ORIGINAL ARTICLE

SHORT-TERM OUTCOME OF CONVULSIVE STATUS EPILEPTICUS IN HOSPITAL ADMITTED CHILDREN: ETIOLOGY AND CLINICAL FEATURE IN NORTH-WEST ETHIOPIA

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ABSTRACT

Background: *Status epilepticus (SE) is a serious neurological problem in children. In sub-Saharan Africa, the high incidence of febrile illnesses and poor health care system influence the outcome of convulsive status epilepticus. However, in Ethiopia there have been few outcome studies of convulsive status epilepticus. Therefore, this study aimed to determine the etiology, clinical features and short-term outcomes of childhood convulsive status epilepticus in Ethiopia.*

Methods: *A cross sectional study based on hospital-based retrospective data from 2013 to 2018 was conducted. A total of 82 children diagnosed with epilepsy were included. The data were collected using a data extraction format and entered into SPSS version 20 for analysis. Descriptive, bivariate and multivariate analyses were applied.*

Results: *Eighty-two patients participated during the study period. Generalized tonic clonic seizure was the commonest type of seizure (86.6%). Pyogenic meningitis and malaria were the leading cause of status epilepticus accounting for 26.9% and 9.8% respectively. The proportion of good recovery was 61% (95% CI; 51.2 to 73%). In the multivariate analysis co-morbidity was found to have significant association with short-term treatment protocols (AOR=3.354; 95%CI: 1.018, 11.045).*

Conclusion*: Acute symptomatic status epilepticus was the common cause in all age groups and most patients arrived late for medical care. Co-morbidity was significantly associated with short-term treatment and poor outcomes. Hence, there is a need for education of caregivers and patients with co-morbidities and indications for extended care. In addition, early diagnosis and management of malaria and meningitis is important.*

Key words: *Status Epilepticus, Childhood, University of Gondar, Ethiopia*

BACKGROUND

Status epilepticus (SE) is a serious neurological problem in children. International Classification of Epileptic Seizures defines SE as a seizure that lasts for a sufficient length of time (30 minutes or longer) or is repeated frequently enough that the individual does not regain consciousness between seizures. It affects people of all ages, though it is more common and causes greater morbidity and mortality in infants. Age, etiology, and the duration of seizure activity correlates with mortality. In the studies that included both types of SE, Non-Convulsive Status Epilepticus NCSE only accounted for up to 6% of SE cases (1).

The overall annual incident of SE in the United States is approximately 110,000-160,000 people per year, or about 7% of all epilepsy cases per year. The most common etiologies include noncompliance

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with prescribed treatments, alcohol-related status epilepticus, drug toxicity, tumors, and trauma (2). The incidence of Convulsive Status Epilepticus (CSE) in London, UK, has been reported as 18–20 per 100 000 per year in children of less than 16 years old, with higher rates in the first few years of life (3). Another study also showed that among children admitted in PICU of Tampere university hospital of Finland, 65 (14%) had SE and the highest incidence was in the first years of life (4).

In a prospective study done in India, SE etiology was acutely symptomatic in 54% of all SE patients, remote symptomatic in 7% and cryptogenic in 19% of all SE patients. Of the acute symptomatic SE, central nervous system infections (CNS) were the provoking risk factor in 52% of patients and encephalitis accounted for 15% of etiology (5). Another prospective study in India also revealed that viral encephalitis was the most common etiology in the acute symptomatic group and constituted 33.3% of all the 70 patients included for the study. This was followed by pyogenic meningitis and tubercular meningitis which accounted for 21% and 15% of diagnoses respectively (6).

Seizures with fever are also common in children admitted to hospitals in sub-Saharan Africa, particularly in malaria-endemic areas where convulsive status epilepticus is well recognized. There are few published data on CSE in children living in Africa, despite the fact that the prevalence of epilepsy is higher than in Europe and North America (7). In a study in Kenya, among all 389 children admitted to Kilfil Health and Demographic Surveillance System (KHDSS), 27% were admitted with seizures. In this study a majority (65%) of the children had a positive malaria slide and malaria was the primary diagnosis for 206 (53%) children (3).

A cross-sectional study at Tikur-Anbesa hospital, Ethiopia indicated that time of arrival for treatment after seizure was less than 2 hours in 28.1% of cases. The most common seizure type observed was generalized seizure (74.2%). The most common cause of SE identified was idiopathic/cryptogenic 40.4% cases, followed by acute symptomatic 30.3%. Among the acute symptomatic cases, pyogenic meningitis was the most common, accounting for 20.2% of patients (8).

Most clinical series of SE involving children and adult shows a mortality rate of 6% to 18% and high incidence of neurological sequelae has been reported (9). Evidence also showed that Central Nervous System (CNS) infection is the leading cause of SE, especially in young age groups among which viral encephalitis, pyogenic meningitis, tuberculosis meningitis and cerebral malaria are the most common causes of SE (6). Ethiopia is one of the sub-Saharan countries with high incidence of infectious disease including malaria, meningitis, HIV, etc which all contribute to high incidence of SE. There is also a limitation in both diagnostic and management modalities of SE.

Gondar is one of the towns of Ethiopia where infectious disease such as malaria is common. This may lead to a high incidence of SE in Ethiopia. Despite this, there is only one study completed to date, which was done in Tikur-Anbessa hospital in Addis Ababa, Ethiopia. Therefore, this study aimed to assess the etiology, clinical features and short-term outcome of status epilepticus in children who were admitted to the University of Gondar (UOG) hospital in North-West Ethiopia.

METHOD

Study setting and period: The study was conducted at the University of Gondar referral hospital in North-West Ethiopia in 2018.

Study design: A hospital-based retrospective cross-sectional study was conducted.

Source and study population: Those children who visited UOG hospital with SE were the source population. Those children with SE admitted from October 2013 to October 2018 at any of pediatric wards fulfilling the inclusion criteria were the study population.

Inclusion criteria*:* Those patients aged 1 month to 18 years who were admitted to the pediatric emergency unit, pediatrics ward and pediatrics ICU with the diagnosis of SE in UOG hospital.

Exclusion criteria: Patients with incomplete chart records and diagnosis

Sample size determination: Since the proportion of CSE is not clearly known, a proportion of 50 % (p=0.5) was selected for the sample size calculation. Considering additional 5% for incomplete charts, the total estimated sample size was 403 patients. Since the study population was less than the computed sample size, all the 82 SE cases admitted at any pediatric ward of University of Gondar referral hospital from 2013 to 2018 that had complete records were considered for this study.

Variables of the study

Dependent variables: *Short-term outcome*

Independent variables

- Socio-demographic factors: Age, sex, address, the month of admission
- Clinical variables: Prior history of epilepsy, comorbidity, duration of seizure before treatment, types of seizure, level of consciousness, length of stay in the hospital
- Etiologic variables: Head trauma, brain tumor, acute meningitis, drug withdrawal, acute encephalitis, febrile status, acute malaria attack

Operational definitions

Confirmed Status Epilepticus: A seizure that lasts for a sufficient length of time (30 minutes or longer in most studies) or is repeated frequently enough that the individual does not regain consciousness between seizures.

Probable CSE*:* Defined by any one of the following criteria: convulsions on arrival to hospital; use of phenytoin or phenobarbital to stop seizures after the failure of two doses of the first-line medication (diazepam, paraldehyde, or both); coma, defined as a Blantyre coma score of 2 or less on admission and a history of more than one seizure in the 30 min before presentation; or coma on admission and a history of more than ten seizures in the 24 h before presentation.

Acute symptomatic SE*:* Status epilepticus in a previously neurologically normal child within a week of an underlying cause including central nervous system (CNS) infection, encephalopathy, traumatic head injury, cerebrovascular disease, and metabolic derangements or toxic injuries.

Short-term outcome*:* The condition of the patient at discharge from the hospital.

Data collection tool and procedures: Data extraction format was prepared from previous studies. The format consisted of 26 items including demographic data, etiologic and clinical variables and short-term outcome variables. After an intensive training, data were collected by three interns and two residents.

Data quality control: The data collection tool was pretested before actual data collection and refinement was done accordingly. The collected data were checked daily for completeness and consistency by supervisors.

Data processing and analysis: The collected data was entered into SPSS version 20, cleaned and analyzed accordingly. Descriptive statistics were computed to characterize the study participants and the

results were presented with frequency and percentages. In addition, bivariable and multivariable binary logistic regression analyses were applied to identify factors associated with the outcome of interest and findings were reported with odds ratio within the 95%CIs. In this study, a significance level of 0.05 was considered acceptable. Results were presented using tables, graphs and narratives.

Ethical consideration: Ethical clearance was obtained from Ethics Review Board (ERB) of the School of Medicine, University of Gondar. In addition, a letter giving permission to gather and record data was obtained from Gondar Hospital's Clinical Director's office. Finally, data was collected anonymously and the information gathered from the chart was treated confidentially.

RESULT

Socio-demographic characteristics of the patients: There were a total of 100 admitted cases with SE. Among these, 82 patients had complete documentation. About 49 (60%) of cases were male. The median age of the patients was 36 months with Inter Quartile range (IQR) of 70. The majority of the patients were age between 13-60 months (43.9%) followed by age greater than 60 months (32.9%). Thirty (36.6%) patients were from Gondar town while the rest lived outside of Gondar. The highest number of admissions was recorded in Kermit (summer) season 26 (31.7%) followed by Tseday (spring) 25 (30.5%) (Table 1).

Etiology: The most common type of CSE was generalized CSE which accounted for 71 patients (86.6%), followed by focal motor epilepsy (FME), of which there were 7 patients (8.5%). Of those 82 patients diagnosed with SE, 50 patients (61%) were diagnosed with acute symptomatic SE and 14 patients (17.1%) were diagnosed with idiopathic/ cryptogenic SE.

Table 1: Socio-demographic characteristics of patients with SE admitted to UoG referral hospital pediatrics ward, 2018 (N=82)

Variable	Frequency	Percent(%)
Sex		
Male	49	59.8
Female	33	40.2
Address		
Gondar town	30	36.6
Out of Gondar town	52	63.4
Age		
1-12 months	19	23.2
13 - 60 months	36	43.9
> 60 months	27	32.9
Season of admission		
Belg (Autumn)	16	19.5
Bega (winter)	15	18.3
Tseday (spring)	25	30.5
Keremt (summer)	26	31.7

Among the acute symptomatic patient; acute pyogenic meningitis was the most common comorbidity (22 patients, or 26.9%), followed by acute malaria attack (8 patients, or 9.8%). Five patients were also found to have remote symptomatic cases and all of them were diagnosed clinically with cerebral palsy.

In children between 1-60 months, acute symptomatic epilepsy was the most common (27 patients, 32.9%) followed by febrile seizure (13 patients, 15.9%). Acute symptomatic and idiopathic/cryptogenic was the most common for children greater than 60 months, accounting for 23 and 3 patients (28% and 3.7%) respectively.

Previous epilepsy disorder was found in 18 cases (22%) among which 8 (44.5%) were on anti-epileptic drugs (AEDs) and 50% of them discontinued AEDs and presented as SE. Comorbidities were identified in 28 patients (34.1%) of which the most common comorbidity was malnutrition (found in 15 patients, 18.3%), followed by Cerebral Palsy (CP) in 5patients (7.3%), Acute Gastro Enteritis AGE 3 (3.7%), pneumonia 1(1.2%) and HIV 1(1.2 (Table 2).

Table 2: Causes of SE identified in children admitted at UoG referral hospital, pediatrics ward, 2018

	Causes	Frequency	Percent (%)
Acute symptomatic (n=50)	Acute pyogenic meningitis	22	26.9
	Acute malarial attach	8	9.8
	Drug withdrawal	4	4.9
	TB meningitis	3	3.7
	Organophosphate poising	3	3.7
	Head trauma	2	2.4
	Brain tumor	2	2.4
	AGN	2	2.4
	Alcohol intoxication	1	1.2
	Encephalitis	1	1.2
	Hypoxic brain injury	1	1.2
	Middle cerebral artery infarction	1	1.2
Remote symptomatic	Cerebral palsy	5	6.1
Idiopathic/cryptogenic	Idiopathic	14	17.1
Febrile SE	Febrile seizure	13	15.8

Treatment: The condition of the patient on arrival was seen as comatose at presentation in 47 (57.3%) of patients. Time of arrival for treatment was less than 2 hours following onset of seizure in 18(22%), 2-12 hours in 22(26.8%) and greater than 12 hours in 42(51.2%). The time taken to control seizures was also analyzed. Seizure was controlled in < 0.5 hours in 25(30.5%) of cases, 0.5-1 hour in 11(13.4%) cases, 1-12 hours in 34(41.5%) cases and the rest was controlled > 12 hours. (Table 3)

Table 3: Time of arrival to the hospital for treatment and time taken to control seizure in patients with SE admitted to UoG referral hospital pediatrics ward, 2018

Variable		Frequency	Percent (%)
Time of arrival for treatment	<2 hr	18	22
	2-12 hr	22	26.8
	>12 hr	42	51.2
Time taken to control seizure	< 0.5	25	30.5
	0.5-1 hr	11	13.4
	1-12 hrs	34	41.5
	>12 hr	12	14.6

The first line drug used to control seizures in the management of CSE was diazepam in 69(84.1%), and phenytoin in 13(15.9%) patients. For those for whom seizures recurred after the first line drug, Phenytoin was used as the second line in 60 (73.2%), and phenobarbital in 3(3.7%) patients. Thirty-three (40.2%) of patients required third AEDs; phenobarbital was used in 32(39%) patients and phenytoin in one patient. All diazepam was given IV except in three patients, where it was given by rectal administration. Phenytoin and phenobarbital were given PO via NGT. The hospital stay ranged from 0.25-79 days with a mean of 8.2± 9.7 days.

Short-term treatment outcomes: In this study, the proportion of good recovery was 61% of participants (95% CI; 51.2 - 73%). The remaining 39% (95% CI; 27- 48.8%) of participants had poor treatment outcomes including severe disability (19.4%), moderate disability (9.8%) and death (9.8%).

Neurologic sequelae (i.e. moderate to severe disability) occurred in 7(36.8%) of participants less than 12 months in age, 8 (22.2%) participants aged 13-60 months and 9 (33.3%) participants aged greater than 60 months. All deaths occurred in participants over than 12 months in age. Neurological sequelae were high in focal motor SE 3(42.9%), followed by generalized CSE 19(26.8%). All deaths occurred in the generalized type of SE.

A total of 47(57.3%) patients presented as comatose among which 15(62.5%) had neurological sequelae and 6(75%) died. Most neurological sequelae (13 cases, 54.2%) and deaths (5 cases, 62.5%) occurred when seizures took more than 2 hours to control.
SE caused by acute symptomatic SE were highly associated with neurological sequelae and death which impacted 18 patients (64.3%) and 7 patients (87.5%) respectively. No neurological sequelae or deaths occurred in febrile SE. More neurological sequelae and deaths were seen when the time of arrival (onset of a seizure before treatment) is greater than 12 hours (16 cases, 66.7% and 5 cases, 62.5% respectively). (Table 4).

Among the total of 8 deaths the cause of death attributed to the underlying disease in 7 cases (87.5%) was due to acute symptomatic SE, the remaining one patient had idiopathic SE and died due to SE and its complications (i.e. hypoxic brain injury and aspiration pneumonia that results in respiratory failure). Seven patients died due to underlying disease (two had acute malarial attacks, two had pyogenic meningitis, and head trauma, retinoblastoma with CNS metastasis, hepatoblastoma with CNS metastasis each accounted for one patient. No deaths were due to febrile SE or remote symptomatic SE.

At 3 months of discharge from the hospital patients were assessed for seizure control, drug adherence, recurrence of SE and neurological sequelae at pediatrics neurologic follow-up clinics and 40(48.8%) patients were found. No seizures were detected in 26 patients (65%), 1-2 episodes had occurred for 10 patients (25%), one patient (2.5%) had weekly seizures and for 3(3.7%) patients subsequent seizure frequency is unknown.

All but one patient of these participants took AEDs adherently. Two patients had one recurrence in 3 months period. The cause for recurrence is drug withdrawal for one of the patient and unknown for the other patient.

Development was abnormal in 5 (12.5%) patients. Three patients were found to have developmental regression: the first infant had motor regression; the other two had motor and language regression. Two patients had developmental delay: one patient had a global developmental delay (motor, language), and the other one had only motor delay.

Short-term treatment outcomes by clinical characteristics: Among the 82 participants, the majority were in the age range of 13-60 months, of whom 24 (66%) had good outcomes from treatment. Among the causes of SE, the majority were acute symptomatic SE, of which half have shown good short-term outcomes. Among the 82 cases, 64 patients have previous history of epilepsy of which 42 had good short-term outcome. Pertaining to co-morbidities, the majority of the patients have no co-morbidities of which 38 have shown good outcome (Table 4).
Factors associated with short-term treatment outcomes: In bivariable binary logistic regression the variables were: the participant's age, the time of arrival for treatment, time to control seizure, previous history of epilepsy, level of consciousness and co-morbidities each showed statistically significant association with short-term treatment outcome of

SE. However, after they were adjusted in multivariable binary logistic regression, only co- morbidity was significantly associated with short-term treatment outcomes. Thus, the likelihood of having good outcomes in patients who had no co-morbidity was 3.35 times higher than those who had some co-morbidity. This association was statistically significant (AOR=3.354; 95%CI: 1.018, 11.045) (Table 5).

Table 4: SE outcome by clinical characteristics in UoG referral hospital, 2018

Clinical characteristics		**Total (N)**	**Outcome**	
			Good	**Poor**
Age in month	1-12	19	12	7
	13-60	36	24	12
	>60	27	14	13
Cause of SE	Acute symptomatic	50	25	25
	Remote symptomatic	5	1	4
	Idiopathic/cryptogenic	14	11	3
	Febrile seizure	13	13	0
Previous history of epilepsy	Yes	18	8	10
	No	64	42	22
Level of consciousness	Conscious	35	24	11
	Unconscious	47	26	21
Co morbidity	Yes	28	12	16
	No	54	38	16
Mechanical ventilation	Yes	5	4	1
	No	77	46	31

Table 5: Bivariate and multivariate logistic regression showing factors associated with short term treatment outcome in patients with CSE admitted in pediatric ward at UoG referral hospital, 2018

Clinical characteristics		**Crude OR(95 %CI)**	**Adjusted OR (95% CI)**
Age in months	1-12	0.628(0.189,2.085)	0.912(0.19,4.330)
	13-60	0.538(0.198,1.500)	1.003(0.270,3.734)
	>60	1	1
Time of arrival for treatment in hours	< 2	1	1
	2-12	0.588(0.145,2.385)	0.627(0.121,3.239)
	>12	2.000(0.632,6.327)	2.170(0.548,8.584)
Time to control seizure in hours	< 0.5	1	1
	0.5-1	0.964(0.197,4.721)	0.828(0.127,5.399)
	1-12	1.800(0.594,5.453)	2.705(0.699,10.477)
	>12	5.143(1.166,22.687)	5.826(1.000,33.930)
Previous history of epilepsy	Yes	2.386(0.824,6.910)	2.011(0.557,7.265)
	No	1	1
Level of consciousness	Conscious	1	1
	Unconscious	1.762(0.705,4.406)	2.382(0.677,8.375)
Co morbidity	Yes	1	1
	No	3.167(1.225,8.183)	3.354(1.018,11.045)

DISCUSSION

This study was conducted to analyze the etiology, clinical features and short-term treatment outcomes of status epilepticus in children admitted to the University of Gondar Hospital, in Northwest, Ethiopia. The majority of the patients were aged between 13-60 months followed by ages greater than 60 months with male predominance, similar to studies done in Ethiopia (8) and Kenya (4). The highest admission rate was recorded in Kermit (summer) followed by Tseday (spring) which coincides with the malaria transmission season in Ethiopia (10).

The most common type of seizure was generalized seizure while the most common cause of SE was acute symptomatic followed by idiopathic/ cryptogenic. The same finding was seen in India (5), Kenya (3) and Turkey (1).

Etiology differences based on age were assessed with acute symptomatic as the most common in all age groups, followed by febrile SE for those less than 60 months. The same findings were also reported in studies conducted in Ethiopia (8), Kenya (7) and India (11).

Among acute symptomatic CNS infections, pyogenic meningitis was the most common followed by acute malarial attack. This was also seen in another study. CNS infections are common in sub-Saharan African countries that have a high incidence of malaria and other infectious diseases and limited treatment access for many lower income patients (12).

In this study time of arrival for treatment to the hospital was greater than two hours in the majority of patients. This treatment delay was seen in the other study done in Ethiopia (8). This could be due to limited referral hospitals in the country and accessible and affordable transport systems.

All deaths in this study occurred in ages greater than 12 months. This is different from the findings of the studies done in Ethiopia (8), Kenya (3) and India (6). This could be explained by the underlying co-morbidity conditions which are not commonly seen in the other settings. The higher percentage of neurologic sequelae (i.e. moderate to severe disability) occurred in age groups less than 12 months in this study which is corroborates with findings in other studies (3, 8, 6).

In this study the overall mortality rate of SE was higher than in the studies in Tikur Anbesa hospital of Ethiopia (8)and India (11). This may be due to differences in the care of patients like using Mechanical ventilation (MV) in patients who has indication which is difficult in our setup because of limited amount of MV.

The time taken to control seizures after admission took more than one hour in the majority of patients in this study. This may be due to the use of oral anticonvulsants which are less effective as compared to other options (13).

In this study, the multivariate binary logistic regression output indicates that, only co-morbidity was significantly associated with short-term treatment outcomes. Thus, the likelihood of having good outcomes in patients with no co-morbidity was 3.35 times higher than those who had co- morbidity (AOR=3.354; 95%CI: 1.018, 11.045). This finding is consistent with other studies (14, 15, 16).

The study findings also revealed that development is abnormal in 12.5% of patients who came for neurologic follow up at 3 months post-discharge. Three of these patients were found to have developmental

regression; one was an infant with motor regression; the other two had motor and language regression. Two patients had developmental delay: one patient only had motor delay and the other had both motor and language delay. Similar percentages were seen in studies done in Kenya 11% (3) and turkey 15% (1).

Limitations of the study: Since the study was retrospective there was difficulty retrieving medical charts and obtaining full information from patients' charts. In addition, lack of investigation modalities like EEG and Neuroimaging makes it difficult to reach to specific etiologies and to ascertain the outcome of SE in this particular study. Though the study included all admitted children in the referral hospital, the sample size was not adequate to test all variables of interest.

CONCLUSION

In the study setting, acute symptomatic SE is the most common cause of SE in all age groups among which pyogenic meningitis being the most common contributor followed by acute malarial attack. Most patients arrived late and for the majority of patients the time of arrival for treatment was greater than 12 hours. The first line drug used to control seizures in the management of CSE was diazepam followed by phenytoin.
In terms of outcomes, more than half of patients had good recovery. Most neurological sequelae and death occurred in those seizures that took more than 2 hours to control. Among the factors, co-morbidity was significantly associated with poor outcomes associated with short-term treatment.

RECOMMENDATION

The findings of this study emphasize the need for education of parents and caregivers as well as community healthcare workers to enable early and aggressive treatment of seizures in order to improve outcomes. In this study also the common cause of SE included infectious diseases like malaria and meningitis which need proper prevention and treatment strategies. Early identification and timely management of comorbidities is also important to improve treatment outcomes.

There is also a need to increase to use diagnostic modalities like EEG and Neuroimaging like CT scans and MRI to reach the specific underlying problems. In addition, policy makers and health care planners should be aware of the need to ensure an adequate supply of different AEDs where children can receive adequate supportive care. Further prospective research is also important to know the etiology, short and long-term outcomes of SE.

What is already known: Though it is not well studied, status epilepticus is one of the most common neurologic emergencies in resource-limited countries where infectious disease like malaria, encephalitis and meningitis are high. The outcome is dependent on the etiology.

What this study adds: Convulsive status epilepticus was not given due attention in Ethiopian children. This study was the first to examine the etiology and clinical features of status epilepticus which will serve as a baseline information to improve the quality of care.

Abbreviations: AEDS: Antiepileptic Drugs, **AGE**: Acute Gastroenteritis, **AGN:** Acute Glomerulonephritis, **CNS:** Central Nervous System, **CP:** Cerebral Palsy, **CT:** Computed Tomography, **EEG:** Electro Encephalography, **ERB:** Ethical Review Board, **GTCS:** Generalized Tonic Clonic Seizure, **HIV**: Human Immunodeficiency Virus, **MRI:** Magnetic Resonance Imaging, **NCSE**: Non-Convulsive Status Epilepticus, **PICU**: Pediatrics Intensive Care Unit, **SE:** Status Epilepticus.

Declarations

Conflicts of Interest: The authors declare that they have no conflicts of interests

Authors' Contributions: OA initiated the research, wrote the research proposal, carried out the data analysis and interpreted the results. AT and ZM were involved in designing the study, revising the proposal, guiding the statistical analysis, and writing the manuscript. All authors read and approved the final manuscript.

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