RELATIONSHIP BETWEEN SERUM IgM LEVELS AND LIVER FUNCTION IN RUBELLA AND MEASLES VIRUS INFECTION IN CHILDREN BELOW FIVE YEARS ATTENDING MATERNAL CHILD HEALTH CLINICS IN SELECTED HOSPITALS IN NAIROBI COUNTY

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2018
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This thesis is my original work and has not been submitted to any other institution for any award.

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DEDICATION

This thesis is dedicated to my parents James Mwangi Kiiru and Mary Nyambura who have been a pillar in my journey of education. My brothers; Joshua Kiiru, Samuel Munga, John Kimaku and my son Marc Mwangi for their unwavering support and encouragement.
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ACRONYMS AND ABBREVIATIONS

ALB  Albumin
ALT  Alanine aminotransferase
ALP  Alkaline phosphatase
AST  Aspartate aminotransferase
CDC  Centre for disease control
CRS  Congenital Rubella Syndrome
DBILI Direct bilirubin
EIA  Enzyme immune assay
ELISA Enzyme immunosorbent Assay
EPI  Expanded programme on immunization
IgM  immunoglobulin M
LDH  lactate dehydrogenase
MDH  malate dehydrogenase
MDG  millennium development goals
MCV  measles containing vaccine
MMR  measles mumps rubella vaccine
MeV  measles virus
NADH nicotinamide Adenine Dinucleotide
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RPM</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>reverse transcriptase polymerase chain reaction</td>
</tr>
<tr>
<td>RuV</td>
<td>rubella virus</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
</tr>
<tr>
<td>TBILI</td>
<td>Total bilirubin</td>
</tr>
<tr>
<td>TP</td>
<td>Total protein</td>
</tr>
<tr>
<td>USA</td>
<td>United State of America</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra violet</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</tbody>
</table>
ABSTRACT

Rubella and measles virus are important diseases of children under five years of age which are preventable through immunization. Despite intervention through immunization, outbreaks of rubella and measles in Kenya are still common. The main objective of current study was to determine the correlation between liver function and IgM levels in children under five years of age in Nairobi County. The study involved 235 subjects and was carried out at Kenyatta National Hospital, Mbagathi District Hospital and Mama Lucy Kibaki Hospital. Out of the 235 study subject who were qualitatively tested for both measles and rubella using IgM assay, only three (1.28%) were positive for measles and five (2.13%) positive for rubella. It was noted that these study subjects had not been vaccinated before for the studied viruses. Liver function tests were analyzed for all the two hundred and thirty five study subjects. Among the liver function parameters, total protein and albumin showed a very strong negative correlation (r = -0.932 and r = -1.000 respectively) with measles IgM concentration; this was statistically significant at p = 0.035 and p = 0.007 respectively. Positive correlation was shown between measles IgM concentration and AST(r=0.247), ALT(r=0.637), ALP(r=0.935), TBILI(r=0.719) and DBILI (r=0.654). This positive correlation was statistically significant for AST (p=0.032) and ALT (p=0.021). TP, ALB, ALP, TBILI and DBILI showed a negative correlation (r=-0.316, r=-0.872, r=-0.804, r=-0.550 and r=-0.404 respectively) with rubella IgM concentration which was statistically significant for TP and ALB at p=0.015 and p= 0.031 respectively. Positive correlation was shown between rubella IgM concentration and AST(r=0.333) and ALT(r=0.360). This positive correlation was statistically significant for AST (p=0.044) and ALT (p=0.028). The study has established that the two diseases still affect children below the age of five years in Nairobi County. The metabolic and excretion functions of the liver for the studied population...
were affected by these viral infections as expressed by an increase in the mean levels of transaminases (AST=206 iu/L, ALT=202 iu/L) and bilirubin (TBILI=43µmol/L and DBILI=30µmol/L) in blood. Similar studies should be undertaken in all the counties in Kenya to establish the status of rubella and measles in children below five years of age since the current study worked on point prevalence of Rubella and measles in Nairobi County. Liver function tests should be included during the baseline study of the suspected cases of rubella or measles infection.
CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Measles is caused by measles virus from the genus *Morbillivirus* for which man is the only reservoir. Measles virus (MeV) is a single-stranded, negative-sense, enveloped RNA virus of the genus *Morbillivirus* within the family *Paramyxoviridae*. Rubella virus (RuV) is the pathogenic agent of the disease rubella, and is the cause of congenital rubella syndrome (CRS) when infection occurs during the first trimester of pregnancy. Rubella virus is the only member of the genus *Rubivirus* and belongs to the family *Togaviridae*, whose members have a genome of single-stranded RNA of positive polarity, which is enclosed by an icosahedral capsid (CDC, 2011). Clinical signs and symptoms of both rubella and measles are almost similar. Rubella infection in women during early pregnancy can severely affect the fetus, resulting in miscarriage, fetal death, or the combination of disabling conditions collectively called congenital rubella syndrome (CRS), which includes heart disease, blindness and deafness (Sugishita *et al.*, 2013)

Measles is highly contagious and can result in complications that include encephalitis, otitis media, blindness, pneumonia and diarrhoea. Mortality is normally high in children with malnutrition particularly in developing countries including Kenya (WHO, 2009). On the other hand; rubella infection causes a relatively mild disease in children. The highly effective, safe and relatively inexpensive measles and rubella vaccines, protect individuals from infection, and their widespread use can completely stop the spread of the viruses in populations and achieve and maintain high levels of immunity. From the reports of WHO some years back (WHO, 2000), it was estimated that 535,000 children died of measles, the majority in developing countries, and
this burden accounted for 5% of all under-five mortalities. Laboratory confirmation of acute infection revealed more cases of rubella virus infection rather than measles (WHO, 2000). Rubella infection has been identified as a leading cause of birth defects commonly known as Congenital Rubella Syndrome (CRS). In the United States of America rubella virus was a common disease that occurred primarily among young children of 6-9 years until the live attenuated rubella vaccine was licensed (WHO, 2000). Introduction of the vaccine has helped to eradicate rubella virus and prevent its congenital infections (Huang et al., 2013). In this outbreak, rubella was detected using the case-based measles surveillance system. The number of confirmed rubella cases in United States was 473 in 2010, 604 in 2011, 300 in 2012, 336 in 2013 and 646 in 2014. (LeBaron et al., 2009).

In developing countries, approximately 10–30% of measles cases require hospitalization, and one in a thousand of these cases among children results in death from measles complications. Improving measles vaccination coverage and reducing measles-related deaths is a global imperative, particularly as it relates to the United Nation’s Sustainable Development Goal 3 (SDG 3) that aims, to ensure healthy lives and promote wellbeing for all at all ages. The United Nations took the initiative to have measles and rubella routine vaccination to reduce child mortality. The infectiousness of measles and rubella easily leads to global spread, and even countries that eliminated their indigenous transmission remain vulnerable to outbreaks. Despite having routine vaccination programmes, measles and rubella viruses continue to cause infections in susceptible persons brought about by low immunization coverage in some countries. Rubella vaccine is not given in most African countries (Hickman et al., 2011).
Between September 2005 and May 2007, a total of two thousand 2544 confirmed measles cases were reported from 71 (91%) of the 78 districts in Kenya. Within this period of measles outbreak, it was observed that April and August 2006 had the highest number of reported cases which were 375 and 332 confirmed cases respectively. New strains of measles viruses identified as genotype B3 and D4 were isolated from eighty Somali immigrants distributed within the seventy-one districts and one from Rift Valley province respectively (CDC, 2007). Measles vaccination campaign was reported to be low in 2009. This was due to limited resources to conduct outreaches and mobile services. The result of the inadequacy was accumulation of large numbers of unvaccinated children which lead to measles outbreak since 2010.

In Kenya, the proportion of children aged 12-23 months that are reported to have received all recommended vaccinations is 77.4%. However, this proportion varies from 48.3% in the North Eastern Province to 85.8% in the Central Province. This geographical inequality in coverage reflects the variation in the influence of determinants of full vaccination across the different provinces. In Nairobi, 73% of children in this age range are reported to have received all vaccination, but estimates in the slums within the city are usually much lower. A study across the slums of Nairobi showed that full vaccination coverage of children was about 44% in these settlements compared to 73% for the whole of Nairobi. Polio and measles vaccinations in these settlements were substantially lower than coverage in Nairobi, but slightly higher than that in the rural areas of Kenya, despite the overall immunization coverage being lower than in the rural areas. Lower immunization coverage rates have also been observed in facilities that serve slums settlements in Nairobi and may be due to missed opportunities among clinic attendees and inappropriately administered vaccines (Mutua et al., 2011). The reports of 2169 cases of measles in 2012 alone from 60% of the country’s districts meant that measles may re-establish in Kenya.
and hence the very need to conduct campaign to protect all the susceptible in the populations under 5 years (WHO, 2012).

In 2011, the proportion of Kenyan children aged 12-23 months that were reported to have received all recommended vaccinations was 77.4%. However, this proportion varied from 48.3% in the North Eastern Province to 85.8% in the Central Province. This geographical inequality in vaccination coverage reflects the variation in the campaign against measles across different provinces. In Nairobi, 73% of children in this age range are reported to have received all vaccination, but estimates in the slums within the city are usually much lower. A study across the slums of Nairobi showed that full vaccination coverage of children was about 44% compared to 73% for the whole of Nairobi. Measles vaccinations in these settlements were slightly higher than that in the rural areas of Kenya (Mutua et al., 2011).

The current study aimed at establishing the status of these two viral infections affecting children below the age of five years in the metropolitan county of Nairobi. On the other hand the study also aims at investigating the effect of these viral infections on liver functions.
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Rubella and Measles diseases

Rubella virus is an enveloped RNA virus of genus *Rubivirus* within the family *Togaviridae*. The virus is relatively unstable and is inactivated by lipid solvents, trypsin, formalin, ultraviolet light, low pH, heat, and amantadine. The incubation period for rubella is 14-20 days in the mother whereby 20-50% of the infections are subclinical. Rubella re-infection is possible in immune suppressed patients. Rubella infections at 8-10 weeks of pregnancy results in 90% damage in the surviving infants, thus leading to common multiple defects which include congenital deafness, congenital heart disease, eye defects and microcephally. If the infection takes place around 11-16 weeks of pregnancy then the fetal damage reduces by 10-20%. Infections after 16 weeks of gestation do not affect the fetus. (LeBaron *et al.*, 2009). Rubella is still very common in developed countries whereby nine cases were reported in England and Wales. Screening for rubella susceptibility was done in this region and the report showed a slight decrease from 98.10% in 2012 to 97.79% in 2013 (Kancherla *et al.*, 2014).

Measles virus is a negative sense single stranded RNA virus within the family *Paramyxoviridae*. The virus lacks neuraminidase enzyme and it is therefore grouped in the genus, *Morbillivirus*. The envelope consists of haemagglutinin protein which acts as a means of attachment to susceptible host cells. The negative-sense, single stranded RNA genome is contained within a helical nucleocapsid in the virion. The genome consists of 15,894 nucleotides. The virus is rapidly inactivated by heat, light, acid pH, ether and trypsin. The virus has a short survival time (less than two hours) in the air, or on objects and surfaces (Czajka *et al.*, 2009).
Measles and Rubella viruses are transmitted through respiratory droplets, or by direct contact with nasal and throat secretions of infected persons. The primary site of infection is the respiratory epithelium. Further viral replication occurs in regional and distal reticulo-endothelial sites. A secondary viraemia occurs 5 to 7 days after initial infection. During this viraemia, there may be infection of the respiratory tract and other organs. On the other hand Rubella virus is usually spread through the air via coughs of people who are infected. (Huang et al., 2013).

After incubation period of 7-14 days, the patient enters the prodromal phase of illness with fever, malaise, sneezing, rhinitis, congestion, conjunctivitis and cough. Koplik’s spots, which are pathognomonic for measles and rubella appear on the buccal and lower labial mucosa opposite the lower molars. Other diseases with similar signs and symptoms include mumps, shingles and small pox have similar clinical manifestations. The distinctive maculopapular rash appears about 4 days after the prodromal phase of illness. This starts behind the ears and the forehead and then the rash spreads to involve the whole body. The virus then affects the hosts by binding onto signaling lymphocyte activation molecule that is expressed on CD46 immune cells (Barreto et al., 2006). Measles virus affects the liver by breaking the cell membrane of the hepatocytes thereby interfering with the metabolic activities. Damage to liver cell membrane integrity results with the elevation of liver enzymes in the extra cellular fluid. Liver enzymes whose concentration is elevated includes: aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) enzymes. (Satoh et al., 2008). Secondary bacterial infection (otitis media and bronchopneumonia), diarrhea, blindness and death commonly occur in measles. Delayed complications of measles in pregnancy include pneumonitis, fetal loss and an increased risk of maternal death. Rubella is a mild illness that
presents with fever and rash. Congenital rubella syndrome (CRS) is an important cause of blindness, deafness, congenital heart disease and mental retardation.

2.2 Laboratory diagnosis of Measles/Rubella

There are several viruses that can cause a rubella/measles like rash so an accurate diagnosis is based on laboratory investigations. These viruses include cytomegalovirus and herpes simplex. Diagnosis of measles and rubella infections is made by detection of measles and rubella specific IgM antibody in single serum samples drawn during the acute phase of infection and within 28 days of rash onset. These immunochemical reaction tests are very selective and sensitive since the principle of detection is based on the fact that the antibodies produced during the infection will form a complex with the specific antigen incorporated in the test reagent. Diagnosis can also be carried out by reverse transcriptase polymerase chain reaction (RT-PCR). More recently, alternative samples (besides serum) such as dried blood spots and oral fluids have been used for diagnosis by antibody detection. Oral fluids can also be used to detect viral ribonucleic acid (RNA) and their use is becoming increasingly common because samples can be obtained safely and non-invasively, without the risks associated with blood collection, and it improves patient compliance with specimen collection, as the procedure is simple and painless. (Semeeh et al., 2016)

2.3 Management of Measles and Rubella

Measles and rubella viruses cause serious infections in the immunocompromised, undernourished individuals and children with chronic debilitating diseases. Such patients can be protected by the administration of human anti-measles/rubella gamma globulin if given within the first 3 days after exposure. Alternatively, the exposed individual can be vaccinated within 72
hours of exposure. Antibiotics may be indicated in cases of secondary bacterial pneumonia or
otitis media. Inactivated and live vaccines are available for children aged less than 1 year who
are most prone to severe complications. In a past study, it was observed that at least 3 doses of
inactivated vaccine were needed to elicit a protective antibody response but the antibody levels
soon waned. This leaves the vaccinated person open to attack by the natural virus. This has led to
withdraw of the killed vaccine in vaccinated programmes (Barreto et al., 2013).

A combination of measles, mumps, and rubella (MMR) vaccine is also available. One dose of
MMR vaccine is about 93% effective at preventing measles, but two doses are effective at 97% protection. In Kenya, the first vaccine of measles is given at 9 months and the second dose given
at 18 months, while rubella vaccine is not part of Kenya Expanded Programme on immunization.
Kenya is among a number of African countries at risk of a measles outbreak because some
parents do not support immunization of their children. The situation is compounded by low
government support to fight the disease. Since the introduction of the second dose of Measles-
Containing Vaccine (MCV2) in 2009, there has been poor response from Kenyan parents and
care givers (Mutua et al, 2011).

In the United States of America (USA), widespread use of measles vaccine has led to a greater
than 99% reduction in measles cases compared with the pre-vaccine era. Since 2000, when
measles was declared eliminated from the U.S.A, the annual number of people reported to have
measles ranged from a low of 37 people in 2004 to a high of 668 people in 2014. Most of these
originated outside the country or were linked to a case that originated outside the country
(Orenstein et. al 2005).
2.4 Vaccination against Measles/rubella and challenges encountered.

The vaccination coverage required to control measles and rubella is well above 90% globally. Measles vaccination coverage in Africa is below 70%, and in East Africa it is 70% (CDC, 2007). In Kenya measles vaccine is given as a single dose at 9 months of age and the coverage is 75%. It is recommended that children should receive a booster at 18 months (CDC, 2007) but this is not done in Kenya. Nevertheless supplemental immunization campaign targeting children aged 5 years and below are conducted in Kenya after every three years. (CDC, 2007). Measles and rubella elimination have been faced by several challenges which include financial constraints in delivering effective routine immunization and elimination of disease. The political instability in Kenya’s neighbouring country, Somalia, has greatly affected the efforts put in place to eradicate measles and rubella due to migrant associated cases. The ever increasing number of legal or illegal immigrants that includes children has a great negative impact on the eradication of rubella and measles infection.

2.5 Statement of the Problem

Despite the immunization programmes by Expanded Programmed on Immunization (EPI) against measles virus, some children under five years still contract the disease in Kenya (Mutua et al, 2011). Immigrant associated cases could compromise the eradication of measles and rubella in children below five years of age. According to the CDC, the measles portion of the MMR vaccine is 93% effective after one dose and 97% after two doses in preventing the disease. One common argument against the MMR vaccine is the claim that the vaccine spreads the measles virus through a phenomenon called “vaccine shedding”. Vaccine shedding refers to the expulsion and release of virus progeny following successful reproduction during a host-cell infection. Once
replication has been completed and the host cell is exhausted of all resources in making viral progeny, the viruses may begin to leave the cell by several methods. (Heather, 2015).

Analysis of Kenya rubella data shows that rubella is endemic throughout the country, and many outbreaks may be underestimated or undocumented. Six percent of all the cases in this outbreak were women of reproductive age indicating that the threat of CRS is real. Routine immunization against rubella virus is non-existent in public hospitals in the country. The high incidence and prevalence of rubella disease in Kenya poses a threat of congenital rubella syndrome (CRS) (Njeru et al., 2014). Immunization is a successful and cost-effective way to save children’s lives. Rwanda has become the first country in sub-Saharan Africa to introduce a dual vaccine to protect children against measles and rubella (Sussane, 2013).

2.6 Justification

Despite the efforts of Expanded Programmed on Immunization (EPI) measles virus continues to cause infection in children below five years of age (Mbugua et al., 2011). There has been a widespread resurgence of measles affecting 28 countries in sub-Saharan Africa since 2009. This has resulted in over 200,000 reported measles cases and over 1,400 reported measles-associated deaths. The true number of measles cases and deaths is estimated to be 10–20 fold higher, recognizing the under reported cases. The underlying cause of these outbreaks is insufficient vaccination, due to both low first-dose coverage (because of weak routine immunization systems) and reduced quality or delayed measles campaigns, which have been exacerbated by major funding gaps. The outbreaks in Africa, together with the continued high numbers of measles deaths occurring in India, threaten the realization of SDG goal 3. There is a great need therefore to establish whether the cause of this challenging measles infection is due to ineffective vaccine or inadequate coverage of the target children intended in measles vaccination. Rubella
vaccination has not been included in the EPI in Kenya despite the fact that this virus is known to cause infection in children. There is need therefore to determine the status of rubella infection in children population with a view to having it included in the immunization programme. The study involved children attending public hospitals in Nairobi County. Laboratory test procedures to confirm acute infection by the two viruses was done at University of Nairobi, Kenyatta National Hospital diagnostic laboratories. Nairobi was an ideal site for this study because of high chances of imported measles and rubella viruses.

2.7 Research Questions

1. Are children below five years of age affected by rubella and measles infection?

2. What are the clinico-pathological effects of measles and rubella?

3. What is the correlation between liver function tests and IgM concentration in measles and rubella infections in children under five years?

2.8 Hypothesis

2.8.1 Null hypothesis

Levels of serum IgM and liver function are not altered by measles and rubella infections in children under five years.
2.8.2 Alternative hypothesis

Levels of serum IgM and liver function are altered by measles and rubella infections in children under five years.

2.9 Objectives

2.9.1 Broad objectives

To determine relationship of serum IgM level and liver function in measles and rubella viruses infections of children under age of five years in Nairobi County.

2.9.2 Specific objectives

- To determine the prevalence of measles and rubella infection in children under five years of age in maternal and child health clinics clinics of Kenyatta National Hospital, Mbagathi District Hospital and Mama Lucy Kibaki Hospital using levels of IgM antibodies
- To determine clinical-pathological effects of measles and rubella virus infection using liver function tests.
- To determine the correlation between liver function tests and serological IgM assays in measles and rubella infections in children under five.
CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site
The study was carried out among children attending maternal child health clinic at Kenyatta National Hospital, Mbagathi District Hospital and Mama Lucy Kibaki Hospital. Clinical examination and recruitment of study subjects was done by a physician at each of the institution. The analytical work was done in Kenyatta National Hospital diagnostic laboratories.

3.2 Study Design
This was a cross sectional descriptive laboratory based study. IgM antibodies and liver function parameters were determined in blood of children suspected to have measles or rubella infection.

3.3 Study population
The study population was children under five years of age brought to the maternal child health clinics at Kenyatta National Hospital, Mbagathi Hospital and Mama Lucy Kibaki hospital.

3.4 Inclusion criteria
The study included all children who presented at the study site with manifesting symptoms and signs consistent with infection of the measles or rubella viruses.

3.5 Exclusion criteria
The study excluded children who did not present with clinical symptoms of measles or rubella.

3.6 Sample size
Sample size was calculated using the formulae of Bartlet et.al 2001.

Calculated sample size for measles is shown below
\[ n = \frac{(p (1-p) z^2)}{d^2} \]

Where \( n \) = sample size

\( p \) = prevalence

\( z \) = confidence interval of 95% (1.96)

\( d \) = precision (0.05)

\( p \) = prevalence for measles virus (6\%=0.06) (Njeru, et. al., 2014)

\[ n = \frac{(0.06(1-0.06)1.96^2)}{0.05^2} \]

\( n = 87 \)

Calculated sample size for rubella is shown below

\[ n = \frac{(p (1-p) z^2)}{d^2} \]

Where \( n \) = sample size

\( p \) = prevalence

\( z \) = confidence interval of 95% (1.96)

\( d \) = precision (0.05)

\( p \) = prevalence for rubella virus is (11.1\%=0.111) (Njeru et.al 2014)

\[ n = \frac{(0.111(1-0.111)1.96^2)}{0.05^2} \]

\( n = 152 \)

Total study sample size for rubella and measles

\( N = 152 + 87 = 239 \)

\( N = 239 \) children.
3.7 Recruitment of study subjects

Guardians /parents who accompanied the children to hospital were explained about the study and requested to give assent for the blood to be withdrawn from the children. Only those guardians/parents whose children had the symptoms consistent with measles and rubella viruses were informed about the study.

3.7.1 Clinical procedures

The physician clinically examined the children for symptoms consistent with rubella and measles infection. The symptoms included the following: Fever, malaise, sneezing, rhinitis, congestion, conjunctivitis and cough and Koplik’s spots (Barreto et al., 2006).

3.8 Personnel collecting blood

Qualified medical laboratory personnel in the hospitals collected blood specimen from all children who met the inclusion criteria in the first appearance within the study period.

3.9 Laboratory procedures

3.9.1 Collection of blood specimens

The upper arm was tied with a tourniquet to locate a vein. A methylated spirit swab was used to sterilize the site for blood collection. A butterfly needle connected to a 5mls syringe was used to draw blood from the vein. Approximately 3 milliliters of blood was drawn from each study subject. The needle was disconnected from the syringe and the blood put into a plain vacuitainer tube labelled with the study subject identification number.

3.9.2 Transport of the specimen

Blood specimen was transported in a cool box and delivered to the laboratory.
3.9.3 Separation and storage of the serum

Clotted blood specimen in the plain vacuittainer were placed in the centrifuge (Haemocentrifuge Hitachi year manufactured:2014)buckets and then centrifuged at 3000 rpm for 3 minutes to separate the serum. (Melissa et al, 2007). The serum was harvested using pastuer pipettes and divided into two equal aliquots for IgM and liver function tests respectively. These serum specimens were stored at 4°C-8°C until the day of analysis. Analysis of the tests were done within seven days after specimen collection.

3.9.4 Laboratory analytical procedures

The study subjects serum was used for the analysis of liver function parameters i.e. total protein (TP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TBILI) and direct bilirubin (DBILI). The IgM immunological analysis was carried out to qualitatively determine the presence or absence of rubella and measles. IgM quantitative determination was carried out on the specimens that were positive for either rubella or measles. Details about these assays are shown in section 3.10.6. The analytical methods and units of measure for the studied parameters are as shown in table 1 below.
Table 1: Analytical methods used for the study parameters of liver function tests and serum immunoglobulin M of measles and rubella patients.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Abbreviation</th>
<th>Units of measure</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>TP</td>
<td>g/l</td>
<td>End point biuret</td>
</tr>
<tr>
<td>Albumin</td>
<td>ALB</td>
<td>g/l</td>
<td>End point bromocresol green</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>ALT</td>
<td>IU/l</td>
<td>1st order Uv kinetic reaction</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td>AST</td>
<td>IU/l</td>
<td>1st order Uv kinetic reaction</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>ALP</td>
<td>IU/l</td>
<td>1st order Uv kinetic reaction</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>TBili</td>
<td>µmol/l</td>
<td>Stabilized diazonium salt end point reaction</td>
</tr>
<tr>
<td>Direct bilirubin</td>
<td>DBili</td>
<td>µmol/l</td>
<td>Stabilized diazonium salt end point reaction</td>
</tr>
<tr>
<td>Immunoglobulin M</td>
<td>IgM</td>
<td>+ve/-ve</td>
<td>immunochemical reaction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>µiu/ml</td>
<td>immunochemical reaction</td>
</tr>
</tbody>
</table>

3.9.5 Analytical Quality control

Internal quality control specimen was analyzed before the study specimen to guarantee that the internal quality control target value had been attained for accuracy and precision. Serological verification of infections was based on commercially procured analytical kits specific for rubella
and measles virus infection. Each kit gave specific IgM cut off to determine infection and IgM assay of rubella and measles. Children with abnormal liver function tests were managed by the physician according to the protocols of in-patients.

3.9.6 Measles/Rubella specific IgM assay

The Enzygnost ® Anti measles virus/IgM immunoassay diagnostic kit (Dade Behring: Germany) and Enzygnost ® Anti Rubella virus/IgM immunoassay diagnostic kit (Dade Behring: Germany) for qualitative detection and quantitative determination of IgM antibodies to measles/rubella virus in human serum and plasma was used for serological ELISA assay of Measles/Rubella IgM antibodies.

Ten microliters of serum sample already diluted with 200 µl of sample buffer was incubated at 18-25°C for 15 minutes. The mixture was then vigorously shaken (on a shaker) and 150 µl of the mixture dispensed in duplicates into measles / rubella antigen and antigen control wells on the 96 well micro titer plastic plate and incubated at 37°C for one hour. It was then washed four times with washing buffer to remove unbound conjugate. Working chromogen substrate (tetramethylbenzidine) was then added at 100 µl and incubated in the dark at 18-25°C for 30 minutes while covered with a foil. Stop solution was added at 100 µl to terminate color development and the optical density read at 450 nm with a reference filter of 650 nm of the ELISA reader machine. All the specimens were run simultaneously with reference positive and negative controls (WHO, 2007).

Conversion of qualitative analytical results to quantitative results was based on the reagent kit specification (appendix iii).
3.9.7 Liver function tests

Serum separated as described in section 3.10.3 was analyzed to test for liver function. The principles of tests for the liver function parameters were applied according to the manufacturer’s instructions provided with kits. Reference ranges for the liver function parameters used to determine the normal and abnormal results were based on the established Kenyan based reference ranges as shown in Table 2 below. Analytical values greater than the upper reference range limit stated were used to determine the pathological effect and the diseases severity. Procedures used for each parameter are described below.

Table 2: Kenyan based reference ranges for the Liver functions parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>unit of measure</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>g/l</td>
<td>56-89</td>
</tr>
<tr>
<td>Albumin</td>
<td>g/l</td>
<td>28-52</td>
</tr>
<tr>
<td>AST</td>
<td>iu/l</td>
<td>3-40</td>
</tr>
<tr>
<td>ALT</td>
<td>iu/l</td>
<td>0-39</td>
</tr>
<tr>
<td>ALP</td>
<td>iu/l</td>
<td>5-227</td>
</tr>
<tr>
<td>TBILI</td>
<td>µmol/l</td>
<td>0-19</td>
</tr>
<tr>
<td>DBILI</td>
<td>µmol/l</td>
<td>0-5.4</td>
</tr>
</tbody>
</table>

(Waithaka et al., 2009)
3.9.8 Total protein (TP)

The concentration of total protein was determined by a timed endpoint biuret method (Ninfa et al., 2009). In the reaction, the peptide bonds in the protein sample bind to cupric ions (cu$^{2+}$) in an alkaline medium to form a peptide/coppper complex. Six microliters of sample was reacted with 300$\mu$l of reagent and the change in absorbance was monitored at 560nm. This change was directly proportional to the concentration of TP in the sample and was used to calculate and express concentration in mmol/L. The reaction took place at 37 °C for four minutes.

Principle of the reaction

Protein + cu$^{2+}$ $\leftrightarrow$ oh- protein-copper complex.

3.9.9 Albumin (ALB)

The concentration of albumin was determined by a timed endpoint method (Bishop, 2000). Albumin combines with bromocresol green (BCG) to form a coloured product. Three microliters of sample was reacted with 300 $\mu$l of reagent and the change in absorbance was monitored at 600nm. This change was directly proportional to the concentration of ALB in the sample and was used to calculate and express concentration in g/l. The reaction took place at 37 °C for one and half minutes.

Principle of the reaction

Albumin +BCG $\rightarrow$ Albumin / BCG complex
### 3.9.10 Alkaline phosphatase (ALP)

Alkaline phosphatase reagent was used to measure alkaline phosphatase activity by a kinetic UV method (Burtis and Carl, 2008) using a 2-amino-2-methyl-1-propanol (AMP) buffer. In the reaction alkaline phosphatase catalyzed the hydrolysis of the colourless organic phosphate ester substrate, p-Nitrophenylphosphate to the yellow colored product, p-nitrophenol and phosphate. The reaction occurred at an alkaline pH of 10.3. Five microlitres of the sample was reacted with 250 µl of the reagent. The change in absorbance was monitored at 410 nm and this change was directly proportional to the activity of ALP. The activity was calculated and expressed in U/L. The reaction took place at 37 °C for three minutes.

**Principle of the reaction**

\[
P\text{-Nitrophenylphosphate} + H_2O \xrightarrow{\text{PH 10.3, Mg}} \text{p-nitrophenol} + \text{Phosphate (colorless) (yellow)}
\]

### 3.9.11 Alanine aminotransferase (ALT)

The ALT reagent was used to measure alanine aminotransferase in the sample by an enzymatic kinetic UV rate method (Xing et al., 2006). In the assay reaction, the ALT catalyzes the reversible transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamine. Pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of β-Nicotinamide Adenine Dinucleotide (reduced form) (NADH) to β-Nicotinamide Adenine Dinucleotide (NAD). Pyridoxal-5-phosphate was required in this reaction as a cofactor for transaminase activity by binding to the enzyme using Schiff-base linkage. 10 microlitres of the sample was reacted with 110µl of the reagent. The change in absorbance was monitored at 340 nm and this change was directly proportional to the activity of
ALT. The activity was calculated and expressed in U/L. The reaction took place at 37 °C for three minutes.

Principle of the reaction

\[
\text{L-Alanine} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{L-Glutamate}
\]

\[
\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+
\]

### 3.9.12 Aspartate aminotransferase (AST)

AST reagent was used to measure aspartate aminotransferase activity by an enzymatic kinetic UV rate method (Xing et al., 2006). In the reaction aspartate aminotransferase catalyzed the reversible transamination of L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate was then reduced to malate in the presence of malate dehydrogenase (MDH) with the concurrent oxidation of reduced β-Nicotinamide adenine dinucleotide (NAD). Ten microlitres of the sample was reacted with 110µl of the reagent. The change in absorbance was monitored at 340 nm and this change was directly proportional to the activity of AST. The activity was calculated and expressed in U/L. The reaction took place at 37 °C for three minutes.

Principle of the reaction

\[
\text{L-Aspartate} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate} + \text{L-Glutamate}
\]

\[
\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}^+
\]

### 3.9.13 Total bilirubin (T BILI)

A stabilized diazonium salt (3, 5-dichlorophenylazonium tetrafluoroborate (DPD), reacts with conjugated bilirubin directly and with unconjugated bilirubin in the presence of an accelerator
(caffeine) to form azobilirubin (purple) (Yuji and Yoshikatsu, 2017). Eight microlitres of sample was reacted with 280 µl of reagent and the change in absorbance was monitored at 578 nm. This change was directly proportional to the concentration of T BILI in the sample and was used to calculate and express concentration in µmol/L. The reaction took place at 37 °C for two minutes.

Nb/ A separate sample blank were performed (set) to reduce endogenous serum interference.

Principle of the reaction

\[
\text{Bilirubin + DPD} \xrightarrow{\text{caffeine}} \text{Azobilirubin}
\]

3.9.14 Direct bilirubin (D BILI)

A stabilized diazonium salt (3, 5-dichlorophenyldiazonium tetrafluoroborate (DPD), reacts with conjugated bilirubin directly in an acidic medium to form azobilirubin (purple) (Yuji and Yoshikatsu, 2017). Five microlitres of sample was reacted with 160 µl of reagent and the change in absorbance was monitored at 546 nm. This change was directly proportional to the concentration of D BILI in the sample and was used to calculate and express concentration in µmol/L. The reaction took place at 37 °C for two minutes.

Principle of the reaction

\[
\text{Bilirubin + DPD} \xrightarrow{\text{H}^+} \text{Azobilirubin (purple colour))}
\]
3.10 Statistical Analysis

The collected data was entered into excel spreadsheet, cleaned and then exported to the Statistical Package for Social Sciences (SPSS version 22) for analysis. Pearson T-test was used to compare the values of each parameter. The tests were conducted at 95% confidence interval and significance level of 5%, p≤ 0.05 was considered significant. The analysed data was presented in tables.

3.11 Ethical considerations

Ethical approval was sought from Kenyatta National Hospital and University of Nairobi ethical research committees. Handling of patients during clinical examination and specimens collection was done by medical personnel in respective hospitals. Permission to collect blood from the children was sought from the parents/guardians and consent form signed (appendix 1). Results obtained from the study were treated with utmost confidentiality.

3.12 Treatment of children without symptoms

Children with normal IgM concentrations and liver function tests were recommended for vaccination if they had not yet received the mandatory immunization. The study results were given to the clinician to do a patient’s follow up.

3.13 Results communication

The IgM and liver function test results were given to the physician for future study subject management and as archive for measles and rubella studies or reference.
CHAPTER FOUR

4.0 RESULTS

4.1 Description of the study subjects

two hundred and thirty five study subjects comprising of 128 (54%) females and 107 (46%) males. The mean age of the study subject was 2.5 years. The distribution of the study subject within the study sites were 184(78%), 29(12%) and 22(12%) from Kenyatta National Hospital, Mbagathi District hospital and Mama Lucy Kibaki Hospital respectively. The number of study subjects from each study site was based on children who attended these sites during the study period. The distribution of the study subject is as shown in figure 1 below.

Figure 1: Distribution of the study subjects within the study sites
4.2 Internal quality control results

This was categorized into two i.e. qualitative and quantitative, based on the analytical procedure carried out. The daily internal quality control for the qualitative analytical procedure for IgM was carried out using a positive and negative control. According to the classification of the reagent kit, a negative results was indicated if the absorbance was less than 0.1 and positive result if the absorbance was greater than 0.2. Any absorbance value between 0.1 and 0.2 was indicated as discordant. The qualitative internal quality control results were achieved through the conversion of the absorbance into quantitative results as per the specification of the reagent kits for both measles and rubella. The quality control results are as indicated in Table 3 below.

The Internal Quality Control multi-sera was used to determine the session quality control results for the specific parameters that constitute the Liver Function Tests i.e. total protein, albumin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, total bilirubin and direct bilirubin. The Internal quality control results are as indicated in Table 3 below.

The session indicates how many times the analysis was carried during the analytical period of the study for both qualitative and quantitative analytical procedures. The immunological analysis was carried out in five sessions whilst the end point and UV enzymatic kinetic reaction analysis was carried out in twenty seven sessions as indicated in Table 3 below.

The assigned quality control result is what is indicated in the reagent kit against which the session internal quality control results are compared with. The study quality control results for the analyzed parameters were within the specified assigned QC range of mean ± 2 standard deviations (SD) (QC range) as shown in Table 3 below.
Table 3: Internal quality control (IQC) for the studied parameters of measles and rubella study in Kenya

<table>
<thead>
<tr>
<th>Parameter/unit</th>
<th>Assigned QC report</th>
<th>Study QC report</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Session</td>
<td>QC range</td>
</tr>
<tr>
<td>IgM(µiu/ml)</td>
<td>5</td>
<td>0.57-1.93</td>
</tr>
<tr>
<td>TP(g/l)</td>
<td>27</td>
<td>63-77</td>
</tr>
<tr>
<td>ALB(g/l)</td>
<td>27</td>
<td>41-49</td>
</tr>
<tr>
<td>AST(iu/l)</td>
<td>27</td>
<td>21-31</td>
</tr>
<tr>
<td>ALT(iu/l)</td>
<td>27</td>
<td>19-31</td>
</tr>
<tr>
<td>ALP(iu/l)</td>
<td>27</td>
<td>78-110</td>
</tr>
<tr>
<td>TBILI(µmol/l)</td>
<td>27</td>
<td>14-28</td>
</tr>
<tr>
<td>DBILI(µmol/l)</td>
<td>27</td>
<td>7-17</td>
</tr>
</tbody>
</table>
4.3 RESULTS FOR OBJECTIVE 1: To determine the status of measles and rubella in the study subjects using ELISA technique

Out of the two hundred and thirty five study subject who were qualitatively tested for both measles and rubella, only three (1.28%) were positive for measles and five (2.13%) positive for rubella. Three study subjects who were positive for measles came from Kenyatta National Hospital. Five Rubella positive cases were distributed as follows: 2 from Kenyatta National Hospital, 2 from Mama Lucy Kibaki Referral Hospital and 1 from Mbagathi District hospital. Positive results were those whose corrected absorbance was greater than 0.2 whilst negative results were those whose corrected absorbance was less than 0.1. The mean corrected absorbance for the measles and rubella positive cases were 0.413 and 0.526 respectively as shown in Table 4 below. Two hundred and thirty two and two hundred and thirty subjects reacted negative for both measles and rubella with corrected mean absorbance of 0.0715 and 0.0703 respectively, as shown in Table 4 below.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Number</th>
<th>Mean absorbance</th>
<th>Mean concentration (µiu/ml)</th>
<th>SD</th>
<th>results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles</td>
<td>232</td>
<td>0.0715</td>
<td>-</td>
<td>0.056</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.413</td>
<td>921</td>
<td>473</td>
<td>positive</td>
</tr>
<tr>
<td>Rubella</td>
<td>230</td>
<td>0.0703</td>
<td>-</td>
<td>0.059</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.319</td>
<td>1470</td>
<td>1271</td>
<td>positive</td>
</tr>
</tbody>
</table>
4.4 RESULTS FOR OBJECTIVE 2: To determine clinical-pathological effects of measles and rubella viruses infections in children under five years of age in Nairobi County.

Liver function tests were normal in two hundred and twenty three out of the two hundred and thirty five study subjects. The mean values for TP, ALB, AST, ALT, ALP, TBILI and DBILI were 65.4 g/l, 40 g/l, 18 iu/l, 28 iu/l, 253 iu/l, 13 µmol/l and 5 µmol/l respectively. (Table 5a). The means value for the TP, ALB, AST, ALT, ALP, TBILI and DBILI for the eight study subjects who were positive for measles and rubella infection were; 67 g/l, 30 g/l, 206 iu/l, 202 iu/l, 246 iu/l, 43 µmol/l and 30 µmol/l respectively (Table 5a).

The mean value for TP, ALB, AST, ALT, ALP, TBILI and DBILI for the 4 out of 227 study subjects who were negative for measles and rubella infection but had abnormal liver function tests results were; 80 g/l, 30 g/l, 295 iu/l, 282 iu/l, 263 iu/l, 18 µmol/l and 4 µmol/l respectively as shown in Table 5a below.

Table 5a: Mean concentration of liver function test parameters of the studied children under five years of age in Nairobi County

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number</th>
<th>TP(g/l)</th>
<th>ALB(g/l)</th>
<th>AST(iu/l)</th>
<th>ALT(iu/l)</th>
<th>ALP(iu/l)</th>
<th>TBILI(µmol/l)</th>
<th>DBILI(µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects without measles and rubella infection</td>
<td>227</td>
<td>65</td>
<td>40</td>
<td>18</td>
<td>28</td>
<td>253</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Subjects with measles and rubella infection</td>
<td>8</td>
<td>67</td>
<td>30</td>
<td>206</td>
<td>202</td>
<td>246</td>
<td>43</td>
<td>30</td>
</tr>
<tr>
<td>Subjects with abnormal LFTS results without measles and rubella</td>
<td>4</td>
<td>80</td>
<td>30</td>
<td>295</td>
<td>282</td>
<td>263</td>
<td>18</td>
<td>4</td>
</tr>
</tbody>
</table>

In order to determine the clinical-pathological effects of measles and rubella infection on liver function, results of study subjects who were negative for measles and rubella infection (n= 227) were statistically compared with those infected (n=8) by the two diseases using paired samples T test. The mean levels of TP, ALB, ALP, TBILI and DBILI in measles and rubella infected and non-infected subjects were not significantly different (p=0.343, p=0.202, p=0.845, p=0.257 and p=0.234 respectively). The means difference of AST and ALT for the infected and non-infected study subject was statistically significant (p=0.002 and p=0.032 respectively) as shown in Table 5b.
Table 5b: Statistical comparison of liver function tests for the study subject infected and those not infected with measles and rubella

<table>
<thead>
<tr>
<th>Study groups compared</th>
<th>Paired Differences</th>
<th>95% Confidence Interval of the Difference</th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Deviation</td>
<td>Std Error Mean</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Pair 1 ntp – aftp</td>
<td>8.28</td>
<td>21.27</td>
<td>8.04</td>
<td>-27.96</td>
<td>11.38</td>
</tr>
<tr>
<td>Pair 2 nalb – afalb</td>
<td>4.00</td>
<td>7.39</td>
<td>2.79</td>
<td>-10.83</td>
<td>2.83</td>
</tr>
<tr>
<td>Pair 3 nast – afast</td>
<td>189.42</td>
<td>92.06</td>
<td>34.79</td>
<td>-274.57</td>
<td>-104.28</td>
</tr>
<tr>
<td>Pair 4 nalt – afalt</td>
<td>159.85</td>
<td>152.69</td>
<td>57.71</td>
<td>-301.07</td>
<td>-18.63</td>
</tr>
<tr>
<td>Pair 5 nalp – afalp</td>
<td>16.42</td>
<td>213.44</td>
<td>80.67</td>
<td>-180.97</td>
<td>213.83</td>
</tr>
<tr>
<td>Pair 6 ntbili – aftbili</td>
<td>30.28</td>
<td>63.97</td>
<td>24.17</td>
<td>-89.44</td>
<td>28.87</td>
</tr>
<tr>
<td>Pair 7 ndbili – afdbili</td>
<td>29.71</td>
<td>59.39</td>
<td>22.44</td>
<td>-84.64</td>
<td>25.21</td>
</tr>
</tbody>
</table>

n=normal, af = affected, df = degree of freedom, std= standard, t = t value
4.5 Objective 3: To determine the correlation between LFTS and IgM concentration in measles and rubella infections.

The correlation of liver function parameters and IgM serum concentration for measles and rubella infected study subjects is summarized in Table 6 below. Total protein and albumin showed a very strong negative correlation \((r = -0.932\) and \(r = -1.000\) respectively) with measles IgM concentration which was statistically significant at \(p=0.035\) and \(p= 0.07\) respectively. Positive correlation was shown between measles IgM concentration and AST \((r = 0.247)\), ALT \((r = 0.637)\), ALP \((r = 0.935)\), TBILI \((r = 0.719)\) and DBILI \((r = 0.654)\). This positive correlation was statistically significant for AST \((p = 0.032)\) and ALT \((p = 0.021)\).

TP, ALB, ALP, TBILI and DBILI showed a negative correlation \((r = -0.316, r = -0.872, r = -0.804, r = -0.550\) and \(r = -0.404\) respectively) with rubella IgM concentration which was statistically significant for TP and ALB at \(p = 0.015\) and \(p = 0.031\) respectively. Positive correlation was shown between rubella IgM concentration and AST \((r = 0.333)\) and ALT \((r = 0.360)\). This positive correlation was statistically significant for AST \((p = 0.044)\) and ALT \((p = 0.028)\).
Table 6: Correlation between the liver function parameters and IgM serum concentration for measles and rubella

<table>
<thead>
<tr>
<th></th>
<th>TP</th>
<th>ALB</th>
<th>AST</th>
<th>ALT</th>
<th>ALP</th>
<th>TBILI</th>
<th>DBILI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>measles</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>conc</td>
<td>-.932</td>
<td>-1.000</td>
<td>.247</td>
<td>.637</td>
<td>.935</td>
<td>.719</td>
<td>.654</td>
</tr>
<tr>
<td>Pearson</td>
<td>-.316</td>
<td>-.872</td>
<td>.333</td>
<td>.360</td>
<td>-.804</td>
<td>-.550</td>
<td>-.404</td>
</tr>
<tr>
<td>Correlation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.035</td>
<td>.007</td>
<td>.032</td>
<td>.021</td>
<td>.231</td>
<td>.489</td>
<td>.546</td>
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</tr>
<tr>
<td><strong>rubella</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>conc</td>
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<td>-.872</td>
<td>.333</td>
<td>.360</td>
<td>-.804</td>
<td>-.550</td>
<td>-.404</td>
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<tr>
<td>Pearson</td>
<td>.015</td>
<td>.031</td>
<td>.044</td>
<td>.028</td>
<td>.101</td>
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<tr>
<td>Correlation</td>
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<tr>
<td>Sig. (2-tailed)</td>
<td>.015</td>
<td>.031</td>
<td>.044</td>
<td>.028</td>
<td>.101</td>
<td>.337</td>
<td>.500</td>
</tr>
<tr>
<td>N</td>
<td>5</td>
<td>5</td>
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</tbody>
</table>

N = number, conc = concentration, Sig = significance
CHAPTER FIVE

5.0 DISCUSSION

Results of this study findings show that there were more children infected with rubella 5 (2.13%) than measles 3 (1.28%) in the study sites. These results represent the point prevalence of rubella and measles for the studied population. Although the results indicate a relatively low prevalence of the two diseases in the two hospitals, it is implied from this study that the two diseases do occur and should be considered in disease control programs, among the other important diseases that cause adverse effects on health status of the children. The low infection could be attributed to improved immunization programmes in Kenya as opposed to other regions quoted in literature. The findings of the current study presented low rate of rubella infection compared with other regions across Africa. A similar study carried out in North Western Nigeria produced a rubella infection rate of 2.6% which was in agreement with the findings of the current study (Semeeh et al, 2016). A higher rate (12 %) of rubella infection was reported in a study carried out in a neighbouring country (Ethiopia) in which the study population was in children below the age of 15 years (Etsehiwot, 2015). Low rubella infection rate of 1.3% was produced by a study carried out in Namibia involving children below five years of age (Emmy, 2015).

Several studies have been reported in literature concentrating on measles infection of children below five years of age. Comparatively, the rate of measles infection expressed in the current study (1.28%) is lower than what has been reported in other studies carried in other regions of Africa. A similar study carried out in Ghana expressed an infection rate of 6.9%, which was far much higher than what was expressed in the current study (Binka et.al, 2007). In 2014, a relatively higher prevalence (2%) of measles in children under five years of age was reported in Nigeria (Chika et al, 2014).
In the present study measles and rubella virus were found to be associated with elevation of liver enzymes namely; alanine transferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Two hundred and twenty five study subjects (94%) had normal liver function tests. Four study subjects who were negative for measles and rubella infection had elevated liver enzymes namely; alanine transferase (ALT), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST). This suggests that there were other factors that caused elevation of liver enzyme in the children included in the study. Our results agree with those of Satoh et al., 2008, who also found that the transaminases were elevated in children who were negative for the studied viruses. Another study carried out in France was also in agreement with the findings of the current study that measles adversely affect the functions of the liver (Aurélien et al., 2013). The findings of these three studies strongly suggest that the studied viruses affect the metabolic function of the liver that take place in the intracellular fluid of the hepatocytes. The appearance of elevated levels of the transaminases in the extracellular fluid further suggests that the viruses compromise the integrity of the cell membrane. Other studies have shown the effect of these diseases on liver function characterized by elevation of the liver transaminases (Ranjan, et al 2017, Masami, et al 2014 and Fisher et al, 2015).

The current study showed that the diseases cause hyperbilubinaemia. This implies a compromised bile excretion by the hepatocytes. Scott (2007) reported similar findings of hyperbilubinaemia in children below five years infected by measles and rubella viruses. This current study did not show any effect of the viruses on the concentration of total protein and albumin among the study subject. There was no significance difference in total protein
(p=0.343) and albumin (p=0.202) concentration of the study subjects found to have rubella and measles when compared statistically with those children who were negative for both viruses. The study showed a positive correlation between some liver function tests and concentration of IgM for the infected subjects. The presence of rubella or measles infection increases the concentration of IgM in the blood of the affected children. The severity of the disorders is consistent with an increase in the blood IgM concentration (Gastañaduy et al., 2016). Total protein and albumin of the study subjects who turned positive for measles showed a very strong negative correlation with measles IgM concentration which was statistically significant suggesting that a rise in IgM does not affect the concentration of proteins of the children with measles infection. The observed strong negative correlation between proteins and IgM blood concentration is in agreement with studies carried out by Sternfeld et al., (2009).

Positive correlation was observed between the transaminases and IgM concentration in measles and rubella infections. It can therefore be stated that the severity of the studied viral infections results in an increase in the blood concentration of the IgM and consequently an increase in the concentration of the transaminases. Satoh et al (2008), also found that all their study subjects who reacted positive for measles infection, showed an elevation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Similarly results of positive correlation between the IgM concentration and transaminases have been expressed in a study by Masami et al (2014). Elevated levels of total and direct bilirubin showed a positive correlation in both infections, but this was not statistically significant. This means therefore that hyperbilirubinaemia has no direct effect on the concentration of IgM.
5.1 CONCLUSION

- The study has established that measles and rubella still affect children below the age of five years in Nairobi County. The rate of infection is low for the two diseases but there occurrence should not be ignored and they should be emphasized in disease control programmes.

- The metabolic and excretion functions of the liver for the studied population are affected by these viral infections as expressed by the elevated levels of transaminases and bilirubin blood levels concentrations. The two disorders have not been seen to affect the synthesis function of the liver for the studied children population since the proteins concentrations in blood are within the reference ranges quoted in literature.

- The current study showed that the studied viruses also compromise the excretory function of the hepatocytes which is expressed by hyperbilubinaemia of the blood specimens of the study subjects.

- Due to the significant positive correlation between the transaminases and the IgM concentration in the studied viral infection, it can be concluded that rubella and measles infection have an adverse effect on the metabolic activities of the hepatocytes of the studied population.
5.2 RECOMMENDATIONS

(1) Similar studies to be undertaken in all the counties in Kenya to establish the status of rubella and measles in children below five years of age.

(2) Any child who present with signs and symptoms of measles or rubella should be investigated to avoid the spread of the virus.

(3) Liver function test should be included during the baseline study of the suspected cases of rubella or measles infection. It should also form part of the investigation to determine the prognosis of the positive cases of both viral infections.
REFERENCES


St Louis, Missouri.


A combined measles, mumps, rubella and varicella vaccine (Priorix-Tetra):

immunogenicity and safety profile. Vaccine. 27 (47): 8-11.


World Journal of Hepatoogy. 6 (9): 632–642


Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) Detection Techniques. Sensors (Basel). 6 (7): 756-782

APPENDICES

APPENDIX I: ASSENT FORM FOR PARENTS/GURDIANS

ASSENT FORM FOR PARENTS/GURDIANS

Title: RELATIONSHIP BETWEEN SERUM IgM LEVELS AND LIVER FUNCTION IN RUBEELLA AND MEASLES INFECTION OF CHILDREN BELOW FIVE YEARS: A PREVALENCE STUDY IN KENYA

This research is being conducted by

Mary Wangui Mwangi (B.Sc.)

Mobile no.0724793327

University of Nairobi

You are requested to allow your child/children to participate in this study which will take place in Kenyatta National Hospital under the following supervisors

i. Prof. George Chege Gitao (BVM, Msc, PhD)

   Department of Veterinary Pathology Microbiology and Parasitology

   University of Nairobi

ii. Prof P .K Gathumbi (BVM, Msc, PhD)

   Department of Veterinary Pathology Microbiology and Parasitology

   University of Nairobi.
iii. Dr. Stanley Kinge Waithaka (PhD)

Department of Medical Laboratory Sciences

Mount Kenya University.

**Participation**

Participation of your child/children in this study is voluntary. We kindly ask you to read the all the information below and ask questions about anything you do not understand before deciding whether to participate or not to participate.

**PURPOSE OF THE STUDY**

To determine measles/rubella infection

**ANTICIPATED BENEFITS TO SUBJECTS**

Your child/children will receive free laboratory diagnosis and in case your child will be found infected with any of the virus under study, he/she will receive treatment free of charge according to national treatment guidelines.

**POTENTIAL RISKS AND DISCOMFORTS**

We do not anticipate any serious risk for your child/children in this study.

Mild pain of short period, bruising around the needle sites may occur.

To minimize risk of microbial infections, the needle site will be sterilized.
CONFIDENTIALITY

When the results of the research are published or discussed in conferences, no information will be included that would reveal the identity of your child/children. If photographs, videos, or audio-tape recordings will be used for educational purposes, the identity of the child/children will be protected during data analysis, only codes instead of names will be used and all the information will be kept in a computer protected with a password.
PARTICIPATION AND WITHDRAWAL

The participation of your child/children in this research is voluntary. If you choose not to allow your children to participate, that will not affect your relationship (Kenyatta National Hospital) or your right or right of the child/children to health care or other services to which your child/children to participate, you are free to withdraw your consent and discontinue your child/children from participation at any time without prejudice.

IDENTIFICATION OF INVESTIGATORS

In the event of a research related injury or your child/children experience an adverse reaction, please immediately contact

Dr. Teresa Kinyari Mwendwa

0707028067

Department of Human Physiology

University of Nairobi

If you have any questions about the research please feel free to contact us using the above address.
RIGHTS OF RESEARCH SUBJECTS

You may withdraw your consent for your child/children at any time and discontinue them from participation without penalty. You are not waiving any legal claims, rights or remedies because of allowing your child/children to participate in this research study. If you have questions regarding your rights or other rights of your child/children as a research subject you may contact.

Dr. Teresa Kinyari Mwendwa

0707028067

Department of Human Physiology

University of Nairobi

Sign …………………..Date…………………………………

SIGNATURE OF THE PARENT/GUARDIAN

I have read the information provided above. I have been an opportunity to ask questions and all of my questions have been answered to my satisfaction. I have been given a copy of this form.

I……………………………………………………(Name of parent or guardian)being 18 years or older and a guardian or parent having fully capacity to consent .I agree my child or children to participate in the study.

Signature…………………………………Date……………………………

Thumb……………………………………………..Date…………………….
Witness: I certify that, the participant have agreed to participate in the study without being forced.

Signature……………………………………..Date………………………………………...
FOMU YA KUKUBALI AU KUKATAA KUSHIRIKI KATIKA UTAFITI

(WAZAZI /WALEZI /WASIMAMIZI)

Tafsiriya Kiswahili

Kichwa cha/jina la utafiti: Udhihirisho wa Ukambi na Rubella kwa watoto chini ya miaka mitano katika kaunti ya Nairobi, Kenya. Unaombwa kuruhusu mwanao/wanao kushiriki katika utafiti huu unafanywa katika kwenye hospitali zifuatazo

✓ Kenyatta National Hospital
✓ Mama Lucy Kibaki Hospital
✓ Mbagathi Hospital

Ushiriki

Ushiriki wa mwanao/wanao katika utafiti huu ni suala la hiari. Soma taarifa zote katika fomu hii na kuwa huru kuuliza swali lolote ambalo huelewi, kabla ya kuamua mwanao/wanaokushiriki au kutoshiriki.

Taratibu ya utafiti

Kama utakubali mwanao/wanao kushiriki katika utafiti huu, watoto wataombwa kufanya yafuatayo

• Mwanao/wanao watachukuliwa damu kutoka katika sehemu ya mkono na kuweka damu kwenye chupa inayotumika kuwekwa damu.
Madhara

Hatutarajii madhara yeyote kwa watoto iwapo watashiriki katika utafiti huu. Mtoto /watoto watapata maumivu kidogo na ya muda mfupi kwenye sehemu ya mkono na wakati mwingine kubadilika rangi. Ilikupunguza maumivu, kazi hii itafanywa na mtalaamu wa maabara. Pia, kuzuia maambukizi katika eneo lililotolewa damu dhidi ya vijidudu vingine vinavyosababisha magonjwa mengine, dawa maalum ya kuwa wadudu utumika kusafisha eneo hilo la kidole kabla ya kuchoma sindano.
Faida ya kushiriki katika utafiti

Mtoto/watoto wako watapata huduma yabure yakupimwa kama wanaugonjwa wa ukambi na rubella. Pia, utafiti huu utasaidia serikali kufanya maamuzi juu ya kutoa matibabu kwa watoto.

Usiriwataarifa

Wakati majibu ya utafiti huu yatakapokuwa tayari kwa kuchapishwa amakuongelewa katika makongamano, hakuna taarifa inayo mhusu/wahusu motto/watoto itakayotolewa hadharani kwa majina yao. Washiriki wote watatambuliwa kwa namba zao nasi kwa majina yao.

Haki yaUshiriki au kujitot katikautafiti

Ushiriki wamtoto katika utafiti huu niuamuzi wako mwenyewe. Kama utaamua mtoto/watowako wasishiriki katika utafiti huu, haitakuzuia kupata huduma zamwanao/wanaokushiriki au kujitoka katika hospitali ya Kenyatta National Hospital, Mama Lucy Kibaki Hospital and Mbagathi district Hospital. Kutoruhusu mwanao/wanaokushiriki au kujitoka katika utafiti hakutasaababisha kupigwa faini au kuchukuliwa hatua zozote zakisheria.

Utambuziwa wautafiti

Kwa suala lolote linalo husiana nautafiti huu ama iwapo motto atapata madhara makubwa kutokana na kutumiadawa au maumivu yeyote makali kutokana na utafitihu, wasiliana na moja kwa moja na:

Dr. Teresa Kinyari Mwendwa

Tel no.0707028067

Human Physiology Department.
University of Nairobi

Sign……………………..Date……………………..
Haki ya mshiriki katika utafiti

Unaweza kuondoa fomu ya mtoto/watoto wako katika utafiti huu wakati wowote nakuwaondoa katika utafiti huu pasipo kutozwa faini yeyote. Hauvunji sharia yeyote kwakuwaondoa kushiriki katika utafiti huu,

Sahihi ya mzazi/msimamizi


Jina la mzazi/msimamizi ............................... Sahihi.........................
Tarehe..............................................................

Sahihi yashahidi......................................................

Sahihi yangu kama shahidi kwamba mshiriki amekubalina kuweka sahihi yake mbele yangu bila kulazimishwa.

Sahihi ..............................................................Tarehe..............................................

Sahihi ya mtafiti......................................................
APPENDIX II: DATA COLLECTING TOOL

MARY WANGUI MWANGI RELATIONSHIP BETWEEN SERUM IgM LEVELS AND LIVER FUNCTION IN RUBELLA AND MEASLES INFECTION OF CHILDREN BELOW FIVE YEARS: undertaking a study on hereby request you kindly the parent/guardian to assist with information regarding your child. You are assured that the information you give will be used for the purpose of the study. You are free to withdraw the information given at any time. You are also informed that the results of the study will be available to you. We therefore request you kindly to give the correct information to the best of your knowledge on the items indicated below.

My contact phone number is 0724793327.

i. Patient identification number ……………………………………………………………

ii. Gender  M/F(tick appropriately)

iii. Date of birth………………………………

iv. Last hospital visit…………………………

v. Age……………………………………

vi. Nationality…………………………

vii. Date of rubella vaccination (evidence by provision of vaccination card)………………………………

viii. Date of measles vaccination (evidence by provision of vaccination card)……………………………………
APPENDIX III: ANALYTICAL REAGENT KIT SPECIFICATIONS FOR IGM ELISA ASSAY.

Both reagents kits for the immunological analysis for measles and rubella had the same constant lot-specific values for both the qualitative and quantitative analytical determinations. The specifications were as follows:

<table>
<thead>
<tr>
<th>Lot</th>
<th>43589</th>
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<tbody>
<tr>
<td>Nominal Value</td>
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<tr>
<td>Upper Margin</td>
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<tr>
<td>Alpha</td>
<td>3.5545</td>
</tr>
<tr>
<td>Beta</td>
<td>0.2131</td>
</tr>
<tr>
<td>Avg ΔAbs</td>
<td>0.8105</td>
</tr>
<tr>
<td>Correction factor</td>
<td>1.407</td>
</tr>
</tbody>
</table>

Corrected

\[ \Delta \text{Abs} = \Delta \text{Abs} \times \text{correction factor} \]

If corrected \( \Delta \text{Abs} > 0.2 \), then positive

Corrected

\[ \Delta \text{Abs}^\beta \]

Log10

\[ \text{mIU/mL} = \Delta \text{Abs}^\beta \times \text{alpha} \]

Qualitative determinations of measles and rubella

Positive test results

An optical density reading greater than 0.200 using 450 nm to 500 nm wavelengths in the spectrophotometer indicated a positive result for the analyzed viruses.
Negative test samples

An optical density reading of less than 0.100 that is required for test to attain quality assurance standards indicated a negative result for the analyzed viruses.

Quantitative determinations

According to the reagent analytical kit, the corrected absorbance that gave the qualitative results was converted using the constant values indicated in the kits specification to produce the quantitative results for both measles and rubella.
APPENDIX IV: ETHICAL APPROVAL LETTER

Dear Mary,

Revised Research Proposal: Application of 1gm and Liver Function Test in Pathological Investigation of Rubella and Measles Infection in Children below Five Years in Nairobi County, Kenya (P322/03/2016)

This is to inform you that the KNH-UoN Ethics & Research Committee (KNH-UoN ERC) has reviewed and approved your above proposal. The approval period is from 15th August 2016 – 14th August 2017.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH-UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period.
(Associate a comprehensive progress report to support the request)
f) Clearance for export of biological specimens must be obtained from KNH-UoN ERC for each batch of shipment.
g) Submission of an executive summary report within 90 days upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

“Protect to discover”
APPENDIX IV (CONT’D)

For more details consult the KNH- UoN ERC website http://www.erc.uonbi.ac.ke

Yours sincerely,

PROF M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
    The Deputy Director, CS, KNH
    The Assistant Director, Health Information, KNH
    The Chair, KNH- UoN ERC
    Supervisors: Prof. George Chege Gitao, Dr. Stanley Kinge Waithaka, Prof. P.K. Gathumbi

“Protect to discover”
APPENDIX V: INVESTIGATOR ANALYSING SPECIMENS