An account of acute febrile viral infections among children below 12 years in Khartoum State

A thesis submitted in partial fulfillment for the requirement of the Degree of Clinical MD in Paediatrics and child health

BY
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Dedication

This study is a gift to my Family

With love
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200 نظير لا يعانون من الحمى مع اشتراط عدم وجود ملاريا في كل من الحالات والمناظرين. 27% من الحالات و 19% من المناظرين تمت دراستهم بالتفصيل.

الالتهابات الفيروسية تم تشخيصها معملا في 64.4% من الأطفال ذوي الحمى.

تم عزل كل من فيروس روبيلانا و انفلونزا (أ) و السيتوميجالوفيروس في 18.5%, 15.5% و 13% من الحالات على التوالي, 11.1% لكل من الأدينوفيروس و فيروس انفلونزا (ب) كما تم عزل فيروس الحصبة في 8.3%, أما فيروسات البارانفلونزا, الأشتيتين بار و الرسبيرونتري سينسيشيال قد تم عزلها في 6.5%, 4.6% و1.9% علي التوالي. بالمقارنة مع المناظرين لكل فيروس أثبتت الدراسة ألا فرق بينهما الا في حالة انفلونزا (أ) الذي وجد أكثر في المناظرين وهو أيضا أكثر الفيروسات تواجدا مع الفيروسات الأخرى. الأصابة بأكثر من فيروس في آن واحد تم تشخيصها في 21 حالة (19.4%).

أثبتت الدراسة أن الالتهابات الفيروسية تمثل 30.5% من التهابات الجهاز التنفسي و 15.1% من النزولات المعوية.

كما وجدت الدراسة أن 1% من فيروس الروبيلا و 13.3% من السيتوميجالوفيروس قد تم عزلها في الأطفال ذوي الأعمار أقل من 3 أشهر مما يرجح انتقال المرض من خلال المشيمة.
أثبتت الدراسة الأفق في نسبة الأصابة بالفيروسات بين الحالات والمناظرين. أيضاً أوضحت أن أعراض المرض بالنسبة للفيروسات موضع البحث لا تختلف مما ذكر سابقاً في المراجع وهي أن معظمهم يحضرون بأعراض التهابات الجهاز التنفسي.

وعليه دعت الدراسة بضرورة وجود فحوصات عملية سريعة لتشخيص الألفيات الفيروسية وذلك لتقليل التكلفة السريرية للنزلاة وتقليل الاستعمالات غير الضرورية للمضادات الحيوية وعلاجات الملاريا.
Abstract

Worldwide viral infection is a common cause of morbidity. In the developing countries, it had been overlooked due to lack of facilities. The objectives of the study were to:

a) improve the clinical diagnosis by investigating the existing infectious viruses in children aged up to 12 years,

b) evaluate the clinical diagnosis of viral fever in relation to serological results and

c) study the clinical presentation of acute viral diseases.

A case–controlled hospital based study was conducted on a four-day week basis during the period from 24th of March 2001 and 13th of August 2002. Febrile children aged less than 12 years who had negative blood film for malaria were selected as cases. A matched afebrile admissions were selected as controls. 108 (27%) out of 400 cases & 38 (19%) out of 200 control were studied.

Viral infection was detected in 64.4% of malaria negative patients (P < 0.05). The incidence of rubella, Influenza A virus, CMV was 18.5%, 15.8% & 13% respectively while that of measles, adenoviruses and Influenza B virus was 8.3%, 11.1% & 11.1% respectively. The incidence of parainfluenza virus, EBV, RSV was 6.5%, 4.6% & 1.9% respectively. Serological titers of cases compared to controls were insignificant except for Influenza A virus which occurred more in afebrile patients (P < 0.05) and it was the most frequent virus encountered in association with other viruses. Mixed viral infections occurred in 21 (19.4%) patients. Viral infection was implicated in 30.5% & 15.1% of respiratory tract infections & gastroenteritis respectively.
About 10% & 13.3% of infants less than three months were found to have significant IgM titers of Rubella & CMV respectively. This raises the possibility of congenital infection. The study revealed that no difference in the incidence of viral infection between febrile and afebrile children (P= 0.3423). Also the study showed that the clinical presentation of certain viral infections e.g. rubella, influenza etc... is not different from that reported in the literature. Mostly presenting with symptomatology of respiratory infections. The latter was the predominant clinical diagnosis in the majority of cases. It is therefore recommended availing rapid viral tests is more appropriate and cost-effective as it lowers the admission rate and the unnecessary use of antibiotics and antimalarials.

List of Abbreviations

Acute respiratory infection  ARI
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Term</th>
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<tr>
<td>CCHF</td>
<td>Crimean – Congo haemorrhagic fever</td>
</tr>
<tr>
<td>CDC &amp; P</td>
<td>Centers of Diseases Controls &amp; Prevention</td>
</tr>
<tr>
<td>CF</td>
<td>Complement fixation</td>
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<td></td>
<td><strong>Cytomegalovirus</strong> CMV</td>
</tr>
<tr>
<td>EBNA</td>
<td>Epstein Barr nuclear antigen</td>
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<tr>
<td>EBV</td>
<td>Epstein Barr virus</td>
</tr>
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<td>EI</td>
<td>Erythema infectiosum</td>
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<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
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<tr>
<td>EM</td>
<td>Electron microscopy</td>
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<tr>
<td>HI</td>
<td>Haemoagglutination</td>
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<tr>
<td>HPIV</td>
<td>Human Parainfluenza virus</td>
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<tr>
<td>ICTV</td>
<td>International committee on Taxonomy of viruses</td>
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<tr>
<td>IF</td>
<td>Immunofluorescence</td>
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<tr>
<td>IM</td>
<td>Infectious mononucleosis</td>
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<tr>
<td>IVIG</td>
<td>Intravenous immunoglobulin</td>
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<tr>
<td>JRA</td>
<td>Juvenile rheumatoid arthritis</td>
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<tr>
<td>MMR</td>
<td>Mumps-Measles-Rubella</td>
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<tr>
<td>PCF</td>
<td>Pharyngoconjunctival fever</td>
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PCR  Polymerase chain reaction
RIA  Radioimmunoassay
RSV  Respiratory Syncytial virus
RV  Rubella virus
SAR  Secondary attack rate
Sfn  Sandfly fever Naples
SFS  Sandfly fever Sicilian
TAC  Transient aplastic crisis
TR-FIA  Time related fluroimmunoassay
URTI  Upper respiratory tract infection
VCA  Viral capsid antigen

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Fig. 9 Clinical presentation of patients with significant RSV titers

Fig. 10 Clinical presentation of patients with significant IgM measles titers
Chapter one

1. Introduction and literature review

Virology has become an increasingly important field of medicine in the past decades. Many infectious diseases were once of unknown etiology can now be linked to specific viral pathogen (1). New methods for diagnosing and treating viral diseases are being developed. In addition, Antiviral therapy or prophylaxis is available for many of these viral infections (1).

1.1. EPIDEMIOLOGY:

There are classification systems based on portals of entry and exit of the agent to and from the host (2). The major division is between direct transmission, which is essentially immediate and indirect transmission, which is delayed (3). Direct contact includes, by extension, vertical transmission, which may take Place transplacentally, during the birth process or in breast milk (4).

Droplets of various sizes are expelled as part of respiratory discharge, the larger droplets, greater than 5 µm, settle out rapidly and do not spread more than 1m, rendering this form of transmission essentially immediate. Thus, even though the previously and the newly infected hosts are not in contact with each other, this is also considered as direct transmission, because of the limited transit time of this class of infected droplets which do not stay in the air for long periods (5). Large droplets spread rhinoviruses. Influenza is spread by both
large droplets and aerosol; a single viral subtype is generally responsible for outbreak (6), in contrast to the relatively limit nature of transmission via a large droplets alone.

The childhood exanthemata, measles, mumps and chickenpox are transmitted mainly by large droplets, which explain why outbreaks may take months to run their course in partially immune population (3).

Indirect transmission covers many diverse mechanism which have in common only the fact that there can be a delay, often prolonged, in the infectious agent reaching a new host. Indirect transmission is divided into three categories; vehicles are varied non-living entities, ranging from inanimate objects to water & biological products such as milk, urine or human tissue on or in which the infectious agent remains in transit to a new host (5). Vector transmission is divided into mechanical & biological. Mechanical transmission implied that the vector is, typically an arthropod, is involved only in providing transport for the agent, i.e. moving it from one site to another without multiplication taking place. It rarely takes place with viruses because the agents would be inactivated by the environmental exposure . No multiplication of the agent occurs, so it is not an efficient form of transmission. In contrast, biological transmission is highly efficient. Most of the large group of viruses transmitted by this mechanism were formerly termed arthropod-borne or arboviruses (7&8).

Infected dusts are rarely involved in transmitting viral infections by the air-borne route because desiccation usually inactivates them if they stay exposed to the environment for
prolonged periods. Certain respiratory viral infection such as influenza & Coxsackie virus A21 are commonly transmitted by small droplets or droplets nuclei (6). The droplet nuclei are formed from large droplets as they evaporate while suspended in the air around a spicule of dust. In most cases it has not been possible to demonstrate by air sampling the presence of virus in aerosols, but this mechanism is assumed to operate because of the ability of a virus to infect large numbers. (9).

1.1.1. Infectious period & secondary attack rate:

The epidemiological measure historically used to approximate indirectly the infectivity of an agent in human is so-called secondary attack rate (SAR)(5). The calculation of the SAR is most appropriate for diseases with relatively long incubation period, such as in Rubella & Mumps. A problem arises with diseases of shorter incubation period (c. 2-4 days), such as Influenza and the common cold, in which it is difficult to distinguish between co-primary & secondary cases because they are only days apart or may be overlap (5). Among infections of high SAR are Measles & Chickenpox. Rubella is less contagious, thus leaving residual susceptible among young woman entering childbearing age (10).

1.1.2. Pathogenecity and virulence:

Pathogenecity some times compares the capacity of different organisms to cause disease, where as virulence compares the severity of disease caused by different strains of the
same organism. Therefore, the virus would be considered of relatively low Pathogenecity but of relatively high virulence (5,11).

1.1.3. Viral infection in person, places, & time
Position in the family affects the occurrence of infections best transmitted in that setting (e.g. the respiratory viruses and enteric agents such as the rotaviruses). An only child in a family will experience lower rate of respiratory infection until other siblings born (12).

Examples of personal factors important in viral illness:

- **Age & gender.**
- **Position in family.**
- **Income.**
- **Education.**
- **Crowding.**
- **Socioeconomic status.**
- **Occupation – exposure.**
- **Genetic and related factor.**
- **Immunity.**
- **Nutrition.**
Place of occurrence of viral infection may be related to purely local biological or physical factors. Regional characteristic such as climate and resident flora and fauna determine whether transmission can take place. Most Arboviruses are found in specific location, determined by the distribution of the appropriate vectors of animal hosts (5).

Many infectious diseases such as rubella do not occur at uniform levels in populations but undergo predictable changes in frequency. Certain terms are used to describe these changes. Seasonal variation describe the phenomenon, well recognized with respiratory viruses, of much higher frequency in colder than in warmer months. Cyclical variation refers to change in frequency over period of years, with gradual increase in intensity of transmission. Hepatitis A, for example, has a cycle of 5-7 years (5).

1.2. History of viruses:

The nature of viruses was not elucidated until well after it was realized that certain diseases of plants & animals could be transmitted by an invisible infective principle that differed considerably from the known parasite (13).

By the end of the 19th century, viruses had been defined solely in terms of their infectivity, filterability & their requirement for living cells as a substrate for growth (13). By the end of the 1st war, it had been established that viruses are much smaller than bacteria, and appear to be capable of growing only in living cells, sometimes leave evidence of their presence in the form of inclusion body. The
main categories of viruses affecting respectively vertebrate, invertebrate, bacteria and plants had been identified, the existence of oncogenic viruses was established, and the outline of the immune response to viruses and other microbes were beginning to take shape (13).

1.3. Physical characteristics of viruses:

1.3.1. Size of the viruses:

Probably because of the difficulty of propagating them in the laboratory, research on the sizing of viruses by filtration and centrifugation was an important focus until well into the 1940s. Thus by 1940, the sizes of about 25 animal viruses ranging from enteroviruses (c. 20nm) to poxviruses (c. 250nm) has been measured with fair accuracy (14). Centrifugation was also useful for purifying viruses and became more so with introduction by Brakke (1953) of sucrose density gradients, in which particles within a mixture can be separated according to their sedimentation rate (13).

1.3.2. Morphology of viruses:

After the interruption of the 2nd world war, new techniques were introduced to exploit the potential of the EM (15). These techniques together with x-ray diffraction studies showed that most
viruses fall into one of two groups: the icosahedral viruses with so-called cubic symmetry; and those with helical symmetry, in which the outer subunits are arranged like the treads in a spiral staircase (13).

1.3.3. Chemical composition of viruses:

Not long after Watson & Crick (1953) solved the structure of DNA, they suggested that viral nucleic acid is protected by a shell of identical protein subunits (13,16).

This notion based on the limited coding capacity of viral nucleic acids, proved to be correct. All viruses were found to have this basic architecture (17), the nucleic acid being, with few exceptions, double-stranded DNA or single-stranded RNA. Casper et al. (1962) proposed the following terminology: the complex of protein shell (capsid) and nucleic acid is the nucleocapsid; the mature nucleocapsid surrounded in some viruses by an outer envelope, is referred to as virion (5).

1.3.4. Replication:

By 1957 Andre Lwoff was able to crystallize the essential features that distinguish viruses from all other organisms: they are strictly intracellular and potentially pathogenic entities with an infectious phase and (1) possess only one type of nucleic acid, (2) multiply in the form of their genetic material, (3) are unable to grow and undergo binary fission, and (4) are devoid of Lipmann system of enzymes for energy production (13).
1.4. Classification of viruses:

The basis for the modern scheme of virus classification was laid down by Lwoff, Horne & Tournier in 1962. This pragmatic scheme gave weight to characteristics considered to be important. These were the nature of the virion nucleic acid –whether RNA or DNA- and the structure of the virion, including its symmetry, the presence or absence of a lipid envelope and its size. The pathway of virus messenger RNA synthesis also form a central feature of the modern scheme of virus classification (18).

Viruses are separated into families on the basis of the type & form of the nucleic acid genome and the size, shape, substructure, and mode of replication of each virus particle. Within each family, classification of genera and species depend on antigenecity in addition to other properties, particularly the base of sequence homology (19). Significant developments in classification and nomenclature of viruses are documented in the reports of the international committee on taxonomy of viruses (ICTV) (19). The ICTV has approved 73 families and groups of viruses. The kind and the strandedness of the nucleic acid and the presence or absence of a lipoprotein envelope are the three key properties that form the basis for the classification of the 73 families and groups (19).

1.5. The laboratory diagnosis of viral infection:

There are four main reasons for diagnosing viral infections: to decide on specific treatment; as a basis for management such as termination of pregnancy; to provide epidemiological information;
or to provide a definite diagnosis and prognosis (20). The true value of definite diagnosis is to allow savings on antibiotics not used, investigation not needed and hospital-days saved through earlier discharge (20). These apply particularly for respiratory infections but, in addition, inappropriate use of antibiotics in gut infection can do serious damage (20).

1.5.1. Principles of diagnosis:

When the patient become infected by the virus, this followed by a very variable incubation period (measured in days to years) at the end of which symptoms and signs appear. Shortly before this the virus will become detectable as a result of multiplication. The patient will become ill as viral multiplication reaches a peak, followed in most cases recovery as the virus declines and the body’s defence mechanisms overcome the invader (20). It is appropriate to look during the early phase for virus or viral components, and during the late phase for an immune response, if any, to such components (20). The appearance of specific antibody follows about a week to 10 days later, initially short-term IgM class and then long (er) term of IgG class which will, in most cases, indicate development of immunity and resistant to reinfection (20).

1.5.2. Rapid viral detection:

Fluorescent antibody techniques or other methods that use antibodies to detect viral antigens in clinical specimens provide rapid identification of viruses (21).
Cytologic examination aids in diagnosis when inclusion bodies or syncytia are found but these methods are not sensitive enough to be relied upon for the diagnosis of life threatening infections such as neonatal herpes. All rapid detection of viruses should be in parallel with and confirmed by viral culture (21).

Viruses require living cells for propagation; the cells used most often are human or animal cell tissue cultures. Because some viruses are difficult to isolate and many require a variety of culture systems, the clinician should describe the clinical signs when the specimen sent to the laboratory (21). There are two components to successful immunofluorescence (IF): (i) an appropriate and adequate specimen that is prepared suitably; and (ii) the staining and the microscopy system (20). If the laboratory is reasonably close, specimens should be transported at 4ºC. Specimen for viral isolation should never be frozen at –20ºC (21).

1.5.3. Electron microscopy (EM):

The electron microscope is the only instrument with which we can see virus particles directly. One typical particle makes the diagnosis but not all viruses are instantly recognizable and it is the number of particles with consistent identifying features that is finally convincing (20). Diagnostic EM requires a stable instrument that is easy to use and capable of straightforward transmission EM at about 50 000 × magnification. A good specimen taken early in the infection will allow the diagnosis easily. Later in the disease, the levels are lower and the diagnosis is more difficult to make. In
these cases, enhancement or other techniques may be necessary (5, 22).

**1.5.4. Viral genome and virion detection:**

New methods for viral diagnosis rely upon the use of probes constructed of sequences complementary to the genome sequences of DNA or RNA viruses. Hybridization of these probes to viral sequences can be detected by southern blot or in situ methods using fluids or swab specimens or tissue sections. Polymerase chain reaction (PCR) detect viral gene sequences by using complementary nucleotides as primers to amplify a conserved region of the genome (21).

**1.5.5. Serologic test:**

Correct diagnosis requires at least two blood specimens: the 1st should be obtained during the early acute phase of the disease (acute serum) and the 2nd (convalescent serum) 14-21 days later. To establish the etiologic diagnosis, it is necessary to demonstrate a four-fold rise in titer of antibody to an agent in the convalescent as opposed to acute phase serum, when all tests are tested together (21). Antibody can be detected by variety of specific serological methods. ELISA & Latex agglutination tests are now used most often to detect antibodies to viral antigens.

**1.5.5.1. Solid-phase immunoassay:**
Radioimmunoassay (RIAs) and Enzyme immunoassay (EIAs) have been used successfully for the detection for viral antigens for over 20 years (20,23).

The basic principles of EIAs is to bind antigen to a solid-phase (Polymerase plate or bead) and detect its presence with a detector antiviral antibody and an antiglobulin antibody to which an enzyme (usually horseradish peroxidase or phosphatase) has been conjugated (20). Successive bonds mean that the enzyme is tethered to the solid phase only if the virus antigen is present. After thorough washing, a colourless substrates added and the enzyme acts on it to produce a clearly visible coloured product whose presence (indicating a positive result) can be read by eye or a spectrophotometer. Difficulties in binding antigen direct from the specimen have prompted variations such as using a capture antibody to pull it onto the plate or bead (20).

The route to success with such assays is attention to details. The constituents and their concentrations in diluents and wash buffers used to rinse the solid phase and the surface properties of the latter, are critical and must be carefully evaluated. The more complex (i.e. the more components) the assay, the more likely are non-specific results, particularly with polyclonal antibodies (20).

Then scope of these assays has been extended with the development of monoclonal antibodies (20). Further modification of solid-phase antigen detection are time-resolved fluroimmunoassay (TR-FIA) and Biotin EIA. Combined with the one-incubation
principle the sensitivity tests improved; the tests are also more practical, with fewer incubation and washing steps (24,25).

The advantages of EIAs and related techniques are (1) specimen transportation is not critical because intact cells are not required; (2) treatment of the specimen is not elaborate; (3) automation allows the test to handle larger numbers; and (4) the cut-off level can be set in advance and the result assessed by computer. The disadvantages of EIAs in antigen detection are the absence of quality control on the specimens and the lack of commercial monoclonal that have been evaluated for this particular use (20).

1.6. Immunoprophylaxis:

The primary objective of viral immunoprophylaxis are to prevent viral infection or to modify viral disease. The two classic approaches to active viral immunization include inactivated viruses or their purified components, and live attenuated vaccines (26).

1.7. Selected viruses:
1.7.1. PARVOVIRUS:

Parvovirus B19 is a member of the genus Parvovirus in the family of Paroviridae (27,28). Parvovirus was discovered by Cossart and Coworkers in 1975 as an anomalous precipitation line during counter immunoelectrophoreses screening of blood for hepatitis B antigen (28,29). The new virus was associated with human disease in 1981 when Pattison and colleagues linked it with the aplastic crisis of sickle cell disease (28).

1.7.1.1. Description of the agent:

B19 virions, as observed by EM, are icosahedral and have a diameter of approximately 26 nm. The viral particles are unenveloped and contain genetic material consisting of ssDNA. They do not appear to contain lipid or carbohydrates and no enzymatic functions have been demonstrated in association with B19 viral particles. The physical properties contribute to viral resistance to heat, solvents and detergent treatment (27,30).

In vivo Parvovirus B19 has a limited host range and its replication requires mitotically active cells (27).

2.1.2. Epidemiology:
Parvovirus is distributed worldwide and is a common cause of human infection. B19 infection can occur in any month of the year in sporadic or epidemic form. In temperate climate, however, epidemic manifestations are more common in late winter, spring and early summer, with epidemic peaks every 2 to 4 years.
B19 epidemics are mainly associated with two of the major clinical manifestations of the infection; Erythema infectiosum (EI) and Transient aplastic crisis (TAC) (27). Antibody prevalence increases with increasing age (28).

Transmission of B19 is by respiratory route, presumably via large droplets spread (27,28,31). Transmission takes place frequently among household and school contacts during outbreaks of B19 infection (31). The transmission rate in households ranges from 15-30%. In susceptible contact; mothers are more commonly infected than fathers (28).

Parvovirus B19 infection can also be transmitted from mother to fetus through the transplacental route and may lead to nonimmune hydrops or fetal death (28). B19 DNA has been detected by PCR in batches of albumin, factor VIII, factor IX, clotting factor concentrates, and immunoglobulins. Hemophiliacs were found to be infected with B19 after receiving dry heat-treated or steam-treated factor VIII and IX or solvent or detergent-treated clotting factor (27,32).

The incubation period for EI ranges from 4-28 days (average 16-17 days). The incubation period for other clinical manifestations, such as TAC is shorter because viremia precedes the rash (28).

2-1-3-Clinical manifestations:

Parvovirus B19 infection can occur totally asymptotically or with mild non-specific symptoms. However, infection also can lead to
numerous clinical; manifestations ranging from acute self-limiting disease to sometimes chronic illness (28).

2.1.3.1. Erythema infectiosum (EI or 5th disease):

The most common clinical manifestation caused by Parvovirus B19 consists of exanthematous illness of childhood, EI also known as 5th disease. These two names together with slapped cheek disease are well known as a childhood disease first recognized to be caused by B19 in 1983 during an outbreak in primary school in London, England (30).

Prodromal symptoms may include low-grade fever, headache and various degree of conjunctivitis, upper respiratory tract complaints, cough, myalgia, nausea and diarrhoea (33). The typical rash of EI occurs in three stages that are not always distinguishable. The initial stage is an erythematous facial flushing (28). The subsequent eruptions became bilaterally symmetric on the arms, legs and trunk but only rarely the palms and soles, has a reticular or lacy pattern, and lasts for about one week (27). The rash resolved spontaneously with desquamation but tends to wax and wane over 1-4 weeks and can reappear in relation to rise in body temperature (fever, heat, exercise and sunlight exposure) (27,28). Lymphoadenopathy and atypical papular, purpuic or vesicular rash are also described (28).

2.1.3.2. Transient aplastic crisis (TAC):

Individuals with chronic haemolytic condition may experience transient red cell aplasia after contact with B19 (28). B19
associated TAC was found in a wide range of patients with chronic haemolytic anaemias or erythroid stress (34). Severe anaemia is associated with thrombocytopenia & granulopenia. B19 induced aplastic crisis can also be associated with flu-like symptoms, gastroenteritis, pallor, dyspnoea, weakness and confusion. Rash manifestations appear to be rare. Congestive heart failure and bone marrow necrosis may develop and the illness can be life threatening (27,35).

Aplastic crisis generally occurs as a single episode in the life of a patient and, although severe, is self limiting because of the appearance of a specific immune response (27).

2.1.3.3. Arthropathy:

Arthritis and arthralgia occur as a complication of 5th disease or as the only clinical manifestation of B19 infection (28). Two large studies in 1985 reported the relation between B19 and arthropathy (27,36,37). B19 arthritis is uncommon in children (less than 10% of cases). (27).

Joint symptoms range from diffuse arthralgia with morning stiffness to frank arthritis. B19 associated arthritis, which is likely to be immune complex mediated, is typically polyarticular and symmetric. The involved joints include the proximal interphalangeal joints of the hands and feet and, less frequently, the wrists, elbows, knees and ankles (27,38). The joint swellings are self-limited and, in the majority of patients, resolved within 2-4 wk. (28).
Although some patients with B19 arthropathy meet the American rheumatism association criteria for a diagnosis rheumatoid arthritis (RA) and criteria for chronic juvenile RA (JRA), recent studies are still not in agreement about the role of Parvovirus B19 in the etiopathogenesis of RA and JRA (39,40). Transient rheumatoid factor positivity is reported in some patients but with no joint destruction (28).

2.1.3.4. Fetal infection:

The incidence of these outcomes after maternal infection is low, estimated at 5% or less (28). The mechanism of fetal disease appears to be viral-induced red cell aplasia at a time when the fetal erythroid fraction is rapidly expanding. This can lead to profound anaemia, high output cardiac failure and hydrops(28).

B19 associated hydrops, which represents 16% to 18% of all idiopathic nonimmune hydrops, most often resolves spontaneously, with the delivery of apparently normal infants. B19 hydrops can lead to fetal loss at an incidence of between 1.66% and 9% (27,41). The 2nd trimester seems to be the most sensitive time, but fetal losses are reported at every stage of gestation (28). Fetal infection has not been associated with other birth defects (28).

2.1.3.5. B19 infection in immunocompromised patients:

Patients with impaired humoral immunity are at risk for chronic infections with Parvovirus B19. Chronic anaemia is the most
common manifestation, sometimes accompanied by other cytopenias or complete marrow suppression (28). In chronically infected immunocompromised patients, B19 viremia can persist at a very low titer (>100,000 genome copies/ul) (42). Immunoglobulin treatment of immunocompromised patients, chronically infected by B19, has been effective in clearing the virus from circulation in some cases. In other cases it has been used to ameliorate the clinical course of persistent B19 infection (27).

2.1.4. Diagnosis:

Methods to detect viral particles or viral DNA such as PCR or DNA hybridization are necessary to make the diagnosis (28). PCR is the most sensitive diagnostic tool for detecting B19 DNA (27). The virus can not be isolated by culture (27,28). The diagnosis is usually based on clinical observation of the typical rash and exclusion of other conditions (28).

Determination of B19 IgM is the best marker of recent or acute infection in a single serum sample. IgM develops rapidly after infection and persists for upto 6 to 8 weeks. Anti B19 IgG serves as a marker of past infection or immunity. Serological diagnosis is unreliable in patients with immunodeficiencies (28).

2.1.5. Management:

No specific antiviral therapy. Commercial lots of intravenous immunoglobulin (IVIG) have been used with some success to treat B19-related episodes of anaemia and bone marrow failure in immunocompromised children (28).
2.2. Cytomegalovirus (CMV):

CMV is a member of the family Herpesviridae, which include EBV, herpes simplex and human herpes virus 6,7,8 (43).

CMV is the most common congenital viral infection, which occasionally causes the syndrome of cytomegalic inclusion disease (hepatosplenomegaly, jaundice, petechiae, purpura and microcephaly). Disease may result from primary or recurrent CMV infection, but the former is a more common cause of severe disease. CMV contains double stranded DNA in a 64 nm core enclosed by an icosahedral capsid composed of 162 capsomeres (44).

Human CMV shows a very strong species specificity, being able to replicate only in human cells (45). HCMV infects multiple cell types throughout the host, and recurrent virus can be shed from multiple sites (46,47). Persistence is characterized by the presence of latent viral genomes that periodically reactivate to produce infectious virus. The mechanism of HCMV persistence are not resolved. Result from in vitro and in vivo studies suggest that the virus utilize a variety of strategies to maintain itself in a host despite vigorous cellular and humoral antiviral immune responses (46). The expression of HCMV genes upon infection is temporally regulated (45). The first genes expressed (immediate-early (IE) genes) are independent of any De novo protein synthesis and encode mainly either regulatory or immune-modulator factors (45).

2.2.1. Epidemiology:
The prevalence of infection, which increases with age, is higher in developing countries and among lower socioeconomic strata of the more developed nations(44). CMV has been detected in 0.2 to 2.5% of newborn infants and is the most common identified cause of congenital infection. Fewer than 5% of congenitally infected infants develop symptoms during the newborn period. Most postnatal infections are acquired by close contact with individuals who are shedding virus. Prolonged shedding of virus after congenital or acquired CMV infection contribute to the ease of virus spread(43). Rates of virus shedding can be as high as 39% in some study populations of healthy individuals(46,48).

Indirect transmission is possible via contaminated fomites such as toys(44). Perinatal transmission is common, reaching 10 – 60% by 6 months of age. Infected infants excrete virus for years in saliva and urine (44).

After transplantation, many patients excrete CMV as a result of infection acquired from the donor organ or from re-activation of latent infection caused by immunosuppression. Seronegative recipients of organs harvested from seropositive donors are at risk for severe disease(44).

CMV is inactivated by a number of physical and chemical treatments, including heat (56°C for 30minutes), low PH, lipid solvents, UV light and cycles of freezing and thawing(43).

2.2.2. Pathology:
Strikingly enlarged intranuclear inclusion-bearing cells that also have cytoplasmic inclusions are pathognomonic for CMV infection (44).

2.2.3. Clinical manifestation:

CMV infections are common and usually asymptomatic, however, the incidence and spectrum of disease in newborns and in immunocompromised hosts establish this virus as an important pathogen (43). In young children, the infection occasionally causes pneumonitis, hepatitis, hepatomegaly and petechial rashes. In older children, adolescents and adult CMV may cause mononucleosis-like syndrome characterized by fatigue, malaise, myalgia, headache, fever, hepatosplenomegaly, abnormal liver function test results and atypical lymphocytosis. It is generally mild and lasting 2-3 weeks (44).

CMV infections are frequent and occasionally severe in children or adult with congenital or acquired defects of cellular immunity such as patients with AIDS, cancer patients (particularly those with leukaemia and lymphoma receiving chemotherapy) (43).

Pneumonia, retinitis and involvement of the C.N.S and Gastrointestinal tract (GIT) are usually severe and progressive. Submucosal ulceration can occur anywhere in the GIT. Haemorrhage and perforation are known complication, as are pancreatitis (44). It is now recognized that even congenitally infected infants who are asymptomatic at birth may develop hearing defects or learning disabilities later in life (43).
In immunosuppressed individuals, CMV pneumonitis, retinitis and GIT diseases are common and can be fatal(44).

2.2.4. Diagnosis:

BY using exfoliative cytologic techniques, a presumptive diagnosis of CMV can be made in 25-50% of cases of symptomatic congenital infection(43). Monoclonal antibodies to CMV can be used for the direct detection of CMV antigen by immunofluorescence tests on tissues obtained by biopsy or at autopsy(49).

The EIA can be used effectively to determine the immune status of a patient(50) and to detect significant rises in antibody titers. Although the detection of CMV specific IgM may be useful in the determination of recent or active infection, the result should be interpreted with caution. Because IgM does not cross the placenta, a positive result from a single serum specimens from an infected newborn is diagnostic (43).

The application of virologic methods including conventional and shell viral culture, rapid direct-detection assays, and serologic testing, should be combined with clinical assessment of the patients to provide an accurate, reliable diagnosis of CMV infection and disease and to allow subsequent prompt appropriate patient management and timely intervention with specific antiviral therapy (43).

2.2.5. Prevention:
Limiting the severity of the HCMV disease that occurs in the non
immune host after prenatal infection or under condition of immunosuppression will require the development of an effective vaccine strategy (51).

2.2.5.1. Passive immunoprophylaxis:

The use of hyperimmune plasma or globulin for prophylaxis of infection in transplant recipients reduces the risk of symptomatic disease but does not prevent infection (44).

2.2.5.2. Active immunization:

Candidates for a CMV vaccine includes seronegative woman of childbearing age, seronegative and transplant recipients. Life-attenuated vaccines are immunogenic but immunity wanes quickly. The vaccine does not protect renal transplant recipients from CMV infection but appears to reduce the virulence of primary infection. Other types of vaccines, such as subunit and recombinant vaccines, are being developed (44).

2.2.6. Treatment:

Ganciclovir has been used to treat life-threatening CMV infections in immunocompromised host. A regimen of 10mg / Kg / 24hr., with individual doses administered at a 12 hours intervals for 2-3 weeks, followed by maintenance dose of 5mg / kg / day administered until the regression of clinical manifestation has had some efficacy(44).

2.2.7. Prognosis:

A key determinant for the outcome of an HCMV infection in these clinical setting is preexisting immunity. The presence of seroimmunity to HCMV prior to conception reduces the frequency of mother-to-fetus viral transmission and, more importantly, decreases the risk of damage in the infected fetus (51).

Patients with mononucleosis-like usually recover fully. CMV infection and disease may be terminal events in individual with increased susceptibility to infections such as those with AIDS(44). The mortality rate in symptomatic CMV congenital infection is around 12%, and most of the surviving infants has permanent sequelae, which include visual deficits, hearing loss, seizure disorders and motor and intellectual retardation (44).

Virtually all children with asymptomatic CMV congenital infection survive, although, in up to 20% hearing deficits and learning problem eventually develop (44).
2.3. Epstein Barr Virus (EBV):

Infectious mononucleosis (IM) is the best-known clinical syndrome caused by EBV (52).

2.3.1. Etiology:

EBV has the characteristic herpetic 120 nm enveloped morphology with 162 capsomeres in icosahedral arrangement (53). Its DsDNA exists both as a linear form in the mature virion and as a circular episomal form in latently infected cells (53).

2.3.2. Epidemiology:

The epidemiology of IM is related to the epidemiology and the acquisition of EBV infection (52). Transmission of EBV requires salivary contact; air-borne or blood-borne transmissions are not important route of infection (53). Familial transmission of EBV infections is common, as demonstrated by intrafamilial sharing of EBV strain (54).

EBV is shed in oral secretion for 6 months or longer after acute infection and then intermittently for life (52). The degree of shedding varies from person to person but remain constant for the same individual (55).

Immunosuppression may permit re-activation of latent EBV; approximately 60% of seropositive; immunosuppressed patients shed the virus (52).

Infection with EBV in developing countries and among socioeconomically disadvantaged population of developed countries usually occurs during infancy and early childhood. In central Africa, almost all children are infected by 3 years of age.

The true incidence of IM is unknown but is estimated to occur in 20-70 of 100,000 persons per year. The prevalence of serologic evidence of past EBV infection increases with age (52).

Childhood infection are mostly asymptomatic; but 50-70% of delayed primary infections in young adult may be associated with classical IM, a self-limiting lymphoproliferative illness ranging from mild to severe (53).

In immunosuppressed patients, EBV-infected lymphoid cells are associated with a number of lymphoproliferative conditions, ranging from benign polyclonal hyperplasia with no cytogenic abnormalities to oligoclonal as well as monoclonal malignant lymphoma (53).

EBV has long been suspected of contributing to the tumorigenesis of Burkitt’s lymphoma, primarily a tumor of children in Africa and New Guinea; and nasopharyngeal carcinoma (NPC), an undifferentiated squamous carcinoma with particularly high incidence among Thousands Chinese. Viral antigens and genome can be detected in both tumors. Serology can be helpful in the management and therapeutic monitoring of patients with either malignancy (53).

2.3.3. Pathogenesis:

Incubation period of IM in adolescent is 30-50days. In children it may be shorter. The majority of cases of primary EBV infection in infants and young children are clinically silent (52).

Most cases of IM can be clinically diagnosed from the characteristic triad of fever, pharyngitis, and cervical lymphadenopathy generally lasting 1-4 week (53).

The physical examination characterized by generalized lymphadenopathy (90% of cases), splenomegaly (50% of cases), and hepatomegaly (10% of cases). Lymphadenopathy occurs most commonly in the anterior and posterior cervical nodes, submandibular lymph nodes (L.N.) and less commonly in the axillary and inguinal L.N. Epitrochlear Lymphadenopathy is particularly suggestive of IM. Symptomatic hepatitis or jaundice is uncommon. Splenomegaly to 2-3 cm below the costal margin is typical; massive enlargement is uncommon (52).

The sorethroat is often accompanied by moderate to severe pharyngitis with marked tonsillar enlargement, occasionally with exudates. Other clinical findings include rashes, oedema of the eyelid. Rashes are usually maculopapular and have been reported in 13-15% of patients. 80% of patients with IM will experience a rash if treated with Ampicillin or Amoxicillin (52).

2.3.4. Clinical manifestation:

2.3.5. Diagnosis:
A presumptive diagnosis may be made by the presence of typical clinical symptoms with atypical lymphocytosis in the peripheral blood (52).

In 85 to 90% of IM patients, Paul Bunnell heterophile antibody tests are positive. False positive may occur in 2 to 3% of patients and can be excluded by EBV-specific serology (53).

Specific EBV antibodies testing is useful to confirm acute EBV infection, especially in heterophile-negative cases, or to confirm past infection and determine susceptibility to future infection (52).

The EB nuclear antigen (EBNA), Early antigen (EA) and viral capsid antigen (VCA) systems are most useful for diagnostic purposes. The acute phase of IM is characterized by rapid IgM and IgG antibody responses to VCA in all cases and an IgG response to EA in most cases. The IgM response to VCA is transient but can be detected for at least 4 weeks and occasionally up to 3 months (52).

Absence of Anti-EBNA when other antibodies are present implies recent infection, while the presence of Anti-EBNA implies infection occurring more than 3-4 months previously (52).

The detection of IgM antibody to VCA is the most valuable and specific serologic test for the diagnosis of acute EBV infection and is generally sufficient to confirm the diagnosis (52).

2.3.6. Treatment:

No specific treatment for IM. Therapy with high doses of I.V. Acyclovir decreases viral replication and oropharyngeal shedding during period of administration but does not affect the severity of symptoms or the eventual clinical course. Short courses of corticosteroids (less than 2 weeks) may be helpful for complications of IM (52).

2.3.7. Prognosis:

The prognosis for complete recovery is excellent if no complications ensue during the acute illness. Second attacks of IM caused by EBV have not been documented (52).

2.4. Influenza viruses:

Influenza viruses A & B are worldwide major causative agents of human acute respiratory infections. Infants, the elderly, and individuals with compromised cardiac, pulmonary or immune systems are at great risk of serious complications from these viruses (57).

2.4.1. Epidemiology:

In region with temperate climate, Influenza viruses cause epidemics almost every year, and many of them are associated with considerable morbidity and mortality. Epidemics typically occur from December to March and from March through August in temperate regions of northern and southern hemisphere respectively. Influenza seasonality in tropical and subtropical climates is less well defined; however, Influenza viruses circulate in these areas throughout the year with peak activity in the summer months or in rainy seasons. Epidemics in community generally last 3-8 weeks (58). The attack rate and frequency of isolation of influenza is highest in young children. As many as 30 –50% of children have serologic evidence of infection in typical year (59).

2.4.2. Etiology:

Influenza viruses are classified as Orthomyxoviridae (59). Influenza A & B viruses contain 8 single-stranded RNA segments that are complementary to mRNA and that encode at least 10 polypeptide, of which 8 are structural viral protein and 2 are found in infected cells (58).

Two distinct types of antigenic variation occur in Influenza viruses and allow them to evade pre-existing immunity. The first is antigenic shift, which involves the appearance of a new subtype of influenza A virus, this abrupt change in antigenicity has been associated with pandemics, as occurred in 1957 with the emergence of the Asian (H2N2) virus and in 1968 with the emergence of Hong Kong (H3N2) strain. The second type of antigenic variation is antigenic drift, which is more gradual and occurs through the accumulation of points mutations in the HA & NA within a subtype, resulting in the
ability of antibody to previous strains to neutralize the mutant virus, this type is responsible for periodic epidemics (5,58).

2.4.3. pathology:

Influenza causes a lytic infection of the respiratory epithelium with loss of ciliary function, decreased mucus production, and desquamation of the epithelial layer. The exact immune mechanisms involved in termination of primary infection and protection against re-infection are not well understood. The extremely short incubation period of influenza and its growth on the mucosal surface pose particular problems for invoking a protective immune response (59).

2.4.4. Clinical manifestation:

The spectrum of illness ranges from asymptomatic Influenza or mild pharyngitis to pneumonia with fatal outcome. Influenza often begins after incubation period of 1-4 days with sudden onset of fever that may be accompanied by sore throat, dry cough, headache, myalgia, malaise or anorexia (58).

The predominant symptoms may localized any where in the respiratory tract, producing an isolated upper or respiratory tract illness, Croup, Bronchiolitis or pneumonia. The children may be highly febrile and toxic in appearance, prompting a full diagnostic workup (59).

Influenza type A and B have been reported to cause myocarditis, and Influenza type B can cause myositis. When Influenza type B is accompanied by the administration of salicylates, the fatty liver, cerebral oedema and mitochondrial changes (WHICH are the hallmarks of Reye syndrome) can be seen (59). Most signs and symptoms resolve within a week; however, cough and malaise may persist (58).

Bacterial superinfection should be suspected with recrudescence of fever, prolonged fever or deterioration in the clinical status (59).

2.4.5. Diagnosis:

The availability of improved drug treatments with neuroaminidase-blocking agents for Influenza viruses has emphasized the importance of rapid laboratory diagnosis. Furthermore, unnecessary use of antibiotics for suspected bacterial infection and prevention of Influenza outbreaks in hospitals and in the community are given increasing priority, which also require efficient diagnostic methods (57). The diagnosis of Influenza depends on epidemiologic and clinical consideration. The laboratory confirmation of Influenza can be made in 3 ways. If seen early in the illness, virus can be isolated from nasopharynx by inoculation of the specimen into embryonated eggs or a limited number of cell lines that support the growth of Influenza (59).

For the quantification of influenza virus specific serum antibody, the hemoagglutination (HI) assay and complement fixation (CF) assay are routinely used. However these assays suffer from some disadvantages. Alternatively, enzyme-linked immunosorbent assays (ELISAs) have been used for the detection of influenza virus-specific antibodies (51). ELISAs measuring influenza virus-specific serum IgG antibodies have been shown to be more sensitive than the HI or the CF assay (60,61,62).

2.4.6. Complication:

The most severe complications contributing to the mortality
associated with influenza are lower respiratory tract infection, which may present as primary viral pneumonia, mixed viral and bacterial pneumonia(58). Acute otitis media can be seen in up to 25% of cases of culture-documented influenza(59).

2.4.7. Prevention:

Although the new antiviral drug zanamivir for influenza A and B and life-attenuated intranasal influenza vaccine were shown to be highly effective in clinical trials, continuous surveillance of influenza viruses is still required in order to properly select the vaccine strain (63). Annual vaccination with trivalent inactivated vaccine is now the primary measure for the control of influenza (58).

Current guidelines include the administration of vaccine intramuscularly to children 6 months of age and older in chronic care facilities; those with chronic disorder of the pulmonary or cardiovascular systems including asthma; those with chronic metabolic diseases (including diabetes mellitus), renal dysfunction, haemoglobinopathies or immunosuppression (including immunosuppression caused by medication) and children receiving long-term Aspirin therapy who may be at risk for Reye syndrome after influenza(59).
2.4.8. Treatment:

Amantidine hydrochloride is the only licensed antiviral drug for influenza type A. It has been prophylactically used in high-risk patients and their care providers during influenza epidemics and in immunodeficient persons and those for whom the influenza vaccine is contraindicated. If given within the first 48 hours, it decreased the severity and duration of influenza symptoms. Confusion and inability to concentrate or sleep are seen in a minority of people given Amantidine hydrochloride (59).

Adequate fluid intake and rest are important components in the management of influenza. Non-salicylate-containing antipyretics can be used for high fever. Bacterial superinfections are common, and antibiotic therapy should be administered (59).

2.4.9. Prognosis:

The prognosis for recovery is excellent although full return to normal level of activity and freedom from cough usually requires weeks rather than days (59).

2-5- Rubella virus:

Rubella virus (RV) is found only in human population & causes German measles (64).

For many years, German measles was frequently confused with measles, scarlet fever & other infectious diseases presented
with rash. Rubella was accepted as a distinct disease by an international congress of medicine in London in 1881(65).

2.5.1. Description of the virus:

RV is classified as a rubivirus & is a member of the family Togaviridae. Only one type of species of RV has been recognized so far, and it is immunologically distinct from all other known viruses. The virus possesses three major structural proteins; two glycoproteins; E1 & E2 are associated with envelope and nucleocapsid protein C is found internally (64). The genome of RV is a single stranded of RNA. This 40s genomic RNA is infectious, but the recovery of infectivity is poor (66). Because of the lipid content of the viral envelope, RV is inactivated by detergent and organic solvents (67). Replication of viral RNA and synthesis of protein occur in the cytoplasm of the cell, and the viral matures by budding into cytoplasmic vesicles or from the marginal plasma membrane (64).

2.5.2. Epidemiology:

Humans are the only natural hosts of RV, which is spread by oral droplets or transplacentally through congenital infection (68). Despite the success of the rubella vaccination program in the USA, a number of cases of congenitally acquired rubella were reported in California and among population in Pennsylvania in 1989/90, this resulting from infection of unvaccinated women (69).

Before the widespread use of rubella vaccine, Rubella was an epidemic disease with 6 to 9 year cycle (64). Seroepidemiological studies showed that the proportion of seropositive people
increased progressively with age, about 50% of 9 to 11 year-old children being immune. Among women of childbearing age, the proportion increased to about 80-85%(59). In 1992, the lowest ever number of cases of rubella were reported in Britain (71,72).

As yet, relatively few virological studies have assessed the impact of congenitally acquired rubella in the developing countries. However, virological studies conducted in parts of South America and India among children who are deaf, blind, or both, suggest that the burden of congenitally acquired rubella may considerable (73).

2.5.3. Immune response to RV infection:

A single attack usually confers permanent immunity (21). However, evidence of reinfection may be obtained by demonstrating a significant increase in antibody concentration following natural & experimental exposure to rubella. Such reinfection is generally asymptomatic (68).

Rubella-specific IgM, IgG&IgA responses develop rapidly after the onset of rash. Rubella-specific IgG persists for life, but may decline to low level in old age (72).

Rubella-specific IgM usually appears within 4 days of onset of rash & persist for 4-12 weeks but detection depends on the sensitivity of the technique employed. Specific IgM may sometimes
persist for up to one year after both naturally acquired & Rubella immunization (72,75).

Serum & nasopharyngeal IgA responses are detectable for at least 5 years after infection. Specific IgD & IgE responses develop rapidly after onset of infection and persist for at least 6 months (68).

2.5.4. Clinical manifestation:

- **2.5.4.1. Postnatal RV infection:** The incubation period of infection is usually between 4 to 12 days (72). Some 25 to 50% of infected children are asymptomatic (64). In young children, the onset of illness is usually abrupt (72). The most characteristic sign is retroauricular, posterior cervical and postauricular adenopathy (68), and patient may complain of tender lymph nodes when or just before the rash appears (72). Lymphadenopathy is evident at least 24 hours before the rash appears and may remain for 1 week or more (68).

  The exanthem is usually discrete, in the form of pinpoint maculopapular lesion; it appears first on the face and spreads rapidly to the rest of the body (72). Mild itching may occur. The eruption usually clears by the 3rd day. Desquamation is minimal (68). Disease without rash may occur in 25% of children (72).

  The pharyngeal mucosa and the conjunctivae are slightly inflamed. The spleen is often slightly enlarged (68).

  Arthralgia occurs commonly in post-pubertal females after administration of rubella vaccine. Thus, joint symptoms may result
from direct infection of the synovial membrane by virus, for RV has been isolated from the joint aspirate of vaccinees with vaccine-induced arthritis (72). Although there is no doubt that RV may be isolated from synovial fluid after naturally acquired infection or vaccination and from occasional patient with chronic inflammatory joint disease, the incidence of vaccine-associated chronic complications has not been determined (76). Thus, there is no convincing evidence that RV plays a causal role in chronic inflammatory joint disease (72). Any joint may be involved, but the small joints of the hands are affected most frequently. Parathesia may be reported (68).

- **2-5-4-2-Congenital rubella:**

  The most common described anomalies associated with congenital rubella are ophthalmologic (cataracts (bilateral or unilateral (68).), microphthalmia, glaucoma and chorioretinitis), cardiac (patent ductus arteriosus, peripheral pulmonary artery stenosis, and atrial or ventricular septal defects), auditory (sensorineural deafness), and neurologic (microcephaly, meningoencephalitis, and mental retardation)(64).

  Blueberry Muffin skin lesions may occur. The infants may have active meningoencephalitis at birth; later sequelae include motor and mental retardation. Persistent infection lead to
pneumonia, hepatitis, bone lucencies, thrombocytopenic purpura, and anaemia in the infant with congenital rubella syndrome (68).

2.5.5. Diagnosis:

- **2.5.5.1. Postnatally acquired infection:**

  A clinical diagnosis of rubella is unreliable and so laboratory confirmation is required, particularly for the diagnosis of rubella-like illness during pregnancy. Because virus is slow to grow in cell cultures and a distinct cytopathic effect is not produced, serological methods are employed. A significant rise in antibody concentration may be detected by a variety of methods, including HAI, EIA, or latex agglutination (LA) titration (72). Rubella-specific IgG and IgM antibodies may be detected in saliva using saline antibody capture radioimmunoassay; results correlate well with serum antibodies (77).

  Virus may also be detected in the stool and urine but excretion occurs over for a shorter time (72).

  Reinfection is associated with a rise in antibody concentration, sometimes to very high level (72). Incidence of reinfection on exposure of individual who are serologically immune to wild virus is 3-10%(68).

  Viraemia may present for about a week before the onset of rash, but as this appears rubella antibodies develop and viraemia terminates (72).
2.5.5.2. Congenital rubella diagnosis:

The diagnosis can be confirmed by finding virus-specific IgM antibody in the neonatal serum or by culturing rubella virus from the infant’s urine or tissues. Prenatal diagnosis of fetal rubella infection can be made either by virus isolation from amniotic fluid or by identification of rubella specific IgM in cord blood (68).

2.5.6. Complication:

Complications are relatively uncommon in childhood (68). Encephalitis and thrombocytopenia are rare complication (64). Encephalitis occurs in about 1/6000 cases (68).

2.5.7. Prognosis:

Prognosis of childhood rubella is good; that of congenital rubella varies with severity of infection. Only about 30% of infants with encephalitis appear to escape residual neuromotor deficit, including an autistic syndrome (68).

2.5.8. Prevention:

The rubella vaccine program in the United states calls for immunization of all boys and girls between the age of 12 and 15 months and pubertal an nonpregnant postpubertal females. Immunization is given as measles mumps, rubella (MMR) vaccine. Since 1979 live-virus vaccine RA27/3 has been used exclusively for active immunization against rubella in the United States. In a
susceptible person, passive protection may be variably afforded by intramuscular injection of immune serum globulin (ISG) given in a dosage (0.25-0.50 ml or 0.12-0.2 ml/lb) within the 1st 7-8 days after exposure (68).

2.5.9. Management:

Unless bacterial complications occur, treatment is symptomatic. Interferon and Isoprinosine have been used with limited success (68).

If a pregnant woman whose immune status is unknown is exposed to rubella, an antibody test should be done as an emergency measure. If determined to be immune, she can be reassured that the pregnancy can be continued without added risk. If she is found to be susceptible and therapeutic abortion is unacceptable or unavailable to her, passive immunization with ISG, 20-30ml intramuscularly should be attempted immediately. Active immunization of pregnant women is not advised (68).

2.6. Human Parainfluenza viruses (HPIV):

Viruses in the Parainfluenza family are common causes of respiratory illness in infants and young children. They cause a spectrum of upper and lower respiratory tract illness, but are particularly associated with laryngotracheitis, bronchiolitis and croup (78).

2.6.1. Etiology:
PIVs belong to the family paramoxyviridae. PIV-1 & PIV-2 belong to the genus paramoxyvirus, while PIV-3; PIV-4A & PIV-4B belong to the genus Rubulavirus. The viruses have a nonsegmental single-stranded RNA genome with negative polarity (79).

2.6.2. Epidemiology:

Parainfluenza viruses spread efficiently and, by 5 years of age, virtually all children have serological evidence of past infection with HPIV-3, 75% with HPIV-1, 60% with HPIV-1 and 50% with HPIV-4 (80).

Infection with HPIV usually occur during seasonal community outbreaks (81). Type 1 & 2 are more seasonal. They occur in the summer and falls and alternate year in which their serotype is most prevalent (78).

PIVs are spread from the respiratory tract by aerosolized secretions or direct hand contact with secretion (78). They are stable for hours, but not days, in the environment (82). Serious illness is seen with PIV type 3 in the immunocompromised child (78). HPIV-4 has less often been detected in patients, presumably because it causes less severe illness (83).

2.6.3. Pathology & pathogenesis:

Illness begins 1-7 days after infection and probably result from combination of cytopathic effect of the virus and the immune or
inflammatory response (or both) to infection (84). The mechanisms by which viral injury occurs are not known. The severity of illness correlates with the amount of viral shedding (78). Virus is usually shed for 3-10 days (84). Reinfection can occur throughout life, the elderly and the immunodeficient patient being at greatest risk of serious complications of infection in adults (85). With 2\textsuperscript{nd} infections, virus is shed for a shorter period, although in immunosuppressed patients it can be shed for prolonged period (84). PIVs replicate in the respiratory epithelium without evidence of systemic spread. The destruction of cells in the upper airways can lead to secondary bacterial invasion and resultant bacterial tracheitis. Eustachian tube obstruction can lead to secondary bacterial invasion of the middle ear space and acute otitis media (21).

2.6.4. Clinical manifestation:

PIV-1 is the principal cause of Croup, although PIV-2, PIV-3 and other infection agents may also cause this disorder (86). HPIV-3 is second only to Respiratory Syncitial Virus as a cause of pneumonia and bronchiolitis in infants and young children (5). Infections with HPIV have been associated with 2-10\% of cases of URTI, 25-50\% of cases of croup and 5-20\% of cases of pneumonia and bronchiolitis, but rarely with meningitis (84).

PIV infections in older children and adults are more likely to be asymptomatic or to result in mild disease resembling the common cold syndrome (86). PIV infections are associated with high fever. A side for low-grade fever, systemic complaints are rare. The
illness usually lasts 4-5 days. Rarely Parainfluenza viruses have been implicated in parotitis (78).

**2.6.5. Diagnosis:**

Infection can be demonstrated by detection of the virus, viral antigens or genome, a diagnostic rise in virus specific antibodies, or the presence of IgM antibodies. To detect a diagnostic rise in antibodies, the acute phase serum specimen should be collected within a week of onset of illness and the convalescent phase specimen > 2 weeks later (84).

The radiographic “steeple” sign of progressive narrowing of the subglottic region is characteristic of PIV infections (78).

**2.6.6. Prevention & treatment:**

No antiviral chemotherapy is approved for use in the treatment of HPIV infections, and in most instances only supportive therapy is needed. Ribavirin has been used to treat immunodeficient patients with severe complication of infection. Live-HPIV-3 vaccines are being developed and tested in clinical trials (87).

**2.6.7. Prognosis:**

The prognosis for full recovery is excellent in the normal child. No long-term pulmonary residua of PIV infection have been described (78).
2.7. Respiratory Syncytial Virus (RSV):

Respiratory syncytial virus (RSV) is the major cause of bronchiolitis & pneumonia in infants younger than 1 year (88).

2.7.1. Description of the virus:

RSV has been assigned to the family Paramyxoviridae, RSV is small, and single stranded negative sense RNA virus. Its genome is linear, with a molecular mass of about 5x 10^6 Da (89).

2.7.2. Epidemiology:

RSV is found in all parts of the world and causes yearly outbreaks in winter months in temperate climates. In the USA, outbreaks occur each year between November & April, the peak month varying between communities (90). Outbreaks in a community last for 2-7 months. The fact that during the same year many strains of RSV can circulate in the same community and different strains in other communities demonstrates that RSV outbreaks affect individual communities rather than whole region or countries (91).

The incubation period from exposure to 1st symptoms is 4 days. By the 1st year of life, 50% of all infants experience a primary RSV infection; by 2 years, nearly all have contracted RSV disease (92).
Nosocomial spread of RSV on pediatric ward is an annual problem encountered by hospital infection control personnel (93). The risk of transmission is especially high in settings where close contact is common, such as households, hospital & daycare centers (88).

Placentally transmitted antibody probably has some protective effect, particularly when present in high concentration. This may account for the fact that severe infections are uncommon in the 1st 4-6 weeks of life (88).

Reinfection occurs at a rate of 10-20% per epidemic throughout childhood (88), despite the presence of RSV-specific local & systemic antibodies and neutralizing antibody (94).

2.7.3. Pathology:

Infection and disease seem to be limited to the respiratory tract except in severely immunocompromised patients (84). Bronchiolitis is characterized by virus-induced necrosis of the bronchiolar epithelium, hypersecretion of mucus, and round cell infiltration and oedema of the surrounding submucosa. These changes result in formation of mucus plugs obstructing bronchioles with consequent hyperinflation or collapse of the distal lung tissue (88).

2.7.4. Clinical manifestation:

The characteristic feature of RSV infection is bronchiolitis, but the most common manifestation of infection is a moderately severe upper respiratory tract infection, with signs such as rhinorrhea,
cough and fever. In the infant & young child, asymptomatic infection is uncommon and 30-50% of infected children have physical signs of lower RTI( wheezing, rales, rhonci) (94). Rhinorrhea persist throughout the illness(88).

Patients with pneumonia also present after several days of URT symptoms, and crackles are heard on auscultation of the chest (89). About 1% of healthy children, who became infected with RSV progress & develop tachypnoea, become hypoxaemic and require hospitalization with either bronchiolitis or pneumonia (84). Another manifestation of RSV disease in infant is apnoea, with or without associated respiratory symptoms. Various studies since the early 1960s have determined the incidence of RSV associated apnoea to be between 16 & 20% (95). It is likely that small portion of deaths included in the category of sudden infant death syndrome are due to RSV infection (88).

Fever is an inconstant sign in RSV infection. Rash and conjunctivitis each occur in a few cases (88).

2.7.5. Diagnosis:

Infection can be demonstrated by detection of the virus, viral antigens or genome, a diagnostic rise in specific antibodies, or presence of IgM antibody (84).

Over the past years, EUA has probably become the most frequently used method of antigen detection for RSV. Although not yet commercially available, PCR technology has been applied to the detection of RSV(89).
2.7.6. **Prognosis:**

The mortality of hospitalized infants with RSV infection of the LRT is about 2%. The prognosis is clearly worse in young, premature infants or those with underlying disease of the neuromuscular, pulmonary, cardiovascular or immunologic system. Many children with asthma have a history of bronchiolitis in infancy (88). Outbreaks of RSV disease in patients after bone marrow transplantation have been reported with mortality rate > 50%(96).

2.7.7. **Prevention:**

Recently several strategies for prophylaxis and treatment of RSV have been developed (97). Passive immunization using RSV immunoglobulin & monoclonal antibodies for prevention of RSV disease in premature infants have provided effective forms of prophylactic intervention for high-risk groups (98).

2.7.8. **Treatment:**

Humidified oxygen is usually indicated for hospitalized infants because most are hypoxic. Bronchodilator should not be routinely used. The antiviral drug Ribavirin, delivered by small-particle aerosol and breathed, along with required concentration of oxygen, for 20 of 24 hour per day for 3-5 days, has a modest beneficial effect on the course of RSV effect (88). Its use is indicated only in high-risk infants, such as those with underlying cyanotic congenital heart
disease, significant bronchopulmonary dysplasia or severe immunodeficiency. It should be administered only in the course of the infection (88).

2.8. Adenoviruses:

Adenoviruses cause 5-8% of acute respiratory disease in infants, plus a wide array of other syndromes including pharyngoconjunctival (PCF) fever, follicular conjunctivitis, epidemic keratoconjunctivitis, haemorrhagic cystitis, acute diarrhoea, intussusception and encephalomyelitis (99). The term adenoviruses was coined in 1956 to describe infectious agents that had been isolated from human adenoids (100).

2.8.1. Description of the virus:

Human adenoviruses are nonenveloped, double-stranded DNA viruses of the family Adenoviridae, genus Mastadenovirus (101). Two genera are now recognized on the presence of a genus-specific antigen: the mastadenoviruses covering 9 groups that infect mammals; and aviadenoviruses with 5 groups infected birds, giving a total of at least 120 different viruses. The most intensively studied are the 47 serotypes isolated from humans (102).

2.8.2. Epidemiology:

Adenovirus infections are widespread and appear to be transmitted primarily by the faecal-oral rather than the respiratory route. Seasonal variation of adenovirus epidemics is well recognized. Most outbreaks of PCF in school-age children occur in summer, in contrast, epidemics in military recruits appear almost
exclusively in the winter. By 5 year of age almost all children have been infected by at least one, generally a low numbered serotype (100). Adenovirus illnesses are endemic throughout the year and occur in all age groups, although they are most common among school-aged children, for whom approximately 50% of infections are asymptomatic. Adenoviruses cause 5 to 15% of cases of gastroenteritis in infants and preschool children. Another epidemiologically important feature is the excretion of virus in the stool for many months without recurrence of symptoms (101).

2.8.3. Pathogenesis & pathology:

In susceptible cells, adenoviruses cause early rounding and aggregation, followed by the appearance of characteristic basophilic nuclear inclusions (100). Adenoviruses replicate in the nucleus and tend to be host species specific. The viruses produce characteristic cytopathic effects (CPE) that are accompanied by accumulation of multiple antigenic components and organic acids in the host cell culture fluids (101).

2.8.4. Clinical manifestation:

Adenovirus infections are mostly asymptomatic but may be associates with diseases of the respiratory, ocular and gastrointestinal systems (100). The importance of adenoviruses in paediatric respiratory tract infection is not as great as that of RSV& Parainfluenza viruses, and it has been estimated that only about 5-10% of paediatric respiratory infection can be attributed to them (103).
Upper respiratory illness caused by adenoviruses can take the form of pharyngitis or tonsillitis and occurs chiefly in infants and young children. It is associated primarily with types 1 to 7. Finding includes coryza, fever, cough, exudate in the pharyngeal walls, a granular appearance of the mucosa, and tender, enlarged cervical nodes (101).

Epidemic keratoconjunctivitis was 1\textsuperscript{st} associated with adenovirus type 8, but other serotypes have also been incriminated in this syndrome. Adenovirus type 11 has been associated with haemorrhagic cystitis in school-age boys, and cases have been reported after renal and bone marrow transplantation (104). A cough syndrome similar to whooping cough has been reported in children following type 5 and rarely type 1 to 3 infection (101).

The role of adenoviruses in gastrointestinal disease is, however, much more important; infection of the colon and the gut can cause severe diarrhoea and acute gastroenteritis, especially in developing countries. Serotypes 40 & 41 seem to be mainly responsible for these outbreaks. Up to 15\% of children hospitalized with acute gastroenteritis can be attributed to these enteric adenoviruses, 2\textsuperscript{nd} only to rotaviruses as the cause of infantile diarrhoea (100).

Reye’s syndrome is infrequently associated with the childhood serotypes, especially type 3. Types 1-3,6,7,12 and 32 have been isolated from cerebrospinal fluid or brain tissue (101).
Extrapulmonary signs such as kidney and liver involvement and encephalomeningitis can occur, especially in infants and immunocompromised patients, and in such patients the generalized disease usually has a fatal outcome (101).

2.8.5. Diagnosis:

Adenovirus infection are ideally diagnosed by isolation of virus from appropriate clinical samples in a variety of sensitive indicator cell cultures such as Hela, Hep2, KB or A549 (100). EIA is much more sensitive than complement fixation and is becoming increasingly used in diagnostic laboratories (101). The most sensitive technique for antigen recognition is the polymerase chain reaction (PCR) (105).

2.8.6. Prevention & treatment:

Vaccines that contain either killed or live virus have been developed to prevent type 4 and 7 infection in military recruits. There are at present no recognized antiviral agents that are effective in treating adenoviruses infections (99).

Fever is defined as a rectal temperature exceeding 38°C (100.4°F). Fever is an elevation of body temperature mediated by an increase of the hypothalamic heat regulatory set-point. The hypothalamic thermoregulatory center controls body temperature by balancing signals from peripheral cold and warm neuronal receptors. The integration of these signals maintains normal core body temperature at the set-point of 37°C (98.6°F). Axillary temperature may be 1 degree lower than core temperature, and
oral temperature may be falsely lowered owing to rapid respiration (106).

Fever occurs when various infectious and non-infectious processes interact with the host’s defense mechanism. Fever in children may be categorized as (1) fever of a short duration with localizing signs for which the diagnosis can be established by clinical history and physical examination, with or without laboratory tests; (2) fever without localizing signs, for which the history and physical examination do not suggest a diagnosis but laboratory tests may establish an etiology; & (3) fever of unknown origin (FUO)(106).

1.8.1. Studies done during outbreaks of fever:

Epidemics of acute illness have been reported to occur following heavy rainfalls in areas where arboviral pathogens are endemic (107). The clinician’s ability to distinguish between parasitic, bacterial and viral such outbreaks is often difficult (108) and the diagnostic dilemma are further complicated by a lack of medical technology in developing countries such as Sudan. Consequently, the relative importance of different infections during epidemics is frequently unknown (109).

A major outbreak of acute febrile illness occurred following heavy rains during August 1988, in the Khartoum province of Sudan. During the weeks following the flooding, the centers for diseases and prevention (CDC &P) reported a marked increase in
cases of acute febrile illness among the displaced persons. Although Malaria was associated with a significant number of the cases, the CDC & P also reported that the etiology was unknown for 52-79% of cases. A total of 200 patients with acute febrile illness and 100 afebrile controls are enrolled in the study during October & November 1988, at the Omdurman Military hospital, Khartoum, Sudan. Sera were tested for IgM & IgG antibodies to six arthropod-borne viruses by an ELISA. Among the acute and convalescent sera collected from 67 febrile patients, 5 cases were caused by Sandfly fever Sicilian (SFS), 6 by sandfly fever Naples (SFN), and 12 by unidentified phlebovirus. Of 233 remaining unpaired acute-phase sera collected from cases & controls, 49(21%) had IgM antibodies to SFS or SFN, West Nile(WN) and chikungunya(CHIK) viruses. 43(22%) of 192 febrile cases & 2 of 100 afebrile controls were positive for Plasmodium falciparum, and bacterial enteropathogens were associated with 25(13%) cases & 4 controls (109). From August through November 1988, 77,500 patients with fever presented to the Municipal hospital and to 8 government health centers in Kassala, the number of febrile patients observed during this period was three times greater than that observed during the same period in 1987. A diagnosis of malaria, based primarily on clinical presentation, was made in 14,395 individuals during this 4-month period; FUO were diagnosed in 29 patients. Sera were collected from 196 febrile patients admitted to the municipal hospital in Kassala; these samples were assayed for arboviruses (Batai virus). IgM antibody against this virus(i.e Batai virus) was detected by ELISA in 7% of the sera from patients with acute fever tested and IgG antibody was detected in 61%(110).
Several arboviruses have been associated with human infections in Sudan. Outbreaks of Rift Valley Fever (RVF) were described during 1973, 1976 %1981 in central Sudan (111,112).

An outbreak of acute febrile illness occurred during August and September 1989 in the northern province of Sudan. An investigation was conducted to determine whether arboviruses were associated with human illness during this outbreak. Sera were obtained from 185 febrile individuals & tested for IgG &IgM antibody to selected arboviruses by EIA. The prevalence of IgG antibody was 59% for WN, 53% for SFS, 32% for SFN, 39% for YF, 24% for DEN-2, 23% for RVF, 12% for CHIK and 5% for Crimean –congo haemorrhagic fever (CCHF) viruses. The prevalence of IgM antibody to SFN was 24% and reciprocal IgM titre exceeded 12 800 for some individuals suggesting that this virus was the cause of recent infection (113).

Epidemic of Malaria-like illness affected several thousand residents of the dam camp, a refugee camp near Hargeysa in Somalia, during 1985, 1986, 1987. The disease was characterized by fever, chills, sweats, headache, back & joint pain for as long as 10 days in some patients. A total of 28 sera were collected from patients who reportedly had acute illness. Additional sera were obtained from 10 of these 38 patients 10 days later. Antibody reactive to dengue 2 virus was detected by IFA in 39%(15/38) and 11 of 29(33%) of the same sera were antibody positive by the HI tests. Also IgG antibody reactive to dengue 2 was demonstrated in 60%(17/28) of the same sera by EIA, and 14%(4/28) were positive for IgM antibody (114).
1.9. Justification and Objectives:

1.9.1. Justifications

- There are many outbreaks of unexplained fever in Sudan and developing countries, which were later on discovered to be viral epidemics.

- Viral diseases are underdiagnosed in Sudan.

- There is overdiagnosis of Malaria and bacterial infection with consequent overuse of antimalarial & antibiotics.
1.9.2. Objectives:

1. To improve the diagnosis of febrile diseases by investigating the existing infectious viruses in children aged up to 12 years.

2. To evaluate the clinical diagnosis of viral fevers in relation to the serological results.

3. To study the clinical presentation of acute viral diseases.
Chapter two

2. Patients & Methods

2.1. Study design:

This is a descriptive, case–control, hospital-based study.

2.2. Duration of the study:

The study was conducted during the period between 24th of March 2001 & 13th of August 2002.

The sample had been collected in four days a week; Saturday, Monday, Tuesday, and Wednesday from 11:00 a.m to 8:00 p.m.

2.3. Study area:

Two hospitals were involved in this study:

1- Khartoum Children Emergency Hospital (KCEH), which lies in the center of Khartoum, it contains two long stay admission wards, malnutrition ward, Quarantine, in addition to three emergency wards; General, ARI & Gastroenteritis wards.
2- Khartoum teaching hospital (KTH) which lies in the center of Khartoum, it is a referral hospitals it contains two medical paediatric wards, one surgical paediatric ward and a nursery unit.

2.4. Study population:

The study includes two groups:

1st) Cases.

2nd) Controls.

The cases were children aged up to 12 year who had been admitted to KCEH and KTH with febrile illness during the study period.

2.4.1. Sample size:

The sample size was calculated according to the following formula & was found to be 400.

\[ N = \frac{Z^2(p.q).d}{E^2} \]

\( N \) = Sample size.
\( Z \) = Constant 1.96 (95% Confidence interval).
\( P \) = Prevalence
\( Q \) = 1-p.
\( E \) = Correction factor.
\( D \) = Design effect.

2.4.2. Inclusion criteria:

The inclusion criteria for the cases:

- Admission to KCEH or KTH.
• Age equals or less than 12 year.
• Temperature more or equal to 38º C at the time of examination.
• Negative blood film for Malaria.

2.4.3. Exclusion criteria for the cases:

* Positive blood film for Malaria.
* Clinically obvious cause of fever e.g. abscesses.
* Frank cases of measles.

The control group were those afebrile children who were matched with the cases for age and sex and who fulfilled the following criteria:

2.4.4. Criteria of the control group (N = 200):

* Fever less than 38º C.
* No skin rash.

2.5. Research team:

The research team was composed of:

* Author.
* Lab technician.
* Two medical students.

2.5.1. Input of the author:

1) Select the cases and controls that fulfilled the inclusion and exclusion criteria.
2) Complete the questionnaires.
3) Perform full physical examination of the cases and control.
4) Collect the blood samples.

2.6. Research tools:

2.6.1. Questionnaire:

A pre-coded questionnaire was completed for every case & control included in the study. It contains the personal data; patient characteristics including the vaccination history, previous childhood diseases, previous febrile illnesses & recent contact with febrile diseases; the social history; the
patient’s presenting complaint; home therapy; thorough physical examination including the anthropometric measures and measurement of the temperature.

The diagnosis of the doctor in charge & the outcome after 24 hours were recorded.

2.6.2. Laboratory materials:

   i. Filter paper blood sample.
   ii. Painless
   iii. Slides for Blood
   iv. Transport media for stools
   v. Batteries of IgM & IgG using ELISA & PCR, for the following viruses:
      1- Respiratory cyncytial virus.
      2- Influenza viruses
      3- Human parvo B19 virus.
      4- Adenovirus type 5.
      5- Parainfluenza A virus.
      6- Rubella virus.
      7- Epstien
      8- Cytomegalovirus.
      9- Influenza B virus.

2.7 Methodology:

Informed consent had been taken from the parents & the responsible consultants.

The questionnaire was then completed and physical examination performed.

Filter paper blood samples of cases with acute fever - with or without rash – and filter papers blood samples of control group admitted without fever or skin rash were collected using painless needles (4 – 8 drops).

The sample were taken from the thumb or hallux in infant and from the medial or lateral aspect of the middle finger in older children.

The filter paper blood samples were kept in a refrigerator at temperature of 4°C and thereafter sent to the Virology laboratory in the Erasmus university of Rotterdam (it is a WHO laboratory which work mainly on measles research), where serological assay were done using ELISA & PCR. The presence of specific IgG & IgM for the above mentioned viruses were determined.

Another 2 drops were taken in slides for thin & thick blood film of malaria & sent to a collaborating laboratory for staining and microscopy.

Stool containers were put beside patients.

The stools when collected were supposed to reach the laboratory within one hour but because of the collaborating laboratory opening hours only 20 samples were examined.
2-8- Data processing and statistical analysis:

Data entered in the computer, simple tabulation was done using EPI info version 6. Chi square test and Odd’s ratio and other tests of significance were used to 95% confidence level.

2-9- Difficulties:

1) Collection of samples especially the stool.
2) Parents’ refusal.

2-10- funding:

This study done in collaboration with Department of Virology in Erasmus University of Rotterdam (EUR) - The Netherlands. Local funding was provided from the measles research project, Department of Paediatrics and Child Health.

Chapter three

3. Results

A total of 600 cases were enrolled in this study. They included 400 febrile cases group and 200 afebrile patient as a control group. Although all the samples were sent to Netherlands for analysis, unfortunately – for some technical reasons – results obtained included 108 from cases and 38 control.

3.1- Characteristics of the cases and control groups:

Because of the maternally-transmitted immunity, the cases and control groups were divided into two age groups; the first group included infants aged up to 6 months, and the second group included children aged more than 6 months up to 12 years.
Table 1 shows the cases and control groups distribution according to age. 40 (37%) cases were below 6 months of age while 68 (63%) cases were between the age of 6 months and 12 years. In control group 10 (26.3%) were below 6 months and 28 (73.3%) were between 6 months and 12 years. There was no significant difference between two groups (p=0.2325).

Table 2 shows the distribution of cases and control groups according to sex. Males were 65 (60.2%) and 26 (68.4%) in cases and control groups respectively, while females were 43 (39.8%) of cases group and 12 (31.6%) of control group. There was no significant difference between the two groups (p=0.3691).

Table 3 shows the vaccination status of cases and control groups. The majority of cases and control groups, 83 (76.9%) and 24 (63%) respectively, were satisfactorily vaccinated. Those who were not vaccinated comprised 16 (14.8%) & 11 (28.9%) in cases and control groups respectively. Unsatisfactorily vaccinated were comprised 8 (7.4%) & 3 (10.1%) of cases & control groups respectively. There was no significant difference between the two groups (p=0.2492).

Fig. 1 shows the distribution of cases group according to clinical diagnosis. 50% of the cases were diagnosed as respiratory tract infection, 23 (21.3%) were diagnosed as Gastroenteritis with dehydration, Malaria was the clinical diagnosis in 8 (7.5%), 6 of Malaria cases (5.6%) were in association with pneumonia, 5 (4.6%) as malnutrition, 4 (3.7%) were diagnosed as anaemia, 3 (2.8%) were cases of CNS infection and 1.9% (n=2) were septicaemia cases.

3.2- Laboratory results:

Table 4 shows the laboratory diagnoses of cases and control groups. Among cases, malaria was detected in 16 (14.8%) and significant titers for one or more than one virus were detected in 58 (53.7%) ; while in control group Malaria was detected in 2 (5.2%) and significant titers for one or more than one virus were detected in 22 (57.9%).

Table 5 shows comparison of viral serology between malaria positive & malaria negative cases. Significant viral titers for one or more than one virus were detected in 5 (31.2%) of malaria positive cases, while 11 (68.8%) had insignificant titers for any of the selected viruses. In malaria negative cases, significant viral titers were detected in 58 (64.4%), while
insignificant titer comprised 32 (35.6%). The difference between the two groups is significant (P < 0.05).

Table 6 shows the serology of malaria-negative cases group. Rubella had the highest frequency 20 (18.5%), Influenza A virus comprised 15 (15.8%), CMV 13 (13%), 12 (11.1%) for each of Adenoviruses and Influenza B virus, IgM measles 9 (8.3%), Parainfluenza virus 7 (6.5%), EBV 5 (4.6%) and 2 (1.9%) cases were significant for RSV. Parvovirus B19 was detected in only one case, this was excluded from the analysis due to the positive Malaria film.

Table 7 shows Rubella titers of cases and control groups. 20 (18.5%) of cases group had significant titers while 64 (59.3%) had insignificant titer. In control group, significant titers were detected in 5 (13.1%) & insignificant titers comprised 25 (65.8%). There was no significant difference between the two groups (p = 0.4190).

Table 8 shows Influenza A virus titers of cases and control groups. IgM & IgG titers were significant in 15 (15.8%) of cases group and 13 (34.2%) of control group. While insignificant titers were comprised 91 (84.3%) & 25 (65.8%) in cases and control groups respectively. The difference between the two groups is significant (p < 0.05).

Table 9 shows Influenza B virus titers of cases and control groups. Significant titers were detected in 12 (11.1%) and 2 (5.3%) of cases and control groups respectively. Insignificant titers comprised 94 (87%) and 36 (94.7%) of cases and control respectively. There was no significance difference between the two groups (P= 0.2811).

Table 10 shows CMV titers of cases and control groups. Among the cases; 13 (13%) had significant titers and 71 (65.7%) had an insignificant titer, while in control group insignificant titer found in 21 (55.3%) & significant titers were detected in 2 (5.3%) of the control group. There was no significant difference between the two groups (P= 0.3540).

Table 11 shows Adenoviruses titers of cases and control groups. In cases group 12 (11.1%) & 97 (87%) had significant and insignificant titer respectively, while in control
group, significant titers were detected in 5 (13.2%) and 33 (86.8%) had an insignificant titer. There was no significance difference between the two groups (P= 0.28119).

Table.12 shows Parainfluenza titers of cases and control groups. The titer were significant in 7 (6.5%) and insignificant in 97(89.8%) of cases group, while no significant titers were detected in control group, insignificant titers comprises 34 (89.5%) of control group (P =0.1218, Odds ratio 0.00 {0.00<OR<0.24}).

Table.14 shows RSV titers of cases and control groups. The titers were not significant in 104 (96.2%) of cases group and 37 (97.4%) of control group, while significant titers were detected in 2 (1.9%) & one (2.6%) in cases and control groups respectively. There was no significant difference between the two groups (p= 0.7834).

Table.15 shows IgM measles titer of cases and control groups. The titer was significant in 9 (8.3%) & 3 (7.9%) of cases and control groups respectively, while the titer was not significant in 59(54.6%) of cases and 27(71.7%) of control groups. There was no significant difference between the two groups (p=0.6541).

3.3- Clinical presentation:

Fig.2 shows clinical presentation of cases with significant Rubella virus titers. 15 (75%) presented with cough, 14 (70%) with poor appetite, 12 (60%) with running nose, shortness of breath and vomiting were the presenting symptoms in 11 (55%), diarrhoea in 10 (50%), earache in 3(15%), abdominal pain in 2(10%) and skin rash in one (5%). Examination revealed crackles in 13 (65%), hepatomegaly in 12(60%), pallor in 11 (55%), dehydration in 8 (40%), lymphadenopathy in 5 (25%) & conjunctivitis in 4 (20%), wheezes in 3(15%), splenomegaly & CNS manifestations in 2 (10%) for each, one of them (5%) was a known case of post-meningetic hemiparesis; and convulsion in one (5%).

Fig.3 shows the clinical presentation of cases with significant Influenza A virus titers. The majority of cases 11 (73.3%) presented with shortness of breath & poor appetite, 10
(66.7%) presented with cough. 7(64.7%) presented with running nose & vomiting; diarrhoea was present in 5 (33.3%) , two of cases (13.3%) presented with convulsion and abdominal pain. Pallor was presented in 11 (73.3%), crackles in 7 (46.7%) while wheezes in 5 (33.3%), hepatomegaly in 7 (46.7%), lymphadenopathy & dehydration were presented in 4 (26.6%) and conjunctivitis was detected in one patient (6.7%).

Fig.4 shows clinical presentation of cases with significant Influenza B virus titers. 10 (83.3%) were presented with cough, 9 (75%) with pallor & crackles, 8 (66.7%) with running nose, diarrhoea, and vomiting; 7(58.3%) with hepatomegaly and dehydration, 6(50%) with shortness of breathing ; 4 (33.3%) with poor appetite; 3 (25%) with lymphadenopathy; 2(16.7%) with splenomegaly while skin rash, signs of heart failure and wheezes were presented in one (8.3%) of patients.

Fig.5 shows the clinical presentation of cases with significant CMV titers. Majority of cases 10(76.9%) presented with cough and running nose & 7 (53.8%) with shortness of breath . Crackles were found in 9 cases (69.2%) while wheezes in 1 (7.6%); 7 (53.8%) of cases were presented with poor appetite and lymphadenopathy; vomiting & diarrhoea were presented in 6(46.1%) . Pallor was detected in 8(61.5%); hepatomegaly in 6(46.1%) while splenomegaly & signs of heart failure were found in one (7.6%) ; abdominal pain & skin rash in 2(15.4%) & convulsions, jaundice & sorethroat were presented in one case (7.6%) .

Fig.6 shows the clinical presentation of cases with significant Parainfluenza virus titers. The main presenting symptoms were cough in 6 patients (85.7%) , running nose, shortness of breath & poor appetite in 5 (71.2%) & diarrhoea in 2 (28.6%) . The majority of cases 6 (85.7%) presented with crackles, while wheezes were found in 2 (28.6%);
hepatomegaly presented in 5 (71.2%), lymphadenopathy & vomiting were presented in 4 (57.1%) while splenomegaly, pallor, jaundice, conjunctivitis, signs of heart failure and skin rash were detected in one case (14.3%) for each. Dehydration was a presentation in two cases (28.6%).

Fig.7 shows clinical presentation of cases with significant EBV titers. All cases presented with poor appetite, 80% with vomiting, 60% with hepatomegaly and pallor, 40% with cough, running nose, shortness of breathing, diarrhoea, jaundice and crackles, 20% with convulsion, signs of heart failure, wheezes, lymphadenopathy, splenomegaly, CNS manifestations and dehydration.

Fig.8 shows the clinical presentation of cases with significant Adenoviruses titers. Most cases 11 (91.7%) were pale, 10 (83.3%) were presented with cough & poor appetite. 7 (58.3%) were presented with hepatomegaly; vomiting & diarrhoea were present in 6 (50%), 4 cases (33.3%) were dehydrated. Lymphadenopathy was detected in 6 (50%), running nose, shortness of breath & crackles were present in 5 (41.7%); wheezes and jaundice in 3 (25%), sore throat in 2 of cases (16.6%) while CNS manifestations, splenomegaly and convulsions were present each in one case (8.3%).

Fig.9 shows the clinical presentation of cases with significant RSV titers (n=2). All cases were presented with pallor, cough, shortness of breath, crackles and wheezes, while one case (50%) presented with running nose, vomiting, diarrhoea and dehydration, poor appetite and lymphadenopathy.

Fig.10 shows the clinical presentation of cases with significant IgM measles titers. Hepatomegaly and poor appetite presented in 8 (88.9%), pallor in 7 (77.8%); running nose, vomiting and lymphadenopathy in 6 (66.7%), diarrhoea in 5 (55.5%), crackles, cough, dehydration and shortness of breathing in 4 (44.4%), skin rash in 3 (33.3%), conjunctivitis and earache and abdominal pain in 2 (22.2%) while convulsion, wheezes, splenomegaly and jaundice were present each in one case (11.1%) case.
3.4- Disease distribution among cases with significant viral titers:

Table.16 shows distribution of cases with significant viral titers with type of diseases. The table shows two percentages columns for each figure, the first percentage was calculated from the whole 108 cases group while the second percentage is the percentage of each disease from the cases with significant viral titers. 33 of cases had respiratory tract infection who were comprised 30.5% of all cases group and 64.9% of cases with significant viral titers. 16 of cases had gastroenteritis who were comprised 15.1% of all cases and 27.6% of cases with significant viral titers.

Table.17 shows the distribution of significant viral titers among those with respiratory tract infection. Rubella virus significant titers were detected in 13 (12%) of cases, CMV & Influenza B virus significant titers were detected in 9 (8.3%) cases for each, Influenza A virus in 7(6.4%), Parainfluenza virus in 6(5.5%), Adenoviruses in 5(4.6%), IgM measles in 4(3.7%) and 2(1.8%) of cases had significant titers for each of RSV & EBV.

Table.18 shows the distribution of significant viral titers among those with gastroenteritis. Rubella titers were significant in 8(7.4%) of cases; Influenza B virus in 7(6.4%). 4(3.7%) of cases had significant titers for each of adenoviruses, IgM measles & Influenza A virus, Parainfluenza virus titers were significant in 2(1.8%) while titers of EBV and RSV were significant in only one (0.9%) case for each.
Table 1: Age distribution of cases and control groups.

<table>
<thead>
<tr>
<th>Age</th>
<th>Cases group n(%)</th>
<th>Control group n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6months</td>
<td>40 (37.0)</td>
<td>10 (26.3)</td>
</tr>
<tr>
<td>&gt;6mo-12yr.</td>
<td>68 (63.0)</td>
<td>28 (73.7)</td>
</tr>
</tbody>
</table>
Total 108(100.0) 38(100.0)

Chi square = 1.43

P = 0.2325

Table 2 Sex distribution of cases and control groups.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Cases n(%)</th>
<th>Control n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>65(60.2)</td>
<td>26(68.4)</td>
</tr>
<tr>
<td>Female</td>
<td>43(39.8)</td>
<td>12(31.6)</td>
</tr>
<tr>
<td>Total</td>
<td>108(100.0)</td>
<td>38(100.0)</td>
</tr>
</tbody>
</table>
Chi square = 0.81  
\[ P = 0.3691 \]

Table 3: Vaccination status of cases and control groups.

<table>
<thead>
<tr>
<th>Vaccination Status</th>
<th>cases group n(%)</th>
<th>Control group n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satisfactory For age</td>
<td>83 (76.9)</td>
<td>24 (63.2)</td>
</tr>
<tr>
<td>Unsatisfactory for age</td>
<td>8 (7.4)</td>
<td>3 (7.9)</td>
</tr>
<tr>
<td>Not Vaccinated</td>
<td>16(14.8)</td>
<td>11(28.9)</td>
</tr>
<tr>
<td>---------------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (0.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>108(100.0)</td>
<td>38(100.0)</td>
</tr>
<tr>
<td><strong>Chi square</strong></td>
<td>4.12</td>
<td></td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.2492</td>
<td></td>
</tr>
</tbody>
</table>

Table.4 Laboratory diagnoses of case and control groups

<table>
<thead>
<tr>
<th>Laboratory Diagnosis</th>
<th>cases group n(%)</th>
<th>Control group n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral</td>
<td>58(53.7)</td>
<td>22(57.9)</td>
</tr>
<tr>
<td>Non malaria</td>
<td>32(29.6)</td>
<td>14(10.5)</td>
</tr>
<tr>
<td>Non viral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malaria</td>
<td>16(14.8)</td>
<td>2(5.2)</td>
</tr>
</tbody>
</table>
Not done     2(0.9)     0(0.0)

Total   108(100.0)   38(100.0)

Chi square = 3.34

P = 0.3423

Table.5 Comparison of viral serology between malaria positive and malaria negative cases

<table>
<thead>
<tr>
<th>Viral Serology</th>
<th>Malaria positive Cases</th>
<th>Malaria negative cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significnt titer</td>
<td>5(31.2)</td>
<td>58(64.4)</td>
</tr>
</tbody>
</table>
Insignificant Titer

11(68.8)  32(35.6)

Total

16(100.0)  90(100.0)

Chi square = 6.15
P < 0.05

* Those with positive malaria film were excluded as being febrile due to viral infection.
Table 6  Viral serology of malaria negative cases group.

<table>
<thead>
<tr>
<th>*IgG&amp;IgM titer</th>
<th>number of cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubella</td>
<td>20</td>
<td>18.5</td>
</tr>
<tr>
<td>Influenza A</td>
<td>15</td>
<td>15.8</td>
</tr>
<tr>
<td>CMV</td>
<td>13</td>
<td>13.0</td>
</tr>
<tr>
<td>Adenoviruses</td>
<td>12</td>
<td>11.1</td>
</tr>
<tr>
<td>Influenza B</td>
<td>12</td>
<td>11.1</td>
</tr>
<tr>
<td>IgM measles</td>
<td>9</td>
<td>8.3</td>
</tr>
<tr>
<td>Parainfluenza</td>
<td>7</td>
<td>6.5</td>
</tr>
<tr>
<td>EBV</td>
<td>5</td>
<td>4.6</td>
</tr>
<tr>
<td>RSV</td>
<td>2</td>
<td>1.9</td>
</tr>
</tbody>
</table>

* Significant titer
Table 7: Rubella titers of cases and control groups.

<table>
<thead>
<tr>
<th>*IgM &amp; IgG titers</th>
<th>cases group n(%)</th>
<th>Control group n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant</td>
<td>20(18.5)</td>
<td>5(13.1)</td>
</tr>
<tr>
<td>Not Significant</td>
<td>64(59.3)</td>
<td>25(65.8)</td>
</tr>
<tr>
<td>Not done</td>
<td>24(22.2)</td>
<td>8(21.1)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>108(100.0)</td>
<td>38(100.0)</td>
</tr>
</tbody>
</table>

Chi square = 0.65

P = 0.4190 (P value compares the significant to the non significant groups).

* Significant titer
Table 8: Influenza A virus titers of cases and control group.

<table>
<thead>
<tr>
<th>*IgM &amp; IgG titers</th>
<th>cases group n(%)</th>
<th>Control group n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant</td>
<td>15(13.9)</td>
<td>13(34.2)</td>
</tr>
<tr>
<td>Not Significant</td>
<td>91(84.3)</td>
<td>25(65.8)</td>
</tr>
<tr>
<td>Not done</td>
<td>2(1.9)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>108(100.0)</td>
<td>38(100.0)</td>
</tr>
</tbody>
</table>

Chi square = 7.14

P < 0.05 (P value compares the significant to the non-significant groups).

* Significant titer
Table 9: Influenza B virus titers of cases and control groups

<table>
<thead>
<tr>
<th>*IgM &amp; IgG titers</th>
<th>cases group (n(%))</th>
<th>Control group (n(%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant</td>
<td>12 (11.1)</td>
<td>2 (5.3)</td>
</tr>
<tr>
<td>Not Significant</td>
<td>94 (87.0)</td>
<td>36 (94.7)</td>
</tr>
<tr>
<td>Not done</td>
<td>2 (1.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>108 (100.0)</td>
<td>38 (100.0)</td>
</tr>
</tbody>
</table>

Chi square \(= 1.16\)

\(P = 0.2811\) (P value compares the significant to the non significant groups).

* Significant titer.
Table 10  CMV titers of cases and control groups

<table>
<thead>
<tr>
<th>*IgM &amp; IgG titers</th>
<th>cases group n(%)</th>
<th>Control group n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant</td>
<td>13 (13.0)</td>
<td>2 (5.3)</td>
</tr>
<tr>
<td>Not Significant</td>
<td>71 (65.7)</td>
<td>21 (55.3)</td>
</tr>
<tr>
<td>Not done</td>
<td>23 (21.3)</td>
<td>15 (39.4)</td>
</tr>
<tr>
<td>Total</td>
<td>108 (100.0)</td>
<td>38 (100.0)</td>
</tr>
</tbody>
</table>

Chi square = 0.86

P = 0.3540 (P value compares the significant to the non significant groups).

* Significant titer.
Table 11: Adenoviruses titers of cases and control groups

<table>
<thead>
<tr>
<th>*IgM &amp; IgG titers</th>
<th>cases group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant</td>
<td>12 (11.1)</td>
<td>5 (13.2)</td>
</tr>
<tr>
<td>Not Significant</td>
<td>94 (87.0)</td>
<td>33 (86.8)</td>
</tr>
<tr>
<td>Not done</td>
<td>2 (1.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>108 (100.0)</td>
<td>38 (100.0)</td>
</tr>
</tbody>
</table>

Chi square = 0.09

P = 0.7641 (P value compares the significant to the non-significant groups).

* Significant titer
Table 12  Parainfluenza virus titers of cases and control group.

<table>
<thead>
<tr>
<th>IgM &amp; IgG titer</th>
<th>cases group n(%)</th>
<th>Control group n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant</td>
<td>7(6.5)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Not Significant</td>
<td>97(89.8)</td>
<td>34(89.5)</td>
</tr>
<tr>
<td>Missed</td>
<td>4(3.7)</td>
<td>4(10.5)</td>
</tr>
<tr>
<td>Total</td>
<td>108(100.0)</td>
<td>38(100.0)</td>
</tr>
</tbody>
</table>

Odds ratio = 0.00 (0.00<OR<2.42)
P = Invalid P value
Chi square = Invalid

* Significant titer
<table>
<thead>
<tr>
<th>*IgM &amp; IgG titers</th>
<th>cases group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant</td>
<td>5 (4.6)</td>
<td>3 (7.9)</td>
</tr>
<tr>
<td>Not Significant</td>
<td>99 (91.7)</td>
<td>35 (92.1)</td>
</tr>
<tr>
<td>Not done</td>
<td>4 (3.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>108 (100.0)</strong></td>
<td><strong>38 (100.0)</strong></td>
</tr>
</tbody>
</table>

Chi square = 0.50

P = 0.4815 (P value compares the significant to the non-significant groups).

* Significant titer.
Table 14 RSV titers of cases and control groups

<table>
<thead>
<tr>
<th>*IgM &amp; IgG titers</th>
<th>Cases group</th>
<th>Control group</th>
<th>n(%)</th>
<th>n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant</td>
<td>2 (1.9)</td>
<td>1 (2.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not significant</td>
<td>104 (96.2)</td>
<td>37 (97.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not done</td>
<td>2 (1.9)</td>
<td>0 (0.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>108 (100.0)</td>
<td>38 (100.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Corrected Chi square = 0.08

P = 0.7834 (P value compares the significant to non significant groups.)

* Significant titer
Table 15 IgM measles titer of cases and control groups.

<table>
<thead>
<tr>
<th>*IgM titer</th>
<th>cases group n(%)</th>
<th>Control group n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant</td>
<td>9 (8.3)</td>
<td>3 (7.9)</td>
</tr>
<tr>
<td>Not Significant</td>
<td>59 (54.6)</td>
<td>27 (71.1)</td>
</tr>
<tr>
<td>Not done</td>
<td>40 (37.0)</td>
<td>8 (21.0)</td>
</tr>
<tr>
<td>Total</td>
<td>108 (100.0)</td>
<td>38 (100.0)</td>
</tr>
</tbody>
</table>

Chi square = 0.20

P = 0.6541 (P value compares the significant to the non significant group).

* Significant titer.
Table 16 Disease distribution of cases with significant viral titers

<table>
<thead>
<tr>
<th>Disease</th>
<th>n</th>
<th>Percentage¹ (n=108)</th>
<th>Percentage² (n=58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARI</td>
<td>33</td>
<td>30.5</td>
<td>56.9</td>
</tr>
<tr>
<td>Gastro-enteritis</td>
<td>16</td>
<td>15.1</td>
<td>27.6</td>
</tr>
<tr>
<td>Others</td>
<td>9</td>
<td>8.3</td>
<td>15.5</td>
</tr>
<tr>
<td>Non: significant</td>
<td>60</td>
<td>46.2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>
**Percentage¹** calculated from the total cases group (n=108)

**Percentage²** calculated from the total cases with significant viral titers (n=58).

Table 17 Distribution of cases of with significant viral titers among those with respiratory tract infection

<table>
<thead>
<tr>
<th>*IgG &amp; IgM titer</th>
<th>n</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubella</td>
<td>13</td>
<td>12.0</td>
</tr>
<tr>
<td>CMV</td>
<td>9</td>
<td>8.3</td>
</tr>
<tr>
<td>Influenza B</td>
<td>9</td>
<td>8.3</td>
</tr>
<tr>
<td>Influenza A</td>
<td>7</td>
<td>6.4</td>
</tr>
<tr>
<td>Parainfluenza</td>
<td>6</td>
<td>5.5</td>
</tr>
<tr>
<td>Adenoviruses</td>
<td>5</td>
<td>4.6</td>
</tr>
<tr>
<td>IgM</td>
<td>4</td>
<td>3.7</td>
</tr>
<tr>
<td>RSV</td>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td>EBV</td>
<td>2</td>
<td>1.8</td>
</tr>
</tbody>
</table>

*Significant titer.
<table>
<thead>
<tr>
<th>*IgG&amp;IgM tites</th>
<th>n</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoviruses</td>
<td>4</td>
<td>3.7</td>
</tr>
<tr>
<td>Influenza A</td>
<td>4</td>
<td>3.7</td>
</tr>
<tr>
<td>Influenza B</td>
<td>7</td>
<td>6.4</td>
</tr>
<tr>
<td>Rubella</td>
<td>8</td>
<td>7.4</td>
</tr>
<tr>
<td>RSV</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>Parainfluenza</td>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td>EBV</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>CMV</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IgM</td>
<td>4</td>
<td>3.7</td>
</tr>
</tbody>
</table>

* Significant titer.
Chapter four

4. Discussion

The study was conducted to identify the circulating viruses that may be a frequent cause of fever in addition to Malaria & bacterial infections in children in Khartoum. Although only one quarter of the samples were analyzed, yet the results give a reflection of the situation of viral infection.

The study groups (cases and controls) showed no significant difference between them with regard to age and sex distribution as well as vaccination status.

4.1. Disease distribution:

The study showed that 14.8% of cases had malaria & had been excluded & and 53.7% of cases had significant titers for one or more than one virus, this percentages is higher than previous studies done in Sudan (109,113,114); this can be explained by the difference in the timing of those studies, also they were conducted during epidemics to detect certain virus genus.

In the control group only two had positive Malaria film & significant virus titers were detected in 57.9%. With exception of
Influenza A virus, all the P values were insignificant and this can be explained by asymptomatic or recent infection (20). This indicates that there is no difference in the incidence of viral infection among febrile and afebrile patients. On the other hands, when comparing the significant & insignificant viral titers in patients with positive & negative Malaria films, it is clear that there was a significant difference (P < 0.05) between malaria negative cases with significant viral titers & malaria positive cases with significant viral titers. This indicate that viral infection is the most likely cause among malaria negative cases (table 5).

Influenza A virus was detected more in control group than in cases (P <0.05). This may be due to the fact that Influenza A virus is often an asymptomatic infection (58), in addition to the high attack rate and frequency (30-50%) of isolation of influenza viruses (59). It may also be due to the small sample size.

One fifth of cases had more than one virus detected on their sera, the most frequent combination were CMV & Rubella virus & the most frequent virus that found in combination with other viruses was Influenza A virus (in 8 cases) followed by Influenza B virus (in 5 cases).

Respiratory tract infection due to viral infection was found in one third of cases. Among them, Rubella virus was detected in 12%, Influenza B virus in 6.4%, Parainfluenza viruses in 5.5%, Adenoviruses in 4.6%, IgM measles in 3.7% & 1.8% for each of RSV & EBV. These percentages simulate to some extent the study done in Taiwan 1997-1999 by Huey-Pin T Sai et al. (2001) where viral titers was significant in 24.8% of hospitalized respiratory tract infections with 1.7% due to RSV, 4% due to Adenoviruses (115). However, the percentages of Influenza A&B viruses were 5.5% & 2% respectively; the higher percentages in this study can be explained by the fact that the analyzed samples were collected during the end of March & April which is a known period of Influenza viruses epidemics (58). Parainfluenza viruses were detected in 2% in Taiwan but it is mentioned in the Literature, PIVs can occur in 5-20% of cases respiratory tract infection (84). Rubella, CMV & EBV were not included in that study.
Gastroenteritis was found to be due to viral infection in 15.1% of patients. Adenoviruses comprised 3.7% of cases which is a little bit lower than the literature where adenoviruses was found in 5-15% of cases of Gastroenteritis in infants and preschool children (101). Early in the study, Latex agglutination test for Rotavirus & Adenoviruses was done for the first 4 children who had watery diarrhoea, 2 patients were negative for both viruses, 1 patient had positive test for Rotavirus & 1 patient had positive test for Adenoviruses.

Other viruses are mainly respiratory viruses and it was hardly to find literature about gastroenteritis, which can be taken as a systemic manifestation of each virus.

4.2. Clinical presentation:

Significant Rubella virus IgG & IgM titers were detected in about one fifth of cases. Recent serologic surveys have similarly indicated that 10-20% of young adult are susceptible to Rubella & the degree of susceptibility is due predominantly to underutilization of vaccine in the population (64); but in Sudan the higher percentage is mainly due to the vaccine unavailability and possibly high rate of circulating virus.

The mean age is 15.8 months. However, 25% of cases were below 6 months of age, 2 of them (10%) were below 2 months of age, this raised the possibility of maternally transmitted Rubella antibodies although these cases had no feature of congenital infection (64).

About two third of the cases presented with evidence of respiratory tract infection e.g. running nose, cough and crackles. More than half of cases presented with hepatomegaly & 20% with splenomegaly. Lymphadenopathy was generalized in 15% and cervical in 10%. Similar clinical presentation
Influenza A virus titers were detected in 13.9% of cases. The main presenting symptoms are those of upper respiratory tract infection e.g. cough & running nose; lower respiratory tract infection were found in half the cases, this is similar to that reported in other studies (59). Other presentation including hepatomegaly (26.6%), lymphadenopathy (26.6%) and convulsion (13.3%) were not mentioned in the literature and this is could explained by the mixed viral infection present in this study.

Significant Influenza B virus titers were found in one tenth of cases. Most cases are presented with cough & running nose. Pneumonia was diagnosed in three quarters while bronchiolitis in 8.3%, this is in agreement with other reports in the literature (59). Similar to Influenza A virus, other findings such as Hepatomegaly, lymphadenopathy, Skin rash & heart failure might most likely be due to the mixed virus infection.

Significant Cytomegalovirus IgG & IgM titers were detected in over one tenth of cases & this represented 8.3% of respiratory viral infection. This is twice the rate reported in study that done in Texas where the virus was detected in 4% of respiratory tract infection (116). Respiratory tract infection and pallor are the presentation of two third of cases. Hepatomegly and cervical lymphadenopathy were present in half of cases with evidence of hepatitis in 7.6%. All the features had been reported in the literature (17,21).

Six of the cases were below 6 months of age and 2 of them were below 3 months of age and this infection most likely raised the possibility of maternally transmitted antibodies.

Among the 12 cases where adenoviruses titers was detected, respiratory tract infection was present in two fifth of
cases with significant adenoviruses titer, gastroenteritis and hepatomegaly in half of them & splenomegaly in 8.3%. CNS manifestation found in 8.3% of cases. All the features had been reported in the literature (101).

Parainfluenza virus was detected in seven cases. The vast majority of cases were presented with cough and running nose. Crackles detected in most of the cases while wheezes in only 14.3%. Other manifestations like hepato-splenomegaly, skin rash & conjunctivitis in 14.3% for each, in addition to lymphadenopathy (57.1%) & heart failure (14.3%) were not mentioned in the literature because PIV are mainly respiratory viruses & systemic manifestations are rare (78). Evidence of hepatitis represent 14.3% of cases. Interestingly, Parainfluenza virus was the only virus which was not detected in association with other viruses, also it was not detected in any of the control group.

Parvovirus was detected in only one case who was excluded from the study because he had a positive Malaria film, he present with high grade fever, shortness of breathing and cough for three days; on examination he was very ill, pale & jaundiced with fine crepitations all over the chest & hepatosplenomegaly.

Although frank cases of measles were excluded from the sampling but IgG & IgM titers were checked to assess the vaccination status of the patients. This is outside the scope of the study but interestingly IgM titer was detected in 8.3% of cases. Among those, 1 case was recently vaccinated, 1 patient was below 6 months of age i.e. not vaccinated yet & 1 patient was not vaccinated. The mean age is 16 months. The majority of cases
presented with vomiting, diarrhoea & running nose. Evidence of pneumonia, hepatomegaly, splenomegaly, lymphadenopathy, & conjunctivitis has been observed in some of the cases. However, skin rash was detected in only one third, this is could be explained by the early presentation of cases. Similar clinical presentations were reported in the literature (117).

EBV was detected in five cases. All cases presented with poor appetite. Hepatomegaly (60%), splenomegaly (20%) & cervical lymphadenopathy (60%). This is unlike report in the literature where patients with infectious mononucleosis have 10%, 50%, & 90% for hepatomegaly, splenomegaly & lymphadenopathy respectively (52), and this could be due to the small sample size in this study.

RSV was detected in two cases. Both of them were cases of Bronchiolitis with typical presentation reported elsewhere (94).
Conclusion

1) Although Sudan is an endemic area for malaria but viral infection was found to be the likely cause of fever in 64.4% of cases who presented with negative blood film for malaria.

2) There is no difference in the incidence of viral infection in febrile and afebrile patients. Possibly due to the small sample size in this study.

3) One third of hospitalized cases have respiratory tract infection due to significant viral titers.
4) 15.1% of cases with gastroenteritis cases were found to be in association with significant viral titers.

5) The most frequent viral infections among cases were rubella, influenza A virus and CMV which were detected in 18.5%, 13.9% and 13% of cases, respectively.

6) Possibility of congenital rubella & CMV was detected in 10% &13.3% of cases respectively.

7) There is evidence of maternally transmitted antibodies (IgG) in cases aged less than six months.
and associated with rubella, CMV and measles with or without acquired antibodies (IgM). This need further research.

**Recommendation**

1) Facilities to investigate viruses in malaria negative patients should be considered to minimize the possibility of overuse of antimalarial treatments & consequent malaria resistant.

2) Investigating those viruses is cost-effective, that is the rate of hospitalization will decrease with consequent decrease in the bed cost per year.

3) Gastroenteritis was found in association with viruses in 15.1 %, although Rotaviruses could not investigated in details. This is an area for further research.

4) MMR vaccine should be considered in the routine immunization program to decrease the incidence of rubella infection.

5) Vaccination against Rubella & CMV for childbearing women is mandatory to minimize the risk of congenital infection.

6) The efficacy of measles vaccine should be assessed.

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A) Personal data:
1. Date [ ] [ ] [ ] [ ] [ ] [ ]
2. Serial number [ ]
3. Name
4. Age (months) [ ]
5. Sex 0= male 1= female
6. Residence
7. Informant 0=mother 1=father 2=relative 8=other

B) Patient characteristics:
8. Complication of pregnancy/birth 0=none 1=yes
   If yes, specify
9. Vaccination status 0=not vaccinated 1=unsatisfactory 2=satisfactory
   for age
10. Breastfeeding duration (in months) [ ]
11. History of illness 0=no 1=yes
    If yes, specify
12. Number of previous hospitalization 0=no 1=yes 2=yes with blood/transfusion
    If yes, specify

C) Social history:
13. Father education (number of school years)--------------------------

14. Father occupation 0=unemployed 1=unskilled laborer 2=skilled laborer 3=government employee/teacher 4=professional/manger 9=other--

15. Mother education (number of school years)------ -----------------

16. Mother occupation 0=housewife 1=unskilled laborer 2=skilled laborer 3=government employee/teacher 4=professional/manger 9=other--

17. Monthly income (Sudanese)

18. Smoking history 0=no 1=father 2=mother 3=both 8=other in household

19. Contact with similar diseases 0=no 1=father 2=mother 3=siblings 8=other

D) Patient complaints

20. Fever 0=no 1=low grade 2=high grade 9=unknown --------------

21. Characterize the fever 0=continuous 1=intermittent 2=remittent 8=other

22. Fever associated with 0=none 1=rigors 2=chills convulsions 8=other

23. Duration of fever (days) -----------------------------------------------

24. Possible cause of fever 0=no idea 1=malaria 8=other --------------
25. Cough 0=no 1=dry 2=productive 8=other

26. Sputum 0=no 1=clear 2=purulent 3=blood-stained 8=other

27. Running nose 0=no 1=clear 2=mucopurulent 3=blood-stained 8=other

28. Sore throat 0=no 1=yes 2=not applicable

29. Earache 0=no 1=yes

30. Wheezing 0=no 1=yes

31. Difficulty of breathing 0=no 1=yes at rest 2=yes at exercise 8=other

32. Vomiting 0=no 1=clear 2=bile-stained 3=blood-stained 8=other

33. Diarrhea 0=no 1=watery 2=mucoid 3=blood-stained 8=other

34. Abdominal pain 0=no 1=yes 2=not applicable

35. How is your appetite 0=poor 1=diminished 2=good

36. Loss of weight 0=no 1=yes 9=unknown

37. Headache 0=no 1=Frontal 2=Occipital 3=Bi-temporal 4=Generalized 5=not applicable 8=other

38. Neck stiffness 0=no 1=yes

39. Pain the joints 0=no 1=big joints 2=small joints 3=generalized
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8=other-------

40. Pain in the bones 0=no 1=localized 2=generalize 8=other -------

41. Pain in the muscles 0=no 1=localized 2=generalize 8=other ---

42. Urine color 0=normal 1=yellow 2=red 3=turbid 8=other --------

43. Urinary symptom 0=no 1=dysuria 2=dripping 3=bedwetting
   8=other---

44. Consciousness 0=alert 1=irritable 2=drowsy 3=unconscious
   8=other-----

45. Home therapy 0=no 1=aspirin 2=paracetamol 3=sponging
   4=antibiotics 5=cough medicine 6=anti-malaria 7=herbal therapy
   8=other

46. Prescribed by 0=no medication 1=parent 2=medical doctor
   3=pharmacist 4=native healer 8=other

47. Family diseases 0=no 1=yes 2=unknown
   If yes , specify:

E) Physical condition

48. General condition 0=well 1=unwell 2=ill 3=very ill

49. Pale 0=no 1=yes 2=cyanosed

50. Jaundice 0=no 1=yes

51. Weight(kg)
52. Length (cm)--------------------------------------------------

53. Temperature (degrees Celsius)------------------

54. Respiration rate 0=normal 1=tachypnea

55. Pulse rate 0=normal 1=tachycardia 2=bradycardia 3=arrhythmia

56. Chest examination 0=normal 1=wheeze 2=crackles 3=wheeze+crackles
   4=diminished air entry 8=other

57. Heart examination 0=normal 1=abnormal
   If abnormal, specify:

58. Enlarged liver 0=no if yes:---------------------

59. Enlarged spleen 0=no if yes:---------------------

60. Ascites 0=no 1=yes

61. Central nervous system 0=normal 1=abnormal
   If abnormal, specify:

62. Skin rash 0=no 1=yes
   If yes, specify:

63. Lymph nodes enlarged 0=no 1=cervical 2=occipital 3=axillary
4=inguinal  5=generalized  8=other  -----------------------------------------

64. Muscle-skeletal examination 0=normal 1=abnormal ----------------
If abnormal, specify: -----------------------------------------------

65. Conjunctiva  0=normal 1=injected 2=sticky 8=other ----------

66. Dehydration 0=no 1=mild 2=moderate 8=other ------------------

67. Clinical diagnosis:---------------------------------------------

68. Malaria result  0=negative 1=positive ------------------------

69. Parasites in stool  0=no 1=yes----------------------------------
If yes, specify-----------------------------------------------------

70. Pus cell in stool/HPF  0=no  if yes: ---------------------------

71. Red blood cell in stool/HPF  0=no  if yes: ---------------------

72. Outcome 1=discharged 2=referred 3=left over 4=died----------