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Detection of Methylene tetrahydrofolate reductase genes (C677T, A1298C) mutations among sickle cell anemia patient among Sudanese population

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Abstract

Introduction: it has been linked that genotypes of MTHFR C677T and A1298C among sickle cell disease with different issues, such as vaso occlusion and levels of vitamin B12 and folic acid, which they affect sickle cell disease patients if not corrected. This study aimed to measure complete blood count and detect MTHFR genotypes C677T and A1298A among patients as well as normal subjects with normal hemoglobin AA.

Method: 200 subjects were enrolled, 100 were SCD and the other were control group. CBC parameters analyzed with hematology analyzer BC3000-Mindray and detection of genotypes through PCR. Data analyzed via SPSS version 20.

Result: A comparison of MTHFR C677T genotypes between SCD and healthy controls. The CC (mutant) genotype was found in 64% of SCD patients compared with 48% of healthy individuals, while the CT (wild type) was more common among healthy participants (52%) than among SCD patients (36%). This difference was statistically significant ($p = 0.023$), the AA genotype was found in 54% of SCD patients and 46% of controls, while the AC genotype was detected in 46% of SCD and 54% of healthy participants. Comparison of the distribution of MTHFR A1298C genotypes between SCD and healthy groups ($p = 0.258$) has no significant difference These results indicate no significant association between the MTHFR A1298C polymorphism and SCD occurrence.

Conclusion: Considering vaso constriction, although existence of genotypes of MTHFR, patients were not at risk of vaso constriction nor normal subjects.

Key words: sickle cell disease, genotypes, Methylene tetrahydrofolate reductase

1. Introduction

A mutation in the gene that codes for beta-globin, a subunit of hemoglobin, the oxygen-carrying protein found in red blood cells, results in sickle cell disease (SCD). Hemoglobin abnormalities in people with

sickle cell disease (SCD) result in sickle-shaped and less flexible red blood cells. SCD is a serious public health issue. Numerous issues, including as organ damage, pain crises, and even death [1]. With an overall observed prevalence of 9.3% HbAS sickle cell trait and 0.21% homozygous HbSS, primarily in the tribal population, sickle cell hemoglobinopathy is a serious public health concern in this region of Central India, where its prevalence ranges from 2 to 34%. Tribal populations residing in isolated catchment areas with much fewer healthcare facilities make up nearly 32% of the state's population [2—5].

Globally, SCD mortality remains a terrifying reality, particularly in African nations where the disease's high prevalence and high death rates are concerning. The South and North regions of Brazil had the lowest mortality rates per 100,000 people, at 0.05 and 0.19, respectively. However, the Midwest area had the highest rate (0.35), with the state of Goiás having the highest concentration (0.48 fatalities per 100,000 residents). The state of Bahia, which has the highest death rate in the northeast with 0.48 fatalities per 100,000 residents, has a similar number [6].

Infants with sickle cell disease (SCD) may not exhibit any symptoms during the first few months of infancy, but with time, the illness typically causes difficulties that worsen with age. Because of the sporadic vaso-occlusive crises, recurrent pain is widespread, mainly in the bones and joints. However, the frequency of this consequence might vary greatly, with some people experiencing up to six or more occurrences per year, while others may not have this symptom or have less frequent pain crises. Almost every organ and system may be affected by SCD symptoms. Anemia, vaso-occlusion, and hemolysis are linked to homozygote sickle cell disease and are exacerbated by oxidative stress, hypercoagulability, inflammatory response, and impaired arginine metabolism [7].

The two most often researched variants of the MTHFR gene, C677T and A1298C, are linked to decreased methylene tetrahydrofolate reductase enzyme activity, which causes homocysteine to build up, particularly in people with low folate levels. Vascular occlusion is linked to major causes of death in people with SCA, including acute chest syndrome and cerebrovascular illness. MTHFR gene polymorphisms have been proposed as a possible risk factor for vaso-occlusive events in SCA patients. Additionally, a common musculoskeletal consequence that lowers the quality of life for people with SCA is avascular necrosis of the femoral and humeral head. Additionally, avascular necrosis in sickle cell anemia has been linked to MTHFR mutations as a potential risk factor [8].

2. Method

This study was conducted among Sudanese patients with sickle cell disease, they were 100 and other 100 normal healthy subjects were recruited to be a control group for comparison. The complete blood count

was conducted for both patients and normal subjects. The SCD patients didn't have vaso-occlusion by any means, not at crisis, they were recruited to detect polymorphism of MTHFR C677T and A1298C by means of polymerase chain reaction (PCR) amplification of MTHFR target regions was performed using specific primers for: C677T (rs1801133) and A1298C (rs1801131)

PCR conditions:

- Initial denaturation: 95°C for 5 minutes
- 35 cycles:
  - Denaturation: 95°C for 30 seconds
  - Annealing: 58–60°C for 30 seconds
  - Extension: 72°C for 45 seconds
- Final extension: 72°C for 7 minutes

Restriction Fragment Length Polymorphism (RFLP)

Amplified PCR products were digested with specific restriction enzymes:

- HinfI enzyme for C677T polymorphism
- MboII enzyme for A1298C polymorphism

Digested products were separated on 2–3% agarose gel, stained with ethidium bromide or a safer alternative, and visualized under UV trans illumination.

Genotypes were recorded as:

- CC, CT, TT for C677T
- AA, AC, CC for A1298C

beside routine check-up for complete blood count. Confirmation of hemoglobin types was conducted as well, via electrophoresis

3. Result

A comparison of MTHFR C677T genotypes between SCD and healthy controls. The CC (mutant) genotype was found in 64% of SCD patients compared with 48% of healthy individuals, while the CT (wild type) was more common among healthy participants (52%) than among SCD patients (36%) as in figure 1. This difference was statistically significant ($p = 0.023$), suggesting that the CC genotype of the MTHFR C677T polymorphism is associated with an increased susceptibility to SCD as in table 1.

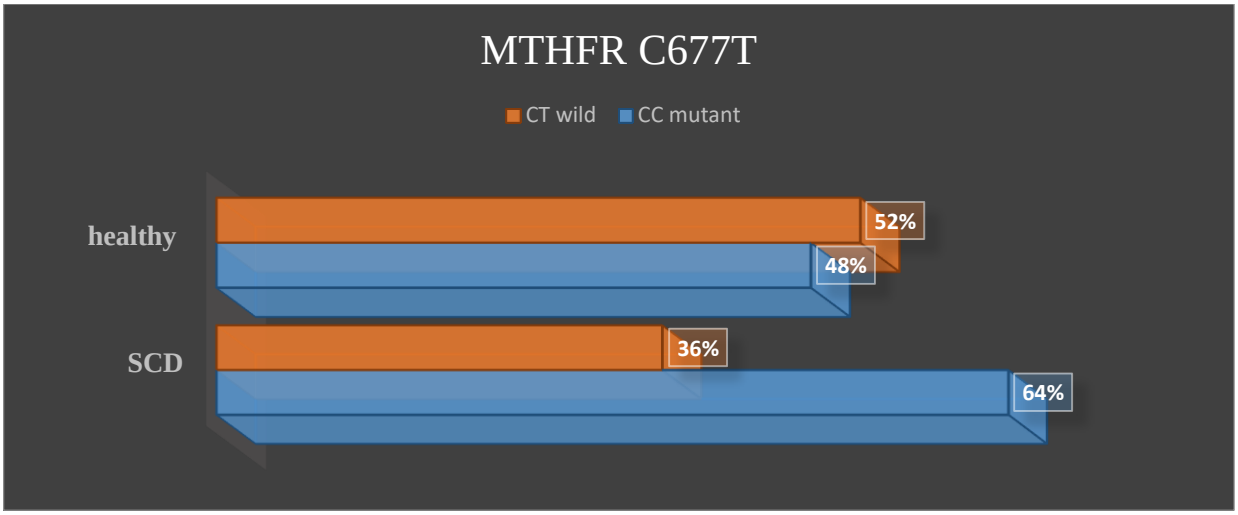

Figure 1: MTHFR C677T mutations among case and control groups

Table1: The distribution of MTHFR C677T genotypes in SCD group compared to Health group.

Genotype	SCD		Health		P Value
	No	%	No	%	
CC (Mutant type)	64	64%	48	48%	**0.023**
CT(Wild type)	36	36%	52	52%	

The AA genotype was found in 54% of SCD patients and 46% of controls, while the AC genotype was detected in 46% of SCD and 54% of healthy participants as in figure 2. Comparison of the distribution of MTHFR A1298C genotypes between SCD and healthy groups (p = 0.258) has no significant difference These results indicate no significant association between the MTHFR A1298C polymorphism and SCD occurrence as in table 2.

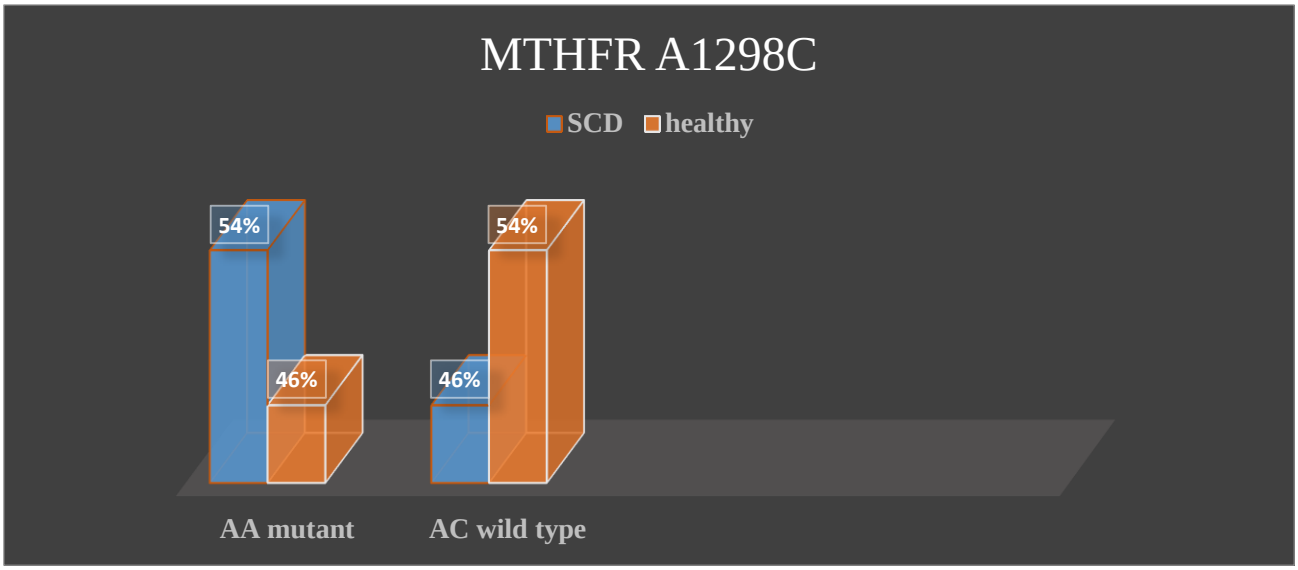

Figure 2: distribution of MTHFR A1298C genotypes

Table (2): The distribution of MTHFR A1298C genotypes in SCD compared to Health group.

Genotype	SCD		Health		P Value
	No	%	No	%	
AA (Mutant type)	54	54%	46	46%	0. 258
AC (Wild type)	46	46%	54	54%	

Table 3: The risk analysis demonstrated that the MTHFR C677T variant was significantly associated with SCD, with an odds ratio (OR) of 1.926 and a 95% confidence interval (CI) of 1.093–3.393. This indicates that individuals carrying the CC genotype have nearly two times higher risk of developing SCD compared with those carrying the CT genotype. In contrast, the MTHFR A1298C variant showed an OR of 1.378 (95% CI: 0.79–2.403), which is not statistically significant, implying that this variant does not contribute significantly to SCD susceptibility in the studied population.

Table 3: The Odd ratios of the two MTHFR C677T, A1298C genotypes among SCD

MTHFR	Odds Ratio	Lower 95%	Upper 95%
C677T	1.926	1.093	3.393
A1298C	1.378	0.79	2.403

Table 4: The risk analysis demonstrated that the MTHFR C677T variant was significantly associated with SCD, with an odds ratio (OR) of 1.926 and a 95% confidence interval (CI) of 1.093–3.393. This indicates that individuals carrying the CC genotype have nearly two times higher risk of developing SCD compared with those carrying the CT genotype. In contrast, the MTHFR A1298C variant showed an OR of 1.378 (95% CI: 0.79–2.403), which is not statistically significant, implying that this variant does not contribute significantly to SCD susceptibility in the studied population.

Table 4: The Odd ratios of the two MTHFR C677T, A1298C genotypes combinations that significantly increase SCD

MTHFR	Odds Ratio	Lower 95%	Upper 95%
C677T	1.926	1.093	3.393
A1298C	1.378	0.79	2.403

Table 5: Within the SCD, comparison between CC and CT genotypes of *MTHFR* C677T, it revealed no significant differences in CBC parameters ($p > 0.05$), except for MCHC a significant difference was obtained ($p = 0.032$), where individuals with the CT genotype had slightly higher MCHC values compared to those with the CC genotype. This suggests that the C677T polymorphism may have a minor influence on red blood cell indices within the SCD population.

Table 5: The Mean difference of CBC Parameters in SCD group compared between MTHFR C677T genotype

Parameters	CC Mean ± SD	CT Mean ± SD	P Value
WBC	8.9± 3.8	8.2 ± 3.2	**0.328**
RBC	3.03 ± 0.7	3.03 ± 0.7	**0.978**
Hb	8.5 ± 2.2	8.6 ± 2.3	**0.850**
PCV	26 ± 6	26 ± 3	**0.927**
MCV	93 ± 6	92 ± 7	**0.595**
MCH	30 ± 1	30 ± 2	**0.624**
MCHC	32 ± 1	33 ± 1	**0.032**
Neutrophil	5.0 ± 3	4.5 ± 2	**0.640**
Lymphocyte	2.3 ± 1	2.4 ± 1	**0.386**
Platelets	346 ± 124	334 ± 114	**0.869**
Total	**64**	**36**	**100**

Table 6: As shown in, there were no significant differences between the AA and AC genotypes of MTHFR A1298C in terms of hematological parameters concentrations ($p > 0.05$). This further supports the lack of a significant role for the A1298C variant in modulating hematological or biochemical profiles among SCD patients.

Table 6: The Mean difference of CBC Parameters in SCD group compared between MTHFR A1298C genotypes

Parameters	AA Mean ± SD	AC Mean ± SD	P Value
WBC	8.2± 3.4	9.2 ± 3.9	0.174
RBC	3.07 ± 0.7	2.98 ± 0.7	0.567
Hb	8.7 ± 2.5	8.3 ± 2.3	0.454
PCV	27 ± 6	25 ± 6	0.305
MCV	92 ± 8	93 ± 7	0.409
MCH	30 ± 2	29 ± 2	0.481
MCHC	32 ± 1	32 ± 1	0.993
Neutrophil	4.5 ± 2	5.2 ± 3	0.329
Lymphocyte	2.3 ± 3	2.5 ± 1	0.209
Platelets	331 ± 120	354 ± 120	0.485
Total	**54**	**46**	**100**

4. Discussion

There was an agreement with a Nigerian cross-sectional study (Adelekan O. O et al 2019) was done among SCA patients attending the Hematology Clinic of the Lagos State University Teaching Hospital (LASUTH), using age and sex matched Hb AA controls. DNA extraction and gene analysis were done. The selective amplification of a particular segment of the DNA by polymerase chain reaction (PCR) was done and subsequent digestion of the amplified MTHFR gene into its various fragments. The overall prevalence of the C677T mutation among participants was 19.3% (37 of 192), while the prevalence of A1298C was 15% (29 of 192). The prevalence of MTHFR C677T was higher than A1298C mutations among sickle cell anemia subjects [8].

A partial agreement with a study was conducted to determine the frequency distribution of the MTHFR C677T and A1298C genotypes in 249 children diagnosed with sickle cell disease, between age group 5–18 years. The demographic and clinical details were entered in a structured questionnaire. Collected blood samples were analyzed for hemoglobin and DNA was extracted for genotypic assay for MTHFR C677T and A1298C single nucleotide polymorphisms (SNPs) by Real-time PCR. The study groups comprised of 218 sickle cell trait (SCT) and 31 sickle cell disease (SCD) children. The caste distribution between the two study groups was quite uniform (X2 31 = 44.21, p = 0.06). Frequencies of homozygous mutants 677TT and 1298CC were 2% and 19.7% respectively. The odds for the variant forms for both SNPs were found to be greater in SCD group. The genotypic and allelic frequencies did not reveal any caste preponderance. The mean age (p = 0.001), weight (p < 0.001), height (p < 0.001), BMI (p < 0.001) and hemoglobin concentrations (p = 0.002) were lower in homozygous 1298CC but not so in 677TT children. A1298C also depicted significant association with BMI and anemia (p < 0.001) [9].

A disagreement with a study conducted on the considering the implication of MTHFR gene mutation as a risk factor for ischemic stroke (IS) in the general population, the study aimed to determine whether the MTHFR. 677C>T variant has linked to an increased risk of IS in different age groups and ancestry groups, through literature review. As of March 2022, 1,925 citations had been identified in electronic databases, of which 96 studies involving 34,814 subjects met our eligibility criteria. A strong link was found between IS and the MTHFR gene rs1801133 (677C>T) polymorphism in all genetic models [10].

A partial agreement with a Sudanese study aimed to determine the frequency of the mutation of MTHFR in patients with sickle cell anemia and to measure the prevalence of MTHFR mutation among the study population, among of 125 patients less than 17 years with sickle cell anemia were examined for the mutation in the mutation in MTHFR in locus A1298C, it found that the frequency of mutation in MTHFR in A1298C was 19% in SCD patient (homozygous was 11.4%, while heterozygous was 7.6 %). significant relationship between mutation in MTHFR and SCD patient (P=001) [11].

5. Conclusion

This study assessed complete blood count and detected of MTHFR genotypes, C677T and A1298C among sickle cell patients and normal healthy subjects, the presence of both genotypes wild and mutant among both patients and healthy subjects, no signs of vaso occlusion. A comparison of MTHFR C677T genotypes between SCD and healthy controls. The CC (mutant) genotype was found in 64% of SCD patients compared with 48% of healthy individuals, while the CT (wild type) was more common among healthy participants (52%) than among SCD patients (36%) as in figure 1. This difference was statistically significant (p = 0.023), suggesting that the CC genotype of the MTHFR C677T polymorphism is

associated with an increased susceptibility to SCD. These results indicate no significant association between the MTHFR A1298C polymorphism and SCD occurrence.

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