Original Research Article

Assess of knowledge level, Prevalence Determination of Distributive Frequency and numerical ratio of Secretion and Non-secretion status of ABH Antigenic Substances using Saliva, Plasma and Urine Samples among Apparently Healthy Individuals in Bamenda II Municipality, Northwest Region, Cameroon.

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ABSTRACT

Background: The indispensable role of genetic predisposition, racial segregation, ethnic variations and population diversity in the secretion and non-secretion status of the ABH antigenic substances have garnered significance interest among researchers. There is greater quest to establish the secretor status of ABH antigenic substances in different human samples in male and female individuals in various cities across the world.

Aim: The current study was aimed at determining the knowledge level, prevalence of distributive frequency and ratio pattern of secretion and non-secretion status of ABH antigenic substances as one of the newest blood group systems among apparently healthy male and female individuals of Bamenda II Municipality, Northwest Region, Cameroon.

Methodology: This cross-sectional study which lasted for one year (2022 to 2023) employed an experimental design with randomized simple sampling and purposeful sample sampling techniques while participants were provided with an opened semi- structural questionnaire after obtaining informed / written consents. Administrative ethical approval was obtained from appropriate authority in accordance with the law in force. Five milliliters of venous blood were collected from the antecubital vein of pre-counseled participants using standard procedures. About five milliliters of urine samples were collected using sterilized universal urinary containers while Three milliliters of saliva samples were collected from the mouth of 630 participants (comprising of 303 (48.1%) samples from the male group (MG) and 327(51.9%) samples from the female group (FG) respectively using a commercial whole-saliva collecting disposable device before transferring into a clean, dried and labeled 16 x 100mm centrifuge Pyrex tubes respectively. Salivary test for the determination of ABH secretor status in plasma, urine and saliva samples were done by Hemagglutination and Inhibition method using Anti-sera H (Lectin) reagent / kit tools which had been manufactured from a purified extract of Ulex *europaeus* seeds and supplied by BIOTEC Laboratories Limited. The manufactural manual was strictly followed in all procedures with inclusion of appropriate standards.

Results: After collation, coding and entry of raw data into IBM-SPSS version 26 for data analysis, the mean age (years) for female was 32.99±1.01 and male 28.95±7.85 with statistically significant difference between ages ($P<0.05$, $t=7.2822$, $P=0.0001$). A total of 546 (86.7%) comprising of 260 (41.3) male and 286(45.4%) female participants had no pre-exiting knowledge (NPEK) of secretor and non-secretor of ABH antigenic substances of study population. Conversely, only 84(13.3%) comprising 43(6.8%) males and 41(6.5%) females had some pre-existing knowledge (SPEK) of secretor and non-secretor ABH antigenic substances of the study population. The prevalence of frequency distribution of secretor and non-secretor status of ABH antigenic substances among apparently healthy individuals in Bamenda II Municipality using saliva samples were 449 (71.3%) secretors [216 (34.3%) males, 233 (36.9%) females] and 181 (28.7%) non-secretors [87 (13.8%) males, 94 (14.9%) females].Using plasma samples there were 467 (74.1%) secretors [221 (35.1%) males, 246 (39%) females] and 163 (25.9%) non-secretors [82 (13%) males, 81 (12.9%) females].Finally, using urine samples there were 542 (86.1%) secretors [255 (40.5%) males, 287 (45.6%) females] and 88 (13.9%) non-secretors [48 (7.6%) males, 40 (6.3%) females] respectively. The Se/Nse Ratio pattern were 3.5:1 and 4.52:1 for both male and female respectively. Statistically significant difference between genders ($P<0.05$), $X^2=3.873$ and $P=0.0491$.

Conclusion: The current study has demonstrated the presence of secreted ABH antigenic substances in saliva, urine and plasma samples and hence these samples can be utilized for the determination of the secretor and non-secretor status of ABH antigenic substance in humans. The study also revealed a highest prevalence rate of (86.1%) secretors and (28.7%) non-secretors of ABH antigenic substances in urine samples, closely followed by (74.1%) secretors and (13.9%) non-secretors in plasma samples and lastly saliva samples having the lowest prevalence of (71.3%) secretors and (25.9%) non-secretors. Majority (71.3-86.1%) of secretor participants across all sample types and female secretors had higher prevalence than males. Our findings were aligned with previous findings cited in other non-Caucasian and Caucasian populations.

KEYWORDS: **knowledge level, Prevalence, Frequency distribution, secretor /non-secretor status of ABH antigenic substances, plasma, saliva and urine samples,**

INTRODUCTION

Historically, the reality and existence of the concept of secretion of ABH antigenic substances have been based on the fact that some individuals in human population are able to secrete these ABH antigenic substances in their body fluid while others cannot **[1].** Consequently, these ABH antigenic substances are present in these individuals body fluids such as saliva, semen, sweat, tears, gastrointestinal juices throughout their lifetime except CSF **[2].** This breakthrough truth was first discovered by Lehrs and Putkonen in 1930 and later demonstrated and expounded by Schiff in 1934 who had been reputed to have designated the secreting pair of genes as "*Se, Se",* or "*Se, se"* and non-secreting pair of genes as "*se, se"* **[3, 4].** Genetically, it has been established that the secretor of ABH antigenic substances or blood group water-soluble antigens are controlled by fructosyltransferase 2 gene (*Fut 2* gene) which is a pair of alleles with the genotype "Se, Se", or "Se, se" which is dominant gene and "se, se" which is recessive gene (amorphic) and are being inherited in Mendelian fashion **[5].** The *Se* locus is located on chromosome 19 at 19q13.3 and is believed to contains two exons that span about 25 kb of genomic DNA. The Se locus encodes a specific fucosyltransferase that is expressed in the epithelia of secretory tissues, such as salivary glands, the gastrointestinal tract, and the respiratory tract **[5].** The fucosyltransferase enzyme it encodes catalyzes the production of H-antigen in bodily secretions. "Secretors" have at least one copy of the Se gene that encodes a functional enzyme—their genotype is Se/Se or Se/se. They secrete H antigen which, depending on their ABO genotype, is then processed into A and / or B antigens. Non-secretors are homozygous for null alleles at this locus (se/se). They are unable to produce a soluble form of H antigen and hence do not produce A and B antigens as shown in **figure I** and **figure 2** below **[6, 7].**The current knowledge of the disease vulnerability and susceptibility of the secretor and non-secretor status of ABH antigenic substances is based on the principle that some individuals in the population are able to secrete their ABO blood group antigens into their body fluids such as saliva, semen, sweat, and gastrointestinal juices **[8]**

Secretor status refers to the presence or absence of water-soluble ABO blood group antigens in a person's bodily fluids, such as saliva, tears, breast milk, urine, and semen. Individuals who secrete these antigens in their bodily fluids are referred to as secretors, while those individuals who do not secrete water-soluble ABO blood group antigens are termed non-secretors. Recent studies have shown that the secretor status is controlled by the FUT2 gene (also called the Se gene), and the secretor phenotype is inherited in an autosomal dominant manner, being expressed by individuals who have at least one functioning copy of the gene. The non-secretor phenotype (se) is a recessive trait [12]. Approximately 80% of Caucasians people are secretors, while 20% are non-secretors [13]. The frequency of the Se gene is approximately 50% in most ethnic groups, but Aboriginal Australians, Inuit, and some Native American and Melanesian groups exhibit a frequency of nearly 100%, while the frequency is only 22% in South India [14]. Non-secretors have reduced susceptibility to

the most common strains of norovirus and are much more vulnerable to other pathological conditions [10]. Expression of the antigens in the Lewis blood group is also affected by secretor status of ABH antigenic substances while non-secretors cannot produce the Le(b) antigens [15].

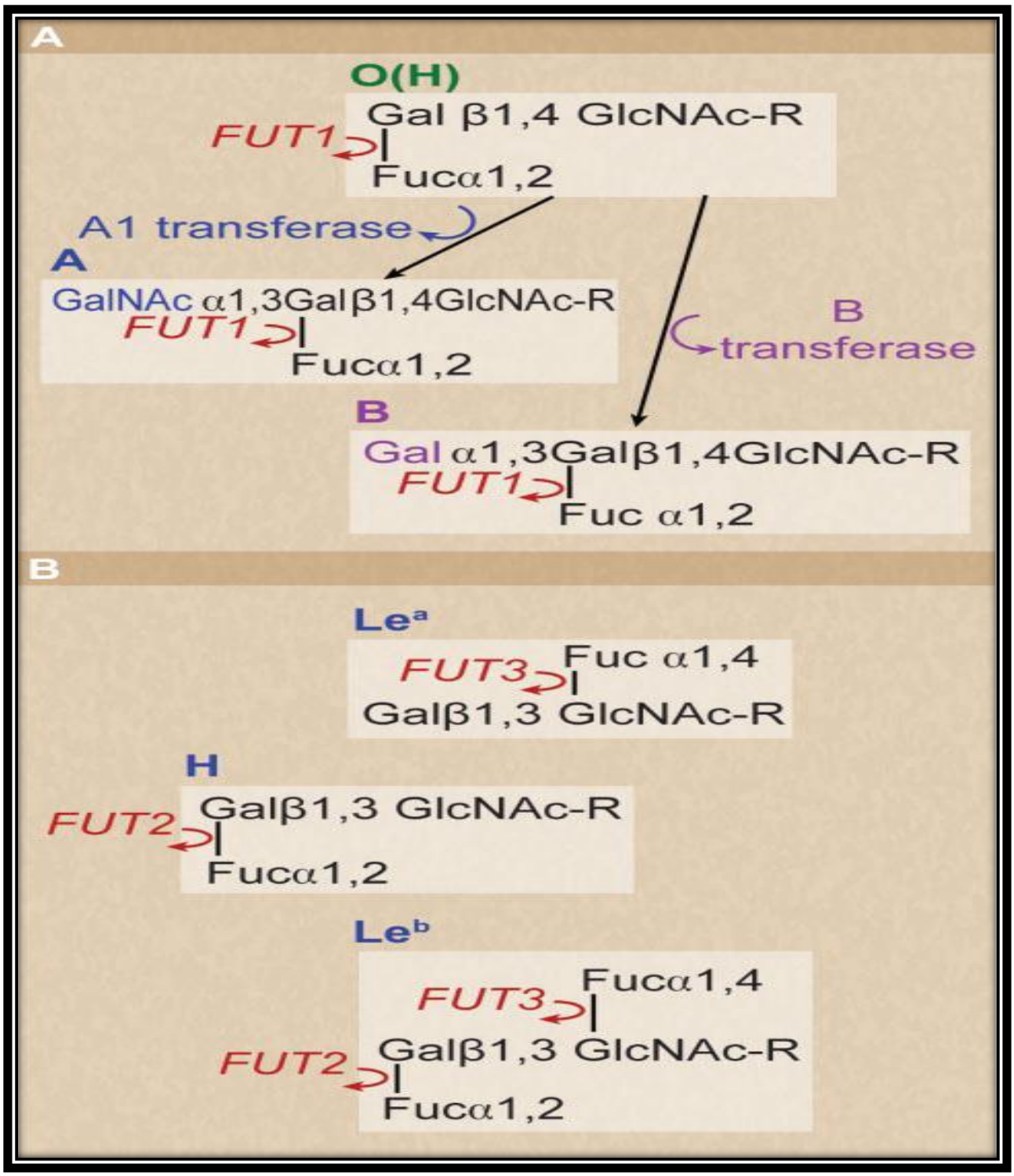

Figure 1: Biosynthesis of ABH, and Lewis substances (antigens**)** (source adapted from *Iizuka and Yoshida, 1987)*

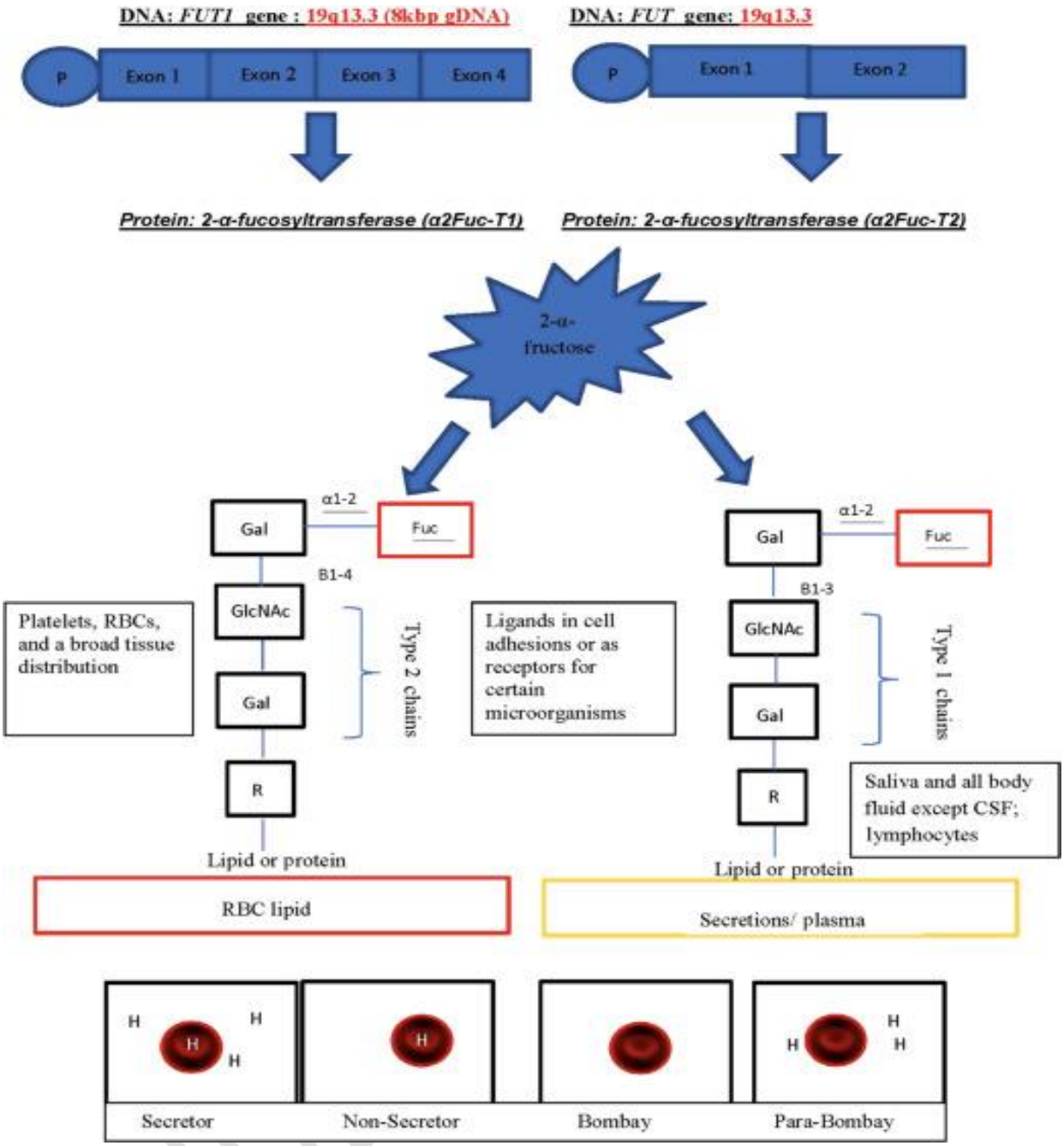

Figure 2: formation and Structure of ABH antigenic substances, various genes, and their products (Reproduced from Schenkel-Brunner, H. Human Blood Groups. Chemical and Biochemical Basis of Antigen Specificity, 2nd ed.; Springer: Vienna (Austria), New York, 2000, with permission from Springer-Verlag)

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1.1) Statement Problem

In the last few decades more studies have shown that the fucosyltranferase 2 gene (*Fut2* Se gene) encodes the fucosyltransferase enzyme that control the formation or result in the expression of secretion status of the ABH antigenic substances. However, in recent times its significant use as a biochemical marker is been gradually recognized in clinical medicine, immunology, serology and genetics. This is due primarily to its association with immune system function and secondly to its association with some pathological conditions and interaction with other blood groups systems including the Lewis blood group system and the ABO blood group system **[9, 10].** However, knowledge about the Secretor Status of the ABH antigenic substances in different human samples such as its presence in plasma, saliva and urine samples are yet to be documented in many Regions of Cameroon.

1.2) **Justification of the study and gaps identification**

The secretion status of ABH substances amongst apparently healthy population though has been studied and well documented in other populations in different cities across many different countries of the world **[11]**, is yet to be studied in the different individual cities, ethnic / tribes and Regions in Cameroon and the targeted population under study. There are no published data that have been documented in the study population or have investigated about secretor status of the ABH antigenic substances in different Regions of Cameroon using human samples such as plasma, saliva and urine samples to the best of our knowledge and at the time of this study.

1.3) Research Questions

1) What will be the prevalence and frequency of distribution of secretor and non-secretor status of the ABH antigenic substances using saliva, plasma and urine samples among apparently healthy apparently healthy male and female individuals in Bamenda II Municipality, Northwest Region, Cameroon.

2) What will be the knowledge level about existence of the secretor status of the ABH antigenic substances among apparently healthy apparently healthy female and male individuals in Bamenda II Municipality, Northwest Region, Cameroon

1.4) Research hypotheses of the study

1)There will be no statistically significant difference between the prevalence rate and frequency of distribution of secretor and non-secretor status of the ABH substances using saliva, plasma and urine samples amongst apparently healthy female and male individuals in Bamenda II Municipality, Northwest Region, Cameroon.

3) There will be no statistically significant difference between the knowledge level of the existence and non-existence of secretor and non-secretor status of the ABH substances in plasma samples among apparently healthy apparently healthy female and male individuals in Bamenda II Municipality, Northwest Region, Cameroon.

1.5) The Main Aim

To determine the knowledge level, prevalence, pattern of secretor /non-secretor ratio, frequency distribution of secretion and non-secretion status of ABH antigenic substances in different human samples such as plasma, saliva and urine samples amongst apparently healthy male and female individuals of Bamenda II Municipal Council, Mezam Division, North West Region of Cameroon.

1.6) Specific Objectives

1) To determine the participation rate with complete submission of their samples from Bamenda II Sub divisional council, North west Region, Cameroon.

2) To determine prevalence of the study population that had no pre-existing knowledge or some pre-existing knowledge about secretor and non-secretor status of ABH substances among apparently healthy male and female Individuals in Bamenda II Municipality.
3) To determine the prevalence of frequency distribution of secretor and non-secretor status of ABH substances between female and male groups with according to their saliva samples from Bamenda II Sub divisional council, North west Region, Cameroon.
4) To determine the prevalence of frequency distribution of secretor and non-secretor status of ABH substances between female and male groups with according to their plasma samples from Bamenda II Sub divisional council, North west Region, Cameroon.
5) To determine the prevalence of frequency distribution of secretor and non-secretor status of ABH substances between female and male groups with according to their urine samples from Bamenda II Sub divisional council, North west Region, Cameroon

1.7) contribution and Significance of the study

1) The findings of this study are expected to generate valuable data on the prevalence and frequency distribution of secretor and non-secretor status of ABH substances between female and male groups with according to their saliva, plasma and urine samples from Bamenda II Sub divisional council, North west Region, Cameroon.
2) The findings of this study will bridge the gap and paucity of knowledge existing in this area and the population under study on the prevalence and frequency distribution of secretor and non-secretor status of ABH substances between female and male groups with according to their, saliva, plasma and urine samples from Bamenda II Sub divisional council, North west Region, Cameroon
3) This study will provide and equip all the various stake holders and actors concerned to take appropriate steps to correct any abnormalities and promptly implement any measure or policy to prevent any risk that may affect the study population.
4) This study is expected to contribute knowledge to scientific community and humanity in general.

3) RESEARCH METHODOLOGY

3.1) Study Setting

The selected as study area of the current research was Municipality of Bamenda II sub-divisional Council, found in Mezam Division, Northwest Region of Cameroon. Administratively, Municipality of Bamenda II sub-divisional Council is an integral of Bamenda City Council (BCC) **[16].** The choice of Bamenda II was based on demographic, historical, and geo-political and administrative reasons. Demographically, Bamenda II council is considered as one of the most urbanized towns in Cameroon, making it a relevant case to explore the livelihood dynamics of fuel vendors in the face of continued urban expansion **[17].** Historically, Bamenda II Council is one of the towns in Cameroon that have witnessed early colonial influence under the German and later British rule. These influences meant that it stood as one of the main towns in the former West Cameroon. Bamenda II Council today plays the role of a primate city in the North-

West Region of Cameroon given its multiple roles as a hub of secondary and higher education and its administrative role as the regional headquarters of the North-West Region and the divisional headquarters of Mezam Division **[18].** Such administrative and social attractions come with urban multiplication or growth which in turn affects the livelihood of inhabitants (especially farmers) at the urban fringes **[19].** Bamenda II Council was created by Presidential Decrees N° 2007/115 of 13 April 2007 and N°2007/117 of 24th April 2007 has quested for development since then. With the acceleration and deepening of the decentralization process in Cameroon by relevant texts i.e. Law N° 2004/017 of 22nd July 2004 on the orientation of Decentralization and Law N° 2004/018 of 22nd July 2004 to lay down rules applicable to Councils respectively as well as subsequent instruments thereto **[20 ,21].**

3.2) Geographical Limitation and map of the study area: The surface area of Bamenda II Council is estimated at 1,482km2. The Bamenda II Council area is delineated by the Bamenda II Sub-division, one of the seven administrative units (Sub-divisions) of Mezam Division in the North West Region of Cameroon. It lies between latitudes 5°52'52" and 6°4'03" North of the equator and longitudes 10°2'02" and 10°10'10" East of the Greenwich meridian. This council area is bordered by six councils, Bafut to the North, Bamenda III and Bamenda I Councils to the East, Santa Council to the South and then Bali and Mbengwi Councils to the West **[22](** See the map of the study area in **Figure 3** below). Bamenda II had a projected population of 261,285 persons in 2019. The population density is approximately 176.3 p/km². The urban area is densely populated and the density decreases towards the urban periphery and rural areas. The high density is associated with the concentration of administrative, health and educational and socio-economic institutions. Bamenda II Council, is made up of four villages (Mankon, Nsongwa, Chomba and Mbatu).

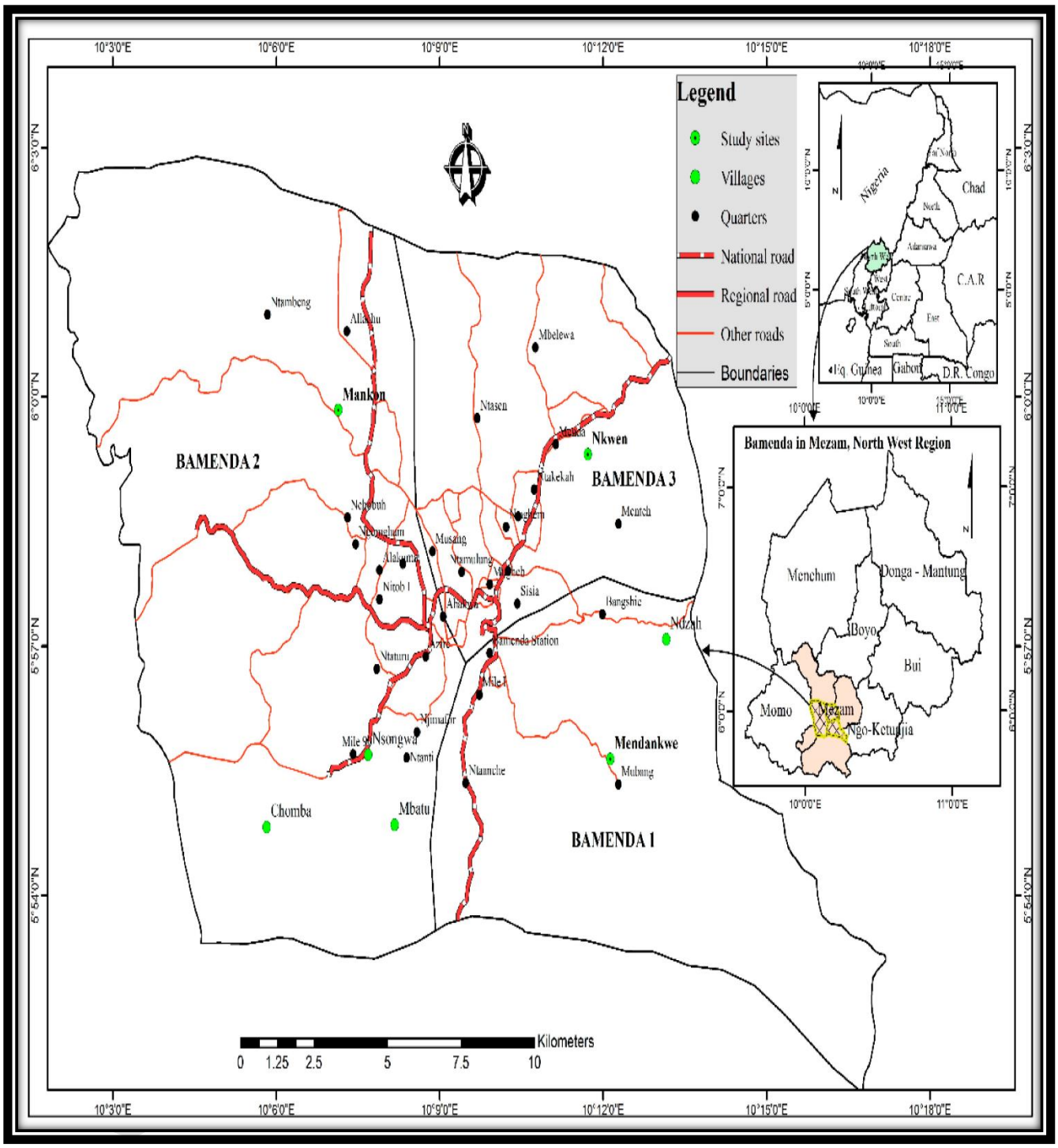

Figure 3. Map of Bamenda city showing the municipality Bamenda I, II, and III Sub-divisional Councils respectively. ***(Source***: adapted from Akhere et al.*, 2020* **[23]** and Compiled from the National Institute of Cartography and the Bamenda City Council)

3.3) Study design: Descriptive and Analytic design was adopted for the study. The duration of the study was from March ,2022 to December ,2023**.** A multi-stage sampling technique was employed in the selection of subjects in population one and two as follows:

3.4) Study populations: Theses were made of study population one and two namely: - study population one (unexposed group). These include all apparently healthy individuals who were not involved in the fuel trade activities and not dealer or retailing of fuels. They must melt the inclusive and inclusive criteria.

3.8) Selection and recruitment of study population two: This was done by selecting the regions in the list of the six regions that have been known to operate the illegal/illicit/informal fuel trade in Cameroon. This was done using the simple random sampling technique through balloting. A basket containing cut pieces of paper of equal sizes was folded and its content was written as Northwest, Southwest, littoral, Adamawa, North and Far North Regions. It was then shaken vigorously and any piece of paper was picked and read. The process was repeated for the Divisions, towns, quarter and streets. Selection of the proportion of respondents from each selected area was done to obtain total sample size of 430 for both exposed and unexposed group. This was done to prevent sample selection bias.

3.11) Subjects Eligibility, Inclusion and Exclusion Criteria

3.11.1) Subject's pre-counseling and recruiting sites

Subject's pre-counseling and recruiting sites were specific fuel filling stations and designated local or informal functioning fuel filling points, streets and roadsides within the selected area of study.

3.11.2) Inclusion criteria for all participants

Pre-counseled prospective subjects were randomly recruited from pre-counseling sites based on Voluntary acceptance to be administration of structural Questionnaire and signing of informed / written consent forms,

3.11.3) Exclusion criteria for recruitment of unexposed subjects

This was based on the on physical state of health. All unexposed subjects were apparently healthy but those suspected of having any form of disease or abnormal medical conditions as evidenced clinically by their medical records were excluded.

3.11.4) Inclusion criteria for recruitment of exposed subjects

This was based on currents employment as fuel attendants/ vendors employed in local fuel filling stations of non-public oil companies but privately self-employed vendors of informal /illicit fuel trade for as least one-year duration.

3.12) Sample size formula and determination of sample size

The sample size for privately self-employed vendors of illegal/illicit/informal fuel trade and formal vendors (both test or exposed subjects and control or unexposed subjects) were calculated using the Cochran's Formula (1963) for sample size determination for proportion in the equation below as quoted by **[24, 25]**.

$$n = \frac{Z\alpha/_2{}^2 Pq}{d^2}$$

In this case P= 50% or 0.5. [This is a virgin study, therefore the prevalence rate of privately self-employed vendors of illegal/illicit/informal fuel trade (both test or exposed subjects and control or unexposed subjects) and formal fuels vendors in this area does not exist for the study population, so P=50% =0.5 **[26,27,28].**

•q = 1-P, and •d is the desired level of precision (usually at 5% for single proportions).

$n= (1.96)^2 (0.5) (1-0.5) / 0.05^2 =$ **384**

- ★ Correction for a small/finite population below 10,000 is given by the formulae
  x =no/1+ (no-1)/N

where x is the corrected sample size, n_0 is the calculated sample size and N is the population size

x =384/1+(384-1)/5650 =**360** = minimum sample size needed

- ★ **Calculation of Non- respondent rate**

Is given by the formula= $\frac{n}{1-non-response\ rate}$

Assuming 10% non-response rate, the minimum estimated sample size will be

=384/1-0.1=384/.9**=426 samples**

Approximately **360** minimum sample size will be needed but 430 samples was employed by convenient sampling techniques after correction of non-respondent rate of 10% for finite population less than 10,000 samples **[29, 30, 31]. Total sample for the two populations were =630**

3,13) Questionnaires forms distribution centers:

These were designated places where there were activities of individuals within the study area.

3.14) Total Numbers of questionnaires forms needed for population one

A total of 327 questionnaire forms were distributed to pre-counseled, screened and registered subjects at least one-year duration of employment of subjects for population one of both male and female sexes and aged 18 years and above years).

3.15) Total Numbers of questionnaires forms needed for population two

A total of 303 questionnaires forms were distributed to the subjects for population two of both male and female sexes and aged 18 years and above year.

3.16) Validation and reliability testing and method of data collection

Familiarization with the sample collection tools were made before a pretesting were carried out in another nearby selected town amongst 17 vendors /attendants (10% of the sample size each for population). This was done to determine the length of questionnaire forms administration and errors to ensure accurate responses of participants.

3.17) Statistical tool for data analysis and data presentation

The raw data was gotten from the results of study for both exposed and unexposed subjects was be subjected to statistical analysis using SPSS software version 26 (SPSS Inc., Chicago, USA). Statistical differences between the populations one and two groups were evaluated using the independent t-test, Chi Squared (X2) tests and ANOVA (analysis of variance). Chi-square was used to determine the associations between independent variables and outcome variables with a test of significance set at a p-value of less than 0.05. The correlation between parameters were determined by Pearson's correlation test and correlation coefficient (r). Socio-Demographical data was presented as the mean value ± SD and the Alpha value of 0.05 was used. This was done using tables but all Categorical variables such as gender, marital status, tribe, profession and denomination was summarized using frequencies and percentages. A two tailed p-value of <0.05 was considered indicative of a statistically significant difference.

3.18) Questionnaire forms

A standardized, close-ended structured and self-administered questionnaire forms were designed and adopted in the current. They were designed based on the previous work of other researchers such as **[32,33,34]** and validated, verified and made available for this purpose. The questionnaire forms consisted of four sections namely: A ,B,C,D and E.

3.19) The response rate was calculated using the formula thus: -

Response rate in percentage (%) = Number of people who actually completed the survey questionnaire forms and returned it / total number of people you sent it to or received it x 100.

3.19.1) The response Completion rate was calculated using the formula thus: -

Completed rate in percentage %= number of people who completed questionnaire forms to the end of the survey by the total number of people who started the questionnaire form **[35]**

3.19.2) Turn Out Rate is defined as the number of people who participated in an event, survey or projected and is calculated by dividing the number of people who come out or shown up for the event by the total number of people projected or invited for the event multiple by 100.

3.20) Ethical consideration and approval

Ethical approval was applied and obtained from the Faculty of Health Sciences international Review Board (FHS-IRB), University of Buea prior to data collection.

3.21) Informed and written consent form

Informed and written consent was sought for and obtained from each participant who was participating in the study. They were also informed about the purpose of the study and their right to withdraw from the study at any point. In other to ensure anonymity and confidentiality, serial numbers were used as identifiers for each questionnaire and they were kept safely after being filled.

3.22) Method for determining the secretor status of ABH antigenic substances in saliva, urine and plasma samples.

Secretor status can be determined through genotyping or through serologic methods. In the serologic method, the person's saliva is boiled, then added to reagents containing antibodies against the A, B, and H antigens. Red blood cells expressing these antigens are then added to the saliva-reagent mixtures. If the person is a secretor, the antibodies will bind to the antigens in their saliva rather than the red blood cells, and will not cause red blood cells to agglutinate. **[36]** Secretor status testing was historically used in forensic science, but this has been made obsolete by advances in DNA testing **[37].** Approximately 80% of Caucasian people possess the Se gene and are secretors; the other 20% are non-secretors **[38]**. The frequency of the Se gene is approximately 50% in most ethnic groups, but Aboriginal Australians, Inuit, and some Native American and Melanesian groups exhibit a frequency of nearly 100%, while the frequency is only 22% in South India **[39].** Individuals who are negative for the H gene express the rare Bombay blood type and a weak variant of the Se gene found mainly in Asian populations results in a Le(a+b+) blood type [1]

3.22.1) Background information about the test

Salivary test for the determination of ABH secretor status was done by Haemagglutination and Inhibition Method used by Walters and adopted by using Anti-H (Lectin) produced from *Ulex europaeus,* manufactured and supplied by BIOTEC Laboratories Limited **[8,40,41].**it is also called Wiener's test ,2008 **[42].**

3.22.2) Principle of the test

It have been shown from recent studies that approximately 80% of the population has the secretor *(Se)* gene are said to secrete water-soluble blood group substances in their salvia and other body fluids while about 20% of the population

has the non-secretor gene denoted by *(se).* Blood Group A individuals secretes A- substance and a small amount H-substance in their salvia and body fluids. On the other hand blood group B individuals secretes B -substance and H-substance in their salvia, similarly, blood group O individuals secretes H-substance only and finally blood group AB individuals secretes A, B and a small amount of H-substance in their salvia and other bodies fluids .To determine if a person is a secretor or non-secretor the principle of agglutination inhibition is utilized, where the presence of agglutination means a negative test and no agglutination is interpreted as a positive result **[39,40,41,42].**

3.22.3) Stages one of the tests

Antibody neutralization: Prepared Saliva samples were mixed with commercial anti- serum (anti-A, anti-B, anti- AB, and anti-H) and appropriate controls and brief incubation was done. If the patient is a secretor, the soluble blood group antigens in the salvia will react with and neutralize the antibodies in the commercial anti-serum. It is necessary, however, to dilute the commercial anti-serum so that its antibody titer more closely matches the antigen level in the salvia. Therefore, doubling dilution of the prepared saliva above was made in normal saline up to a dilution titer of 1024**.** An undiluted saline was regarded as the neat. One drop of anti-A, anti-B, and anti-H (diluted 1in 32) and anti-A not diluted according to the manufacturer instruction was added separately to each dilution and left at room temperature for about 10 minutes for any neutralization reaction to occur.

3.22.4) Stage two of the test

Agglutination inhibition: One drop each of pre-prepared and washed blood group A-cells, B-cells and O-cells were added to each of the corresponding dilution in the test tube and both solutions were well mixed and left at room temperature for one hour. Each dilution in the test tube was examined for agglutination both macroscopically and microscopically to determine the titer at which neutralization have taken place. Similar procedure was carried out for the control group's samples. When commercial RBC of the appropriate blood group were then added to the test mixture, there should be no free antibody to agglutination them if the participant was a secretor. This is because the antibodies have already reacted with the blood group antigens in the salvia. The reaction will be negative for agglutination, but is interpreted as positive for secretor status. If the participants is a non-secretor, there will be no blood group antigens in the saliva, the antibodies in the anti-serum will not be neutralized and will be free to react when the test cells are added. Therefore, agglutination is a negative test for secretor status.

3.22.5 Procedure of the test

1) Collect 2 to 3mls salvia in clean 16 x 75mm tube of 5ml capacity. Use paraffin wax or clean rubber bands to stimulate secretions do not use gum. Those who could produce salvia were exempted from the study.

2)Place in a boiling water bath for 10 minutes. This inactivates enzymes that the might otherwise to destroy blood group substances.

3) Allowed to cool briefly, then transferred to 12x 75mm tube of five milliliters by volume

4) Centrifuge at least 5 minutes

5) Label three 12 x 75mm of five milliliters by volume test tubes as follows A-test, B-test and C-test

6)Label three more tubes as follows A-control, B-control and C-control (These are diluted control to ensure the antisera was not diluted beyond its capacity to agglutinate).

7)Add one drop of the appropriate diluted antiserum to cash tube: One drop dilutes anti-A to the A test and A control tube, One drop of anti-B to the B test tube and B control and One drop anti-H to the H test and H control.

8) To each Test tube, add one drop of clear salvia

9) To each control tube, add one drop of saline

10) Mix and incubate at room temperature 10 minutes

11) Add one drop of the appropriate reagent red cells to each tube as follow A1-cells for A test and control, B-cells for the B test and control, Screening cells I or II for the H test and control

12) Mix and incubate at room temperature 10 minutes

13 Centrifuge the length of time for saline reactions in the serofuge

14) Using the lighted agglutination viewer, read grade and record the reactions

15) the control tube should have agglutination for the test to be valid

16) Urine and plasma samples were treated similarly to the saliva after centrifugation

3.22.6) Interpretation of the test

1) No agglutination in test tube 1 and agglutination in test tube 2 is interpreted as A secretor (A group).

2) Agglutination in test tube 2 and no agglutination in tube one is interpreted as B secretor (B group)

3) No Agglutination in tube 1 and 2 both test tubes is interpreted as AB secretor (AB group).

4) Agglutination in both test tubes is interpreted as AB non-secretor (AB group)

5) Agglutination in tube 5 is interpreted as O non- secretor (O group)

6) No Agglutination in tube 6 is interpreted as O secretor (O group)

3.22.7 Quality control and quality assurance of the test.

1) Prior to the test participants were not allowed to eat, drinks, brush mouth or smoke or chew gum.
2) Prior to the test the mouth of the participants was rinsed thoroughly with bottled water to remove all cells and others debris and food particles that could interfered and influenced the result negatively.
3) The salvia samples collected must be boiled at appropriate temperature and time to destroy all interfering enzymes.
4) The test must be conducted under standard procedure as recommended by the manufacturer in test manual.
5) The strength of the anti-A, B, AB and H reagents chosen for the test was moderate with a titration value of 32 and as more potent sera will decrease the sensitivity of the test.

3.22.8) Calculation of prevalence rate of among ABH secretors and non-secretors:

PR (%) = Total numbers of participants who have ABH Secretors or Non -41secretors x 100 / Total number of participants recruited **[39]**

4) RESULTS

Table 1 below shows the actual number of respondents who voluntarily participated in the current study by completing and returning their consent forms and questionnaires forms as well as offering full acceptance for collection of saliva and urine samples. A total number of 303 (48.1%) samples were collected from the male group (MG) and 327(51.9%) samples were collected from the female group (FG) respectively. There was statistically insignificant difference between the numbers of samples collected from the two groups of populations. Calculated Chi Square test (X^2) =2.5374, at degree of freedom (df) =1, Total of sample collected (N) =630, alpha value =0.05, and Chi Square test (X^2) critical value or Table value =3. 841. The obtained Chi Square test (X^2) value (2.5374) was lesser than the critical value (3.841) or (X^2) calculated value > X^2 table value) and right-tail p-value is 0.1112 (P>0.05).

Table 1. Results of the total number of respondents who willingly participated with complete collection and submission of their saliva, plasma and urine samples among Apparently Healthy male and female Individuals in Bamenda II Municipality

Name of Variable	Full Participation and Completion Rates with samples		Chi-Square without Yates Correction Statistical Tool test		
Targeted Population Type	(f)	(%)	X^2 -value	*p*-Value	Remarks
male Group (MG)	303	48.1	2.5374	0.111	NS* $p>0.05$
female Group (FG)	327	51.9			
Total number of samples (N)	630	100			

Frequency = f, Percentage = %, Total numbers of sample collected (N)=630. NS* statistically insignificant difference between the numbers of samples collected from the two groups of populations (P>0.05).

Table 2 shows results of respondent's group numbers and age ranges among apparently healthy male and female individuals in Bamenda II Municipality. The most predominant group number was 2 in both groups and the predominant age range was between 21- 30 years in both group with a total of 137 in the control group representing (45.2%) of respondents and total of 169 representing (51.7 %) of respondents in the test group respectively. The least group number 6 with age range between 61-70 years had 26 respondents thus representing 8.6 % of respondents in the control group. Similarly group number one with age range between 18 - 20 years also had the least number of respondents (20) in the control group representing (6.1%) of respondents. There were statistically insignificant differences observed between the two group of populations (P>0.05). P-value < 0.8805, and t-value =0.1542, at df=10, alpha level of 0.05.

Table 2. Results of age ranges for respondents among apparently healthy male and female individuals in Bamenda II Municipality

Age Variable		Male Group n=303 (48.1%)	Female Group n=327 (51.9%)	Independence t-Test Statistical Tool		
Group Category	Age Range in years	f(%)	f (%)	t – value	p-value	Remark
1	18 - 20	41 (13.5)	55(16.8)	0.1542	0.881	NS* ($p>0.05$)
2	21- 30	137(45.2)	169(51.7)			
3	31- 40	113(37.3)	97(29.7)			
4	41-50	54(17.8)	59(18)			
5	51- 60	32(10.6)	27(6.3)			
6	61-70	26(8.6)	20(6.1)			
Total samples (N)		**630 (100)**				

Frequency = f, Percentage = %, Total numbers of samples collected (N)=630
***NS** =Not statistically significant differences observed between the two group of populations

Table3 below shows the Mean age range value was =32.99 ± 1.014 years for male group and 28.95±7.845 years for female group respectively, there were statistically significant differences observed between the two group of populations (p<0.05). p-value < 0.0001, t = 7.2822, df = 428 and standard error of difference = 0.555,

Table 3. Comparison of mean age range for apparently healthy male and female individuals in Bamenda II Municipality

Name of Variable	Male Group **n=303 (48.1%)**	Female Group **Group n=327 (51.9%)**	Unpaired *t* -Test Statistical Tool		
Mean age	Mean value ±SD	Mean Value ±SD	t- value	*p*-value	Remarks
Mean age range value (MARV) /year	32.99 ± 1.014	28.95±7.845	7.2822	0.0001	S* *(P<0.05*
Total sample size (N)	630 (100.0)				

***S** =statistically significant differences observed between the two group of populations **(**P<0.05)

In **Table 4** comparative results of the knowledge about secretor and non-secretor status of ABH antigenic substances for both study populations are shown. A total of 546 (86.7%) comprising of 260 (41.3) male and 286(45.4%) female participants. had no pre-exiting knowledge (NPEK) of secretor and non-secretor of ABH antigenic substances of study populations. Conversely, only a of total 84(13.3%) comprising 43(6.8%) male and 41(6.5%) had some pre-existing knowledge (SPEK) of secretor and non-secretor ABH antigenic substances of the study populations.

The secretor to non- secretor ratio (Se/nse) was approximately 4.54:1 for female, 3.72:1 for male and 4.13:1 total respectively.

Table4: Comparatives results of the study population that had no pre-existing knowledge or some pre-existing knowledge about secretor and non-secretor status of ABH substances among apparently healthy male and female Individuals in Bamenda II Municipality.

Study population Variable		Male Group (n=303)* X±SD	Female Group (n=327)* X±SD	Total Male + female	Chi-square without Yates correction		
Secretor Status (SS) Parameter	Code	Frequency (percentage %)	Frequency (percentage %)	Frequency (percentage %)	Chi-value	p–value	Remarks
Had no pre-exiting knowledge of Secretor and Non-secretor	NPEK	260(41.3)	286(45.4)	546 (86.7)	0.664	0.4152	NS**
Had some pre-exiting knowledge knowledge of Secretor and Non-secretor	SPEK	43(6.8)	41(6.5)	84(13.3)			
Total (N)		303 (48.1)	327 (51.9)	630 (100)			
Secretor to non-secretor ratio	Se/nse	3.72:1	4.54:1	4.13:1			

Source: Generated from the field survey by researcher, 2023).

NS * = No statistically significant differences(P>0.05). **X±SD*** = Mean value plus or minus two standard deviation for the test group and the control group. Total number of sample (N) =630, n= sample size for both male and female groups. **NS **** = No statistically significant differences (P>0.05). between the mean (X±SD) value of frequency distribution of respondents with secretor status and non-secretor status in percentage (%) for both male group and the female group. Using Chi squared statistical tool, the Chi-value was =0.664 and the p –value was = 0.4152. at 1 degree of freedom, alpha level =0.05 and N=630

In the **Table 5,** the comparative results of the mean prevalence rate and frequency distribution of secretor and non-secretor status of ABH antigenic substances among apparently healthy male and female individuals using saliva sample in Bamenda II Municipality are shown .The mean prevalence rate and frequency distribution were 216 (34.3%) for Male Secretors group and 233(36.9%) female Secretors group and total 449(71.3) respectively while there were 87(13.8%) male non-secrete Secretors and (94(14.9%) female non secretor group and total 181(28.7) respectively.

Table5: Comparative results of presence, prevalence rate and frequency distribution of secretor and non-secretor status of ABH substances among apparently healthy male and female Individuals saliva samples in Bamenda II Municipality

Parameters/variables considered		Male Group (n=303) (X±SD)*	Female Group (n=327) (X±SD)*	Total Male +fe	Chi-square without Yates correction¥		
	Secretor Status	Frequency (percentage %)	Frequency (percentage %)		Chi value (X^2)	p-value	Remark
saliva samples	Secretor	216 (34.3)	233(36.9)	449 (71.3)	3.873	0.0491	S** (P<0.05)
	Non-Secretor	87 (13.8)	94 (14.9)	181(28.7)	0.562	0.4534	NS*** (p>0.05)
Total (N)		303	327	630			
Secretor to non-secretor ratio	Se/nse	3.72:1	4.54:1	4.13:1			

NS ** = No statistically significant differences between male and female secretors (P>0.05). **S***** = statistically significant differences between the Mean value (X ±SD) of secretors and non-secretors for the male group and the female group (P<0.05 **¥** = Chi-square with Yates correction and Chi-square without Yates correction

In the **Table 6,** the comparative results of the mean prevalence rate and frequency distribution of secretor status of ABH antigenic substances among apparently healthy male and female individuals plasma samples in Bamenda II Municipality are shown .The mean prevalence rate and frequency distribution plasma samples were 221(35.1%) male Secretors and (246(39%) female secretor and 467(74.1%) respectively while the mean prevalence rate and frequency distribution plasma samples were 82 (13%) for Male non-secretors and 81 (12.9%) female Secretors group and 163(25.9%) for total respectively.

Table 6: Comparative results of the presence, prevalence rate and frequency distribution of secretor status and non-secretor of ABH substances among apparently healthy male and female using plasma samples in Bamenda II Municipality.

Parameters /variables considered		Male Group (n=303) (X±SD) *	Female Group (n=327) (X±SD) *	Total Male +female	Chi-square without Yates correction¥		
Type of sample collected/ submitted	Secretor Status	Frequency (percentage %)	Frequency (percentage %)	Frequency (percentage %)	Chi value (X^2)	p-value	Remark
plasma	Secretor	221(35.1)	246 (39)	467 (74.1)	3.873	0.0491	S**(p<0.05)
	Non Secretor	82(13)	81(12.9)	163(25.9)			
Total (N)		303 (48.1)	327 (51.9)	630			

(Source: Generated from the field survey by researcher, 2023). **NS *** = No statistically significant differences between male and female secretors (P>0.05). **S**** = statistically significant differences between the Mean value (X ±SD) of secretors and non-secretors for the male group and the female group (P<0.05 **¥** = Chi-square with Yates correction and Chi-square without Yates correction

In the **Table 7,** the comparative results of the mean prevalence rate and frequency distribution of secretor and non-secretor status of ABH antigenic substances among apparently healthy male and female individuals using urine samples in Bamenda II Municipality are shown .The mean prevalence rate and frequency distribution using urine samples were 255(40.5%) male Secretors and (287(45.6%) female secretor and 542(86.1%) for total respectively while the mean prevalence rate and frequency distribution using urine samples were 48(7.6%) for Male non-secretors and 40(6.3%) female non-secretors group and 88(13.9%) for total respectively.

Table7: Comparative results of the presence, prevalence rate and frequency distribution of secretor and non-secretor status of ABH substances among apparently healthy male and female using urine samples in Bamenda II Municipality.

Parameters /variables considered		Male Group (n=303) (X±SD) *	Female Group (n=327) (X±SD) *	Total Male +female	Chi-square without Yates correction¥		
Type of sample collected/ submitted	Secretor Status	Frequency (percent %)	Frequency (percent %)	Frequency (percent %)	Chi value (X^2)	p-value	Remark
Urine	Secretor	255(40.5)	287(45.6)	542 (86.1)	3.873	0.0491	S**(p<0.05)
	Non-Secretor	48 (7.6)	40(6.3)	88(13.9)			
Total (N)		303	327	630 (100			

(Source: Generated from the field survey by researcher, 2023).

NS * = No statistically significant differences(P>0.05) between the mean prevalence and frequency distribution male and female secretors group. **S**** = statistically significant differences between the Mean value (X ±SD) of secretor and non-secretor status for the male and the female groups (P<0.05) . **¥** = Chi-square with Yates correction and Chi-square without Yates correcti

5) DISCUSSION

The general aim of the current study was to determine the knowledge level , prevalence, pattern of secretor /non-secretor ratio, frequency distribution of secretor and non-secretor status of ABH antigenic substances in different human samples such as plasma, saliva and urine samples amongst apparently healthy male and female individuals in Bamenda II Municipal Council, Mezam Division, North West Region of Cameroon.

In **Table 1** the actual number of respondents who voluntarily participated in the current study by completing and returning their consent forms and questionnaires forms as well as offering full acceptance for collection of their blood, saliva and urine samples are shown. A total number of 303 (48.1%) samples were collected from the male group (MG) group and 327(51.9%) samples were collected from the female group (FG) respectively. There was statistically insignificant difference between the numbers of samples collected from the two groups of populations (p>0.05). Calculated Chi Square test (X^2) =2.5374. Total of sample collected (N) =630, alpha value =0.05, and Chi Square test (X^2) critical value or Table value =3. 841. The obtained Chi Square test (X^2) value (2.5374) was lesser than the critical value (3.841) or (X^2) calculated value > X^2 table value) and right-tail p-value is 0.1112 (p>0.05). There were more female in our study than male participants and these results of gender inequality were in line with the observations and have been reported by study of **[43,44, 45 ,46,47]** who have also carried out studies on the same study population and pointed out huge differences between male and female gender. The result in **table 1** also show a full completion rate, high participation and high response rates reflecting the reports of **[48,49,50].**

Table 2 shows results of respondent's group numbers and age ranges among apparently healthy male and female individuals in Bamenda II Municipality. The most predominant group number was 2 in both groups and the predominant age range was between 21- 30 years in both group with a total of 137 in the control group representing (45.2%) of respondents and total of 169 representing (51.7 %) of respondents in the male group respectively. The least group number 6 with age range between 61-70 years had 26 respondents thus representing 8.6 % of respondents in the male group. Similarly group number one with age range between 18 - 20 years also had the least number of respondents (20) in the male group representing (6.1%) of respondents. There were statistically insignificant differences observed between the two group of populations (P>0.05). P-value < 0.8805, and t-value =0.1542, at df=10, alpha level of 0.05. These results were inconsistent with those early reported by **[51]** who carried a similar study in Bamenda and had the similar observations on the differences in the age group. However, these results were higher than those earlier reported by **[52]** who conducted a study with three state universities in Cameroon. The reason for the differences in our results were due to crisis in the northwest and southwest regions of Cameroon effecting the study area.

In **Table 4** the comparative results of the knowledge about secretor and non-secretor status of ABH antigenic substances for both study populations were shown. A total of 546 (86.7%) comprising of 260 (41.3) male and 286(45.4%) female participants. had no pre-exiting knowledge (NPEK) of secretor and non-secretor of ABH antigenic substances of study populations. Conversely, only a of total 84(13.3%) participants comprising 43(6.8%) male and 41(6.5%) had some pre-existing knowledge (SPEK) of secretor and non-secretor ABH antigenic substances of the study populations. These results and findings were expected and obvious. This is due to the fact that the secretor and non-secretor status of ABH antigenic substances still remain very new and unpopular in the study population and in the general public of Cameroon. This explain why more than 50% have never heard about this new blood group system.

The results in **Table 5** show the serological typing results of the secretor and non-secretor status of ABH antigenic substances for both male and female groups of the study populations using saliva samples. The mean prevalence rate and frequency distribution were 216 (34.3%) for male secretors and 233(36.9%) female secretors and total of 449(71.3%) respectively while there were 87(13.8%) male non-secretors and (94(14.9%) female non-secretor and total 181(28.7) respectively. Using Chi squared statistical tool, the Chi-value was =0.664 and the *P* –value was = 0.4152. Hence there was no statistically significant differences (P>0.05) between the mean (±SD) value of frequency distribution of respondents with secretor status and non-secretor status in percentage (%) for both male and the female groups. These results were in complete agreement with that of **[53]** who worked in the population of Karachi in Pakistan and obtained similar findings. These results were also consistent with report of **[54]** who had similar findings in the residents of Sokoto in the Northern Nigeria. More so, the secretor to non-secretor ratio (Se/nse) was approximately 4.52:1 and this result agreed with those early studied and published by **[55,56]** in the population of residents in the Calabar Municipality or Cannaan City, Cross Rivers State Nigeria.

In **Table 6** the results of the serological typing of the comparative results of the mean prevalence rate and frequency distribution of secretor status of ABH antigenic substances among apparently healthy male and female individual using plasma samples in Bamenda II Municipality are shown. The mean prevalence rate and frequency distribution using plasma samples were 221(35.1%) male secretors and 246(39%) female secretor and total was 467(74.1%) respectively. while the mean prevalence rate and frequency distribution using plasma, samples were 82 (13%) for male non-secretors and 81 (12.9%) female non-secretors groups and 163(25.9%) for total respectively. These results

are similar to those reported by **[57]** who has reported the frequency of ABH secretor status: a cross-sectional study in Lagos, Southwestern Nigeria and **[54]** who had also reported on the status of secretor and non–secretor with respect to ABO blood group system in young population in Karachi-Pakistan and **[59]** in the population of the Calabar of the Calabar Municipality or Cannaan City, Cross Rivers State Nigeria. These results were in line with those reported by **[54]** in Lagos, Southwestern Nigeria and **[55]** and **[56]** in the population of the Calabar Municipality or Cannaan City, Cross Rivers State Nigeria.

In the **Table 7** the comparative results of the mean prevalence rate and frequency distribution of secretor and non-secretor status of ABH antigenic substances among apparently healthy male and female individuals using urine samples in Bamenda II Municipality are shown. The distribution and presence of the secretor and non-secretor status of ABH antigenic substances into and other body fluid have long been reported by **[53,54,55,56]**. However, the various prevalence rates and the frequency distribution using urine sample have not be done extensively. In the current study the mean prevalence rate and frequency distribution using urine samples were 542(86.1%) comprising of 255(40.5%) male secretors and 287(45.6%) female secretors respectively. while the mean prevalence rate and frequency distribution urine samples were 88(13.9%) comprising of 48 (7.6%) for Male non-secretors and 40 (6.3%) female non-secretor group respectively. These results are similar to those using saliva and plasm that have reported by **[9,10,11,12,13**] and those reported by others researchers such as **[60,61,62,63,64]**

5.1) CONCLUSION

The current study has demonstrated the presence of secreted ABH antigenic substances in both saliva, urine and plasma samples and can be utilized for the determination of the secretor and non-secretor status of ABH antigenic substance in humans. However, findings revealed a highest prevalence rate of (86.1%) secretors and (28.7%) non-secretors of ABH antigenic substances in urine and closely followed by (74.1%) secretors and (13.9%) non-secretors in plasma samples and lastly saliva samples having the lowest prevalence of (71.3%) secretors and (25.9%) non-secretors. The mean prevalence secretor and non-secretor participants range from (71.3 to 86.1%) and (13.9 to 28.7%) across all sample types and female secretors had higher prevalence than males. A total of 546 (86.7%) comprising of 260 (41.3) male and 286(45.4%) female participants had no pre-exiting knowledge (NPEK) of secretor and non-secretor of ABH antigenic substances of study populations. Conversely, less than (13.3%) comprising 43(6.8%) males and 41(6.5%) females had some pre-existing knowledge (SPEK) of secretor and non-secretor ABH antigenic substances of the study populations. These results were aligned with previous findings cited in other non-Caucasian and Caucasian populations.

5.2) RECOMMENDATIONS

1) A larger samples size can be use in other populations to compare the presence results of the current study
2) More future study should be carried out in other part of Cameroon so that people may know their secretor and non-secretor status of ABH antigenic substances
3) Public awareness about the advantages and disadvantage of knowing the secretor and non-secretor of ABH antigenic substances in populations should be created and population sensitized

8) AVAILABILITY OF DATA AND MATERIALS: Datasets generated and analyzed in this study are available from the corresponding author on request.

10) CONSENT FOR PUBLICATION: Not applicable.

12) DISCLAIMER (ARTICIAL INTELLIGENCE)

Author(s) hereby declare that No generative AI technologies such as Large Language Models, ChatGPT, COPILOT etc.) and text-to-image generators have been used during the writing or editing of this manuscript .

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