(RESEARCH ARTICLE)

Association of Methylenetetrahydrofolate Reductase (*MTHFR*) Gene C677T (rs1801133) polymorphism among Sudanese patients with chronic myelogenous leukemia

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Abstract

Background: Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme regulating the intracellular folate metabolism which plays an important role in carcinogenesis through DNA methylation.

Objectives: The current study aimed to determine the association between MTHFR C677T polymorphisms and the risks of chronic myeloid leukemia (CML).

Material and methods: This are hospital based cross sectional case control study, a total of 170 Sudanese subjects were enrolled, and 75 patients with chronic myeloid leukemia and 75 age- and sex matched healthy volunteers as a control group. Genomic DNA was extracted by sodium chloride method and The SNPs genotypes were determined using polymerase chain reaction followed by restriction fragment length polymorphism method. (PCR- RFLP). Data of this study were collected using a structured interview questionnaire and analyzed by statistical package for social sciences (version 21).

Results: The frequency of the CC genotype was higher in the control group compared with patients (100%,96%), while of the CT genotype was higher in the case (4%) than in control (0.0%); the TT genotype was absent in both study groups, the frequencies T and C allele were 0.02 and 0.98 in CML patient group and 0.00, 0.100 respectively in the control group. There was no clinically significant association between age (P.value= 0.3), gender (P.value= 0.4) and genotype, also there was no statistically significant association when compare case with control genotype (p. value 0.08).

Conclusion: C677T MTHFR polymorphism is not associated with the risk of CML among the Sudanese population

Keywords: Leukemia; Methylenetetrahydrofolate reductase; Polymorphism; Sudan

1. Introduction

CML is a myeloproliferative malignancy characterized by accumulation of myeloid precursors in bone marrow, peripheral blood and body tissues [1]. Studies have shown that 95% of patients with CML possess the Philadelphia chromosome which results from a reciprocal translocation t (9;22) (q31;q11) between chromosome 9 and chromosome 22. The translocation results in the formation of a fusion gene that encodes Bcr Abl protein, which possesses aberrant protein tyrosine kinase activity. This, in turn, activates its downstream signaling pathways involving cell proliferation,

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metastasis, cell survival, and differentiation. Progression of CML patients is divided into three phases, namely chronic phase, accelerated phase9 and blast crisis phase. CML patients with no treatment will develop fatal blast crisis phase within 5 years [2,3,4.5]. Although the biological and clinical aspects of CML have been well documented, little is known about the mechanism underling the genesis of Philadelphia chromosome and the factors influencing individuals' susceptibility to CML. To date, there have been no reports on familial, geographic, hereditary, ethnic and economic association with the pathogenesis of CML, and the mechanisms behind the disease progression haven't been fully understood as well [6]. It is recognized that leukemia is derived from abnormally proliferating hematopoietic tissue with greatest DNA synthesis, and thought the pathogenesis of CML would be associated with the metabolic fate of folic acid [7]. Methylene Tetrahydrofolate Reductase (MTHFR) is the rate-limiting enzyme in the methyl cycle, and it is encoded by the MTHFR gene [8,9]. The MTHFR gene is located on the short (p) arm of chromosome 1 at position 36.3[9]. It is spans approximately 2.2Kb and consist of 11 exons [10]. MTHFR enzyme is composed of an N-terminal catalytic domain and a C-terminal regulatory -domain. MTHFR has at least two promoters and two isoforms (70 k Da and 77 k Da) [11]. Methylenetetrahydrofolate reductase catalyzes the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine re-methylation to methionine [12]. The (C677T, rs1801133) polymorphisms in MTHFR gene have been described: First, C to T transition at nucleotide 677 (C677T, rs1801133), which causes the substitution alanine with valine at codon 222, and decreases in the enzymatic activity, and subsequently increases the level of homocysteine and altered distribution of folate [13] .The mutant gene produces an inactive enzyme that has been associated with uracil misincorporation into DNA, DNA double strand breaks during uracil excision repair, thus increasing the risk of chromosomal aberrations and malignancy[14].

2. Methodology

This is an analytical case-control study, conducted at Khartoum state, Sudan, in the period from July 2017 to july 2022. In which, 75 patients were confirmed diagnosis of CML and 75 age- and sex-matched apparently healthy volunteers- as a control group- were enrolled. Venous blood samples were collected from all participants in Ethylenediamine tetra acetic acid (EDTA). Genomic DNA was isolated from peripheral leucocytes manually by using sodium chloride method and stored at -30°C until PCR is carried out. MTHFR C677T polymorphism was analyzed by Allele-Specific Polymerase Chain Reaction (AS-PCR) followed by restriction enzyme. A reaction mixture of 20µl was prepared for each sample, containing 5µl genomic DNA, 1µL of each of the forward (5'GCAAGTCCCCCAAGGAGG-3'), reverse (5'GGTCCCCACTTCCAGCAT-3'), (Rabab M Aly et al; 2014) (MACROGEN, KOREA), 5µL master mix (MAXIME PCR PRE-MIX KIT (I-TAQ), INTRON, KOREA), and 8µL sterile distilled water. The amplification program consists of initial denaturation at 95ºC for 5 minutes; then 40 cycles [each consists of denaturation at 95ºC for 30 second , annealing at 60ºC for 1 minutes, and extension at 72ºC for 1minutes], and a final extension at 72ºC for 7 minutes. PCR products were incubated over night with HINF restriction enzyme (restriction enzyme was prepared by 7.5 µl of DW, 2 µl of enzyme buffer and 0.5 µl l of enzyme) then the product was separated on 3% agarose gel electrophoresis containing ethidium bromide with a 100 bp DNA ladder (SOLIS BIODYEN, ESTONIA) run with each batch of samples and the size of the fragments was determined under UV transilluminator (SYNGENE, JAPAN). A PCR fragment of 175 bp, 23bp, indicates the presence TT genotype, while a fragment of 175, 198 bp and23bp indicates to CT genotype and the wild type (CC state) remains undigested, preserving the original 198 bp fragment.

Patient's data were collected using a structured interview questionnaire and analyzed by statistical package for social science (SPSS), version 21. The qualitative data were presented as frequency and percentage. Quantitative data were presented as Mean±SD. Association between qualitative variable was tested by Chi-square (X2) and Fisher's exact tests. Multivariate logistic regression analysis was used for the examination of interaction between the polymorphism and MI risk factors. The allele frequency and Hardy Weinberg Equilibrium (HWE) were calculated using the conventional formulas.

The study was approved by the scientific research committee, faculty of medical laboratory sciences, Karary University Khartoum, Sudan.

3. Results

A total of 150 Sudanese subjects were enrolled in this study, 75 patients with chronic myeloid leukemia, and 75 healthy volunteers as a control group. The patients' and control age were ranged from 20 to 70 years (44.5 ± 16.1) and age of the controls was ranged from 29 to 70 years (38.5 ± 12.1) with no previous history and risk factor of CML as shown in (Table 1), the patients age divided as group,(20-30y) (37%), (31-40) (43%), (41-50) (33%), (51-60) (24%) and more than(60y) (13%) as shown in (Figure 3.1) , fourty three (57%) of patients were male and thirty tow (43%) were female in compersion to control, male were fourty four (59%) and other part (41%) were female, the most common sign of

patients was Spleenomegaly (48%), fatigability (43%), fever (33%) huge Spleenomegaly (31%) and abdominal pain (25%) as shown in (Figure 2). According to genotype, the analysis of result showed that For C677T polymorphism, the results of PCR amplification yielded one amplicons, length 198 bp and Post digestion documentation was done on gel electrophoresis, the results yielded 198bp indicate CC genotype, 198, 175 and 23bp for CT genotype nobody with TT genotype were found in both patient and control groups as shown in (Figure 3). There was no clinically significant association between age (*P.value*= 0.3) as in (Table 2), gender (*P.value*= 0.4) as in (Table 3) and genotype, there was no statistically significant association when compare patients with control genotype (*p. value* 0.08) as shown in (Table 4). The distribution of allele frequency within each group in Hardy-Weinberg equilibrium was found, the prevalence of the C allele was 0.98 in the patients and100 % in the control group, where T allele was 0.02 in patients and 0.00 in control group as shown in (Table 5). This was indicates no a significant association of the T allele with CML patients in contrast to control. No statistically significant association was observed between the *C677T MTHFR* polymorphism and CML risk and this variant may not be a genetic risk factor for CML susceptibility in Sudan.

Table 1 Characteristic of patient and control

Characteristic	Patients	Control
N	75	75
Age	44.5 ± 16.1	38.5 ± 12.1
Sex(male: female)	43:32	44:31

Figure 1 PCR-base restriction analysis of *MTHFR* C677T polymorphism shown on 3% agarose electrophoresis. The polymorphic region was amplified by PCR resulting in digestible fragment length 198 bp indicated for CC genotype in lane 1,2,3,4,5,6,7,8 , L: ladder, 100 bp ladder (Fermentas, Germany).
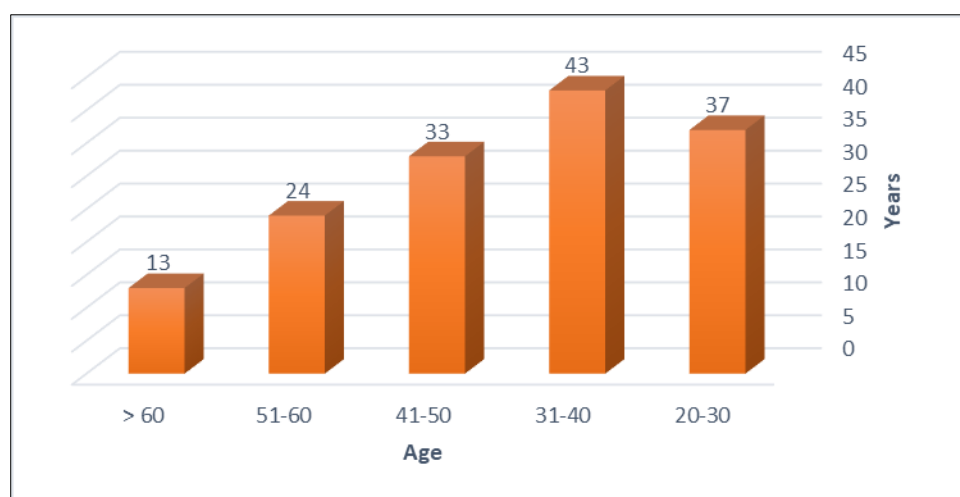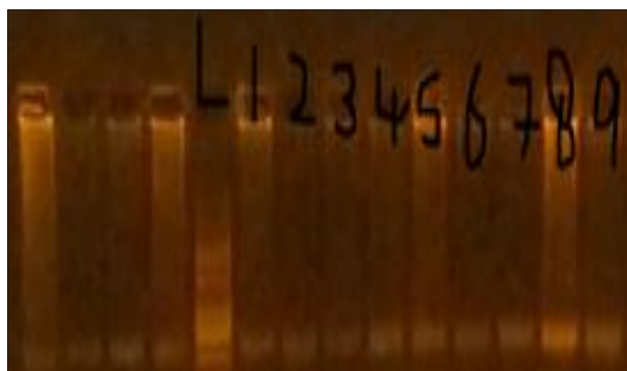


Figure 2 Age group of patients
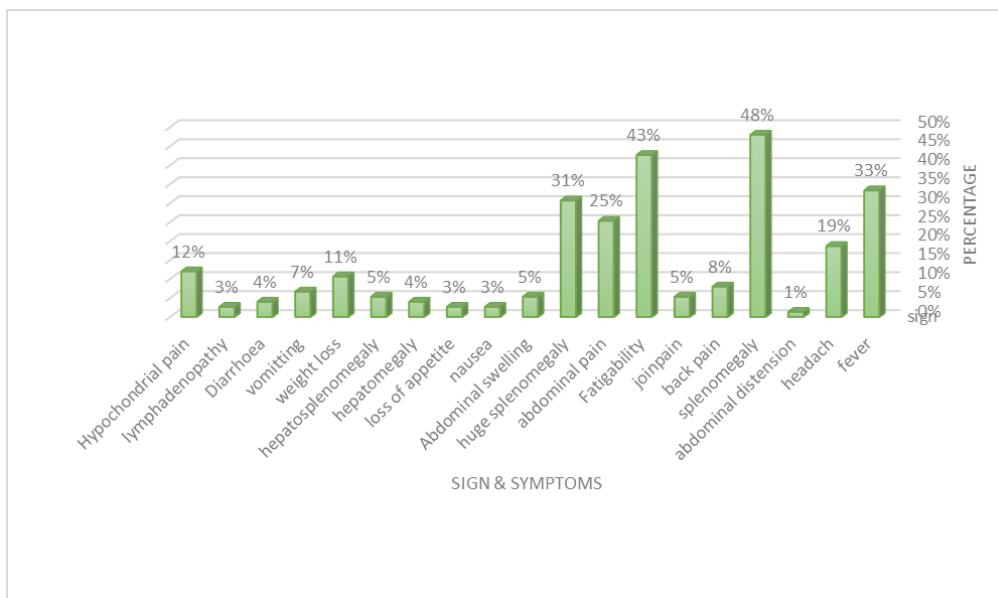
Figure 3 Frequency of sign and symptoms among patients

Table 2 Comparison between patient's genotype and age group

Hinf		Age					P.value
		20-30	31-40	41-50	51-60	> 60	
CC	Count	13	18	16	13	12	
	%	100.0%	94.7%	88.9%	100.0%	100.0%	
CT	Count	0	1	2	0	0	0.3
	%	0.0%	5.3%	11.1%	0.0%	0.0%	
Total	Count	13	19	18	13	12	
	%	100.0%	100.0%	100.0%	100.0%	100.0%	

Table 3 Comparison between patient's genotype and gender

Hinf		Gender		P. value
		male	Female	
CC	Count	42	30	
	%	97.7%	93.8%	
CT	Count	1	2	0.4
	%	2.3%	6.3%	
Total	Count	43	32	
	%	100.0%	100.0%	
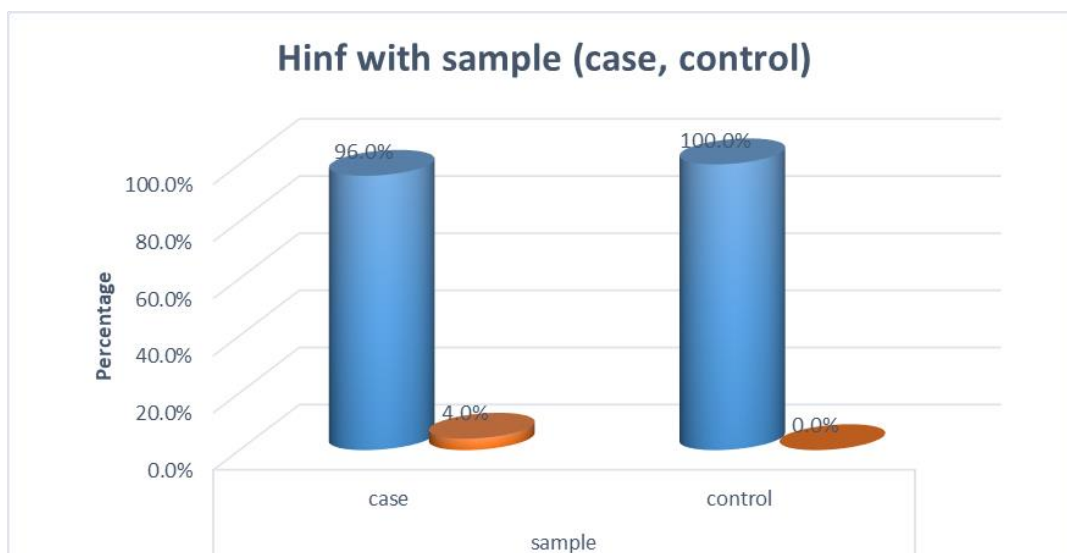
Figure 4 Frequency of genotype in patients and control

Table 4 Comparison between patients and control genotype

Hinf		Sample		Total	P. value
		Case	control		
CC	Count	72	75	147	
	%	96.0%	100.0%	98.0%	
CT	Count	3	0	3	0.08
	%	4.0%	0.0%	2.0%	
Total	Count	75	75	150	
	%	100.0%	100.0%	100.0%	

Table 5 T and C allele Frequency

MTHFR(C677T)	Patients	Control
C	0.98	100
T	0.02	0.0

4. Discussion

This a case control study was conducted to investigate the association of methylene tetra hydro folate reductase enzyme polymorphism C677T among Sudanese patients with chronic myeloid leukemia confirmed. This study was carried on 150 Sudanese individual, seventy five apparent healthy as control and seventy five as CML, fourty three of patients were male and thirty two were female, fourty four of control were male and Thirty one were female. The result of current study showed there was no clinically significant association between age (*P.value*= 0.3) , gender (*P.value*= 0.4) and genotype and this agree with Rabab M Aly et al in 2014 no statistical difference for age when compared CML patients with genotype [15]in addition reveled that there was no difference for sex distribution in respect to C677T genotype and this completely agree with the current study in contrary Lordelo in 2012 et al that the C677T more associated with male than female [16] regarding the allele and genotype frequency, the CC genotype was more in control group than patients (100%, 96%) respectively and the CT was more common in patients than control (4%, 0.0%) respectively with no significance difference when compare them (*P.value*= 0.08) , this agree with Rabab M Aly et al in 2014 The frequency

of the MTHFR 677CC genotype was higher among controls (45.0%) when compared to CML patients (35.2%). In addition, for CML patients, the frequency of the 677CT genotype (51.7%) was higher than control group (49.0%) but with no statistical significance (P = 0.981) also Lordelo in 2012 reveled that CC genotype more common in control than patients and this similar with the finding of present study in contrast to the current study was showed CT also more common in control[16]. The present study finding which showed there was no association between C677T polymorphism and risk for CML seemed to contradict Rabab M Aly et al in 2014 an association between *MTHFR* polymorphisms (677TT) and risk of developing CML, also Barbosa and his colleagues did not find any significant association between both *MTHFR* polymorphisms and CML[17] in addition Lordelo showed that there was no association between C677T and CML patients (Lordelo et al; 2012) and Pazhakh Vahid also agree with the present study which found that the polymorphism do not contribute to an inherited genetic susceptibility of CML [18,19], while Robin *et al*. who investigated the relationship between *MTHFR* polymorphisms and the risk of relapse after hematopoietic cell transplantation for CML, observed a decrease in the relapse risk of patients with variants of both *MTHFR* polymorphisms [20] also in study performed in Jordan by Ismail said in 2009 there result conflict with present study which showed that a significant association of c677T with CML risk [21].

5. Conclusion

The homozygous C/C genotype was more frequent in the patient and the healthy controls, while the T/T genotype was completely absent in both study groups. However, there was no statistically significant association between MTHFR C1677T polymorphism and risk of CML among the Sudanese population.

Compliance with ethical standards

Disclosure of conflict of interest

There was no conflict of interest in this study. Statement of informed consent Informed consent was obtained from all individual participants included in the study.

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References

[1] Deininger MW, Goldman JM, Melo JV. The molecular biology of chronic myeloid leukemia. Blood 2000;96:3343-3356.

[2] Faderl S, Talpaz M, Estrov Z et al. The biology of chronic myeloid leukemia. N Engl J Med 1999;341:164-172.

[3] Ren R. Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenousleukaemia. Nat Rev Cancer 2005;5:172-183.

[4] Melo JV, Barnes DJ. Chronic myeloid leukaemia as a model of disease evolution in human cancer. Nat Rev Cancer 2007;7:441-453.

[5] Quintas-Cardama A, Kantarjian H, Cortes J. Flying under the radar: the new wave of BCR-ABL inhibitors. Nat Rev Drug Discov 2007;6:834-848.

[6] Quintas-Cardama A, Cortes JE. Chronic myeloid leukemia: diagnosis and treatment. Mayo ClinProc 2006;81:973-988.

[7] Lunt SY, Vander Heiden MG. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. Annu Rev Cell Dev Biol 2011;27:441-464.

[8] Goyette P, Sumner JS, Milos R, Duncan AM, Rosenblatt DS, Matthews RG, Rozen R (June 1994). "Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification". Nat. Genet. 7 (2): 195–200

[9]   Goyette P, Sumner JS, Milos R. (August 1994). "Human methylenetetrahydrofolate reductase: isolation of cDNA mapping and mutation identification". Nat. Genet. 7 (4): 551.

[10]   Thivaratana Sinthuwiwat,1 Phanasit Poowasanpetch,1 Angsana Wongngamrungroj,1 Kamonwan Soonklang,2 Somying Promso,3 Chirayu Auewarakul,4 and ChintanaTocharoentanaphol Published online Feb 29, 2012.Dis Markers. 2012; 32(2): 115–121.

[11]   Tran P, Leclerc D, Chan M. (September 2002). "Multiple transcription start sites and alternative splicing in the methylenetetrahydrofolate reductase gene result in two enzyme isoforms". Mamm. Genome 13 (9): 483–92.

[12]   Wang H, Wang J, Zhao L, et al. Methylenetetrahydrofolate reductase polymorphisms and risk of Chronic myeloid leukaemia-evidence From an updated meta-analysis including 35 studies. BMC Med Genet. 2012;13:77. Doi: 10.1186/1471-2350-13-77.

[13]   Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995;10(1):111–3.

[14]   Yuan Jiang, Jing Hou, Qiang Zhang, Shu-Ting Jia, Bo-Yuan Wang, Ji-Hong Zhang,Wen-Ru Tang,Ying Luo.Asian Pacific Journal of Cancer Prevention, Vol 14, 2013

[15]   Rabab M Aly1, Mona M Taalab2, Hayam F Ghazy. MTHFR A1298C and C677T gene polymorphisms and susceptibility to chronic myeloid leukemia in Egypt Int J Clin Exp Pathol 2014;7(5):2571-2578.

[16]   G.S. Lordelo1,2, A.L. Miranda-Vilela1, A.K. Akimoto1, P.C.Z. Alves1, C.O. Hiragi1, A. Nonino2,3, M.B. Daldegan2, M.N. Klautau-Guimarães1 and C.K. Grisolia. Association between methylene tetrahydrofolate reductase and glutathione S-transferase M1 gene polymorphisms and chronic myeloid leukemia in a Brazilian population genetics and Molecular Research 11 (2): 1013-1026 (2012)

[17]   Barbosa   CG,   Souza   CL,   Neto   JPM,   Arruda   MGB, Barreto JH, Reis MG, Goncalves MS. Methylenetetrahydrofolate reductase polymorphisms in myeloid leukemia patients from Northeastern Brazil. Genet and Mol Biol 2008; 31: 29-32

[18]   Pazhakh Vahid, MSc, Razmkhah Farnaz, MSc, Farhad Zaker, PhD, Atashrazm Farzaneh, MSc, Rasighaemi Parisa. Methylenetetrahydrofolate Reductase Gene Polymorphisms and Risk of Myeloid Leukemia. LABMEDICINE Volume 41 Number 8 August 2010

[19]   Abd Elgadir A Altoum, Ahmed L Osman, Asaad MA Babker. Impact of body mass index in malondialdehyde, antioxidant vitamins A, E, C and plasma zinc among type 2 diabetic patients. Kuwait Medical Journal 2019; 51 (1): 16 - 20

[20]   Robien   K,   Ulrich   CM,   Bigler   J,   Yasui   Y,   Gooley   T, Bruemmer B, Potter JD, Radich JP. Methylenetetrahydrofolate reductase genotype affects risk of relapse after hematopoietic cell transplantation for chronic myelogenous leukemia. Clin Cancer Res 2004; 10: 7592-98.

[21]   Said I. Ismail,*1 Nida A. Ababneh, 2 Abdallah Awidi 2 Methylenetetrahydrofolate Reductase (MTHFR) Genotype Association   with   the   Risk   of   Chronic Myelogenous Leukemia. (J Med J 2009; Vol. 43 (1): 8-14)