TSP-1 level with (rs222826 T/C) Polymorphism in acute myeloid leukemia in Iraqi population

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Abstract

Purpose: Acute myeloid leukemia patients from Iraq were studied to determine the prevalence and correlation of Thro mbospondin 1 (THBS1) gene single nuc leotide poly morphisms (SNPs). Method: Amplification Refractory Mutation System Polymerase Chain Reaction genotyping of the THBS1 gene SNPs (rs2228261 C>T) in a prospective case-control study of individuals with acute myeloid leukemia and healthy controls (ARMS PCR). Result : conducted a case– control study on 60patients and 30 healthy groups from the country of Iraq. Individuals was genotyped for SNPs (rs2228261 C > T) in THBS1 . The genotypic distributions of THBS1 in the case and control groups were examined for links to the development of cancer. Acute myeloid leukemia with odd ratio was show to be statistically significantly related with homozygous CC of THBS1 rs2228261 T > C, according to compared between the patients and control groups (0.068) ,while no found significant different in heterozygote pattern TC between patients with acute myeloid leukemia than control group with odd ratio (0.30).Also result found the level of TSP-1 show a significant decrease (p≤ 0.00) in the patients with AML as compared to the control. Conclusion: Iraq population genotyped for THBS1 gene SNPs using ARMS PCR only showed genetic link in homozygote pattern CC, which was more common in control groups than patients.

1. Introduction

Leukemia is a type of bl0od cancer that originated when a blood cell's life cycle or certain aspects of its division are not adequately controlled (Chaudhari et al.,2014). From a single cell, the cell starts to multiply uncontrollably, creating a large cell population (Stock and Hoffman.,2000). hematological disease known as acute myeloid leukemia (AML) is phenotypically and genetically heterogeneous. that can be cured in 30–50% of people under the age of 50. (Dohner et al.,2015 and Dohner et al.,2009). AML clinical risk assessment now relies mostly on cytogenetic and molecular genetic findings (Saygin and Carraway.,2017) (Marcucci et al.,2012).

Many mutations in leukemia cells have been discovered in recent years, including recurring lesions in the nucleophosmin gene (NPM1), F LT3-IT D, CE BPA, T ET2, and others. It has been established that extensive genetic anomaly performance a vital part in patient diagnosis, risk assessment and prognosis prediction. The risk classification of AML can be split into good, intermediate, and bad prognosis, in relation to the US National Compreh ensive Cancer Network (NCCN) guidelines (Shimizu et al.,2018) Acute myeloid leukemia (AML) is a hematological disease that is phenotypically and genetically diverse.

Haematological malignancies are cancers of the blood, bone marrow, and lymph nodes. This classification includes myeloma, acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic lymphocytic leukemia (ALL), and lymphomas (Taylor.,2017) (Enad and Al-Amili, 2019).

Other research found that leukemia cell dissemination can harm normal BM microenvironments and that the severity of the damage varies with cell engraftment. Additionally, it was found that reconstruction of new niches can promote leukemia cell growth, proving the significance of the AML BM niche for tumor cell proliferation and expansion (Duan et al.,2014). Exploring the components of the BM microenvironment, as well as the interaction between leukemia cells with stromal cells, was crucial to understanding leukemogenesis.

A great matrix glycoprotein with anti-angiogenesis activity is called thrombospondin-1 (THBS1). It is thought to be included in the regulation of motility, proliferation and cell adhesion (Sargiannidou et al., 2001; Lawler, 2002). According to in vivo and in vitro research, THBS1 plays a critical role in the progression and development of cancer (Hawighorst et al., 2002; Kazerounian et al., 2008). THBS1 expression in gastric cancer is associated with the expression of transforming growth factor beta, microvessel density, and vascular endothelial growth factor, and has proven a prognostic indicator for patients with advanced gastric cancer that has increased survival rates (Nakao et al., 2011).

However, there is no genetic indication that it contributes to cancer. On chromosome 15q15, THBS1 is a 16.39 kb gene. THBS1-associated single-nucleotide polymorphisms (SNPs) have been linked to a variety of disorders in recent years, according to multiple studies (Hannah et al., 2003; Stenina et al., 2004, Valdes et al., 2005; Zwicker et al., 2006; Sfar et al., 2009). Various studies have looked into whether there is a link between THBS1 SNPs and the chance of acquiring cancer (e.g., prostate cancer and col0rectal ca ncer), however the results have been mixed (Sfar et al., 2007, 2009; Forsti et al., 2010)

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2. Materials and Methods

Acute myeloid leukemia patients and controls are included in this prospective case-control study to investigate for THBS1 gene single nucleotide polymorphisms (SNPs). The study included 60 patients with acute myeloid leukemia (40 men and 20 women), with ages (mean SD) of (40.8615.60). (Ranging from 18-68 years). These patients were sent to the Hematology Consultation Clinic because they have AML. Those AML cases then had been analyzed via a specialized haematologist . Diagnosis was based on peripheral blood smear of blood sample revealing at least 10% blast cell and other diagnostic (TSP-1) . The study included 40 patients who received first-line chemotherapy as first-line therapy and 20 with newly diagnosed AML . The healthy group consisted of 30 people, 15 males and 15 females, with ages (mean S.D.) of (40.7314.99). (range 18 to 68) the control group, which did not have any illnesses, was matched with the patient group . Two milliliters (2 mL) of venous blood were drawn from each case control. Using the Blood Genomic DNA Extraction kit (TaKaRa), genomic DNA was isolated from the cell pellets collected from whole blood and kept at - 20C until genotypic analysis. Standard proteinase K-phe nol,chlor0form,ethanol extraction techniques were used to isolate the DNA. Using an Eppendorf Biophotometer and UV spectrophotometry at 260 nm, DNA concentrations were indomitable. The A260/A280 ratio was used to assess the quality of the DNA.

Amplification Refractory Mutation System Poly merase Chain Reaction (ARMS PCR) was used to examine previously discovered THBS1 gene SNPs (rs2228261, C>T). The THBS1 gene polymorphism as mentioned below was intended to be analyzed via Tetra ARMS PCR genotyping. Two primer pairs are used in the Tetra primer ARMS approach to simultaneously amplify the two distinct alleles of a particular SNP (Since these genotypes are single nucleotide polymorphisms, presence of either nucleotide, T or C). Different individuals within a society may have various genotypes while remaining healthy . With two outer primers amplifying the SNP's area and two inner allele-specific primers, two allele-specific amplifications take place in opposite directions using this technique. The allele-specific primers used in this technique intentionally mismatch twice, the second time being at position 2 from the 3' terminus. To ensure that specificity arises from variations in extension rates rather than hybridization rates, the inner primers have an average length of 28 bases to reduce the difference in stability of primers annealed to the target and non-target alleles. The initial primer design analysis and optimization process is necessary for the tetra primer ARMS PCR technology to attain the desired degree of reliability and repeatability. Primer1 software created four primers for each SNP. According on the SNPs and primers employed, the common fragment length differed [Table 1]. After being run through a horizontal 3 percent agarose gel, PCR results were seen under a UV light source.

Table (1): Primers of TSP-1 (rs2228261T/C)

TSP-1 (rs2228261T/C)	Sequence	Product size
Forward inner primer(C allele)	TCTGCAACTCTCCCAGCCCCCAGATGCAC	160(C allele
reverse inner primer (T allele)	CGCGCTTCGCCTTCACAGGGTTTCACA	139(T allele
Forward outer primer	AACAGGATGGTGGCTGGAGCCACTGGTCC	243 bp (from two outer primers)
reverse outer primer	CAGATGCCAGGCAACCAGCTGGGCAG	

3. Statistical analysis

the Hardy– Weinberg equilibrium (HWE)These tests evaluate the relationship between genotypic distributions and the emergence of AML and the control group. By computing odds ratios (ORs) and their 95% confidence intervals (CIs) with logistic regression analyses and adjusting for age and gender as co-variables, the connections between the genotypes of SNPs and AML were evaluated. The following models, each assuming either codominant inheritance (i.e., where the relative hazard varied between subjects with heterozygote and those with minor allele homozygote), dominant inheritance (i.e., where subjects with one or two minor alleles had the same relative hazard for the disease), recessive inheritance (i.e., where subjects with two minor alleles were at increased risk of the disease), or recessive inheritance and codominant inheritance or or additive inheritance (the risk is increased r-fold for subjects with one minor allele and 2r-fold for subjects with two minor alleles).

4. Results

The general characters of study groups

Table 1: General characteristic of the patients and healthy groups		
Patients)N= 60(	Healthy Control)N= 30(	Character
(18-68) (40.86±15.60)	(18-68) (40.73±14.99)	Age (years)mean ±S. D(
40/60(66.6%) 20/60(33.3%)	15/30 (50%) 15/30(50%)	Gender (NO%) Male Female
25/40(62.5%) 12/20(60%) 15/40(37.5) 8/20(40%)	8/15(53.33%) 9/15(60%) 7/15(46.66%) 6/15(40%)	Hypertension)NO(%, . Male with hypertension Female with hypertension Male without hypertension Female without hypertension
Residence		
42/60(70%)	19/30(63.33%)	Urban
18/60(30%)	11/30(36.66%)	Rural
		Family history
13/60(21.66%)	8/30(26.66%)	Present
47/60(78.33%)	22/30(73.33%)	Absent

This study showed that the majority of patients with acute myeloid leukemia are (40.86±15.60) years and the percentage of males was (66.6%) while (33.3%)) are females , (62.5%) of males are hypertension while the proportion (53.33%) of healthy male . also the results of study revealed (60%) of female patient are hypertension while it was (60%) in healthy female , as shown in table (1)

Table (2): The genotype distribution of TSP1 gene in patients and control

genotype	Patients%	Control%	OD ratio	*CI 95%*	P-value	Allele Frequency	
CC	6	13	0.068	(0.01-0.28)	0.00	**Patients**	**Control**
TC	27	13	0.30	(0.08-1.06)	0.06	**T (0.67)**	**T(0.65)**
TT	27	4	Reference group			**C (0.33)**	**C(0.35)**

The genotype distribution of TSP1 in acute myeloid leukemia patients and control

The homozygote pattern CC were more frequent in control groups than patients with acute myeloid leukemia with odd ratio (0.068),while the heterozygote pattern TC were more frequent in the patients with acute myeloid leukemia than control group with odd ratio (0.30) as shown in table (2).

The genotype distribution of TSP1 in treatment acute myeloid leukemia patient and new diagnosis patients.

The homozygote pattern CC were more frequent in treated patients than new diagnose patients with odd ratio (1.18) , while the heterozygote pattern TC were more frequent in the treated patients than new diagnose patients with odd ratio (1.16) as shown in table (3).

Table (3): The genotype distribution of TSP1 in treatment acute m myeloid leukemia patient and new diagnosis patients.

genotype	New diagnose patient(%)	Treated patient(%)	OD ratio	*CI 95%*	P-value	Allele Frequency	
CC	2	4	1.18	(0.17-7.84)	0.85	**New Patients**	Treated patient
TC	9	18	1.16	(0.37-3.75)	0.76	**T (0.675)**	**T(0.7)**

The result show the homozygote pattern CC was more frequent in control than patient (male and female) with AML, while heterozygote pattern (TC) was more frequent in patient(male and female) with AML than control as shown in table (4).

Table (4): The genotype distribution of TSP-1 according to gender between patient with acute myeloid leukemia and control group.

Group	Patient		P valve	control		P valve
	male	Female		Male	female	
CC	4	2	1.00	6	7	0.46
TC	20	7	0.55	8	5	0.22
TT	18	9		1	3	

Serum protein (TSP-1) levels in acute myeloid leukemia patients and healthy group (mean ± S.D)

The results of serum protein analysis showed the presence of a significant difference between the groups of the study, where the results recorded a significant decrease at level (p≤ 0.00) in the concentration of (TSP-1) in patients compared with healthy people.

Table (5) : level TSP-1 in acute myeloid leukemia patients and healthy control groups (Mean ± S.D).

Study groups				Variable
Patient(N=60)			Healthy (N=30)	
Sig	New diagnosis Patients (N=20)	Treated Patients)N= 40(		
0.00	13.05±20.45	28.02±53.07	116.19±95.72	TSP1(ng/ml)

Serum proteins level in acute myeloid leukemia patients and healthy group according to gender (mean ± S.D)

The results of the study indicate a significant decrease (p≤ 0.01) in (TSP1) in acute myeloid leukemia patients' group (male and female) compared control groups. summarized for these results in table (6)

Table (6) : levels of TSP-1 in acute myeloid leukemia Patients and healthy group according to gender

sig value (female)	sig value (male)	patients (N=60		Healthy control (N=30)		Groups variable
		Female	male	female	Male	
0.01*	0.00*	35.06±58.26	17.01±36.49	130.56±100.37	79.82±66.11	TSP1(ng/ml)

5. Discussion

1.Age

The current study shown that acute myeloid leukemia is common in the all ages, but is mainly in elderly patients, as the ages of patients ranged (18.68) years table (1). Agree with (Seer., 2004) show elder patient is common infected with AML. Also (Appelbaum., et al.2006) found that elderly individuals had greater comorbidity and have a lower performance status (PS) upon diagnosis. Less than 5% of AML patients in this age range have a 5-year survival rate, compared to 40% in young people, which is a very bad prognosis (Alibhai.,et al.2009 ; Menzin.,et al.2002) . The causes of poor outcomes in the elderly include patient- and disease-related given that persons in the Western world should expect to live another 20 years after they are 65 and another three years after they turn 80 (WHO. 2016). Frailty and comorbidities are frequently associated with advanced age, and these factors have a significant impact on these patients' tolerance for rigorous treatment methods (Mohammadi et al.,2015). Elderly patients are more likely to have undergone prior cytotoxic treatments or to have previous hematologic disorders, such as myeloproliferative neoplasms, myelodysplastic (MDS), and radiation [Mohammadi., et al. 2015]. In addition to these clinical characteristics, elder AML patients also have a different cytogenetic profile from younger patients, with a higher prevalence of numerous chromosomal abnormalities (Leith.,1997)

2.Gender

Results in this study recorded that males are more affected than females table (1). The reason why males are more likely than females to get acute leukemia at almost any age is unknown, however it's possible that the ABO blood group distributions of males and females with acute myeloid leukemia are different. They suggest that group O women are protected from AML, at least in our population, by the presence of a "sex-responsive" gene close to the ABO gene locus on chromosome 9 in females with AML. Another reason why males are more prone to acquire acute myeloid leukemia and probably other childhood cancers is that such a gene may exist. Additionally, some risk factors, such smoking or occupational exposure to carcinogens, could contribute to the male preponderance in adults. (Jackson et al., 1999) and could be different according to and may be due to differ in hormones between male and female patients.

3. TSP1 (rs 222826) polymorphisms between patients with AML and control.

In this study we examined the impact of TSP1 rs

222826 polymorphisms on the risk of acute myeloid leukemia in a sample of Iraqi population. The results showed found genetic association in homozygote pattern CC were more frequent in control groups than patients with acute myeloid leukemia with odd ratio (0.068) between SNPs and Acute myeloid leukemia in our study, while no found genetic association in heterozygote pattern TC were more frequent in the patients with acute myeloid leukemia than control group with odd ratio (0.30) as shown in table (2). A person's genetic propensity to a specific disease or occurrence can be predicted using genotype frequency, commonly known as genomic profiling.

Cancer has become a public health concern, affecting millions of people in both industrialized and developing countries. In most developing countries, cancer is expected to be the leading cause of morbidity and mortality (Wang et al, 2016). To improve this humiliating scenario, cancer risk factors must be detected quickly and successfully managed. Cancer is caused by a combination of hereditary and environmental factors. As a result, understanding the role of genetic variables in cancer will be beneficial. A family of five THBS genes encodes thrombospondins in vertebrates. Although THBS1 is seldom altered in most malignancies, its expression is influenced by a number of tumor suppressor genes, as well as active oncogenes and promoter hyper methylation. As a result, thrombospondin-1 expression is frequently lost during cancer development and is associated with a poor prognosis in several malignancies. Thrombospondin-1 is a secreted protein that inhibits angiogenesis, regulates antitumor immunity, stimulates tumor cell motility, and regulates the activities of extracellular proteases and growth factors in the tu032..66662mor microenvironment. Differential effects of thrombospondin-1 on normal vs malignant cell sensitivity to ischemia and genotoxic stress also control tumor responses to therapeutic radiation and chemotherapy (Jeffrey et al.,2020)

In addition, some research discovered that SNPs linked to these genes are crucial in the development of myocardial infarction, coronary artery disease, and early cardiovascular disease (Stenina et al., 2005, 2007; Koch et al., 2008). Recently, THBS1 rs2228262 and the risk of prostate cancer have been linked (Sfar et al., 2007, 2009). Thrombospondin-1 is a key player in the tumor microenvironment, although it has complex and even opposing effects on tumor growth. Early studies on people with breast, lung, gastrointestinal, and even gynecological cancers revealed higher TSP-1 levels (Tuszynski et al., 1992; Nathan et al., 1994).

4. TSP1(Thrombospondin-1) Serum protein level in acute myeloid leukemia patients and healthy group. In this study the result show level of TSP1 in patients with acute myeloid leukemia was significant decrease (p≤ 0.01) compared to control, and also show the level of TSP1in new diagnose patients and treated patients was found significant decrease (p≤ 0.01) compared to control. Additionally, in our investigation, serum THBS1 was found to be a promising predictor for patients. THBS1 is a member of the thrombospondin (TSP) family of adhesion proteins that are produced and interact with other proteins in vivo by endothelial cells, macrophages, platelets, monocytes, and other cells in the matrix. Additionally, it contributes to the formation of new blood vessels, cell migration, and other vital life activities (Akil et al.,2015 and Abu Zaid et al.,2017).

The fact that THBS1 was less expressed in the serum of AML patients may be due to the fact that their methylation levels were higher than those of healthy controls. Using data from the MSP and GEO datasets, it has been shown that treatment with hypomethylating agents may increase the expression of THBS1 and lower its methylation levels. This revealed a connection between THBS1 gene methylation and AML patients' prognoses (Zhu et al.,2020).

The current study contains a number of drawbacks. First off, because our study's sample size was too small to allow for a thorough examination of the impacts of genotypes, the findings need be verified in a bigger study. Second, no information regarding the subjects' genotypes or history of environmental exposure was available, making it unable to investigate gene-environment interactions. The research described here suggests that genetic variation in THBS-1 may have a significant role in the development of AML, albeit our findings need to be verified in a bigger investigation.

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