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HEMATOLOGICAL NEOPLASMS IN ECUADORIANS: MOLECULAR AND CYTOGENETIC CHARACTERIZATION

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ABSTRACT

In terms of cancer, haematological malignancies are consistently ranked in the top ten most frequent cancers that occur each year in Ecuador. In this multicenter study project, the participants were patients who had been diagnosed with a variety of haematological illnesses between 1984 and 2012. It was revealed that aberrant chromosomes were present in 45.9% of the instances including 1886 individuals. In addition to conventional cytogenetics, the use of fluorescence in situ hybridization (FISH) and reverse transcription-PCR (RT-PCR) enabled the identification of genetic rearrangements. FISH and RT-PCR, both of which reported more positive occurrences, pointed to the same conclusion. We investigated fusion genes that resulted from 11q23 rearrangements, translocations (8;21), translocations (15;17), inversions (16), translocations (9;22), translocations (4;11), and t (1;19). The frequency of fusion gene transcripts was of particular interest as a result of the fact that our results were different from those of earlier studies. The fusion gene BCR-ABL was found in the b2/a2 transcript in 95% of CML patients, while the b3/a2 transcript contained BCR-ABL in the remaining 5% of CML patients. Additionally, the expression of the PML-RARA fusion gene transcript was distinct. This fusion gene had the bcr2 transcript at a frequency of 36% and the bcr3 transcript at a frequency of 64%; however, the bcr1 transcript was not discovered in our sample group. Every instance of the CBFB-MYH11 fusion gene was discovered to include the F transcript. In addition, this translation is quite uncommon all across the world. Patients with the MLL-AF4 fusion gene always have the E7-E8 transcript present in their genomes. It is possible that discrepancies in the frequency of many fusion gene subtypes in the Ecuadorian population, which is mostly made up of mestizos, were caused by a combination of genetic and environmental factors.

KEYWORDS: - Hematological Diseases; Chromosomal Aberration; BCR-ABL; PML-RARA; CBFB-MYH11; MLL-AF4; Geographical Heterogeneity

INTRODUCTION

The World Health Organization (WHO) categorizes tumors that may be found in myeloid and lymphoid tissues; some types of these tumors can be recognized by certain genetic abnormalities. This is how haematological neoplasms are classified generally speaking. The finding of clonal abnormalities, which provides invaluable information for both prognosis and treatment, might lend credence to the hypothesis of a neoplastic or premalignant condition. The World Health Organization (WHO) has defined a growing number of haematological neoplasms as a result of genetic anomalies, which has led to an increase in the number of specific therapy options that directly or indirectly target genetic disorders. Utilizing genetic analysis for illness diagnosis, categorization, and prognostication, as well as monitoring the body's reaction to treatment, is thus very necessary. In recent years, a number of cytogenetic diagnostics, including as chromosomal banding and fluorescence in situ hybridization (FISH), have become more important in the therapeutic management of patients. In the testing that is done in the future, technologies based on arrays and processes that sequence the whole genome will take the place of karyotyping and FISH. In today's world, genetic abnormalities are analyzed utilizing a wide variety of techniques, either alone or in combination. Even though they are not being used in diagnostic settings at the present time, new technologies that can simultaneously detect copy number alterations, structural differences, and mutations are now available. It is anticipated that this approach will be extensively used in the future even though it is not being used in diagnostic settings at the present time. This study does not include further suggestions for molecular testing since doing so would go beyond the scope of the project. It is essential that a comprehensive report be delivered, one that takes into account cytogenomic as well as molecular genetic information. This material need to contain prognostic information derived from cytogenetics, provided that it is appropriate.

LITERATURE REVIEW

Yassmine M. N. Akkari (2022) - Since the field of cytogenetics was first developed, it has been very significant in the process of diagnosing hematologic tumors. Chromosome banding experiments have shown that variations in copy quantity and structural features throughout the whole genome may lead to carcinogenesis, provide insight into the nature of diseases, and direct treatment. The advancements in sequencing technologies that have occurred in recent years ushered in the present era of clinical genomics. We, an international consortium of laboratory geneticists, pathologists, and oncologists, share our thoughts on crucial next steps to implement these novel technologies in a global clinical setting for the purpose of producing more accurate diagnoses. In this article, we describe the benefits and drawbacks of both conventional chromosome banding and novel sequencing technologies, as well as their similarities and differences. We provide some things to think about regarding the expansion of cytogenetic testing all over the globe, taking into account the clinical, logistical, technical, and economic sides of the matter.

K. A. Rack (2019) - K. A. Rack Cytogenomic investigations, such as chromosomal banding and fluorescence in situ hybridization (FISH), are becoming more important in the therapeutic care of patients who are afflicted with haematological tumors. This is because these types of research may help clinicians better target their treatment. Even though cytogenomic testing may be carried out using a number of distinct approaches, the widespread use of this technique in the field of genetic diagnosis has brought into sharper focus the need for expert guidance about the criteria that are considered to be the most essential. In order for laboratories to carry out their operations in accordance with the standards that are generally recognized and to provide a service of sufficient caliber, the aforementioned recommendations give a document that is current, applicable, and easy to understand.

Aikaterini Koutsi (2018) - There are a number of chromosomal abnormalities as well as epigenetic alterations that have the potential to induce haematological disorders. The sequencing of the genome and the exome have been found to be highly successful in identifying a large number of variations that are connected to haematological conditions including sickle cell anemia and acute lymphoblastic leukemia. knowledge is mounting that genetic information may be used to a wide variety of therapeutic practices, such as diagnosis, prognosis, and the prediction of a patient's reaction to treatment. Patients suffering from haematological diseases may benefit from novel personalized medications as a result of this knowledge.

Shailendra Dwivedi 2017 - The study of genomics has shifted its emphasis to the identification of pathogenic occurrences at the level of the genome as a result of the recent proliferation of molecular technology and the cross-disciplinary interaction between a number of different fields. The approaches of structural and functional genomics have brought to light the technical challenges involved in the identification of disease-related genes, as well as the identification of structural anomalies or the clarification of gene function. Utilizing mutation scanning and the technology behind DNA chips, structural genomics is now creating a large library of disease genes, genetic alterations, and other genetic variants. As a component of functional genomics (hybridization, PCR, and sequence-based technologies), as well as the two-hybrid technique, next generation sequencing in conjunction with bioinformatics and computational biology is now being investigated as well. Because of advancements in "chip" technology known as microarrays, it is now feasible to examine the expression patterns of thousands of genes in parallel at the same time. Single nucleotide polymorphisms, sometimes known as SNPs, are being found at a pace that has never been seen before because to the abundance of data that has been collected from the genomes of a large number of individuals. The method of phage display as well as the generation of genes that encode antibodies have both contributed to the revolution that has taken place in immunoassay biotechnology. One of the primary responsibilities of biotechnology is the creation of diagnostic tests that may be used in the event of an outbreak or other medical emergency. In addition, there is a need to handle the commercialization and widespread transmission of genetic information that is produced by the biotechnology industry, as well as the production and marketing of diagnostic services. The pharmaceutical industry has a significant challenge when it

comes to the use of genetic factors for the selection of patients and the individualization of assessments of the dangers and benefits of treatment. As a result of the revolutionary character of this field, it is possible that new approaches to the treatment of sickness may become available in the near future.

RESEARCH AND METHODOLOGY

Between the years 1984 and 2012, a total of 4108 people from ten institutions located in a variety of places in Ecuador who had been referred to the genetic study were investigated for their various haematological cancers. The haematological illnesses were classified according to WHO guidelines when they were examined and diagnosed. As soon as the bone marrow cells were extracted from the body, they were put through a cytogenetic analysis to determine their genetic make-up. In order to categorize the morphological and numerical anomalies that were discovered by the G-banding method, the International System for Human Cytogenetic Nomenclature was used.

The results of the cytogenetic tests were categorized as either having a normal karyotype, being hyperdiploid, being hypodiploid, having a translocation, or having a difficult karyotypy. Numerous rearrangements, such as t (8;21), t (15;17), and 11q23 (t (1;19) and t (9;22), are associated with ALL, AML, and CML. This is the case with many of the rearrangements. Because chromosomal abnormalities with three or more alterations are so rare, this group of haematological malignancies has been given the name "complex karyotype" to reflect their unusual nature. It was agreed to separate the patients into two unique groups, each of which would be based on their respective ages. Patients who were less than 15 years old were placed in one group, known as the pediatric group, while patients who were more than 16 years old were placed in the adult group.

It was discovered that the fusion genes were found utilising the t (8;21) (q22; q22), t (15;17) (q21; q22), inv (16/t (16;16) (p13 q22), t (9;22) (q34 q11.2), t (1;19) (q23 q13.3), t (12;21 (p13 q22) and translocations involving 11q23 and 8q24. LSI PBX1 Dual Color, Dual Fusion Translocation Probe; LSI TCF3/PBX1 Dual Color, Dual Fusion Translocation Probe; Vysis LSI ETV6(TEL)/ RUNX1(AML1) ES Dual Color Translocation Probe Set; Vysis LSI MLL Dual Color, Break Apart Rearrangement Probe; and Vysis LSI MYC Dual Color Break Apart Rearrangement Probe (Abbott). Each sample was evaluated using two hundred cells, with normal blood cells serving as a negative control. We used previously established techniques to extract total and messenger RNA from bone marrow samples [24]. Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to identify the junction location of the chimeric gene in the RNA of the samples.

DATA ANALYSIS

Males outnumbered females by a margin of 56.7 percent to 1780 (43.3 percent) of the 4108 newly diagnosed patients with various haematological malignancies. An average patient age was 43,

however the variation was wide. There were 715 acute myeloid leukaemia (AML), 1260 acute lymphoblastic leukaemia (ALL), 168 myeloproliferative disorder (MPD), 945 CML, 15 polycythemia vera (PV), 63 essential thrombocythemia (ET) and 2 monoclonal gammopathy of undetermined significance (MGUS) (Figure 1). Figures 2 and 3 depict the cases, which were sorted according to gender and age.

Conventional Cytogenetics

We analysed a minimum of 20 metaphases per patient using conventional cytogenetics in 4108 individuals. A total of 1886 (45.9 percent) individuals showed chromosomal abnormalities, whereas 1405 (34.2 percent) had a normal karyotype.

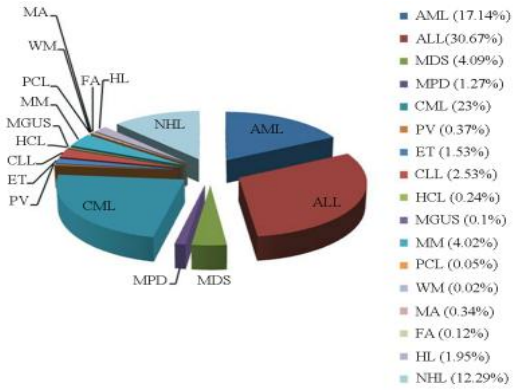

Figure 1. Types of hematological diseases in which genetic study was conducted in Ecuadorian population, 1984-2012.

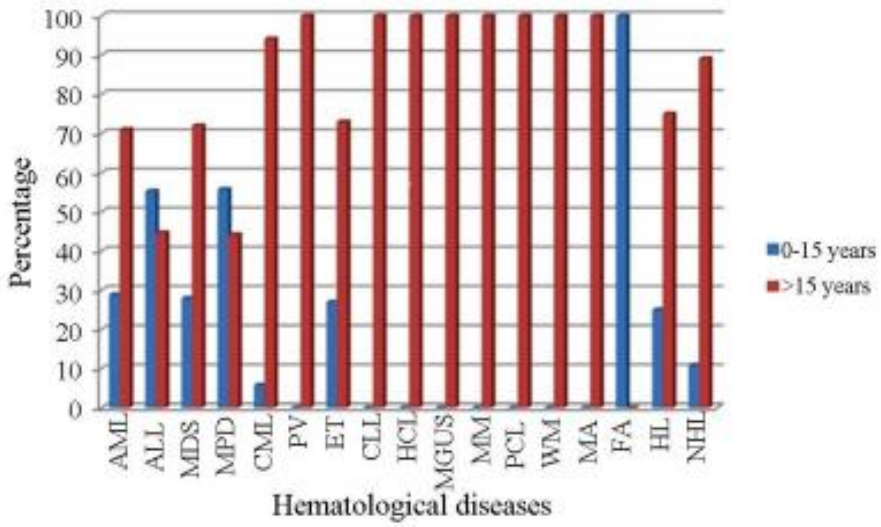

Figure 2. Hematological diseases analyzed by group of age in the Ecuadorian population.

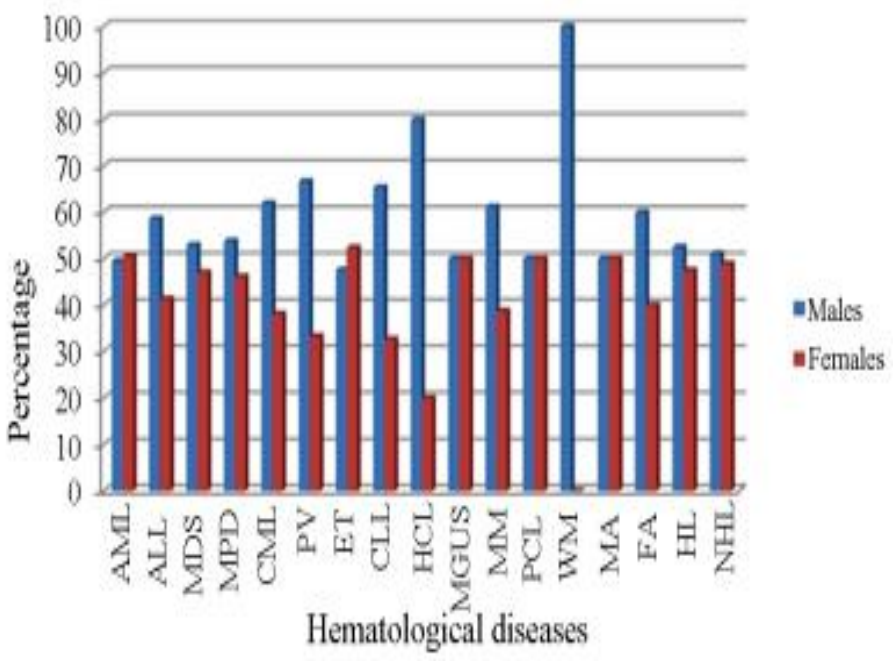

Figure 3. Hematological diseases analyzed by gender in the Ecuadorian population.

Numeric and structural chromosomal abnormalities were found in 24.6 percent of individuals, as well as karyotypes with multiple chromosomes (24.8 percent patients). Only a small percentage of the samples were able to be categorised using this method. Most prevalent translocations in structural chromosomal modifications were t (8;21), t (15;17), 11q23 rearrangements, t (1;19) and t (9;22) and were linked to the development of cancers of the blood.

Table 1. Cytogenetics findings in Ecuadorian patients with hematological neoplasm.

Hematological Diseases[a]	Cases (n)	Normal Karyotype		Altered Karyotype								No Methaphases	
				Hyperdiploid		*Hypodiploid*		*Translocation*		*Complex*			
		n	%	n	%	n	%	n	%	n	%	n	%
AML	715	259	36.2	66	9.2	57	8.0	68	9.5	110	15.4	155	21.7
ALL	1260	419	33.3	102	8.1	92	7.3	99	7.9	191	15.2	357	28.3
MDS	168	75	446	15	8.9	9	5.4	4	2.4	20	11.9	45	26.8
MPD	52	26	50.0	8	15.4	3	5.8	3	5.8	8	15.4	4	7.7
CML	945	100	10.6	6	0.6	6	0.6	756	80.0	10	1.1	67	7.1
PV	15	9	60.0	1	6.7	1	6.7	0	0	0	0	4	26.7
ET	63	35	55.6	0	0	1	1.6	1	1.6	4	6.3	22	34.9
CLL	104	46	44.2	7	6.7	5	4.8	2	1.9	15	14.4	29	27.9
HCL	10	5	50.0	1	10.0	0	0	0	0	0	0	4	40.0
MGUS	4	3	75.0	0	0	0	0	0	0	0	0	1	25.0
MM	165	63	38.2	14	8. 5	7	4.2	8	4.8	30	18.2	43	26.1
PCL	2	1	50.0	0	0	0	0	0	0	0	0	1	50.0
WM	1	1	100.0	0	0	0	0	0	0	0	0	0	0
MA	14	5	35.7	0	0	0	0	0	0	2	14.3	7	50.0
FA	5	2	40.0	0	0	0	0	0	0	3	60.0	0	0
HL	80	56	70.0	3	3.8	2	2.5	0	0	9	11.3	10	12.5
NHL	505	300	59.4	30	5.9	27	5.3	14	2.8	66	13.1	68	13.5
Total	4108												

[a]ALL, Acute Lymphoblastic Leukemia; AML, Acute Myeloid Leukemia; MDS, Myelodysplastic Syndromes; MPD, Myeloproliferative Disease; CML, Chronic Myeloid Leukemia; PV, Polycythemia vera; ET, Essential Thrombocythemia; CLL, Chronic lymphocytic leukemia; HCL, Hairy cell leukemia; MGUS, mono-clonal gammopathy of undetermined significance; MM, Myeloma Multiple; PCL, Plasma Cell Leukemia; WM, Waldenstrom Macroglobulinemia; FA, Fanconi Anemia; MA, Medullary Aplasia; HL, Hodgkin Lymphoma; NHL, Non-Hodgkin Lymphoma.

Among the most common chromosomal abnormalities in leukaemia have been found in this investigation. The following are some of the most significant findings:

Trisomy 8 (4%) was the most common chromosomal abnormality detected in AML, followed by t (8;21) (4.3%), t (15;17) (3%) and inv (16) (1.3%) (5.3 percent). 11q23 rearrangements, t (9;22) (15.5%), t (1;19) (4.8%), t (4;11) (1.2%), and t (8;14) were the most common in ALL (0.4 percent each). Eighty percent of CML patients have t (9;22). Chromosome 14 rearrangements were found in 8.5% of MM patients. In NHL, translocations were found in 20% of the changed metaphases.

FISH Evaluation

Samples from patients without metaphases or with normal karyotypes were utilised for FISH, using probes specific to the most common leukaemia rearrangements. 13/715 AML patients, 31/1260 ALL patients, and 7/945 CML patients had changes that boosted our favourable outcomes by 2 percent, 2.5 percent and 1 percent correspondingly (Table 2).

Molecular Study

The results of the FISH experiment were consistent with those obtained from the molecular analysis (Table 2). It was discovered that there was an increase in good outcomes from conventional cytogenetics in 22/715 AML patients, 29/1260 ALL patients, 65% of CML patients, and 3.1 percent of CML patients who did not have any metaphases in their cancerous cells.

Products of the AML1-ETO RT-PCR were discovered in instances in whom the t (8;21) translocation was established by FISH. It was discovered that AML patients with PML-RARA rearrangements had the bcr2 and bcr3 transcripts in 36% and 64% of the cases, respectively. There was not a single one of these people who had the bcr1 transcript. Seven patients who had ALL have the CBFB-MYH11 fusion transcript, and all of these patients had the type F transcript. RTPCR was able to identify the BCR-ABL fusion gene in every single one of the t (9;22) positive ALL patients, and every single sample showed evidence of the e1-a2 transcript. Four of the ten patients with ALL who had 11q23 rearrangements were found to have the t (4;11) within the MLL-AF4 fusion gene. In each and every case, the e7-e8 transcript was found to be present. The E2APBX1 fusion gene discovered was designated by the symbol t (1;19). In CML patients who had the BCR-ABL fusion gene, the b2a2 and b3a2 fusion transcripts were detected in 95% and 5% of those individuals, respectively.

Table 2. Positive results in AML, ALL and CML cases without metaphases and normal karyotypes.

FISH		**RT-PCR**		
AML				
Probe	No. Cases[a]	**Fusion Gene**	No. Cases[b]	**Transcripts**
t (8;21)	4	AML1-ETO	4	
t (15;17)	5	PML-RARA	11[c]	4 (bcr2) & 7 (bcr3)
Inv (16)	1	CBFB-MYH11	7[d]	7 (type F)
(v 11q23)				
ALL				
Probe	No. Cases[e]	**Fusion Gene**	No. Cases[f]	**Transcripts**
t (9;22)	9	BCR-ABL	18[g]	18 (e1a2)
(v 11q23	10	) MLL-AF4	4[h]	4 (e8-e7)
t (1;19)	1	E2A-PBX1	1	7 (type F)
t (12;21)	9			
t (8;14)	2			
CML				
Probe	No. Cases[i]	**Fusion Gene**	No. Cases[j]	**Transcripts**
t (9;22)	7	BCR-ABL	65[k]	62 (b2-a2) & 3(b3a2)

[a]FISH analysis in 13 cases; [b]RT-PCR analysis in 22 cases; [c,d]These results included the positive cases by FISH and other cases without cytogenetic result; [e]FISH analysis in 31 cases; [f]RT-PCR analysis in 29 cases; [g]This result included the positive cases by FISH and other cases without cytogenetic result; [h]This result shown the specific fusion gene in 4/10 cases with rearrangement of 11q23 determined by FISH; [i]FISH analysis in 7 cases; [j]RT-PCR analysis in 65 cases; [k]This result included the positive cases by FISH and other cases without cytogenetic result.

The RT-PCR analysis was not carried out in patients with AML who had 11q23 rearrangements and ALL patients who had t (12;21) and t (8;14).

CONCLUSION

Hematological illnesses are a leading cause of death in Ecuador. A wide range of chromosomal abnormalities and molecular haematological issues have been documented, however there is a lack of knowledge in Ecuador. Patients with hematologic illnesses who were submitted for genetic testing had their chromosomes characterized in this study, allowing researchers to learn more about the course of the disease and its prospects for recovery. Since 1984, when cytogenetic research started in Ecuador, we offer data from genetic investigations undertaken in three reference institutions in Quito. In addition to patients sent from 10 different Ecuadorian cities, these facilities studied their own patients. It is important to note that 45.9 percent of patients have chromosomal abnormalities, which might affect their prognosis and how well they respond to treatment. FISH probes were used in situations where a karyotype could not be obtained or a normal karyotype was obtained. We were able to identify an average of 1.8% more changes, which resulted in an increase in the number of individuals being categorised as having a blood disorder. Leukemia-associated translocations accounted for the majority of these mutations. By employing RT-PCR, we were able to confirm FISH-positive instances. Indigenous Amerindians and Europeans have coexisted peacefully in Ecuador for 500 years. Consider the possibility that the discrepancies in the frequency of distinct transcripts in the Ecuadorian population compared to other populations may be the consequence of a different genetic component in the Ecuadorian population [35,36]. When traditional cytogenetics couldn't solve a problem, FISH and RT-PCR procedures were utilised to provide findings that could be used to help patients' diagnoses. Fusion genes demonstrate distinct genetic behaviour than previously reported genes linked to disorders such cystic fibrosis, meningioma, and hemochromatosis, such as hRAD54 in meningioma.

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