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# Prevalence of dengue fever virus antibodies and associated risk factors among residents of El-Gadarif state, Sudan

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## Abstract

**Background:** Dengue fever, caused by dengue virus (DENV), has become one of the most important mosquito-borne viral diseases with a steady rise in global incidence, including the Sudan. Sporadic cases and frequent acute febrile illness outbreaks, compatible with Dengue fever, have been reported in El-Gadarif State, Sudan. However, diagnosis was based almost exclusively on clinical signs without confirmatory laboratory investigations. Despite the magnitude of the problem in El-Gadarif State, no information is currently available with regard to the epidemiology of the disease in this State. El-Gadarif State is one of the largest commercial centers in the Sudan. The objective of the present investigation is to estimate the prevalence of DENV antibodies, and determine the potential risk factors associated with seropositivity among residents of El-Gadarif State.

**Methods:** A cross sectional study was conducted in a total of 701 residents randomly selected from all 10 localities in El-Gadarif State. The sera from the 701 residents were tested for the presence of DENV-specific immunoglobulin G (IgG) antibodies using a commercially available Anti-dengue IgG enzyme-linked immunosorbent assay (ELISA).

**Results:** Among the 701 residents, 334 residents (47.6%) were seropositive for DENV. Mosquito control (OR = 2.73, CI = 1.37–5.87,  $p$ -value = 0.001); low income (OR = 2.31, CI: 1.71–6.36,  $p$  value = 0.032); sleeping out-doors (OR = 3.73, CI = 2.63–6.23,  $p$ -value = 0.013), and localities were determined as potential risk factors for contracting DENV infection.

**Conclusions:** The prevalence rate of DENV antibodies among residents of El-Gadarif State is significantly high (47.6%). Further epidemiologic studies including, distribution of mosquito vectors and implementation of improved surveillance are urgently warranted for better prediction and prevention of a possible DENV outbreak in El-Gadarif State, Sudan.

**Keywords:** Epidemiology, Survey, Dengue fever, ELISA, Sudan

## Background

Dengue fever is a cosmopolitan mosquito-borne viral disease, and is the most important human arbovirus disease [1]. Dengue fever is caused by dengue virus (DENV), a member of the genus *Flavivirus* of the family *Flaviviridae*. The virus is transmitted by the infected female of the primary vector *Aedes aegypti* mosquitoes [2]. Infections can also be transmitted through blood

transfusion, organ transplantation and possibly vertically from mother to child [3–7]. DENV infections often result in an acute, self-limiting, viral disease. However, the infection can be devastating and progresses to a fatal clinical disease characterized by substantial increase in vascular permeability resulting in shock [3]. Currently, DENV infection is increasingly recognized as one of the world's emerging infectious diseases [8]. The detection of DENV-specific antibodies and circulation of the virus in infected people is well documented along the Red Sea coast of Eastern Sudan [9–13]. Recently, Kordufan region in western Sudan has witnessed several dengue fever outbreaks due to social unrest and war in the region [14, 15].

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In the Sudan, the first outbreak of DENV was reported in 1986 among febrile patients in the Red Sea State, and DENV serotypes 1 and 2 were identified as the causative agents of the outbreak [16]. Serologic surveys have detected DENV antibodies in humans from different States of the Sudan, including Port Sudan, Kassala, Khartoum, and Kordufan [16–18]. A recent seroepidemiologic survey reported high prevalence of DENV-specific IgG among febrile patients in Kassala State in Eastern Sudan, suggesting significant circulation of DENV in the area, instead of being restricted to a particular point in the past [19, 20]. The recently reported high prevalence of DENV-specific antibodies in Kassala State was attributed to the constructed irrigated agricultural schemes and development of extensive urbanization. The presence of DENV-specific antibodies, and subsequent recovery of several DENV serotypes from infected patients have been reported in various regions of the Sudan [16–22]. The high prevalence of Dengue in the different states of the Sudan made its control a top priority. Epidemiologic studies, including implementation of improved surveillance, are extremely warranted and urgently needed for better prediction of the prevalence of this important arbovirus pathogen in the study area. The increasing incidence of dengue outbreaks in the Sudan warranted the current study to provide some information about the epidemiology of the disease in the study area. Dengue has become of great concern because of the frequent occurrence of sporadic cases and outbreaks among residents of urban areas [1, 3, 8]. Except for the DENV studies conducted along the Red Sea borders, very little information is available about the epidemiology and disease potential in the Sudan. The current investigation was conducted to elucidate knowledge on the epidemiology of the disease, and determine the risk factors associated with the prevalence of DENV seropositivity among residents of El-Gadarif, one of the largest commercial State in the Sudan.

The objective of the present study is to estimate the prevalence of dengue fever by the detection of DENV-specific Ig G antibodies, and determine the potential risk factors associated with the infection with the DENV arbovirus among residents of El-Gadarif State, Sudan. It is quite probable that this study would assist in reducing the impact of infection on the livelihood of urban communities and prevent a possible DENV outbreak of the disease among residents in the study area of El-Gadarif State, eastern Sudan.

## Methods

### Study area

This community-based, cross-sectional study was conducted in the urban area of El-Gadarif, one of the largest commercial States of the Sudan, during the period between August, 2016 and May, 2017. The state is

characterized by vast areas of landscape suitable for agriculture, mainly depending on rainfall irrigation. The state is a strategic center that has the largest market for sesame and sorghum crops, and is considered as one of the pillars for food security, and a source of national economy in the Sudan; being the second largest commercial State in the country. El-Gadarif has a total population of 1,400,000 and covers an area of approximately 75, 263 km<sup>2</sup>. The State is located between latitudes 14° 02' - 5° 75' N and longitudes 35° 36' - 23° 03.8' E in the semi-desert tropics. The climate is hot and rainy in summer and cold in winter. The rainy season extends for 4 months, with an average annual rainfall of 700 to 900 mm. In the rainy season, large pools of water and green meadows with various types of *acacia* trees cover the land. The average temperature ranges from 21 °C in winter and 47 °C in summer. The mean annual evaporation rate is 7.7 mm/day, and the average relative humidity ranges from 21 to 44%. The localities included in the current investigation in El-Gadarif State are shown in Fig. 1.

### Method of the study

A cross sectional study was conducted to estimate the prevalence rate of DENV-specific IgG antibodies and determine the potential risk factors associated with the disease. As shown in Fig. 1 - El-Gadarif State has 10 localities, which include; Al-Showak (Al-Fashaga), El-Fau (El-Fau), Center Gadaref, El-Sobak (Butana), (Gadaref) El-Gadarif City, El-Hawatah (Al- Rahad), Kassab (Al-Gadaref Western Locality, Gala El-Nahal (Gala El-Nahal), Doka (Bassunda), and Al-Qurayshah locality (Al-Qurayshah).

A total of 701 randomly selected participants from all 10 localities were included in the current investigation using the multistage probability sampling method. Briefly, all localities of El-Gadarif state were considered in the study. Two administration units were selected randomly from each locality. Five villages were selected from each administrative unit, which accounts for 10 villages per locality. Finally, 70 participants were selected from each of the 10 villages using simple random sampling. Thus, 700 samples were collected from the 10 localities of El-Gadarif State. However, it should be noted that an additional blood sample was collected from El-fashaga locality, which resulted in a total number of 701 blood samples. All samples were collected using simple random sampling method. The participants included in this study were adults of over 18 years old.

### Study inclusion and exclusion criteria

We included in the study any individual aged ≥5 years old. The participants were members of a randomly selected household and residents in El-Gadarif State, with



**Fig. 1** A map showing the localities included in the study area of El-Gadarif State, Sudan

or without symptoms. We excluded from the study those who have history of travelling to endemic area within last 2 weeks.

**Sample size**

The prevalence of Dengue fever in Elgadarif State is unknown, therefore we assumed the.

seroprevalence of dengue antibodies to be 50%. The required sample size at 95% confidence and 50% prevalence and 0.04 absolute precision was calculated to be 600. A design effect of 2 and a non-response rate of 10% were considered to adjust for the sampling technique. The formula for the calculation of the sample size was applied as described by Martin et al., [23].

**Ethics approval and consent to participate**

The ethical clearance for this study was obtained from the Ethics committee, Deanship of Scientific Research, Al-Neelain University, Sudan. All participants were provided with a written consent for the purpose of this study. The study objectives were explained to all

participants before obtaining the informed consent. The structured questionnaire was employed to collect the risk factor information.

**Questionnaire**

The collection of required data was made possible using a structured questionnaire through. Interviews were conducted for all participants from all above mentioned 10 localities. Interviewers were trained before conducting the survey to ensure that the questionnaires were well understood by the surveyors, avoiding the differences in the definitions and interpretations of the concepts used. All participants included in this study responded to the questionnaire, which covers socio-demographic characteristics including; age (young age,  $\geq 5$  and  $< 18$  years-old; old age  $> 18$ ); gender (male and female); disease awareness (yes, no); work (employed, unemployed); education (illiterate, primary, secondary, University); household income which includes, low income (less than 1,800 Sudanese Ginah (SDG), medium income (more than 1800 but less than

3000 SDG) and high income (more than 3000 SDG); presence of clean water containers (yes, no), mosquito vector (present or absent), mosquito control (practiced or not practiced).

### Laboratory investigations

Blood samples were collected from the participants, and sera were separated and kept frozen at  $-20^{\circ}$  C until used for the detection of DENV-specific IgG antibodies by the enzyme-Linked Immunosorbent Assay (ELISA). The ELISA assay was performed using a commercially available non structural protein 1 (NS1) DENV ELISA Kit (Euroimmun AG, Luebeck, Germany), in accordance with the manufacturer's specifications. The test samples were considered positive if the optical density was  $\geq 50\%$  of the mean of the negative controls.

### Statistical analyses

Statistical package for social sciences (SPSS) software package for window (version 21.0) was employed to enter the data in the computer. The univariable analysis using Chi-square ( $\chi^2$ ) test was used to determine the associations between the outcome variable (status of dengue seropositivity) and its potential risk factors. Significant association between dengue fever and risk factor was initially considered when  $P$  value  $< 0.25$  (two tailed;  $\alpha = 0.25$ ). The results of the univariable analysis were further subjected multivariable analysis using logistic regression. Results were expressed as odd ratios (OR) with 95% confidence intervals (C.I) for each risk factor.  $P$  value less than 0.05 ( $p < 0.05$ ) represents significant association between the dengue fever seropositivity and associated risk factors [24].

### Results

DENV-specific IgG antibodies were detected in 334 out of 701 participants as determined by the competitive ELISA. This study recorded a prevalence rate of 47.6% for DENV antibodies among residents of El-Gadarif State. The highest and lowest rates of DENV seropositivity were 65.7 and 22.9%, respectively, which were recorded in Bassunda and Al-Qurayshah localities. Seven risk factors with  $p$ -value  $< 0.25$  (two tailed;  $\alpha = 0.25$ ) were reported to be significantly associated with DENV-antibodies as calculated in the  $\chi^2$  test. These risk factors included locality ( $p$ -value = 0.001), age ( $p$ -value = 0.123), gender ( $p$ -value = 0.145), income ( $p$ -value = 0.039), sleeping out-doors ( $p$ -value = 0.013), mosquito nets ( $p$ -value = 0.112), and mosquito control practice ( $p$ -value = 0.001). The results of the univariate analysis are presented in (Table 1). The significant results of univariate analysis were further analyzed in the final model of multivariate analysis using logistic regression to exclude confounding factors. The final model of DENV

infection indicated that four potential independent risk factors were statistically significant. These potential risk factors included, mosquito control practice (OR = 2.73, CI = 1.37–5.87,  $p$ -value = 0.001); Low income (OR = 2.31, CI: 1.71–6.36,  $p$  value = 0.032); sleeping out-doors (OR = 3.73, CI = 2.63–6.23,  $p$ -value = 0.013), and localities. The six localities associated with high DENV seropositivity included Bassunda (OR = 4.09, CI: 1.35–6.91,  $p$  value = 0.007); Gadarif (OR = 2.42, CI: 1.94–4.99,  $p$ -value = 0.013), El-Rahad (OR = 2.51, CI = 1.61–5.54,  $p$ -value = 0.015); West Gallabat (OR = 2.13, CI = 1.05–4.31,  $p$ -value = 0.036); East Gallabat (OR = 2.73, CI = 1.30–5.71,  $p$ -value = 0.004); and El-Fashaga (OR = 3.27, CI = 1.58–6.76,  $p$ -value = 0.001). The significant association between DENV seropositivity and potential risk factors in the final model are shown in (Table 2). Age, gender, work, education, water containers, and mosquito vector were not significantly associated with DENV seropositivity.

### Discussion

Dengue is a mosquito-borne virus is of high morbidity rate causing economic losses in many tropical and subtropical regions of the world [25–27]. Dengue virus (DENV) is rapidly spreading as the result of urbanization, climatic changes and increased human movements. DENV has emerged as the most common vector-borne viral infection in the current century. The virus occurs primarily in rural areas, but has recently become of urban distribution due to development of extensive urbanization in rural areas [28]. In addition, dengue was also reported in many African countries neighboring the Sudan including, Southern Sudan; Ethiopia, Eritrea, Uganda, Kenya, Democratic Republic of Congo, Egypt and Libya [29]. In recent years, the distribution and nature of DENV and other hemorrhagic fever viruses including, Rift Valley fever virus (RVFV) and Crimean Congo hemorrhagic fever virus (CCHFV) have changed substantially [30, 31]. In previous epidemiological surveys, high prevalence rates (71.7%) of DENV seropositivity were reported among residents of Kassala [11]. Other studies reported prevalence rates of 9.4% in the same state [9, 19]. In the Red Sea State of eastern Sudan, the prevalence rate was reported to be 12.8% among pregnant women [10]. In the present study, the prevalence of DENV-specific antibodies in residents of El-Gadarif State was estimated to be 47.6%, which is significantly high among the population of the state.

The high sero-prevalence rate could be attributed to the newly constructed irrigation projects and agricultural schemes in this state, which provides a suitable habitat for the survival of the adult and larval stages of *Aedes* vectors in this region. In addition, the heavy rainfall in

**Table 1** Univariate analysis for the association between potential risk factors and DENV infection among residents in El-Gadarif State, Sudan, using chi-square test

Risk factors	Cases tested	Cases affected (%)	df	$\chi^2$	<i>p</i> -value
<b>Locality</b>					
Gadaref	70	21 (30%)	9	59.5	0.001
Center Gagarif	70	37 (52.9%)			
Butana	70	19 (27.1%)			
E Ifau	70	37 (52.9%)			
Al Rahad	70	41 (58%)			
Bassunda	70	46 (65.7%)			
West Galabat	70	34 (48.6%)			
East Galabat	70	39 (55.7%)			
Qurayshah	70	16 (22.9%)			
Elfashaga	71	44 (62%)			
<b>Age</b>					
young	176	91 (51.7%)	1	1.55	0.123
Old	525	243(46.3%)			
<b>gender</b>					
female	282	127(45%)	1	1.29	0.145
male	419	207(49.4%)			
<b>Education</b>					
illiterate	186	90(48.4%)	4	2.02	0.732
primary	154	75(48.7%)			
secondary	199	95(47.7%)			
university	107	45(42.1%)			
informal study	55	29(52.7%)			
<b>Income</b>					
low	489	245(50.1%)	2	6.4	0.039
medium	153	59(38.6%)			
high	59	30(50.8)			
<b>Work</b>					
unemployed	345	166(48.1%)	1	0.06	0.806
employed	356	168(47.2%)			
<b>Disease awareness</b>					
no	645	308(47.8%)	1	0.03	0.849
yes	56	26(46.4%)			
<b>Sleeping outdoor</b>					
no	324	138(42.6%)	1	6.16	0.013
yes	377	196(52%)			
<b>Mosquito net use</b>					
No	400	201(50.2%)	1	2.52	0.112
yes	301	133(44.2%)			
<b>Presence of Clean water container</b>					
No	31	12(38.7%)	1	1.03	0.308
Yes	670	322(48.1%)			

**Table 1** Univariate analysis for the association between potential risk factors and DENV infection among residents in El-Gadarif State, Sudan, using chi-square test (*Continued*)

Risk factors	Cases tested	Cases affected (%)	df	$\chi^2$	<i>p</i> -value
<b>Mosquito control</b>					
No	313	126(40.3%)	1	12.38	0.001
yes	388	208(53.6%)			

this State was suggested as a contributory factor to high prevalence of the disease. The wide circulation of DENV in El-Gadarif State and the risks that these virus serotypes pose on the health and welfare of the residents of this State warrant an extensive and improved surveillance system for this arbovirus pathogen in the Sudan.

In the present study area (El-Gadarif State), DENV seropositivity increased among low-income participants. Low income residents are at 2 fold risk of contracting DENV infection compared to high income residents (OR = 2.31, CI: 1.71–6.36, *p* value = 0.032). The neighborhood in which people had a lower socio-economic status (unemployment), had higher dengue prevalence rates, suggesting that the socio-economic status of the population is a potential risk factor for dengue prevalence. This could be attributed to the fact that unemployment may pose a lifestyle behavior that provides a suitable habitat for the breeding of the mosquito vector, thus increasing the risk of mosquito contact. Sleeping out-doors was also recorded

**Table 2** Multivariate analysis using logistic regression model for significant association (*p* > 0.05) between risk factors and DENV seropositivity among residents in El-Gadarif State, Sudan

Risk factors	OR	95%CI	<i>P</i> -Value
<b>Mosquito control</b>			
yes	Ref		
no	2.73	1.37–5.87	0.001
<b>Sleeping out-doors</b>			
Yes	Ref		
No	3.75	2.63–6.23	0.013
<b>Income</b>			
high	Ref		
low	1.61	1.71–6.36	0.032
<b>Locality</b>			
Elguresha	Ref		
Gadarif	2.42	1.94–4.99	0.013
Elrahad	2.51	1.16–5.54	0.015
Bassunda	4.09	1.35–6.61	0.007
West Galabat	2.13	1.05–4.31	0.036
East Glatat	2.73	1.30–5.71	0.004
Elfashaga	3.27	1.58–6.76	0.001

as a potential risk factor for contracting DENV infection. Residents sleeping out-doors were at approximately 4 fold risk of becoming infected with DENV (OR = 3.73, CI = 2.63–6.23,  $p$ -value = 0.013), due to the increased contact and bites of the infected mosquito vector (*Aedes aegypti*) causing DENV infection. There was also association between DENV seropositivity and the six localities of El-Gadarif states including, Bassunda (OR = 4.09, CI: 1.35–6.91,  $p$  value = 0.007); Gadarif (OR = 2.42, CI: 1.94–4.99,  $p$ -value = 0.013), El-Rahad (OR = 2.51, CI = 1.61–5.54,  $p$ -value = 0.015); West Gallabat (OR = 2.13, CI = 1.05–4.31,  $p$ -value = 0.036); East Gallabat (OR = 2.73, CI = 1.30–5.71,  $p$ -value = 0.004); and El-Fashaga (OR = 3.27, CI = 1.58–6.76,  $p$ -value = 0.001). This is probably due to high rain fall in these localities, which provide suitable habitat for the mosquito vector, which transmits the disease. Residents of Bassunda locality are at 4 fold risk of becoming infected with DENV compared to other localities, suggesting increased endemicity of this locality with DENV infection. There was strong association between DENV seropositivity and routine mosquito control practice. Application of mosquito control measures, such as spraying with insecticides protects against DENV infection and decreased the DENV seropositivity by approximately 3 folds (OR = 2.73, CI = 1.37–5.87,  $p$ -value = 0.001). It is, therefore, recommended that routine application of insecticides or insect repellents should be considered for the prevention of DENV infection. It is worth mentioning that the development of a vaccine against DENV infection is in progress but has not yet been produced on commercial basis [32, 33]. Therefore, vector control by residual spraying of houses with insecticides, education and extension programs for public awareness about dengue and its prevention or reduction of its prevalence are considered as important components in the prevention of DENV infection. Monitoring and elimination of mosquito breeding sites is an important preventive measure that should also be considered. The results obtained in the current investigation are highly suggestive that during or before dengue epidemics; rigorous efforts are to be directed towards neighborhoods of lower socioeconomic status, and/or areas with large vegetation coverage around individual houses.

The risk assessment studies indicated that there was no significant association between DENV seropositivity and the rest of the risk factors including, work, education, water containers, mosquito vector (present or absent), included in the study. It is worth mentioning that gender has no significant difference for DENV seropositivity, and both sexes are equally susceptible to infection with DENV. Likewise, there was

no significant difference related to age of the participants, suggesting that all ages are susceptible for DENV infection. Lack of sufficient awareness of dengue may have contributed significantly to the undermining of the danger of the disease by health care workers. In addition, dengue is not a reportable disease in the Sudan, and its surveillance and diagnosis are not widely or consistently implemented throughout the country. Moreover, reports for surveillance and other research activities pertaining to dengue in Africa are also limited and are not always available [29]. In the Sudan, acute febrile diseases of unknown etiology are very common, particularly in the urban areas, and malaria is usually considered to be the primary cause [34–37]. In Sudan and most African countries, dengue virus infection is not enlisted among the differential diagnosis of the acute febrile diseases. It is, therefore, recommended that the practicing physicians in rural hospitals and clinics of El-Gadarif State should consider DENV in their differential diagnosis in patients showing symptoms indicative of acute febrile illness or hemorrhagic fevers.

**Limitation of the study:** In the present study, important information regarding the prevalence and risk factors of the disease was made available for the first time in Elgadarif state. However, one of the limitations of the study is reflected by the fact that the participants were selected regardless of the presence of an active infection or clinical symptoms of the disease, which makes it difficult to differentiate between primary and secondary dengue infections. In addition, seropositivity was assessed by detecting dengue IgG antibody levels. It is well documented that detection of Ig G is useful in an epidemiological survey to identify previous or past infections. Therefore, detection of recent Dengue virus infection is likely to be missed by the described ELISA assay. Detection of recent infection necessitates the screening for Ig M antibodies. Moreover, Household density and screens in the window were not added in the model. The household density is always associated with poverty and low socioeconomic status, particularly in developing countries, such like Sudan, which could be considered as another social predictor for dengue infections. In this study, we have included socioeconomic status but household density and screens in the window were not included in the model. It is recommended that additional models such as house hold density and window screens should be included in a future study plan as they are expected to be potential risk factors for contracting dengue infections. Furthermore, virus isolation attempts and subsequent sequencing of the serotypes responsible for the secondary and severity of the infection have not been assessed. Further study should be conducted in the future to overcome this limitation of the study.

## Conclusions

In conclusion, the results obtained from the current study were indicative of circulation of DENV in El-Gadarif State, Sudan, and that the residents of the State are at risk of developing the disease. The prevalence of Dengue fever is significantly high (47.6%) among residents of this State. Mosquito control, socio-economic status (income), sleeping outdoors and localities were recorded as potential risk factors for contracting Dengue fever. However, outbreaks of dengue hemorrhagic fever have not yet been reported in this state of the Sudan. The specific DENV serotypes circulating in the area remain to be identified. Virus isolation attempts and subsequent molecular characterization studies (viral genome sequencing and phylogeny) are deemed necessary for tracing the movement of the virus in the region and to determine the genetic lineages of DENV serotypes circulating in Sudan. Surveillance for Dengue among residents of the State and the distribution of mosquito vectors should continue for better understanding of the epidemiology of the disease, and to provide public health authorities an opportunity for anticipation and preparation for a possible DENV outbreak in El-Gadarif State, Sudan.

## Abbreviations

CI: Confidence interval; DEN: Dengue; DEN-V: Dengue virus; ELISA: Enzyme-linked immunosorbent assay; HRP: Horse radish peroxidase; Ig G: Immunoglobulin G; OR: Odd ratio; RT-PCR: Reverse transcriptase polymerase chain reaction; MI: Microliter

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## Availability of data and materials

Data and materials are available upon request from the corresponding author.

## Authors' contributions

MHE, GKA, RAB, FK, IAA helped with the collection of blood samples, extracted the DNA, optimized the polymerase chain reaction-based detection assay, editing of sequences and helped with the manuscript writing. OHO, MEA, SLB, analyzed the ELISA results; EMH, HAA, AEK, HAS, designed the experiment and helped with the preparation of the final manuscript. IEA designed the experiment, help with data analysis and prepared the final manuscript. All authors read and approved the final version of the manuscript.

## Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of, Al-Neelain University, Khartoum, Sudan. Participation was on a voluntary basis, and the residents were randomly selected and a written informed consent was obtained from all participants after explanation of the study objectives and prior to the study procedures and blood collection. The risk factors information was obtained from the residents through the structured questionnaire form, permitting the use of their blood samples for diagnostic and research purposes.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests. The result of this study does not reflect the opinion of the funding sources. All authors have read and approved the final version of this manuscript.

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