



بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ



Shendi University

**Faculty of Graduate Studies and Scientific
Research**

**Frequency of Viral Infection in Congenital Neonates in
Khartoum State**

*A Thesis Submitted in Fulfillment for the Requirements of M.Sc. Degree
in Medical Microbiology*

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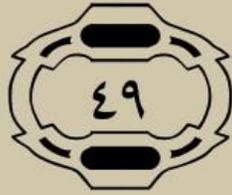
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الذُّكُورَ



صدق الله العظيم

الشوري 49

Dedication

*I dedicate this research to
my mother,*

*Who she always making
Du,aa for me*

To my late father.

To my wife and to my son.

Acknowledgement

I'm very grateful to my supervisor Dr. Leila Mohammed Ahmed who spends her time to help me in finishing this research, also I thanks my uncle's Dr. Motaz Ibrahim Hassan.

Also thanks for all laboratory team of Shendi university whom I spend with them happy time.

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List of Abbreviations

Abbreviations	Explanation
Abs	Antibodies
AIDS	Acquired immunodeficiency syndrome
AoHV-1	Aotine herpesvirus 1
AoHV-3	Aotine herpesvirus 3
AP	Alkaline phosphatase
BCIP	Build in Canada innovation program
BSA	Bovine serum albumin
CAKUT	Congenital anomalies of the kidney and urinary tract
CCMV	Chimpanzee CMV
CDC	Center for Disease Control and Prevention
CeHV-5	Cercopithecine herpesvirus 5
CeHV-8	Cercopithecine herpesvirus 8
CMV	Cytomegallo virus
CRS	Congenital rubella syndrome
D.W	Distilled Water
DDT	Dithiothreitol
DNA	Deoxy ribonucleic acid
EPV	Epstein–Barr virus
HCMV	Human cytomegalovirus
HHV-5	Human herpes virus-5
HHV-5	Human herpesvirus 5
HIV	Human immunodeficiency virus
HLA-A1	Human leukocyte antigen –A1
HRP	Horseradish peroxidase
HSV	Herpes simplex virus
IgG	Immunoglobulin G

IgM	Immunoglobulin M
Iqs	Institute quimic de serria
KD	Kilo Dalton
MMR vaccine	Measles , mumps and rubella vaccine
mRNA	Messenger ribosomal nucleic acid
MuHV-2	Murid herpes virus 2
N TD	Neural tube deformity
NC	Nitrocellulose
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate Buffer Saline
PCR	Polymerase chain reaction
PoHV-2	Panine herpesvirus 2
PoHV-4	Pongine herpesvirus 4
PPM	Part per million
PVDF	Polyvinylidenedifluoride
Rh CMV	Rhesus CMV
SCCMV	Simian CMV
SDS	Sodium dodecyl sulfate
SES	Socioeconomic status
STI	Sexually transmitted infection
TBS	Tris Buffered Saline
TORCH	Toxoplasma, Rubella, Cytomegallo virus and Herpes virus
VZV	Varicella-zoster virus
WHO	World health organization

Glossary

- **Congenital disorder:** A medical condition that is present at or before birth.
- **Acquired:** A first time of infection.
- **Recurring:** When the patient is already infected, the virus is dormant and then becomes active due to a weak immune system.
- **Congenital:** A condition occurs during pregnancy and affects the unborn child.
- **Teratogen:** Substances whose toxicity can cause congenital disorders.
- **Congenital physical anomaly:** A abnormality of the structure of the body part.
- **Malformation:** A associate with disorder of tissue development and occur in the first trimester
- **Dysplasia:** Disorder at the organ level that is due to problems with tissue development.
- **Deformation:** A condition arising from medical stress to normal tissue, occur in the second or third trimester.
- **Coryza:** Cold like symptoms.

Abstract

Back ground: This is a cross-sectional descriptive study to detect and determine the type of viruses (Rubella, CMV, and HSV-1 & HSV2).

Objectives: To detect the HSV, Rubella, and CMV in patient's serum (IgG /IgM), and to correlate the viral infections with age and gender.

Methodology: This study done from 2016-2017 in medical military hospital, department of pediatric of congenital babies in Khartoum State.

About 100 serum sample of congenital babies (62 male and 38 female) in different Ages (neonate to five years) were collected, A simple random sample was collected, five milliliters venous blood samples was taken in plane container and used Euroline Blot Technique for detection.

Results: We found Rubella IgM in (10) cases and IgG in (90) cases, Cytomegallo virus IgM in (10) cases and IgG in (90) cases, HSV-1 IgM in (20) cases and IgG in (95) cases, HSV-2 IgM in (0) cases and IgG in (10) cases.

All viruses in study assimilation (40%) of congenital babies causative Agents.

Rubella IgM (10%), Cytomegallo virus IgM (10%), HSV-1 IgM (20%), HSV-2 IgM (0%).

In Rubella 8 of 10 cases (IgM) positive there were maternal Antibodies (IgG). (80% has maternal Abs and 20% hasn't maternal Abs).

In Cytomegallo virus (7) of 10 cases (IgM) there were maternal Antibodies (IgG). (70% has maternal Abs and 30% without maternal Abs).

In (HSV-1), all cases (20) (IgM) have maternal Antibodies (IgG). (100% has maternal Abs).

Also Rubella represents (25%) from the viruses, CMV represent (25%), HSV-1 represent (50%) and HSV-2 represent (0%)

Conclusions: A congenital disorder is a medical condition that is present at or before birth. These conditions also referred to as birth defects, can be acquired during the fetal stage of development or from the genetic makeup of the parents.

All viruses in study Assimilation 40% of congenital babies causative Agents. And we found Rubella IgM in (10) cases and IgG in (90) cases, Cytomegallo virus IgM in (10) cases and IgG in (90) cases, HSV-1 IgM in (20) cases and IgG in (95) cases, HSV-2 IgM in (0) cases and IgG in (10) cases.

الخلاصة

المقدمة: -تعتبر هذه الدراسة من الدراسات الوصفية العرضية وتقوم علي تحديد وتقييم الفيروسات كمسبب من المسببات الرئيسية للأمراض المنقولة للجنين من الأم .

الأهداف: الأهداف التي تقوم عليها الدراسة هي تحديد الفيروسات كعامل مسبب للأمراض المنقولة من الأم للجنين و تحديد نسبة كل فيروس كمسبب اساسي للمرض وتحديد علاقة الفيروسات بالنسبة للجنس والعمر.

المنهجية: اجريت هذه الدراسة في الفتره من سبتمبر 2016 وحتى ديسمبر 2017 بمستشفى (عدة ايام وحتى السلاح الطبي قسم الاطفال. مائة عينة جمعت من اطفال تتراوح اعمارهم ما بين خمسة سنوات) وكانوا كالاتي (اثان وستون ذكور وثمانية وثلاثون اناث).

الغرض من هذه الدراسات تحديد الفيروسات المسببة للامراض المنقولة من الام للجنين .

النتائج: وكانت نتائج البحث كل الفيروسات المطروحة في البحث تمثل (40%) من العوامل المسببة للامراض المنقولة من الام الي الاطفال.

الحصبة الالمانية تمثل (10%) ، الفيروس المضخم للخلايا يمثل (10%) ، الحلا او العقبولة النوع الاول تمثل (20%).

وجد ان (80 %) من حالات الحصبة الالمانية عندهم اجسام مضادة من الام ، وجد ان 70% من حالات الفيروس المضخم للخلايا عندهم اجسام مضادة من الام ،كما وجد ان 100% من حالات الحلا او العقبولة النوع الاول عندهم اجسام مضادة من الام .

وجد ان (26) حالة من نصيب الذكور و(14) حالة من نصيب الاناث .

ايضا وجد ان (29) حالة لاطفال اعمارهم اقل من عامين (11) حالة لاطفال اعمارهم اكبر من عامين.

الاستنتاجات: الامراض الوراثية هي التي تحدث قبل او اثناء عملية الولادة كما يمكن ان تحدث اثناء فترة تكوين وتطور الجنين .

الفيروسات في هذه الدراسة تمثل (40 %).

الحصبة الالمانية تمثل (10%) ، الفيروس المضخم للخلايا يمثل (10%) والحلا او العقبولة النوع الاول تمثل (20%).

ايضا الحصبة الالمانية تمثل (25%) ، الفيروس المضخم للخلايا يمثل (25%) اما الحلا او العقبولة فيمثل (50%) من نسبة الفيروسات .

الخاتمة: التشوهات الوراثية او الخلقية هي تغيرات تحدث اثناء او قبل الولادة . ويمكن ان تكتسب اثناء مراحل تطور الجنين او نتيجة للتركيب الجيني .

كل الفيروسات في هذه الدراسة تمثل (40%) من اسباب التشوهات الوراثية .
وجد ان الحصبة الالمانية تمثل (10%) ، الفيروس المضخم للخلايا يمثل 10% والحلا او العقبولة النوع الاول يمثل (20%).

Chapter one

- **Introduction**
 - **Rational**
- **Objectives**

1.1 Introduction

A congenital disorder is a medical condition that is present at or before birth. These conditions also referred to as birth defects, can be acquired during the fetal stage of development or from the genetic makeup of the parents.

1.1.1 Infections:

A vertically transmitted infection is an infection caused by bacteria, viruses or in rare cases, parasites transmitted directly from the mother to an embryo, fetus or baby during pregnancy or childbirth. It can occur when the mother gets an infection as an intercurrent disease in pregnancy.

Congenital disorders were initially believed to be the result of only hereditary factors. However, in the early 1940s, Australian pediatric ophthalmologist Norman Gregg began recognizing a pattern in which the infants arriving at his surgery were developing congenital cataracts at a higher rate than those who developed it from hereditary factors. On October 15, 1941, Gregg delivered a paper which explained his findings-68 out of the 78 children who were afflicted with congenital cataracts had been exposed in utero due to an outbreak in Australian army camps. These findings confirmed, to Gregg, that there could, in fact, be environmental causes for congenital disorders.

Rubella is known to cause abnormalities of the eye, internal ear, heart, and sometimes the teeth. More specifically, fetal exposure to rubella during weeks five to ten of development (the sixth week particularly) can cause cataracts and microphthalmia in the eyes. If the mother is infected with rubella during the ninth week, a crucial week for internal ear development, there can be destruction of the organ of Corti, causing deafness. In the heart the ductus arteriosus can remain after birth, leading to hypertension. Rubella can also lead to atrial and ventricular septal defects in the heart. If exposed to rubella in the

second trimester, the fetus can develop central nervous system malformations. However, because infections of rubella may remain undetected, misdiagnosed, or unrecognized in the mother, and/or some abnormalities are not evident until later in the child's life, precise incidence of birth defects due to rubella are not entirely known. The timing of the mother's infection during fetal development determines the risk and type of birth defect. As the embryo develops, the risk of abnormalities decreases. If exposed to the rubella virus during the first four weeks, the risk of malformations is 47 percent. Exposure during weeks five through eight creates a 22 percent chance, while weeks nine to twelve a seven percent chance exists, followed by a percentage of six if the exposure is during the thirteenth to sixteenth weeks. Exposure during the first eight weeks of development can also lead to prematurity and fetal death. These numbers are calculated from immediate inspection of the infant after birth. Therefore, mental defects are not accounted for in the percentages because they are not evident until later in the child's life. If they were to be included, these numbers would be much higher (Atkinson., 2007).

Other infectious agents include cytomegalovirus, the herpes simplex virus, hyperthermia, toxoplasmosis, and syphilis. Mother exposure to cytomegalovirus can cause microcephaly, cerebral calcifications, blindness, chorioretinitis (which can cause blindness), hepatosplenomegaly, and meningoencephalitis in fetuses (Atkinson., 2007). Microcephaly is a disorder, in which the fetus has an atypically small head, (Immunisation Handbook 2006). Cerebral calcifications means certain areas of the brain have atypical calcium deposits (Smith., 1881) and meningoencephalitis is the enlargement of the brain. All three disorders cause abnormal brain function or mental retardation. Hepatosplenomegaly is the enlargement of the liver and spleen which causes digestive problems (Hess *etal.*, 1914). It can also cause some kernicterus and petechiae. Kernicterus causes yellow pigmentation of the skin,

brain damage, and deafness. Petechiae are when the capillaries bleed resulting in red/purple spots on the skin (Hanshaw., 1985) However, cytomegalovirus is often fatal in the embryo.

The herpes simplex virus can cause microcephaly, microphthalmus (abnormally small eyeballs) (Pan American Health Organization., 1998) retinal dysplasia, hepatosplenomegaly, and mental retardation. (Atkinson., 2007). Both microphthalmus and retinal dysplasia can cause blindness. However, the most common symptom in infants is an inflammatory response that develops during the first three weeks of life (Atkinson., 2007). Hyperthermia causes anencephaly, which is when part of the brain and skull are absent in the infant (Atkinson., 2007).

Mother exposure to toxoplasmosis can cause cerebral calcification, hydrocephalus (causes mental disabilities), and mental retardation in infants. Other birth abnormalities have been reported as well, such as chorioretinitis, microphthalmus, and ocular defects. Syphilis causes congenital deafness, mental retardation, and diffuse fibrosis in organs, such as the liver and lungs, if the embryo is exposed (Atkinson., 2007).

The common virus that cause congenital abnormality HSV, Rubella and cytomegalovirus (Donald., 2015).

1.1.2 HSV:

The herpes simplex virus, also known as HSV, is an infection that causes herpes. Herpes can appear in various parts of the body, most commonly on the genitals area and mouth. There are two types of the herpes simplex virus. HSV-1, also known as oral herpes, can cause cold sores and fever blisters around the mouth and on the face. HSV-2 is generally responsible for genital herpes outbreaks (Pan American Health Organization PAHO., 2015).

1.1.3 Rubella:

Rubella is a mild and preventable disease caused by a virus. If you catch it you may feel unwell, with swollen glands, a slight temperature, or a sore throat and rash but some people have no symptoms at all and so are unaware that they may be infectious and may be passing on the disease (Edlich., 2005).

Rubella is very serious if a pregnant woman catches it in the early stages of her pregnancy because it can profoundly damage the development of her unborn child. It can result in deaf blindness or raise the possibility of a termination (Pan American Health Organization PAHO., 2015). Ensuring that children are routinely vaccinated helps to protect pregnant women and their babies..

1.1.4.Cytomegalovirus:

It is a common herpes virus; many people do not know they have it, because they may have no symptoms. But the virus, which remains dormant in the body, can cause complications during pregnancy and for people with a weakened immune system.

The virus spreads through bodily fluids, and it can be passed on from a pregnant mother to her unborn baby.

Also known as HCMV, CMV, or Human Herpes virus 5 (HHV-5), cytomegalovirus is the virus most commonly transmitted to a developing fetus (Robert *etal.*, 2006).

There are three main types of CMV infections: acquired, recurring, or congenital.

- Acquired, or primary, CMV is a first-time infection.
- Recurring CMV is when the patient is already infected. The virus is dormant and then becomes active due to a weak immune system.
- Congenital CMV is when infection occurs during pregnancy and affects the unborn child.

CMV is generally not a problem, except when it affects an unborn child or a person with a weak immune system, such as a recent transplant recipient or a person with human immunodeficiency virus, or HIV.

A pregnant woman has a very small risk of reactivation infecting her developing baby. If infection is suspected, she may consider amniocentesis, which involves extracting a sample of amniotic fluid to find out whether the virus is present (Robert ., 2015).

1.2 Rationale:

Congenital abnormality is one of the phenomenon's which spread recently in Sudan and become one of the problems which uncontrolled and consider one of diseases which have many causative agents, so this study aim to know the viruses as causative Agents or a predisposing factor for congenital abnormality so as to treat and to prevent the babies from the viral infection.

1.3 Objectives

1.3.1 General Objective:

To detect present of the common viral infection as causative agent in congenital babies.

1.3.2 Specific Objectives:

1. To detect HSV in patient's serum (IgG /IgM).
2. To detect Rubella in patient's serum (IgG /IgM).
3. To detect CMV in patient's serum (IgG /IgM).
4. To correlate the viral infections with age &gender.

Chapter two

- **Literature & Review**

Literature Review

2.1 Congenital disorder:

Also known as congenital disease, birth defect or anomaly (Neighbors *etal.*, 2010) is a condition existing at or before birth regardless of cause. Of these diseases, those characterized by structural deformities are termed "congenital anomalies" and involve defects in a developing fetus. Birth defects vary widely in cause and symptoms. Any substance that causes birth defects is known as a teratogen. Some disorders can be detected before birth through prenatal diagnosis (screening).

Birth defects may be the result of genetic or environmental factors. This includes errors of morphogenesis, infection, epigenetic modifications on a parental germ line, or a chromosomal abnormality. The outcome of the disorder will depend on complex interactions between the pre-natal deficit and the post-natal environment (Neighbors *etal.*, 2010). Animal studies indicate that the mother's (and likely the father's) diet, vitamin intake, and glucose levels prior to ovulation and conception have long-term effects on fetal growth and adolescent and adult disease (Lambert, *etal.*, 2015).

Animal studies have shown that paternal exposures prior to conception and during pregnancy result in increased risk of certain birth defects and cancers. This research suggests that paternal food deprivation, germ line mutations, alcohol use, chemical mutagens, age, smoking habits and epigenetic alterations can affect birth outcomes (Lambert *etal.*, 2015. Atkinson *etal.*, 2011. Huong McLean., 2014. cdc., 2015).

However, the relationship between offspring health and paternal exposures, age, and lifestyle are still relatively weak. This is likely because paternal exposures and their

effects on the fetus are studied far less extensively than maternal exposures. Birth defects are present in about (3%) of newborns in USA (cdc., 2014).

Congenital anomalies resulted in about 632,000 deaths per year in 2013 down from 751,000 in 1990. The type with the greatest numbers of deaths are congenital heart disease (323,000), followed by neural tube defects (69,000) (PAHO., 2015)

2.1.1 Definitions of congenital disorder:

A congenital disorder is a medical condition that is present at or before birth. These conditions also referred to as birth defects, can be acquired during the fetal stage of development or from the genetic makeup of the parents. Congenital disorders are not necessarily hereditary, since they may be caused by infections during pregnancy or injury to the fetus at birth. If the lamina (bony arch) to the spine does not fully develop, this is known as spina bifida and is the most common congenital disorder.

2.1.2 Classification:

Much of the language used for describing congenital conditions predates genomic mapping, and structural conditions are often considered separately from other congenital conditions. It is now known that many metabolic conditions may have subtle structural expression, and structural conditions often have genetic links. Still, congenital conditions are often classified in a structural basis, organized when possible by primary organ system affected.

2.1.3 Primarily structural:

Several terms are used to describe congenital abnormalities. (Some of these are also used to describe non congenital conditions, and more than one term may apply in an individual condition.)

2.1.4 Congenital anomaly:

A congenital physical anomaly is an abnormality of the structure of a body part. An anomaly may or may not be perceived as a problem condition. Many, if not most, people have one or more minor physical anomalies if examined carefully. Examples

of minor anomalies can include curvature of the 5th finger (clinodactyly), a third nipple, tiny indentations of the skin near the ears (preauricular pits), shortness of the 4th metacarpal or metatarsal bones, or dimples over the lower spine (sacral dimples). Some minor anomalies may be clues to more significant internal abnormalities. Birth defect is a widely used term for a congenital malformation, i.e. a congenital, physical anomaly which is recognizable at birth, and which is significant enough to be considered a problem. According to the CDC, most birth defects are believed to be caused by a complex mix of factors including genetics, environment, and behaviors (Lambert., 2015) though many birth defects have no known cause. An example of a birth defect is cleft palate, which occurs during the fourth and seventh week of gestation (Edlich *et al.*, 2005) Body tissue and special cells from each side of the head grow toward the center of the face. They join together to make the face. ("Pan American Health Organization (PAHO., 2015)

A cleft means a split or separation; the "roof" of the mouth is called the palate."Public Health Image Library (PHIL)" CDC. 1966. Retrieved., 2015).

A congenital malformation is a congenital physical anomaly that is deleterious, i.e. a structural defect perceived as a problem. A typical combination of malformations affecting more than one body part is referred to as a malformation syndrome.

2.1.5 Some conditions are due to abnormal tissue development:

2.1.5.1 Malformation

Is associated with a disorder of tissue development (Robert., 2015).

- Malformations often occur in the first trimester.

A dysplasia is a disorder at the organ level that is due to problems with tissue development (Donald., 2015).

2.1.5.2. Deformation:

Is a condition arising from mechanical stress to normal tissue (Robert., 2015).

Deformations often occur in the second or third trimester, and can be due to oligo hydramnios. A disruption involves breakdown of normal tissues (*Public Health Image Library (PHIL)*" 1966. Retrieved., 2015).

When multiple effects occur in a specified order, it is known as a sequence.

When the order is not known, it is a syndrome.

2.1.6. Examples of primarily structural congenital disorders:

A limb anomaly is called a dysmelia. These include all forms of limbs anomalies, such as amelia, ectrodactyly, phocomelia, polymelia, polydactyly, syndactyly, polysyndactyly, oligodactyly, brachydactyly, achondroplasia, congenital aplasia or hyperplasia, amniotic bands syndrome, and cleidocranial dysostosis.

Congenital anomalies of the heart include patent ducts arteriosus, arterial septal defect, and ventricular septal defect .

Congenital anomalies of the nervous system include neural tube defects such as spina bifida, meningocele, meningomyelocele, encephalocele and anencephaly. Other congenital anomalies of the nervous system include the Arnold - Chiari malformation, the Dandy Walker malformation, hydrocephalus, microencephaly, megalencephaly, lissencephaly, polymicrogyria, holoprosencephaly, and agenesis of the corpus callosum. Congenital anomalies of the gastrointestinal system include numerous forms of stenosis and atresia, and perforation, such as gastro schisis.

Congenital anomalies of the kidney and urinary tract (CAKUT) include renal parenchyma, kidneys, and urinary collecting system. (*"Public Health Image Library (PHIL)" 1966., Retrieved., 2015).*

Defects can be bilateral or unilateral, and different defects often coexist in an individual child

A congenital metabolic disease is also referred to as an inborn error of metabolism. Most of these are single gene defects, usually heritable. Many affect the structure of body parts but some simply affect the function.

Other well defined genetic conditions may affect the production of hormones, receptors, structural proteins, and ion channels.

2.2. Causes of congenital disorder:

2.2.1 Infections:

Main article: Vertically transmitted infection A vertically transmitted infection is an infection caused by bacteria, viruses or, in rare cases, parasites transmitted directly from the mother to an embryo, fetus or baby during pregnancy or childbirth. It can occur when the mother gets an infection as an undercurrent disease in pregnancy.

Congenital disorders were initially believed to be the result of only hereditary factors. However, in the early 1940s, Australian pediatric ophthalmologist Norman Gregg began recognizing a pattern in which the infants arriving at his surgery were developing congenital cataracts at a higher rate than those who developed it from hereditary factors. On October 15, 1941, Gregg delivered a paper which explained his findings-68 out of the 78 children who were afflicted with congenital cataracts had been exposed in utero due to an outbreak in Australian army camps. These findings confirmed, to Gregg, that there could, in fact, be environmental causes for congenital disorders.

Rubella is known to cause abnormalities of the eye, internal ear, heart, and sometimes the teeth. More specifically, fetal exposure to rubella during weeks five to ten of development (the sixth week particularly) can cause cataracts and microphthalmia in the eyes. If the mother is infected with rubella during the ninth week, a crucial week for internal ear development, there can be destruction of the organ of Corti, causing deafness. In the heart

the ductus arteriosus can remain after birth, leading to hypertension. Rubella can also lead to arterial and ventricular septal defects in the heart. If exposed to rubella in the second trimester, the fetus can develop central nervous system malformations. However, because infections of rubella may remain undetected, misdiagnosed, or unrecognized in the mother, and/or some abnormalities are not evident until later in the child's life, precise incidence of birth defects due to rubella are not entirely known. The timing of the mother's infection during fetal development determines the risk and type of birth defect. As the embryo develops, the risk of abnormalities decreases. If exposed to the rubella virus during the first four weeks, the risk of malformations is 47 percent. Exposure during weeks five through eight creates a 22 percent chance, while weeks nine to twelve a seven percent chance exists, followed by a percentage of six if the exposure is during the thirteenth to sixteenth weeks. Exposure during the first eight weeks of development can also lead to prematurity and fetal death. These numbers are calculated from immediate inspection of the infant after birth. Therefore, mental defects are not accounted for in the percentages because they are not evident until later in the child's life. If they were to be included, these numbers would be much higher (Atkinson., 2007).

Other infectious agents include cytomegalovirus, the herpes simplex virus, hyperthermia, toxoplasmosis, and syphilis. Mother exposure to cytomegalovirus can cause microcephaly, cerebral calcifications, blindness, chorioretinitis (which can cause blindness), hepatosplenomegaly, and meningoencephalitis in fetuses (Atkinson., 2007). Microcephaly is a disorder in which the fetus has an atypically small head, (Immunisation Handbook 2006). cerebral calcifications means certain areas of the brain have atypical calcium deposits, (Smith, J. 1881) and meningoencephalitis is the enlargement of the brain. All three disorders cause abnormal brain function or mental retardation. Hepatosplenomegaly is the enlargement of the liver and spleen which causes

digestive problems (Hess *etal.*, 1914). It can also cause some kernicterus and petechiae. Kernicterus causes yellow pigmentation of the skin, brain damage, and deafness. Petechiae is when the capillaries bleed resulting in red/purple spots on the skin (Hanshaw., 1985) However, cytomegalovirus is often fatal in the embryo.

The herpes simplex virus can cause microcephaly, microphthalmus (abnormally small eyeballs) (Pan American Health Organization.,1998) retinal dysplasia, hepatosplenomegaly, and mental retardation (Atkinson., 2007). Both microphthalmus and retinal dysplasia can cause blindness. However, the most common symptom in infants is an inflammatory response that develops during the first three weeks of life (Atkinson., 2007). Hyperthermia causes anencephaly, which is when part of the brain and skull are absent in the infant (Atkinson., 2007).

Mother exposure to toxoplasmosis can cause cerebral calcification, hydrocephalus (causes mental disabilities), and mental retardation in infants. Other birth abnormalities have been reported as well, such as chorioretinitis, microphthalmus, and ocular defects. Syphilis causes congenital deafness, mental retardation, and diffuse fibrosis in organs, such as the liver and lungs, if the embryo is exposed (Atkinson., 2007).

2.2.2 Fetal alcohol exposure:

- Main articles:

- a) Fetal alcohol spectrum disorder and Fetal alcohol syndrome

The mother's consumption of alcohol during pregnancy can cause a continuum of various permanent birth defects : crano facial abnormalities ("Public Health Image Library (PHIL)". . 1966. Retrieved 2015).

- b) brain damage, intellectual disability (Edlich, *etal.*, 2005).

- b)The prevalence of children affected is estimated at least 1 percent in U.S. (De Santisetal., 2006).

c) as well in Canada.

d) Very few studies have investigated the links between paternal alcohol use and offspring health.

2.2.3 teratogenes:

Substances whose toxicity can cause congenital disorders are called "teratogens", and include certain pharmaceutical and recreational drugs in pregnancy as well as many environmental toxins in pregnancy.

A review published in 2010 identified 6 main teratogenic mechanisms associated with medication use: folate antagonism, neural crest cell disruption, endocrine disruption, oxidative stress, vascular disruption and specific receptor- or enzyme-mediated teratogenesis (Honey man *etal.*, 1975).

It is estimated that 10% of all birth defects are caused by prenatal exposure to a teratogenic agent. (*Best* (2007).).

These exposures include, but are not limited to, medication or drug exposures, maternal infections and diseases, and environmental and occupational exposures. Paternal smoking use has also been linked to an increased risk of birth defects and childhood cancer for the offspring, where the paternal germ line undergoes oxidative damage due to cigarette use (Stegmann & Carey., 2002).

Teratogen-caused birth defects are potentially preventable. Studies have shown that nearly 50% of pregnant women have been exposed to at least one medication during gestation (Dayan *et al.*, 2006).

During pregnancy, a female can also be exposed to teratogens from the contaminated clothing or toxins within the seminal fluid of a partner (Donald *etal.*, 2015) (Frey., 1994). An additional study found that of 200 individuals referred for genetic counseling for a teratogenic exposure, 52% were exposed to more than one potential teratogen. (By the Institute for Clinical Systems Improvement 2010).

2.2.4 Medications and supplements:

The most notorious teratogenic drug is the thalidomide, developed at the end of 1950 by Chemie Grünenthal as a hypnotic and antiemetic and therefore frequently prescribed to pregnant women in almost 50 countries worldwide between 1956 - 1962.(Khandekar., 2007). Until William McBride published the study leading to its withdrawal from the market at 1961, about 8-10 000 severely malformed children were born. The most typical disorder induced by thalidomide were reductional deformities of the long bones of the extremities (phocomelia), otherwise a rare deformity, which therefore helped to recognize the teratogenic effect of the new drug. Among other malformations caused by thalidomide were those of ears, eyes, brain, kidney, heart, digestive and respiratory tract. 40% of the prenatally affected children died soon after birth (Khandekar., 2007). As thalidomide is used today as a treatment for multiple myeloma and leprosy, several births of affected children were described in spite of the strictly required use of contraception among female patients treated by it.

Several anticonvulsants are known to be highly teratogenic. Phenytoin, also known as diphenylhydantoin, along with carbamazepine is responsible for the fetal hydantoin syndrome, which may typically include broad nose base, cleft lip and/or palate, micro cephalia, nails and fingers hypo plasia , intrauterine growth restriction and mental retardation. Trimethadione taken during pregnancy is responsible for the fetal trimethadione syndrome, characterized by craniofacial, cardiovascular, renal and spine malformations, along with a delay in mental and physical development. Valproate has anti-folate effects, leading to neural tube closure-related defects such as spina bifida. Lower IQ and autism have recently also been reported as a result of intrauterine valproate exposure (Khandekar., 2007).

Hormonal contraception is considered as harmless for the embryo. Peterka and Novotna (Khandekar., 2007), do however state that synthetic progestin's used to

prevent miscarriage in the past frequently caused masculinization of the outer reproductive organs of female newborns due to their androgenic activity. Diethylstilbestrol is a synthetic estrogen used from the 1940s to 1971 when the prenatal exposition has been linked to the clear-cell adenocarcinoma of the vagina. Following studies showed elevated risks for other tumors and congenital malformations of the sex organs for both sexes.

All cytostatics are strong teratogenes, abortion is usually recommended when pregnancy is found during or before chemotherapy. Amino pterin, a cytostatic drug with anti-folate effect, was used during the 1950s and 1960s to induce therapeutic abortions. In some cases the abortion didn't happen, but the newborns suffered a fetal aminopterin syndrome consisting of growth retardation, cranio synostosis, hydrocephalus, facial dismorphities, mental retardation and/or leg deformities (Khandekar., 2007) (Reef *et al.*, 2002).

2.2.5 Environmental toxic substances:

Drinking water is often a vessel through which harmful toxins travel. Studies have shown that heavy metals, elements, nitrates, nitrites, fluoride can be carried through water and cause congenital disorders. Nitrate, which is found mostly in drinking water from ground sources, is a powerful teratogen. A case-control study in rural Australia that was conducted following frequent reports of prenatal mortality and congenital malformations found that those who drank the nitrate-infected groundwater, as opposed to rain water, ran the risk of giving birth to children with central nervous system disorders, muscoskeletal defects, and cardiac defects. Chlorinated and aromatic solvents such as benzene and trichloroethylene sometimes enter the water supply due to oversights in waste disposal. A case-control study on the area found that by 1986, leukemia was occurring in the children of Woburn, Massachusetts at a rate that was four times the expected rate of incidence. Further investigation revealed a connection between the high occurrence of leukemia and an

error in water distribution that delivered water to the town with significant contamination manufacturing waste containing trichloroethylene (Plotkin., 2001). As an endocrine disruptor, the DDT was shown to induce miscarriages, interfere with the development of the female reproductive system, cause the congenital hypothyroidism and susceptibly childhood obesity (Khandekar., 2007). Fluoride, when transmitted through water at high levels, can also act as a teratogen. Two reports on fluoride exposure from China, which were controlled to account for the education level of parents, found that children born to parents who were exposed to 4.12 PPM fluoride grew to have IQs that were, on average, seven points lower than their counterparts whose parents consumed water that contained 0.91 PPM fluoride. In studies conducted on rats, higher PPM fluoride in drinking water lead to increased acetyl cholinesterase levels, which can alter prenatal brain development. The most significant effects were noted at a level of 5 PPM (Cooper., 1975). The fetus is even more susceptible to damage from carbon monoxide intake, which can be harmful when inhaled during pregnancy, usually through first or second-hand tobacco smoke. The concentration of carbon monoxide in the infant born to a non-smoking mother is around 2%, and this concentration drastically increases to a range of 6% - 9% if the mother smokes tobacco. Other possible sources of prenatal carbon monoxide intoxication are exhaust gas from combustion motors, use of dichloromethane (paint thinner, varnish removers) in enclosed areas, defective gas hot water heaters, indoor barbeques, open flames in poorly-ventilated areas, atmospheric exposure in highly polluted areas. Exposure to carbon monoxide at toxic levels during the first two trimesters of pregnancy can lead to intrauterine growth restriction, leading to a baby that has stunted growth and is born smaller than 90% of other babies at the same gestational age. The effect of chronic exposure to carbon monoxide can depend on the stage of pregnancy in which the mother is exposed. Exposure during the embryonic stage can have neurological consequences,

such as telencephalic dysgenesis, behavioral difficulties during infancy, and reduction of cerebellum volume. There are also possible skeletal defects that could result from exposure to carbon monoxide during the embryonic stage, such as hand and foot malformations, hip dysplasia, hip subluxation, agenesis of a limb, and inferior maxillary atresia with glossoptosis. Also, carbon monoxide exposure between days 35 and 40 of embryonic development can lead to an increased risk of the child developing a cleft palate. Exposure to carbon monoxide or polluted ozone exposure can also lead to cardiac defects of the ventricular septal, pulmonary artery and heart valves (Danovaro *et al.*, 2000). The effects of carbon monoxide exposure are decreased later in fetal development during the fetal stage, but they may still lead to anoxic encephalopathy (Reef., 2006). Industrial pollution can also lead to congenital defects. Over a period of 37 years, the Chisso Corporation, a petrochemical and plastics company, contaminated the waters of Minamata Bay with an estimated 27 tons of methyl mercury, contaminating the local water supply. This led to many people in the area developing what became known as the “Minamata Disease.” Because methyl mercury is a Teratogen, the mercury poisoning of those residing by the bay resulted in neurological defects in the offspring. Infants exposed to mercury poisoning in utero showed predispositions to cerebral palsy, ataxia, inhibited psychomotor development, and mental retardation. ([http://www.thelancet.com/journals/lancet/article/PIIS01406736\(14\)60712-1/fulltext](http://www.thelancet.com/journals/lancet/article/PIIS01406736(14)60712-1/fulltext)).

Another issue regarding environmental justice is lead poisoning. If the fetus is exposed to lead during the pregnancy, this can result in learning difficulties and slowed growth. A lot of paints (before 1978) and pipes contain lead. Therefore, pregnant women who live in homes with lead paint will inhale the dust containing lead, leading to lead exposure in the fetus. When lead pipes are used for drinking water and cooking water, this water is ingested, along with the lead, exposing the

fetus to this toxin. This issue is more prevalent in poorer communities. This is because more well off families are able to afford to have their homes repainted and pipes renovated (Best., 2005).

2.2.6 Paternal smoking:

Paternal smoking prior to conception has been linked with the increased risk of congenital abnormalities in offspring (Huong., 2014). Smoking causes DNA mutations in the germ line of the father, which can be inherited by the offspring. Cigarette smoke acts as a chemical mutagen on germ cell DNA. The germ cells suffer oxidative damage, and the effects can be seen in altered mRNA production, infertility issues, and side effects in the embryonic and fetal stages of development. This oxidative damage may result in epigenetic or genetic modifications of the father's germ line. Research has shown that fetal lymphocytes have been damaged as a result of a father's smoking habits prior to conception (Donald., 2015) (Watson *et al.*, 1998). Correlations between paternal smoking and the increased risk of offspring developing childhood cancers (including acute leukemia, brain tumors, and lymphoma) before age five have been established. However, further research is needed to confirm these findings. Little is currently known about how paternal smoking damages the fetus, and what window of time in which the father smokes is most harmful to offspring (Watson *et al.*, 1998).

2.2.7 Lack of nutrients:

Further information: Nutrition in pregnancy

For example, a lack of folic acid, a vitamin B, in the diet of a mother can cause cellular neural tube deformities that result in spina bifida. Congenital disorders such as a neural tube deformity (NTD) can be prevented by 72% if the mother consumes 4 milligrams of folic acid before the conception and after 12 weeks of pregnancy. Folic acid, or vitamin B₁₂, aids the development of the foetal nervous system. ("Viral Zone". ExPASy. Retrieved 2015.)

Studies with mice have found that food deprivation of the male mouse prior to conception leads to the offspring displaying significantly lower blood glucose levels. (Virus Taxonomy: 2014 ")

2.2.8 Physical restraint:

External physical shocks or constraint due to growth in a restricted space, may result in unintended deformation or separation of cellular structures resulting in an abnormal final shape or damaged structures unable to function as expected. An example is Potter syndrome due to oligohydramnios. This finding is important for future understandings of how genetics may predispose individuals for diseases like obesity, diabetes, and cancer.

For multicellular organisms that develop in a womb, the physical interference or presence of other similarly developing organisms such as twins can result in the two cellular masses being integrated into a larger whole, with the combined cells attempting to continue to develop in a manner that satisfies the intended growth patterns of both cell masses. The two cellular masses can compete with each other, and may either duplicate or merge various structures. This results in conditions such as conjoined twins, and the resulting merged organism may die at birth when it must leave the life-sustaining environment of the womb and must attempt to sustain its biological processes independently.

2.2.9 Genetic causes:

Main article: Genetic disorder

Genetic causes of congenital anomalies include inheritance of abnormal genes from the mother or the father, as well as new mutations in one of the germ cells that gave rise to the fetus. Male germ cells mutate at a much faster rate than female germ cells, and as the father ages, the DNA of the germ cells mutates quickly (CDC., 2014) (Stegmann & Carey., 2002).

If an egg is fertilized with sperm that has damaged DNA, there is a possibility that the fetus could develop abnormally (CDC., 2014) (Forrest *et al.*, 2002).

Genetic disorders or diseases are all congenital, though they may not be expressed or recognized until later in life. Genetic diseases may be divided into single-gene defects, multiple-gene disorders, or chromosomal defects. Single-gene defects may arise from abnormalities of both copies of an autosomal gene (a recessive disorder) or of only one of the two copies (a dominant disorder). Some conditions result from deletions or abnormalities of a few genes located contiguously on a chromosome. Chromosomal disorders involve the loss or duplication of larger portions of a chromosome (or an entire chromosome) containing hundreds of genes. Large chromosomal abnormalities always produce effects on many different body parts and organ systems.

2.2.10. Socioeconomic status:

A low socioeconomic status in a deprived neighborhood may include exposure to “environmental stressors and risk factors (Ryan *et al.*, 2004) socioeconomic inequalities are commonly measured by the Cartairs-Morris score, Index of Multiple Deprivation, Townsend deprivation index, and the Jarman score. The Jarman score, for example, considers “unemployment, overcrowding, single parents, under-fives, and elderly living alone, ethnicity, low social class and residential mobility. In Vos’ meta-analysis these indices are used to view the effect of low SES neighborhoods on maternal health. In the meta-analysis, data from individual studies were collected from 1985 up until 2008. Vos concludes that a correlation exists between prenatal adversities and deprived neighborhoods. Other studies have shown that low SES is closely associated with the development of the fetus in utero and growth retardation. (Koichi., 2007). Studies also suggest that children born in low SES families are “likely to be born prematurely, at low birth weight, or with asphyxia, a birth defect, a disability, fetal alcohol syndrome, or AIDS (Koichi.,

2007). Bradley and Corwyn also suggest that congenital disorders arise from the mother's lack of nutrition, a poor lifestyle, maternal substance abuse and "living in a neighborhood that contains hazards affecting fetal development (toxic waste dumps) (Koichi., 2007). In a meta-analysis that viewed how inequalities influenced maternal health, it was suggested that deprived neighborhoods often promoted behaviors such as smoking, drug and alcohol use (Ryan., 2004). After controlling for socioeconomic factors and ethnicity, several individual studies demonstrated an association with outcomes such as perinatal mortality and preterm birth.

2.2.11 Role of radiation:

For the survivors of the atomic bombing of Hiroshima and Nagasaki, who are known as the Hibakusha, no statistically demonstrable increase of birth defects/congenital malformations was found among their later conceived children, or found in the later conceived children of cancer survivors who had previously received radiotherapy (Kendall., 2014).

The surviving women of Hiroshima and Nagasaki who were able to conceive, though exposed to substantial amounts of radiation, later had children with no higher incidence of abnormalities/birth defects than in the Japanese population as a whole (Kendall., 2014).

Relatively few studies have researched the effects of paternal radiation exposure on offspring. Following the Chernobyl disaster, it was found that the germ line of irradiated fathers suffered minisatellite mutations in the DNA, which was inherited by descendants (Frey., 1994).

Animal studies have shown that the X-ray irradiation of male mice resulted in birth defects of the offspring (Donald., 2010).

In the 1980s, a relatively high prevalence of pediatric leukemia cases in children living near a nuclear processing plant in West Cumbria, UK, led researchers to investigate whether the cancer was a result of paternal radiation exposure. A

significant association between paternal irradiation and offspring cancer was found, but further research areas close to other nuclear processing plants did not produce the same results (Donald & McNeil., 2015) (Frey., 1994).

2.3. Herpes Simplex: Herpes Type 1 and 2:

Herpes simplex viruses – more commonly known as herpes - are categorized into two types: herpes type 1 (HSV-1, or oral herpes) and herpes type 2 (HSV-2, or genital herpes). Most commonly, herpes type 1 cause's sores around the mouth and lips (sometimes called fever blisters or cold sores). HSV-1 can cause genital herpes, but most cases of genital herpes are caused by herpes type 2. In HSV-2, the infected person may have sores around the genitals or rectum. Although HSV-2 sores may occur in other locations, these sores usually are found below the waist.

2.3.1 Life cycle :

- The lytic cycle (entry, uncoating, viral transcription and DNA replication in nucleus, particle assembly, exit from the cell).
- Some viral enter the sensory neuron terminals and travels retrograde to nucleus where it establishes latency.
- Periodic reactivation result in intergraded transport of viral particles , shedding from neuron and re infection of epith cells which lead to asymptomatic shedding or recurrent

2.3.2 Transmission of Herpes Infections:

Herpes simplex type 1, which is transmitted through oral secretions or sores on the skin, can be spread through kissing or sharing objects such as toothbrushes or eating utensils. In general, a person can only get herpes type 2 infection during sexual contact with someone who has a genital HSV-2 infection. It is important to know that both HSV-1 and HSV-2 can be spread even if sores are not present.

Pregnant women with genital herpes should talk to their doctor, as genital herpes can be passed on to the baby during childbirth.

For many people with the herpes virus, which can go through periods of being dormant, attacks (or outbreaks) can be brought on by the following conditions:

- General illness (from mild illnesses to serious conditions).
- Fatigue
- Physical or emotional stress
- Immunosuppression due to AIDS or such medications as chemotherapy or steroids
- Trauma to the affected area, including sexual activity
- Menstruation

2.3.3 Signs and symptoms of Herpes Simplex:

It is important to understand that although someone may not have visible sores or symptoms, they may still be infected by the virus and may transmit the virus to others. Some of the symptoms associated with this virus include:

1. blistering sores (in the mouth or on the genitals)
2. pain during urination (genital herpes)
3. itching

Additionally, you may experience many symptoms that are similar to the flu. These symptoms can include fever, swollen lymph nodes, headaches, tiredness, and lack of appetite. HSV can also spread to the eyes, causing a condition called herpes keratitis. This can cause symptoms such as eye pain, discharge, and a gritty feeling in the eye.

2.3.4 Herpes Simplex Diagnosed:

Often, the appearance of herpes simplex virus is typical and no testing is needed to confirm the diagnosis. If a health care provider is uncertain, herpes simplex can be diagnosed with lab tests, including detect Antibodies in serum-DNA -- or PCR -- tests and virus cultures.

2.3.5 Herpes Simplex Treatment:

Although there is no cure for herpes, treatments can relieve the symptoms. Medication can decrease the pain related to an outbreak and can shorten healing time. They can also decrease the total number of outbreaks. Drugs including famvir, zovirax, and vortrex are among the drugs used to treat the symptoms of herpes. Warm baths may relieve the pain associated with genital sores.

Person at Risk of Developing Herpes Simplex Infections:

Anyone can be infected with HSV, regardless of age. Your risk is determined almost entirely based on exposure to the infection.

In cases of sexually transmitted HSV, people are more at risk when they participate in risky sexual behavior without the use of protection, such as condoms. Other risk factors for HSV-2 include:

- having multiple sex partners
- being female
- having another sexually transmitted infection (STI)
- having a weakened immune system

If a mother is having an outbreak of genital herpes at the time of childbirth, it can expose the baby to both types of HSV, and may put them at risk for serious complications.

2.3.6 Oral Herpes:

More than 50 percent of the adult population in the United States has oral herpes, typically caused by herpes simplex virus type 1 (HSV-1). Most people contract oral herpes when they are children by receiving a kiss from a friend or relative.

Oral herpes is commonly referred to as “cold sores” and “fever blisters.” While symptoms of oral herpes most commonly appear on or around the lips, oral herpes is not always limited to this area. For some, symptoms may appear between the upper lip, on or inside the nose, or on the chin or cheek. In these instances, herpes is

referred to as oral-facial herpes. You have most likely seen someone experiencing an oral herpes outbreak before.

Oral herpes is transmitted through direct contact between the contagious area and broken skin (a cut or break) and mucous membrane tissue (such as the mouth or genitals). Herpes can also be transmitted when there are no symptoms present. There are several days throughout the year when the virus reactivates yet causes no symptoms (called asymptomatic shedding, viral shedding, or asymptomatic reactivation).

If a person is experiencing symptoms orally, we recommend abstaining from performing oral sex and kissing others directly on the mouth until signs have healed and the skin looks normal again. Because most adults have oral herpes, we do not advise that a person stop giving or receiving affection altogether between outbreaks (when there are no signs or symptoms) simply because they have oral herpes. However, using a barrier (such as a dental dam) or condom when performing oral sex (even though there are no symptoms present around the mouth) can reduce the risk of contracting genital herpes.

By performing oral sex on someone who has genital herpes, it would be possible to contract oral herpes – but this is rare. Most cases of genital herpes are caused by HSV-2, which rarely affects the mouth or face. Also, and even more importantly, most adults already have oral HSV-1, contracted as a child through kissing relatives or friends.

2.4 Rubella:

It is a mild and preventable disease caused by a virus. If you catch it you may feel unwell, with swollen glands, a slight temperature, or a sore throat and rash. But some people have no symptoms at all and so are unaware that they may be infectious and may be passing on the disease.

Rubella is very serious if a pregnant woman catches it in the early stages of her pregnancy because it can profoundly damage the development of her unborn child. It can result in deaf blindness or raise the possibility of a termination.

Ensuring that children are routinely vaccinated helps to protect pregnant women and their babies.

2.4.1 German measles:

German measles is a common term used to describe rubella.

Rubella, also known as German measles or three-day measles (Centers for Disease Control and Prevention (CDC., 2005). Is an infection caused by the rubella virus.

This disease is often mild with half of people not realizing that they are sick (Khandekar *et al.*, 2007) (Weisinger & Pesudovs., 2002). A rash may start around two weeks after exposure and last for three days. It usually starts on the face and spreads to the rest of the body. The rash is not as bright as that of measles and is sometimes itchy. Swollen lymph nodes are common and may last a few weeks (Weisinger., 2002). A fever, sore throat, and fatigue may also occur (Weisinger., 2002). In adults joint pain is common. Complications may include bleeding problems, testicular swelling, and inflammation of nerves (Weisinger., 2002)

Infection during early pregnancy may result in a child born with congenital rubella syndrome (CRS) or miscarriage. Symptoms of CRS include problems with the eyes such as cataracts, ears such as deafness, heart, and brain. Problems are rare after the 20th week of pregnancy.(Health Care Guideline: Routine Prenatal Care., 2010)

Rubella is usually spread through the air via coughs of people who are infected. (Health Care Guideline: Routine Prenatal Care., 2010) (Reef *et al.*, 2002). People are infectious during the week before and after the appearance of the rash. Babies with CRS may spread the virus for more than a year (Weisinger., 2002). Only humans are infected. Insects do not spread the disease.(Weisinger & Pesudovs., 2002). Once recovered, people are immune to future infections. Testing is available

that can verify immunity. (Centers for Disease Control and Prevention (CDC), 2005). Diagnosis is confirmed by finding the virus in the blood, throat, or urine. Testing the blood for antibodies may also be useful (Weisinger., 2002).

Rubella is preventable with the rubella vaccine with a single dose being more than 95% effective (Centers for Disease Control and Prevention (CDC., 2005). Often it is given in combination with the measles vaccine and mumps vaccine, known as the MMR vaccine (Centers for Disease Control and Prevention (CDC., 2005). With a population vaccination rate of less than 80%, however, more women might make it to childbearing age without developing immunity and issues could increase (Lambert., 2015) Once infected there is no specific treatment (Freij., 1988).

Rubella is a common infection in many areas of the world (Huong., 2014). Each year about 100,000 cases of congenital rubella syndrome occur (Lambert (2015).¹ Rates of disease have decreased in many areas including the Americas as a result of vaccination (Khandekar., 2007) (Freij., 1988). There are ongoing efforts to eliminate the disease globally (Centers for Disease Control and Prevention (CDC) .,2005). The name "rubella" is from Latin and means little red. It was first described as a separate disease by German physicians in 1814 resulting in the name "German measles (Atkinson., 2011) (Plotkin., 2001).

2.4.2 Life cycle:

- RV virion attaches to cell surface and translocated to coated pit.
- The coated pit pinches off to form a coated vesicle that contains the virion.
- The virion passes through a series of endosome with progressively acidic PH until it arrives at endosome where the environment is sufficiently acidic to trigger the uncoating process. The capsid proteins undergo conformational changes that result in the release of viral genomic RNA in cytoplasm.

- Release of viral RNA triggers transformation of the endosome, and vesicles are induced to form within the endosome. This lead to formation of replication complex
- The RER (rough endoplasmic reticulum) migrate to the vicinity of virus modified endosome.
- At this early stage of the infection , the RER is associated with the side of the vacuole where the vesicles are located
- As infection progresses, the RER surrounds the entire vacuole, which is lined internally with vesicles. While these events are occurring the virus modified endosome fuses to lysosome as part of its life cycle.
- The replication complex continuous in its life cycle as virus modified lysosome and eventually expels its lysosomal contents, including the vesicles, after fusion of lysosomal vacuole membrane to the plasma membrane.

2.4.3 Signs and symptoms:

Rubella has symptoms that are similar to those of flu. However, the primary symptom of rubella virus infection is the appearance of a rash (exanthem) on the face which spreads to the trunk and limbs (Appendix-1) and usually fades after three days (that is why it is often referred to as three-day measles). The facial rash usually clears as it spreads to other parts of the body. Other symptoms include low grade fever, swollen glands (sub occipital & posterior cervical lymphadenopathy), joint pains, headache, and conjunctivitis (Danovaro *et al.*, 2000).

The swollen glands or lymph nodes can persist for up to a week and the fever rarely rises above 38 °C (100.4 °F). The rash of German measles is typically pink or light red. The rash causes itching and often lasts for about three days. The rash disappears after a few days with no staining or peeling of the skin. When the rash clears up, the skin might shed in very small flakes where the rash covered it. Forchheimer's sign

occurs in 20% of cases, and is characterized by small, red papules on the area of the soft palate (Reef., 2006).

Rubella can affect anyone of any age and is generally a mild disease, rare in infants or those over the age of 40. The older the person is the more severe the symptoms are likely to be. Up to 60% of older girls or women experience joint pain or arthritic type symptoms with rubella (Robert & Jarrett., 2015).

In children rubella normally causes symptoms which last two days and include (Ackerknecht., 1982).

- Rash beginning on the face which spreads to the rest of the body.
- Low fever of less than 38.3 °C (101°F).
- Posterior cervical lymphadenopathy

In older children and adults additional symptoms may be present including (Ackerknecht & Erwin., 1982).

Swollen glands

- Coryza (cold like symptoms)
- Aching joints (especially in young women)

Serious problems can occur including the following:

1. Brain infections
2. Bleeding problems.
3. Birth Defects (Congenital)
4. Cataracts
5. Glaucoma
6. Heart Defects
7. Hearing Loss

Coryza in rubella may convert to pneumonia, either direct viral pneumonia or secondary bacterial pneumonia, and bronchitis (either viral bronchitis or secondary bacterial bronchitis) (Atkinson., 2007).

2.4.4 Congenital rubella syndrome:

Rubella can cause congenital rubella syndrome in the newborn. The syndrome (CRS) follows intrauterine infection by the rubella virus and comprises cardiac, cerebral, ophthalmic and auditory defects (Immunisation Handbook 2006). It may also cause prematurity, low birth weight, and neonatal thrombocytopenia, anemia and hepatitis. The risk of major defects or organogenesis is highest for infection in the first trimester. CRS is the main reason a vaccine for rubella was developed (Smith., 1881).

Many mothers who contract rubella within the first critical trimester either have a miscarriage or a still born baby. If the baby survives the infection, it can be born with severe heart disorders (Patent ductus arteriosus being the most common), blindness, deafness, or other life-threatening organ disorders. The skin manifestations are called "blueberry muffin lesions" (Smith., 1881) for these reasons, rubella is included on the TORCH complex of perinatal infections.

About 100,000 cases of this condition occur each year.

2.4.5 Causes of Rubella:

The disease is caused by Rubella virus, a toga virus that is enveloped and has a single-stranded RNA genome (Hess & Alfred., 1914). The virus is transmitted by the respiratory route and replicates in the nasopharynx and lymph nodes. The virus is found in the blood 5 to 7 days after infection and spreads throughout the body. The virus has teratogenic properties and is capable of crossing the placenta and infecting the fetus where it stops cells from developing or destroys them. During this incubation period, the patient is contagious typically for about one week before he/she develops a rash and for about one week thereafter.

Increased susceptibility to infection might be inherited as there is some indication that HLA-A1 or factors surrounding A1 on extended haplotypes are involved in virus infection or non-resolution of the disease (Hanshaw., 1985).

2.4.6 Diagnosis:

Rubella virus specific IgM antibodies are present in people recently infected by Rubella virus but these antibodies can persist for over a year and a positive test result needs to be interpreted with caution (Pan American Health Organization. 1998). The presence of these antibodies along with, or a short time after, the characteristic rash confirms the diagnosis.

2.4.7 Prevention:

Rubella infections are prevented by active immunization programs using live, disabled virus vaccines. Two live attenuated virus vaccines, RA 27/3 and Cendehillstrains, were effective in the prevention of adult disease. However their use in prepubertile females did not produce a significant fall in the overall incidence rate of CRS in the UK. Reductions were only achieved by immunization of all children.

The vaccine is now usually given as part of the MMR vaccine. The who recommends the first dose be given at 12 to 18 months of age with a second dose at 36 months. Pregnant women are usually tested for immunity to rubella early on. Women found to be susceptible are not vaccinated until after the baby is born because the vaccine contains live virus.

The immunization program has been quite successful. Cuba declared the disease eliminated in the 1990s and in 2004 the Centers for Disease Control and Prevention announced that both the congenital and acquired forms of rubella had been eliminated from the United States.

Screening for rubella susceptibility by history of vaccination or by serology is recommended in the United States for all women of childbearing age at their first

preconception visit to reduce incidence of congenital rubella syndrome (CRS). It is recommended that all susceptible non-pregnant women of childbearing age should be offered rubella vaccination. Due to concerns about possible teratogenicity, use of MMR vaccine is not recommended during pregnancy. Instead, susceptible pregnant women should be vaccinated as soon as possible in the postpartum period.

2.4.8 Treatment:

There is no specific treatment for Rubella; however, management is a matter of responding to symptoms to diminish discomfort. Treatment of newborn babies is focused on management of the complications. Congenital heart defects and cataracts can be corrected by direct surgery.

Management for ocular congenital rubella syndrome (CRS) is similar to that for age-related macular degeneration, including counseling, regular monitoring, and the provision of low vision devices, if required.

2.4.9 Prognosis:

Rubella infection of children and adults is usually mild, self-limiting and often asymptomatic. The prognosis in children born with CRS is poor.

2.4.10 Epidemiology:

Rubella is a disease that occurs worldwide. The virus tends to peak during the spring in countries with temperate climates. Before the vaccine to rubella was introduced in 1969, widespread outbreaks usually occurred every 6-9 years in the United States and 3-5 years in Europe, mostly affecting children in the 5-9 year old age group. Since the introduction of vaccine, occurrences have become rare in those countries with high uptake rates.

Vaccination has interrupted the transmission of rubella in the Americas: no endemic case has been observed since February 2009. Since the virus can always be reintroduced from other continents, the population still needs to remain vaccinated

to keep the western hemisphere free of rubella. During the epidemic in the U.S. between 1962-1965, rubella virus infections during pregnancy were estimated to have caused 30,000 still births and 20,000 children to be born impaired or disabled as a result of CRS. Universal immunization producing a high level of herd immunity is important in the control of epidemics of rubella.

In the UK, there remains a large population of men susceptible to rubella who has not been vaccinated. Outbreaks of rubella occurred amongst many young men in the UK in 1993 and in 1996 the infection was transmitted to pregnant women, many of whom were immigrants and were susceptible. Outbreaks still arise, usually in developing where the vaccine is not as accessible.

In Japan, 15,000 cases of rubella and 43 cases of congenital rubella syndrome were reported to the National Epidemiological Surveillance of Infectious Diseases between October 15, 2012, and March 2, 2014 during the 2012–13 rubella outbreaks in Japan. They mainly occurred in men of ages 31 to 51 and young adults aged 24–34.

2.4.11 Etymology:

The name rubella is sometimes confused with rubeola, an alternative name for measles in English-speaking countries; the diseases are unrelated (Ryan *et al.*, 2004). In some other European languages, like Spanish, rubella and rubeola are synonyms, and rubeola is not an alternative name for measles (Koichi *et al.*, 2007). Thus, in Spanish, "rubeola" refers to rubella and "sarampión" refers to measles.

2.5 . Cytomegalovirus:

Cytomegalovirus (from the Greek cyto-, "cell", and megalo-, "large") is a genus of viruses in the order Herpes virales, in the family Herpes viridae, in the subfamily Beta herpesvirinae. Humans and monkeys serve as natural hosts. There are currently eight species in this genus including the type species human herpes

virus 5 (HHV-5). Diseases associated with HHV-5 include glandular fever, and pneumonia.(Centers for Disease Control and Prevention (CDC., 2005). (Health Care Guideline: Routine Prenatal Care. 2010). It is typically abbreviated as CMV.

The species that infects humans is commonly known as human CMV (HCMV) or human herpesvirus-5 (HHV-5), and is the most studied of all cytomegalo viruses (Weisinger., 2002) Within Herpesviridae, CMV belongs to the Beta herpesvirinae subfamily, which also includes the genera Muro megalovirus and Roseolo virus (HHV-6 and HHV-7) (Freij *etal.*, 1988). It is related to other herpes viruses within the subfamilies of Alpha herpesvirinae that includes herpes simplex viruses (HSV)-1 and -2 and varicella-zoster virus (VZV), and the Gamma herpesvirinae subfamily that includes Epstein–Barr virus (Weisinger., 2002).

All herpes viruses share a characteristic ability to remain latent within the body over long periods. Although they may be found throughout the body, CMV infections are frequently associated with the salivary glands in humans and other mammals (Freij *etal.*, 1988). Other CMV viruses are found in several mammal species, but species isolated from animals differ from HCMV in terms of genomic structure, and have not been reported to cause human disease.

2.5.1 Species:

Several species of Cytomegalovirus have been identified and classified for different mammals (Freij *etal.*, 1988). The most studied is Human cytomegalovirus (HCMV), which is also known as Human herpes virus 5 (HHV-5). Other primate CMV species include Chimpanzee cytomegalovirus (CCMV) that infects chimpanzees and orangutans, and Simian cytomegalovirus (SCCMV) and Rhesus cytomegalovirus (RhCMV) that infect macaques; CCMV is known as both Panine herpes virus 2 (PaHV-2) and Pongine herpesvirus-4 (PoHV-4). SCCMV is called Cercopithecine herpesvirus-5 (CeHV-5) and RhCMV, Cercopithecine herpes virus 8 (CeHV-8). A further two viruses found in

the night monkey are tentatively placed in the Cytomegalovirus genus, and are called Herpesvirus 1 and Herpesvirus 3. Rodents also have viruses previously called cytomegalovirus's that are now reclassified under the genus Muromegalovirus; this genus contains Mouse cytomegalovirus (MCMV) is also known as Murid herpesvirus 1 (MuHV-1) and the closely related Murid herpesvirus 2 (MuHV-2) that is found in rats. In addition, there many other viral species with the name Cytomegalovirus identified in distinct mammals that are as yet not completely classified; these were predominantly isolated from primates and rodents.

2.5.2 Structure:

Viruses in Cytomegalovirus are enveloped, with icosahedral, Spherical to pleomorphic, and Round geometries, and T=16 symmetry. The diameter is around 150-200 nm. Genomes are linear and non-segmented, around 200kb in length (Lambert *etal.*, 2015).

2.5.3 Life cycle:

Viral replication is nuclear, and is lysogenic. Entry into the host cell is achieved by attachment of the viral glycoproteins to host receptors, which mediates endocytosis. Replication follows the dsDNA bidirectional replication model. DNA templated transcription, with some alternative splicing mechanism is the method of transcription. Translation takes place by leaky scanning. The virus exits the host cell by nuclear egress, and budding. Human and monkeys serve as the natural host. Transmission routes are contact, urine, and saliva (Centers for Disease Control and Prevention (CDC., 2005).

2.5.4 Genetic engineering:

The CMV promoter is commonly included in vectors used in genetic engineering work conducted in mammalian cells, as it is a strong promoter and drives constitutive expression of genes under its control (Reef *et al.*, 2002).

Chapter Three

- **Materials & Methods**

3. Materials and methods

3.1 Study design:

This is a cross-sectional, descriptive study.

3.2 Study area:

The study was conducted in Khartoum State in *Medical Military Hospital*.

Khartoum State is located in the middle of the populated areas in Sudan almost northeast center of the country between 16 degrees latitude north and 15 degrees latitude south and longitude 21 degrees west and 24 degrees longitude east, and expands an area amounting to 20,736 km (12884 Mile). Most of the population works in government service, the private sector, and banking. There are also a large number of merchants, and migrants and displaced people working in marginal activities.

3.3 Study duration:

The study was carried out during the period from September 2016 to December 2017.

3.4 Ethical Considerations:

This study was approved by the research committee - College of Medical Laboratory Sciences - Shendi University. Informed consent will be obtained from each participant before taking the samples.

3.5 Sample Size:

The study included 100 congenial infected babies.

3.6 study population:

Congenital babies (males and females) differ in age (few days to five year)

3.7 Sampling technique:

A simple random sample will be collected; five milliliters venous blood samples were taken in plane container.

3.8 Sample Separation:

The serum samples separated after collection. Then samples will be centrifuged for 5 minutes at 3000 rpm, the serum will be immediately transported to labeled Eppendorf safe-lock tubes with cap.

3.9 Sample Storage:

Serum samples kept in a laboratory refrigerator (4 - 8 °C) till time of working.

3.10 Data collection:

Personal data obtained by reviewing standard questionnaire.

3.11 data analysis:

The data was analyzed by using SPSS.

3.12 Laboratory tests

Identification of the viral Agent that caused the infection using Euroline Technique.

It's a new Technique for extensive Abs profile.

3.12.1 Euroblot master which used in this technique consist of: (Appendix -2)

- Cover
- Aspirating and dispensing head
- Priming tube
- Rocking Shaker
- LCD display
- Keyboard
- Housing
- Tubes (A-Z)
- Dispensing pumps (A-Z)
- Reagent bottle
- West bottle
- Measuring cylinder
- West and vacuum tube

3.12.2 Principle of Euroline Technique: (Appendix3)

- Membrane strips coated with parallel lines of several purified, biochemically characterized antigens are used as the solid phase, and the membranes are fixed on to synthetic foil.
- If sample is positive, specific antibodies in the diluted serum attach to antigen coupled to the solid phase.
- In second incubation step the attached antibodies react with alkaline phosphatase labeled anti human antibodies.
- In third step the bound Abs are stained with chromogen substrate solution which promotes a color reaction.
- On intense dark band at the line of the corresponding Ag appears if the serum sample contains specific Ab.
- Depending on the spectrum of Antigen used, it's possible to analyze several Abs next to each other and simultaneously under identical conditions.

3.12.3 Procedure of (Euroline technique):-

A. Preparation: (Appendix-4)

- 1- Open the power
- 2- Choose the test which you want to do (TORCH)
- 3- Write number of test samples you want to do (up to 30)
- 4- Determine type of Igs. (IgG or IgM or together)
- 5- Prepared adequate amount of conjugate and substrate, wash buffer and D.W according to number of samples
- 6- Put the strips in suitable position (IgG/IgM)
- 7- Choose start the test

B. testing: (Appendix-5)

- 1- Pre test (moist the strips) by using sample buffer for 5 min
- 2- Fill each channel with 1.5 ml of diluted serum (1:100).
- 3- Incubate at room temperature for 30 min on rocking shaker.
- 4- Aspirate off the liquid from each channel and wash 3 times with wash buffer.
- 5- Pipette 1.5 ml of diluted enzyme conjugate (IgG/IgM) into each channel
- 6- Incubate for 30 min at room temperature on each channel.
- 7- Aspirate off the conjugate from each channel and wash 3 times with wash buffer.
- 8- Pipette 1.5 ml of substrate each channel on each channel
- 9- Incubate about 10 min at room temperature
- 10- Aspirate off the substrate from each channel and wash 3 times with distilled water. (stop solution).
- 11- Air dry
- 12- Place test strips on scanner and evaluate by protocol.

Chapter Four

- **Results**

4. Result:

In this study (100) serum sample of congenital babies (62 male and 38female) in different Ages (neonate to five years) (66 less than 2year & 34more than 2 year) were collected, and we found Rubella IgM in (10) cases and IgG in (90) cases, Cytomegallo virus IgM in (10) cases and IgG in (90) cases, HSV-1 IgM in (20) cases and IgG in (95) cases, HSV-2 IgM in (0) cases and IgG in (10) cases (Table 4-1).

All viruses in study Assimilation (40%) of Congenital babies causative Agents. (Figure 4-1)

Rubella IgM (10%), Cytomegallo virus IgM (10%), HSV-1 IgM (20%), HSV-2 IgM (0%) (Figure 4-2).

In Rubella (8) of (10) cases (IgM) positive there were maternal Antibodies (IgG). (80% has maternal Abs and 20% hasn't maternal Abs)

In Cytomegallo virus (7) of (10) cases (IgM) there were maternal Antibodies (IgG). (70% has maternal Abs and 30% hasn't maternal Abs).

In HSV-1 all (20) cases (IgM) there were maternal Antibodies (IgG). (100% has maternal Abs) (Figure 4-3).

Also Rubella represent 25% of viruses, CMV represent 25% and HSV-1 represent 50%.

The viruses found in male (26) case which represent 65%, in female (14) case which represent 35%.

According to age we found 29 cases in less than 2 year which represent (72.5%), and 11 cases in more than 2 year which represent (27.5%).

(Table 4-1) Frequency of IgM & IgG viruses among cases

Rubella IgM	Rubella IgG	CMV IgM	CMV IgG	HSV-1 IgM	HSV-1 IgG	HSV-2 IgM	HSV-2 IgG
10	90	10	90	20	95	0	10

(Table 4-2) Distribution of viruses according to gender

	Descriptive	Frequency	Percent
	Male	62	62.0
	Female	38	38.0
	Total	100	100.0

(Table 4-3) Show the distribution of viruses according to age

	Descriptive	Frequency	Percent
	less than 2year	66	66.0
	more than 2 year	34	34.0
	Total	100	100.0

(Table 4-4) Relationship between age & viruses

Age	viruses +ve				Viruses -ve	Total
	Rubella	CMV	HSV-1	HSV-2		
less than 2yer	4	8	17	0	37	66
more than 2 year	6	2	3	0	23	34
Total	10	10	20	0	60	100

(Table 4-5) show Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	40.860 ^a	3	.000
Likelihood Ratio	53.954	3	.000
Linear-by-Linear Association	32.986	1	.000
N of Valid Cases	100		

a. 2 cells (25.0%) have expected count less than 5. The minimum expected count is 3.80.

P.value (.000) sig

(Table 4-6) Relationship between Gender & viruses

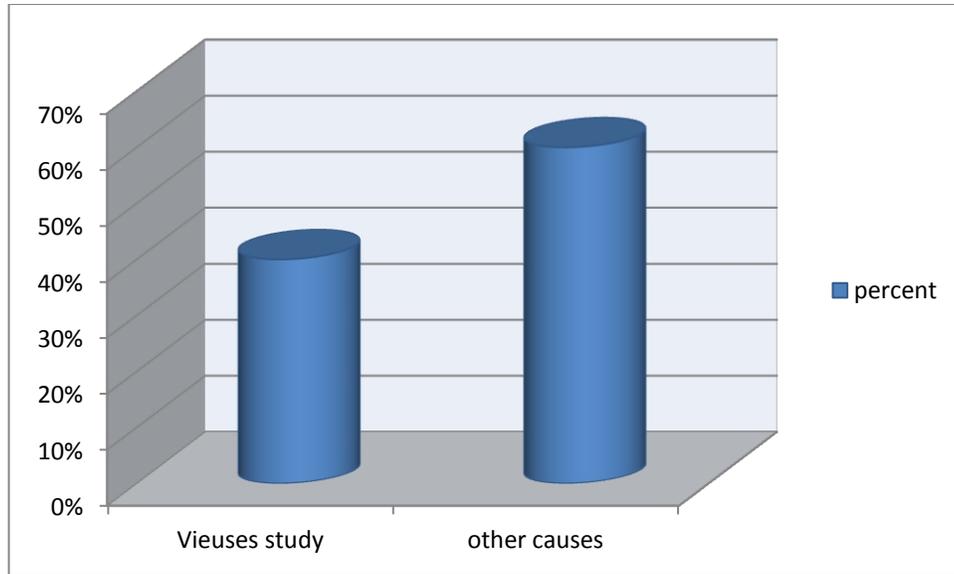
	viruses +ve				Viruses -ve	Total
	rubella	Cmv	hsv-1	hsv-2		
Male	7	5	14	0	36	62
Female	3	5	6	0	24	38
Total	10	10	20	0	60	100

(Table 4-7) show Chi-Square Tests

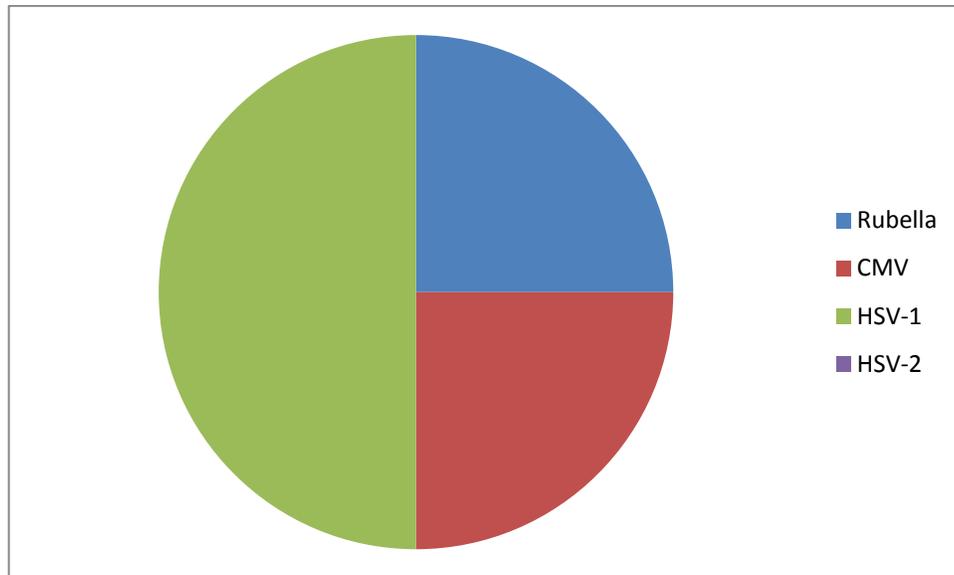
	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	34.343 ^a	3	.000
Likelihood Ratio	46.099	3	.000
Linear-by-Linear Association	27.725	1	.000
N of Valid Cases	100		

a. 2 cells (25.0%) have expected count less than 5. The minimum expected count is 3.40.

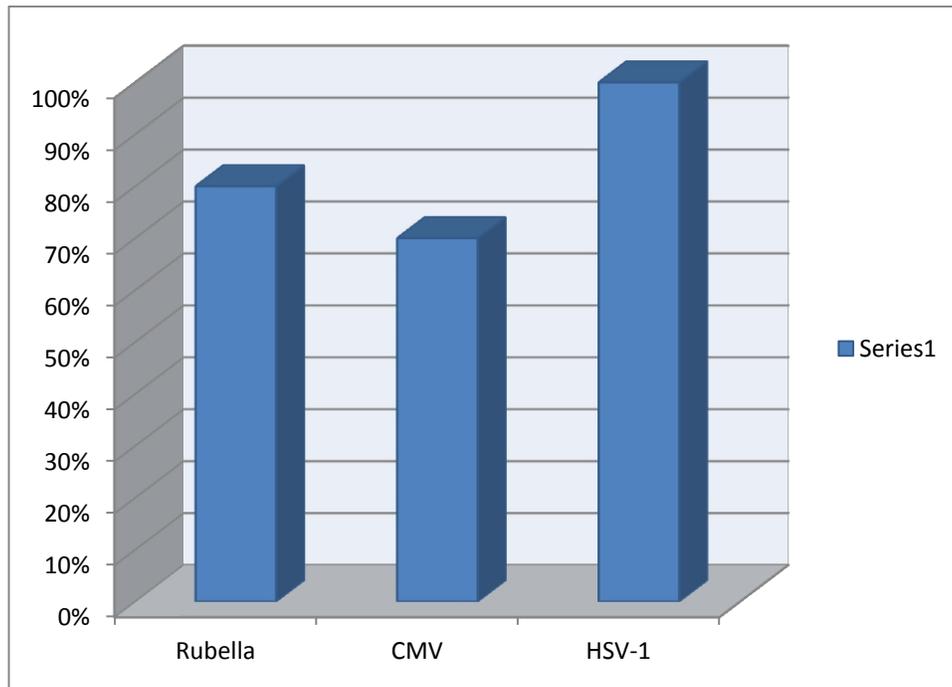
P.value (.000) sig



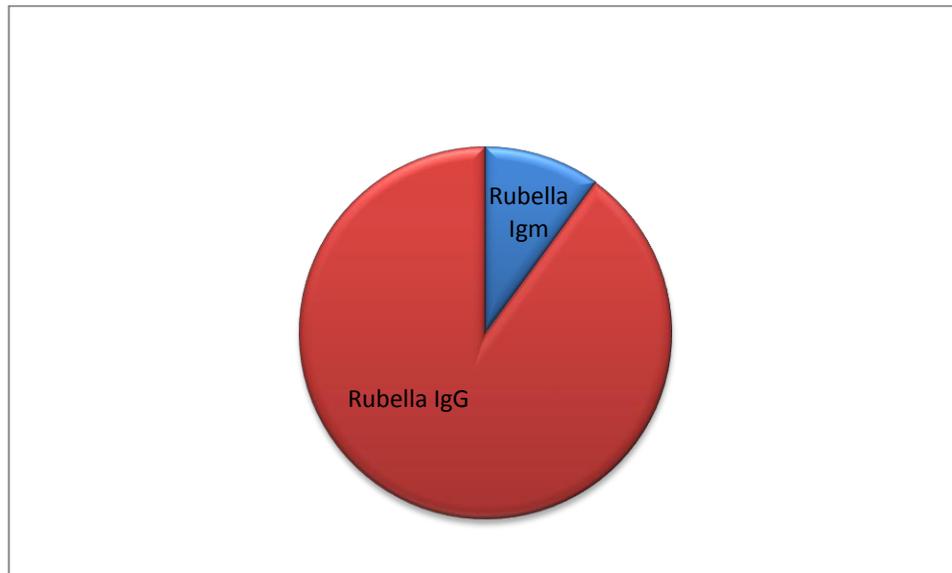
(Figure4-1) viruses and other causative Agents.



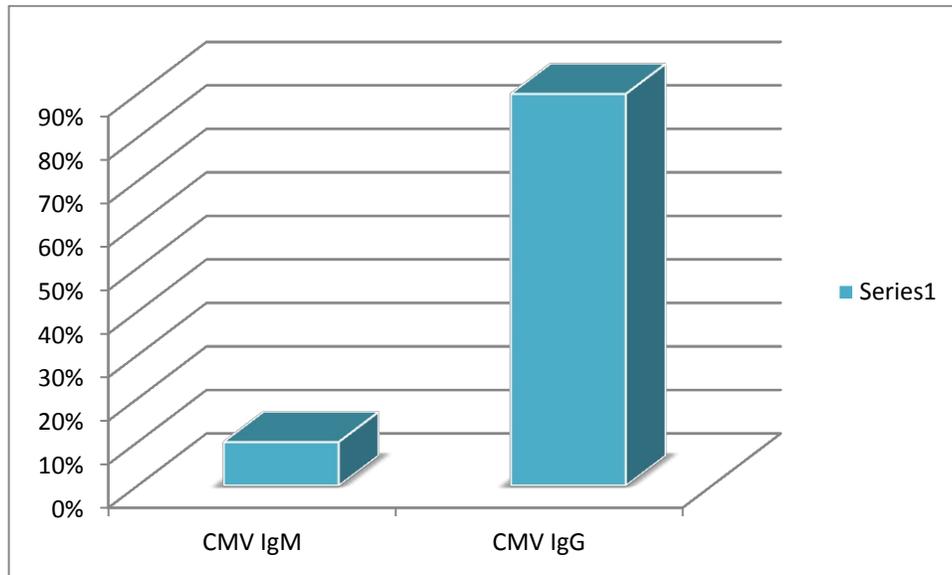
(Figure 4-2) IgM positive Viruses



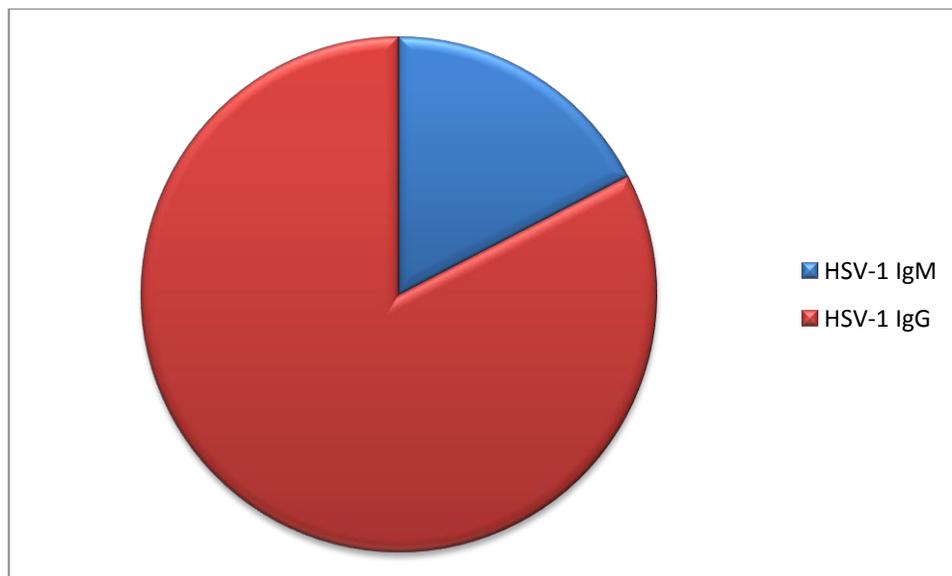
(Figure 4-3) Maternal Abs in IgM positive cases.



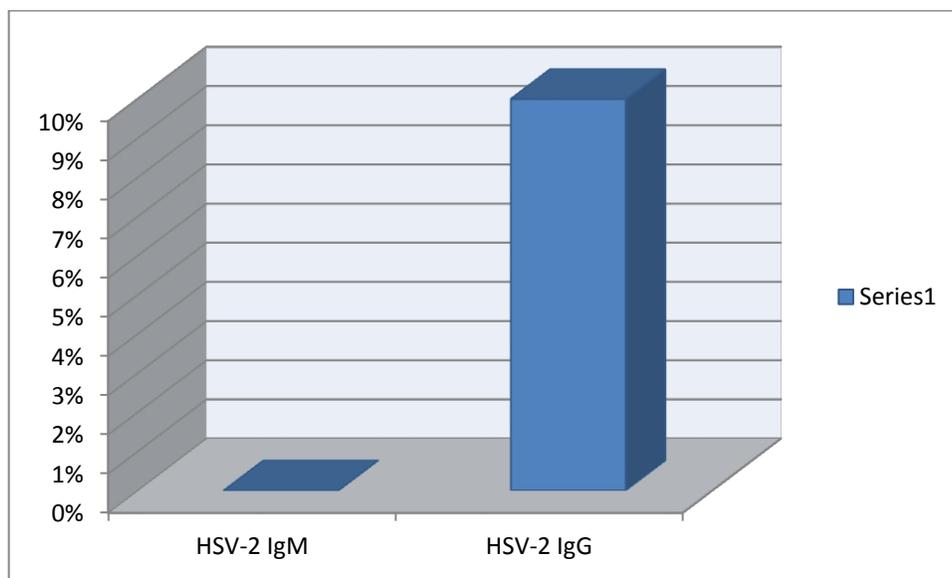
(Figure 4-4) Rubella IgM and IgG



(Figure 4-5) IgM and IgG CMV



(Figure 4-6) Show IgM and IgG HSV-I



(Figure 4-7) IgM and IgG in HSV-2

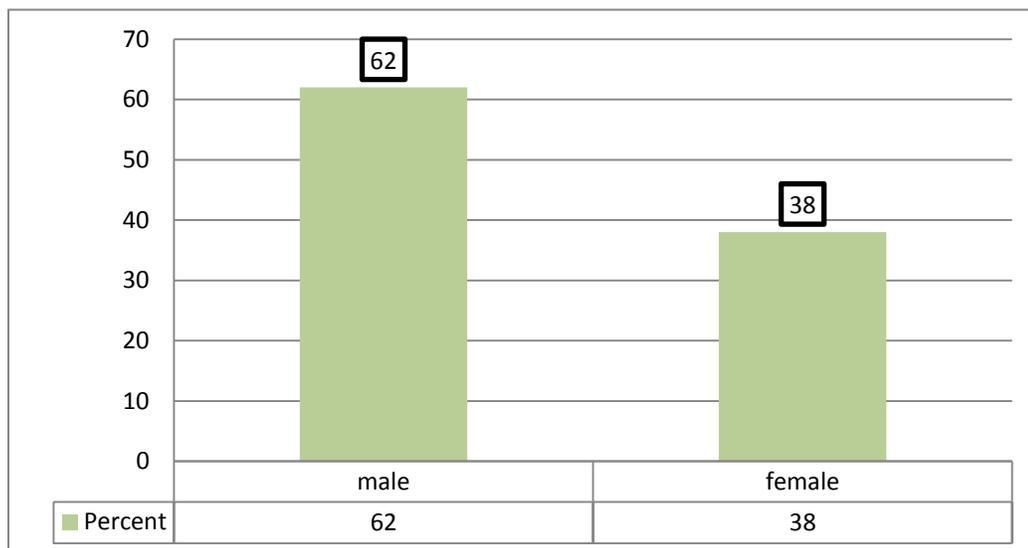


Figure (4-8) Show the distribution of viruses according to gender

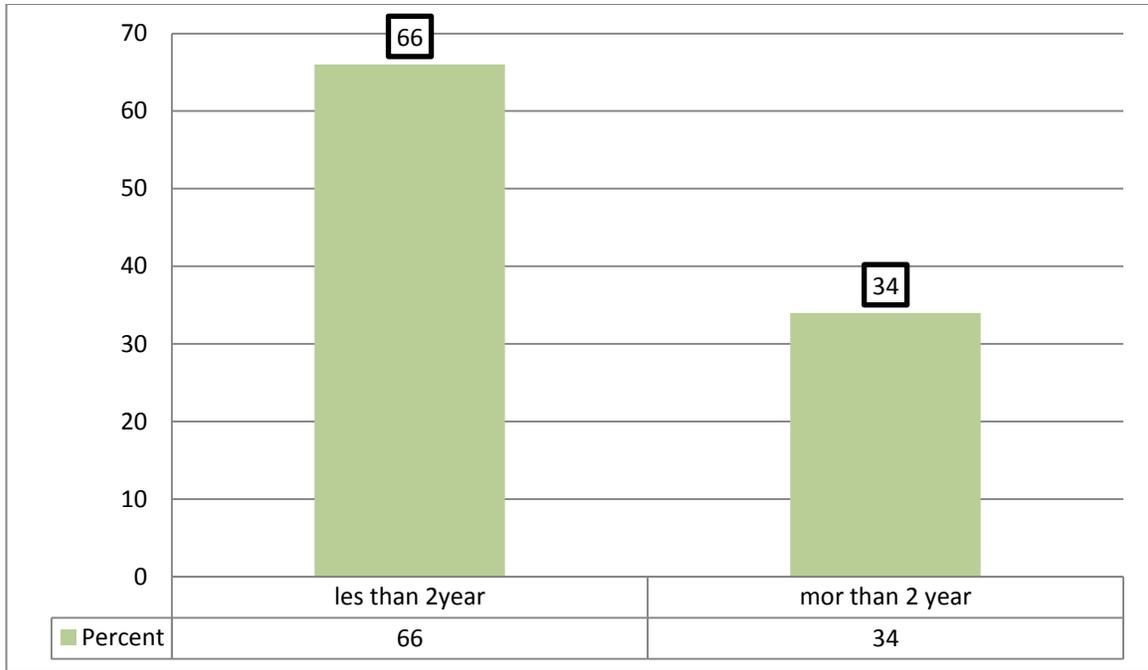
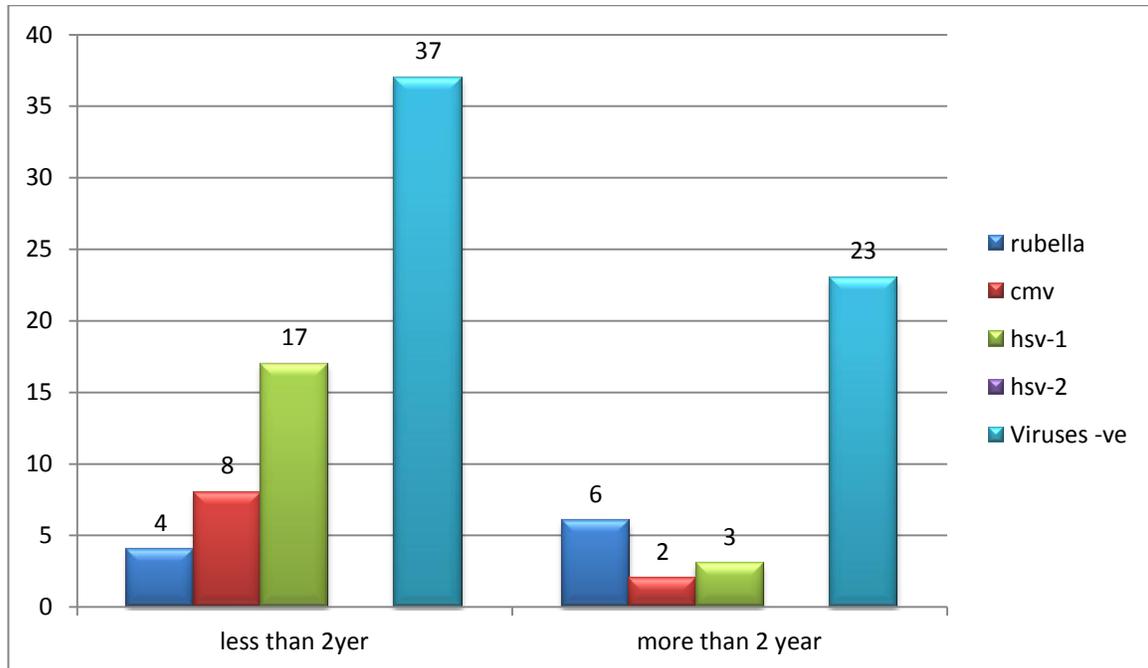
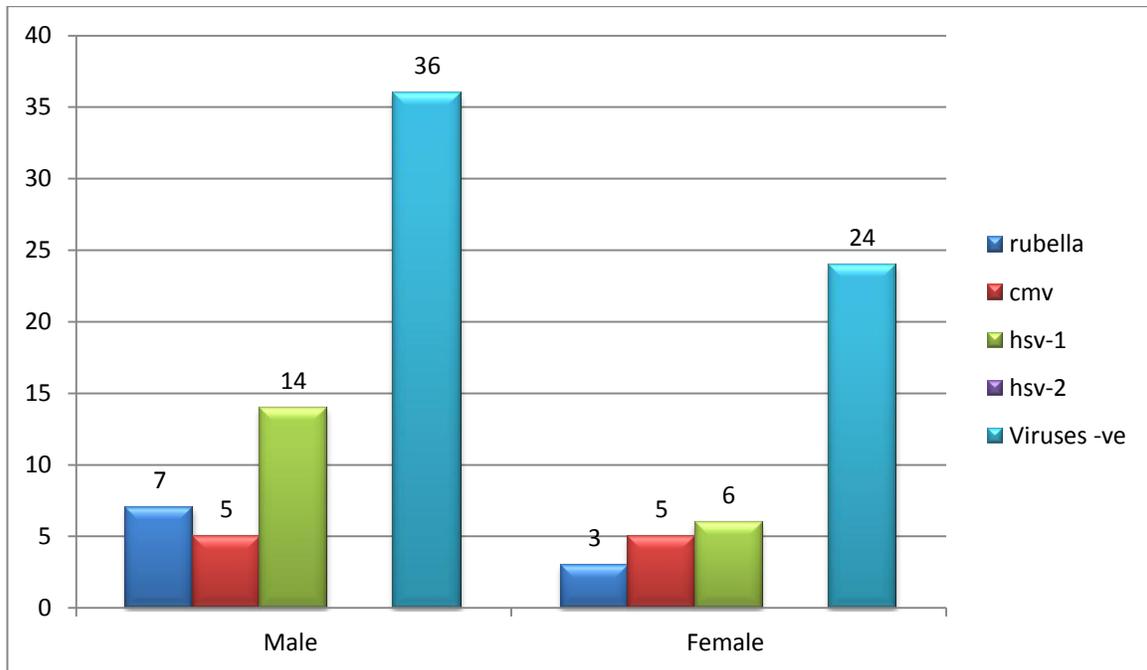


Figure (4-9) Distribution of viruses according to age



(Figure4-10) Relationship between age & viruses



(Figure 4-11) Relationship between Gender & viruses

Chapter Five

- **Discussion**
- **Conclusion**
- **Recommendation**

5.1 Discussion

This study was performed in medical military hospital, department of pediatric. From September 2016 to December 2017.

One hundred congenital babies (male and female) in different ages (neonate to five years), to detect the common viral infection as causative agent in congenital babies, to detect the presence of Rubella CMV HSV-1 and HSV-2 in patient's serum (IgG /IgM), and to correlate the viral infections with age and gender.

We found Rubella IgM in (10) cases and IgG in (90) cases, Cytomegallo virus IgM in (10) cases and IgG in (90) cases, HSV-1 IgM in (20) cases and IgG in (95) cases, HSV-2 IgM in (0) cases and IgG in (10) cases.

All viruses in study Assimilation 40% of Congenital babies causative Agents.

It's agreed with research (child cause of death) who 2000-2005 reports the other causative agent (not biological agent) represent high percent of congenital infection. Rubella IgM (10%), Cytomegallo virus IgM (10%), HSV-1 IgM (20%), HSV-2 IgM (0%).

This agree with the research done in Canada by (Brown et al. 2003) they report HSV-1 more likely than HSV-2 to transmitted to the neonate.

This is disagree of research done in united state by (Brown, *et al.*, 2005) they report HSV-2 more likely than HSV-1.

Also it disagrees with research done by (Jones., 1996) (HSV in Neonate. clinical presentation and management .neonatal Net w). Which report (most of congenital HSV infection caused by HSV-2 which represent 70%, HSV-1 represent 30%).

In Rubella 8 of 10 cases (IgM) positive there were maternal Antibodies (IgG) in (80% has maternal Abs and 20% hasn't maternal Abs)

In Cytomegallo virus (7) of (10) cases (IgM) there were maternal Antibodies (IgG). (70% has maternal Abs and 30% hasn't maternal Abs)

In HSV-1 all (20) cases (IgM) there were maternal Antibodies (IgG). (100% has maternal Abs).

This study also agree with the research (infectious etiology of congenital cataract based on torch screening in a tertiary eye hospital in chenni, Tamil Nadu, India) done by (Mahalakshmi *et al.*, 2010).

Another agree of this study with the research (Antenatal screening for congenital infection with Rubella, CMV and Toxoplasma) done by (Sfameni *et al.*, 1986).

According to age we found 29 cases in less than 2 year which represent (72.5%), and 11 cases in more than 2 year which represent (27.5%).

This agree with research done by (Claire *et al.*, 2002) long term outcomes of congenital CMV infection in Sweden and in the United Kingdom) they report congenital CMV incidence in neonate more likely than children.

The viruses found in male (26) case which represent 65%, in female (14) case which represent 35%.

This agrees with research done by (Green., 1992) which report male predominance in the incidence of symptomatic disease has been reported for some infectious agents.

5.2 Conclusion:

Congenital infection is one of the common recent infections which may lead to death of children.

Rubella is very serious if a pregnant woman catches it in the early stages of her pregnancy because it can profoundly damage the development of her unborn child.

The viral that cause congenital infection represent (40%).

HSV-1 is the common cause of congenital viral infection which represents (20%), also can be caused by Rubella (10%, and CMV (10%).

Most of viruses' causative agent hasn't treatment, but it treat according to symptoms and signs.

5.3 Recommendation:

- The correct diagnoses of infection are necessary to limit the prevalence of the disease.
- Treatment of most viral causative agent according to signs and symptoms.
- Treatment as acyclovir, valtrex give to relief pain and reduce period of infection.
- Treatment with hyper immune globulin in mothers with CMV infection has been should be effective in preventing congenital disease.
- Vaccination of mothers can protect the children from the congenital viral infection (Rubella), especially when vaccination before pregnancy which lead to formation of antibodies (IgG).
- To reduce neonatal infections all pregnant women should be screened for the congenital infections at the first antenatal visit within the first trimester and again in late pregnancy.
- Throughout the pregnancy, practice good personal hygiene, especially hand washing with soap and water, after contact with diapers or oral secretions. Sharing of food, eating and drinking utensils and contact with toddler's saliva should be avoided.

Chapter Six

- **References**
- **Appendix**
- **Questionnaire**

6.1 References

- Ackerknecht, Erwin Heinz (1982).** A short history of medicine. *Baltimore*: Johns Hopkins University Press. p.129.
- Atkinson, W; Hamborsky;McIntyre, L; Wolfe, S, eds. (2007).** "12. Rubella" (PDF). *Epidemiology and Prevention of Vaccine-Preventable Diseases* (10th ed).
- Atkinson, William (2011).** *Epidemiology and Prevention of Vaccine-Preventable Diseases* (12 ed). *Public Health Foundation*. pp. 301–323.
- Atreya CD, Mohan KV, Kulkarni S (2004).** "Rubella virus and birth defects: molecular insights into the viral teratogenesis at the cellular level". *Birth Defects Res. Part a Clin. Mol. Teratol.* 70.
- Best JM (2007).** "Rubella". *Semin Fetal Neonatal Med.* 12 (3): 182–92.
- Best, J.M.; Cooray, S.; Banatvala, J.E. (2005).** "45. Rubella". *Topley and Wilson's Microbiology and Microbial Infections. 2 Virology.* pp. 960–992.
- Brown ZA ,Wald A, Morrow RA ,Selk S, Zeh J, et al. 2003** Effect of serologic status and Cesarean delivery on transmission rate of herpes virus from mother to infant .page:203-209.
- Cdc. GOV (2014) Centers for Disease Control and Prevention (CDC) (2005).** "Elimination of rubella and congenital rubella syndrome—United States, 1969–2004". *MMWR Morb. Mortal Wkly. Rep.* 54 (11): 279–82. PMID 15788995.
- Centers for Disease Control and Prevention. Retrieved2007-07-03.**

Cooper, L.Z. (1975). "Congenital Rubella in the United States". In Krugman S Gershon A. Symposium on Infections of the Fetus and Newborn Infant. New York: Alan R. Liss. pp. 1.

Dangerous Pregnancies: Mothers, Disabilities, and Abortion in Modern America Leslie J. Reagan (2010).

Danovaro-Holliday MC, LeBaron CW, Allensworth C, et al. (2000). "A large rubella outbreak with spread from the workplace to the community". *JAMA*. 284(21): 2733–9.

Dayan GH, Castillo-Solórzano C, Nava M, et al. (2006). "Efforts at rubella elimination in the United States: the impact of hemispheric rubella control". *Clin. Infect. Dis.* 43 (Suppl 3): S158–63.

De Santis M, Cavaliere AF, Straface G, Caruso A (2006). "Rubella infection in pregnancy". *Reprod. Toxicol.* 21 (4): 390–8.

Donald G. McNeil Jr. (2015). "Rubella Has Been Eliminated From the Americas, Health Officials Say".

Edlich RF, Winters KL, Long WB, Gubler KD (2005). "Rubella and congenital rubella (German measles)". *J Long Term Eff Med Implants.* 15 (3):28.

EPI Newsletter (1998) (PDF). XX (4). *Pan American Health Organization.* August. Retrieved 2007-07- 03.

Forrest JM, Turnbull FM, Sholler GF, et al. (2002). "Gregg's congenital rubella patients 60 years later". *Med. J. Aust.* 177 (11-12).

Freij BJ, South MA, Sever JL (1988). "Maternal rubella and the congenital rubella syndrome". *Clin Perinatol.* 15 (2): 247–57.

Frey TK (1994). "Molecular biology of rubella virus". *Adv. Virus Res.* 44: 69–160.

Health Care Guideline: Routine Prenatal Care (2010). Fourteenth Edition. By the Institute for Clinical Systems Improvement.

Hess, Alfred Fabian (1914). "German measles (rubella): an experimental study". *The Archives of Internal Medicine*. Chicago. 13: 913–6.

Honeyman MC, Dorman DC, Menser MA, Forrest JM, Guinan JJ, Clark (1975). "HLA antigens in congenital rubella and the role of antigens 1 and 8 in the epidemiology of natural rubella". *Tissue Antigens*. 5 (1): 12–8.

http://kidshealth.org/parent/infections/skin/german_measles.html

<http://www.elespectador.com/noticias/salud/colombia-fue-declarada-libre-de-sarampion-y-rubeola-articulo-470243>

<http://www.eltiempo.com/vida-de-hoy/salud/colombia-libre-de-sarampion-y-rubeola-13396295-4>

[http://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(14\)60712-1/fulltext](http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(14)60712-1/fulltext)

Huong McLean (2014). "3 Infectious Diseases Related To Travel". CDC health information for international travel 2014.

ICTV. "Virus Taxonomy: 2014 Release". Retrieved 15 June 2015.

Immunisation Handbook (2006). Ministry of Health,. ISBN 0-478-29926 5. Retrieved 2007-07-03.

J.B. Hanshaw, J.A. Dudgeon, and W.C. Marshall (1985). Viral diseases of the fetus and newborn. W.B. Saunders Co., Philadelphia.

Kendall Morgan for Addgene Blog. Apr 3, (2014).

Khandekar R, Sudhan A, Jain BK, Shrivastav K, Sachan R (2007). "Pediatric cataract and surgery outcomes in Central India: a hospital based study". *Indian J Med Sci*. 61 (1): 15–22.

Koichi Yamanishi; Arvin, Ann M; Gabriella Campadelli-Fiume; Edward Mocarski; Moore, Patrick; Roizman, Bernard; Whitley, Richard (2007). Human herpesviruses: biology, therapy, and immunoprophylaxis. Cambridge, UK: *Cambridge University Press*.

Lambert, N; Strebel, P; Orenstein, W; Icenogle, J; Poland, GA (2015). "Rubella.". *Lancet*. 385: page 2297–307.

Mahalakshmi B, et al (2010). India J Med Res.

Marissa Selner; Winnie Yu (2012). "German Measles (Rubella)".*Health line*.

Michael, M. (1908). "Rubella: Report of Epidemic of Eighty Cases". In Treat, E.B. *Archives of Pediatrics*. p. 604.

Neighbors, M; Tannehill-Jones, R (2010). "Childhood diseases and disorders".Human diseases (3rd ed). Clifton Park, New York: Delmar, Cengage Learning. pp. 457–79.

PAHO (2015).Americas region is declared the world's first to eliminate rubella". Retrieved 6 May 2015.

Plotkin SA (2001). "Rubella eradication". *Vaccine*. 19 (25-26): 3311–9.

Public Health image library (PHIL) 1966 Retrived 24-may 2015.

Public Health Image Library (PHIL)". Center for Disease Control and Prevention. 1966. Retrieved 2015.

Reef S (2006). "Rubella mass campaigns". *Curr. Top. Microbiol. Immunol*. 304: 221–9.

Reef SE, Frey TK, Theall K, et al. (2002). "The changing epidemiology of rubella in the 1990s: on the verge of elimination and new challenges for control and prevention". *JAMA*. 287 (4): 464–72.

Robert Kliegman, Waldo E. Nelson, Hal B. Jenson, Karen J. Marcadante, M.D., Richard E. Behrman. Nelson (2006), *Essentials of Pediatrics*, page 467.

Robert kligmanwaldoE.Nelsonetal. Nelson Essentials of pediatric, page 467(1987)

Robert R. Jarrett (2015) .Numbered Diseases of Childhood: THIRD Disease – Rubella". Pediatric House Calls.

Rubella (German Measles, Three-Day Measles)". *cdc.gov*. (2014) Retrieved 30 March 2015.

Rubella: Complications". Diseases and Conditions. Mayo Foundation for Medical Education and Research. 13 May 2015

Ryan K J, Ray CG, eds. (2004). *Sherris Medical Microbiology* (4th ed.).

Sfameni SF, *et al* (1986). Aust N Z J *ObstetGynaecol*.

Smith, J. L (1881). Contributions to the study of Rötheln. Trans. *Int. Med. Congr.* Phil.4, 14.

Stegmann B J, Carey J C (2002). "TORCH Infections. Toxoplasmosis, Other (syphilis, varicella-zoster, parvovirus B19), Rubella, Cytomegalovirus (CMV), and Herpes infections". *Curr Women's Health Rep.* 2 (4): 253–8.

Viral Zone". ExPASy. Retrieved 15 June 2015.

Watson J C, Hadler S C, Dykewicz C A, Reef S, Phillips L (1998). "Measles, mumps, and rubella—vaccine use and strategies for elimination of measles, rubella, and congenital rubella syndrome and control of mumps: recommendations of the Advisory Committee on Immunization Practices (ACIP)". *MMWR Recomm Rep.* 47 (RR-8): 1–57.

Weisinger H S, Pesudovs K (2002). "Optical complications in congenital rubella syndrome". *Optometry*. 73 (7): 418–24.

Wellington, N Z. (2006) — Rubella" (PDF) Chapter 11.

Wesselhoeft C (1949). "Rubella and congenital deformities". *N. Engl. J. Med.* 240 (7): 258–61.

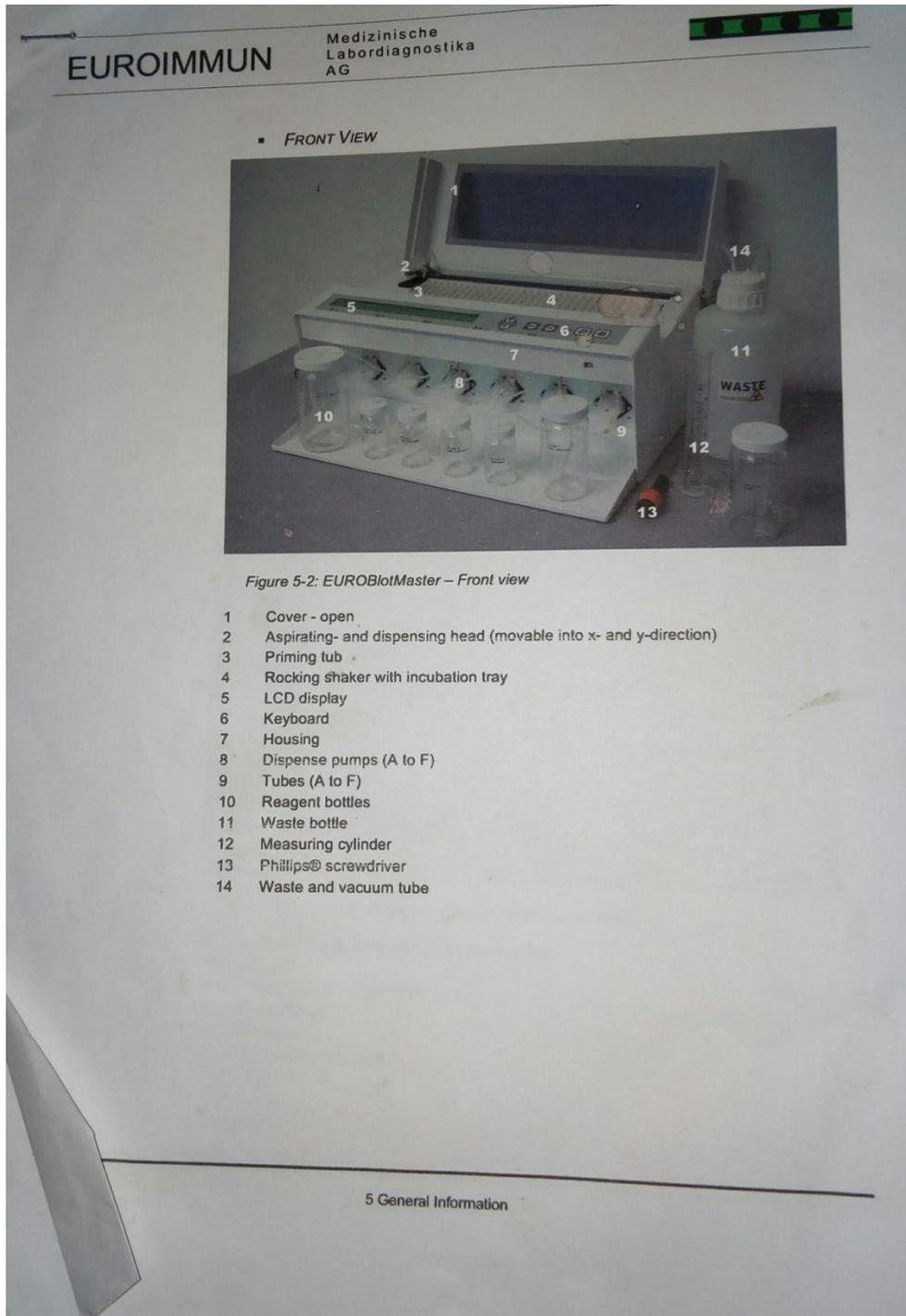
Wkly (2011). *Epidemiol Rec* . 86 (29): 301–16. 15 PMID 21766537.

6.2Appendix

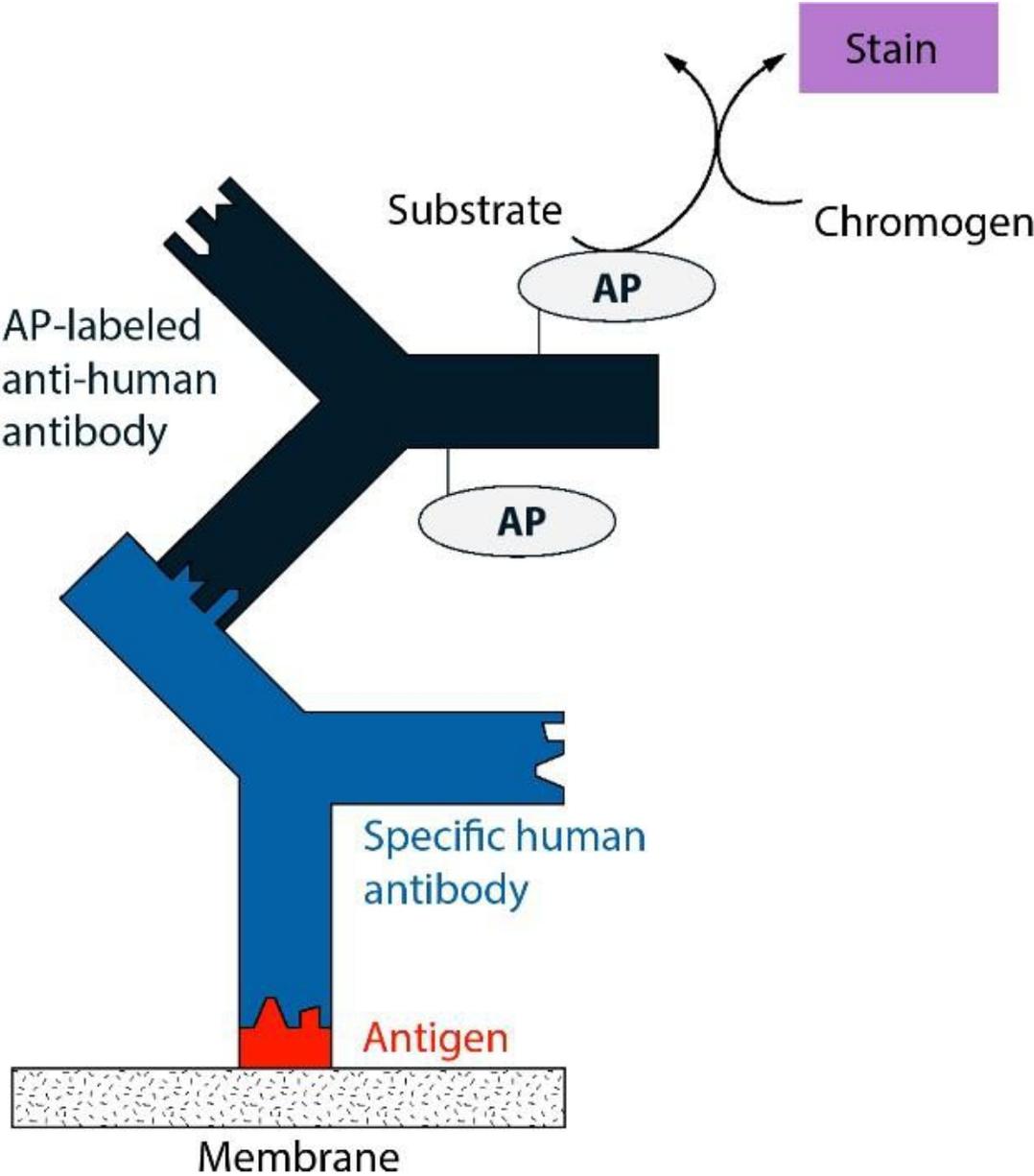
Appendix I



Appendix II



Appendix III



Appendix IV

ROIMMUN Medizinische
EUROIMMUN Medizinische Labordiagnostika AG

7.5.2 How to Start a Test Run

Switch on device → Switch on the system (power switch on the back left-hand side). The display reads:

Main menu:
List of assays
Confirm with

Select test programme
List of assays:
01 Euro01 AAb EL30
Select test using or
Confirm with

Please see chapter 7.4 for further information about test programmes.

Enter number of strips
Please enter the number of strips which shall be processed:
No. of strips: 00 Max: 30
Enter number of strips using
Confirm with

i If more than one conjugate is used in a test run, the number of strips per conjugate will be requested at this point. For details see chapter 7.5.3.

Load fluid
Pump A: Wash buffer xx ml
- Priming, + Postpone, ENTER Ready
Use to fill tube with reagent (push key twice)
Confirm with

Reagent type and volume for channel A is shown in the top line on the display. The requested volume is the volume needed for the test run. Dead volume of containers and tubes as well as the priming volume are not taken into account.

i It is recommended to prepare 5ml reagent in addition for priming and in order to compensate for the dead volume of the containers.

There is the possibility to postpone the reagent insertion by using the Yes-key (+ Postpone). The control LED is switched off. The reagent is requested 5 minutes before its application. The function is usually available for substrate only.

Repeat procedure for remaining channels.

Pump B: IgA Conjugate xx ml
- Priming, + Postpone, ENTER Ready
Use to fill tube with reagent (Push key twice)
Confirm with

... (intermediate steps) ...

- 28 - 7 Operating Instructions

After the priming of each channel, the display reads:

Is waste bottle empty?

Check waste container.
Confirm with 

Start test run

Start assay?
01 Euro01 AAb EL30

Confirm with 

The EUROBlotMaster is performing the test run.



The upper line of the display shows the description of the current assay step: "S" stands for "step" and "A" for "assay". The numbers and letters give the approximate period of time (hours and minutes) until the completion of the assay step or test run (hh:mm).

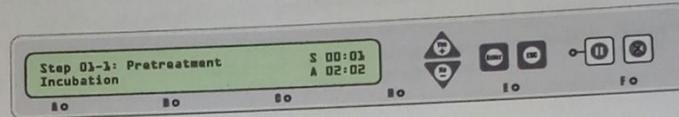


Figure 7-3: Display of assay step and duration

Insert strips

Place the strips into the channels of the incubation tray, when requested to do so by a visual and audible signal. Always start with the first position.

Step 01-1: Strips S 00:01
Insert strips! Compl.? A 01:53

Insert strips into tray.
Confirm with 



Use moderate pressure on the sticky end of the strips to fix them to the bottom of the tray.

Some intermediate steps follow:

... (intermediate steps) ...

Add samples

The EUROBlotMaster fills the channels with diluent buffer. Add undiluted samples to each channel, when requested to do so by a visual and audible signal.

Step 04-1: Sample incub. S 00:30
Add 15ul sample! Compl.? A 01:47

Pipette undiluted sera.
Confirm with 

... (intermediate steps) ...

Appendix V

EUROIMMUN Medizinische Labordiagnostika AG

Incubating the EUROLINE/Westernblot/EUROLINE-WB
using the EUROBlotMaster or manually on a rocking platform

		membrane strip incubation channel
Pipette:	1.5 ml per channel	buffer
Incubate:	5-15 min shaking, depending on test system	
Aspirate off:		
Pipette:	1.5 ml per channel	diluted samples
Incubate:	30 min shaking	
Wash:	1.5 ml buffer, 5 min incubation, aspirate off	3X buffer
Pipette:	1.5 ml per channel	enzyme conjugate
Incubate:	30 min shaking	
Wash:	1.5 ml buffer, 5 min incubation, aspirate off	3X buffer
Pipette:	1.5 ml per channel	chromogen/substrate solution
Incubate:	10 min shaking	
Wash:	rinse with distilled water, aspirate off	
Evaluate:	with EUROLineScan or visual assessment	

Applicable for most EUROLINE/Westernblot/EUROLINE-WB test kits

بسم الله الرحمن الرحيم

University of Shendi

Faculty of Graduate Studies and Scientific Research

Questionnaire

Name.....Date...../...../.....

Age < 2 year more than 2 year

Gender male.....female.....

Previous infections of children yes.....no.....

If mother suffer from any symptoms yes.....no.....

If mother vaccinated

Before pregnancy ()

After pregnancy ()

No vaccinated ()

