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STUDY OF HEMATOLOGICAL NEOPLASMS IN AN ECUADORIAN POPULATION: MOLECULAR AND CYTOGENETIC CHARACTERIZATION

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ABSTRACT

When it comes to cancer, haematological malignancies are among the top 10 most prevalent cancers in Ecuador each year. Patients diagnosed with various haematological diseases between 1984 and 2012 were studied in this multicenter research. In 1886 patients, chromosomal abnormalities were found in 45.9% of cases. The employment of FISH and RT-PCR methods, in addition to traditional cytogenetics, allowed the detection of genetic rearrangements. Increased positive instances were found using FISH and RT-PCR, respectively. We examined fusion genes originating from translocations (8;21), translocations (15;17), inversions (16), translocations (9;22), 11q23 rearrangements, and translocations (4;11) and t (1;19). Because our findings differed from those of previous research, the frequency of fusion gene transcripts was of special interest. 95 percent of CML patients had the fusion gene BCR-ABL in the b2/a2 transcript, whereas the remaining 5 percent had the fusion gene BCR-ABL in the b3/a2 transcript. PML-RARA fusion gene transcript expression was also different. The bcr2 (36%) and bcr3 (64%) transcripts were present in this fusion gene, while the bcr1 transcript was not found in our sample group. The F transcript was found in all CBFB-MYH11 fusion gene instances. As a bonus, this transcript is quite rare in the globe. MLL-AF4 fusion gene patients all have the transcript E7-E8 in their genomes. Genetic and environmental factors may have contributed to differences in the frequency of several fusion gene subtypes in the Ecuadorian population, which is mostly mestizo.

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

INTRODUCTION

The World Health Organization (WHO) classifies tumours in myeloid and lymphoid tissues, and some of them are identified by specific genetic anomalies. Haematological neoplasms are categorised in this way. A neoplastic or premalignant state may be supported by the discovery of clonal anomalies, which gives valuable prognostic and therapeutic information. As the number of WHO haematological neoplasms classified by genetic abnormalities rises, so does the number of particular therapeutic options that target genetic problems directly or in some way. It is thus critical to use genetic analysis for diagnosis, classification, prognostication, and monitoring of disease response to therapy. A variety of cytogenetic tests, including chromosomal banding and fluorescence in situ hybridization (FISH), have become more significant in the therapeutic care of patients in recent years. In the future, karyotyping and FISH will be replaced by array-based methods and genome-wide

sequencing procedures in testing. Genetic anomalies are now evaluated using various procedures, either alone or in combination. Although they are not presently being utilised in diagnostic settings, new technologies that can concurrently detect copy number changes, structural variations, and mutations are now available, and it is envisaged that this method will be widely employed in the future. Other molecular testing recommendations aren't included in this paper since they're beyond its scope. An overall complete report should be provided that incorporates both cytogenomic and molecular genetic data. If applicable, this material should include prognostic information from cytogenetics.

LITERATURE REVIEW

Yassmine M. N. Akkari (2022) - Since its inception, cytogenetics has played an important role in the diagnosis of hematologic cancers. Genome-wide copy number and structural variation have been proven to cause carcinogenesis, characterise illnesses, and guide therapy via chromosome banding investigations. The current age of clinical genomics was ushered in by advances in sequencing technology. We, an international consortium of laboratory geneticists, pathologists, and oncologists, describe herein the advantages and limitations of both conventional chromosome banding and novel sequencing technologies and share our thoughts on crucial next steps to implement these novel technologies in the global clinical setting for a more accurate diagnosis. We present considerations for the worldwide development of cytogenetic testing, taking into account the clinical, logistical, technological, and economical aspects.

K. A. Rack (2019) - It has become more significant in the therapeutic care of patients suffering from haematological tumours to conduct cytogenomic studies such as chromosomal banding and fluorescence in situ hybridization (FISH). Although cytogenomic testing may be performed using a variety of methods, its extensive use in genetic diagnosis has emphasized the need for advice on the most important criteria to follow. In order for labs to operate within accepted norms and provide a quality service, these suggestions offer an updated, practical and simply accessible document.

Aikaterini Koutsi (2018) - There are several genetic abnormalities and epigenetic changes that may cause haematological diseases. Genome and exome sequencing were shown to be quite effective in finding numerous variants linked to haematological disorders, such as sickle cell anaemia and acute lymphoblastic leukaemia. Patients with haematological illnesses may benefit from new tailored therapies based on emerging evidence that genetic data may be used in many areas of clinical practise including diagnosis, prognosis, and response prediction.

Shailendra Dwivedi (2017) - Because of the present emergence of molecular technologies and the cross-disciplinary interaction of several areas, genomics has developed to focus on the detection of pathogenic events at the genome level. The structural and functional genomics techniques have now highlighted the technological difficulty in the discovery of disease-related genes and the detection of structural abnormalities or clarification of gene function. Structural genomics is now building a big library of disease-genes, genetic changes, etc., using mutation scanning and DNA chip technology. Next generation sequencing with Bioinformatics and computational

biology is also being explored as a part of the functional genomics (hybridization, PCR and sequence-based technologies) as well as two-hybrid technology The parallel examination of gene expression patterns for thousands of genes concurrently has been made possible by advances in microarray "chip" technology as microarrays. SNPs (single nucleotide polymorphisms) are being discovered at an unprecedented rate because to a wealth of data gathered from the genomes of many people. The phage display technique and the creation of antibody-encoding genes have also revolutionized immunoassay biotechnology. Developing diagnostic tests to respond to an epidemic or emergency illness need is a major function of Biotechnology. In addition, the commercialization and mass dissemination of genetic information generated by the biotechnology sector, as well as the creation and marketing of diagnostic services, must be addressed. The pharmaceutical business has a huge problem in the implementation of genetic criteria for patient selection and personalized evaluation of the risks and benefits of therapy. Because of its revolutionary nature, this area might offer up new avenues in illness treatment in the future.

Catharina Brant Campos (2015) - Acute leukaemia therapy relies heavily on cytogenetics and molecular genetics. Patients with acute myeloid leukaemia (AML) or acute lymphoblastic leukaemia (ALL) in Brazil were studied for genetic changes that were associated with clinical and laboratory data. One hundred fifty-five patients with acute myeloid leukaemia and 161 patients with de novo acute lymphoblastic leukaemia (ALL) were studied. More than a third of patients with acute myeloid leukaemia have t(15;17) (19.4 percent), whereas in the general population, the most common results are t(1;19) (9.7 percent), high hyperdiploidy (18.7 percent), and low hyperdiploidy (9.7 percent) (8.2 percent). PML-RARa 21.9 percent, RUNX1-RUNX1T1 7.1 percent, CBFB-MYH11 and MLL-AF9 2.6 percent, FLT3-ITD 14.2 percent, NPM1mut 13.6 percent were the most common gene fusions and mutations in AML. TCF3-PBX1 was found in 10.8% of cases, ETV6-RUNX1 and BCR-ABL in 11.5%, and ETV6-RUNX1 and MLL-AF1 in 1.5% of ALL. There were 3.6 per cent of instances where the RT-PCR and CC results did not agree. PML-RARa, FLT3-ITD, and NPM1mut were all related with younger ages, lower WBC and platelet counts, and longer ages and normal karyotypes. Age was connected with BCR-ABL; MLL-AF1 with WBC and EGIL BI-subtype; and EGIL BI was associated with BCR-ABL. Some AML abnormalities were more common than previously reported in the literature from other countries. For the diagnosis of leukemias to be accurate, it is necessary to use both conventional and polymerase chain reaction (PCR).

RESEARCH AND METHODOLOGY

4108 individuals with diverse haematological malignancies were studied between 1984 and 2012 from ten institutions in different cities in Ecuador who were referred to the genetic research. The WHO classification was used to diagnose the haematological disorders. Bone marrow cells were subjected to a cytogenetic examination as soon as they were removed from the body. The International System for Human Cytogenetic Nomenclature was used to classify the structural and numerical abnormalities identified by the G-banding procedure.

The findings of the cytogenetic tests were divided into five categories: normal karyotype, hyperdiploid, hypodiploid, translocation, and complicated karyotypy. Many of them are related with the ALL, AML, and CML, such as the t (8;21), t (15;17), and 11q23 rearrangements (t (1;19) and t (9;22). This category of haematological cancers was dubbed "complex karyotype" because of the uncommon occurrence of chromosomal aberrations with three or more changes. It was decided to split up the patients based on their age into two distinct groups. Patients under the age of 15 were divided into a paediatric group, while those beyond the age of 16 were divided into an 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. Use of the primers previously published for the AML1-ETO, PML-RARA, CBFB-MYH11, BCR-ABL, E2A/PBX1, and AF4-MLL assays.

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.

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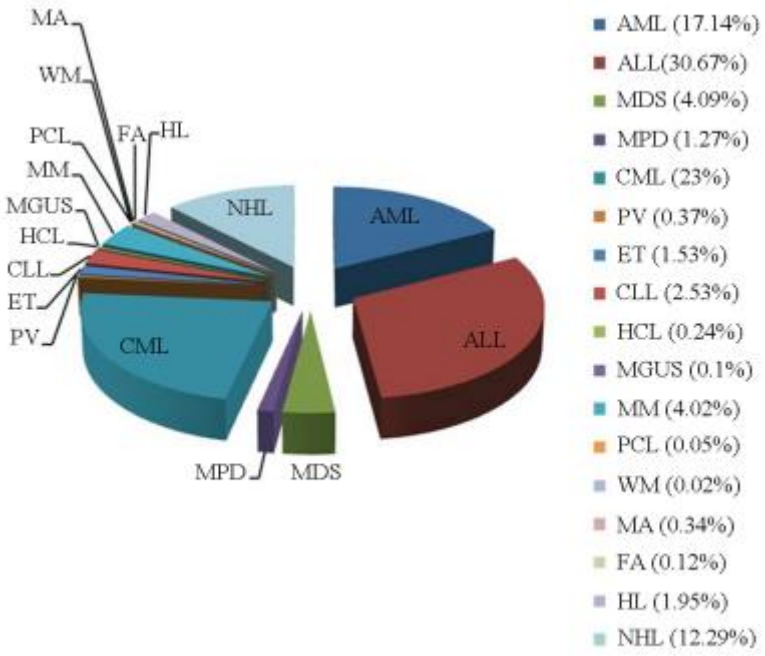

Figure 1. Types of hematological diseases in which genetic study was conducted in Ecuadorian population, 1984-2012.

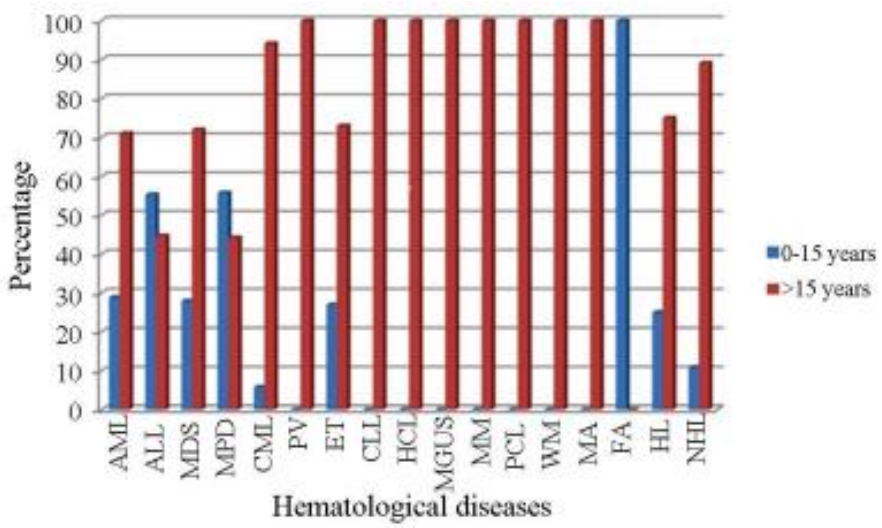

Figure 2. Hematological diseases analyzed by group of age in the Ecuadorian population.

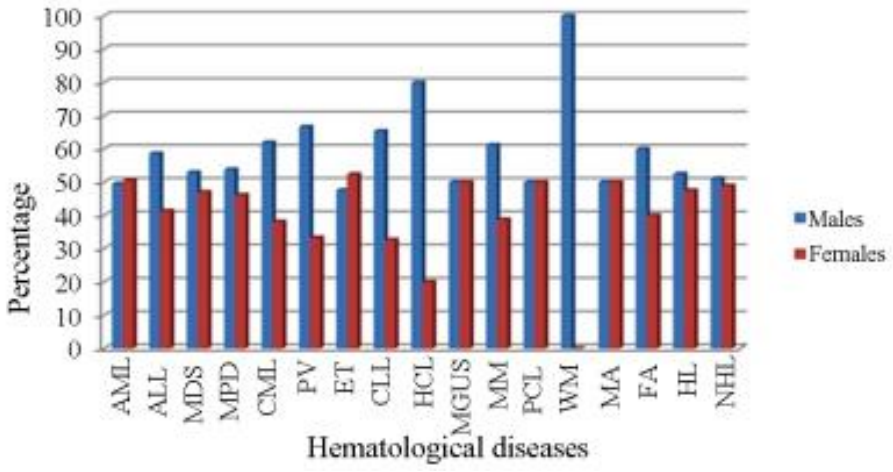

Figure 3. Hematological diseases analyzed by gender in the Ecuadorian population.

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 FISH findings matched those from the molecular analyses (Table 2). It was found in 22/715 AML patients; 29/1260 ALL patients; 65% of CML; and 3.1 percent of

CML patients with no metaphases that there was an increase in positive outcomes from conventional cytogenetics.

AML1-ETO RT-PCR products were found in cases where t (8;21) was identified by FISH. The bcr2 and bcr3 transcripts were found in 36% and 64% of AML patients with PML-RARA rearrangements, respectively. The bcr1 transcript was not present in any of these individuals. Seven individuals with ALL had the CBFB-MYH11 fusion transcript, all of whom had the type F transcript. All t (9;22) positive ALL cases had the BCR-ABL fusion gene discovered using RTPCR; all of the samples revealed the e1-a2 transcript. The t (4;11) inside the MLL-AF4 fusion gene was discovered in four of the 10 patients with ALL who had 11q23 rearrangements. In all instances, the transcript e7-e8 was detected. t (1;19) was the E2APBX1 fusion gene that was found. The b2a2 and b3a2 fusion transcripts were found in 95% and 5% of CML patients with the BCR-ABL fusion gene, 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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