HUMAN RESPIRATORY SYNCYTIAL VIRUS INFECTION AMONG CHILDREN 1-5 YEARS IN ZARIA, KADUNA STATE, NIGERIA

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DECLARATION

I declare that the work in this thesis entitled 'Human *Respiratory Syncytial Virus* Infection among Children 1-5 years In Zaria' has been performed by me in the Department of Microbiology under the supervision of Prof. A. A. Ahmad and Dr H. W. Idris. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

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Aliyu A'ishatu Muhammad Signature Date

CERTIFICATION

This thesis entitled "HUMAN RESPIRAYORY SYNCYTIAL VIRUS INFECTION AMONG CHILDREN 1-5 YEARS IN ZARIA" by Aliyu A'ishatu Muhammad meet the regulations governing the award of the degree of Master of Science in the Department of Microbiology, Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation

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DEDICATION

This work is dedicated to my beloved and caring husband, Bashir Tijjani Babajo and my lovely children Habiba, Khadija, Hafsat, Aisha, Fatima (Khairat),Ismail and Bashir.

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ABSTRACT

Serological survey was carried out among children in Zaria to determine the level of Respiratory syncytial virus IgG antibodies. Blood samples of children aged 12 –60 months were collected in three out patient clinics of Ahmadu Bello University Teaching Hospital Zaria. Respiratory syncytial virus IgG antibody level was measured using commercial ELISA kit obtained from DEMEDITEC DIAGNOSTIC GmbH Germany. Three hundred and eighty four blood samples, were collected how ever 379 were analyzed due to lyses of some. Of the 379 blood samples analyzed, 353 (93.1%) were seropositive for Respiratory Syncytial Virus. There was female predominance, with 178 (94.2%) being positive compared to males 175(92.1%) but the difference was not statistically significant (P value >0.05). However, statistical association was observed between age and seropositivity (P value<0.05). The seroprevalence was highest (98.7%) in age group 49-60 months and lowest in age group 0-12 months (78.6%). There was no association between the presence of cough, catarrh and/or running nose with seropositivity (P value >0.05). The result also shows no association with the presence of sickle cell disease and parental smoking with seropositivity (p value >0.05). This study has revealed that children ages 1-5 years have very high prevalence of RSV infection in Zaria, and therefore, highlighting the need for the younger age group to be targeted for prevention programme against RSV infection.

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CHAPTER ONE

1.0 INTRODUCTION

Acute lower respiratory tract infection is one of the major causes of mortality and morbidity in young children world wide (Weber *et al.,* 1999; Openshaw and Tregoning, 2005). Respiratory syncytial virus (RSV) is the single most important viral cause of lower respiratory tract infection during infancy and early childhood world wide (Glezen, 1987; De Graaft *et al.,* 2005).

Most children with RSV infection suffer from mild upper respiratory tract infection; however up to 40% of children infected with RSV develop serious lower respiratory tract disease requiring hospitalization (Tripp, 2004). The magnitude and intensity of infection and the host response to RSV infection determine the severity and intensity of the disease (Domachowske and Rosenberg, 1999).

Respiratory syncytial virus belongs to the Pneumovirinae subfamily of the Paramyxoviridae family of enveloped single stranded negative sense RNA viruses (Carpenter *et al.*, 2002). The RSV infection rate in young children approaches 70% in the first year of life, with peak incidence occurring between ages 2 - 4 months (Domachowske and Rosenberg, 1999).

World wide, RSV is the leading cause of infant morbidity from respiratory infections and is so highly contagious that by age two years nearly all children have been infected. Respiratory syncytial virus infection in infancy cause severe bronchiolitis and pneumonia. The virus

accounts for approximately 50% of all pneumonia and up to 90% of the reported cases of bronchiolitis in infancy (Glezen, 1987). It may also predispose children to subsequent development of asthma, the most common chronic illness of childhood (Johnston 1997; Tekkanat *et al.,* 2001).

Out breaks of RSV disease are abrupt in onset and can last up to 5 months. These epidemics occur annually at regular, predictable intervals. In temperate climate where there is an annual epidemics during the winter months. In tropical climate, infection is most common during the raining season (Hall *et al.,* 1990). The virus was also reported to be the common cause of admission to the pediatric intensive care unit due to respiratory failure in infancy (Eisenhut, 2006). Nosocomial RSV infections are frequently reported and tend to be more severe, because of co morbidity (Thwaites and Piercy, 2006).

The virus is spread from respiratory secretions through close contact with infected persons or contact with contaminated surfaces or objects. Infection can occur when infectious materials come into contact with mucous membrane of the eyes, mouth, or nose and possibly through the inhalation of droplets nuclei generated by sneezing or coughing. Symptoms begin most frequently with fever, running nose, cough and sometimes wheezing (Simoes, 1999; Uzel *et al* 2000). Recovery from RSV infection is mediated largely by the host immune system. Secretory antibodies, serum antibodies, major histocompatibility complex, class 1 restricted cytotoxic T lymphocytes and in the very young maternally derived antibodies serve as

specific effectors for protection against RSV infection (Bangham *et al.*, 1986; Watt *et al.*, 1986).

1.1 STATEMENT OF THE PROBLEM

Respiratory syncytial virus bronchiolitis is one of the most important causes of morbidity and death in infants less than 6 months of age. Prematurity, pre-existing cardiopulmonary disease, parental smoking and atopy are some predisposing factors to severe bronchiolitis and death from RSV infections (Smyth *et al.*, 1997; Groothius *et al.*, 1998).These viruses are prevalent in young children and almost all have serological evidence of infection by the age of 2 years (Smyth *et al*, 1997).

The virus is ubiquitous in all parts of the world, avoidance of infection is not possible epidemiologically, and vaccine development has been fraught with spectacular failure (Domachowske and Rosenberg, 1999; McCarthy and Hall, 2003).

Respiratory syncytial virus accounts for unscheduled visits to a health care provider for respiratory illness and was associated with unnecessary antibiotic prescriptions that may contribute to antibiotic resistance.

1.2 JUSTIFICATION

The radiological features of lower respiratory tract disease due to RSV may vary from a virtually normal appearance to one which mimics bacterial pneumonia. Respiratory syncytial virus infection must be distinguished from bacterial pneumonia because antibiotic treatment for viral infection does not help. The etiological diagnosis can be determined by serological

test or by viral culture. Improving the etiological diagnosis of viral infection might avoid unnecessary therapy with antibiotics (Gulen *et al.,* 2007*).*

1.3 AIM

The aim of this research is to determine the prevalence of Respiratory syncytial virus infection among children 12 – 60 months attending out patient clinics of ABUTH Zaria, Kaduna State.

1.4 SPECIFIC OBJECTIVES

1. To determine the level of IgG antibodies in children 12 – 60 months attending the out patient clinics of ABUTH Zaria.
2. To determine the relationship if any between serum IgG level among children With respiratory symptoms and those without respiratory symptoms
3. To determine the possible association of serum IgG level in these children and the Variables of age, sex, parental smoking and sickle cell disease.

CHAPTER TWO

2.0 LITERATURE REVIEW

Respiratory syncytial virus (RSV) is the leading viral cause of lower respiratory tract infection in infants and young children in both developing and developed countries (Sullender, 2000). RSV infects approximately 60 million people and is responsible for an estimated 160,000 deaths annually world wide according to the World Health Organization (MacLellan *et al.*, 2007).

2.1 HISTORICAL BACKGROUND

The significance of RSV infection in the young pediatric population has been recognized many decades ago, but only recently has it been confirmed as an important cause of respiratory tract illness throughout life (McCarthy and Hall, 2003).

The virus was first isolated from a chimpanzee with common-cold-like illness in 1956 as the chimpanzee coryza agent (CCA) during an outbreak in Baltimore. It was subsequently isolated from infants who had respiratory symptoms and was renamed Respiratory syncytial virus in 1957, because of the characteristic syncytial pattern observed in tissue culture (Mclntosh, 1997; Tripp, 2004).

2.2 VIROLOGY OF RESPIRATORY SYNCYTIAL VIRION

The RSV virus is a pleomorphic enveloped virus, has a single, negative-strand genome of 5 X 10^6 kilo Dalton (KD). The genome encodes 10 mRNAs each of which codes for an individual

protein (McCarthy and Hall, 2003). It is classified within the Pnuemovirineae subfamily of the paramyxoviridae family order Mononegavirales (Collins and Murphy, 2001). The virus attaches to host cells via the glycoprotein. This is followed by the fusion of the virion envelop with the cell membrane of the host by the action of the fusion glycoprotein F1 cleavage product. If the Fo precursor is not cleaved it has no fusion activity. Virion penetration does not occur and the virus particle is unable to initiate infection. Fusion by F1 occurs at the neutral PH of the extra cellular environment, allowing release of the viral nucleocapsid directly into the cell. This enables the virus to bypass internalization through endosomes (Jawetz *et al.,* 2004). After replication the virus matures by budding from the cell surface. Progeny nucleocapsids form in the cytoplasm and migrate to the cell surface. The M protein is essential for particle formation, probably serving to link the viral envelop to the nucleocapsid. During budding, most host proteins are excluded from the membrane. If appropriate host cell proteases are present, Fo proteins in the plasma membrane will be activated by cleavage. Activated fusion protein will then cause fusion of adjacent cell membranes, resulting in formation of large syncytia (Prescott *et al* 1996; Jawetz *et al.,* 2004).

Respiratory syncytial virus grows optimally at a PH of 7.5, and inactivated at temperatures between 50-60ºC. Although temperature –sensitive, it is recoverable for hours from countertops and for more than 1 hour from rubber gloves contaminated with RSV-infected nasal secretions (McCarthy and Hall, 2003). It is readily inactivated with soap and water and disinfectants.

2.3 THE STRUCTURE OF RESPIRATORY SYNCYTIAL VIRUS

Respiratory syncytial virus is a medium-sized (120-200nm) enveloped virus that contains a lipoprotein coat and a linear minus-sense RNA genome. The genome contains 10 separate genes, each coding for a separate protein, and the gene order has been established unambiguously as 3′-NS1-NS2-N-P-M-SH-G-F-M2-L-5′. The internal helical nucleocapsid contains the linear viral genome to which are complexed many molecules of the nucleocapsid (N) protein, fewer molecules of the phosphoprotein (P) and putative viral polymerase (L). The outer envelop is lined internally with matrix (M) protein and is studded externally with fusion (F) and attachment (G) glycoprotein projections. Another protein (M2) is also present in the viral envelop. Three other proteins, nonstructural proteins 1and 2 (NS1, NS2) and small hydrophobic (SH) are in a position that remains to be determined. The SH protein was shown to be a third integral membrane glycoprotein that is inserted into the outer membrane of infected cells (Mclntosh., 1997). The entire genome of RSV is composed of approximately 15,000 nucleotides long (Dickens *et al.*, 1984).

2.3.1 STRUCTURAL PROTEINS

There are three transmembrane glycoproteins, two matrix proteins and three proteins associated with the nucleocapsid. The transmembrane proteins are the attachment glycoprotein (G), the fusion protein (F), and the small hydrophobic protein (SH). The two matrix proteins are M and M2. The three proteins associated with the nucleocapsid are N, P,

and L. The fusion protein of RSV is similar to that of the other paramyxoviruses in structure and function. The F protein is a type 1 transmembrane glycoprotein with a

Cleaved N-terminal signal sequence and a transmembrane anchor near the C terminus. It is cleaved into two subunits, F1 and F2 that are linked by disulfide bonds, after synthesis and modification by the addition of N-linked sugars (Collins *et al.,* 1996).

The G protein is of particular interest because variability in this protein is greater than that in the other proteins both between and within the major antigenic groups of RSV. The G protein is presumed to be the attachment protein of RSV by analogy to the other Paramyxoviruses and on the basis of experimental data (Levine *et al.,* 1987). However, it is said to lack the hemagglutinin or neuraminidase activity of the other Paramyxovirus attachment proteins. The G protein also differs in size and biochemistry from the other attachment proteins of other Paramyxoviruses (Sullender and Wertz, 1991). Antigenic dimorphism between the subgroups of RSV A and B is mainly linked to the G protein. The A2 strain isolate of RSV, a prototype of the group A viruses, has a G mRNA of 918 nucleotides. The major open reading frame encodes a 298-amino-acid type II membrane protein (Sullender and Wertz, 1991). The G proteins are important antigenically because they stimulate the production of protective immune responses. The G protein is recognized by neutralizing antibodies but does not stimulate significant cytotoxic T-lymphocyte responses (Sullender, 2000).

The nucleocapsid-associated proteins include the major nucleocapsid protein (N), the phosphoprotein (P), and the RNA polymerase (L). These proteins together, have been

demonstrated to function as the RSV replicase (Grosfeld *et al.,* 1995). The M2 mRNA contains two overlapping translational open reading frames, (ORF) 1 and 2 (Collins *et al.,* 1990). The M2 open reading frame 1 has historically been considered a second matrix protein but has recently been found to colocalise with the nucloecapsid proteins N and P (Fearns *et al* 1997). The M2 open reading frame 1 protein is a necessary component of the viral replicase, since it ensures efficient production of full-length mRNA. The protein appears to be a negative regulatory factor (Hardy and Wertz 1998).

2.3.2 NON-STRUCTURAL PROTEINS

There are 2 nonstructural proteins (NS) 1 and 2. These proteins are found in negligible amounts within the virion and are thought to regulate RNA synthesis or the host's innate immunity (MacLellan *et at*., 2007).

2.4 EPIDEMIOLOGY

RSV has a worldwide presence with predictable, yearly epidemics. In temperate climates, RSV usually appears in the community in November or December and persists until April or May. In tropical climates infection is most common during the rainy season (Constantopoulos *et al.,* 2002; McCarthy and Hall, 2003; Jawetz *et al.,* 2004). According to World Health Organization (WHO), RSV infects approximately 60 million people and is responsible for an estimated 160,000 deaths annually worldwide. There are two major strains, A and B. Both strains circulate concurrently, with the A strains usually dominating. Several distinct genotypes within these strains predominate within a community. The dominant strains shift yearly, suggesting a mechanism for frequent reinfections by evasion of immunity induced by

previous strains (Hall, 2001). Fluctuations in the circulating strains of RSV may contribute to the variation seen in the severity of the annual outbreaks caused by the virus (McConnochie *et al* 1990, Opavsky *et al* 1995).

Reinfection occurs frequently, but resulting symptoms are those of a mild upper respiratory infection. In families with an identified case of Respiratory syncytial virus infection, virus spread to siblings and adults is common. The virus causes nosocomial infections in nurseries and on pediatric hospital wards. Transmission occurs primarily via hospital staff members (Jawetz *et al.*, 2004).

Seventy percent (70%) of infants are infected by age 1 and almost all by age 2 years. Serious bronchiolitis or pneumonia is most apt to occur in infants between the ages of 6 weeks and 6 months with peak incidence at 2 months. Respiratory syncytial virus is the most common cause of viral pneumonia in children under age 5 years but may also cause pneumonia in elderly or in immunocompromised persons. RSV infection of older infants and children results in milder respiratory tract infection than in those under age 6 months (Jawetz *et al.*, 2004).

2.5 PATHOGENESIS

Viral transmission occurs by direct inoculation of contagious secretions from the hands or by large-particle aerosols into the eyes, nose, and rarely the mouth (Hall, 2001). High viral titers are present in respiratory tract secretions from infected young children. Inoculum size is an

important determinant of successful infection in adults, and possibly in children as well (Jawetz *et al.,* 2004). The prolonged survival of RSV on skin, cloth, and other objects emphasized the importance of fomites in nosocomial spread and of hand washing in controlling infection (Hall *et al.,* 1980).

The incubation period of RSV is two to eight days. Respiratory syncytial virus replicate in the nasopharyngeal epithelium, with spread to the lower respiratory tract one to three days later. The characteristic pathological features of RSV bronchiolitis are inflammation, with edema, and increased secretion of mucus, necrosis and sloughing of the epithelium of the small airways, which obstructs flow in the small airways. The resulting clinical findings of pneumonia with interstitial infiltration, alveolar fillings, and consolidation are the hallmarks of bronchiolitis, especially in adults who die of the disease (Hall, 2001).

Clinical recovery from RSV bronchiolitis may occur in the presence of continued virus shedding from upper respiratory tract (Damachowske and Rosenberg, 1999). However, some studies have demonstrated that virus shedding stops coincident with the emergence of specific secretory immunoglobulin A (IgA) at the time of clinical recovery (Meert *et al.,* 1994).

An intact immune system seems to be important in resolving an infection, as patients with impaired cell mediated immunity may become persistently, infected with Respiratory syncytial virus and shed virus for months (Jawetz *et al.,* 2004).

2.6 IMMUNITY

Protection against and recovery from RSV infection are mediated largely by the host immune system. Secretory antibodies, serum antibodies, major histocompatibility complex, class I-restricted cytotoxic T lymphocytes (CTLs), and in the very young, maternally derived antibodies serve as specific effectors (Bangham *et al.,* 1986; Watt *et al* 1986). The importance of the host immune system in the eradication of RSV is exemplified by the observation that children with primary or acquired immunodeficiency diseases have difficulty in eradicating the virus and shed virus for many months rather than the typical interval of 1 to 3 weeks (Fishaut *et al.,* 1980; Chadwani *et al.,* 1990; Taylor *et al.,* 1990).

The F and G protein are important antigenically because they stimulate the production of protective immune responses. Responses of the F protein include humoral and cytotoxic T-lymphocyte responses (Cherie *et al.,* 1992).

Humoral immunity

Specific RSV-neutralizing antibodies are present in the sera of all full-term newborns by virtue of the transplacental transfer of maternal antibodies. The measurable antibody titres in the newborn are similar to the maternal levels and decline slowly during the first few months of life. After 7 months of age, detectable neutralizing antibody is usually the result of natural infection. Breast-fed infants have the added benefit of maternal antibodies in the form of colostrums (Domachowske and Rosenberg, 1999).

Both serum and secretory antibodies are made in response to RSV infection. Primary infection with one subgroup induces cross reactive antibodies to virus of the other subgroup. Younger infants have lower IgG and IgA secretory antibody responses to RSV than do older infants. An association has been noted between virus-specific IgE antibody and severity of disease. Viral secretory IgE antibodies have been correlated with occurrence of bronchiolitis (Jawetz *et al.*, 2004).

Cellular immunity

Cell- mediated immune response, a probably most important in recovery and viral clearance. The fact that patients with compromised cellular immunity have severe, prolonged disease indicates the importance of CD4 and CD8 T cells in controlling infection. The exaggerated cellular response engendered by formalin-inactivated vaccine, consisting of eosinophilia and hemorrhagic necrosis that apparently arise from the response of types 1 and 2 helper T cells, has been reproduced in animals (Hall, 2001).

In direct contrast to both parainfluenza and influenza virus infections, RSV infection frequently fails to induce a detectable level of interferon in nasal secretions of infected patients. This observation supports a minor role, if any, for interferon in recovery from RSV infection (Domachowske and Rosenberg, 1999).

It is apparent that immunity is only partially effective and is often overcome under natural conditions; reinfections are common, but the severity of ensuing disease is lessened (Jawetz *et al.*, 2004).

2.7 CLINICAL MANIFESTATIONS (PULMONARY)

RSV infection is associated with a wide spectrum of symptoms. In young children, illness frequently begins with cough, nasal congestion, and fever. The risk of developing lower respiratory tract disease with a primary infection is at least 50%. After several days of upper respiratory tract symptoms, infants may develop tachypnea, dyspnea, and intercostal retractions. Difficulty in feeding, features of otitis media and hypoxemia are common (McCarthy and Hall, 2003). The primary manifestations of lower respiratory tract disease in infants are bronchiolitis and pneumonia. These two syndromes may be difficult to distinguish and may occur simultaneously. The characteristic clinical features of bronchiolitis are wheezing, rales and rhonchi may be heard in infants. Apnea may be the presenting manifestation of RSV disease, particularly in infant born preterm (McCarthy and Hall, 2003). Symptoms include; difficulty in breathing, which may include breathing more rapidly than normal, wheezing, coughing that, is getting worse. A child may choke or vomit from intense coughing that may be dry or loose (producing mucus). Lethargy, increased tiredness, decreased interest in surrounding or loss of interest in food (Simoes, 1999).

Recurrent infections with RSV continue throughout life, although disease typically is milder and generally localized to the upper respiratory tract. Cough may be more severe and persistent, than that observed with other common cold agents, such as rhinovirus. Significant lower respiratory tract disease is uncommon, but can occur in healthy older children and adults, and infection may be followed by a prolonged period of airway hyperactivity. In the

elderly, particularly those who have chronic illness, RSV may cause significant lower respiratory tract illness (McCarthy and Hall, 2003).

2.7.1 EXTRAPULMONARY MANIFESTATIONS OF SEVERE RSV INFECTION

Extrapulmonary manifestations suggest that RSV may infect organs other than the lung. It is unlikely that systemic co-infection with bacterial pathogens is responsible for most extrapulmonary manifestations. Previous studies have shown that serious bacterial infection is present in 0.6 to 1.2% of children admitted with RSV infection (Randolph *et al*., 2004). Extrapulmonary presentations of severe RSV infection were first highlighted in a report on an epidemic affecting infants admitted to a children's hospital in Cleveland, USA (Njoku and Kliegman., 1993).

RSV and the cardiovascular system

Extrapulmonary manifestations of RSV reported include cardiovascular failure with hypotension and inotrope requirement associated with myocardial damage as evident from elevated cardiac troponin levels.

The first report of clinically symptomatic myocardial involvement during RSV bronochiolitis was that of a case of fatal interstitial myocarditis in a child in 1972 (Eisenhut, 2006). Other early reports include also the development of a second degree heart block during the disease. Subsequently there were reports of an RSV- associated multifocal arterial tachycardia (Armstrong and Menahem, 1993; Donnerstein *et al.,* 1994). Other forms of supraventricular tachycardia was also reported to be associated with RSV infection; these

findings seemed to occur in patients with structurally normal hearts and were not associated with hypoxia or beta-agonist therapy (Playfor and Khader., 2005).

Central nervous system manifestations of RSV

Acute neurological signs and symptoms such as central apneas, seizures, feeding/ swallowing difficulties, muscle tone abnormalities, as well as abnormalities of the cerebrospinal fluid (CSF), and electroencephalogram (EEG) abnormalities were found in some patients with RSV infection (Kho *et al.,* 2004).

Central apnea

Respiratory arrest for more then 20 seconds and/or bradycardia with accompanying cyanosis or oxygen desaturation below 90% have been found in 16 to 21% of children admitted to hospital with RSV infection. The most important risk factor associated with apnea in RSV infected patients was age below two months. Apnea on admission is associated with increased risk for recurrent apneas, and these children did have a significantly increased probability of requiring mechanical ventilation (Kneyber *et al.,* 1998).

Seizures

Seizure types found to be associated with RSV infection include both generalized tonic-clonic and partial seizures with altered consciousness and focal motor features or eye deviation (Sweetman *et al.,* 2005).

Endocrine effects of RSV infection

Antidiuretic hormone

Antidiuretic hormone (ADH) which is responsible for regulating the level of sodium in the blood was found to be higher in patient with RSV bronchiolitis. Hyponatraemia (a serum sodium level of less than 136 mmol/l) was found in infants requiring intensive care with RSV infections. Increasing ADH levels were associated with high arterial partial pressure of co_2 and hyperinflation on a chest X-ray (Hanna *et al.*, 2003).

Other hormonal imbalance

A prospective study comparing ventilated infants with RSV infection and patients admitted to the ward showed that ventilated patients had higher prolactin and growth hormone levels and significantly lower leptin and insulin-like growth factor-1 levels. Cortisol levels were not different. The leptin and prolactin levels accounted for 57% of the variation in lymphocyte count, which was significantly lower in ventilated patients with RSV infection (Tasker *et al.*, 2004).

Respiratory syncytial virus-associated hepatitis

The virus has been found to be associated with viral hepatitis resulting in increased transaminase level in children. Serum transaminase specific to the liver were found to be significantly elevated in 46 to 49% of ventilated children with RSV bronchiolitis. Severe hepatitis with alanine aminotransferase levels of nearly 3, 000lu/I have been noted and this was associated with coagulopathy. Respiratory symptoms were more severe in infants with

elevated transaminase levels. The prevalence of hepatitis was 80% in children with congenital heart disease. This was a significantly higher prevalence than the one found in children without congenital heart disease. Direct invasion of the liver in an immunocompetent infant with RSV infection has been documented by the successful culture of RSV from material of a liver biopsy (Nadal *et al.,* 1990). Hepatic involvement in the form of fatty changes was described in a fatal case of Reye's syndrome associated with RSV infection (Griffin *et al.,* 1979).

Other extrapulmonary manifestations of RSV bronchiolitis

Several other extrapulmonary manifestations of RSV infection have been described but most of them only in single case reports and none of them seem to be life threatening. They include hypothermia (Njoku and Kliegman 1993), exanthems involving the trunk and face in the form of a finely granular, scarlatiniform rash (Unger *et al.,* 1982), thrombocytopenia and conjunctivitis (Fujishima *et al.,* 1995).

2.8 DIAGNOSIS

Respiratory syncytial virus often is diagnosed accurately in young children based on the season plus a typical history and finding on physical examination. In older children and adults, however, the signs and symptoms of RSV are less characteristic and may easily be attributed to other pathogens. A definitive diagnosis of RSV may not be necessary for children who have mild disease, laboratory confirmation however is important for

hospitalized patient and those at increased risk for severe disease (McCarthy and Hall, 2003). The diagnosis can be by antigen detection by viral isolation, and other serological techniques.

Antigen detection: Direct identification of viral antigen in clinical samples is rapid and sensitive. Immunoflurescence on exfoliated cells or ELISA on nasopharyngeal secretions is commonly used. A nasal wash or nasal aspirate is a good source of virus. Large amounts of virus are present in nasal washes from young children (10^3-10^8 plaque forming units (PFU) per millitre), but much less is present in specimens from adults (<100PFU/ml). ELISA kits are useful for rapid diagnosis (Jawetz *et al.,* 2004).

Isolation and identification of virus: Respiratory syncytial virus can be isolated from nasal secretions. It is extremely labile. Samples should be inoculated into cell cultures immediately; freezing of clinical specimens may result in complete loss of infectivity. Human heteroploid cell lines Hela and HEP-2 are the most sensitive for viral isolation. The presence of RSV can usually be recognized by development of giant cells and syncytia in inoculated cultures. It may take as long as 10 days for cytopathic effects to appear.

Definitive diagnosis can be established by detecting viral antigen in infected cells using a defined antiserum and the immunofluorescence test. More rapid isolation of RSV can be achieved by spin-amplified inoculation of vials containing tissue cultures growing on cover slips. Cells can be tested 24-48 hours later by immunoflourescence. Detection of RSV is

strong evidence that the virus is involved in a current illness because it is almost never found in healthy people (McCarthy and Hall, 2003; Jawetz *et al.*, 2004).

Serology: serum antibodies can be assayed in a variety of ways- Immunofluoresence, ELISA, and NT tests are all used. Measurements of serum antibody are important for epidemiologic studies but play only a small role in clinical decision making (Jawetz *et al.*, 2004).

Nucleic acid detection: polymerase chain reaction assays are useful for subtyping RSV isolates and for the analysis of genetic variation in outbreaks (Jawetz *et al.*, 2004).

2.9 TREATMENT

Therapy for RSV remains largely supportive. These supportive measures mainly involved the use of supplemental oxygen and mainly fluid and electrolytes balance as well as close monitoring of the patients for disease progression. In those with severe disease, drug treatment can be considered (McCarthy and Hall, 2003).

Ribavirin, a synthetic nucleoside delivered as a small particle aerosol, was approved for the treatment of RSV lower respiratory tract infection and licensed in 1985 for use in hospitalized children in the United States (Domachowske and Rosenberg,1999). Ribavirin which is a broad spectrum antiviral agent interferes with expression of mRNA and inhibits protein synthesis (McCarthy and Hall, 2003). It is administered as aerosols via oxygen hood, tent, or

mask until improvement is seen (Usually 3 to 7 days). Longer courses may be helpful in immunocompromised patients who have more severe disease and prolonged shedding of the virus. Ribavirin may be administered to a patient on a ventilator, but special care must be taken because deposition of drug in the system may cause clogging of small airways (McCarthy and Hall, 2003). Toxicity has not been observed with therapy in infants, although further studies of the use of ribavirin for treatment of RSV infections are needed. The American Academy of Pediatrics (AAP) has recommended that decision about ribavirin therapy be based on the individual clinical situation and the physician's experience.

A variety of different structural formats have been explored in the past few years. Interesting leads for future discovery and lead development include a group of biphenyl relatives (represented by CL-387626) that bind to RSV fusion (F) protein; 2-5A-antisense oligonucleotides that target the RSV genomic RNA. Rho A-derived peptides that block Rho A GTPase interaction with RSV fusion (F) protein; and several compounds of presently unknown mechanism of action, such as benzodithiins (Torrence, 2000).

2.10 COMPLICATIONS

Children at increased risk for severe RSV disease include infants born preterm and infants who have chronic lung disease or functionally important congenital heart disease. Children with certain neurological disorder, who are immunocompromised, or have multiple congenital anomalies (Jawetz *et at.,* 2004). Respiratory Syncytial Virus infection is associated with most frequent complications, which include:

Apnea: This is where breathing stops for about 15 to 20 seconds. This usually occurs only in babies who were born prematurely (McCarthy and Hall, 2003).

Severe bronchiolitis: This is as a result of inflammation of the small air passages (bronchioles) that usually affects children younger than 2 years.

Pneumonia: This is inflammation of the lungs; it causes difficulty in breathing and reduces oxygen supply to the bloodstream (Selwyn, 1990; Jawetz *et al.,* 2004).

Otitis media: this could be due to RSV or a superimposed bacterial pathogen (McCarthy and Hall, 2003).

Asthma: RSV bronchiolitis has been associated with the development of recurrent episodes of bronchiolar obstruction, specific IgE production, and establishment of asthma (Welliver *et al.,* 1981; Sigurs *et al.,* 1995; Richardson *et al.,* 2005; Hegele *et al.,* 2008; Mohapatra and Boyapalle, 2008).

2.10.1 RSV IN IMMUNOCOMPROMISED PATIENTS

Immunocompromised adults and children, especially those undergoing bone marrow transplantation are at high risk of severe and often fatal RSV disease (McCarthy and Hall 2003). In severely immunocompromised children and adults, mortality rates from RSV can nearly reach 90% (Tristram *et al* 1998). Respiratory syncytial virus infections account for about one-third of respiratory infections in bone marrow transplant. Pneumonia develops in about one-half of infected immunocompromised children and adults, especially if infection occurs in the early post-transplant period. Infections in the elderly may cause symptoms

similar to Influenza virus disease. Pneumonia may develop in 10-20% of those infected and mortality rates of 2-5 % (Jawetz, *et al* 2004).

2.11 PREVENTION

Various strategies can be used to prevent RSV transmission in the community and the hospital. Close contact with individuals who have respiratory symptoms or fever should be avoided when possible and frequent hand washing is advised. Patients who have suspected RSV infection should be identified on admission and placed in contact isolation. Good hand washing is critical and the most important single method of infection control. Staff should be educated about the modes of spread of RSV and that shedding of RSV typically lasts about 3 to 8 days, but may persist for weeks, particularly in immunosuppressed patients (McCarthy and Hall, 2003).

Prophylactic administration of antibody to RSV has been shown to decrease severe disease. Currently two products have been approved for use in selected children at high risk for RSV disease. The first licensed product was Respiratory syncytial virus intravenous immune globulin (RSV-IGIV) (RespiGam). Approval of this agent in 1996 followed completion of the multicenter prevent trial. The other approved agent for prophylaxis is palivizumab (Synagis), a humanized IgG-1 monoclonal antibody that binds to the F protein of RSV. It is estimated to have 50 to 100 times more activity than RSV-IGIV and is administered intramuscularly. The Unites States food and drug administration approved its use in 1998

following a placebo controlled, multicenter trial (the IMpact study) involving high–risk children similar to those studied in the prevent trial (Atkins *et al.*, 2000; Sharland and Bedford-Russell, 2001; McCarthy and Hall, 2003).

The American Academy of Pediatrics recommends that palivizumab and RSV-IGIV be considered for prophylaxis against RSV in children who are younger than 2 years of age, have chronic lung disease, and have received medical therapy for it, in the prior 6 months, as well as infants born at 32 or fewer weeks gestation. None of the two products are currently licensed for use in infants who have congenital heart disease (McCarthy and Hall, 2003).

2.11.1 VACCINE DEVELOPMENT

The reasons why an RSV vaccine is not yet available arise from multiple problems with its development. The first, and most important, is the possibility that vaccination will potentiate naturally occurring RSV disease, as observed with the formalin-inactivated vaccine (Domachowske and Rosenberg, 1999). Newborns and very young infants may not mount a protective immune response because of relative immunologic immaturity or because of suppression of their immune response due to circulating maternally derived anti-RSV antibodies (Collins *et al.*, 1996; Falsey and Walsh, 1996). Another important consideration in the development of an effective RSV vaccine is the need to provide protection against multiple antigenic strains of RSV in the two major groups, A and B. A number of strategies have been implemented recently to generate safe and effective sub unit, inactivated, and live attenuated virus vaccines (Falsey and Walsh, 1997). Currently, the two most promising

candidate vaccines to be studied in clinical trials are an RSV F subunit vaccine (for immunization of patients who have already had their primary RSV infection, such as the elderly and older RSV seropositive children with conditions predisposing them to severe RSV disease (Hildreth and Paradiso, 1993, Paradiso *et al* 1994, Piedra *et al* 1996, Falsey and Walsh 1997, Groothius *et al* 1998). And cold passaged, temperature-sensitive (cpts) attenuated RSV strains (Karron *et al.,* 1997). While this cpts mutant appears immunogenic, retained virulence has been observed in older children, precluding the study of this variant in infants (Domachowske and Rosenberg, 1999). Another possibility is to immunize infants with an attenuated RSV vaccine to optimize the Th1-type response and then to immunize them with a subunit vaccine to boost both anti-F and anti-G neutralizing antibody production (Domachowske and Rosenberg, 1999).

CHAPTER THREE

METHODOLOGY

3.1 Study Design

This research work is a descriptive cross sectional study. Children attending out patient clinics of Ahmadu Bello University Teaching Hospital Zaria (GOPD, Institute of Child Health, ABUTH Zaria and Primary Health Clinic Sabon Gari Zaria) from 1-5 years of age were recruited for the study.

3.2 Area of Study

The study area comprises 3 hospitals located in Zaria and Sabon Gari Local Government Areas, of Kaduna State.Zaria Local Government has a population of about 369,800 inhabitants (Population Census, 1991), It is located on longitude 8^0 and latitude 9^0. It is an urban set up bounded on the east by Soba Local Government, on the West by Giwa Local Government, on the North by Sabon Gari Local Government, and on the South by Igabi Local Government. Sabon Gari Local Government is an urban set up with a population of 350,000 inhabitants (population census, 1991). It is along longitude 8^0 and 9^0 latitude 10^o and 11^0. It is bounded by Soba Local Government on the East, Giwa Local Government on the West, Makarfi Local Government on the North, and Zaria Local Government on the South. The hospitals include ABUTH Shika, Institute of Child Health Banzazzau and Primary Health Clinic Sabon Gari Zaria

3.3 Preliminary Investigation

Preliminary visits were paid to the out patient clinics (Shika, Banzazzau and Sabon Gari PHC) of ABUTH to discuss the feasibility of the study and seek the cooperation of the management, clinicians (pediatricians) and laboratory staff. Ethical approval was obtained from ABUTH (Appendix III).

3.4 Sample Size

Due to the difficulty in finding records of any research on Respiratory syncytial virus infection been carried out in this state or any other part of the country, a prevalence rate of 50% was used to calculate a sample size with the equation below: As recommended by ((Lwanga and Lemeshow, 1991; Tolley, 1999; Sarmukaddam *and Garad*, 2006).

$$n = \frac{Z^2 pq}{L^2}$$

Where ‘n is the sample size

'z' is the standard normal distribution at 95% confidence interval =1.96

'p' is the prevalence rate, which is taken as 50% =0.5

'q' is 1-P =1-0.5=0.5

'L' is the allowable error, which is taken as 5% =0.05

Therefore,

$$n = \frac{(1.96)^2 x0.5x0.5}{(0.05)^2}$$

= 384.16

Three hundred and eighty four samples were collected from the three hospitals. Seventy seven samples were collected from General Out Patient Department ABUTH, 205 samples were collected from Institute of child health Banzazzau and 102 samples were collected from Sabon Gari Primary Health Clinic. The number of samples collected from each hospital was on the basis of statistical data of patients attendance for each hospital obtained from Medical Record and Statistic Department ABUTH.

3.5 SAMPLE COLLECTION AND PROCESSING

Children 12-60 months of age were all included in the study. The samples were collected only from children whose parents/guardians gave a written informed consent (Appendix II). Dry sterile plastic syringe (2ml capacity) with 23SWG needle attached to was used for blood collection. Blood was collected by applying soft tubing tourniquet on the arm of the patient to enable the veins to be seen and felt. The site was cleaned using methylated spirit and allowed to air dry. The needle was inserted to the selected straight vein with the bevel of the needle directed upward in the line of the vein. Steadily the plunger of the syringe was withdrawn until 2ml of blood was obtained. The tourniquet was loosed and the needle was removed from the punctured vein. Pressure was applied to the punctured site to secure haemostasis. The needle was removed from the syringe and the blood was transferred to a clean dry plain specimen bottle and labelled. The used syringes and needles were disposed appropriately. The blood samples were centrifuged at 1500 revolution per minute for five minute (1500 rpm/min for 5min) and the serum collected into a clean and dry plain specimen bottles using

clean and dry Pasteur pipettes and stored at -20^{0}c until needed for analysis (Cheesbrough, 2000). All tests were carried out in the Department of Microbiology, Ahmadu Bello University Zaria. Using Enzyme Linked Immunosorbent Assay (RSV IgG ELISA DEMEDITEC, GmbH Germany), samples were assayed for specific IgG antibody against the RSV. Manufacture's instructions were duly followed. The optical density (OD) values were read at 450nm, using Sigma Diagnostic EIA Multi well Reader II. The kit has 100 % specificity and 100% sensitivity.

3.6 Interview

Information was collected by means of specially designed proforma for this study. The informants were the parents or adult care givers of the children under study. The Age, Sex of the child, occupational and educational level of the parents was enquired for and filled into appropriate section of the proforma (Appendix 1).

3.7 PRINCIPLE OF THE ASSAY

The RSV IgG antibody assay is based on the principle of the enzyme Immunoassay (EIA) RSV antigen is bound on the surface of the micro titer strips. Diluted patient serum or ready to use standard are pipetted onto the wells of the micro titer plate. Binding between the IgG antibodies of the serum and the immobilized RSV antigen takes place. After one hour incubation at room temperature, the plate was rinsed with wash solution, in order to remove unbound material. Then ready to use anti-human-IgG peroxidase conjugate was added and incubated for 30 minutes at room temperature. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of blue dye in the wells. The color development is terminated by the addition of a stop solution,

which changes the color from blue to yellow. The resulting color is measured spectrophotometrically at the wave length of 450nm. The concentration of the IgG is directly proportional to the intensity of the color.

3.8 ASSAY PROCEDURE

The procedure according to manufacturer instruction was used as follows: sufficient amount of microtitre wells for the standards, controls; samples as well as substrate blank were prepared in duplicate. About 10µl each of diluted samples and ready-to-use standards and controls respectively were pipetted into the wells. The first well was left empty as blank, mixed by gentle shaking and incubated for one hour at room temperature. The wells emptied after incubation and washing solution were added into the wells. This procedure was repeated three times. The rest of the washing solution was afterwards removed by gentle tapping of the microtitre plate on a tissue paper. About 100µl each of ready-to-use conjugate were added into the wells. This time also the substrate blank was left empty. The plates were incubated at room temperature for 30 minutes, the same procedure for washing was followed, and about 100µl each of the ready-to-use substrate was pipetted into the wells. This time the substrate was pipetted into the blank, incubated at room temperature for 20 minutes. About 100µl of stop solution was finally pipetted into the wells to terminate the substrate reaction. After thorough mixing and wiping the bottom of the plate, the reading of the absorption at 450nm was made using, ELISA reader machine (sigma diagonistic).

3.9. CALCULATION, INTERPRETATION AND QUANTITATIVE ESTIMATION OF RSV IgG RESULT

The mean values for the measured absorption were calculated after subtraction of substrate blank value. The calculated absorptions for the sera were compared with the value for the cut-off standard. A positive result was considered when the value for the sample was higher than the cut-off value and negative when the value was below the cut-off standard.

For a quantitative evaluation, the absorptions of the standards and controls were graphically drawn against their concentrations. From the resulting reference curve, the concentration values for each patient sample were extracted in relation to their absorptions. Quantitative antibody titers < 8 IU/ml were reported as negative and >12 IU/ml as positive.

3.10 STATISTICAL ANALYSIS

The prevalence of RSV antibodies for both sex, age groups were calculated. Chi-square was used at 95% confidence interval ($P< 0.05$) to test whether there is association between sex, age, respiratory tract symptoms and the presence of RSV antibodies among the studied population. All statistical analysis was performed using (SPSS version 10). The results were expressed as descriptive study regarding the prevalence of IgG antibody.

CHAPTER FOUR

RESULTS

A total of 384 blood samples were collected for the detection of Human Respiratory Syncytial Virus IgG antibody. However, 5(1.3%) of the blood was observed to be lysed hence only 379 sera were tested. Of the 379 screened for IgG RSV antibody, 353(93.1%) were seropositive (Table 4.1).

4.1 Distribution of Respiratory Syncytial Virus IgG Antibody According to Age Group

Table 4.1 shows the seroprevalence of RSV IgG antibody among the studied population with respect to age group. The seroprevalence was highest (98.7%) in age group 49-60 months and lowest in age group 12 months (78.6%). The result shows slight increased seropositivity with age. ($X^2 = 27.010$, P=0.000).

TABLE 4.1: Distribution of Respiratory Syncytial Virus IgG Antibody Seropositivity According To Age Group

Age Group (Months)	No of Children Tested	No of Seropositive	% seropositive For each age grp	
0-12	56	44	78.6%	(11.6%)
13-24	117	107	91.5%	(28.2%)
25-36	78	76	97.4%	(20.0%)
37-48	54	53	98.1%	(14.0%)
49-60	74	73	98.6%	(19.3%)
Total	379	353		93.1%

X^2 =27.010 p=0.000 at 95% CI.

4.2 Profile of Respiratory Syncytial Virus Antibody Titre According to Age Group

Table 4.2 indicates that 26 (6.9%) of the children had no detectable RSV IgG antibodies with a titer less than 8IU/ml and a total number of 8 (2.1%) had a maximum level of 301-350IU/ml a relatively low level of RSV antibody was found in 9 (2.4%) with a titer of 10-50 IU/ml, 10 (2.6%) with a titer of 51-100IU/ml, 20(5.3%) with a titer of 101-150IU/ml, 40 (10.6) with a titer of 151-200 IU/ml, 130 (34.3%) with a titer of 201-250 IU/ml and 136 (35.9%) with a titer of 251-300 IU/ml

TABLE4.2: Profile of Respiratory Syncytial Virus IgG Antibody According to Age

Titer (Iu/ml)

Age (mths)	Subject	<8	10-50	51-100	101-150	151-200	201-250	251-300	301-350
0-12	**56**	**12**	**5**	**5**	**9**	**8**	**9**	**7**	**1**
13-24	**117**	**10**	**1**	**3**	**6**	**16**	**39**	**41**	**1**
25-36	**78**	**2**	**2**	**1**	**3**	**6**	**26**	**36**	**2**
37-48	**54**	**1**	**1**	**1**	**2**	**5**	**23**	**18**	**3**
49-60	**74**	**1**	**-**	**-**	**-**	**5**	**33**	**34**	**1**
Total	**379**	**26**	**9**	**10**	**20**	**40**	**130**	**136**	**8**

KEY:

Iu/ml = International unit/ml

Where <8 Iu/ml is negative

> 12 Iu/ml is positive

4.3 Distribution of Respiratory Syncytial Virus IgG Antibody According to Sex

Of the total number of 379 children tested, 190 (50.1%) were males and 189 (49.9%) were females. Seropositivity was observed in 178 (94.2%) females and 175 (92.1%) males. This shows that there was slight female predominance over male, the difference was not statistically significant, **(**X^2 =0.638, P value=0.276) (Table 4.3).

TABLE 4.3: Distribution of Respiratory Syncytial Virus IgG Anttibody According To Sex

Sex	Number Tested	Seropositive	% seropositive
Male	190	175	92.1%
Female	189	178	94.2%
Total	379	353	93.1%

X^2 =0.638 P value =0.276

4.4 Distribution of Respiratory Syncytial Virus IgG Antibody Based on Presence of Catarrh/Running nose

Of the total 379 children tested, 268 (70.7%) were with symptoms of catarrh/running nose and 111 (29.3%) were those without catarrh/ running nose. Seropositivity was observed in 247 (92.2%) of those with catarrh/running nose and 106 (95.5%) of those without catarrh/running nose. The difference was not statistically significant ($X^2 = 1.363$, P value = 0.173) (Table 4.4).

TABLE 4.4: Distribution of Respiratory Syncytial Virus IgG Antibody Based on Presence of Catarrh/Running nose

Catarrh /R.Nose	No. tested	Seropositive	%seropositive
Presence	268	247	92.2%
Absence	111	106	95.5%
Total	379	353	93.1%

X^2 =1.363, P value =0.173

4.5 Distribution of Respiratory Syncytial Virus IgG Antibody with the Occurrence of Cough

Of the total number of 379 children tested, 266 (70.2%) had cough. Among the 266, 247 (92.9%) are seropositive of RSV antibody but the difference was not statistically significant, (X^2= 0.112, P value = 0.466) (Table 4.5).

TABLE 4.5: Distribution of Respiratory Syncytial Virus IgG Antibody With The Occurrence of Cough

Cough	Number tested	Seropositive	% seropositive
Present	266	247	92.9%
Absent	113	106	93.8%
Total	379	353	93.1%

X^2= 0.112 P value = 0.466

4.6 Prevalence of Respiratory Syncytial Virus IgG Antibody in Children with Sickle Cell Disease

Of the total number of 379 children tested, 9 (2.4%) have sickle cell disease, and all (100%) were seropositive but the difference was not statistically significant, (X^2 =0.679, P value=0.524) (Table 4.6).

TABLE 4.6: Prevalence of Respiratory Syncytial Virus IgG Antibody In Children With Sickle Cell Disease

Sickle cell disease	Number tested	Seropositive	% Seropositive
Present	9	9	100%
Absent	370	344	92.9%
Total	379	353	93.1%

X^2 =0.679, P value=0.524

4.7 Prevalence of Respiratory Syncytial Virus IgG Antibody in Children whose Parent Smoke Cigarette

Of the 379 children tested, 46 (12.1%) have one of their parent being a smoker. Among the 46, 42 (91.3%) were tested positive to RSV antibody but the difference was not statistically significant ($X^2 = 0.276$, P value = 0.390) (Table 4.7).

TABLE 4.7: Prevalence of Respiratory Virus Antibody in Children Whose Parent Smoke Cigarette

Parental smoking	Number tested	Seropositve	% seropositve
Present	46	42	91.3%
Absent	333	311	93.4%
Total	379	353	93.1%

X^2 = 0.276, P value = 0.390

CHAPTER FIVE

DISCUSSION

Respiratory syncytial virus is the leading viral cause of death in children under 1 year of age and is an increasing cause or morbidity and mortality in transplant patients and the elderly (Richardson *et al.,* 2005). The virus is a precipitant of asthma attacks, and infection may predispose susceptible children to recurrent wheezing later in life (Pullen and Hey, 1982; Stein *et al.,* 1999; Morrison *et al.,* 2007).

In the present study, IgG antibody was measured, to determine the seropositivity and the level of the antibody with respect to age, sex, and presence of respiratory symptoms, sickle cell disease and the presence of parental smoking.

It was found in this study that 353 (93.1%) of the subjects were seropositive for RSV IgG antibodies. This conforms to the reports of Glezen *et al., (*1981) Shier and Badr, (2000) which stated that by 2 years of age; almost every child would have been infected with 95% of children RSV seropositive. But in contrast to the work of (Bha*ttarakosol et al.,* 2003) who reported 68.91% in Thai children. This could be due to the period of the research because the disease is highly prevalent in the winter months (December-April).

The high prevalence in this study could be due to the seasonal influence (raining season), since RSV infection is more prevalent in this season in tropical countries.

The study also reveals that there is increase in seropositivity with age, of which could be due to reinfection of older children with the same virus, resulting in boaster effect on the immune system of the children. Children of older ages developed higher antibodies which protect them from severe form of recurrent infection with RSV. This conforms to the findings of (Hazlett *et al.,* 1988; Bhattarakosol *et al*., 2003)

The profile of RSV antibody titer indicates that 8(2.1%) had maximum level of antibody titre, 136 (35.9%) had antibody level between 251-300 and 130 (34.3%) had 201-250 level of antibody. This high antibody titer in this number of subjects indicates the possibility of reinfection which has boaster effect on the immune system.

In assessing the sex distribution of RSV antibodies, seropositivity was also observed to be high in females (94.2%) than in males (92.1%) although the difference was not statistically significant. It could be due to the high seroprevalence (93.1%) which shows that every child has a risk of having the infection regardless of sex. This is in contrast with the work of (Shier and Badr, 2000) who recorded increased percentage of male RSV positive cases (55%) more then female cases (45%). The report of (Denny and Clyde, 1986; Kristensen *et al.,* 1998) also stated that there was male predominance with respiratory tract infections.

The results revealed that of the 9 subjects tested that had sickle cell disease all were positive (100%) but the difference was not statistically significant ($p>0.05$). This could be due to the small number of children with sickle cell disease tested. The presence of RSV in sickle cell patient will further aggravate the condition and may require immediate medical attention.

The finding of 65.2% seropositivity to RSV in those with cough in the present study was not of statistical significance, probably due to the fact that other microbial agents may be responsible. Also RSV has a predilection of the lower respiratory tract.

The results also showed that 46 (12.1%) have been exposed to tobacco smoke from there parents/or relatives. 42 (91.3) were seropositive for RSV antibody, but the difference was not statistically significant which could be due to the small number of children who are exposed to tobacco smoke tested. This is in contrast to the findings of (Shier and Badr, 2000; Lanari *et al.,* 2002) who identified tobacco smoke as an environmental risk factor for RSV disease. Smoke destroys ciliated epithelial cells which make it easy for the organism to invade the tissue.

In assessing the symptom of catarrh/running nose distribution of RSV antibodies, seropositivity was observed to be (92.2%), although the difference was not statistically significant ($P>0.05$) this could be due to other microbial agents that might be responsible for the condition rather than the virus.

CONCLUSION

This study shows the importance of RSV as an etiological agent of respiratory tract infection in children, with seroprevalence of 93.1%; it also shows that seropositivity increases with age which shows possible reinfection. It therefore reinforces the need for a rapid and reliable diagnosis of RSV infection in children. This is very important for clinical management of children with respiratory tract infection. This study has also provided epidemiological data that may be useful in control effort and vaccine trials.

RECOMMENDATION

Respiratory Syncytial Virus infection is found to have high prevalence in the present study. This reinforces the need for the development of an effective vaccine to protect this particular age group from the severe form of the disease.

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APPENDIX I

APPENDIX II

APPENDIX III

APPENDIX IV

ABBREVIATIONS

AAP	American Academy of Pediatrics
ADH	Antidiuretic Hormone
CCA	Chimpanzee Coryza Agent
CPTS	Cold Passaged Temperature Sensitive
CSF	Cerebrospinal fluid
CTL	Cytotoxic T-Lymphocyte
EEG	Electroencephalogram
F	Fusion Protein
G	Glycoprotein
EIA	Enzyme Immunoassay
GOPD	General Out Patient Department
L	Polymerase
M	Matrix Protein
MMOL	Milimoles
mRNA	messenger Ribonucleic Acid
N	Nucleocapsid Protein
NS	Nonstructural Protein
OD	Optical Density
ORF	Open Reading Frame
P	Phosphoprotein

PFU Plaque Forming Unit

PHC Primary Health Clinic

RSV Respiratory Syncytial Virus

SH Small Hydrophobic Protein

SPSS Statistical Package for Social Science

TMB Tetramethyl benzedine