaluation information: For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another EUROIMMUN recommends to retest the sample.

For the interpretation of borderline results an investigation using further tests (e.g. avidity determination of antibody class IgG) can be helpful.

A negative serological result does not exclude an infection. Particularly in the early phase of an infection, antibodies may not yet be present or are only present in such small quantities that they are not detectable. In case of a borderline result, a secure evaluation is not possible. If there is a clinical suspicion and a negative test result, we recommend clarification by means of other diagnostic methods and/or the serological investigation of a follow-up sample. A positive result indicates that there has been contact with the pathogen. In the determination of pathogen-specific IgM antibodies, polyclonal stimulation of the immune system or antibody persistence may affect the diagnostic relevance of positive findings. Significant titer increases (exceeding factor 2) and/or seroconversion in a follow-up sample taken after 7-10 days can indicate an acute infection. To investigate titer changes, sample and follow-up sample should be incubated in adjacent wells of the ELISA microplate within the same test run. For diagnosis, the clinical picture of the patient always needs to be taken into account along the serological findings.

Test characteristics

Calibration: The calibration is performed in international units (IU) using the international reference serum W1044 (Anti-Varicella Zoster Serum, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands). The W1044 serum contains 50 IU/ampoule by definition and was resuspended in a concentration of 50 IU/ml.

For every group of tests performed, the extinction values of the calibrators and the international units and/or ratios determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature (+18°C to +25°C) during the incubation steps, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The antigen used are highly purified VZV proteins (strain "Ellen") from infected, human fibroblasts (NHDF).

Linearity: The linearity of the Anti-VZV ELISA (IgG) was determined by assaying 4 serial dilutions of different patient samples. The coefficient of determination R^2 for all sera was > 0.95. The Anti-VZV ELISA (IgG) is linear at least in the tested concentration range (12 IU/l - 5000 IU/l).

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-VZV-ELISA (IgG) is 3 IU/l.

Cross reactivity: The quality of the antigen used ensures a high specificity of the ELISA. Sera from patients with infections caused by various agents were investigated with the EUROIMMUN Anti-VZV ELISA (IgG).

Antibodies against	n	Anti-VZV ELISA (IgG)
Adenovirus	12	0%
Bordetella pertussis PT	7	0%
Bordetella FHA	12	0%
Chlamydia pneumoniae	9	0%
CMV	12	0%
EBV-CA	12	0%
Helicobacter pylori	7	0%
HSV-1	12	0%
Influenza virus A	12	0%
Influenza virus B	12	0%
Measles virus	12	0%
Mumps virus (AT)	12	0%
Mycoplasma pneumoniae	12	0%
Parainfluenza virus Pool	12	0%
Parvovirus B19	9	0%
RSV	12	0%
Rubella virus	12	0%

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-assay variation, n = 20		
Serum	Mean value (IU/l)	CV (%)
1	269	2.3
2	335	2.5
3	2442	6.1

Inter-assay variation, n = 4 x 6		
Serum	Mean value (IU/l)	CV (%)
1	347	5.4
2	383	4.4
3	2983	4.9

Specificity and sensitivity: A panel of 333 clinically characterised patient samples (interlaboratory test samples from INSTAND, Labquality Finland, UK NEQUAS, MQ Schweiz) was examined with the EUROIMMUN ELISA. The test shows a sensitivity of 100% and a specificity of 100%.

n = 333		INSTAND, Labquality, NEQAS, MQ Schweiz		
		positive	borderline	negative
EUROIMMUN Anti-VZV ELISA (IgG)	positive	291	1	0
	borderline	4	1	1
	negative	0	0	35

Correlation study: 170 patient samples were investigated with the EUROIMMUN Anti-VZV ELISA (IgG) and the Wampole Anti-VZV ELISA (IgG). The EUROIMMUN ELISA showed a sensitivity of 100% at a specificity of 95% with respect to the Wampole ELISA.

n = 170		Wampole ELISA IgG		
		positive	borderline	negative
EUROIMMUN Anti-VZV ELISA (IgG)	positive	127	1	2
	borderline	0	2	0
	negative	0	0	38

Reference range: The levels of the anti-VZV antibodies (IgG) were analysed with this EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off of 100 IU/l, 97.4% of the blood donors were anti-VZV positive (IgG), which reflects the known percentage of infections in adults.

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Clinical significance

Varicella zoster virus (VZV), synonyme: human herpes virus 3 (HHV3), is the causative agent of chickenpox (varicella) after which it establishes latency and can subsequently reactivate to cause Herpes zoster (shingles) [1, 2]. The virus is strictly human [3]. Chickenpox – a very contagious disease – has traditionally been regarded as a benign, inevitable disease among children (25% in 1-4-year-olds, 43% in 5-8-year-olds, 27% in 9-18-year-olds) with typical blister-like rash of the entire skin [1, 4, 5]. Now we know varicella can also be a serious infection, even in childhood, but especially in young and older adults and during pregnancy [6, 7, 8, 9].

Zoster is the endogenous recurrence of an earlier varicella infection or the result of a reinfection with existing residual immunity [2]. The average incidence of herpes zoster in Europe is 3 per 1000 people per year in the total population and more than 10 per 1000 people per year in those aged >80 years [10]. The entire virus genome is present in the latently infected ganglia. VZV is latent in multiple ganglia along the entire human neuraxis [1].

In zoster, the rash affects the spreading area of one or several sensitive nerve roots, especially T3-L3 and N. trigeminus [2, 3]. Central nervous system (CNS) com-plications can follow both primary infection and reactivation of VZV [8, 11]. The more serious mani-festations arise when VZV invades the spinal cord or cerebral arteries after reactivation of the virus, causing diseases such as myelitis, focal vasculopathies, and encephalitis [7, 11].

Varicella also causes serious infections during pregnancy with severe consequences of maternal varicella for the infant [6, 12, 13]. At birth, maternal infection with the VZV poses a truly life-threatening risk to the newborn. The neonatal mortality rate is up to 20-30%, if the maternal VZV-infection occurs between day 4 ante partum and day 2 post partum [14]. Patients with congenital varicella syndrome (CVS) typically show clinical symptoms such as skin lesions, neurological defects, eye diseases, and/or limb hypoplasia. In rare cases, isolated manifestations in the brain or eye have been reported [15].

Antibodies against varicella zoster viruses (IgA, IgG, IgM) can be found in the serum of almost all subjects during and after a varicella infection. They can be verified by ELISA and IIFT. IgG- and IgM-antibodies against VZV are markers to confirm suspected VZV-infections, especially VZV-IgG-antibody titers during pregnancy [14, 16, 17, 18]. IgA-titers are typical for a reinfection (zoster) [19].

In addition to classic serodiagnosis of VZV, especially IgG and IgM antibodies suggestive of acute infection, measurement of VZV-IgG-avidity provides information making it possible to distinguish exactly between acute and chronic infection, as determination of avidity in other virus infections demonstrates [20]. Therefore it is of particular interest in pregnant women. Avidity describes the binding strength of specific antibody to antigen. It was found to be low in the first phase after primary infection but then to increase over time. Based on this additional information, repeated testing and unnecessary anxiety in patients can be avoided, which also is the case in the serological diagnostic of other virus infections [19, 20, 22, 23].

VZV myelitis or VZV encephalitis are diagnosed by the determination of antibodies against VZV in CSF and serum using ELISA [11]. CNS involvement results in the intrathecal synthesis of antibodies against VZV in cerebrospinal fluid (CSF). Due to the fact that specific antibodies can pass from the serum through the blood-brain barrier into the CSF by diffusion, a relative CSF/serum quotient (CSQrel., synonym: antibody specificity index) [20] is determined. The quotient is calculated from the amount of specific IgG antibodies in total CSF IgG in proportion to the amount of specific IgG antibodies in total serum IgG. During conversion the CSF/serum quotient of the pathogen-specific IgG-antibody concentrations $CSQ_{path.-spec.}$ (IgG) is put into relation to the CSF/serum quotient of the total IgG concentrations CSQ_{total} (IgG) [20]. A relative CSQ result above 1.5 indicates the production of specific antibodies in the CNS and the involvement of the CNS in the disease [24, 25, 26].

Generally, a life-long immunity develops, and this is also the case after a successful protective vaccination [17, 27, 28]. A passive immunisation with specific immunoglobulins is often given to immunocompromised seronegative people, such as tumour patients and recipients of transplants, as well as seronegative pregnant women after exposure to the virus [17, 29].

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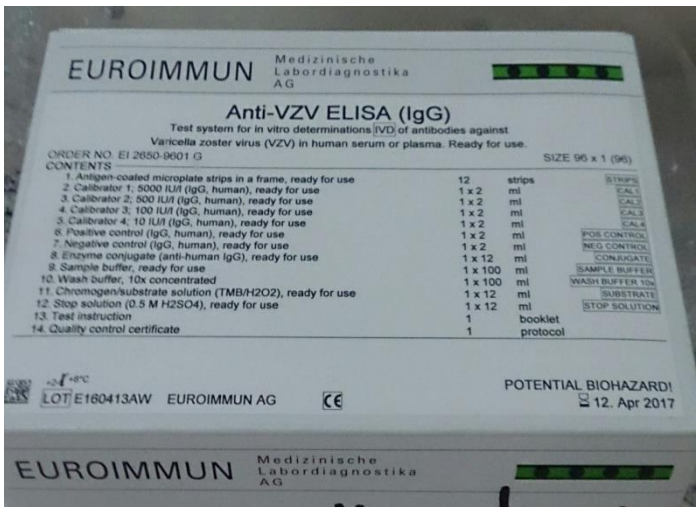
Appendix 2

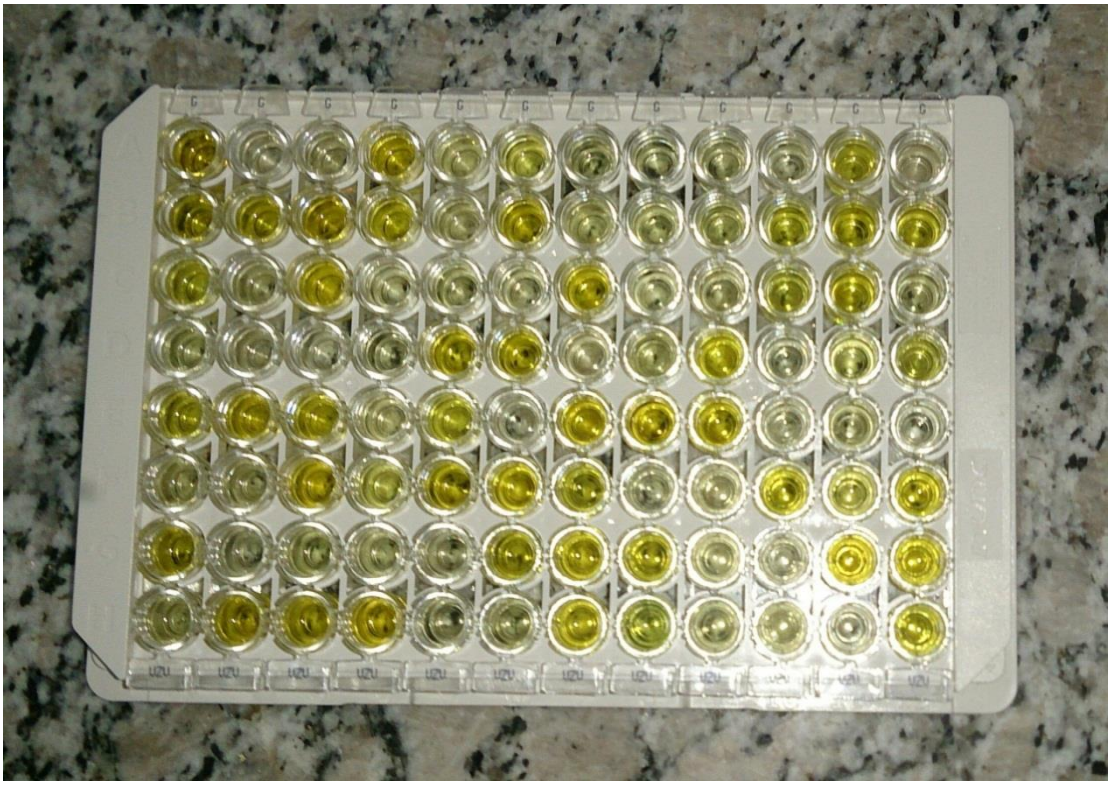
Anti-VZV ELISA IgG kit



TECAN ELISA washer

TECAN ELISA reader

Microtitre plate



Appendix 3

Questionnaire

Sudan University of Science and Technology

Collage of graduate studies

Microbiology Department

By: Yosra Abdelrahman

Supervisor: Yousif Fadllala

Title: Seroprevalence of Varicella-zoster virus among pregnant women in Al-Turkey Teaching Hospital, Khartoum state, Sudan.

Date**: …….**

Name:……..

Age: ……..

Residence: …….. Marital status: ……..

Job:

History of chickenpox: Yes ☐ NO ☐

Abortion: Yes ☐ NO ☐

Gestational stage:

First trimester ☐

Second trimester ☐

Third trimester ☐

Child with congenital abnormalities: Yes ☐ NO ☐

إقرار

أقر أنا _____

بالموافقة علي المشاركة في هذا البحث مع العلم بالمنافع والمضار المترتبة علي المشاركة واعلم بأنني لست ملزمة للمشاركة في هذا البحث وأن عدم موافقتي للمشاركة لا يؤثر علي الخدمة الطبية المقدمة لي .

الاسم:

_____

التوقيع:

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