World Academy of Science, Engineering and Technology
International Journal of Medical and Health Sciences
Vol:6, No:3, 2012

Genetic Polymorphism of the Acute Lymphoblastic Leukaemia and Hyperhomocysteinemia its Relation with the for a Group of Children in the East of Algeria

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***Abstract*—**A lot of recent research have spoken on the relation between the increase of the homocysteinemia and some kinds of cancer . For that, our study was based on the research of a possible relation between the increase of the concentration of this amino-acid in the plasma and the appearance of the disease of the Acute Lymphoblastic Leukaemia in a part of Algerian children with Berber origin in the East of Algeria . The study has done on 47 ill persons with an average age of (09±06) years , with whom the disease has diagnosed by blood and marrow examination in the hospital of blood diseases in the CHU of Batna, and on 194 healthy witnesses of the same age. The two groups were benefited by a dosage of the concentration of the homocysteine vitamin B9 ,vitamin B12 , and also of the study of special polymorphisms of indispensable enzymes in the metabolism of this acid , and that by the use of the method (Light cycler) Real time PCR , on the following enzymes : MS (C2756G), MSR (A66G) ,MTHFR1 (C677T) and MTHFR2 (A1298C). The obtained results have revealed that the rate of the homozygote muted genotype is the less frequent in the two groups , and that exist at list one genotype of each enzyme in the ill group and in which the percentage exceed with remarkable way the same genotype in the healthy group and we notice specially the muted genotype GG of -the methionine synthetase-and the form TT of the enzyme – methyline tetra hydrofolate reductase – We notice the existence of considerable number of genotypes in the ill group lied with characteristic increase of this Amino-acid ,and that for the reduction of the biologic activity of these enzymes which become inefficient in the transfer of the homocysteine into the methionine and cause the diminution of the biologic activity of these enzymes and with consequence the reduction of the percentage of methylic radicals in the DNA of studied genes and that lead to the increase of the activity and the capacity of transcription , and it's so probably that this last one is one of the factors of this disease especially if we know that the specific check-up of vitamins is normal and similar in the two groups , which ovoid the hypothesis of the reduction of vitamins . We notice also that the heterozygote genotype is the less in the sick category except the MTHFR2. Wild genotype is more frequent in the witness group except MSR. Even these results are partials; they open a new way in the genetic diagnosis of this malicious disease which allow a precocious diagnosis and the use of an effective and appropriated treatment in the same time.

***Keywords*—**Genetic polymorphism, Acute Lymphoblastic Leukaemia, Biomarkers, Metabolism of homocystein

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I. Introduction

CANCEROUS diseases kill every year thousands of people in the world. These diseases are characterized by its variation and they affect all the categories without distinction, and in spite of the development of scientific research but the most of these diseases stay without treatment.

The most cases of death by the cancer is essentially caused by the late diagnosis of the disease, and in the aim of a precocious diagnosis of this pathology in order to combat it with an effective treatment; many studies used the bio-markers of the cancer which represent nowadays one of the most important domains.

And in spite of the determination of the nature of these bio-markers in some types of cancers such as the increase of alpha feto protein AFP in the case of the liver cancer and PS1 in the case of the prostate cancer and erb 2 / Neu in the case of the breast cancer and others [1] - [2]-[3] but many of them are in the course of study. And for that, our study is based on one of the blood cancers which is the acute lymphoblastic leukaemia at the children and the biomarker used in this study is the hyperhomocysteinemia.

II. Materials And Methods

A. Patients and methods

Our study was conducted on 47 children of Berber origin in the East Algerian with an average of age of (9 ±6) years, the acute lymphoblastic leukaemia was diagnosed at them by analyses of the blood and of the marrow in the service of hematology in the CHU of Batna, against 194 healthy witnesses whose age is close to the age of the first group.

B. Sampling and collection

Sample of the blood were collected in tubes containing of the EDTA, immediately centrifuged and aliquots stored at -70°C for future use.

C. Method of the polymorphism study

The ADN is extracted from leukocytes by Kit BACC3 Nucleon in order to study the specific polymorphisms of enzymes entering in the metabolism of this amino acid by the use of the PCR in real time method (light cycler 480) which allows to multiplying the ADN's chains and its analysis by a micro spectro fluorimeter associated to the machine. This method is based on the use of Probs which carry fluorescent

elements steered towards the polymorphism which we want to study. Besides, this machine is equipped with a microcomputer which allows a precise programming of the various stages of this operation.

We indicate that the studied primers are as follows:

Primers MTHFR1 forward (sens) C677T:
5'TGG CAGG TTA CCC CAA AGG3' ; Primers MTHFR1 reverse (anti-sens) C677T : 5'TGA TGC CCA TGT CGG TGC3' ; Primers MTHFR2 forward A1298C: 5'CTT TTG GGA GCT GAA GGA CTA CTA CTAC3'
Primers MTHFR2 reverse A1298C: 5'CAC TTT GTG ACC ATT CCG GTT TG3'; Primers MS forward A1256G: (5µM) 5'TAT GGC TAT CTT GCA TTT TCA G3' Primers MS reverse A1256G: 5'TTT ACA CTC CTC AAA ACC ATT3'; Primers MSR forward A66G: 5'GCA AAG GCC ATC GCA GAA GAC3'; Primers MSR reverse A66G: 5'GTG AAG ATC TGC AGA AAA TCC ATG TA3'.

D.Homocysteine and vitamins Dosage

The homocysteine concentration was measured in all samples by immunological assay using polarization of fluorescence on the IMX for the quantitative measure of total L-homocysteine in the plasma. Folates and B12vitamin were measured by immunoassay in a simulTRAC-SNB. The sensitivity of the assay was 7nmo1/L and 100 pmo1/L respectively established by the WHO.

E. *Statistical analyses*

The data were expressed in percentages and allelic frequencies for the quantitative information and in averages for the qualitative data. Paired sample t-test was used to compare the qualitative variables and Chi square analysis was used to compare the qualitative variables. Significance was accepted when P was < 0.05.

III. Results And Discussion

For the variant MTHFR1 (C677T), it results from a punctual mutation by the changing of the nitrogenous base cytosine with the thymine in the gene of enzyme, and consequently the amino acid Alanine is replaced by the Valine on the protein in the position numbered A222V [4] - [5].

According to our results illustrated in tables 1 and 2, the muted homozygous genotype TT is the most frequent at the leukaemic group, and this genotype is the most connected to the considerable decrease of the activity of the enzyme which affects a percentage of 80 %, on the other hand the genotype CT is more increased at the witness's but its effect is less than the preceding and it leads to a decrease of 35 % [6].

The wild genotype CC is the most found at the witness' group and without any significant difference in the concentration of homocysteine for the two groups. Our results were in agreement with several studies as [7] - [8].

A. The variant MTHFR2 (A1298C)

This variant is appeared by a change of the Adenine with the Cytosine in the gene what's led to the appearance of the amino acid Alanine in the place of the Glutamate in the place organizer of the enzyme. According to these results it is clear that the muted homozygote genotype CC and the wild one AA are less frequent at the ill group, on the other hand , the heterozygote structure AC is increased at the same group (Table1).

Several studies showed that this mutation is not connected to the increase of the homocysteine [8] [9] contrary to what shows our study especially for structures AA, AC (Table 2), and it may be that the presence of MTHFR1 and MTHFR2 together especially for the homozygote has more effect in the decrease of the activity of the MTHFR enzyme [5]- [10].

B. The variant MS or MTR (A2756G

This structure results from the change of the Adenine by the Guanine in the position 2756 of the nucleotide chain what led to the replacement of the aspartic acid by the glycine in the region of the link of the enzyme with the B vitamin.

According to our results obtained in the table 1, the healthy genotypes, homozygote (AA) and heterozygote (AG) were the least found at the ill group, but the muted type (GG) was the most found. Besides, all the genetic structures cited have a significant increase of the homocysteine in the ill group especially (GG) and (AG), and they are the same results quoted in several studies [7] - [11] but they oppose other studies which do not see any relation between this polymorphisms and the appearance of the acute lymphoblastic leukaemia ([12], and we can explain that by the difference of the origin and the vitaminic ration.

C. The MSR or MTRR Polymorphism (A66G)

This mutation is a change of the adenine at the ADN's chain by the guanine in the position 66 and that leads to the appearance of the methionine in the place of the isoleucine in the protein.

TABLE I
THE GENOTYPES 'PERCENTAGE OF THE ILL GROUP AND THE WITNESSES

	Patients			Witnesses		
	gen	N	%	gen	N	%
MS	AA	29	61.7%	AA	129	66.6%
	AG	13	27.6%	AG	59	31.1%
	GG	05	10.6%	GG	6	2.2%
MSR	AA	22	46.8%	AA	70	35.5%
	AG	19	40.4%	AG	90	46.6%
	GG	06	12.7%	GG	34	17.7%
MTHFR1	CC	20	42.5%	CC	87	44.4%
	CT	18	38.2%	CT	89	45.5%
	TT	09	19.1%	TT	18	10%
MTHFR2	AA	27	57.4%	AA	124	64.4%
	AC	19	40.4%	AC	59	30%
	CC	01	2.1%	CC	11	5.5%

gen: the genotype; N: the number, %: the percentages

Our study showed that genetic structure (GG) and (AG) appeared with a lower percentage at the leukaemic subjects contrary to the structure (AA) (Table1) and that they are connected to a modest increase of the homocysteine at the same group. These results are in agreement with the results of two other researchers [7] – [13].

IV. Conclusion

Obtained results showed that the genetic structure TT specific to the variant MTHFR1 is the most frequent among muted types at the leukaemic group and that is connected to the excess of homocysteine in the blood, also the structure CT for the same enzyme and the specific structures AA and AC in the MTHFR2. In spite of, all the specific genotypes in the enzyme MS and MSR had a significant increase of the biomarkers to the same group.

TABLE II
THE HOMOCYSTEINE CONCENTRATION FOR VARIOUS GENOTYPES AT THE ILL GROUP AND THE WITNESSES

	The patients		The witnesses	
	gen	Homocy (µmol/L)	gen	homocy (µmol/L)
MS	AA	8.81±1.69	AA	6.99±0.64
	AG	13.12±2.48	AG	7.15±0.92
	GG	14±6.31	GG	6.86±4.79
MSR	AA	13.83±4.78	AA	6.57±0.80
	AG	10.70±2.55	AG	7.35±0.85
	GG	9.95±3.35	GG	7.14±1.02
MTHFR1	CC	7.95±2.73	CC	6.79±0.71
	CT	12.20±2.24	CT	7.23±0.76
	TT	15.78±2.50	TT	7.55±3.49
MTHFR2	AA	13.45±3.72	AA	7.28±0.65
	AC	12.10±3.15	AC	6.44±0.92
	CC	8.5±0.0	CC	7.32±2.50

n: the genotype; Homocy: The rate of homocysteine (µmol/L)

This increase can be a cause of the decrease of the rate of homocysteine which is considered as an important source of methyl's radical in the body, these radicals play a very important role in the organization of the genic expression. Besides, the increase of homocysteine has a role in ADN's instability what led to the loss of the certain nucleotide especially at the marrow cells causing the elongation of the life's cycle of the stem lymphatic cells and not the differentiation towards other terminal cellular elements of the blood. Even our results are partial but altogether they show the presence of a possible genetic relation between the homocysteine and the acute lymphoblastic leukaemia what opens a new way for the precocious diagnosis of this fatal disease.

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