Online Journal of
Clinical and Medical Case Reports

LAURIN PUBLISHERS

Research Article

The Relationship Between some Macro and Micro-nutrient Status on Gut Dysbiosis Pattern in HIV Patients in the South West and Littoral Regions of Cameroon

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Submission: November 25, 2024; Published: January 08, 2025

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ABSTRACT

Background: Malnutrition leads to an alteration of the digestive microbiota with a disappearance of methanogenic arches that are intolerant to oxygen, a depletion of anaerobic bacteria and a relative proliferation of Proteobacteria, Streptococcus and Staphylococcus aureus which are oxygen tolerant bacterial groups containing many pathogens potentially responsible for infectious diarrhoea and immune system alterations.

Aim: The aim of this study was to investigate the impact of nutritional deficiency on the microbiota in HIV Positive Patients on ART.

Materials and Methods; Blood and stool samples were collected from 143 HIV positive participants in a period of 3 months. Stools culture was done to identify gut bacteria. Blood samples were analysed for micro (calcium and magnesium) and macronutrient (albumin, and glucose). Questionnaires were used for sociodemographic data while anthropometric data was measured using standard methods.

Results: The prevalence of wasting and obesity was 4.2% (6) and 18.9% (27) respectively. Calcium deficiency was 28.7% (41) and magnesium deficiency was 13.3% (19). With respect to macronutrient deficiency,

hypoalbuminaemia was 17.7% (25) while hypoglycaemia was 16.1 % (23). Proteobacteria, Enterococcus 57% (82) and Fusobacteria 78% (112) had increase prevalence. Gut Firmicute (lactic acid bacteria), actinobacteria and Bacteroidetes which are desirable flora had reduced prevalence. We had a Firmicute to Bacteroidetes ratio of 10:8. Micronutrient deficiency was mostly associated with increase prevalence of Klebsiella spp ($p = 0.04$), Citrobacter

spp ($p = 0.04$) and Enterobacter spp ($p = 0.01$) while macro-nutrient deficiencies were associated reduce Bifidiobacterium spp ($p = 0.02$) and Lactobacillus ($p = 0.02$). However, both were associated with increased proteobacteria. Low income was the most likely reason for poor feeding which resulted to malnutrition.

Conclusion: Malnutrition is still a major problem in the management of HIV and the prevention of AIDS in Cameroon. Our findings uncover dysbiotic changes in gut microbiome in HIV infected persons with malnutrition and are associated with increase susceptibility to infection by opportunistic microbes. Familiarity with these associations will be of tremendous use to the practitioner as well as the patient.

KEYWORDS

HIV/AIDS, Malnutrition, Gut Microbiome, Nutrient deficiency, Macro and Micronutrient

LIST OF ABBREVIATIONS

HIV: Human Immunodeficiency Virus; AIDS: Acquired Immune Deficiency Syndrome; OC: Oral Candidiasis; HAART: Highly Antiretroviral Therapy; PLWHA: People Living with HIV/AIDS; CD4: Cluster of Differentiation; SCFAs: Short Chain Fatty Acids

INTRODUCTION

Background for study

Malnutrition is a severe non-communicable disease in low- and middle-income countries, two forms exist, namely undernutrition (stunting and wasting) and over nutrition (obesity). World Health Organization (WHO) reported prevalence of stunting and wasting among 160 million adults and 50 million children respectively, worldwide [1]. Sub-Saharan Africa has the highest prevalence estimates of undernourishment in the world, with 23.2% of its population affected [2]. Nutritional issues such as specific nutrient deficiencies, less ideal diet composition, and excess calorie consumption are a challenge in developed countries [3]. Sub- Saharan Africa has the highest burden of Human Immunodeficiency Virus (HIV) infection, constituting 54% (20.7 million) of the estimated people living with HIV globally in 2018 with Cameroon having an estimate of 3.4% of HIV infection in 2019[4].

The human gut microbiome is composed of an estimated 1014 microbes representing approximately 1000 species and including archaea, eukaryotes and predominantly, members of the 5 bacterial phyla of Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Verrucomicrobia [5]. These microbiomes collectively act like

a bioreactor for fermenting dietary macronutrients to health-promoting metabolites, such as, short chain fatty acids (SCFAs) [6], amino acids (AAs) [7] and vitamins. Gut bacteria educate the immune system and help control harmful pathogens[8]. However, distinctive compositions of this complex community have been associated with pathologies that are of increased prevalence in HIV infection, including metabolic disorders, wasting/malnutrition [9-11], and susceptibility to opportunistic infections [12]. Quantitation of the relative proportions of each phylum and more-specific taxonomic ranks have identified consistent phenotypes present in the setting of HIV infection, malnutrition, and states of persistent systemic inflammation and adaptive immune activation. Alterations of the gut microbial population in people with HIV, including those in whom HIV is well controlled with antiretroviral therapy (ART) has been reported. Microbial translocation and its association with immune activation and chronic inflammation during HIV disease progression highlights the role of microbiota in HIV pathogenesis [13]. An altered gastrointestinal microbiome appears to occur early in the course of HIV-infection and may contribute to, or is at least correlated with mucosal inflammatory activity, mucosal CD4+T-cell depletion, and peripheral CD8+T-cell activation [14]. These findings do not relate with ART, possibly because of persistent presence of HIV at the mucosal surface or the lasting depletion of gastrointestinal CD4+T cells and other immune effectors [5,15].

HIV infection results in functionally defective metabolic ability at the individual level to absorb, store and utilize nutrients thus resulting in nutrient deficiencies, compromised immunity and increased risk of acquiring infectious diseases. Insufficient food intake, together or with malabsorption, result in further progression of HIV/AIDS [16]. Between 2010 and 2018 there has been more than a two-fold increase in the number of HIV-positive people receiving antiretroviral therapy (ART), which reached 10.3 million in Eastern and Southern Africa, the world's most affected regions. Reports revealed that in 2018, 79% were on treatment (equivalent to 67% of all people living with HIV in the region), and 87% of those on treatment had achieved viral suppression (equivalent to 58% of all people living with HIV in the region) [4]. Three key factors contributing to malnutrition in patients with HIV/AIDS include inadequate intake, malabsorption, and increased energy expenditure [17]. It has been shown that malnutrition leads to an alteration of the digestive microbiota with a disappearance of methanogenic arches that are intolerant to oxygen, a depletion of anaerobic bacteria and a relative proliferation of Proteobacteria, Streptococcus and Staphylococcus aureus which are oxygen tolerant bacterial groups containing many pathogens potentially responsible for infectious diarrhoea [1]. To be understand the correlation between nutritional status and the alteration in gut microbiome in HIV Positive patients, the is a need to carry out a study aimed at investigating the impact of nutritional deficiency on the microbiota in HIV Positive Patients on ART. Thus, the main objective of this study was to evaluate the effect of macro and micro-nutrient deficiency on the gut microbiome dysbiosis pattern in HIV Positive Patients on ART in the Southwest and Littoral Regions of Cameroon. We specifically wanted to know the prevalence of malnutrition among HIV positive patients on ART, identify the bacteria phylum (microbiome dysbiosis) that is more prominent in the gut of HIV Positive Patients on ART and to determine the relationship between macro and micro-nutrient deficiencies with gut

microbiome dysbiosis pattern in HIV positive.

MATERIAL AND METHODS

Study design, area, and population

The study was a cross-sectional study. The participants were recruited from the months of April to July 2020. The study population comprised HIV positive patients on ART who visited the hospitals and HIV treatment centres within Fako division in the Southwest region and Mungo division in the Littoral Region. Areas where the participants were recruited include Tiko, Bota (Limbe), Kompina, Idenau and some HIV support groups located in Tiko.

Inclusion and exclusion Criteria

All HIV positive patients on ART aged 18 years and above who visited the selected treatment centres and support groups, and who accept to sign a written informed concern were recruited in to the study. All HIV positive patient with severe comorbidities other than TB and HIV positive pregnant women, were excluded from the study.

Sample size

A sample size of 142 was obtained using the formula described by Swinscow [1] and 10.3% prevalence of malnutrition in IV positive patients from a South African study [16].

Ethical consideration

Ethical clearance was obtained from the Faculty of Health Sciences Institutional Review Board (IRB/FHS). Administrative authorisation was obtained from the Southwest Regional Delegation of Public Health where the study was done. Hospital authorization was obtained from Cameroon development corporation (CDC) and each volunteer signed a written informed consent form before recruitment into the study.

Sampling Technique and Sample Collection

Sampling technique

Clients visiting the Tiko central clinic who met the inclusion criteria were recruited after signing an informed consent form. Other participants were recruited from support groups by visiting, educating, and sensitizing them on the importance of good nutrition and about the project, explaining the benefits involved in taking part in the study. Participants from Bota, Idenau and Kompina were mobilized by Health workers in the various health centres. Consenting participants were assisted in completing a structured questionnaire on demography, socio-economic status, and nutritional status. Weight and height were measured from each participant.

Collection of stool and blood Samples

Stool was collected from each study participants into a dry and sterile container and 4 mL blood samples were

collected into dry tubes only from those who fasted overnight (8-14 hours). The blood samples were transported on ice packs to the laboratory for processing and analyses. The blood samples were centrifuged at 5000 rpm for 5mins to obtain serum. The serum was aliquot into Eppendorf tubes and stored in duplicates at about -20°C and -80°C. The samples were later analysed in batches.

Analysis on blood samples for measurement of nutrients

The stored serum was used for the analysis of macro (Albumin and glucose) and micronutrients (calcium and magnesium) using colorimetric method. A normal quality control serum was used with every batch of analysis.

Culturing and identification of culturable gut flora

Culture plates were prepared following the manufacturer's instruction. Stool was inoculated on; Brain Heart Infusion (BHI)+ 5% human blood + chloramphenicol at 37°C aerobically, BHI + 5% human blood + chloramphenicol at 37°C anaerobically, BHI + chloramphenicol at 37°C aerobically, BHI + gentamicin at 37°C anaerobically, BHI chloramphenicol + gentamicin at 37°C anaerobically, BHI agar at 37°C aerobically, BHI agar ¬+ 5% human blood at 37°C aerobically, BHI agar ¬+ 5% human blood at 37°C anaerobically and Mueller Hinton agar at 37°C aerobically, for culture of Bacteroides, Bifidobacterium, and Fusobacterium (Figure 1). Stool was also inoculated on deMan Rogosa Sharpe (MRS) supplemented with tween, Sabouraud dextrose agar, and Mannitol salt agar (MSA) for identification of Enterococcos, yeast, Clostridrium, Lacobaccilus and Staphylococcus (Figure 2). Some of the stool was inoculated on MacConkey agar at 37°C aerobically and anaerobically for identification of enteric bacteria (Figure 3). The culture was carried out for 24 – 48 hours and the bacteria characterized using Gram staining and bbiocemical tests (catalase, oxidase and Enterosysterm 18R). Direct stool microscopy was done using normal saline and Lugol's iodine solution within two hours of stool collection immediately after inoculation on culture plate. The sample was smeared on a slide with a drop of normal saline solution (for the detection of trophozoites of parasites) and a drop of Lugol's iodine solution (for detection of cyst of the parasites).

Data analysis

The data obtained was recorded in the respective logbooks, entered Microsoft excel and analysed using the statistical package SPSS version 25 for data analysis. Mean and standard deviations were used while Multinomial regression and Chi-square tests were used to assess the associations between nutrient levels and the gut microbiome frequencies. A p-value < 0.05 was considered statistically significant.

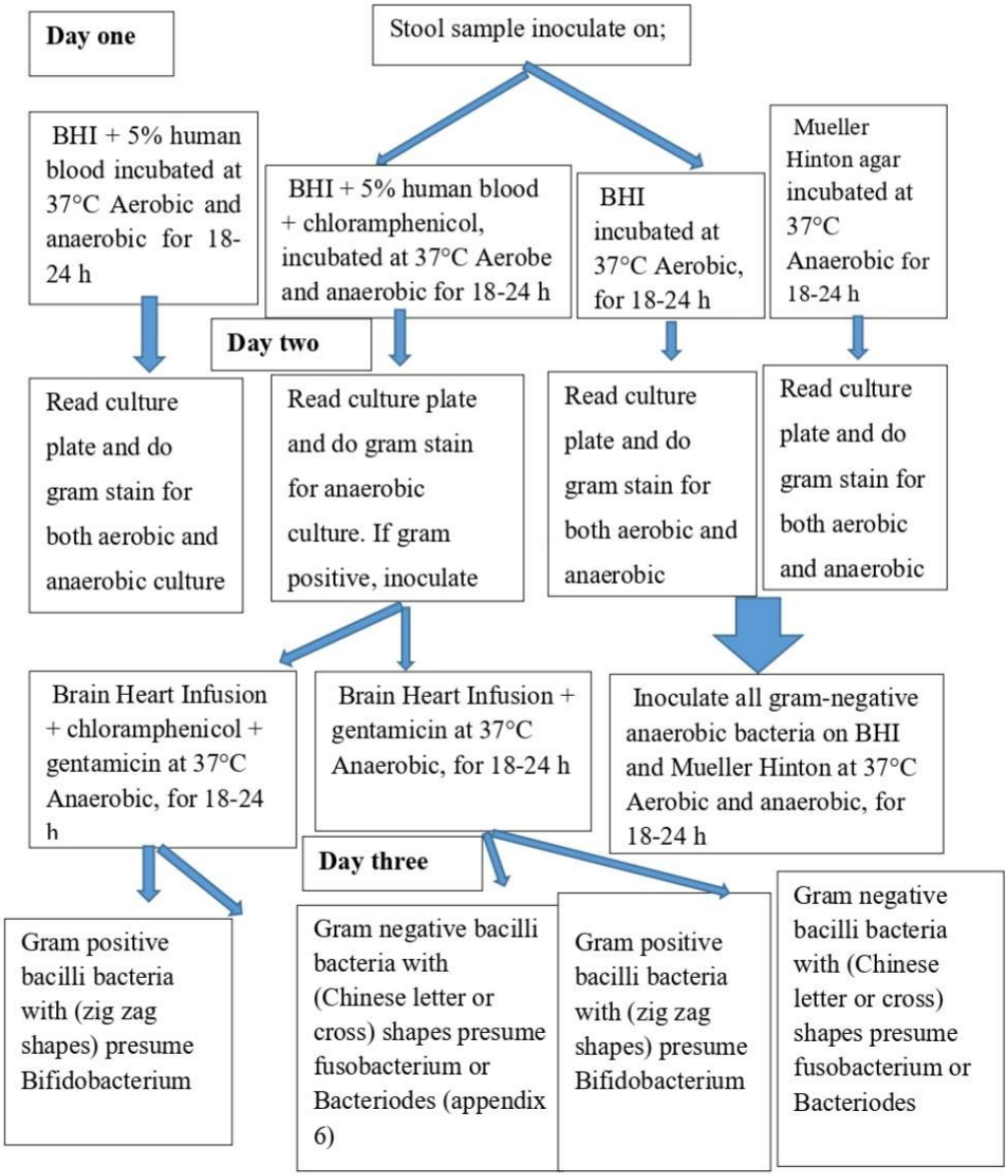

Figure 1: Identification of Bacteroides, Bifidobacterium, and Fusobacterium.

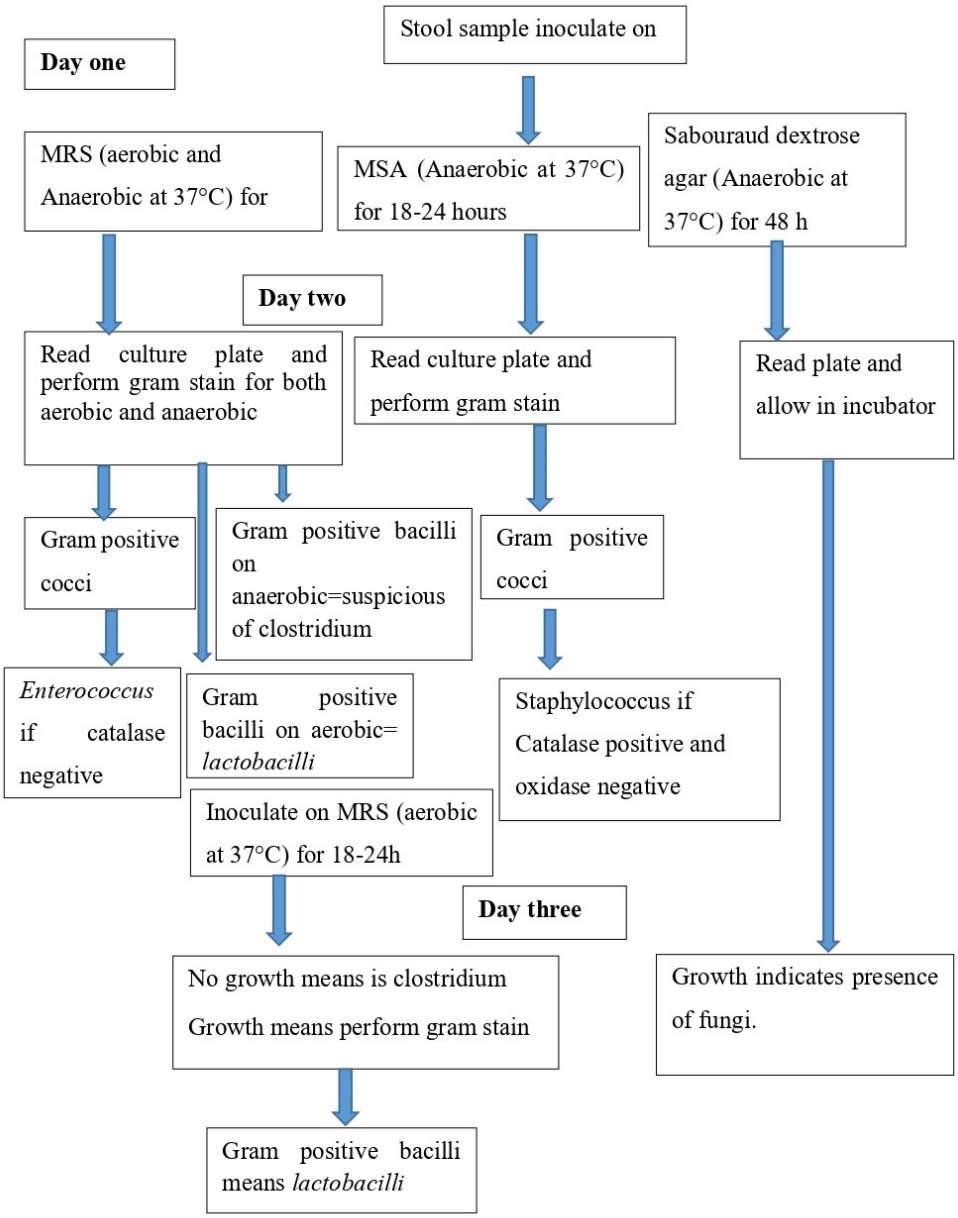

Figure 2: Identification of Enterococcus, yeast, Clostridium, Lactobacillus and Staphylococcus.

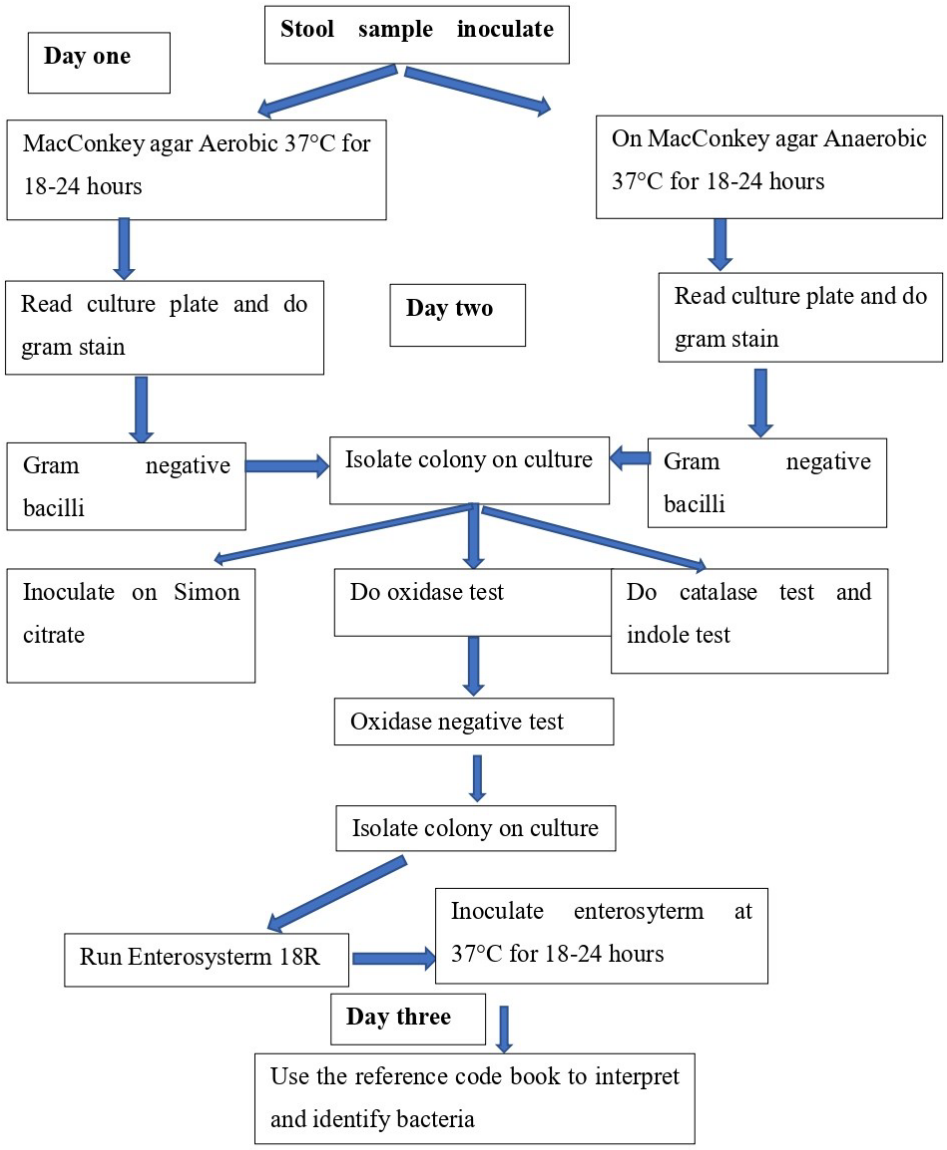

Figure 3: Identification of enteric bacteria

RESULTS

Sociodemographic characteristics of study participants

A total of 143 participants were recruited into this study, 90 (62.95%) were females while 53 (37.06%) were males. Those ≤ 46 years of age were 68 (48.23%) while 73 (51.77 %) were above 46 years, with mean and median ages of 46.24 and 47 respectively (Table 1). Most of our participants 81(56.64%) had at least completed primary school, 54 (37.76%). Participants with no employment were 44 (30.77%), while 98 (58.53) were CDC employed workers. With respect to income, 108 (84.38%) had monthly incomes of less than 100,000 frs CFA while 18 (14.06%) earned between 100,000-200,000 frs CFA, and just 2 (1.65%) earned above 200,000 frs CFA per month. Also had 45(31.69%) of the participants were unmarried, 58 (40.85%) married, 8 (5.63%)

divorced and 31 (21.83%) widows.

Prevalence of macro and micronutrient deficiencies among HIV positive patients

Macro and micronutrients play significant role in gut microbiome colonialization. using spectrophotometric techniques, of 143 participants, a total of 41 (28.7%) had hypocalcemia, 19 (13.29%) had hypomagnesemia, 25 (17.5%) had hypoalbuminemia while 23 (16.08%) had hypoglycemia (Table 1)

Micro and Macronutrients (reference range)	Category	Frequency n (%)	95% lower Confidence Level	95% Upper Confidence Level
Calcium (8.6-10.2 mg/dL)	Low	41 (28.7)	3.40	12.48
	Normal	89 (62.2)	81.65	92.92
	High	13 (9.1)	1.99	9.83
	Total:	143		
Magnesium (1.6-2.5 mg/dL)	Low	19 (13.3)	8.19	19,97
	Normal	120 (83.9)	77.64	90.10
	High	4 (2.8)	0.43	6.01
	Total:	143		
Albumin (3.5-5.0 g/dL)	Low	25 (17.5)	0.43	6.01
	Normal	96 (67.1)	92.03	98.86
	High	22 (15.4)	0.17	4.96
	Total:	143		
Glucose (74-110 mg/dL)	Low	23 (16.1)	10.48	23.15
	Normal	109 (76.2)	69.15	83.55
	High	11 (7.7)	3.40	12.48
	Total:	143		
Triglycerides (<150 mg/dL)	Normal	125 (87.4)	75.14	95.44
	High	18 (12.6)	7.63	19.16
	Total:	143		
Total Cholesterol (<200 mg/dL)	Normal	128 (89.6)	72.2	93.1
	High	24 (16.8)	11.06	23.94
	Total:	143		

Table 1: Prevalence of macro and micronutrient in HIV positive patients.

Dietary quality

A total of 103 (73.57%) of our participants ate proteins daily, 31 (22.14%) ate 4-5 times weekly, 4 (2.86%) ate 2.3 times weekly and only 2 (1.43%) ate weekly. Participants that ate fruits on a weekly basis were 89 (65.44%) while 44 (32.35%) ate 4-5 times weekly, 2 (1.47%) and 1 (0.74%) ate 203 times weekly and ones a week respectively. 139 (97.89%) of participants ate carbohydrates daily while only 3 eat it 4-5 times a week. This study also revealed that 21 (16%) of participants had feeding difficulties.

The mean of BMI was 25.12 ±5.57 SD. A total of 6 (4.20 %) were underweight, 35 (24.48 %) were overweight of which 27 (18.88 %) were obese.

Prevalence of culturable bacteria isolated among HIV Positive Patients.

Stool from participants were cultured and biochemical characterization done to identify some culturable bacteria in the gut, Shigella 4 (2.80%), Klebsiella 10 (6.99%), Salmonella 32 (22.38%) and Proteus 33 (23.08%) where the least occurring microbe in the gut, while Fusobacteria 112(78.32%), Bifidobacterium 106 (74.33%), Bacteroides 102 (71.1%) and Enterococcus 82(57.34%) where the most prominent bacteria genus isolated (Table 2). We also isolated 54 (38 %) of *candida spp*

Bacteria	Frequency n (%)	95% lower Confidence Level	95% Upper Confidence Level
Escherichia spp	49 (34)	26.54	42.66
Shigella spp	4 (3)	0.77	7.01
Klebsiella spp	10 (7)	3.40	12.48
Proteus spp	33 (23)	16.45	30.85
Enterobacter spp	77 (54)	45.32	62.21
Citrobacter spp	72 (50)	100.00	27.19
Salmonella spp	32 (22)	15.84	30.10
Clostridium spp	71 (50)	41.19	58.13
Enterococcus spp	82 (57)	48.81	65.57
Lactobacillus spp	77 (54)	45.32	62.21
Staphylococcus spp	78 (55)	46.01	62.88
Bacteroides spp	102 (71)	63.18	78.58
Fusobacterium spp	112 (78)	70.66	84.77
Bifidobacterium spp	106 (84)	66.14	81.08

Table 2: Frequency of Bacteria isolated.

All participants had at least a member of the Gut phylum Proteobacteria, 128 (90.1%) had Firmicutes, 112 (78.3%) had Fusobacteria, 106 (74.13%) had Actinobacteria, while 102 (71.3%) had Bacteroidetes and 54 (37.8%) had Ascomycota.

Effect of macro and micro-nutrient deficiencies on gut microbiome

Gut microbiomes play a very important role in immunity, digestion, and absorption of nutrients. Using culture dependent techniques and biochemical characterization and by measuring levels in blood of micronutrients through spectrophotometry, we observed that Enterobacter (p=0.01) had statistically significant association with hypomagnesemia (19), Escherichia, Shigella, Clostridium and Lactobacillus (p=0.08, 0.09, 0.06, 0.07 respectively) had p values slightly higher in those with hypomagnesaemia (Table 3)

	Magnesium				
	Low (n =19) n (%)		Normal (n =120) n (%)		
Bacteria Genus/ Fungi	**Present**	**Absent**	**Present**	**Absent**	**P value**
Escherichia spp (49)	9 (18.4)	10 (10.3)	10 (75.6)	83 (88.3)	0.08
Shigella spp (4)	2 (50)	17 (12.2)	2 (50)	118 (8.9)	0.09
Klebsiella spp (10)	0 (0)	19 (14.3)	10 (100)	10 (82.7)	0.35
Proteus spp (33)	5 (15.2)	14 (12.7)	28 (84.8)	92 (83.6)	0.52
Enterobacter spp (77)	5 (6.5)	14 (21.1)	71 (92.2)	49 (74.2)	**0.01**
Citrobacter spp (72)	10 (4)	15 (18.3)	33 (94)	63 (78.5)	0.82
Salmonella spp (32)	5 (15.6)	14 (12.6)	27 (84.4)	93 (83.8)	0.52
Clostridium spp (71)	5 (7)	14 (19.4)	63 (88.7)	57 (79.2)	0.06
Enterococcus spp (82)	13 (15.9)	6 (9.8)	67 (81.7)	53 (86.9)	0.56
Lactobacillus spp (77)	6 (7.8)	13 (19.7)	68 (88.3)	52 (78.8)	0.07
Staphylococcus spp (78)	8 (10.3)	11 (16.9)	68 (87.2)	52 (80)	0.49
Bacteroides spp (102)	14(13.7)	5 (12.2)	85 (83.3)	35 (85.4)	0.96
Fusobacterium spp (112)	14 (12.5)	5 (16.1)	95 (85.8)	25 (80.6)	0.85
Bifidobacterium spp (106)	15 (14.2)	4 (10.8)	87 (82.1)	33 (89.2)	0.41
Candida spp (54)	5 (9.3)	14 (15.7)	46 (85.2)	54 (83.1)	0.18

Table 3: Association between Magnesium deficiency and gut bacteria.

There was a significant association between calcium deficiency (hypocalcaemia) with presence of Klebsiella (p = 0.04) and Citrobacter (p = 0.04) (all of whom are members of the phylum Proteobacteria) (Table 4). This suggests a strong association between protobacteria with calcium levels in blood.

Bacteria Genus/ Fungi	Calcium				
	Low (n = 41) n (%)		Normal (n = 89) n (%)		
	Present	**Absent**	**Present**	**Absent**	**P value**
Escherichia spp (49)	12 (24.5)	28 (30.9)	34 (68.5)	55 (58.5)	0.41
Shigella spp (4)	2 (50)	38 (28.1)	2 (50)	87 (62.6)	0.57
Klebsiella spp (10)	1 (10)	40 (30.1)	6 (60)	83 (62.4)	**0.04**
Proteus spp (33)	10 (30.3)	32 (28.2)	19 (57.6)	70 (63.6)	0.073
Enterobacter spp (77)	21 (27.3)	20 (30.3)	46 (59.7)	43 (65.2)	0.22
Citrobacter spp (72)	2 (13)	17 (34.4)	47 (68)	73 (59.1)	**0.04**
Salmonella spp (32)	11 (34.3)	30 (27.0	17 (53.1)	71 (64.9)	0.46
Clostridium spp (71)	21 (29.6)	20 (29.8)	48 (57.7)	41 (66.7)	0.29
Enterococcus spp (82)	23 (28)	18 (29.5)	52 (63.4)	37 (20.7)	0.92
Lactobacillus spp (77)	23 (33.8)	18 (22.7)	43 (58.5)	46 (69.7)	0.23
Staphylococcus spp (78)	25 (31.1)	16 (24.6)	47 (60.3)	42 (64.6)	0.56
Bacteroides spp (102)	29 (28.4)	12 (29.3)	64 (62.7)	25 (61)	0.98
Fusobacterium spp (112)	34 (30.4)	7 (22.6)	68 (61.6)	20 (65.5)	0.56
Bifidobacterium spp (106)	32 (30.2)	9 (21.3)	63 (59.4)	26 (70.3)	0.45
Candida spp (54)	13 (31.5)	28 (57.3)	38 (24.1)	50 (70.4)	0.25

Table 4: Association between calcium deficiency and gut bacteria.

Effect of macro-nutrient deficiency on gut bacteria

Macronutrients play a role in the development of the gut microbiome. By measuring the levels of albumin (the

most abundant protein in blood) and through culture dependent techniques, and biochemical characterization for the identification of culturable gut flora, Lactobacillus (0.02), Staphylococcus (0.02) and Bifidobacterium (0.02) were significantly reduced in persons with hypoalbuminemia 25 (17.5%); while Shigella (p=0.009) was more prevalent in hypoalbuminemia (Table 5). Bifidobacterium (p=0.02) decreased significantly in people with hypoglycaemia 23 (16.1%) (Table 6).

Bacteria Genus/ Fungi	Albumin				
	Low (n = 25)		Normal (n = 96)		
	Present	Absent	Present	Absent	P value
Escherichia spp (49)	8	17	34	62	0.92
Shigella spp (4)	3	22	1	95	**0.009**
Klebsiella spp (10)	1	24	8	88	0.67
Proteus spp (33)	3	22	25	71	0.33
Enterobacter spp (77)	14	11	49	47	0.56
Citrobacter spp (72)	9	14	38	71	0.79
Salmonella spp (32)	4	21	23	73	0.7
Clostridium spp (71)	9	16	53	43	0.16
Enterococcus spp (82)	17	8	37	44	0.45
Lactobacillus spp (77)	11	13	59	37	**0.02**
Staphylococcus spp (78)	8	17	60	36	**0.02**
Bacteroides spp (102)	16	9	72	24	0.38
Fusobacterium spp (112)	21	4	72	24	0.38
Bifidobacterium spp (106)	15	10	78	18	**0.02**
Candida spp (54)	8	17	37	59	0.7

Table 5: Association between albumin deficiency and gut bacteria.

Bacteria Genus/ Fungi	Glucose				
	Low (n=23) n (%)		Normal (n=109) n (%)		
	Present	Absent	Present	Absent	P value
Escherichia spp (49)	5 (10.2)	18 (19.1)	39 (79.6)	70 (74.5)	0.31
Shigella spp (4)	1 (25)	22 (15.8)	2 (50)	107 (77)	0.34
Klebsiella spp (10)	1 (10)	22 (16.5)	9 (90)	100 (75.2)	0.51
Proteus spp (33)	7 (21.2)	16 (14.5)	25 (75.8)	84 (76.4)	0.38
Enterobacter spp (77)	12 (15.6)	11 (16.7)	58 (75.3)	51 (77.3)	0.80
Citrobacter spp (72)	9 (18.0)	14 (15.1)	38 (76)	71 (76.3)	0.78
Salmonella spp (32)	6 (18.8)	19 (15.3)	25 (78.1)	84 (75.7)	0.52
Clostridium spp (71)	14 (19.7)	9 (12.5)	53 (74.6)	56 (77.8)	0.37
Enterococcus spp (82)	13 (15.9)	10 (16.4)	61 (74.4)	48 (78.7)	0.56
Lactobacillus spp (77)	14 (18.2)	5 (13.6)	59 (76.4)	50 (75.5)	0.40
Staphylococcus spp (78)	13 (15.7)	10 (15.4)	61 (79.5)	47 (72.3)	0.17
Bacteroides spp (102)	18 (17.6)	5 (12.2)	77 (75.5)	32 (78)	0.64
Fusobacterium spp (112)	16 (14.3)	7 (22.6)	86 (76.8)	23 (74.2)	0.35
Bifidobacterium spp (106)	21 (19.8)	2 (5.4)	80 (75.5)	29 (78.4)	**0.02**
Candida spp (54)	11 (20.4)	12 (13.5)	39 (72.2)	70 (78.7)	0.55

Table 6: Association between glucose and gut bacteria.

DISCUSSION, CONCLUSION AND RECOMMENDATION

DISCUSSION

Prevalence of malnutrition

This study illustrates the effects of nutritional status on the culturable gut microbiota of HIV positive patients

in the Fako Division (Southwest region) and Mungo division (Littoral region) of Cameroon. This study found malnutrition among HIV positive patients at 4.20 % and 24.48 % overweight, of which 18.88 % were obese. This finding was similar to that reported in 2019 by UNICEF which saw wasting at 5.2% but had a low rate of overweight at 8.3% [19]. Malnutrition is often reflected in low socio-economic status of individuals or households, this study found out that 84.4% of participants were leaving on less than 100,000 frs CFA. With small income, individuals and household could therefore hardly have a good and consistent dietary intake. This study also revealed that 16% of participants had feeding difficulties, similar to a study in a study by Uthman et al. [16]. HIV infections result in feeding difficulties as well as malabsorption, resulting in malnutrition. Bartlett et al. [17] also showed that inadequate food intake, malabsorption and increase energy expenditure are among the factors associated with increase malnutrition among HIV infected persons. Obesity has long been thought to be caused solely by energy is stored in adipose tissue when caloric intake exceed expenditure. However, this has been slightly altered, because gut microbiota has been found to be a contributing factor to the pathophysiology of obesity. Several studies such as that by Haro et al. in 2016 have suggested that changes in the gut microbiota trigger pathogenic mechanisms to promote the development of obesity [20]. We could thus suggest that the increase prevalence of obesity in our study could be due to changes in gut microbiome and poor feeding habits.

This study showed an overall increase in the presence of gut flora for proteobacteria 100% and a decrease in the occurrence of gut firmicute bacteria 90.1, which was in concordance with the findings of Arumugam et el. in 2013 [23]. HIV infection has been associated with alterations in intestinal structure and immunity, Dien et al. in 2013 in their study showed that HIV resulted in microbial translocation which could be a reason for increase in the prevalence of gut proteobacteria, a phylum with potentially harmful bacteria [24]. We also observed a decrease in gut firmicutes to Bacteroidetes ratio. Several studies such as that done by Yang et al in 2017 also showed that HIV infection and those on ART therapy had a considerable decrease in Bacteroides and a slight increase in gut firmicutes including increase in members that are aerotolerant (facultative aerobes) like *Enterococcus spp*, *Staphylococcus spp* [25]. This could be due to inflammatory changes that occur in the gut mucosal because of HIV infection. This study showed that *Bifidobacterium spp* from the phylum actinobacteria, *Bacteroides spp* from the phylum *Bacteroidetes* and *Enterococcus spp* from phylum firmicutes were the most abundant gut microbes. This was similar to studies carried out by Ako et al. in the Southwest region of Cameroon in 2019, Chow et al 2011 and Yang et al 2017 [26,27]. *Bifidobacterium spp* have been used as probiotics and have several beneficial effects, including preventing translocation of enteropathogenic *E. coli O157* and strengthening epithelial barrier function in human ulcerative colitis patients [28]. Moreover, studies by Schroeder et al suggested that some *Bifidobacterium spp* bind to mucus proteins *in vitro* [28]. It is therefore possible that Bifidobacterium species exert part of their probiotic action by stimulating colonic mucus growth which is very important in preventing colonisation by pathogenic growth as well as inflammation [28]. There

was also a significant increase in gut Fusobacteria which has been associated with pathogenic potentials in the gut, notably by its association to human colonic cancer as observed by Part et al in 2016 [29].

Calcium deficiency was associated with an increase prevalence of Enterococcus (a potentially harmful bacterium) and Bacteroides. These findings were like a study carried out by Yang et al. [30]. Calcium increases the microbial diversity and hypocalcemia has been associated with increased firmicutes and Bacteroidetes ratio [30]. The reason for this association is not clear yet. We observed a slight increase in bacteria diversity with people who had hypomagnesemia, with increase in proteobacteria, fusobacterium and lactobacilli and a decrease in Bacteroides, these results were similar to the study carried out by Wang et al. 2018 and Pachikian et al. in 2010 [31,32]. Magnesium deficiency is associated with several metabolic disorders, systemic inflammation has been observed in subjects with hypomagnesemia. Magnesium is required more by Gram-positive than for Gram-negative bacteria, thus could explain the slight association that we observed between hypomagnesemia and *lactobacillus spp*.

Hypoglycaemia was found to negatively impact Bifidobacterium. A study by Schroeder et al in 2018 found similar results [28]. This could be as a result of the role the bacterium plays in carbohydrate absorption in the gut. Low Albumin was associated with increase detrimental bacteria such as Escherichia and Shigella as well as decrease in gut useful bacteria such as Lactobacillus, and Bifidobacterium. The low protein levels could be as a result of poor intake due to poverty and also as a result of increase proteobacteria that could impair their absorption, these findings are similar to those of Yang et al. 2020 and Kostovcikova et al. 2019 [30,33]. Low albumin also showed a strong association with yeast. Hypoalbuminemia has been strongly associated with ART. Leal et al. in 2018 in their study showed that hypoalbuminemia was significantly associated with ART [34]. Valdes et al. in 2018 in their study deduced that the quality and quantity of protein eaten affects the type of bacteria present in the gut. For example, he observed that proteobacteria, Enterococcus and other detrimental bacteria were present more in those with increase intake of animal proteins [35]. The change in gut bacteria as a result of hypalbuminaemia could be due to both the quality and quantity of protein intake.

CONCLUSION

Malnutrition is still a major problem in the management of HIV and the prevention of AIDS in Cameroon. The overall prevalence of wasting was 4.20% while obesity stood at 18.88%. hypocalcaemia was 28.7%, while hypomagnesaemia was 13.3%. For macro-nutrient deficiency, hypoalbuminaemia and hypoglycaemia were 25 (17.7%) and 23 (16.1 %) respectively. An overall increase in the prevalence of gut proteobacteria as well as other potentially harmful bacteria such as enterococcus and fusobacteria and decrease in prevalence of gut firmicute, Bifidobacterium and Bacteroides was observed, the gut Micro nutrient deficiency was mostly associated with increase prevalence of *Klebsiella spp*, *Citrobacter spp* and *Enterobacter spp*, while macro-nutrient deficiencies were associated with reduce *Bifidiobacterium spp* and *Lactobacillus spp*, However, both were associated with increased proteobacteria.

AUTHORS' CONTRIBUTIONS

ADT, KKP NRV and HDM conceived and designed the study: KKP and STN implement the study: ADT, HDM and AEA supervised the study: KKP conducted data analysis: KKP, NRV and HDM interpreted study results: KKP, ADT and NRV wrote the draft manuscript, ADT and NRV reviewed and corrected the manuscript. All authors approved the final copy

FUNDING

This study was fully funded by CANTAM as a Post-Doctoral Career development fellowship to Dr Anye Delphine Tangoh, supervised by Prof Achidi Eric Akum.

CONFLICTS OF INTERESTS

The authors declare no competing interest.

ACKNOWLEDGEMENTS

We are grateful to all who participated in this research

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