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Original Research Article

Clinico-Epidemiology and Molecular Detection of Zika Virus in Kassala, Eastern Sudan

Yasir B. Ahmed[1], Alzubair M. Ahmed[2], Abdualmoniem O. Musa[2], Hasan A. Ibrahim[3], Doaa Mohammedelhassan Burei Musa[4,5], Abubaker A. Ramli[6], Mona M. S. Salama[7], Dr. Nadir Abuzeid[8,9*]

[1]Department of Microbiology, Albandr Clinic Complex, Altaif City, Kingdom of Saudi Arabia
[2]Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, University of Kassala, Kassala State, Sudan
[3]Department of Medical Microbiology, Ministry of Health and Social Department Kassala State, Kassala Teaching Hospital, Kassala, Kassala State, Sudan
[4]Department of Microbiology, Alnoor medical complex, Oman City, Sultanate of Oman
[5]Department of Medical Microbiology, Postgraduate College, Karrai University, Khartoum, Sudan
[6]Shamekh Medical Complex, Al Baha, Saudi Arabia
[7]Department of Haematology, currently achieved PHD from Al-Neelain University, Sudan
[8]Department of Medical Microbiology, Programme Faculty of Medical Laboratory Science, Delta College of Science and Technology, Khartoum, Sudan
[9]Department of Medical Microbiology, Faculty of Medical Laboratory Science, Omdurman Islamic University, Khartoum, Sudan

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***Corresponding Author:** Dr. Nadir Abuzeid
Department of Medical Microbiology, Programme Faculty of Medical Laboratory Science, Delta College of Science and Technology, Khartoum, Sudan

Abstract

Zika is a disease transmitted to humans by mosquitoes in Africa, Asia, and America, it's caused by Zika virus (ZIKV) which is a RNA virus, ZIKV is classified within the family Flaviviridae, genus Flavivirus. The monitoring and diagnosis of the viruses early with specific molecular techniques will reduce the severity of viruses. This study aimed to detect the prevalence and molecular detection of ZIKV, and relation of age, sex, locality and other biomarkers to ZIKAV in Kassala State, Eastern Sudan. This was a cross-sectional study conducted in Kassala Teaching Hospital, Kassala State in Eastern Sudan, 286 samples for ZIKV analyzed using Real Time Polymerase chain reaction (RT-PCR), patients data were collected from questionnaire, from the Health Research Ethics Committee of the Ministry of Health and Social Development Kassala State ethical approval was achieved, and informed consent was obtained from participants or their guardians, statistical significance was determined using chi-square with significant set at $P < 0.05$. Out of 286 participants, 9 patients were positive for ZIKV, ZIKV positive was determined using RT-PCR, 2(22.2%) were males and 7(77.8%) were females, there was no a statistically significant association between gender and infection ($\chi^2 = 3.1$, $P = 0.1$). The symptoms of positive patients were fever 9(100%) headache 9(100%), joint pain 9 (100%), back pain 9(100%), loss of appetite 9(100%), sweating 9(100%), chill 9(100%), vomiting 6(2.1%), skin rash 3(33.3%) and bleeding 0 (0%) and Poly arthralgia 8(88.9%). There were no a statistically significant differences between these symptoms and ZIKV positive patients. The haematological parameters in this study showed normal and abnormal, the most abnormal was low (thrombocytopenia, leucopenia, low RBCs), there was no a statistically significant differences with ZIKV infection (($\chi^2 = 0.66$, $P = 0.717$), ($\chi^2 = 0.43$, $P = 0.803$), ($\chi^2 = 0.191$, $P = 0.909$)) respectively. The study concluded no statistically significant differences were found among the symptoms of ZIKV-positive patients. Patients not used mosquito net, from family with same signs and past infection were a statistically significant differences with ZIKV infection (($X^2 = 4.1$, $P = .008$), ($X^2 = 8.1$, $P = .011$), ($X^2 = 9.4$, $P = .014$)) respectively There were no a statistically significant differences between thrombocytopenia, leucopenia and low RBCs concerning ZIKV infection (($\chi^2 = 0.66$, $P = 0.717$), ($\chi^2 = 0.43$, $P = 0.803$), ($\chi^2 = 0.191$, $P = 0.909$)) respectively.

Keywords: ZIKV, Clinico, Epidemiology, Kassala, Sudan, RT-PCR, Prevention.

INTRODUCTION

Arthropod-borne viruses (Arboviruses) cause many infectious diseases spread in the world. One of these is ZIKV present within tropical and subtropical regions and other world. It causes infectious diseases and can be clinically diagnosed certainly in endemic countries (Wilder and Byass, 2016).

ZIKV is a RNA virus single strand of the family Flaviviridae, genus Flavivirus. It composed of three structural proteins capsid, membrane, and envelope, and seven non-structural proteins (NS1, NS2A, B, NS3, NS4A, B, and NS5) (Strottmann *et al*., 2019). Transmitted by Aedes mosquitoes, also sexually and through blood transfusions (Fauci & Morens., 2016).

The symptoms of ZIKV are fever, conjunctivitis, muscle pain, headaches, and a rash. Microcephaly in fetus when pregnant woman infected (Cook., 2016).

Diagnosis of ZIKV by detection of antibodies to the virus and detection of viral RNA using enzyme-linked immunosorbent assays (ELISA) and RT-PCR respectively (Eldigail *et al*., 2020).

The first indication of Zika virus circulation in Sudan was documented in 1981 in El Gezira state, located in central Sudan. A recent study found a significant exposure rate to ZIKV, with 62.7% of 845 blood samples collected from various regions of the country testing seropositive via ELISA, although only one sample was confirmed positive by the more specific neutralization test. Given the recent link between ZIKV infection in pregnant women and congenital defects in their infants, along with the virus's presence in seven of Sudan's 18 states, research on ZIKV and its epidemiology in Sudan remains notably limited (Ahmed *et al*., 2020). However, a few studies have been investigated in eastern Sudan by using real-time PCR. Therefore the aims of the study is to investigate the epidemiological and molecular detection of ZIKV, to identify areas with high risk for infection and to associate relative risk age, sex, locality and others biomarkers with infection.

MATERIAL AND METHODS

A cross-sectional study was conducted from 2021 to 2023 at Kassala Teaching Hospital, Eastern Sudan. A total of 286 participants were included in this study according to the formula estimated by proportion and confidence interval 95%, ethical approval was obtained from the Health Research Ethics Committee of the Ministry of Health and Social Development, Kassala State, and informed consent was obtained from participants or their guardians.

5 mL of blood was collected aseptically into EDTA vacutainer tubes. They were then centrifuged at 3000 rpm for 5 minutes. RNA was extracted and stored at -20°C.

ZIKV RNAs were extracted from plasma samples using a Patho Gene-spinTM DNA/RNA Extraction Kit (Intron Biotechnology, korea) is designed for rapid and sensitive isolation of DNA or RNA from a variety of pathogens, uses advanced silica-gel membrane technology for rapid and effective purification of DNA or RNA without organic extraction or ethanol precipitation. The procedure followed according to the manufacturer's.

RT-PCR was carried out to evaluate the extracted RNA, use of the Aridia Zika/Dengue/CHIK Real-Time PCR Test (CTK Biotech, Inc., Poway, California, USA kit), is based on the real-time amplification of the ZIKV NSP1 gene in a one-step format. The RNA is transcribed into complementary DNA (cDNA) by reverse transcription, followed immediately by PCR within the same well.

The Aridia Zika/Dengue/CHIK Real-Time PCR test comes ready-to-use. All real-time PCR components, including DNA polymerase, reverse transcriptase, primers, probes, and dNTPs, are stabilized within each reaction well. In addition, an Internal Control is included within each well for monitoring PCR reaction inhibition, the assay is based on 5’ nuclease chemistry which utilizes ZIKV-specific primers and hydrolyzable fluorogenic probes to detect the accumulation of amplified target sequences during the PCR reaction. Upon extension of the primers by DNA polymerase, the fluorogenic probes are hydrolyzed by the 5’ to 3’ exonuclease activity of the polymerase, causing spatial separation of the reporter and quencher. The resulting increase in ZIKV-specific fluorescence signal is measured by the real-time PCR thermocycler and is proportional to the amount of amplified product.

The RT-PCR thermocycler protocol was programmed as follows: an initial reverse transcription step was conducted at 50°C for 15 minutes, followed by an initial denaturation at 95°C for 2 minutes. Subsequently, 45 amplification cycles were performed, each consisting of denaturation at 95°C for 10 seconds and annealing/extension at 60°C for 50 seconds. The PCR thermocycler used was a Bio-Rad machine, model NO. CFX96 Optics Module, with serial NO. 785BR32294, Singapore.

Data Analysis

Data were analyzed using version 28 of the Statistical Package for Social Sciences (SPSS). The results were interpreted through cross-tabulation analysis, with a chi square employing tests to evaluate the relationships between variables. Statistical significance was determined using chi square with a threshold set at $P < 0.05$. Numerical data were expressed in terms of frequencies and proportion, while

quantitative data were assessed through recurrent responses, calculating both the mean and standard deviation.

RESULTS

A total of 286 participants from Kassala State, Eastren Sudan, were included in this study. The mean age of the participants, with standard error, was 30.3±.789 years, with ages ranging from 1 to 73 years. Among the participants 146 (51%) were males and 140 (49) were females. The prevalence of ZIKV was found to be 3.15% (see Figure 1).

Of the nine participants who tested positive for ZIKV, 2(22.2%) were males and 7(77.8%) were females indicating a higher rate of positivity among females (X^2 = 3.1, P = 0.1). However, no a statistically significant difference was observed between gender and ZIKV infection (Table 1).

Regarding age groups positive results were mostly found among participants aged 40-50 years, account 5 participants (55.6%) in this category. A statistically significant association was found between the age group and ZIKV infection (X^2 = 48.3, P = .003) (Table 1).

The analysis of residence, occupation, and marital status showed no a statistically significant differences between these variables and positive ZIKV patients, the educational level was a statistically significant difference (X^2 = 7.7, P = .029) (Table 1).

Symptoms collected in patients were fever 9 (100%), headache 9 (100%), joint pain 9 (100%), back pain 9 (100%), loss of appetite 9 (100%), sweating 9 (100%), chill 9 (100%), vomiting 1(11.1%), skin rash 3 (33.3%) and bleeding 0 (0.0%) and Poly arthralgia 8 (88.9). There was no a statistically significant difference between symptoms and viral infection (Table 2).

Patients not used mosquito net, from family with same signs and past infection were a statistically significant differences with ZIKV infection ((X^2 = 4.1, P = .008), (X^2 = 8.1, P = .011), (X^2 = 9.4, P = .014)) respectively (Table 2).

In terms of haematological parameters in this study showed normal and abnormal findings, commonly abnormal was low (thrombocytopenia, leucopenia, low RBCs), there was no a statistically significant differences with ZIKV infection (Table 3).

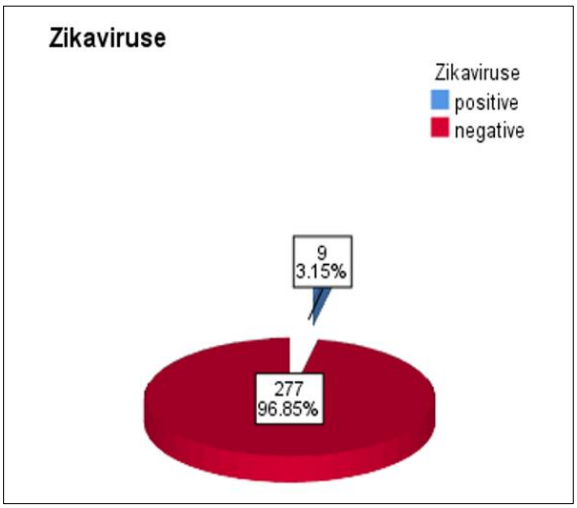

Figure 1: Frequency and percentage of positive and negative ZICKV

Table 1: Association of Sociodemographic data with ZIKV infection among patients

Viruses		**ZIKAV**				
Variables	**Types**	**Positive N(100%)=9**	**Negative N(100%)=277**	**Total 286(100%)**	**X^2**	**P**
Sex	Males	2(22.2)	144(52)	146(51)	3.1	.1
	Females	7(77.8)	133(48)	140(49)		
Age group	0-1	0(0)	3(1.1)	3(1)	48.3	.003
	1-10	0(0)	11(4)	11(3.8)		
	10-20	2(22.2)	37(13.4)	39(13.6)		
	20-30	1(11.1)	89(32.1)	90(31.5)		
	30-40	0(0)	82(29.6	82(28.7)		
	40-50	5(55.6)	34(12.3)	39(13.6)		
	50-60	0(0)	12(4.3)	12(4.2)		
	60-70	0(0)	9(3.2)	9(3.1)		
	70-80	1(11.1)	0(0)	1(0.3)		

Viruses		ZIKAV				
Variables	**Types**	**Positive N(100%)=9**	**Negative N(100%)=277**	**Total 286(100%)**	**X^2**	**P**
Educational Level	Illiterate	3(33.3)	30(10.8)	33(11.5)	7.7	.029
	Primary	1(11.1)	62(22.4)	63(22)		
	Secondary	5(55.5)	104(37.5)	109(38.1)		
	University	0(0)	81(29.2)	81(28.3)		
Residence	Rural	0(0)	41(14.8)	41(14.3)	1.6	.367
	Urban	9(100)	236(85.2)	245(85.7)		
Occupation	Selfemployed	2(22.2)	112(40.4)	114(39.9)	2.7	.608
	Housewife	5(55.6)	94(33.9)	99(34.6)		
	Student	1(11.1)	51(18.4)	52(18.2)		
	Farmer	0(0)	4(1.4)	4(1.4)		
	Others	1(11.1)	16(5.8)	17(5.9)		
Marital status	Single	1(11.1)	86(31)	87(30.4)	1.6	.201
	Married	8(88.9)	191(69)	199(69.6)		

Table 2: Associations of Symptoms data with ZIKA infection among patients

Viruses		ZIKV				
Variables	**Types**	**Positive N(%)=9**	**Negative N(%)=277**	**Total 286(100%)**	**X^2**	**P**
Symptoms						
Headache	Yes	9(100)	275(99.3)	284(99.3)	.07	1.00
	NO	0(0)	2(.7)	2(.7)		
Fever	Yes	9(100)	277(99.3)	286(100)	A	A
	NO	0(0)	0(0)	0(0)		
Joint pain	Yes	9 (100)	266(96)	275(96.2)	.372	1.000
	NO	0(0)	11(4)	11(3.8)		
Vomiting	Yes	1(11.1)	5(1.8)	6(2.1)	3.6	.176
	NO	8(88.9)	272(98.2)	280(97.9)		
Back pain	Yes	9(100)	275(99.3)	284(99.3)	.07	1.00
	NO	0(0)	2(.7)	2(.7)		
Skin rash	Yes	3(33.3)	52(18.8)	55(19.2)	1.9	.381
	NO	6(66.7)	225(81.2)	231(80.8)		
Loss appetite	Yes	9(100)	265(95.7)	274(95.8)	.41	1.00
	NO	0(0)	12(4.3)	12(4.2)		
Sweating	Yes	9(100)	253(91.3)	262(91.6)	.85	.181
	NO	0(0)	24(8.7)	24(8.4)		
Chills	Yes	9(100)	275(99.3)	284(99.3)	.07	1.00
	NO	0(0)	2(.7)	2(.7)		
Bleeding	Yes	0(0)	19(6.9)	19(6.6)	. 7	1.00
	NO	9(100)	258(93.1)	267(93.4)		
Polyarthraglia	Yes	8(88.9)	168(61.6)	176(61.5)	2.9	.16
	NO	1(11.1)	109(39.4)	110(38.5)		
Mosquito net use	Yes	6(66.7)	246 (88.8)	252(88.1)	4.1	.08
	NO	3(33.3)	31(11.2)	34(11.9)		
Mosquito control	Yes	0(0)	5(1.8)	5(1.7)	.17	1.000
	NO	9(100)	272(98.2)	281(98.3)		
Disease awareness	Yes	0(0)	8(2.9)	8(2.8)	.26	1.000
	NO	9(100)	269(97.1)	278(97.2)		
Family with same signs	Yes	6(66.7)	68(24.5)	74(25.9)	8.1	.011
	NO	3(33.3)	209(75.5)	212(74.1)		
Past infection	Yes	4(44.4)	30(10.8)	34(11.9)	9.4	.014
	NO	5(55.6)	247(89.2)	252(88.1)		

Table 3: Associations of Hematological and biomarkers data with ZIKV infection among patients

Viruses		ZIKV				
Variables	**Types**	**Positive N(%)=9**	**Negative N(%)=277**	**Total 286 (100%)**	**X^2**	**P**
CRP	Normal	0(0)	16(5.8)	16(5.6)	.55	1.00
	Abnormal	9(100)	261(94.2)	270(94.4)		
ESR	Normal	8(88.9)	168(61.6)	176(61.5)	2.9	.16
	Abnormal	1(11.1)	109(39.4)	110(38.5)		
Hb	Normal	5(55.6)	165(59.6)	170(59.4)	.0584	1.00
	Abnormal	4(44.4)	112(40.4)	116(40.6)		
Abnormal	Low	4(44.4)	103(37.2)	107(37.4)	.44	. .802
	High	0(0)	9(3.2)	9(3.1)		
RBCs	Normal	7(77.8)	224(80.9)	231(80.8)	.054	.685
	Abnormal	2(22.2)	53(19.1)	55(19.2)		
	Low	2(22.2)	50(18.8)	52(18.2)	.191	.909
	High	0(0)	3(1.1)	3(1)		
TWBCs	Normal	4(44.4)	150(54.2)	154(53.8)	.33	.51
	Abnormal	5 (55.6)	127(45.8)	132(46.2)		
Abnormal	Low	3 (33.3)	86(31)	89(31.1)	.43	.803
	High	2(22.2)	42(15.2)	44(15.4)		
Lymphocyte	Normal	5(55.6)	113(40.8)	118(41.3)	.78	.5
	Abnormal	4(44.4)	164(59.2)	168(58.7)		
Abnormal	Low	1(11.1)	110(39.7)	111(38.8)	3. 7	.205
	High	3(33.3)	53(19.1)	56(19.6)		
Platelets	Normal	4(44.4)	153(55.2)	157(54.9)	.410	.736
	Abnormal	5(55.6)	124(44.8)	129(45.1)		
Abnormal	Low	5(55.6)	119(43)	124(43.4)	.66	.717
	High	0(0)	5(1.8)	5(1.7)		
Neutrophiles	Normal	4(44.4)	97(30.1)	101(35.3)	.339	.725
	Abnormal	5 (55.6)	180(65)	185(64.7)		
Abnormal	Low	1 (11.1)	26(9.4)	27(9.4)	.44	.801
	High	4(44.4)	154(55.6)	158(55.2)		
MCV	Normal	9(100)	228(82.3)	237(82.9)	.92.23	.366
	Abnormal	0(0)	49(17.7)	49(17.1)		
Abnormal	Low	0(0)	40(14.7)	40(14.4)	1.92	.383
	High	0(0)	9(3.2)	9(3.1)		
MCH	Normal	7(77.8)	145(52.3)	152(53.1)	.262	.181
	Abnormal	2(22.2)	131(47.3)	134(46.9)		
Abnormal	Low	2(22.2)	131(47.3)	133(46.5)	2.48	.289
	High	0(0)	4(1.4)	4(1.4)		
MCHC	Normal	2(22.2)	86(31)	88(30.8)	.62	.726
	Abnormal	7(77.8)	191(69)	198(69.2)		
Abnormal	Low	7(77.8)	186(67.1)	193(67.5)	.529	.768
	High	0(0)	5(1.8)	5(1.7)		

DISCUSSION

A total of 286 participants from Kassala State, Eastren Sudan, were included in this study, RT-PCR investigate 9 patients were positive for ZIKV.

The aim of study to assess epidemiological and molecular detection of ZIKV in Kassala State, Eastern Sudan and to determine it's association with demographic, hematological and clinical parameters.

A study by Mota *et al*., (2021) studied 182 samples and observed 35 cases (19.23%) were positive for ZIKV analyzed by RT-QPCR. The frequency of positive ZIKV in comparison with participants is unlike our study. Carrillo-Hernández *et al*., (2018) from 157 patients found 29 patients positive for ZIKV. Study by Marinho *et al*., (2020) tested 102 samples for Arbovirus, they did not detect ZIKV, Silva *et al*., (2019) studied of 948 participants, 247 (26.1%) positive for viruses, of which ZIKV was 13 (1.4%). Some studies have found that the frequency has increased. This may be due to travellers from active transmission areas who came to the area of study, sexual transmission, outbreaks, and the spread of mosquitos. The low frequency of ZIKV infection in studies perhaps attributed to asymptomatic patients infected with ZIKV.

In the present study the typical age group positive for ZIKV was 40-50 years, and ZIKV cases showed a statistically significant association difference between age and infection ($X^2 = 48.3$, P = .003). Like our study, Carrillo-Hernández *et al*., (2018) observed typical mean age for ZIKV and a statistically significant association difference between age and infection. Also the females more affected than males.

Like our result Farias *et al.,* (2023) observed ZIKV cases mainly in females but disagree in age group from the 0–9 and 20–39 years. Many studies reported females affected more than males, this findings need concern about this population.

The residence, occupation, and marital status also showed no a statistically significant differences between these variables and ZIKV while the education level was a statistically significant differences, meta-analysis study by Power *et al*., (2022) indicated weak or no statistical evidence of an association between education and Arboviral infection, also evidence that having been married, including currently or previously (ie, divorced or widowed), was associated with an increase infection risk.

The symptoms in patients were fever 9(100%), headache 9(100%), joint pain 9(100%), back pain 9(100%), loss of appetite 9(100%), sweating 9(100%), chill 9(100%), vomiting 1(11.1%), skin rash 3(33.3%) and bleeding 0(0.0%) and Poly arthralgia 8(88.9).. Mota *et al*., (2021) observed symptoms of fever (82.85%), exanthema (77.14%), headaches (77.14%), myalgia (71.42%), arthralgia (54.28%) and pruritus (54.28%) for patients positive ZIKV similar to present study in some symptoms like fever and headache. Jimenez *et al*., (2016) noted the clinical features in the 93 cases were fever (96.6%), rash (93.3%), non-purulent conjunctivitis (88.8%), headache (85.4%), and myalgia (84.3%). Like our study there were no a statistically significant differences between symptoms and viral infection.

Patients not used mosquito net, from family with same signs and past infection were a statistically significant differences with ZIKV infection, its normally to report patients not used mosquito net increase incidence of infection due to increase mosquito bites and transmission infection so the prevention and control important to control disease. Past infection increase incidence to disease un less develop antibodies. UN like our study public health reported antibodies from past infection protects people against the disease, as they stop the virus from entering our cells.

The haematological parameters in this study showed normal and abnormal, the most abnormal was low, similar findings by Brasil *et al*., (2016) observed the leucocyte count in ZIKV patients was decreased but disagree in platelet count was normal because our study detected 55.6 had thrombocytopenia.

Prevention and control measures take place to reduce vector density, mosquito control, are essential, habitat control involves draining swamps and removing other pools of stagnant water, take personal protective measures seriously.

CONCLUSION

The frequency of ZIKV was 3.15%, the females infected were higher than males ($X^2 = 3.1$, P = 0.1) but no a statistically significant difference between gender and ZIKV.

The common age group positive for ZIKV was 40- 50 year age group ZIKV cases showed a statistically significant difference in age.

Fever, headache, back pain, loss of appetite, sweating and chills were reported in all patients but there were no a statistically significant differences between these symptoms and ZIKV infection.

The residence, occupation, and marital status showed no a statistically significant differnces between these variables and positive ZIKV patients, the educational level was a statistically significant difference ($X^2 = 7.7$, P = .029).

Patients not used mosquito net, from family with same signs and past infection were a statistically significant differences with ZIKV infection (($X^2 = 4.1$, P = .008), ($X^2 = 8.1$, P = .011), ($X^2 = 9.4$, P = .014)) respectively.

The haematological parameters in this study showed normal and abnormal, the most abnormal was low (thrombocytopenia, leucopenia, low RBCs), there was no a statistically significant differences with virus.

Recommendation

Further studies with a larger sample size are warranted. A meta-analysis should be conducted to compile data from all previous ZIKV studies in Sudan, encompassing screenings from both eastern and western regions in Sudan.

Vector control measures, especially mosquito control, are essential, habitat control involves draining swamps and removing other pools of stagnant water, take personal protective measures seriously.

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