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# Short term optimization of glycaemic control using insulin improves sympatho-vagal tone activities in patients with type 2 diabetes

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ABSTRACT

*Introduction:* Diabetic cardiac autonomic neuropathy (CAN) is potentially life threatening and its severity might further be aggravated by poor glycaemic control. A decrease in Heart rate variability (HRV) is the earliest finding of CAN even at the sub clinical stage. While intensive glycaemic control prevents the development of CAN in patients with type 1 diabetes, it is not known whether the intensification of glycaemic control using insulin would improve cardiovascular autonomic functions in type 2 diabetes patients. This study aimed to determine the short term effects of optimizing glycaemic control using insulin on the HRV in type 2 diabetes patients.

*Methods:* We conducted a single arm open label clinical trial. Participants were poorly controlled non-insulin treated type 2 diabetes mellitus patients (HbA1c $\geq$ 7%). The intervention lasted 60 days and consisted in the intensification of glycaemic control through the initiation of a basal plus insulin regimen with titration of insulin to protocol defined glycaemic targets which were; fasting glycaemia: 0.70–1.30 g/L and post prandial glycaemia <1.80 g/L. Long term HRV measurement was done using a 24-h ambulatory electrocardiographic (ECG) recording on day 0 and day 60. Wilcoxon signed rank test was used to compare differences in HRV parameters before and after the intervention.

*Results:* A total of 29 (14 males and 15 females) consenting type 2 diabetes mellitus patients without clinical signs of CAN were enrolled and allocated to intervention (14 males and 15 females). The median age was 52 [43–59] years, and duration of diabetes 3.0 [0.6–6.7] years. The intervention induced a reduction in HbA1c from 10.1 [9.1–11.9]% to 6.7 [5.9–6.9]% ($p < 0.001$) without severe hypoglycaemic events. Concerning HRV parameters, there was

*Abbreviations:* Bpm, beats per minutes; HbA1c, glycated hemoglobin; HR, heart rate; HRV, heart rate variability; HF, high frequency; LF, low frequency; ms, milliseconds; PNN 50, percentage of normal to normal intervals differing by more than 50 ms; RMSSD, root-mean-square of successive differences; SDNN, standard deviation of all normal R-R intervals; SDANN, standard deviation of average normal to normal R-R intervals; T2DM, type 2 diabetes mellitus

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a significant improvement in markers of the parasympathetic tone (PNN50: 5.7 [3.6–10.3]% to 8.1 [3.1–16.9]%, p = 0.008) and sympathetic tone (SDNN: 102.01 [90.45–111.05] ms to 122.40 [91.70–135.95] ms, p = 0.01).

*Conclusion:* The optimization of glycaemic control using a basal plus insulin regimen while inducing a significant reduction in glycated hemoglobin, significantly improves 24-h ambulatory ECG derived sympathetic and parasympathetic activities. This suggests that tight glycaemic control using insulin may revert cardiac autonomic neuropathy in type 2 diabetes mellitus patients.



## 1. Introduction

Cardiovascular death is the leading cause of mortality amongst patients with diabetes mellitus (DM). Apart from coronary artery disease, cardiac autonomic neuropathy (CAN) is amongst the leading causes of death in patients with DM especially in poorly controlled patients [1]. The pathophysiology of CAN results from a cascade of complex mechanisms that result in oxidative stress and the toxic effects of glycosylation products that results in neuronal dysfunction and death. CAN is a progressive disease that initially affects the vagal branch, but can progress to affect the sympathetic branch causing more severe manifestations. CAN has been linked to postural hypotension, exercise intolerance, increased incidence of asymptomatic ischemia, myocardial infarction and decreased likelihood of survival after myocardial infarction. Among patients with diabetes, the 5-year mortality rate is 5 times higher in those with autonomic involvement than in those without. However, treatment is based on early diagnosis, optimizing glycaemic control, life style changes and management of cardiovascular risk factors [2]. Heart rate variability (HRV) is the most commonly used diagnostic method to assess cardiac autonomic regulations because it is non-invasive and provides information about CAN at an early stage. Other tests are minimally invasive and include: microneurography, scintigraphy evaluation of the sympathetic innervation of the heart and the measure of the vascular flow [2]. Since the diagnosis of diabetes is late in Africa, most patients already present complications at the time of diagnosis [3,4].

HRV refers to the beat to beat alteration in heart rate and provides a tool for exploring the autonomic nervous system with the advantage of being non-invasive and may be analyzed from long term or short term measures. The 24-h indices appear to be stable and reproducible over short term measures, thus may be ideal to assess intervention therapies [5]. A decrease in HRV is one of the earliest findings of CAN even at subclinical stage. Studies have shown that poor glycaemic control could negatively impact cardiac autonomic modulations leading to early cardiac autonomic dysfunction [6]. This suggests that optimal glycaemic control using insulin treatment can improve cardiac autonomic functions. Moreover, with regards to the potential effects of glucotoxicity, it will be interesting to know whether short term improvement of glycaemic control in patients with Type 2 DM has an effect on cardiac autonomic functions.

## 2. Participants and methods

### 2.1. Study design and population

We conducted a single arm open label clinical trial (before and after) at the National Obesity Centre of the Yaoundé Central hospital in Cameroon. Participants were poorly controlled type 2 diabetes patients with HbA1c $\geq$ 7% on oral hypoglycaemic agents only [1]. Patients were excluded if they had changed their anti-diabetic medications or received insulin therapy within the last 3 months, presented with clinical signs of CAN such as tachycardia, orthostatic hypotension, resting tachycardia and exercise intolerance. Significant renal impairment (Clearance of creatinine < 60 ml/min), any abnormal ECG finding that could affect HRV analysis, treated hypertension or measured blood pressure $\geq$ 140/90 mmHg) were also criteria for exclusion.

Sample size calculation was done using Whitley and Ball's formula which is one used for calculation for a difference in means [n = (2/d2) × Cp] [7].

Where d, the standardized difference = target difference/SD and Cp is a constant defined from statistical tables.

Using that, we needed at least 25 subjects to have 80% power to detect a mean difference of 20 ms between the groups using a two tailed test with a significance level of 0.05

### 2.2. Outcomes

The primary end point was a change in HRV parameters from baseline to day 60.

The secondary endpoints included changes in heart rate, fasting glycaemia, HbA1C, weight and the percentage of participants experiencing one or more episodes of symptomatic confirmed hypoglycaemic events reported during the intervention. HbA1C was used since it assesses the glycaemic control over the preceding 8–12 weeks [8].

### 2.3. Procedure and investigations

This study was conducted in six visits: first visit for assessment of inclusion criteria, second visit for HRV measurement,

third visit for initiation of insulin therapy, fourth and fifth visits were follow up visits and the sixth visit was for assessment of outcome variables.

### 2.4. Clinical examination

The study included a screening phase during which patients were approached during external consultations. A self-administrated questionnaire was used to assess the identification, medical history, smoking habit and alcohol consumption. Other clinical parameters such as height, weight, heart rate and blood pressure sitting and standing were measured in search of orthostatic hypotension. Resting electrocardiographic recording was done in search of findings which may alter HRV such as arrhythmias, bundle branch blocks and myocardial infarction. Blood samples were collected to evaluate their biochemical status (HbA1c and serum creatinine). Participants were instructed to abstain from the intake of caffeinated drinks, alcohol, smoking and strenuous exercise 12 h prior to the visit with regards to the 24-h ambulatory electrocardiographic recording for the next day. Participants otherwise continued with their usual lifestyle measures for the management of their diabetes.

### 2.5. Assessment of heart rate variability

The recommendations of the Task Force on HRV were followed for recording long term HRV [5]. We used a 3 channel ECG EKG recorder holter monitor TLC 9803. The holter monitor was connected to the patient's chest via a series of wired non-invasive pre-gelified electrodes (Tiga-med© deutschland gmbh, tiga-med EKG-klebeelectroden). These electrodes were firmly fixed to the chest at 5 specific positions. The participants were advised to carry on their usual daily activities except bathing and swimming. They were also asked to refrain from any strenuous exercise, smoke, nor drink caffeinated drinks and alcohol. Electrical signals originating from the heart were recorded via electrodes into a digital flask memory which is used to store the ECG data. The holter device was worn by the participants during their normal daily activity for a period of 24 h. Data recorded was uploaded to a computer containing the corresponding software for further processing and analysis. Artifacts and ectopic from the RR interval series were cleared up with the help of a cardiologist. HRV was then computed in the time and frequency domain ranges. This automatically provided values for parameters for the time domain range and the frequency domain range. In the time domain range, standard deviations of RR intervals (SDNN), the square root of the mean squared difference of successive RR intervals (RMSSD), and the percentage of adjacent NN intervals differing by more than 50 ms (pNN50) were analyzed. The RMSSD and pNN50 are associated with the parasympathetic activity, whereas SDNN is correlated with the sympathetic branch. In the frequency domain range, we analyzed the low frequency (LF) an index of both sympathetic and parasympathetic activity but more strongly correlated with the parasympathetic activity, and high frequency (HF) which represents the vagal activity. The LF/HF ratio (the sympathovagal balance) was also calculated. A decrease in any of these parameters represents a dysfunction of the corresponding autonomic branch [2].

Measurement of HR was performed according to the recommendations of the long term method published by the Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology for measurement and analysis of HRV. Participants arrived at 8 am and the ECG holter device was placed for 24 h. Participants were encouraged and advised to undertake their usual daily activities except bathing and swimming. They were also asked to refrain from any strenuous exercise, smoke, nor drink caffeinated drinks and alcohol. The HR data recorded was uploaded to a computer containing the corresponding software for further processing and analysis. Artifacts and ectopics from the RR interval series were cleared up with the help of a cardiologist. HRV was then computed in the time and frequency domain ranges. The software used was that of the holter system 3 channel ECG EKG recorder monitor TLC 9803 analyzer software.

### 2.6. Intervention

The intervention lasted 60 days and consisted in the optimization of glycaemic control through the initiation of a basal plus insulin regimen to existing oral anti-diabetic agents. Participants were given a glucose meter, strips and lancets and were educated in their use, including the recording of self-blood glucose monitoring (SMBG) results in a diary. They were educated on signs of hypoglycaemia and its management. Insulin glargine was administered subcutaneously once daily at bedtime, starting at 0.3 IU/Kg/day and titrated via phone calls done every 48 h to determine the most effective dose of basal insulin. Participants performed four points self-blood glucose monitoring profiles every 2 days till glycaemic targets were achieved, then once per week till the end of the study. These targets were: fasting glycaemia between 0.70 and 1.30 g/L and post prandial glycaemia <1.80 g/L [1]. Adjustments were restricted by protocol to changes by 2 or 4 units. Meal time insulin was introduced at the main meal whenever fasting blood glucose was within targets but post prandial glycaemia remained $\geq$1.80 g/L. Insulin aspart was initiated at a fixed initial dose of 4 IU and titration performed based on SMBG using fixed dose increments of 1 IU, till glycaemic targets were reached. Hypoglycaemia was defined as an event with or without symptoms consistent with hypoglycaemia and associated with a blood glucose concentration <0.70 g/L [1]. Participants were educated on signs of hypoglycaemia and how this complication could be managed.

Follow up visits were done on the 15th and 30th day after initiation of insulin. On day 60, HRV was analyzed, HbA1c, fasting blood glucose and clinical parameters measured again so as to assess the differences induced by the intervention.

### 2.7. Statistical analysis

Data was recorded and analyzed using SPSS version 20. Continuous variables were expressed as median while categorical variables in the form of percentages. Wilcoxon signed rank test was used to assess statistical significance. Analysis was

done as "on-treatment". The significance level was set at 5% such that a p-value less than 0.05 were considered significant.

## 3. Results

### 3.1. General characteristics

A total of 31 consenting type 2 diabetes mellitus patients without clinical signs of CAN (palpitations, resting tachycardia, orthostatic hypotension, and self-reported exercise intolerance) were enrolled and allocated to intervention (17 females). We recorded 2 losses to follow-up. Results from 29 participants (15 females) were analyzed. The median age was 52 [43–59] years, and known duration of diabetes 3.01 [0.56–6.71] years. At baseline, median HbA1C was 10.1 [9.1–11.9]% with a range from 7.9 to 12.6%, and median weight was 75 [69–84] Kg. As seen on Table 1, all the 29 participants were on 2000 mg of metformin; 16 (55.2%) of these participants were on a combination metformin and sulphonylureas (13 participants on gliclazide 60 mg/day and 3 on glimepiride 4 mg/day); and 1 (3.4%) on metformin plus GLP 1 receptor agonist. No patient was on SGLT 2 inhibitors.

**Table 1 – Baseline clinical characteristics of study participants.**

| Characteristics | Type 2 diabetes (HbA1c ≥ 7%) |
|---|---|
| Age (years) | 52 [43–59] |
| Sex: | |
| Female n (%) | 15 (51.7) |
| Duration of diabetes (years) | 3.01 [0.56–6.71] |
| Duration of current oral anti-diabetic medication (years) | 0.83 [0.38–2.03] |
| Oral anti-diabetic medication: | |
| Metformin, n (%) | 29 (100) |
| Sulfonylurea, n (%) | 16 (55.2) |
| GLP1 agonist n (%) | 1 (3.4) |

Values are given in M [Q1–Q3]: median [25th–75th percentile] unless otherwise stated.

Table 2 shows the analysis of HRV parameters before and after 60 days of addition of insulin. The intervention induced a significant increase in markers of the parasympathetic (PNN50: 5.70 [3.55–10.25]% to 8.12 [3.05–16.90]%, p = 0.008) and sympathetic activities (SDNN: 102.01 [90.45–111.05] ms to 122.40 [91.70–135.95] ms, p = 0.01). There was no significant change in any of the elements of the time domain range and heart rate.

The intervention led to a significant decrease in fasting glycaemia from 1.96 [1.83–2.13] g/L to 1.04 [1.01–1.07] g/L and glycated hemoglobin from 10.1 [9.1–11.9]% to 6.7 [5.9–6.9]%, p < 0.001 Figure 1.

As shown in Figure 2, there was a significant positive correlation between RMSSD, a parameter of the time domain measure of HRV representing parasympathetic activity and the dose of insulin used during the intervention. We did not find a significant correlation between the dose of insulin used and the weight gain, reduction in HbA1c and the other HRV parameters.

### 3.2. Adverse effects

Two out of 29 (6.9%) participants reported having 2 episodes or less of symptomatic confirmed hypoglycaemia less than 0.70 g/L but greater than 0.50 g/L. Therefore, no severe hypoglycaemic episode was noted.

Concerning weight gain, a significant increase in weight from a median of 75 [69–84] Kg to 77 [71–86] Kg, p < 0.001

**Table 2 – Clinical and HRV parameters before and after the intervention.**

| Characteristics | T2DM patients | | P-value |
|---|---|---|---|
| | Day 0 M [Q1–Q3] | Day 60 M [Q1–Q3] | |
| SBP (mmHg) | 126 [121–130] | 128 [120–136] | 0.194 |
| DBP (mmHg) | 74 [67–81] | 76 [67–84] | 0.632 |
| Weight (Kg) | 75 [69–84] | 77 [71–86] | **<0.001** |
| BMI | 26.57 [24.56–29.62] | 26.95 [25.38–31.04] | **<0.001** |
| Heart rate ECG holter (bpm) | 79 [77–83] | 82 [76–86] | 0.13 |
| Markers of sympathetic activity | | | |
| SDNN (ms) | 102.01 [90.45–111.05) | 122.40 [97.01–135.95] | **0.01** |
| SDANN (ms) | 88.04 [72.95–99.70] | 89.70 [74.01–109.71] | 0.47 |
| LF ($ms^2$) | 259.90 [177.75–632.20] | 214.20 [115.257–13.90] | 0.35 |
| Markers of parasympathetic activity | | | |
| PNN50 (%) | 5.70 [3.55–10.25] | 8.11 [3.05–16.90] | **0.008** |
| RMSSD (ms) | 47.06 [39.70–73.15] | 47.40 [29.75–86.95] | 0.24 |
| HF ($ms^2$) | 238.90 [112.05–689.85) | 228.50 [100.40–765.05] | 0.16 |
| Markers of sympatho vagal balance: | | | |
| LF/HF | 0.95 [0.77–1.42] | 0.99 [0.81–1.63] | 0.35 |

M [Q1–Q3]: median [25th–75th percentile]

Bpm: beat per minute, ms: millisecond, $ms^2$: millisecond square, HR: heart rate, bpm: beats per minutes, SDNN: standard deviation of all normal to normal interval, SDANN: standard deviation of average normal to normal interval, RMSSD: square root of the mean of the sum of the squares of differences between adjacent normal to normal intervals, PNN50: Percentage of normal to normal intervals differing by more than 50 ms, LF: low frequency, HF: high frequency.

Boldface type indicates statistical significance where p < 0.05.

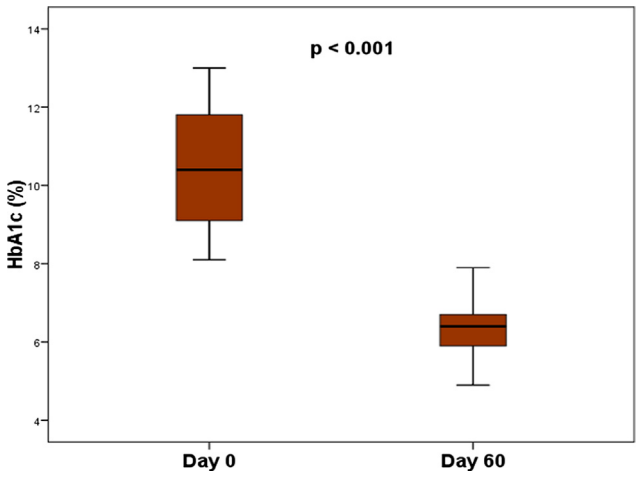


**Fig. 1 – Comparison of glycated hemoglobin before and after the intervention.**

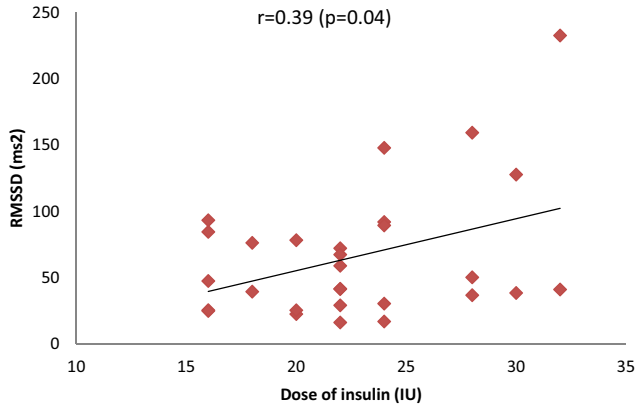


**Fig. 2 – Correlation between RMSSD (a parameter representing the parasympathetic branch) and dose of insulin used.**

was noted after 60 days of initiation of a basal plus insulin regimen.

## 4. Discussion

This study aimed to determine the effects of optimization of glycaemic control using insulin in poorly controlled type 2 diabetes mellitus patients. Our results show that heart rate variability parameters representing the overall variability and that representing the parasympathetic branch were significantly improved after 60 days of initiation of a basal plus insulin regimen. However, additional research is needed to determine if the increase in HRV parameters is due to insulin use or the good glycaemic control or both.

The intervention induced a significant increase in elements of the time domain range; SDNN a marker of overall HRV by 19.6% and PNN50 a marker of parasympathetic activity by 42.1%. This is in accordance with Peter Gaede et al. and Verma S et al, who provided evidence that an intensive insulin treatment in type 2 diabetes patients could slow down the development of autonomic neuropathy [9,10]. The increase in PNN50 (HRV parameter representing the parasympathetic activity) in our study is explained by the fact that bringing exogenous insulin put the β- cells to rest and improved the activities of the parasympathetic branch which is responsible for insulin secretion [11].

Improving glycaemic control is a challenging task for clinicians. The results of this present study showed that a significant reduction in 3.4 points of HbA1c was achieved after 60 days of initiation of a basal plus insulin regimen. Likewise, Bolli et al. in 2015 showed a reduction in HbA1c of 1.6 points after 6 months of treatment and Janka et al. in 2005 showed a reduction of 1.64 points after 24 weeks of addition of insulin glargine to the oral hypoglycaemic agents [12,13]. A possible explanation for this slight difference is the fact that our sample size was smaller and study duration shorter (29 participants and 60 days respectively), thus allowing a close follow-up of the participants. Furthermore, SMBG was done once every 2 days allowing for a rapid adjustment of the insulin dose whenever required, hence permitting a tight glycaemic control during the 60 days. In the other studies, monitoring was done mainly during follow up visits. This is important because the benefits of self-monitoring of blood glucose in insulin treated patients are already known [14]. This goes to emphasize on the use of SMBG whenever possible since it allows targets to be achieved within a short period of time.

Concerning side effects, significant weight gain of 2 kg was observed after 60 days of intervention. Corroborating with our study, Lankisch et al in 2015 demonstrated a significant increase in 1.2 kg of body after 6 months of initiation on a basal plus insulin regimen [15]. Since insulin is an anabolic hormone amongst other reasons, slight weight gain is usually possible especially at the beginning of the therapy and this weight gain may vary depending on the insulin regimen. Only 3 of the 29 participants necessitated the addition of a meal time insulin in this study. However, participants were poorly controlled patients who had not modified their anti-diabetic drugs in the past 3 months and reported with polyuria, nocturia and weight loss. The weight gain might be related to the better glycaemic control. Therefore, the mean increase in 2 kg of weight cannot be attributed to insulin alone. Two out of the 29 participants reported with symptomatic confirmed hypoglycaemia less than 0.7 g/L but greater than 0.50 g/L. Therefore, no severe hypoglycaemic episode was noted. This is in line, with the study carried out by Yki Jarvinen *et al.* in the United Kingdom Prospective Diabetes Study (UKPDS), which supported the fact that insulin glargine plus oral therapy may provide better post dinner glucose control as well as less hypoglycaemic events [16]. For many years, the cardiovascular safety of insulin was unclear but large outcome trials have helped to improve our understanding on the subject. Although the ACCORD trial showed increased mortality in the intensive group when compared to the conventional group, ADVANCE and ORIGIN showed the neutral effect of insulin on the cardiovascular system, thus providing evidence for the cardiovascular safety of insulin [17].

Our result suggests that optimizing glycaemic control using insulin improves the overall HRV represented by SDNN, and PNN50, which represents the parasympathetic branch.

This means that optimizing glycaemic control using insulin improves the parasympathetic activity of the autonomic nervous system which tends to be the first branch affected by neuropathy [2]. There is evidence that parasympathetic branch of the autonomic nervous system plays an important role in mediating both pre and post prandial glycaemia by increasing insulin secretion and insulin sensitivity [18]. However, there was no significant shift of the sympatho-vagal balance represented by the LF/HF. This therefore stresses on the importance of ongoing strategies to manage glycaemic control in patients with type 2 diabetes mellitus.

### 4.1. *Limitations*

The major limitation of this study is the absence of a control group. However, this quasi experimental design study did not aim to assess the effect of insulin on HRV but that of improving glycemic control using insulin on the HRV which reflects cardiovascular autonomic functions. The beneficial effect observed over this short period is an important finding although we cannot conclude whether these effects are due to insulin alone, the improvement of glycemic alone or both.

The choice of the method for HRV measurement was a limiting factor for analysis. The use of HRV parameters of the frequency domain range (LF, HF and LF/HF) is recommended whenever short term measurement is done. The long term recording (24-h ambulatory EKG recording) was used because of the possibility of measuring long range heart rate fluctuations, better reproducibility and sensitivity which are major advantages over short term recording in the assessment of HRV. Mind full of the fact that the duration of recording is dictated by the nature of each investigation, the task force standards of measurement on HRV recommends the use of 24 h indices to assess intervention therapies since they are more stable. Also, using a larger sample size might have shown a significant improvement in all HRV parameters. In addition, the follow up of 60 days was short and long term effects cannot be guaranteed from this. Despite this, we had marked effects on both the glycaemic control and in some HRV parameters, suggesting that these effects could be better on the long term. However, these findings should be interpreted cautiously, as the absence of a control group limits our ability to claim causality.

## 5. Conclusion

Short term optimization of glycaemic control using a basal plus insulin regimen while inducing a significant reduction in glycated hemoglobin, improves the sympathetic and parasympathetic activities measured by 24-h ambulatory ECG. This suggests that tight glycaemic control using insulin may revert CAN in patients with type 2 diabetes.

## 6. Ethical considerations

The study was approved by the Centre Regional Ethical Committee (Authorization No 0110/CRERSHC/2016) and the Institutional Ethical Research Committee of Université des Montagnes in Cameroon (Authorization No 2016/071/UdM/PR/CAB/CIE) and was conducted in accordance with the guidelines of the Helsinki Declaration. All participants provided written informed consent prior to inclusion.

## 7. Authors' contributions

Study conception and design: CMM, CNN, MAK, JCM, ES.

Data collection: CMM, CNN, AKM, MYD.

Statistical analysis: CMM, CNN.

Drafting: CMM, CNN.

Critical discussion and manuscript revision: CMM, CNN, MAK, LMK, MYD, ES, JCM.

All the authors approved the final version of the manuscript.

## Declaration of Competing Interest

The authors declare that they have no competing interests.

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