

Endorsing Improvements of AFP and Measles Surveillance Performances after the First COVID-19 Pandemic Lockdown (2020-2021), a Comparative Analysis Study Design: The Case of Sidama Province, Ethiopia

Amenu Wesen Denegetu^{1*}, Eshetu Wassie Asemahaegn¹, Tadesse Gossaye Birru²

¹Department of Medical Physiology and Pharmacology, Haramaya University, Dire Dawa, Ethiopia; ²Department of Medical Physiology and Pharmacology, The Catholic University of Malawi, Nguludi, Malawi

ABSTRACT

Introduction: Following the declaration of COVID-19 as a global public health threat, disease surveillance activities were crippled in 2020. Documenting surveillance improvements of Sidama province in 2021 has to be endorsed to boost the performances.

Methods: A comparative analysis study design of acute flaccid paralysis and measles surveillance data of 2020 and 2021 for Sidama province was used. The non-polio AFP rate and stool adequacy rate were used to assess the sensitivity of the AFP surveillance. Whereas, the non-measles febrile rash rate was used to assess the measles surveillance.

Results: A total of 91 AFP cases in 2021 and 44 in 2020 have been detected and investigated for polio analysis. All cases were discarded in both years. The stool adequacy rate for 2021 was 90.1%; whereas, 86.4% in 2020. Annualized non-polio AFP rate was 2.2/100,000 for 2020 and 3.8/100,000 for 2021, which the latter is much higher to the minimum expected rate in outbreak areas of 3/100,000.

A total of 154 suspected measles cases in 2021 and 111 in 2020 have been investigated for IGM analysis. In 2020, 64 and 11 in 2021 IGM positive for measles were identified. The non-measles febrile rash rate for 2020 was 1.1/100,000; whereas, 2.5/100,000 for 2021.

Conclusion: There is an improved in the sensitivity of AFP and measles surveillance performance for Sidama province in 2021. Sustaining high quality measles and AFP surveillance is suggested to maintain measles and polio free statuses.

Keywords: Polio eradication; Non-polio AFP rate; Non-measles febrile rash; Acute flaccid paralysis; Measles

Abbreviations: AFP: Acute Flaccid Paralysis; CDC: The US Centers for Disease Control and Prevention; FP: Focal Person; GPEI: Global Polio Eradication Initiative; HC: Health Center; HOSP: Hospital; IDSR: Integrated Disease Surveillance and Response; IGM: Immunoglobulin M, the largest antibody (a substance in the blood that attacks harmful bacteria) in the human circulatory system; MCV: Measles Containing Vaccine; MCHN: Maternal Child Health and Nutrition unit; NMFR: Non-Measles Febrile Illness; nOPV2: novel Oral Polio Virus type 2, is a new oral vaccine developed to build immunity against the most prevalent type of circulating VDPV; NPAFP: Non-Polio AFP rate; nPEI: national Polio Eradication Initiative; NPEV: Non-Polio Enterovirus; PHEM: Public Health Emergency Management; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus-2; SNNPR: Southern

Correspondence to: Amenu Wesen Denegetu, Department of Medical Physiology and Pharmacology, Haramaya University, Dire Dawa, Ethiopia; E-mail: denegetu@gmail.com

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Nations and Nationalities Peoples Region, a province in Southern Ethiopia; VDPV: Vaccine Derived Polio Virus; VPD: Vaccine Preventable Disease; WHO: World Health Organization; WPV: Wild Polio Virus.

INTRODUCTION

Sidama is one of the 13 provinces/provinces in Ethiopia; established just less than two years ago following residents' referendum. The province has an estimated total population of 4.47 million, which is around 5% of the national population. It is divided into 38 Woredas/districts and 626 Kebeles (smallest administrative unit). Hawassa city is the seat of the province government and the largest city in the area. Sidama is one of the most densely populated areas in Ethiopia.

There are a total of 708 health facilities in the province; *i.e.*, one specialized referral hospital, four general hospitals, thirteen primary hospitals and 690 primary health care centers (137 health centers and 553 health posts). There are also more than 170 private health facilities in the province.

The backbones of Vaccine Preventable Diseases (VPD), including Acute Flaccid Paralysis (AFP) and measles, surveillance in the province are Woreda health offices; which are dependent on their respective major health facilities; *i.e.*, Health Centers (HCs) and Hospitals (Hosps). All HCs and Hosps have designated surveillance officers who should have a basic training on disease surveillance, sometimes called Integrated Diseases Surveillance and Response Focal Person (IDSR FP). On the other hand, each Woreda has a Public Health Emergency Management Focal Person (PHEM FP), who co-ordinates all surveillance sites (HCs and Hosp) in the respective Woredas. Woreda PHEM FPs have a surveillance structure, which lists all health facilities (both for profit and nonprofit) and community informant sites which the community perceive for their health seeking behavior. Hence, all suspected diseases of surveillance interest which occurs at any of the regular health care system or in one of the community sites, including rumors, will be reported to Woreda PHEM FP. Then the FP will verify, if it satisfies the case definitions of interest, case investigation and specimen collection will be done while being reported to provincial level for a routine line-listing for further analysis, interpretation and communication for appropriate timely response interventions.

Following the declaration of COVID-19 as a global public health emergency threat, almost all non-emergency essential health services have been crippled including disease surveillance activities in the country including Sidama province for nearly one year. At which time, the AFP and measles surveillance performances have been drastically dropped. However, with better understanding and response of the COVID-19 outbreak, new directions and recommendations have been released for integrated COVID-19 and essential health services, including the routine AFP/measles surveillance activities. Hence, well documented improvements have been achieved in Sidama province just within a year following this integrated COVID-19/surveillance activity. This improvement has to be endorsed to

boost the performances that accelerate the road to global polio eradication.

The main source of detecting poliovirus circulation is through surveillance of all AFP cases among children aged <15 years by testing stool specimens for laboratory confirmation of poliovirus. Two important indicators, the Non-Polio AFP (NPAFP) rate and the percentage of stool specimens collected within 14 days from AFP patients that are received in the lab in good condition, measure the sensitivity and quality of polio surveillance; rate is defined as the number of NPAFP cases per 100,000 children aged [1].

Surveillance for detecting the transmission of poliovirus is critical to reach global polio eradication, as high-quality surveillance permits the timely detection of poliovirus transmission due to Wild Poliovirus (WPV), Vaccine-Derived Polioviruses (VDPVs), and Sabin-like viruses [2].

Worldwide, the number of AFP cases reported from January to September in 2020 has declined by 33% compared to the same time period in 2019; from 81,439 to 54,631 [3].

On January 30, 2020, the World Health Organization (WHO) declared Coronavirus disease 2019 (COVID-19) a public health emergency of international concern [4]. On March 24, 2020, the Global Polio Eradication Initiative (GPEI) suspended all polio supplementary immunization activities and recommended the continuation of polio surveillance [5].

In April 2020, GPEI developed a revised polio surveillance guideline in the context of the COVID-19 pandemic, focusing to reduce the risk of transmission of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), to health care providers and communities by minimizing personal contacts, through hand hygiene and use of personal protective and somehow loosening movement restrictions, while continuing essential polio surveillance functions [6]. However, the adoption of the new protocol seemed very low as health workers at health facility levels got very little training or orientation on the revised guideline due to suspension of all trainings until the last months of 2020.

The key objective of measles surveillance is to identify areas of measles virus transmission and immunity gaps. This will guide effective public health responses to achieve elimination of endemic measles and sustain elimination in post-elimination settings [7].

Objectives of the study

General objective: To assess the performances of AFP and measles surveillance indicators of Sidama province during and after the lockdown by COVID-19.

Specific objectives:

- To compare the performances of NP-AFP rate and stool adequacy of AFP surveillance key indicators for the years 2020 and 2021.
- To compare the performances of NMFR of measles surveillance for the years 2020 and 2021.
- To endorse and document the improvements in surveillance of AFP and measles for Sidama province.

MATERIALS AND METHODS**Study design**

A comparative analysis study design of AFP and measles surveillance data received from all Woredas/districts of Sidama province was used to assess the improvements in the AFP/measles surveillance performance after the initial outbreak year for the same time period of January to December 2020 and 2021.

The review of weekly AFP and measles surveillance performance key indicators were used from January to December of 2020 and 2020 keeping in mind the initial and post COVID-19 setting. Key AFP and measles surveillance indicators were used for all cases which had been investigated and specimen collected for testing in the measles or polio laboratories. This was to compare the sensitivity of the surveillance systems during and after normalizing COVID-19 outbreak.

Confirmed weekly update information from the national Polio and measles control data center was processed for the same period to understand the performances of key indicators. The annual reported AFP and measles surveillance performances were compared for the two years. The differences were subjected to statistical tests for ascertaining levels of significance.

Study population

There are two groups of study populations:

- All case reports of fewer than 15 children who developed sudden onset of flaccid paralysis involving any of the extremities (AFP) from Sidama province in 2020 and 2021 are one group of the study population.
- All case reports of persons of any age with signs and symptoms for suspected measles from Sidama province in 2020 and 2021 are the second group of the study population.

Note: The study does not involve contacting individual patients. Only retrospective data, which is available at provincial and national levels, has been utilized for analysis.

Hence, the cumulative AFP and suspected measles cases reported at the end of 2020 (31 December 2020), which is during the initial lockdown of COVID-19 setting, were compared with the cumulative caseloads of suspected measles and AFP cases reported at the end of 2021 (31 December 2021), which is after the lockdown of COVID-19 setting, from all districts of Sidama province, Ethiopia.

The World Health Organization (WHO) standard case definitions for both AFP and suspected or confirmed measles case definitions were used for this study. An AFP case is defined

as a child less than 15 years of age with sudden onset of floppy paralysis or weakness of any of the limbs, irrespective of the causes. Whereas, a suspected measles case is defined as any person of any age with fever, maculopapular rash and cough or conjunctivitis or coryza (the 3Cs); whereas a confirmed measles case is laboratory confirmed case, i.e., a suspected measles case with measles Immunoglobulin M (IgM) positive. All AFP cases with, if any, circulating vaccines derived polioviruses and those reported as compatible with polio were all included in the study. Those AFP cases classified as “not AFP” were excluded for this study. Besides, those suspected measles cases who were investigated and blood specimens collected for laboratory analysis were only included in this study.

Key parameters

The WHO main surveillance indicators were used as parameters to assess the effects of the COVID-19 pandemic on the AFP and measles surveillance systems. The scope of parameters covered from investigation of the reported AFP and suspected measles cases to the release of final laboratory results by the national polio lab and/or provincial measles laboratories. The Non-Polio AFP rate (NP-AFP) and stool adequacy rate were mainly used to assess the sensitivity of the AFP surveillance systems. Whereas, the Non-Measles Febrile Rash rate (NMFR) was mainly used to assess the sensitivity of measles surveillance system.

The stool adequacy of reported AFP cases was measured by the percentage of AFP cases investigated with two stool specimens collected within two weeks of onset of weakness or paralysis. On the other hand, the NP-AFP rate was measured by the number of non-polio AFP cases detected per 100,000 under 15 populations in a certain district or province. In addition, the completeness of 60 days follow-up examination was also used to assess the percentage of follow-up examinations conducted for all inadequate AFP cases within two months of onset of paralysis.

Data collection and analysis

We retrieved the weekly AFP epidemiological update of 2020 and 2021, which is compiled by national Polio Eradication Initiative (nPEI) data control room of WHO Ethiopia, to get the line-listing of all AFP cases reported from Sidama province [8,9]. Similarly, data for measles surveillance trend was retrieved from measles and neonatal tetanus weekly update_week_52_2020 and 2021, which is compiled by national measles elimination control room, WHO Ethiopia [10,11].

The data obtained from the nPEI (Ethiopia) control room for AFP and measles surveillance was directly used for this analysis. In addition, semi analyzed data for key indicators for both AFP and measles were directly utilized for this study. Both AFP and measles surveillance data for Sidama province was filtered for the purpose of this study. Data were analyzed manually and presented in tables accordingly on the selected thematic areas.

RESULTS

The descriptive findings of both AFP and suspected measles cases with blood specimens for both 2020 and 2021 will be described below.

AFP cases descriptive findings for 2020 and 2021, Sidama province

2020: A cumulative total of 44 AFP cases were reported from Sidama province at the end of 2020 (Table 1), all laboratory results were known and categorized. Of which, 27 were male and 17 were female; whereas, 23 cases were below 5 years and 21 cases were 5 years-15 years. Of the expected 23 districts (old number of districts in the province before restructured to

become 38 districts in 2021) to report at least one AFP case during the year from the province, 16 districts reported, which is 70% of Woredas reported at least one AFP case at the end of 2020.

2021: A cumulative total of 91 AFP cases were reported from Sidama province at the end of 2021 (Table 1), all laboratory results were known and categorized. Of which, 60 were male and 31 were female; whereas, 47 cases were below 5 years and 44 cases were 5 years-15 years. Of the expected 31 districts to report at least one AFP case during the year from the province, 27 districts reported, which is 87% of Woredas reported at least one AFP case at the end of 2021.

Table 1: AFP surveillance performance indicators for 2020 and 2021, Sidama province.

Year	Total cases	Adequate cases	Stool adequacy rate (%)	Annualized NPAPF rate per 100,000	Wild/CVDP cases	Overall performance status
2020	44	38	86.4	2.2	0	High NPAPF and high stool adequacy rate
2021	91	87	90.1	3.8	0	High NPAPF and high stool adequacy rate

Suspected measles cases descriptive findings for 2020, Sidama province

2020: A cumulative total of 111 suspected measles cases with blood specimens were reported from Sidama province at the end of 2020 (Table 2), all laboratory results were known and categorized. Of the expected 34 districts (these are Woredas identified as densely populated, previous outbreak areas and low Measles Containing Vaccine (MCV) coverage) to report at least one suspected measles case with blood specimen during the year from the province, 17 districts reported, which is 50% of woredas reported at least one suspected measles case at the end of 2020.

2021: A cumulative total of 149 suspected measles cases with blood specimens were reported from Sidama province at the end of 2021 (Table 2), all laboratory results were known and categorized. Of the expected 37 districts to report at least one suspected measles case with blood specimen during the year from the province, 18 districts reported, which is nearly 50% of woredas reported at least one suspected measles case at the end of 2021.

Table 2: Measles surveillance performance indicators for 2020 and 2021, Sidama province.

Year	Total cases with blood specimen	Adequate cases	Adequacy rate (%)	Annualized NMFR rate per 100,000	Measles IGM+	Measles detection rate	Incidence rate per 100,000
2020	111	108	97.3	1.1	64	2.8	17
2021	149	148	99.3	2.5	7	2.7	2

Key performance indicators for AFP surveillance, comparing 2020 with 2021

In 2020, a total of 44 AFP cases had been detected, investigated and specimens were collected for polio analysis. All cases were discarded and no wild or vaccine derived polio viruses were identified. The number of adequate cases was 38, which gave us a stool adequacy rate of 86.4%. The NP-AFP rate for the year was 2.2/100,000, which was to the global standard, but less than

the minimum rate expected in outbreak areas, 3/100,000. However, the provinces' overall performance was high NP-AFP rate and high stool adequacy.

In 2021, a total of 91 AFP cases have been detected, investigated and specimens were collected for polio analysis. All cases were discarded and no wild or vaccine derived polio viruses were identified. The number of adequate cases was 82, which gave us a stool adequacy rate of 90.1%. Annualized NP-AFP rate was

3.8/100,000, which was much higher to the global standard and above the minimum rate expected in outbreak areas, 3/100,000. Hence, the provinces' overall performance was high annualized NP-AFP rate and high stool adequacy.

Key performance indicators for measles surveillance, comparing 2020 with 2021

In 2020, a total of 111 suspected measles cases with blood specimens had been reported, investigated and specimens were collected for measles and rubella analysis. A total of 64 confirmed IGM positive for measles cases were detected and four rubella IGM positive cases identified. The proportion of IGM positive for measles and rubella was 57.7% and 9.1%, respectively. The remaining 44 cases were discarded; *i.e.*, became negative for any of the antigens. Two confirmed measles cases were epidemiologically linked to previous outbreaks in the province and three measles compatible cases categorized. The incidence rate for the year was 17/100,000, which means insensitive to detect all outbreaks in the province. Besides, adequacy rate was 97.3% and overall measles detection rate for the province was 2.8%. The NMFR rate for the year was 1.1/100,000, which was below the standard of sensitive measles surveillance system.

In 2021, a total of 154 suspected measles cases with blood specimens have been reported, investigated and specimens were collected for measles and rubella analysis. A total of 11 confirmed IGM positive for measles cases were detected and 7 rubella IGM positive cases identified. The proportion of IGM positive for measles and rubella, was 7% and 5.1%, respectively. The remaining 137 cases were discarded; *i.e.*, became negative for any of the antigens. No measles cases were epidemiologically linked to any of previous outbreaks in the area and one measles compatible case was categorized. The incidence rate for the year was 2/100,000, which means very sensitive to detect most outbreaks in the province. Besides, adequacy rate was 99.3% and overall measles detection rate for the province was 2.7%. The NMFR rate for the year was 2.5/100,000, which is above the minimum standard of sensitive measles surveillance system.

Males were more likely to have AFP in both 2020 and 2021; 61.4% and 66%, respectively. AFP cases reported below five years and between 5-14 age groups in both 2020 and 2021 were at almost equal proportion.

Regarding, limb paralysis, in 2020, 11 (25%) AFP cases involved at least one of the upper limb; whereas, 41 (93%) of the reported AFP cases involved at least one of the lower limb of the total 44 AFP cases for the year. Similarly, in 2021, only 31 (34%) AFP cases involved at least one of the upper limb and 85 (93.4%) cases involved at least one of the lower limbs of the total 91 AFP cases reported during the year. Besides, 17 (39%) in 2020 and 19 (21%) of the reported AFP cases developed quadriplegic.

The non-polio enterovirus isolation rate for both 2020 and 2021 was above 10%; *i.e.*, 11% for 2020 and 10% for 2021.

Concerning reporting period of AFP cases, a monthly variation was observed being highest in April 2020 (10 cases) and no cases

in September 2020. On the other hand, the highest number of AFP cases reported in 2021 was in November (16 cases), this was mainly because of enhanced surveillance conducted during nOPV2 house-to-house supplementary immunization campaign, and the lowest with no case was in January 2021, which has no any known explanation. But overall, majorities (64%) of AFP cases in 2020 were reported during the first half of the year; whereas, around 55% of AFP cases in 2021 were reported during the second half of the year (July-December).

DISCUSSION

An increased in the number of reported AFP cases in 2021 was clearly indicated and attributed to the loosening of lockdown due to COVID-19 pandemic, at which time, measures such as active case search, surveillance training, capacity building of health facilities with specimen collection materials, facilitation of specimen collection, packaging and transportation to laboratory, use of personal protective materials during investigation and specimen collection, resuming clinician sensitization and laboratories started receiving specimens. Besides, with better response lessons, directions and recommendations from the global health regulators, integrated COVID-19 and routine surveillance activities has resulted in marked improvements in all performance indicators of both AFP and measles surveillance in 2021 [12]. In addition, the WHO interim guidelines of continuation of the immunization services and diseases surveillance was implemented by countries [13,14]. On the other hand, the decrease in the number of AFP cases in 2020 is mainly attributed to COVID-19 epidemiology and associated restrictions on movement of polio staff members, diversion of resources from polio to the COVID-19 response, or the emergence and spread of type 2 circulating vaccine derived-poliovirus outbreaks.

The NPAFP rate for 2021 has improved significantly to 3.8/100,000 in 2021 compared with 2.2/100,000, an improvement of 73%; which shows the surveillance became very sensitive and might not miss any polio outbreaks in the province. Both the number of AFP cases detected and eventually the NPAFP for the preceding year (during the lock down had been low).

The finding in 2021 (3.8/100,000) meets the surveillance indicator standard in outbreak setting; *i.e.*, ≥ 3 NPAFP rate. According to the polio weekly, Ethiopia, week 51, 2021 update, there were 6 Vaccine Derived Polio Virus type 2 (VDPV2), 12 cases of cVDPV2 and 1 environmental cVDPv2 in Ethiopia including at two bordering provinces, Oromya and Southern Nations and Nationalities Peoples Region (SNNPR) to Sidama. A report by MMWR, during January 2020-June 2021, there were 38 cVDPV2 emergences in active transmission in 34 countries; 28 (82%) of these countries are in Africa including Ethiopia.

Studies show that interruptions to poliovirus surveillance might have negative consequences on detection of poliovirus circulation and hence continued analysis of AFP reporting trends is necessary to better understand the long-term impact to the eradication initiative. This decline has been indicated as well in other studies in East and Southern African countries sub-

province for March to December 2020 compared to the same period in 2019. The improvement in case detection in 2021 can also be attributed to the deployment of polio consultants for the province by global polio eradication initiative partners, strengthening capacity of local surveillance staffs through training and supportive supervision.

Besides, the stool adequacy rate has improved slightly from 86.4% to 90%, showing a 4.2% improvement. This progress is also attributed similarly to the NPAFP rate improvement explained above.

In both 2020 and 2021, males were more likely affected by AFP than females, with significant proportion of around 64% in 2021. This finding is consistent with similar studies in Nigeria, Ghana, Iran, Italy and India in which higher frequency of AFP was observed among boys than girls. Male majority in the incidence of symptomatic infectious diseases in children may be due to reduced immunity, effect of sex hormones, genetic influence or exposure connected reasons, which clearly need further study.

Quite majority (93%), if not all, of the AFP cases developed lower limb (s) paralysis in both 2020 and 2021. This finding is similar to the study finding in East and Southern African countries from 2012-2019.

The non-polio enterovirus isolation rate for both 2020 and 2021 was above 10%, indicating the quality of specimen collection, packaging and transportation seem satisfactory. This finding is better than a 4 % isolated Non Polio Enterovirus (NPEV) found in a study in Palestine.

The NMFR rate indicates the sensitivity of the surveillance in detecting suspected measles cases in an area, and the minimum surveillance target is 2/100,000 population. In this study, a total of 154 suspected measles cases with blood specimens were reported in 2021; whereas, in 2020, 111 cases with specimens had been reported; showing an increase in case load of almost 1.4-fold. The NMFR rate, which is the prime performance indicator for measles surveillance has been significantly improved to make the sensitivity of the surveillance more indicative of early outbreaks in the province.

CONCLUSION

Surveillance performances recorded in 2021 for both measles elimination and polio eradication strategies should be continued to be implemented effectively in the province to maintain the current status of polio eradication and measles elimination goals in Sidama province. Besides, other neighboring provinces (Oromya, SNNPR and South West) ought to buy the good experiences of surveillance activities from Sidama province so as for a synergistic effect for the whole country.

Regardless of the global significant reduction in the case load of wild poliomyelitis, AFP surveillance continues to be the gold standard for detecting cases of poliomyelitis. The global wild polio case narrowed down to remain only in two endemic countries, Pakistan and Afghanistan. However, there is a recent case surge of vaccine derived polio viruses in developing

countries particularly in Africa. The current outbreak of VDPV in Ethiopia urge to strengthen AFP surveillance activities more than ever while implementing other concurrent polio eradication strategies. Both the NPAFP rate and stool adequacy, the two most important surveillance indicators, are achieved in the province to the best of the national figure, which need to be maintained in subsequent years.

Measles surveillance is in general dependent on investigating all suspected cases with blood specimens to identify measles viruses. Hence, the key indicator is NMFR rate. The global measles elimination target is to achieve 2/100,000, for which the finding in this study for Sidama province is 2.5/100,000. This indicates the existence of sensitive marker to detect any outbreaks in the province, which again needs to be maintained.

Finally, maintaining high quality measles and AFP surveillance together with high immunization coverage in the province are mandatory strategies to maintain measles and polio free statuses. In addition, sensitive surveillance indicators help to identify quickly importation of polio viruses and detect measles cases rapidly and help for early warning and response interventions accordingly.

RECOMMENDATIONS

- Sustain polio eradication partners (the US centers for Disease Control and Prevention (CDC) and WHO) presence in the province, who help in capacity building, monitoring and supervision of surveillance activities.
- Clinician sensitization has to be done regularly at major health facilities.
- Establishing community informant structure at all districts is an urgent priority.

DECLARATIONS

The following declarations have been fulfilled to conduct and publish this study.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Before conducting this study, ethical clearance has been obtained from Sidama province health bureau ethical clearance committee. All methods were carried out in accordance with relevant guidelines and regulations. It was informed for all participants to understand the value of the study and how the information will be used before they give their consent. Written informed consent has been obtained from all study subjects. Participation in the study was voluntary and participants were informed of their right to withdraw from the study even if they had initially given their written consent. For participants younger than 18, consent was obtained from their legal guardians, but discussions were held mainly with the patients themselves under the support of their guardians.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

FUNDING

The authors declare that there is no fund received to conduct the study.

AUTHORS' CONTRIBUTIONS

AWD designed the study, the analysis, interpretation, discussion, and organized the manuscript for publication. EWA critically reviewed the manuscript and participated in the data analysis. TGB reviewed the manuscript critically. All authors have read and agreed to the final manuscript.

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