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Chromosomal aberrations in Nigerians with haematological malignancies: Preliminary Report

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Summary

Cytogenetic studies were carried out on the blood/bone marrow samples of three patients with chronic granulocytic leukaemia (CGL) and one patient with Burkitt's lymphoma. The karyotypes determined were 46, XY, Ph+ for patient O. A; 46, XY, t(9q$^+$, 22q$^-$) for T. A. and 46, XX, t(9q$^+$, 22q$^-$) for patient E. O. Patient O. L. with Burkitt's lymphoma presented two karyotypes: 46, XY and 46, XY, + 18. The results showed that Philadelphia (Ph^1) chromosome was detected in all the patients with CGL and a chromosome marker for African Burkitt's lymphoma in the boy with the disease.

Resume

Nous avons mene des etudes cytogenetiques sur les prelevements de sang/moelle de trois patients souffrand de leucemie granulocytique chronique (LGC) et un autre patient qui souffre de lymphone de Burkitts. Nous avons pu relever le chromosome de Philadephie (Ph) chez tous les patients avec LGC et un indicateur de chromosome de lymphone de Burkitts chez le patient atteint de cette maladie.

Introduction

The cytogenetic basis of leukaemia is now quite well understood although fine details are still unfolding[1-4]. Chromosome banding techniques were used in the identification of the Philadelphia (Ph^1) chromosome as a chromosome 22 which has lost a substantial part of its long arm (22q$^-$). The Ph^1 chromosome has become a biological marker in leukaemia and some other human syndromes[1,5,6].

Apart from the well-known translocation between chromosomes 22 and 9 t(9q$^+$; 22q$^-$) many complicated chromosome interchanges have been documented in leukemias and lymphomas. In acute myeloid leukemia (AML), t(8q$^-$; 21q$^+$), t(15q$^+$; 17q$^-$), - 7, + 8, -X, -Y are some of the chromosome rearrangements and aneuploids known[2]. In chronic myeloid leukemia (CML) the typical rearrangement is t(9q$^+$; 22q$^-$). This rearrangement was first described by Nowell and Hungerford[5] and identified by Rowley[7]. It is now accepted that the Ph^1 chromosome can be found in chronic and acute leukemias and in a variety of myeloproliferative disorders[2]. However, it is normally found in about 90% of patients with typical chronic granulocytic leukaemia (CGL).

In addition to the chromosome rearrangements aneuploids like + 7, t22q$^-$ (an additional Ph^1, +ii (17q) (an additional isochromosome of the long arm of chromosome 17) have been found in GCL[2].

Karyotypes with X 5, -7, +8, +19 and even t(9q$^+$; 22q$^-$) have been reported in acute non lymphocytic leukaemia (ANLL)[1,4]. Similarly Manolov and Manolova[8] described t(8q$^-$; 14q$^+$) with the variants t(2p$^-$; 38q$^+$) and t(8q$^+$; 22q$^-$) in Burkitt's lymphoma. Kristoffersson *et al* [9] reported that chromosomes 1, 3, 6, 7, 12 and 14 were involved in aberrations in malignant lymphomas. A t(4; 11) was reported in childhood acute lymphocytic leukemia[1].

We are not aware of any cytogenetic work on leukaemia in Nigeria. Adeyinka [10] however illustrated the value of karyotype analysis in patient management citing a case of Down's syndrome with 46, XY+G and a Turner with a mosaic: 45 X/46XX. This preliminary report presents gross chromosomal patterns in four patients: three with CGL and one with Burkitt's lymphoma (Table 1). The results give an indication of the chromosome patterns that are likely to show in leukemia and lymphomas in Nigeria.

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Table 1: Karyotype states in the patients studied

	Patient	No. metaphase cells	Karyotypes	Proportion	Diagnosis
1.	E. O. (No. 100427) M. 38 yrs.	36	46, XY, t(9q$^+$; 22q$^-$.)	100%	CGL
2.	T. A. (No. 76494) F. 12 yrs.	40	46, XX, t(9q$^+$; 22q$^-$.)	100%	CGL
3.	O. A. (No. 100299) M. 46 yrs.	30	46, XY, Ph^1+	100%	CGL
4.	O. L. (No. 109595) M. 12 yrs.	60	46, XY 46, XY, + 18	90% 10%	Burkitts Lymphoma

Materials and methods

Collection of blood

Blood samples were obtained as peripheral and bone marrow blood from patients in heparinized bottles. The samples were allowed to settle in the laboratory at room temperature for 1 hour.

Preparation of culture

The medium used is McCoy's 5A medium supplemented with L- glutamine (Gibco), 10cm^3 of medium at room temperature were dispended into sterilized culture flasks. About 0.5 cm^3 of buffy coat was dispensed into each other culture bottle with 4 drops of fetal calf serum. The culture was incubated at 37°C for 23 hours. 2 drops of colcemid (10 micrograms/cm^3) were added per 10cm^3 media and the culture was allowed to incubate for one more hour at 37°C.

Harvesting of leucocytes

Flask contents were poured into 15cm^3 centrifuge tubes and centrifuged at 1200 rpms for 5 minutes. The supernatant was discarded and the pellet was immediately resuspended by agitation in residual medium. 10cm^3 of 0.74MKCl at 37°C was added to the resuspended pellet and allowed to stand at room temperature for 10 minutes. Centrifugation was done at 1200 rpms for 5 minutes to recover the pellets. The fixing procedure was repeated two more times after which the cells were stored overnight in the refrigerator.

Spreading the cells

The next day, cells were centrifuged at 1000 rpms. The cells were resuspended and 5cm^3 of 3:1 methanol-glacial acetic acid were added. Cleaned slides kept in distilled water and stored in refrigerator overnight were used for spreading cell. Suspension was dropped under gravity on the slides to ensure good spread and the slides were appropriately labelled and dried on slide drier overnight. Slides were stained in FLP orcein[11].

Karyotypes

Good metaphase spreads were photographed under phase contrast with a Leitz Dialux research microscope. The final prints were done at an enlargement factor of 7 and the karyotypes were constructed from very good spreads. The karyotypes are described according to the International system for Human Cytogenetic Nomenclature[12].

Patients

(i) E.O. (No. 100427)

A 34 year old Civil Servant from Ilesa. He presented to the Casualty on 7/11/86 with sustained priapism of 36 hours duration. Further history, physical examination and laboratory investigations confirmed Ph' positive CGL in accelerated phase. He had a massive splenomely of 25cm below the costal margin, haematocrit was 0.15L/L, platelets of 236 x 10^9/L and leucocytes of 528 x 10^9/L. He had immediate bilateral cavernostomy and packed red cells in addition to other resuscitative measures. He was also put on busulphan and allopurinol tables. As at 29/1/87 (when he was last seen) his haematological parameters had improved with haematocrit of 0.40L/L, platelets 216 x 10^9/L and leucocytes of 60 x 10^9/L. His spleen went down to 5cm. He was yet to regain penile erection. This patient is presumed dead.

(ii) T. A. (No. 76494)

A 12 year old first year secondary school girl from

Ilesa. First seen 14/7/86. She was discovered to have CGL during investigation for a febrile illness and tenderness on the left hip. Full examination and laboratory investigations confirmed Legg-calve-pethes disease with Ph'+ve CGL[13]. Her haematocrit at diagnosis was 0.43L/L, platelets 193 x 10^9/L and leucocytes 104 x 10^9/L with steady state distribution. She never had splenomegaly nor hepatomegaly, she did well on busulphan. She is still in remission and has been off busulphan or any other chemotherapy for the past six months.

(iii) O. A. (No. 100299)

A 46 year old agricultural extension officer from Akure, Ondo State. He presented on 30/10/86 with a 9 month history of weakness, recurrent fever and insomnia. Routine haematological investigation confirmed steady state Ph'+ve CGL with a haematocrit of 0.39L/L, platelets of 114 x 10^9/L and leucocytes of 132 x 10^9/L. There was sternal tenderness. Spleen and liver were not palpably enlarged. He responded well to busulphan and he is currently in remission.

(iv) O. L. (No. 109595)

He was a 12 year old boy from Ile-Ife. He presented to the childrens' emergency room on 19/12/87 with a 4 weeks history of rapidly progressive abdominal swelling. Physical examination and laboratory investigations confirmed stage D Burkitt's lymphoma (abdominal and bone marrow involvement). The jaws were grossly normal. He showed an initial good response to chemotherapy but he later succumbed to central nervous system relapse on 21/12/88.

Results and observations

Table 1 shows karyotype states in the patients studied. Patients E. O. and T. A. have a distinctly heteromorphic pair of chromosome 9 each with the longer member showing a distinctly heteropycnotic distal end of its long arm (9q) as shown in Plates 1C and 2 for E. O. and in Plate 3 for T. A. These chromosomes were monitored in all the cells studied in these patients as shown in Table 1. The karyotype of patient O. A. (Plate 4) does not reveal a distinct heteromorphic state for chromosome 9 but the Ph^1 chromosome was distinct in all the cells studied as in the case of patients E. O. and T. A. The karyotype of patient O. A. is therefore designated 46, XY Ph^1 +.

Patient O. L. presents a different karyotypic picture. Two distinct clones were observed --46, XY in 90% of the cells studied (Plates 1A and 5) and 46, XY, + 18 in 10% of the cells studied (Plates 1 B and 6). The 46, XY karyotype of the patient was carefully examined in all the spreads photographed to ensure that it does not represent a pseudodiploid. As Plate 5 shows, the chromosomes are well matched, barring cryptic structural rearrangements. It is also evident that the cells of the patient are Ph^1 negative. It would seem therefore that the major abnormality in this patient is a trisomy for chromosome 18.

Plate 1: Metaphase spreads in some patients studied

A. Normal karyotype in patient O.L. Arrow shows a chromosome touching an undivided cell.

B. Karyotype of patient O.L. showing 47, XY, +18. Arrows show the three copies of chromosome 18. The broken circle shows some artefact.

C. Karyotype of patient E.O. The solid arrows show the 2 copies of chromosome 9. Note the deeply stained distal end of the long arm (9q) of the long chromosome 9. The broken arrow shows the Ph^1 chromosome.

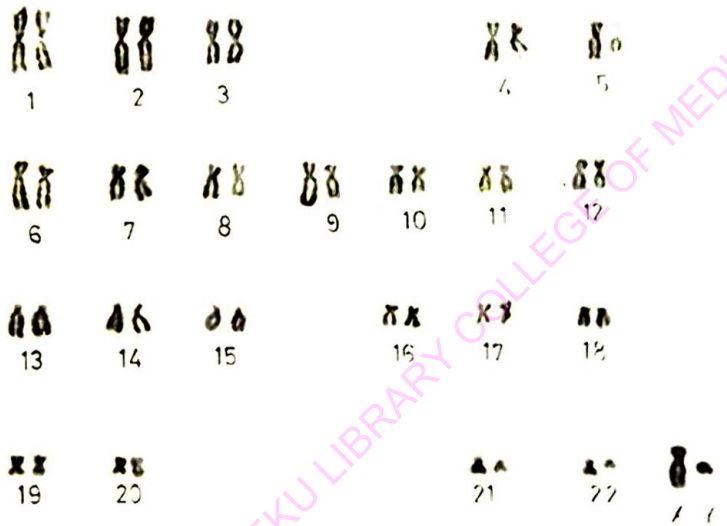

Plate 2: The karyotype of patient E.O. 46, XY, t(9q+ ; 22q-). Note the distinct heteromorphic chromosome 9.

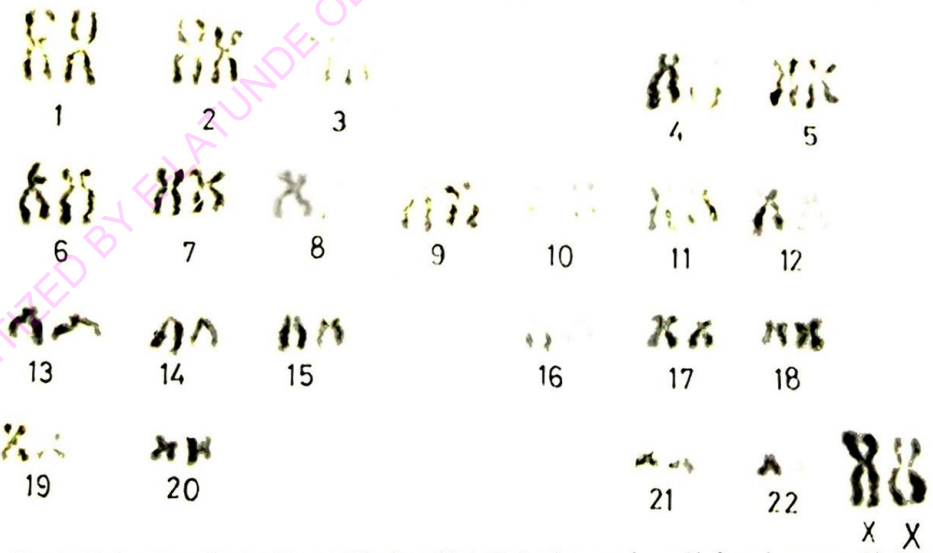

Plate 3: The karyotype of patient T.A. 46, XX, t(9q+ ; 22q-). The break on one chromatid of one chromosome 1 is due to treatment effect.

Plate 4: Karyotype of patient O.A. 46, XY, Ph^1+.

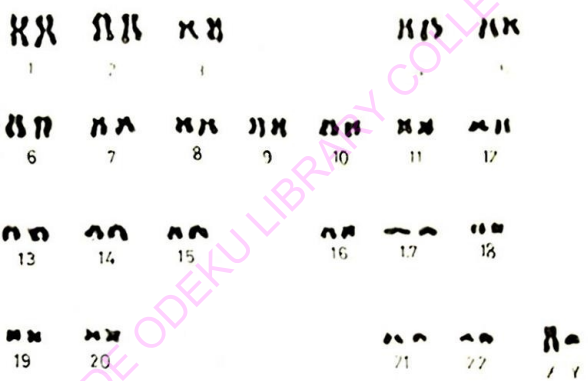

Plate 5: Karyotype of patient O.L. 46, XY.

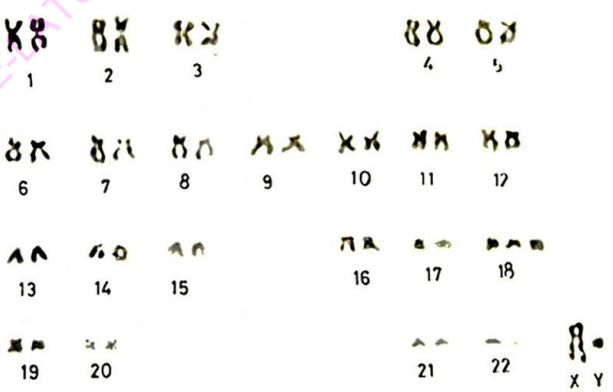

Plate 6: Karyotype of patient O.L. 46, XY, +18.

The three patients with CGL had Ph^1 (Plates 1, 2, 3 and 4) and the sample from the single patient with Burkitt's lymphoma revealed a marker chromosome for the disease (Plate 6). The karyotypes presented in these photomicrographs were from bone marrow cells except patient E. O. whose karyotype was obtained from peripheral blood. In all the patients with CGL both peripheral and bone marrow karyotypes correlated. However, abnormal karyotypes were not seen in the peripheral blood of the Burkitt's case.

Discussion

The chromosome patterns reported in this study are similar to those generally encountered in leukaemia and lymphomas — simple to complicated rearrangements associated or not associated with Ph^1, rearrangements with or without Ph^1 accompanied with loss or gain of autosomes and/or sex chromosomes, and normal karyotypes[1,2,7,9]. The karyotypes encountered in this study fall in these categories except that none lacked sex chromosomes.

The trisomy seen in patient OL is similar to a marker chromosome which McCaw *et al*[3] reported was of the size of chromosome 18 in African Burkitt's lymphoma. No rearrangement could be detected in this karyotype because no banding was done.

All the patients with CGL examined in this work have the Ph^1. Their haematological and clinical manifestations are similar to documented cases of classical (i.e. Ph + ve) CGL[14]. This further confirmed the diagnostic importance of this chromosome in patients with typical CGL, irrespective of race or region.

With the exception of Ph^1 positive CGL patients managed with allogeneic bone marrow transplantation[14] and some of those treated with alpha interferon[15], induction of remission with conventional chemotherapy (as in our patients) hardly ever reverts the marrow karyotypes to normal[14]. Hence none of our two patients who are still in remission had a repeat karyotyping. Further more, Ph^1 + ve in CGL is no longer considered to be of any prognostic importance[14], the favourable prognostic factors are not identified as younger age at presentation, absence of splenomegaly, female sex and low blast counts at diagnosis[15]. Two of our patients — T. A. and O. A., especially the former had these positive criteria which may contribute to their apparent long remission. Patient E. O. presented in accelerated phase and hence his early death.

The boy with Burkitt's lymphoma behaved as expected. Prognosis in this disease depends largely on the clinical stage at diagnosis rather than on the chromosomal pattern. However, it would have been revealing to see whether the chromosomal aberration would disappear if he had survived long enough — say uninterrupted 2 years remission.

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