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ANALYSIS OF CYP2C19POLYMORPHISMS AS GENETIC RISK FACTORS FOR HEMATOLOGICAL MALIGNANCY OF THE POPULATION SOUTHERN IRAQ

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ABSTRACT

The present study aimed to detect the role of CYP_2C_{19} gene and its relationship in the incidence of hematological malignancies of the Population Southern Iraq, through the use of PCR- RFLP technique, were collected 120 blood samples (from oncology center/AL-Habboubi Teaching Hospital in Thi-Qar and specialized center of hematology and oncology cancer/Al-Sadr Teaching Hospital in Basra) in tubes EDTA from patients with hematological malignancies ages ranging between (15 - 90 years) and 50 samples from healthy people ages ranging between (15 – 80 years) and saved directly at a temperature of - 20 ° C Later to be used for the extraction DNA and the PCR technique results of statistical analysis.

Showed no correlation between polymorphism of the gene CYP_2C_{19} and the incidence of hematological malignancies when compared to patients a range of comparison And each of the mutant homogenized style (AG) (OR=0.64 ; 95%CI=0.31-1.30)The style of the mutant differential (GG) (OR= 0.42; 95%CI=0.21-0.83). It was evident from the results that females who have the mutant heterozygous (AG) has increased their risk of congenital heart defects by deference (OR = 1.86) and the period of confidence (0.65 - 5.27), and the results also showed that those who have the heterozygous differential (AG) and category age (month - 9 years) have increased the risk of congenital heart defects by teams (OR = 3) and the period of confidence (0.8 – 12). and the results showed no correlation between smoking and multiple genotypes of the gene CYP_2C_{19}.

KEYWORDS: Cytochrome P450, CYP2C19, Polymorphism, Hematological Malignancy

INTRODUCTION

Cancer is a growing health problem at global level in terms of the number of new cases, cost of care and death toll. It is estimated that the global annual number of new cases of cancer is more than 10 million and the annual number of deaths is about 6 million deaths. In addition, more than 24 million people are living with cancer. Despite the great advances in science and technology, the etiology of many types of cancer is still obscure and the role of specific risk factors in the causation of certain cancers is unresolved with substantial variation across the world[1]. Hematological malignancies are diseases originating in the bone marrow and lymph nodes. They include leukemia, lymphomas and myeloma. The etiology of most hematological malignancies is not yet known, ionising radiation, exposure to chemicals, industrial exposures including benzene, viral infections, genetic predisposition and Down's syndrome are all associated with an increased risk of one or several of these diseases, for most patients, however, there is no identifiable cause[2]. Hematological malignancies accounted for 8% to 9% of all malignant disease. WHO predicts that the number of blood-related cancer cases would increase about 48% in less developed countries by 2030[3]. The situation in Iraq as a whole and

in Southern provinces in particular has been especially complicated further by the harsh condition to which the country was exposed for the last three decades. Wars, economic sanctions and the violence has struck all infrastructure in the country and lead to deterioration in the health indicators. As a result, the health status of the population was under a high risk of various diseases including cancer[4].

Hematological malignancies can cause many different signs and symptoms, depending on where it is in the body. In some cases it might not cause any symptoms until it grows quite large. Common signs, and symptoms include enlarged lymph nodes, swollen abdomen (belly), chest pain or pressure, shortness of breath or cough, fever, weight loss, night sweats, fatigue (extreme tiredness), bruising or bleeding and low red blood cell counts (anemia)[5].

Aims of the Study

There are several studies indicated the correlations between genetic polymorphism (CYP_2C_{19})and risk of hematological malignancies, but these correlations differ from country to another in the world with different environmental factors. In southern Iraq, there is no previous study conducted in genetic field and risk of hematological malignancies, so, that aim of the present study is:

- To determine the impact of (CYP_2C_{19}) genetic polymorphism in risk of hematological malignancies in patients and controls.
- The correlation between various etiological factors and risk of hematological malignancies.
- The relation among all these factors.

MATERIALS AND METHODS

Samples Collection

One hundred and twenty blood samples were collected from patients with hematological malignancies from the oncology center/AL-Habboubi Teaching Hospital in Thi-Qar and specialized center of hematology and oncology cancer/Al-Sadr Teaching Hospital in Basra. The patients ages range between 15 - 90 years (71 males, 49 females). Other 50 samples healthy volunteers, were selected during the period at October 2014_ March 2015. The blood samples from both groups were the size (2-3)ml per person and were preserved in tubes containing EDTA under temperature (-20) to extract DNA.

The information collected was based on a questionnaire containing a lot of information (sex, age, smoking, residence place, family history, accompany the blood disease).

Isolation of DNA from Human blood

Genomic DNA was isolated according to the procedure of[6].

Polymerase Chain Reaction (PCR)

Reagents

- DNA templet.
- Primers (forward and reverse).

- Go Taq Green Master Mix.
- Sterilized distilled water.

Genotyping for the *CYP$_2$C$_{19}$* gene amplification was performed by Polymerase Chain Reaction (PCR) techniques based on the method described by[7]. Briefly, genomic DNA was amplified using the primer given in table PCR reaction mix and condition are given in (Table 1).

Table 1: Oligonucleotide Primer Sequences Used for PCR Amplification of *CYP2C19*

Gene	Primer Sequences	Length	Tm	Ta
CYP$_2$C$_{19}$	F-5AAA TTG TTT CCA ATC ATT TAG CT	22	64	58
	R-5ACT TCA GGG CTT GGT CAA TA	22	66	58

Table 2: The Reaction Mix (20 µl) For *CYP$_2$C$_{19}$* Gene

Chemicals	Volume
Go Taq Green Master Mix	5 µl
Primer Forward	1 µl
Primer Reverse	1 µl
DNA	5 µl
D.W.	8 µl
Total Volume	**20 µl**

Table 3: PCR Condition for *CYP$_2$C$_{19}$* Gene

St No.	Stage	Temperature	Time	No 0f cycles
1	Denaturation 1	94.0 C^o	5 min	1
2	Denaturation 2	94.0 C^o	1 min	35
3	Annealing	54.0 C^o	1 min	
4	Extension 1	72.0 C^o	1 min	
5	Extension 2	72.0 C^o	5 min	1

The PCR product was restricted using BamH1 restriction enzyme which cuts the normal allele into segments (175 bp and 96 bp) but doesn't cut the abnormal allele. The CYP_2C_{19} mutant homozygote should yield one band (271 bp). The CYP_2C_{19} heterozygotes should produce three bands (271 bp, 175 bp and 96 bp).

Statistical Analysis

Descriptive statistic has been used to describe patients characteristics by using mean, standard deviation, and percentage. Chi-Square test is used to determine the difference in the characteristics between cases and control using SPSS version 21. ORs and the 95% Cls were calculated and $p \leq 0.05$ were considered significant.

RESULTS AND DISCUSSIONS

The present study has been conducted to determine the association (polymorphism of *CYP$_2$C$_{19}$*) with prevalence to hematological malignancies in Southern Iraq, through the analysis of questionnaire to patients (n = 120) and controls (n = 50) group, which included several questions: age, sex, diagnosis, smoking, residence and family history as in appendix No.1, the P value was calculated between cases and control groups. A total of 120 diagnosed hematological malignancy cases of these, patients aged between 15 to 80 years, 63 were males (52.5%) and 57 females (47.5%).

State-Wise Distribution of the Cases

Most of the patients were from Basra province (55%), Nassiriyha province (37.5%), and Missan province (7.5%) as shown (Table 4). The results showed an elevation of hematological malignancies among people who live in Basra more than Nassiriyha and Missan. The reasons maybe due to the high number of people that live in Basra compared to Nassiriyah and Missan, and many cases from inhabitant of Nassiriyah and Missan may consult sources of care in Basra. However, it has been clearly shown that there has been an increase in malignant tumours in Basra province after the second Gulf war[8]. The environmental pollutants play an important role to increase this type of cancer, Uranium is one of these compounds which enters the human body by ingestion with food and drinks or by respiratory system since it is containing air particles or aerosols[9]. So, may be increased the cancer in Basra due to the pollution with uranium in wars compare to other cities.

Table 4: State-Wise Distribution of Cases and Controls in the South of Iraq

State	Nassiriyha	Basra	Missan
Cases (%)	45 (37.5%)	66 (55%)	9 (7.5%)
Controls (%)	35 (70%)	9 (18%)	8 (16%)

NHL was the most frequent HM (31.67%) in South of Iraq with a median age of 55 years. This was followed by HL (22.5%), median age 29 years, AML (20.38%), median age 40 years, CLL (11.66%), median age 62 years, ALL (9.17%) median age 24 years, CML (4.17%), median age 53 years as shown (Table 5). These findings are consistent with the study in Pakistan, NHL is the most prevalent type of HM [10], in US, NHL is the commonest cancer among HM, which is 1.5 times that of all leukemias[11]. As well as Japan, Korea and Singapore, NHL is the most frequent hematological malignancies[12].

Table 5: Distribution Cases, Median Age at Diagnosis, Male and Female Ratio of Hematological Malignancies in South of Iraq

HM type	Cases	Distribution %	Median age	Male %	Female%
AML	25	20.38%	40	9 (%)	16 (%)
ALL	11	9.17%	24	7 (63.6%)	4 (36.3%)
CML	5	4.17%	53	3 (60%)	2 (40%)
CLL	14	11.66%	62	7 (50%)	7 (50%)
HL	27	22.5%	29	16 (59.2%)	11(40.7%)
NHL	38	31.67%	55	22(75.9%)	16(42.1%)
Total	**120**	**100%**	**43.83**	**63**	**57**

Residence Distribution of Cases and Controls

The resident distribution founds that shows that (80%) of cases were urban and20% of the cases were from rural areas, while the control groupwas90% of the urban areas and10%from rural areas as shown (Table 6). In the present study there was an association between dwellings neighboring a petrol station, oil refineries car repairing garage and heavy traffic roads which are benzene emitting sources and the risk of HM, this result is supported by a study in California and Britain[13].

Therefore, the proximity to main roads, gasoline stations, or railways to indicate air pollutant exposure and reported results that were suggestive of an increased risk of HM.

Table 6: Residence Distribution of the Cases and Controls

Residence	Cases (%)	Controls (%)
Rural (%)	24 (20%)	5 (10%)
Urban (%)	96 (80%)	45 (90%)
Total	**120 (100%)**	**50 (100%)**

Age Distribution of the Cases And Controls

Results of the present study showed that the highest percentage of patients were within the age group (15 – 25) reached 31%, while the lowest percentage of infection occurred within the age group (26 – 36) reached5.83% as shown (Table 7). Reason is related to the underreporting cases of older individuals possibly because of several socioeconomic and cultural reasons, The reasons behind this phenomenon are unclear. However, it is likely that the multiple factors including genetic, infections and other environmental factors might play crucial role in this young age phenomenon in Asia, Another study, which found that residential petrochemical exposure was a significant risk factor for HM in persons of age 20–29 years[14].

Table 7: Age Distribution of the Cases and Controls

Age group	15 – 25	26 - 36	37 - 47	48 _ 58	59 - 69	70 – 80
Cases	38(31.66%)	7(5.83%)	20(16.66%)	22(18.33%)	19(15.83%)	14(11.66%)
Controls	11(22%)	17(34%)	10(20%)	8(16%)	3(6%)	1(2%)

Sex Wise Distribution of the Cases and Controls

HM affects predominantly male population, Male: female ratio differs from country to country. As illustrated in (Table 8), 52.5% of the cases were males and the rest were females indicating the predominance of this disease amongst males. Man traditionally had increased exposures to putative hematological malignancies carcinogens found in the workplace and in smoke.

Studies all over the world have revealed that hematological malignancies is gender-skewed, often affecting men more than women, the higher prevalence of HM in males might be the result of increased exposure to environmental and occupational risk factors, smoking, alcohol consumption as well as different hormonal and genetic background of males and females[14]**.** HM are more prevalent among males in Asian countries, including Iraq**[15],** while in Jordan ,the percentage offer males was higher than in male**[16].**

Table 8: Sex-Wise Distribution of the Cases and Controls

Six	No. of cases (%)	No. of controls (%)
Male (%)	63 (52.5%)	27 (54%)
Female (%)	57 (47.5%)	23 (46%)
Total (%)	**120 (100%)**	**50 (100%)**

Polymorphisms in *Cyp*$_2$*C*$_{19}$ Gene

The interest in human genetic diversity for understanding disease etiology, toxicology and response to therapy has grown in recent years into an active field of research. In cancer epidemiology, and especially in the field of molecular epidemiology, a great deal of attention has been paid to the role of common population polymorphisms in genes controlling carcinogens metabolism. These genes have low penetrance with respect to cancer risk and their precise role with respect to interactions with environmental agents is under intensive investigation[17]. To our knowledge, the

association between polymorphisms *CYP$_2$C$_{19}$* and hematological malignancy has not been studied before in southern Iraq, therefore, this study was designed. Genetic polymorphism of CYPs can lead to severe toxicity or therapeutic failure of medications as well as to a possible increase in an individual's susceptibility to certain types of chemotherapy induced cancers and other diseases[18]. *CYP$_2$C$_{19}$* homozygous (EM) allele frequency was respectively (60%) in control group and (61.6%) in the HM cases while heterozygous (HEM) allele frequency was respectively (38%) in control group and (35%) in HM cases. The poor metabolizer mutant genotype frequency in the control group was (2%) and in the cases was (3.3%) (Table 9).The comparison of *CYP$_2$C$_{19}$* gene in cases and controls, did not show a significant statistical difference associated between HEM genotype with the overall risk of hematological malignancies with an (OR= 0.8962; 95% CI= 0.4503-1.7835), whereas there was a 2-fold nearly increased risk of hematological malignancies with PM genotype with an (OR=1.6216; 0.1740 – 15.1115) as seen in (Table 18). Among enzymes phase I, the *CYP$_2$C$_{19}$* is an important enzyme of Cytochrome P450 enzymes which Involved in metabolize approximately 20% of clinically used drugs**[19],** *CYP$_2$C$_{19}$* represents about 3% of total hepatic CYPs[20].

This result is similar to other studies which did not show any association between *CYP$_2$C$_{19}$* and occurrence hematological malignancies**[21].** *CYP$_2$C$_{19}$* polymorphism, even though not significantly associated with hematological malignancies, they may predispose to other types of malignancies, Research on the role of *CYP$_2$C$_{19}$* polymorphisms in hematological malignancies is not in the last stages, because many genes are involved in the initiation and progression of the hematological malignancy the reason why it is called a multi factorial disease (i.e., determined by a combination of multiple factors, genetic and environmental), and the *CYP$_2$C$_{19}$* polymorphism could be one step in this process**[22].**

Table 10: Distribution of Polymorphism of *CYP$_2$C$_{19}$* Gene among Hematological Malignancies Cases and Controls

Genotype	Controls (%)	Cases (%)	OR	95% CI
EM	30 (60%)	74 (61.66%)	1.0	---
HEM	19 (38%)	42 (35%)	0.8962	0.4503 - 1.7835
PM	1 (2%)	4 (3.33%)	1.6216	0.1740 – 15.1115

EM: Homozygous (Extensive Metabolizers)

HEM: Heterozygous (Hetero extensive Metabolizer)

PM: Poor Metabolizer

Concerning the tobacco smoking of patients, the *CYP$_2$C$_{19}$*polymorphism did not appear to be a factor affecting hematological malignancies (Table 11).

This result is similar to other findings which did not report any interaction between tobacco use and *CYP$_2$C$_{19}$* polymorphism**[23].** showed that smoking habit need not be taken into account when humans are phenotype for *CYP$_2$C$_{19}$*. *CYP$_2$C$_{19}$* does not influence the disposition of nicotine or nicotine dependency, therefore, this gene is not likely to have a major influence on tobacco smoking**[24].** Conversely, it has been found**[25]**that there is a significant association between *CYP$_2$C$_{19}$*polymorphism and smoking.

Table 11: OR of Developing For *CYP$_2$C$_{19}$* Genotypes Stratified by Status of Smoking

Smoking status	EM	HEM	OR	95% CI
Non-smokers	56 (61.5%)	35(38.46%)	1.0	-------
Smokers	18 (72%)	7 (28%)	0.6222	0.2359 - 1.6411

The frequency of the HEM genotype according to the age of patients showed an increased risk of developing hematological malignancies in a group of 15-25 year old patients, while in a group of 26-36 find there arenotany situation (OR=0.0968; 95%CI=0.0051-1.8214) (Table 12).

Table 12: The Relationship between *CYP$_2$C$_{19}$* Genotype and Age Group in Hematological Malignancies Cases

Age group	EM	HEM	OR	95% CI
15 – 25	22 (18.9%)	15 (12.9%)	1.0	------
26 – 36	7 (6%)	-	0.0968	0.0051 – 1.8214
37 – 47	9 (7.7%)	11 (9.4%)	1.7926	0.5976 – 5.3774
48 – 58	14 (12%)	7 (6%)	0.7333	0.2393 – 2.2471
59 – 69	14 (12%)	3 (2.5%)	0.3143	0.0768 – 1.2862
70 – 80	8 (6.8%)	6 (5.1%)	1.1000	0.3166 – 3.8220

It is apparent in (Table 13) that the *CYP$_2$C$_{19}$* HEM genotype had no significant association for the hematological malignancies in urban residence with an OR=0.7583(95%CI=0.3651-1.5746), while there was found an increased of hematological malignancies in rural residence by 7-fold (Table 14).

Table 13: The Relationship between *CYP$_2$C$_{19}$* Genotype and Urban Residence in Cases and Controls

Genotype	Controls (%)	Cases (%)	OR	95% CI
EM	25 (55.56%)	59 (61.45%)	1.0	-----
HEM	19 (42.22%)	34 (35.42%)	0.7583	0.3651 – 1.5746
PM	1 (2.22%)	3 (3.13%)	1.2712	0.1260 – 12.8198

Table 14: The Relationship between *CYP$_2$C$_{19}$* and Rural Residence in Cases and Controls

Genotype	Controls (%)	Cases (%)	OR	95% CI
EM	5 (100%)	14 (58.34%)	1.0	-----
HEM	-	9 (37.5%)	7.2069	0.3558 – 145.9880
PM	-	1 (4.16%)	1.1379	0.0400 – 32.3624

Aftergeneamplificationtechnology*CYP$_2$C$_{19}$*conducteddetect the presence of the gene by electro phores is relay product PC Ron the gel Aga rose concentration2%, where the gene package appeared at the271-base pair as shown in Figure.1

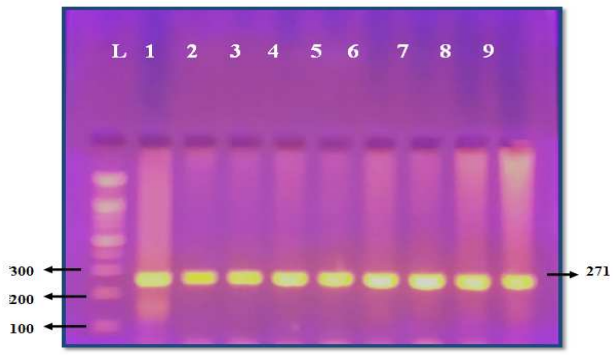

Figure 1: PCR-RFLP Analysis for *CYP$_2$C$_{19}$*gene

Lane L: Marker

Lane 1 – 4: Cases

Lane 5 – 9: Controls

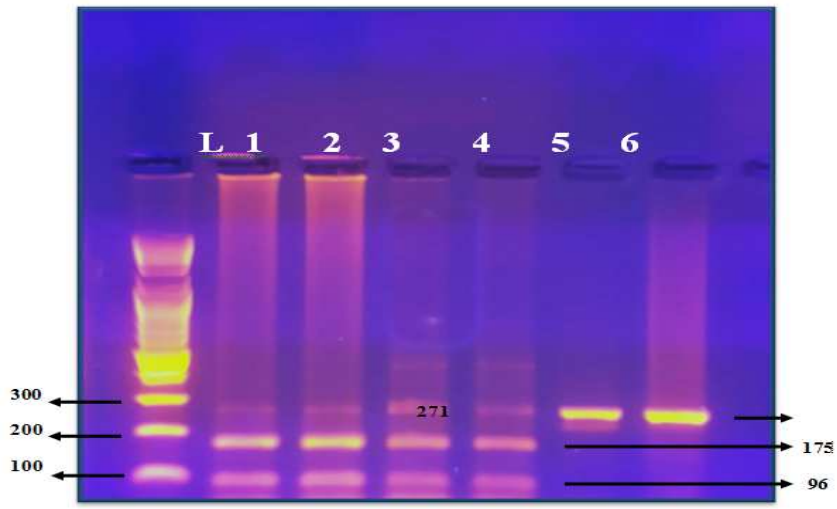

Figure 2: PCR Product Analysis Using Bamh1 Restriction Enzyme

Lane L: Marker

Lane 1,2: EM

Lane 3,4: HEM

Lane 5,6: PM

CONCLUSIONS

This study showed no correlation between polymorphism of the gene CYP_2C_{19} and the incidence of hematological malignancies when compared to patients a range of comparison And each of the mutant homogenized style(AG) (OR=0.64 ; 95%CI=0.31-1.30)The style of the mutant differential (GG) (OR= 0.42; 95%CI=0.21-0.83) . It was evident from the results that females who have the mutant heterozygous (AG) has increased their risk of congenital heart defects by deference (OR = 1.86) and the period of confidence (0.65 - 5.27), and the results also showed that those who have the heterozygous differential (AG) and category age (month - 9 years) have increased the risk of congenital heart defects by teams (OR = 3) and the period of confidence (0.8 – 12). and the results showed no correlation between smoking and multiple genotypes of the gene CYP_2C_{19}.

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