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Hematology

Prevalence of Leukaemia among Patients Attending the Haemato-Oncology Unit of Ahamdu Bello University Teaching Hospital Zaria

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Abstract | **Original Research Article**

To detect and characterize leukaemia in the peripheral blood of subjects suspected of leukaemic disorders at the haemato-oncology unit of Ahamdu Bello University Teaching Hospital Zaria, Kaduna State. Suspected leukaemia patients attending haemto-oncology clinic of Ahmadu Bello University Teaching Hospital Zaria, during March 2015 to October 2018 were included in the study. Platelet count, wbc count, pcv and Hb estimation were determine through an automated haematology analyzer for detection of leukaemia hematological parameters. Also, the pattern of leukaemia DNA was confirmed using polymerase chain reaction assay. A total of 500 subjects were enrolled for the study and amplification with specific primers gave rise to 28 leukemic positive cases with DNA fragment patterns of (AML), (ALL), (CML) and (CLL) genomic DNA. However, the prevalence rate was 30 (6%) by cytomorphological examination of well Lieshman-stained slides. The males had higher prevalence rate of 20 (4%) however, the difference was statistically insignificant ($p \geq 0.288$). The age group 32- 41 years had the highest number of leukaemia infection. The results showed that the educated patients had the highest percentage of leukaemia, 22(78.5%). The geographic areas at risk were patients residing in Kaduna north and Kaduna south senatorial districts with 14 (50%) and 9 (22%) respectively. Those who had tertiary education had the highest prevalence rate of 12(6%) ($p \leq 0.046$). Also, the farmers had the highest prevalence rate of 8(11.4%) ($p = 0.0186$). Chronic myeloid leukaemia had the highest percentage of (3%). Leukemia was significantly associated with occupational status, educational status and senatorial districts. Government should mobilize human and financial resources towards leukemia prevention by increasing understanding of the economic impact of the disease, Leukemia patients are advised to go for early diagnosis and treatment in any approved haemato-oncology centre.

Keywords: Hematological markers, deoxyribonucleic acid (DNA), white blood cell (WBC), pack cell volume (PCV), hemoglobin (HB).

INTRODUCTION

The developing world, Africa inclusive is witnessing an alarming upsurge of Leukaemia incidence. The annual number of new cancer cases is expected to double by 2020 [1]. Leukaemias are neoplastic clonal disorder of haemopoietic tissues in which a stem cell or other precursor of blood cells in the bone marrow or lymphoid tissues proliferate [2]. Molecular epidemiology is the study of the genetic and environmental causes of disease and both their interactions together to understand clinical risks, outcome and prevention of disease [3]. Initially, while investigation began into the molecular epidemiology of leukaemia, one of the approaches was to explore specific candidate genes that could contribute to leukaemia risks, using technological approaches available such as folate metabolism, genetic instability, bone marrow failure, immunodeficiency syndromes and transcription factor syndromes [4]. Applications of molecular methods of leukaemia diagnosis could resolve many problems associated with the cytomorphological method. The exquisite sensitivity and specificity of many molecular methods allow the accurate detection of Leukaemia even in early stage of infections [4].

Hematological markers abnormalities such as low platelet count and hemoglobin can predispose patients from minor bleeding to massive life-threatening hemorrhage [5]. Also, leukaemia can be measured with very high white blood cell count (leukocytosis), this is indicative of low immunity and can lead to secondary infection to patients [6]. The presence of blast cells and smudge cells in peripheral blood stained film is

evidence of leukemia infection and these parameters are used as prognostic indices of leukaemia[7]. Thrombocytopenia is a common future of liver diseases in leukemia infection seen in 30 to 64% [8]. Spleenomegaly is considered the main cause of low platelet count in acute leukemia. Polymerase chain reaction (PCR) assay with specified markers detects DNA which relates to hematological parameters in leukemia patients are consideredas evidence of the infection [9, 10]. In this study, haematological abnormal parameters and DNA in leukemia patients were investigated.

MATERIALS AND METHODS

The study Design

This is a cross sectional, Centre controlled study.

Study Area

The study was carried out in the haemato-oncology of Ahmadu Bello University Teaching Hospital, (ABUTH) Shika, Zaria, and Kaduna State, Nigeria. ABUTH Shika, Zaria in Kaduna State is a referral center for the Northern States as well as some Neighboring states. Kaduna State is in the North – Western geo-political zone of Nigeria with a population of 6,066,512. It is made up of 23 local government areas which are further grouped into 3 senatorial districts (North, Central and South). It is a metropolitan as well as a cosmopolitan industrialized state, with over 80 commercial and manufacturing industries. It is one of the educational centers in Nigeria [11].

Study Population

A total of 550 subjects were studied. These consist of 500 (300 males and 200 females) patients, and 50 (25 males and 25 females) apparently healthy subjects as controls. The total populations were subjects aged 2 to 60 years reporting to haemato-oenology Unit of ABUTH, Shika, Zaria.

Ethical approval

Approval for the study was obtained from the ethical committee of ABUTH, Shika Zaria in accordance with Helsinki declaration. Patient's anonymity was maintained and the findings treated with utmost confidentiality.

Sample Size Determination

The minimum sample size was determined using this formula described by Ahmed [12].

$$n = \frac{z^2 p(1-p)}{d^2}$$

Where:
n = minimum sample size
p = expected prevalence (which is 6.8% by [13])
z = the standard normal distribution at 95% confidence interval (which is equal to 1.96).
d = precision/allowable error (which is 5% = 0.05)

Thus,

$$n = \frac{(1.96)^2 \times 0.068 \times (1 - 0.068)}{(0.05)^2}$$

$$= \frac{3.8416 \times 0.068 \times 0.932}{0.0025}$$

$$= 97.4 \leq 97$$

In order to have a more representation of the study population, 500 blood samples were collected.

Inclusion Criteria

Subjects aged between 2 and 60 years of either sex presenting to the above mentioned unit of Ahmadu Bello University Teaching Hospital, Zaria with features suggestive of leukaemia such as anaemia, fever, bleeding, lymph-node, Spleenomegaly and hepatomegaly were included in the study. Apparently healthy individuals aged 2 to 60 years among staff and students of ABUTH Zaria without anyone of the features suggestive of leukaemia were considered as controls and included in the study.

Exclusion Criteria

All patients who declined to give consent for inclusion were excluded from the study and those without any of the features of leukaemia infection.

Sampling Techniques

A consecutive sampling method was used. All the leukaemia patients were recruited from the population of patients attending the Haemato-oncologyclinic of ABUTH Zaria. Informed consnt of the patients was obtained. Full medical history was obtained from the patients such as clinical information, socio-demographic data and information on risk factors likely to be associated with leukaemia.

Blood Collection and Processing

This was carried out based on standard procedures described by Dacie and Lewis [14]. All selected leukemic patients were investigated for hematological abnormalities by determining their total white blood cell count, platelet count, pack cell volume, differential leucocytes count and polymerase chain reaction assay (DNA). All these tests were performed at Ahmadu Bello University Teaching Hospital research Laboratory Zaria and DNA assay at Biotechnology laboratory ABU Zaria. Platelet count, wbc count, pcv and Hb estimation were determine through an automated haematology analyzer (sysmexKx-21, manufactured by sysmex cooperation Kobe, Japan). It determines all the hematology parameters automatically in few minutes after whole blood sample (5µl) was sucked through suction inlet (nozzel). These samples were analyzed on the same day and two milliliters (2ml) of the whole blood were kept at –20ºC for polymerase chain reacting (PCR) assays.

Molecular Analysis

All the samples (30)diagnosed of leukaemia from full blood count and Leishman stained blood films reading were tested for the presence of leukaemia DNA using specific polymerase chain reaction (PCR) assay at the Centre for biotechnology research and training ABU Zaria, Nigeria.

Extraction of the Deoxyribonucleic (DNA)

The genomic DNA was extracted from the whole blood according to the standard phenol-chloroform extraction [15]. Briefly two hundred micro liters (200µl) of whole blood was mixed with 400µl of the lysing fluid, vortexes and incubated at 65 ºC for a minimum of 1 hour. Exactly, 400µl of phenol-chloroform was then added vortexed and centrifuged at 14,000 revolutions per minutes (rpm) for 10 minutes. The supernatant was harvested into fresh empty micro tubes by pipetting out. To the supernatant 400µl of chloroform was added, vortexed and spun at 13,000 rpm for 5 minutes. The supernatant fluid was again harvested into fresh microtubes. Furthermore, 1,000µl of absolute ethanol and 40µl of sodium acetate were added to the supernatant fluid and mixed by inversion. The eluted DNA was stored at -20ºCover night following the instruction of DNA purification kit obtained from Promega Company in USA.

Polymerase Chain Reaction Assay (DNA amplification)

Specific PCR was performed using the following primers designed by Williams *et al.*[15] as shown in Table 1.

Procedure

The PCR master-mix was prepared by adding 2µl of genomic DNA, 1µl of forward primer, 1µl of reverse primer and 16µl of deionized water to PCR premix kits containing 1.5 Mm Mg^{2+} and 250µm of each dNDP lyophilized unto the surface of the hot start appendoff tubes. The PCR was then carried out by incubation at 94 ºC for 5 minutes, denaturation at 94 ºC for 30 seconds, annealing and extension were repeated 35 times. In the final cycle, an additional 4 minutes extension step at 72 ºC was included for the leukaemia types (OPA-09 AML, ALL, CML and CLL).

The PCR products were analyzed by gel electrophoresis using1.5% agarouse stained with ethidum biomide. The bands were then read using the 100bp plus ladder as a marker and visualized using ultraviolet (UV) light and photographed.

Polymerase chain reaction assay

The following genes of leukemia types were used as targets for amplification. This was carried out in a DNA thermal cycler type480^{T0} (Pakon Elmer, countaboeuf France). Specific PCR was performed using the primers designed by Williams [16], electrophoretic patterns of leukemia DNA were analyzed by gel electrophoresis using 1.5% agarous, stained with ethidumbromide. The bands were read using the 100bp plus ladders as marker and vissualised using altraviolet (UV) light [16]. Simple frequencies, percentage, correlation and association between haematological abnormal parameters and DNA assay using polymerase chain reaction at 95% confidence interval.

Ethical approval

Ethical approval for the study was obtained from the ethics review committees (ERC) of the Ahmadu Bello University Teaching Hospital Shika Zaria.

Data Analysis

Results were analyzed by using statistical package for social science (SBSS) version 16. The Pearson chi-square test was used to determine the association between the variables at 95% confidence interval. A p-value of equal to or less than 0.05 ($p \leq 0.05$) were considered as statistically significant.

Table-1: Leukaemia Primer Sequences

Leukaemia	Gene	Primer Sequence (OPA -09)	Size (bp) PCR Product
OPA – 09 AML	DNMT3A	AGTTCGGAGC	980
OPA – 09 ALL	PAX5	AGTTAGATTC	2187
OPA – 09 CML	B.CR-ABL1	TAGTCTATAC	3162
OPA – 09 CLL	RFC1	AAGCTTACCT	1659

Key: OPA=Optimization primer amplification; AML=Acute myeloid leukemia; ALL=Acute lymphocytic leukemia; CML=Chronic myeloid leukemia; CLL=Choronic lymphocytic leukemia

RESULTS

A total of 500 suspected lymphoid disorder subjects were studied comprising of 300 (60%) males and 200 (40%) females. Out of these, 28 (5.6%) were confirmed to be leukaemic by PCR assay while 30 (6%) were detected by cytomorphological method. Prevalence of leukaemia with respect to gender shows that the males had higher prevalence rate of 20 (71.4%) and females had a lower prevalence rate of 8(28.6%). However, the difference was statistically insignificant ($p \geq 0.288$).

The result of the diagnosis of leukaemia among patients in relation to educational status, showed that those who had tertiary education had the highest prevalence rate of 12(6%) followed by those with informal education with 8 (5.7%) while those with

primary education had 10(2%). The difference was statistically significant ($p \leq 0.046$).

Molecular prevalence of leukaemia in relation to the occupational status in the study area showed that farmers had the highest prevalence rate of 8(11.4%), followed by business cadre with 5(6.2%) and civil servant with 10 (5.5%) while unemployed and artisans had no prevalence. The difference was statistically significant ($p = 0.0186$).

Prevalence of leukaemia in Kaduna State in relation to some social habits showed that those who drink alcohol had a higher prevalence rate of 8(10%), those who smoke had 12 (6%), while those who do not drink or smoke had 8 (4.4%). However, statistical analysis shows that the difference was not significant ($p \geq 0.707$).

Molecular prevalence of leukaemia in relation to the senatorial districts showed that the highest prevalence rate of 14 (2.8%) was found in Kaduna north, 9(1.8%) in Kaduna south districts and 5(1%) in Kaduna central district. The difference was significant ($p \leq 0.0416$).

Table-2: Molecular prevalence of leukaemia among patients in relation to socio economic and demographic factors in the study area

Factors	Number examined	PCR Positive (%)	Prevalence	P. value $p \leq 0.05$
Sex	300	20	4	0.228
Male	200	8	1.6	
Female				
Educational status				
Informal	140	8	5.7	0.046
Primary	10	2	2.0	
Secondary	150	6	4	
Tertiary	200	12	6	
Occupation status				
Unemployed	20	0	0	0.0186
Student	100	5	5	
Civil servants	180	10	5.5	
Business	80	5	6.2	
Artisans	50	0	0	
Farmers	70	8	11.4	
Social factor (habit)				
Smoking	200	12	6	0.707
Drinking of alcohol	120	6	10	
None	180	8	4.4	

Table-3: Molecular and cytomorphological prevalence of leukaemia among patients in Kaduna State in relation to senatorial districts

Senatorial Districts	Number examined	PCR positive (%)	p. value
Kaduna north	220	14(2.8%)	0.0416
Kaduna south	180	9(1.8%)	
Kaduna central	100	5(1%)	
Total	500	28(5.6%)	

The DNA fragments amplification indicated different base pairs (fig1 A). The base pairs ranged from 980 to 3162 which was used to group or determine leukaemia pattern. Results showed 3 chronic CML which amplified at the same level with the ladder at 3162bp. Six patients each indicated ALL and CLL while three patients showed AML disease. Generally high CML was observed in the study area within the period under review.

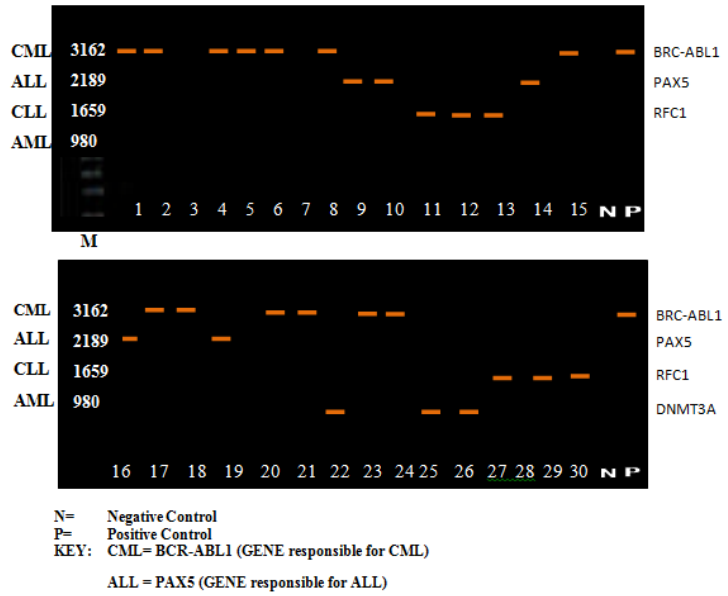

Fig-1A: DNA fragments amplification using OPA-09 primers in 30 leukaemic patients

DISCUSSION

The molecular prevalence of leukaemia 28(5.6%) by polymerase chain reaction (PCR) obtained in the present study, though not very high is a reflection of the presence of leukaemia in Kaduna state. This result is in agreement with the 6.2% reported by Ahmed *et al.* [12] among leukaemia patients in the university of Maiduguri teaching hospital, 7.3% in the southern region of the country by Degos *et al.*[17] reported in Uganda the prevalence rate of 3% leukaemia in children respectively. These values were however by far lower than 34% reported by Omoti [18] in University of Benin teaching hospital Edo state and 32% by Durosimi [19] in Ile Ife south western region of the country on leukaemic patients.

This variation in the prevalence rate of leukaemia from different regions of the country is a reflection of the level of leukaemia in these parts of the country. The regional differences in these countries could be attributed to the variation in the predisposing factors such as environmental conditions, climatic condition, and life style of people, the study population and the diagnostic methods used in the study. Similar conclusions have been made by previous researchers [18] who stated that infectious diseases such as tuberculosis, malaria, chronic infections, sickle cell disease as well as oncogenic virus, and also environmental factors are predisposing factors for leukaemia infections.

The preponderance of the infection in the males is consistent with the assertion that in Africa males are considered more vulnerable to disease than females [18]. However, the prevalence is statistically insignificant ($p>0.05$). Also, the mechanism of increase could be some home racial and physiological factors involved since Hofbrand [20] reported that genetic makeup plays a significant role in male with leukaemia and hence they have higher rate of depression than their female counterparts.

In this study, close observation revealed that the patients who had tertiary education recorded the statistically significant higher percentage of leukaemia. This is in consonance with the observation of Albano *et al*. They observed that level of education was releted to exposure to important risk factors of cancer such as smoking and obesity [21].

The higher prevalence among the farmers is attributable to the exposure to hazards such as environmental pollution and yet to be confirmed toxic waste deposition and radioactive waste product which have contributed to substantial number of lymphoid disorders. Also, there are various agents to which subsistence farmers and artisans are exposed that have been implicated in aetiology of leukaemia. Also, researchers in Italy have demonstrated that farmers and industrial workers have a significant risk for haematological malignancies [22].

With respect to social habits of patients, those who were drinking alcohol had the highest prevalence followed by those that smokes cigarette while those who do not smoke nor drink alcohol had the least prevalence. This is not surprising because alcohol acts and precipitates DNA thereby leading to transformation of genetic materials. This observation is in agreement with the work of Omoti[11] which stated that drinking of beer or alcohol is positively associated with leukaemia. Also, the study of Melo *et al.*[23] showed that prolonged smoking of cigarette and meat intakes were risk factors for AML.

The prevalence rate of leukaemia in different senatorial districts of Kaduna state is a reflection of the level of leukaemia in this part of the country. These regional differences could be attributed to variation in the predisposing factors such as environmental factors, study population and diagnostic test method used in this study. The senatorial districts of the state at risks were Kaduna north and south senatorial districts with highest prevalence. This is in agreement with some researchers [18] who stated that chemicals and petrochemical industries, as well as ionizing radition, certain drugs and viruses are risky factors for leukaemia infection. Kaduna north and the south district have a good number of chemical industries and hospitals.

The findings from this study indicated a higher percentage of 15 (3%) of the patterns of chronic myeloid leukaemia by cytomorphological method, followed by Acute lymphoblastic leukaemia 6 (1.2%) as well as chronic lymphocytic leukaemia but Acute myeloid leukaemia had the least percentage of 3 (0.6%). This observation was also recorded by Omoti *et al.*[18] who stated that chronic myeloid leukaemia were the most common among other leukaemias in the southern part of Nigeria. Also, 3.8% of CML was reported in Thailand. In West Indies, 0.6% was recorded as compared to 0.8% recorded in France by Benson *et al.*[22].

Conclusion

Out of the 500 blood samples studied, the overall prevalence rate was 30 (6%) by cytomorphological examination of lieshman-stained blood films and 28 (5.6%) by PCR assay. Leukemia was significantly associated with occupation status, educational status and senatorial districts. Based on the findings in this study, I wish to recommend that government should upgrade the haematology daycare unit so that tissue transplantation and weekly monitoring of anaemia can be done to improve leukemia patient's conditions. Furthermore, government should mobilize human and financial resources towards leukemia prevention by increasing understanding of the economic impact of the disease, Leukemia patients are advised to go for early diagnosis and treatment in any approved haemato-oncology centre. Also, Leukemia therapy should be made as safe as possible so that side effects will not be a discouraging phenomenon to the patients. Other aspects of the epidemiology of leukemia especially factors responsible for low on moderate prevalence in the area should be extensively studied as the findings may help towards the reduction or control of the infection in areas with high prevalence rate.

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