1. Introduction

1.1 Background

West Nile virus (WNV) is by far the most widely distributed arbovirus. It belongs to the Japanese encephalitis antigenic complex of the family Flaviviridae. It is a mosquito-borne virus that circulates among birds, but can also affect other species, particularly humans and horses (Hayes *et al*; 2005).

West Nile Virus was first recognized in 1937 after it was isolated from blood of

a febrile woman in the West Nile District of Uganda (Smithburn*et al*;1940).

West Nile Virus infection in humans causes a spectrum of manifestations from subclinical infection to death (Petersen and Marfin, 2002).

Most infections are subclinical but occasionally clinical manifestations will develop 2–21 days after infection. Cases lacking neurologic manifestations generally do not require hospitalization, and are termed ‘‘West Nile fever’’ (WNF). Neurologic cases usually involve meningoencephalitis, and have been termed ‘‘West Nile meningoencephalitis’’ (WNME) (Asnis*et al*; 2000).

Although it has been reported that all ages are equally susceptible to infection with WNV, elderly persons (over 60

years) are at greater risk of neurological complications and death (Campbell*et al*;2002). Transmission of WNV in Southern Africa and ofKunjin virus in Australia increases in the early months of the year after heavy spring and summer rainfall(Hall *et al;* 2002). The most important risk factor for acquiring WNV infection is exposure to infected mosquitoes (Han *et al*;1999).

Enzyme-linked immunosorbent assays (ELISA) was use to detect IgM and IgG antibodies in serum and CSF (IgM antibodies are responsible for the early immune response and indicate an early infection, whereas IgG antibodies correspond to the secondary immune response) (Murgue*et al*; 2001).

Treatment of WNV infection issupportive. Controlled studies to evaluate specific therapies for WN virus infection have not been completed (Sejvar*et al*; 2003;Gea-Banacloche *et al*; 2004).

1.2 Rationale

West Nile virus infection are subclinical or asymptomatic. Most symptomatic persons experience an acute systemic febrile illness that often includes headache, myalgia, or arthralgia; gastrointestinal symptoms and a transient maculopapular rash also are commonly reported (Watson *et al*; 2004, Hayes *et al*; 2005b).Which is similar to symptoms of malaria which is endemic disease in Sudan, thus could be

miss diagnosed by physician in malaria negative patient.

Little is known about the serodetectionof WNV among patients in WadMadeni district.So this study aimed to focus on the possibility of WNV existence in negative malaria patient.

Assessing the detection WNV among patient is necessary for the planning of health control measure in primary and secondary prevention.

1.3 Objectives

1.3.1 General objective

To serodetectof West Nile Virus among febrile patient at WadMadeni Teaching Hospital.

1.3.2 Specific objective

1. To detect WNV IgG and IgM antibodies among febrile non malarial patients.

2. To identify the major possible risk factor associated with West Nile fever(gender, age, contact with birds and residence).

3. To know the prevalence of WNV among febrile patients.

2. literature review

2.1 Introduction

West Nile virus (WNV) is a mosquito borne, neurotropic, positive-stranded, enveloped RNA virus in the Flaviviridae family. WNV is related genetically to other viruses that cause severe visceral and central nervous system (CNS) diseases in humans including dengue(DENV), yellow fever (YFV), Japanese encephalitis (JEV), and tick-borne encephalitis (TBEV) viruses. WNV is maintained in an enzootic cycle between mosquitoes and birds, but also infects and causes disease in vertebrate animals including horses and humans. WNV is transmitted primarily by Culex species mosquitoes and the virus amplifies in bird reservoirs, with humans and horses largely considered as dead-end

hosts (Hayes *etal*; 2005).

Although human cases occur primarily after mosquito inoculation, infection after blood transfusion, organ transplantation, and intrauterine transmission has been reported (Hayes *etal*; 2005).

2.2 History

WNV was first recognized in 1937 after it was isolated from blood ofa febrile woman in the West Nile District of Uganda (Smithburn*et al*;1940). It became known as the etiologic agent of West Nile fever andwas occasionally isolated from febrile children in North Africa andthe Middle East beginning in the 1950s (Hayes; 2001). The occurrenceof a dozen WNV encephalitis cases among elderly victims in Israel in1957 was the first indication that WNV could cause serious centralnervous system infections (Spigland*et al*;1958).In 1974, thelargest known outbreak of WNV disease caused approximately10,000 human fever cases in South Africa (Jupp, 2001; McIntosh*et al*; 1976). In 1996, WNV emerged as a major cause of arboviralencephalitis in Romania, where an outbreak led to 393 recognizedhuman cases of encephalitis, with 16 deaths (Tsai *et al*; 1998). After1996, outbreaks of West Nile viral encephalitis in people and horseswere reported with increasing frequency in the Mediterranean Basin(Hubalek and Halouzka, 1999; Triki*et al*; 2001), Russia (Platonov*et al*; 2001) and Australia (Brown *et al*; 2002). An identical strain emerged in New York City in 1999 (Roehrig*et*

al; 2002).

After 1999, WNV continued to cause sporadic equine and human disease in the United States (CDC, 2002a;Marfin*et al*; 2001)

2.3Etiology

West Nile virus is an arbovirus in the Flavivirus genus of the family Flaviviridae. It belongs to the Japanese encephalitis virus complex or serogroup. The two most common genetic lineages of WNV are lineage 1, which contains 3 clades (1a, 1b and 1c), and lineage 2. Both lineages contain virulent viruses, as well as strains that usually cause asymptomatic infections or mild disease. Many of the virulent viruses from recent outbreaks belonged to clade 1a, which is widespread NY99 has continued to evolve in the Americas, where it has been replaced by its variants, especially WN02. Clade 1b consists of Kunjin viruses, a subtype of WNV found in Australia, and clade 1c consists of some West Nile viruses found in India.

Several additional WNV lineages also exist or have been proposed. Under some taxonomic schemes, it might be possible to classify this virus into as many as eight lineages by including Koutango virus, a related virus that circulates in Africa, and elevating clades 1b and 1c to lineages. (Bentler *et al;* 2007).

2.4 Characteristics of West Nile Virus

WNV is an arbovirus in the family Flaviridae. Its spherical, enveloped capsid has a diameter of ≈50 nm and contains single-stranded RNA that encodes the capsid (C), envelope (E), and premembrane (prM) proteins, as well as 7 nonstructural proteins that likely contribute to viral replication(Beasley *et al;* 2004).

2.5 Virology and Pathogenesis

Although cellular receptors have not yet been identified definitively, studies suggest that WNV enters cells by endocytosis and fusion with the early endosome (Chambers *et al*;1990 ; Brinton ,2002). Following fusion between the viral and endosomal membranes, the nucleocapsid is released into the cytoplasm and 11 kilobase viral genomic RNA associates with endoplasmic reticulum (ER) membranes. The single open reading frame is translated into a polyprotein and enzymatically processed into three structural proteins (capsid (C), pre-membrane (prM)/membrane (M), and envelope (E)) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). Negative strand viral RNA then is synthesized and serves as a template for positive strand RNA synthesis (Mackenzie and Westaway;2001) . Positive strand RNA is packaged in progeny virions, which bud into the ER to form enveloped immature virions. A maturation step, cleavage of the prM protein to the membrane M protein, occurs in the trans Golgi network by furin-like proteases (Elshuber*et*

al;2003 ; Stadler *et al*; 1997) and results in a reorganization of E proteins on the virus surface into a homodimeric array (Mukhopadhyay *et al*;2003); these virions are secreted into the extracellular space by exocytosis.

Following mosquito inoculation into the skin, it is believed that WNV replicates within epidermal keratinocytes and Langerhans cells(Byrne *et al*;2001 ; Lim *et al*;2011). Migratory Langerhans dendritic cells enter afferent lymphatics and travel to draining lymph nodes(Byrne *et al*;2001). Here, infection and the risk of dissemination are countered by the rapid development of an early immune response including type I and II IFN production and the effector functions of innate immune cells (NK cells, neutrophils, macrophages, and IgM-secreting B cells) (Bourne *et al*;2007;Bai *et al*; 2010). Virus produced in the lymph node can enter circulation via the efferent lymphatic system and thoracic duct, and viremia allows spread to secondary lymphoid and visceral organs including the spleen and kidney(Diamond *et al*; 2003;Eldadah and Nathanson; 1967) . In peripheral tissues, infection is restricted by innate and adaptive immune responses including serum IgM (Diamond *et al*; 2003), IFN (Samuel and Diamond, 2005), IFN) (Wang *et al*; 2003;Shrestha*et al*; 2006)., cytolytic CD8+ T cells (Shrestha*et al*; 2012), and cell-intrinsic IRF-3-dependent antiviral responses (Daffis*et al*; 2007)

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2.6 Transmission

WNV is transmitted to humans primarily through the bite of infected mosquitoes (Campbell *et al*; 2002). However, person-to-person transmission can occur through transfusion of infected blood products or solid organ transplantation (Pealer*et al*; 2003, Iwamoto *et al*; 2003). Intrauterine transmission and probable transmission via human milk also have been described but appear to be uncommon (O'Leary *et al*; 2006, Hinckley *et al*; 2007). Percutaneous infection and aerosol infection have occurred in laboratory workers, and an outbreak of WNV infection among turkey handlers also raised the possibility of aerosol transmission (CDC 2002b).

2.7 West Nile virus propagation in the mosquito host

Female *Culex*spp. mosquitoes acquire WNV while feeding on infected viraemic birds. WNV replicates in the midgut epithelial cells of the mosquito and spreads via the haemolymph to the salivary glands and other organs12. A key step in WNV transmission and vector competence is the midgut barrier, which acts as a physical and immune barrier through the production of antimicrobial peptides and a peritrophic matrix (composed of chitin, proteins, glycoproteins and proteoglycans), which together limit viral replication and spread within the insect (Moskalyk *et al* ; 1996).

2.8Clinical Presentation

An estimated 70-80% of human WNV infections are

subclinical or asymptomatic (Mostashari*et al*; 2001).

2.8.1West Nile fever

Most symptomatic persons experience an acute systemic febrile illness that often includes headache, myalgia, or arthralgia; gastrointestinal symptoms and a transient maculopapular rash also are commonly reported (Watson *et al*; 2004).

2.8.2 neuroinvasive disease

Less than 1% of infected persons develop neuroinvasive disease, which typically manifests as meningitis, encephalitis, or acute flaccid paralysis (Hayes *et al*; 2005b). WNV meningitis is clinically indistinguishable from aseptic meningitis due to most other viruses (Sejvar and Marfin, 2006). Patients with WNV encephalitis usually present with seizures, mental status changes, focal neurologic deficits, or movement disorders (Sejvar and Marfin,2006). WNV acute flaccid paralysis is often clinically and pathologically identical to poliovirus-associated poliomyelitis, with damage of anterior horn cells, and may progress to respiratory paralysis requiring mechanical ventilation (Sejvar and Marfin,2006). WNV-associated Guillain-Barré syndrome has also been reported and can be distinguished from WNV poliomyelitis by clinical manifestations and electrophysiologic testing (Sejvar and Marfin,2006).

2.8.3Infection During Pregnancy

A causal relationship between WN virus and fetal abnormalities has not been proven. In 2002, there was a single report of a woman who had WN virus encephalitis during the 27th week of her pregnancy and subsequently delivered a term infant with chorioretinitis and laboratory evidence of congenitally acquired WN virus infection (Paisley *et al*; 2006).

2.8.4 Other clinical features

Cardiac dysrhythmias, myocarditis, rhabdomyolysis, optic neuritis, uveitis, chorioretinitis, orchitis, pancreatitis, and hepatitis have been described rarely with WNV infection (Hayes *et al*; 2005b).

2.9Incubation period

The typical incubation period for infection ranges from 2 to 14 days, although longer incubation periods have been observed among immunosuppressed hosts (Pealer*et al*;2002).

2.10 Risk Factors

The principal risk factor for infection was geographic location because WNV was noted to be active in certain well-defined locations within specific countries. It was also noted that advanced age was the principal risk factor for severe human disease. (Han *et al*;1999) found that time spent out-doors and in flooded basements were risk factors for infection during the 1996 outbreak in Bucharest,

Romania. (Bin*et al*; 2001) reported that close contact with sick geese was a risk factor for human infection in Israel in 1999, but not residence in areas along bird migration routes.

In New York City in 1999, the initial series of eight patients hadclustered residences within a 2-mile radius in Queens, and all hadoutdoor exposure (Asnis*et al*; 2000). An analysis of the full series of59 hospitalized cases determined that all had disease onsets betweenearly August and late September (Nash *et al*; 2001). Thus, risk isgreatest in the New York City region during the third quarter ofthe year coincident with the seasonal blood-feeding by mosquitoes.This seasonality of risk would be less restricted where mosquitoblood-feeding is extended or occurs year-round.Because WNV is known to cause viremia in humans, blood transfusion was considered a potential risk factor for WNV infection after the1999 epidemic in New York City. The theoretical risk of transmissionfrom donors was estimated at 1.8:10,000 (Biggerstaff and Petersen,2002).

Other risks that emerged in 2002 besides infection by mosquito bite in-cluded organ transplantation, pregnancy (risk to developing fetus),breastfeeding (risk to infant), and occupation (laboratory workers thatcontact WNV directly). No risk of WNV infection has been described forthe following potentially risky behaviors: caring for human cases,sexual contact, bird feeding, handling live birds or other vertebrates,eating bird-derived foods, and handling of

infected animal carcasses(outside the laboratory) (Biggerstaff and Petersen,2002).

2.11 Geographic Distribution

The geographic distribution of WNV is known from human andequine outbreaks, avian epizootics (particularly in North America)and serosurveys of vertebrate hosts (Hayes, 1989; Hubalek andHalouzka, 1999; Komar, 2000). In Africa, WNV is known from mostcountries where arbovirus studies have been conducted, from SouthAfrica and Madagascar in the extreme south to Morocco, Algeria,Tunisia, and Egypt in the north. Its range extends from Africa east-ward through the Middle East into south Asia, where it is knownfrom Pakistan and India. It extends northward into southernRussia, and westward through southern Europe. A variant of WNvirus, Kunjin virus, is present in Australia and contiguous regions ofSoutheast Asia (Hall*et al*; 2002; Scherret*et al*; 2001).In North America through the end of 2002, WNV had spread to everycontinental U.S. state except Oregon, Utah, Nevada, and Arizona(CDC, 2002b); the Canadian provinces of Saskatchewan, Manitoba,Ontario, Quebec, and Nova Scotia (P. Buck, personal communication);the Mexican states of Coahuila (Blitvich*etal*; 2003) and Yucatan(Loron ˜o-Pino*et al*; 2003); the Cayman Islands (CDC, 2002a), Jamaica(DuPuis*et al*; 2003), and the Dominican Republic (Komar*et al*;2003b) .

2.12Molecular epidemology

WNV is aFlavivirus (familyFlaviviridae). Its structure and size aresimilar to other flaviviruses, including the prototype, yellow fevervirus. A large body of knowledge of the molecular biology of WNVhas recently been reviewed(Brinton, 2002). Numerous strains ofWNV have been isolated, separated by time and space, since 1937. At least two separate genetic lineages of WNV have been described (Berthet*et al*; 1997; Burt et *al*; 2002; Scherret*et al*; 2001). place all of the European, Middle Eastern, South Asian, Australian (Kunjin virus), and North American strains in Lineage 1. This lineage includes the strains that have caused encephalitis outbreaks in humans and horses. It also includes some African strains. Lineage 2 includes southern African strains, including some from central Africa and the Ugandan prototype strain isolated in 1937. Although Lineage 2 viruses have not been associated with outbreaks of severe disease, one South African strain was responsible for the largest WNF outbreak recorded, with over 10,000 mild fever cases in 1974. However, these strains have caused only isolated cases of human encephalitis and hepatitis (Burt*et al*;2002). (Scherret*et al*; 2001) suggest that four or five separate WNV subgroups may be recognized. In this phylogeny, the south Asian, Malaysian, and Australian strains form three additional subgroups in addition to the Lineage 1 and 2 groups.

2.13 Laboratory findings

WNV infections can be diagnosed by isolating the virus, detecting viral antigens or RNA, or using serological methods. The usefulness of the various techniques varies with the level of virus replication in the host (Lanciotti *et al*; 2000).

2.13.1 Viral isolation &nucleic acid testing

2.13.1.1in-vivo culture

Virus isolation in Vero cell culture remains the standard for confirmation of WNV positive pools (Beaty*et al*; 1989, Savage *et al*; 1999, Lanciotti*et al*; 2000). Virus isolation provides the benefit of detecting other viruses that may be contained in the mosquitoes, a feature that is lost using test procedures that target virus-specific nucleotide sequence or proteins. However, Vero cell culture is expensive and requires specialized laboratory facilities; thus, nucleic acid assays have largely replaced virus isolation as detection and confirmatory assay methods of choice. Virus isolation requires that mosquito pools be ground in a media that protects the virus from degradation such as BA-1 (Lanciotti*et al*; 2000), and preservation of an aliquot at -70oC to retain virus viability for future testing.

2.13.1.2nucleic acid testing (NAT)

Use of nucleic acid testing (NAT) in serum or CSF may be valuable in severely immunocompromised patients, who may have absent IgM antibody. " It is also possible to isolate WN

virus or to detect viral antigen or nucleic acid in CSF, tissue, blood, or other body fluids, although the low sensitivities of these methods preclude their use as routine screening tests. Viral culture of CSF or brain tissue in humans had very low yield among patients in the United States; nucleic acid amplification testing such as real-time PCR has been positive in up to 55 percent of CSF samples and 10 percent of serum samples (New York City Department of Health, 2001).

Nucleic acid test (NAT) screening of blood donors using newly developed investigational screening assays was introduced in 2003 . In one study, the analytical sensitivity of this approach was effective in testing of individual samples, but was compromised by the use of minipool donations, particularly in the setting of low-level viremia (Busch *et al*; 2005a).

In contrast, the false positivity and unit discard rate for blood donations screened by individual lNAT was higher than for minipool screening (Kleinman*et al*;2005).

2.13.1.3Molecular methods

detection of nucleic acidSeveral PCR methods have been described for the identification of WNV and some are available as commercial kits. Included here is a real-time RT-PCR with the capacity to detect both lineage 1 and lineage2 WNV (Busch *et al*; 2005a).Additionally, a conventional, gel-based RT-PCR designed to detect lineage

1North American strains is described (Scherret*et al*; 2001). Both assays have been successfully employedwith field-collected samples. Lineage 1 WNV from France, Egypt, Israel, Italy, Kenya, Mexico and Russiademonstrate a highly conserved nucleotide sequence in the target region, regardless of species of origin.While the laboratory practices required to avoid contamination in a nested methodare stringent, there is higher sensitivity for detection of North American strains of WN RNA with theconventional nested procedure. (Lanciotti*et al*; 2000).

The following method was developed by (Busch *et al*; 2005a).for concurrent identification of lineage 1and lineage 2 WNV. Strain identification may be achieved by sequencing of the resultant amplicon andalignment with WNV reference strains. The procedure has been slightly modified from the publishedmethod and included here are the primers and probe directed to the NS2A region of the WNV genome.

2.13.2 Serological tests

Antibody can be identified in serum by IgM capture enzyme-linked immunosorbent assay (IgM capture ELISA), haemagglutination inhibition (HI), IgG ELISA, plaque reduction neutralisation (PRN), and microtitre virusneutralisation (VN) (Beaty*et al*; 1989; Hayes; 1989).

The PRN test is the most specific amongWNV serological tests; when needed, serum antibody titers against related flaviviruses can be tested in parallel.

2.13.2.1Captureenzyme-linked immunosorbent assay (MAC-ELISA)

The IgM antibody captureenzyme-linked immunosorbent assay (MAC-ELISA)is optimal for IgM detection because it is simple, sensitive, and applicable to serum and CSF samples. Testing of serum or CSF is available commercially, and also can be obtained through local or state health departments. The timing of the IgM antibody response in patients with WN fever has not been completely determined (New York City Department of Health , 2001).

A rise in WN virus-specific neutralizing antibody titer in acute andconvalescent sera is confirmatory of acute infection. Clinicians should also bear in mind that IgM antibody to WN virus may persist for six months or longer. Since most infected persons are asymptomatic, residents in endemic areas may have detectable IgM antibody from previous WN virus infection that is unrelated to their current clinical illness (Roehrig*et al*; 2003).

2.13.2.2Indirect and competitive ELISAs

Numerous indirect and competitive commercial and in-house ELISAs have been developed and are used todetect WNV antibodies. While competitive assays are applicable for sera of all species, indirect assaysrequire enzyme-labelled species-specific antibodies. Both ELISA techniques lack specificity as they crossreactwith antibodies directed to

other flaviviruses especially those of the Japanese encephalitis serogroup.

They are very useful for epidemiological and surveillance purposes as well as a screening method. Apositive ELISA result however should be confirmed by a more specific test such as VN or PRNT. Mostindirect or competitive ELISAs detect antibodies of any immunoglobulin class (IgM, IgG, etc) (Roehrig*et al*; 2003).

2.13.2.3EIA test

testing with EIA for the detection of IgM antibody to WN virus. A positive test in a patient with suggestive clinical features of the disease has a high predictive value for the diagnosis of WN virus infection, although false positive tests can occur. A negative test, however, does not rule out infection. In patients who present with suspected meningitis, encephalitis, or acute flaccid paralysis, we recommend a lumbar puncture and testing of the CSF for detection of IgM antibody as well as serologic testing (Roehrig*et al*; 2003).

2.13.2.4Neutralisation

2.13.2.4.1Plaque reduction neutralisation

(PRNT), the most specific test for the arthropod-borne flaviviruses, can help distinguish false positive results of MAC-ELISAor other assays (eg, indirect immunofluorescence, hemagglutination inhibition). The PRNT may also help distinguish serologic cross-reactions among the flaviviruses,

although some degree of cross-reaction in neutralizing antibody may still cause ambiguous results(Weingartl*et al*; 2003).

2.13.2.4.2Virus neutralization-microtitre format

The microtitre VN assay is capable of identifying and quantifying antibodies against WNV present in testsamples. Its performance is comparable to the PRNT; however, it requires less sample volume than PRNTand is more suitable when only small volumes of samples are available (Weingartl*et al*;2003). Appropriateprecautions are necessary to prevent human exposure when using live WNV in unsealed microtitre plates.

2.14Treatment

Treatment of WN virus infection is supportive. Controlled studies to evaluate specific therapies for WN virus infection have not been completed. Uncontrolled studies or case reports suggesting treatment efficacy should be cautiously interpreted, since the clinical course and outcomes with WN virus neuroinvasive disease are highly variable (Sejvar*et al*; 2003;Gea-Banacloche *et al*; 2004).

Interferon — The rationale for the use of alfainterferon is based upon evidence of efficacy against WN virus in vitro and in animal models (Anderson and Rahal, 2002).

Ribavirin — The antiviral agent ribavirinhas demonstrated in vitro activity against WN virus, but therapeutic efficacy has

not yet been demonstrated in animal models (Morrey *et al*; 2004).

intravenous immunoglobulin G (Omr-IgG-am) containing high anti-West Nile virus antibody titers in patients with, or at high risk for progression to West Nile virus (WNV) encephalitis and/or myelitis. Sponsored by: National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health.

A randomized double-blinded, placebo-controlled trial of alpha-interferon (Alferon) therapy for West Nile meningoencephalitis (Protocol WN-102).

An exploratory study of the safety, tolerability, pharmacokinetics and potential effectiveness of AVI-4020 injection in patients presenting with presumptive acute neuroinvasive West Nile virus (WNV) disease (Sayao *et al*; 2004).

2.15 Prevention

Prevention of infection includes personal protection measures, mosquito control programs, and blood donor screening. It is important to drain standing water where mosquitoes are likely to breed. In a case control study examining risk factors, only spending increased amounts of time outdoors and the presence of flooded basements correlated with infection (Han *et al*, 1999)

Blood donor screening programs — Blood donor screening for WN virus has greatly reduced, but not eliminated, the risk of transfusion transmission (Petersen and Epstien; 2005; Stramer*et al*; 2003 ;Busch *et al*; 2005a).

WN virus infection should be considered in recent transfusion recipientsk,with unexplained, compatible illness.

Vaccine development — There has been great interest in a WN virus vaccine and studies of different vaccines in animals suggest efficacy.

Human vaccines are unlikely to be available for at least several years (Pletnev*et al*; 2003).

To prevent WN virus infection in humans, extensive early season larval control has been recommended and undertaken, as have the development and dissemination of public health messages for reducing personal exposure to mosquito bites (Petersen and Epstien; 2005). The efficacy and cost-effectiveness of these prevention measures, along with application of pesticides to control adult mosquitoes, require further evaluation. These evaluations are likely to be hindered by the sporadic nature of human WN epidemics. Given our incomplete and evolving knowledge of the ecology and public health impact of WN virus in the Americas, as well as the efficacy of control efforts, the virus will remain an important public health challenge in the next decade (Busch *et al*;

3. MATERIALS AND METHODS

3.1 Study approach

Qualitative approach

3.2 Study design

Discreptive cross-sectional study

3.3 study area

This study was conducted at Wad Madeni Teaching Hospital**.**

3.4 Study duration

This study was carried out in period from July 2013 to September 2014.

3.5 Study population

Febrile patients males and females who attended at Wad Madeni Teaching Hospital for medical investigation.

3.6 Inclusion criteria

Febrile patients males and females were included in this study.

3.7 Exclusion criteria

All non febrilepatient were excluded.

3.8 Sampling technique

Simple random clustering technique.

3.9 Sample size

Ninety one venous blood samples were collected from febrile patients.

3.10 Data analysis

Collected data were analyzed by a computer system using Statistical Package for social science (SPSS) program using the Chi square test and crosstabulation. Statistical significant was set at p-values < 0.05.

3.11 Ethical consideration

Permission to carry out the study was obtained from the College of Graduate Studies, Sudan University for Science and Technology. All human examined were informed for the purpose of the study before collection of samples, and verbal consent was taken from them.

3.12 Data collection

Personal and clinical data were collected by direct interviewing questionnaire from each subject.

3.13 Specimen collection

Under sterile condition, 3ml of venous blood were collected in sterile plain containers and allowed to clot at room temperature. The sera were obtained by centrifugation of the blood at 3000 rpm for 5 minutes. The sera were separated from the clot and transferred into new sterile

labeled plain containers and stored at -20 ºC until used.

3.14 Laboratory method

Enzyme-Linked Immunosorbent Assay (ELISA) was used to detect anti-WNV IgM and IgM antibodies.

3.14.1 ELISA for detection of WNV IgG antibodies

3.14.1.1 Principle

Serum antibodies combine with purified and inactivated antigen coated on the polystyrene surface of the microwell test strips (assay plate). Residual serum is removed from the assay plate by washing and HRP-conjugated anti-human IgG is added. After incubation, the microwell are washed and a colourless substrate system, tetramethylbenzidine/hydrogen peroxide (TMB/H_2O_2),is added. The substrate is hydrolysed by the HRP, if present, and the chromogen changes to a blue colour. After stopping the reaction with acid, the TMB become yellow. Colour development is indicative of the presence of WNV antibodies in the test sample.

3.14.1.2 Assay procedure

Commercial ELISA Kits (Panbio diagnosis) were used as described by manufacture.

All reagent and samples were brought at room temperature before beginning the procedure. The samples diluted (1:100) with serum diluents and washing solution diluted (1:20) with

distilled water,other reagents were ready for use.

The strips needed were set in strip holder and sufficient number of wells included negative control (N), positive control (P) , calibrator (CAL) in triplicate and samples. 100µl of diluted patient sample, control and calibrator sera were added into their respective microwells. Covered plate and incubated for 30 minutes at 37 ºC ± 1 . The plate cover was removed and discarded.The wells were washed six (6) times by diluted wash buffer using ELISA washer (see appendix).then 100µl HRP conjugated anti-human IgG were added to each well. The plate was Covered and incubated for 30 minutes at 37 ± 1 . Then The plate cover was removed and discarded.The wells were washed six (6) times by diluted wash buffer,an enzyme substrate reagent (TMB) was added(100µl/well) and the plate was incubated 10 minutes at room temperature (20-25 ºC). A bluecolour was develop.the blue colour changed to yellow after adding of stop solution(100µl/well). The optical density (OD) in microplate reader was read within 30 minutes at 450 nm.

3.14.1.3 Calculation of control values and cutoff

Cut-off value (COV) =Mean absorbance of calibrator × calibration factor

calibration factor = 2.0

Panbio Unit =index value × 10

3.14.1.4. Interpretation of results

Index	Panbio Unit	Result
<1.4	<14	Negative
1.4 – 1.6	14 – 16	Equivocal
>1.6	>16	Positive

3.14.2.ELISA for detection of WNV IgM antibodies

3.14.2.1. Principle

Serum antibodies of the IgM class combine with anti-human IgM antibodies attached to the polystyrene surface of the microwell test strips (assay plate). Residual serum is removed from the assay plate by washing. WNV antigen combined with HRP conjugated monoclonal antibody (MAb) is added to the assay plate. After incubation, the microwells are washed and a colourless substrate system, tetramythylbenzidine/hydrogen peroxide (TMB/H2O2) is added. The substrate is hydrolyzed by the HRP if present, and the chromogen change to a blue colour. After the stopping the reaction with acid, the TMB becomes yellow. Colour development is indicative of the presence of IgM WNV antibodies in the test sample.

3.14.2.2. Assay procedure

Commercial ELISA Kits (Panbio diagnosis) were used as described by manufacture.

All reagent and samples were brought at room temperature before beginning the procedure. The samples diluted (1:100) with serum diluents and washing solution diluted (1:20) with distilled water,other reagents were ready for use. All the steps for the procedure used above for the detection of WNV IgG, were followed step by step for detection of WNV IgM.

3.14.2.3.calculation

Cut-off value (COV) =Mean absorbance of cut-off calibrator

Panbio Unit =index value × 10

3.14.2.4. Interpretation of results

Index	Panbio Unit	Result
<0.9	<9	Negative
0.9 – 1.1	9 – 11	Equivocal
>1.1	>11	Positive

4.RESULT

4.1 Detection of WNV IgG among febrile patients

Out of 91 febrile patients tested, 32 patients (35.2%) were positive , while59 patients(64.8%) were negative.(**table 4.1**).

Table 4.1 : Detection of WNV IgG among febrile patients

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Negative	59	64.8	64.8	64.8
	Positive	32	35.2	35.2	100.0
	Total	91	100.0	100.0	

4.2 Detection of WNV IgM among febrile patients

Out of 91 febrile patients tested, 12 patients (13.2%) were positive , while 79 patients(86.8%) were negative.(**table 4.2**).

Table 4.2 : Detection of WNV IgM among febrile patients

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Negative	79	86.8	86.8	86.8
	Positive	12	13.2	13.2	100.0
	Total	91	100.0	100.0	

4.3 The effect of gender on WNV IgGserodetection

The study demonstrated that the gender had no effect (p>0.05) on WNV IgG 5 males and 27 females were positive(**table 4.3**)**.**

Table 4.3 The effect of gender on WNV IgGserodetection

Gender	IgM		
	Positive	Negative	Total
Male	5	18	23
Female	27	41	68

Total	32	59	91

***P* value=0.119**

4.4 The effect of gender on WNV IgMserodetection

The study demonstrated that the gender had no effect (p˃0.05) on WNV IgM, 2 males and 10 females were positive for IgM (**table 4.4**)**.**

Table 4.4: The effect of gender on WNV IgMserodetection

Gender	IgM		
	Positive	Negative	Total
Male	2	21	23
Female	10	58	68
Total	12	79	91

***P* value=0.461**

4.5. The effect of contact wih birds on WNV IgG serodetection

The results revealed that there was significant difference (p<0.05) between contact with birds on WNV IgG ,the patients in contact with birds were found to have more IgG antibodies (**table 4.5**)**.**

Table 4.5 The effect of contact with Birds on WNV IgG serodetection.

Contact with birds	IgG		
	Positive	Negative	Total
Yes	19	11	30
No	13	49	61
Total	32	59	91

***P* value=0.0**

4.6 The effect of contact wih Birds on WNV IgMserodetection

The results revealed that there was significant difference (p<0.05) between contact with birds on WNV IgM .The patients who were in

contact with birds were found to have more IgM antibodies (7 out of 12 were positive) (**table 4.6**).

Table 4.6 The effect of contact Birds on WNV IgM serodetection

Contact with birds	IgM		
	Positive	Negative	Total
Yes	7	23	30
No	5	56	61
Total	12	79	91

***P* value=0.045**

4.7.The effect of Residence on WNV IgGserodetection

The study demonstrated that the Residence had no effect (p>0.05) on WNV IgG (**Table 4.7**).

Table 4.7 The effect of Residence on WNV IgG serodetection

Residence	IgG

	Positive	Negative	Total
Rural	22	33	55
Urban	10	26	36
Total	32	59	91

***P* value=0.232**

4.8. The effect of Residence on WNV IgMserodetection

The study demonstrated that the Residence had no effect *(p*˃0.05) on WNV IgM (**table 4.8**)**.**

Table 4.8 The effect of Residence on WNV IgMserodetection

Residence	IgM		
	Positive	Negative	Total
Rural	9	46	55
Urban	3	33	36
Total	12	79	91

***P* value=0.268**

4.9 .The effect of Age on WNV IgG serodetection

The study demonstrate that the Age had no effect (*p*>0.05) on WNV IgG. The patients of age between 21-40 (**table 4.9**)**.**

Table 4.9 The effect of age on WNV IgGserodetection

Age group	IgG

	Positive	Negative	Total
10-20	4	7	11
21-30	6	5	11
31-40	6	14	20
41-50	5	9	14
51-60	5	11	16
Above 61	6	13	19
Total	32	59	91

***P* value=0.811**

4.10.The effect of Age on WNV IgMserodetection

The study demonstrate that the age had no effect (p˃0.05) on WNV IgM. The IgM was greater in patients of age 21-40 (**table 4.10**).

Table 4.10The effect of age on WNV IgMserodetection

Age group	IgM		
	Positive	Negative	Total
10-20	2	9	11
21-30	8	3	11

31-40	3	17	20
41-50	1	13	14
51-60	2	14	16
Above 61	1	18	19
Total	12	79	91

***P* value=0.594**

5. Discussion

West Nile Virus is found worldwide and is associated with a febrile illnessthat occasionally causes neuroinvasive disease,particularly in the elderly or immunosuppressed host.While the risk is highest in the elderly, severe disease does occur among young adults and children(O'Leary *et al* ; 2006; Hayes and O'Leary, 2004).

Many cases in Africa that present with fever are documented as fever of unknown origin, especially if they fail to respond to antimicrobial drugs. The majority of these conditions remain undiagnosed. However, several arboviruses are

frequently considered in the etiology of acute febrile illness(Nur*et al*; 1999).

The present study revealed that the WNV seroprevalence among febrile patients at WadMadeni Teaching Hospital was 35.2%and 13.2% for WNV IgG and IgM respectively. This study is in agreement with Karoo and the Orange River study which revealed that incidence of WNV in adults in Karoo and the Orange River was 13% and the incidence in adults in Cape Province was 24%(Murgue*et al*; 2002).

The rate of WNF IgG antibodies was however found to be higher in our study (35.2%) compared to that obtained by Luke in Kenya (9.2%) (Luke *et al;* 2011).

The results of this study do not agree Nur et al (1999) who found high prevalence of IgM 66%(Nur*et al*; 1999).

These differences could possibly ,be attributed to the facts that other investigators had used different samples size , or different techniques that used to detect WNV. Moreover, these variation might also due to different study area.

There was significant association between birds contact on WNV infection.this is in line with Eidson(Eidson *et al*; 2001a, 2001b).

However the age was found as an insignificant risk factor (p>0.05) that is agree with hayes *et al*,(2005) who reported in the United States, persons of all ages appear to be equally susceptible to WNV infection.

Data on the incidence of WNV in most of the world are not readily available, and little information is documented in Sudan. Hence, the aim of this research work was to generate preliminary information about WNV infection in febrile patients and to establish and improvelaboratory diagnosis in Sudan .

5.2 Conclusion

this study conclude that, there is a considerable WNV infection in Wad Madeni indicated by the high prevalence of anti-WNV IgG and IgM antibodies, it is also found contact with birds has a significant association with the infection.

5.3 Recommendations

1) West Nile Virus should be strongly considered in patients who have the onset of unexplained febrile

illness, encephalitis and or meningitis, or flaccid paralysis during mosquito season.

2) Use of large sample size is critical for better result.
3) PRNT and PCR are essential for accurate identification

Increase awareness of WNV infection amongst physicians to enhance its diagnosis and reporting , in order to facilitate early epidemiological investigation and outbreak detection.

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Appendix

Sudan university of Science and Technology College of Graduate Studies

<u>Questionnaire</u>

Serodetection of West Nile Virus among febrile patient at Wadmadani Teaching Hospital.

1.Date……

2.Serial No……

3. Sex ……

A.Male b. female

4. Age……

5. Residences……

a. Rural b. Urban

6.Contact With Birds

a. Yes b.No

7.Signs and Symptoms……

……

8. Investigation Result:

.....