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Study of Methylenetetrahydrofolate Reductase C677T gene polymorphism and some biochemical markers for patients with hypertension

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Abstract

The study aims to evaluate the levels of some biochemical in hypertension patients, find out MTHFRC677T gene polymorphism exist, and whether they are a risk factor for hypertension. This case-control study, included 120 participants, the biochemical analyses were measured by ELISA technique. Genetic analysis was performed by Tetra ARMS-PCR, Chi-square and odds ratio used for data analysis. The study found an increase in the level of homocysteine; a decrease in the levels of MTHFR and vitamin B12; a non-significant difference in folic acid levels in hypertension patients without treatment when compared with controls. There is a non-significant difference in the levels of this markers in patients on angiotensin receptor blocker (ARBs) valsartan drugs when compared with hypertension patients without treatment. In MTHFRC677T gene polymorphism, the most prevalent genotype is CT (56%), CC, and TT with (32%) and (12%) for each of them in succession with OR of 1.18 ,the T allele is present (40%) in case group and (24%) in control group. In conclusion, hyperhomocysteinemia decrease in the levels of MTHFR enzyme and vitamin B12 may be one of the underlying cause of hypertension, there is no relationship between the level of folic acid in serum and hypertension.

Keywords: Hypertension; Methylenetetrahydrofolate reductase enzyme; MTHFRC677T gene polymorphism.

1. Introduction

Hypertension is both a disease and a major risk factor for other diseases[1].Hypertension (HTN) has been determined to be the leading cause of death, Early detection of undetected hypertension can help prevent the disease's progression and avoid serious health risks including heart disease and stroke [2]. Normal blood pressure (BP) is characterized by a

systolic BP below 120 mmHg and a diastolic BP below 80 mmHg without any antihypertensive medication [3]. Homocysteine (Hcy) is an amino acid that contains Sulphur and it represents an intermediary in the metabolism of methionine but it is neither found in dietary protein nor required for its endogenous production [4]. Methionine is an essential amino acid that cannot be synthesized from metabolic intermediates by humans or other animals. The human body does not contain the metabolic pathways necessary for the synthesis of essential amino acids, thus they must be obtained through an external diet [5]. The link between methionine, Hcy, folate, vitamin B12 is shown in the figure below.

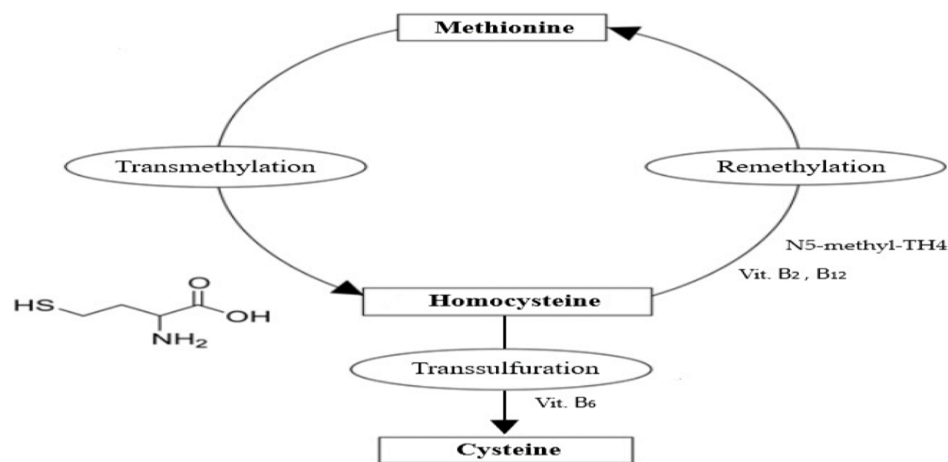


Figure 1. Overview of homocysteine metabolism[6],Dietary methionine is the exclusive source of HCy. A transsulfuration pathway can catabolize it to cysteine or remethylate it to methionine. Vitamins from the B group are very important in this process. These include riboflavin, cobalamin, vit. B12, pyridoxine, vit. B6, and N5-methyl-tetrahydrofolate (N5-methyl-TH4), which is vit. B9.

Hyperhomocysteinemia (HHcy) has been identified as a new risk factor related hypertension [7]. Hcy improves the formation of collagen, increase smooth muscle cell proliferation, and increase blood viscosity [8]. Hcy, on the other hand, raise oxidative stress, damage endothelial repair, induce endothelial dysfunction, and promote vascular remodeling, all of which may accelerate hypertension [9]. HHcy lowering NO's bioavailability, which plays a significant role in its vasodilator action[10]. Hcys increases the expression of endothelin-1, a strong vasoconstrictor[11]. Methylenetetrahydrofolate reductase enzyme (MTHFR) is responsible for keeping the equilibrium between homocysteine and methionine in order to avoid cellular dysfunction[12]. methionine synthase (MS), which uses B12 and 5-methylTHF (made by

MTHFR) to convert Hcy to methionine[13]. Elevated plasma Hcy levels are one sign of MTHFR enzyme malfunction [14]. Elevated homocysteine levels are linked to a higher risk of hypertension [15]. Folic acid is vitamin soluble in water, FA is also known as vitamin B9. Its natural form is called folate. Human blood serum should contain between 2 and 15 ng/mL of FA at normal levels[16]. 5-methyltetrahydrofolate (5-MTHF) is the active form of folic acid , 5-MTHF is involved in numerous metabolic processes as a methyl donor, such as the transformation of homocysteine into methionine [17]. Folate has an impact on eNOS activity directly as well as through increasing tetra-hydro biopterin (BH4), a cofactor of eNOS,'s availability, Nitric oxide (NO) is vasodilator it production is enhanced by folic acid [18].One cause of HHcy is a folate deficits [19]. Vitamin B12 is a Water-soluble vitamin [20]. also referred to as cobalamin[21]. Methionine synthase uses it as a cofactor to help convert homocysteine to methionine [22]. It is necessary for maintaining human cardiovascular health by avoiding an increase in homocysteine levels [23]. It is now well acknowledged that a complex interaction between several hereditary and environmental variables leads to hypertension[24]. The MTHFR gene has been associated to high blood pressure[25]. The MTHFR gene expresses MTHFR an enzyme that is involved in the methyl cycle [26].This enzyme's gene can be found on chromosome 1's short arm. (1p36.3)[27]. It cooperates with methionine synthase reductase (MTRR) to maintain the body's regular homocysteine (Hcy) levels and the folate metabolism [28,29].Increased homocysteine can raise blood pressure, and one possible cause of this condition is polymorphism in the MTHFR gene [30].

2. Materials and Methods

The present study was designed as a case control study. The period extended from 1st of October 2023 until the 1st of May 2024. This work was done in the Department of Biochemistry, the practical side of the study was performed at the laboratory of Imam Sadiq Teaching Hospital (Hilla City), laboratories medical chemistry department in medicine collage university of AL-Qadisiyah, Al-Hashimiya Hospital internal medicine clinic, Mirgan Teaching Hospital and Shaheed Almuhrab center in Hilla city, Iraq. Sample size was calculated according to Daniel sample size formula equation. These study included 120 participants , for biochemical analysis there was sixty subjects (32 males, 28 females) who were apparently healthy control that enrolled in this study were referred to as G1 matched with patients in sex and age and 60 patients with hypertension were enrolled in this study, there were 30 males and 30 females ,these 60

patients were segregated into two subgroups:30 of them were hypertension patients without treatment were referred to as G2, and 30 of them were taking Angiotensin receptor blocker(ARBs) Valsartan hypertension treatment were referred to as G3. For genetic analysis there was 44 apparently healthy control and 44 hypertension patients. The exclusion criteria were included many cases of hypertension and selected only patients with hypertension without treatment and patients with Angiotensin receptor blocker (ARBs)Valsartan Treatment, any subject with diabetes mellitus or other chronic diseases, autoimmune diseases and other endocrinopathy were excluded from present study.

Inclusion criteria: The following were the inclusion criteria:

- Both sexes with hypertension and do not take any medication.
- Both sexes with Hypertension and take Angiotensin receptor blockers (ARBs) **Valsartan medication**.

-Age range between (30-70) years.

Exclusion criteria: The following were the exclusion criteria:

- Any subject with Kidney disease, heart disease, Asthma, cancer pulmonary disease, diabetes mellitus or other chronic diseases.
- Any subject with Endocrine diseases such as hyper or hypothyroidism, hyperparathyroidism, Cushing syndrome, feochromocytoma.
- Any subject with Anemia, pregnancy and oral conceptive that contain estrogen
- Anyone who smokes.

Body mass index (BMI) was calculated using the formula [BMI = Weight (kg) / $[\text{height}]^2$ (m)].

2.1 Collection of Samples

Each patient had 5 milliliters of blood extracted from a vein. The blood was then divided into two test tubes: 2 milliliters were placed in a tube containing EDTA for gene polymorphism analysis, and 3 milliliters were placed in a gel tube for biochemical analysis. the blood samples contained in gel tubes are centrifugation was performed for 10 minutes at a force of 3000 times the acceleration due to gravity (xg) in order to acquire an adequate quantity of serum. The serum is thereafter distributed into three distinct Eppendorf tubes and preserved all samples at a temperature of -20C in the deep freezer until the analysis is carried out.

2.2 Analysis of Biochemical Parameters

Determination of homocysteine, MTHFR enzyme, Folic acid and vitamin B12 levels in patient

and control groups were done by use BT lab ELISA kits and according to manufacturer manual.

2.3 DNA Extraction

Request The PrepTM Genomic Mini Kit was used to extract genomic DNA from frozen human blood according to the manufacturer's instructions. Around 200 microliters of thawed cryopreserved blood from an EDTA tube was placed place into a 1.5 milliliter microcentrifuge tube. Subsequently, the sample underwent incubation with 30 µL of proteinase K in the incubator, which was set at temperature of 60 degrees Celsius for a duration of 15 minutes.

Upon the addition of 200 µL of FABG Buffer for cell lysis, the blood was observed to turn greenish-black, following which the sample was incubated at 70 °C and subjected to vortexing or shaking to ensure complete lysis of the cells. To create a new sample, the inversion of the sample was done every three minutes. The Elution Buffer was placed in an incubator set to 70 °C for DNA elution. To prevent the formation of precipitation during DNA binding, 200 µL of ethanol (96-100%) was added to the sample and vortexed for 10 seconds. The FABG column was then carefully transferred to the sample, and the resulting mixture was centrifuged at a speed of 14,000 revolutions per minute for a period of 60 seconds. The Flow-Through was discarded, and the FABG column was secured in position using2ml of new collection tubes. After adding 400 µL of W1 Buffer to the FABG tube, the tube was subjected to centrifugation at a speed of 14000rpm for a duration of 30 seconds. The flow-through was discarded, and the FABG column was then reinserted into the collection tube. To eliminate moisture from the column, 600 µL of wash Buffer was added to the FABG tube and centrifuged at a speed of 14000rpm for 30 seconds. Subsequently, the liquid that passed through was thrown away, and the FABG tube was put into the collection tube and was subjected to an additional 3minutes of centrifugation. Afterwards, the dehydrated FABG column was moved to a fresh 1.5 ml microcentrifuge tube, and 100 µL of either hot elution buffer or TE was promptly introduced to the FABG column membrane. The DNA was extracted after 10 minutes of incubation at 37 °C, by subjecting the mixture to centrifugation at 14000 rpm for 1 minute. The DNA was then kept at 4 °C until it was needed (favergene, 2023).

2.4 The Estimation of DNA concentration and purity

A spectrophotometer [nanodrop] was used to determine the quantity and integrity of DNA as follows:

1. For empty appliances, add 1µl of TE solution to the lens; be careful not to touch the

lens.

2. After that, 1µl of the DNA sample was added to the lens, and the absorption was read at 260 nm. An OD of one corresponds to almost 50 µL/ml of double-stranded DNA. The accepted ratio of DNA purity is [1.8 ± 0.2].

2.5 Genetic Analysis

Tetra-ARMs PCR (tetra amplification -refractory mutation system) was used to the genotype of the MTHFR gene. They designed four primers (Forward Outer, Forward Inner, Reverse Inner, and Reverse Outer). The set of Primer is explained in Table (1) [32].The preferred name for the SNPs being used in the study of Methylenetetrahydrofolate reductase genotyping is MTHFRC677T(rs1801133).

Table1. MTHFR (rs1801133) polymorphism primer sequences

Mutation	**Primer sequence**
MTHFR (rs1801133) 677 C>T	**Outer F: 5′-CCC AGC CAC TCA CTG TTT TAG TTC AGGC-3′**
	Outer R: 5′-GGT GAG AGT GGG GTG GAG GGA GCT TAT-3′
	R inner (C allele): 5′-CAA AGA AAA GCT GCG TGA TGA TGA AATAG G-3′
	F inner (T allele): 5′-TTG AAG GAG AAG GTG TCT GCG GGC GT-3′

F(forward), R(reverse)

The following temperature profile was used for the PCR: 95 °C for 5 minutes for the initial denaturation phase, 33 cycles of denaturation (95 °C for 25 seconds), annealing (60 °C for 30 seconds), and extension (72 °C for 25 seconds), and finally, final extension (72 °C for 10 minutes) for rs1801133. Present study was approved by the local ethics committee. All persons participating in this study provided consent.

Data Analysis

The statistical analysis was done with SPSS software 21. conducted the statistical analysis using SPSS software 21. Used frequencies and percentages to describe categorical variables. (means ± SD) was used to show continuous variables. Applied a student t-test to compare the means of two groups. The assessed the relationship between two continuous variables using the Pearson correlation coefficient the receiver operating characteristic (ROC) curve to determine the sensitivity and specificity of biochemical parameters, and calculated the optimal cutoff according

to the "Youden index" by selecting the point closest to the top-left corner of the ROC curve, giving equal weight to sensitivity and specificity when choosing a cut-off pointe. People frequently refer to this concept as the Youden index. [33]. A P value ≤ 0.05 was considered significant.

3. Results and Discussion

3.1 General characteristics

3.1.1 Gender distribution of the participants under study.

Among the 60 patients with hypertension who contributed to this study, there were 30 (50%) males and 30 (50%) females, and among the 60 controls who contributed to this study, there were 32 (53%) males and 28 (47%) females. Patients included in this study had an age range of 29–72 years, with a mean± SD of 51.27±10.21 years. The control group was with an age range of 33–77 years and a mean± SD of 50.3±10.95 years. The study found no significant difference in the average age between patients and the control group, possibly due to careful selection to avoid influencing other variables. The patients' BMI average was 24.5±3.87, while the control group's average was 23.4±4.3, indicating no significant difference (P ≤ 0.05).

3.2 Biochemical Parameters

The study variables for both apparently healthy control G1 and hypertension patients without treatment G2 are presented in Table (2), The table provides a comprehensive overview of the statistical outcomes for each variable, including means ± SD, and test statistics with p-values. Table (3) provides comprehensive statistics and the results of various statistical tests performed on two distinct groups: hypertension patients without treatment (G2) and patients on Angiotensin receptor blocker (ARBs) Valsartan Treatment (G3). The mean ±SD of Hcy in G1 was (8.1±1.93 mmol/ml), and the mean ±SD of Hcy in G2 was (9.8±2.91 mmol/ml). The results revealed a significantly different increase in the level of Hcy in G2 when compared with G1 (P≤0.05), as shown in Table 2. Furthermore, Table 3 demonstrates that there are insignificant disparities in Hcy levels between G2 and G3, with a significance level of P≤0.05. The mean ±SD of MTHFR in G1 was (5.8±2.62ng/ml), and the mean ±SD of MTHFR in G2 was (4.4±2.63ng/ml). The results revealed a significantly different decrease in the level of the methylenetetrahydrofolate reductase in G2 when compared with G1 (P≤0.05), as shown in Table 2. Also, there are non-significant differences in MTHFR levels between G2 and G3 (P≤0.05), as shown in Table 3. The mean ±SD of folic acid in G1 was (86.4±50.28 nmol/L), and the mean ±SD of folic acid in

G2 was (71.6±50.86 nmol/L). The findings demonstrated a non-significant difference in folic acid levels between G1 and G2 (P≤0.05), as shown in Table 2**.** Also, there are non-significant differences in folic acid levels between G2 and G3 (P≤0.05), as shown in Table 3. The mean ±SD of B12 in G1 was (93.9±48.92 ng/L), and the mean ±SD of B12 in G2 was (56.8±29.42 ng/L). The results revealed a significant difference decrease in the B12 level in G2 when compared with G1 (P≤0.05), as shown in Table 2. Also, there are non-significant differences in B12 levels between G2 and G3 (P≤0.05), as shown in Table 3.

Table 2. Comparison of biochemical parameters between "control G1" and " hypertension patients without treatment G2" participants.

Variables	**G1** ***n* = 60**	**G2** ***n* = 30**	***p***
Homocysteine (mmol/mL)			
Mean ±SD	8.1±1.93	9.8±2.91	0.008 **S**
MTHFR (ng/ml)			
Mean ±SD	5.8±2.62	4.4±2.63	0.04 **S**
Folic acid (nmol/L)			
Mean ±SD	86.4±50.28	71.6±50.86	0.26 **NS**
Vitamin B12 (ng/L)			
Mean ±SD	93.9±48.92	56.8±29.42	0.002 S

n, refers to number of participants; p, refers to p-value it was statistical significance when(p≤0.05); S, refer to statistical significant p-value; NS, refers to statistical non-significant p-value and SD, refer to standard deviations.

Table 3. Comparison of biochemical parameters between hypertension patients without treatment G2 and patient with Angiotensin receptor blocker(ARBs) valsartan hypertension treatment G3.

Variables	**G2** ***n* = 30**	**G3** ***n* = 30**	***p***
Homocysteine (mmol/mL)			
Mean ± SD	9.8 ±2.91	9. 7±3.63	**0.906 NS**
MTHFR (ng/ml)			
Mean ± SD	4.4 ±2.63	4.7±2.94	**0.724 NS**

Folic acid (nmol/L)			
Mean ±SD	71.6±50.86	102.4±79.66	**0.134 NS**
Vitamin B12 (ng/L)			
Mean ± SD	56.8±29.42	66.6±35.72	**0.323 NS**

n, refers to number of participants; p, refers to p-value it was statistical significance when($p \leq 0.05$); S, refer to statistical significant p-value; NS, refers to statistical non-significant p-value and SD, refer to standard deviations.

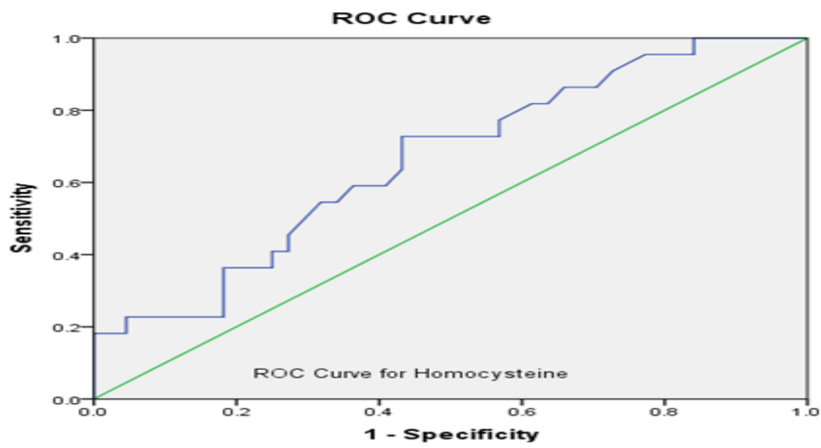
3.3 Receiver Operating Characteristic Curve (Roc) for Serum Homocysteine, MTHFR, Vitamin B12

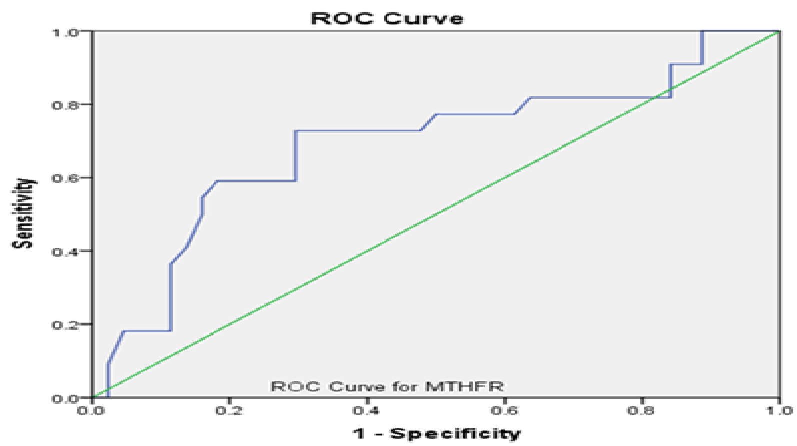
Receiver operating characteristic(ROC) is statistical analysis curve was used to evaluate the diagnostic value of Homocysteine, Methylenetetrahydrofolate reductase and Vitamin B12. The sensitivity and specificity of biochemical parameter were measured and the optimal cutoff was calculated according to "Youden Index" by Choose the point that is nearest to the upper-left corner of the ROC curve giving equal weight to sensitivity and specificity when picking a cut-off point is a typical practice. This idea is often referred to as the Youden Index [33]. The calculation of the area under the curve (AUC) serves as a valuable method for comparing various biochemical parameters. The biochemical parameters evaluated in this study included Homocysteine, MTHFR and Vitamin B12. Homocysteine, with a sensitivity of 72% and specificity of 57%, showed a diagnostic cutoff value of more than 8.25 mmol/ml, contributing to an AUC of 0.661. MTHFR had a sensitivity of 72%, a specificity of 70%, and a cutoff value of less than 4.5 ng/mL, leading to an AUC of 0.692. Vitamin B12 exhibited a sensitivity of 55%, a specificity of 93%, and a cutoff value of less than 47.7 ng/L, resulting in an AUC of 0. 776.Detailed findings can be found in **Table (4), Figure (2).**

Table 4. Receiver operating characteristic curve analysis for Serum Homocysteine, MTHFR and Vitamin B12.

Variable	AUC	Threshold	P-value	SN	SP	Accuracy	PPV	NPV
Hcy	0.661	> 8.25	0.03	72%	57%	29	47%	81%
MTHFR	0.692	< 4.5	0.01	72%	70%	42	57%	83%
B12	0.776	< 47.7	0.001	55 %	93 %	48	78 %	78 %

SN=sensitivity, SP=specificity, PPV= positive predictive value, NPV= negative predictive value, AUC= area under the curve value ≤ 0.05 was significant.





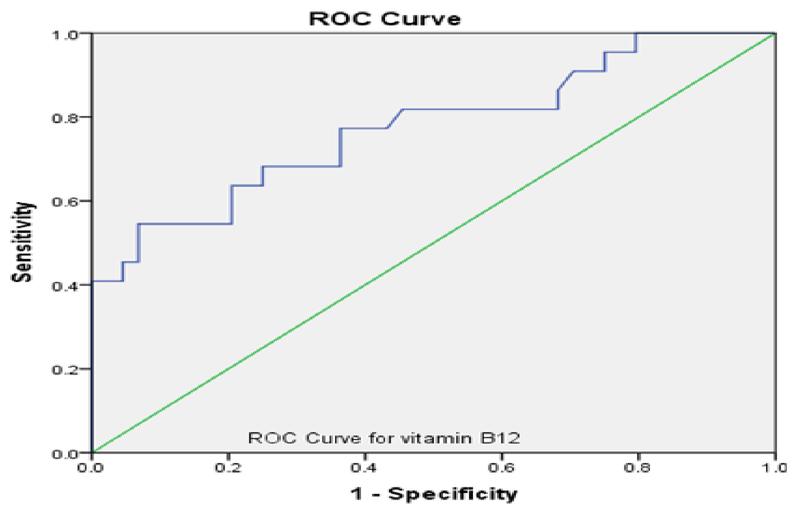


Figure 2. Receiver operating characteristic curve for Serum MTHFR, Homocysteine and Vitamin B12.

For vitamin B12 surfaced as the most effective biomarker according to the AUC; present results stated that it has fair diagnostic value in the predictive and diagnosis of hypertension; While homocysteine and methylenetetrahydrofolate reductase according to the AUC; present results stated that it had poor diagnostic value in the predictive and diagnosis of hypertension.

3.4 Pearson Correlations of MTHFR with other parameters.

In hypertension patients without treatment G2, there is a negative correlation between MTHFR and homocysteine (-0.573, p=0.001), as shown in table (5), Figure (3) A. This result may be clarified through that MTHFR enzyme is key enzyme in the metabolism of homocysteine (MTHFR enzyme contribute convert homocysteine to methionine), MTHFR enzyme deficiency lead to a defect in metabolism of homocysteine and causes hyperhomocysteinemia which increase the risk of hypertension. There is no correlation between MTHFR with age, BMI, Folic acid, vitamin B12, as shown in table (5), Figure (3) B, C, D, E respectively.

Table 5. Correlations between biochemical parameters.

Biomarkers		**MTHFR (ng/ml)**
Age (years)	***r***	0.189
	p	0.28
BMI	***r***	-0.092

(Kg/m^2)	***p***	0.22
Hcy (mmol/ml)	***R***	-0.573
	p	0.001
Folic Acid (nmol/L)	***r***	-0.100
	p	0.06
B12 (ng/L)	***r***	-0.094
	p	0.34

r, Pearson correlation coefficient. *P,* P-value(P≤0.05) was significant. MTHFR, Methylenetetrahydrofolate reductase. BMI, Body mass index. Hcy, Homocysteine. B12, vitamin B12.

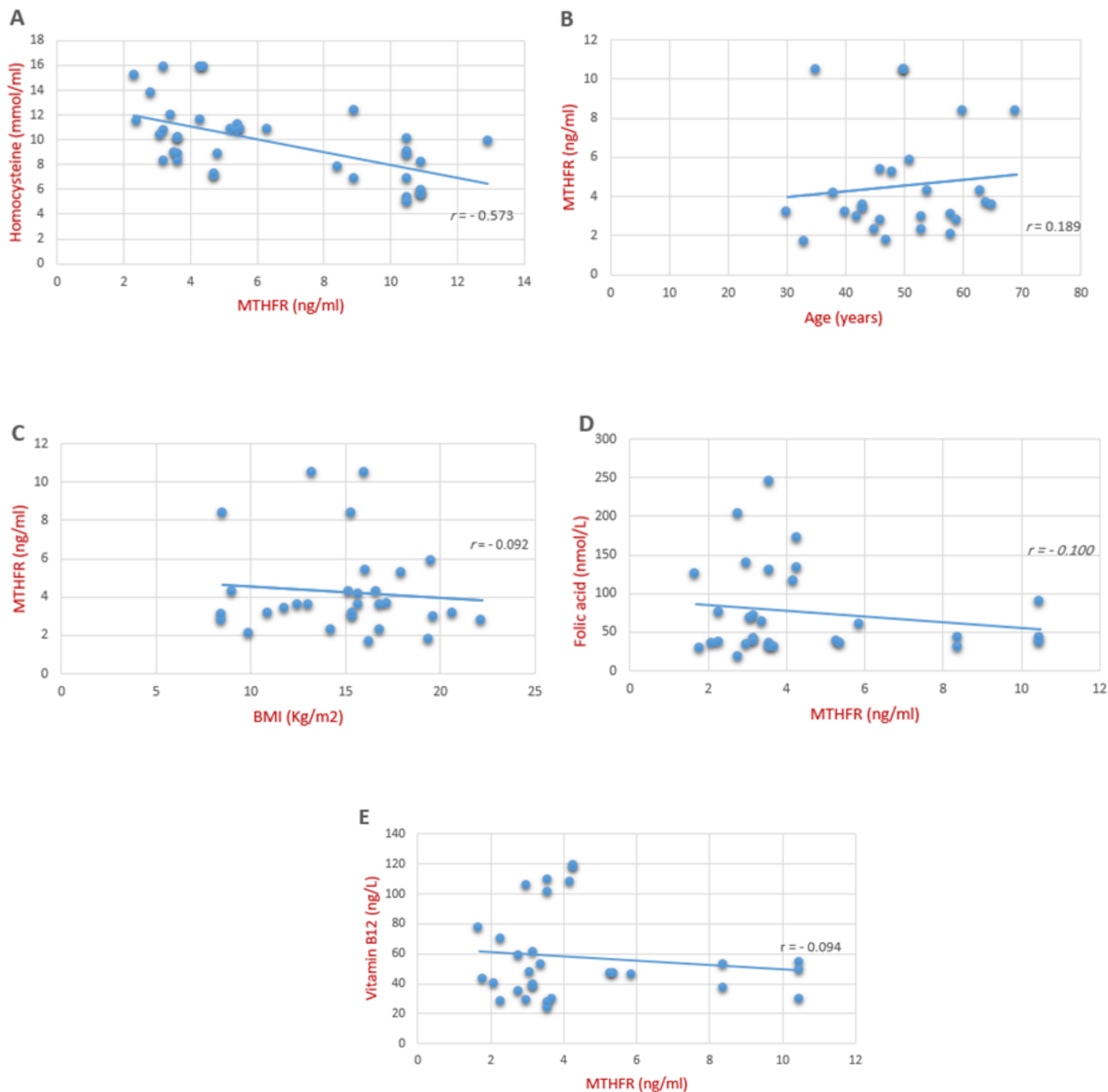


Figure 3. The Pearson correlation of MTHFR with other parameters

3.5 Results of Gel electrophoresis

MTHFRC677T genotype distribution in the case group is CC (n=14, 32%), CT (n=25, 56%), and TT (n=5, 12%), and in the control group CC (n=26, 59%), CT (n=15, 34%), and TT (n=3,7%). The distribution of the C allele is 53 (60%) in the case group and 67 in the control group (76%), while the T allele distribution is 35 (40%) in the case group and 21 (24%) in the control group (Table 6**).** significant relationships between MTHFRC6777T and hypertension as well as, MTHFRC677T is a risk factor for hypertension were revealed by statistical analysis (p=0.03, OR=1.18).

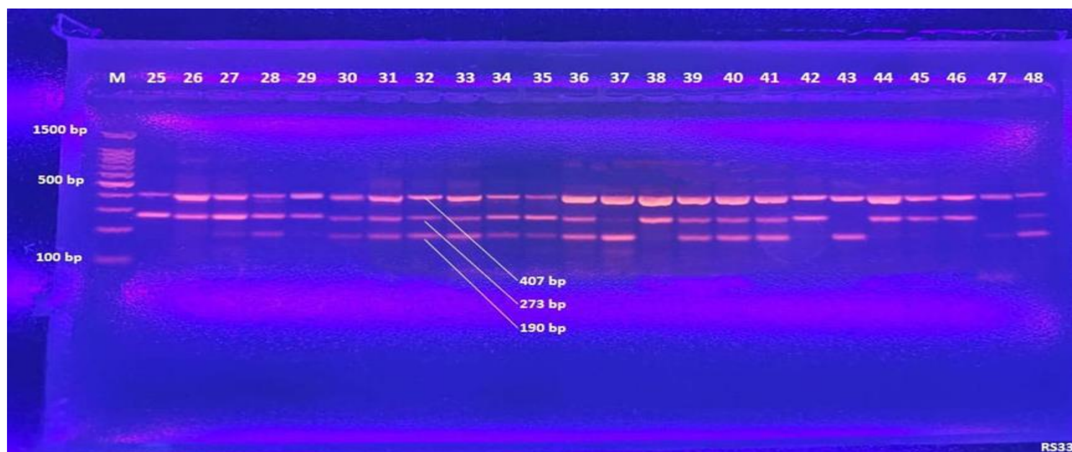


Figure 4. (1.5 %) Agarose gel electrophoresis at 80 volts for 80 minutes of, (25-48) represented samples, M (DNA molecular size marker (100 bp ladder).

Table 6. The distribution of genotype & allele frequency MTHFR rs1801133 SNP between study groups.

MTHFR		**Patients (No.= 44)**		**Control (No.= 44)**		**P-Value**	**OR (95% CI)**
		Fr.	**%**	**Fr.**	**%**		
Genotype	**CC**	**14**	**32**	**26**	**59**	**0.03***	**1.18 (0.46 – 2.56)**
	CT	**25**	**56**	**15**	**34**		
	TT	**5**	**12**	**3**	**7**		
Allele	**C**	**53**	**60**	**67**	**76**	**0.02***	**2.1 (1.11 – 3.97)**
	T	**35**	**40**	**21**	**24**		

Chi-Square test for genotypes frequency to determine significance. P ≤ 0.05 were significant. Fr: frequency; MTHFR: methylene tetrahydrofolate reductase; OR: Odd ratio; SNP: Single nucleotide polymorphism; Cl (95%): confidence interval.

Table (6) presents the distribution of genotype and allele frequencies for the MTHFR rs1801133 SNP between two study groups, each consisting of 44 participants. The table lists frequencies and percentages of genotypes (CC, CT, TT) and alleles (C, T) for both the patient and control groups. The results show that the genotype CC is more prevalent in the control group (59%) compared to the patient group (32%), with a statistically significant P-value of 0.03*. Similarly, allele C shows a higher frequency in the control group (76%) compared to the patient group (60%), also reaching statistical significance with a P-value of 0.02*. The odds ratios (OR) with 95% confidence intervals (CI) are also provided for comparative analysis.

Discussion

The two studied group were well matched regarding sex distribution (P≤0.05).

hyperhomocysteinemia has perhaps proven to be the most difficult risk factor to establish a strong association with hypertension. Inverse results have been found in numerous clinical and epidemiological investigations examining the connection between elevated homocysteine levels and hypertension. Thus, the question of whether hyperhomocysteinemia and hypertension are causally related remains unanswered [34]. The primary theory explaining how hyperhomocysteinemia causes hypertension is that homocysteine damages endothelial cells and vascular smooth muscle, impairing the structure and function of the arteries. The underlying mechanisms include increased production of pro-oxidants, vascular smooth muscle cell proliferation, increase collagen formation, and coagulation [35]. It has been proposed that hyperhomocysteinemia may increase the risk of developing hypertension via the following mechanisms: (1) raising arterial blood pressure; (2) causing endothelial dysfunction by increasing oxidant stress and decreasing nitric oxide release, which impairs vasodilation; (3) causing oxidative damage to vascular endothelial cells and obstructing the endothelium's ability to synthesis nitric oxide, a powerful vasodilator; or (4) increasing platelet adhesion to endothelial cells, which promotes the proliferation of vascular smooth muscle cells [36]. Homocysteine Increasing the expression of endothelin-1, a vasoconstrictor that causes severe and prolonged vasoconstriction [37]. In 2017, Yang and colleagues report in their work "Interactions of Homocysteine and Conventional Predisposing Factors on Hypertension in Chinese Adults,"

which is pertinent to the ongoing discussion on the role of homocysteine in hypertension. In their study, which was a case-control study demonstrated a significant correlation between hypertension and elevated homocysteine levels. Additionally, the interaction between hyperhomocysteinemia and hypertension was found to be significantly influenced by various factors, including age, vitamins, family history of hypertension. These findings support the authors' conclusion that there is substantial evidence linking homocysteine's interaction with these cofactors to hypertension. They also raise the possibility that the relationship between hyperhomocysteinemia and hypertension is not direct or linear but instead involves, more complex relationship include the input of other essential factors in select populations [38].Another study found that early HHcy identification may help reduce high levels of morbidity associated with HTN and its side effects, such as stroke like a results of [15].There was also another study that was discovered a positive correlation between homocysteine and hypertension like a results of [39],Our results agreed with the results of previously cited earlier research. According to the results of our research Present study suggest that hyperhomocysteinemia may be one of the underlying causes of hypertension. Methylenetetrahydrofolate reductase (MTHFR) is an essential enzyme involved in the metabolism of homocysteine and is intimately linked to the occurrence of hypertension [40],[41]. Severe MTHFR deficiency can result in hyperhomocysteinemia [42]. Higher homocysteine levels are associated with an increased risk of hypertension [15],[43]. Although the fact that folic acid is necessary for the metabolism of homocysteine, little information is known about the relationship between serum folic acid levels and the risk of hypertension. The level of Folic acid did not association with the prevalence of hypertension in the present study. In the present study, discovered that is significant association between vitamin B12 and hypertension. This may be results from vitamin B12 deficiency lead to hyperhomocysteinemia, because vitamin B12 is a cofactor for methionine synthase enzyme that convert homocysteine to methionine, and this increase in homocysteine, in turn, may be lead to hypertension. The reduction of homocysteine levels associated with vitamin B12 supplementation is thought to positively regulate blood pressure[44].In another study, the relationship between dietary consumption of vitamin B12 and the prevalence of hypertension was significantly and negative association like a results of [45].Our results disagree with those of previous studies that did not clearly identify a link between plasma vitamin B12 levels and hypertension like the results of [39]. Genetic and

environmental variables can have an impact on the complicated multifactorial state known as hypertension[46].The MTHFR gene has been associated to high blood pressure[25].The MTHFRC677T gene polymorphism decreases the activity of MTHFR enzyme by 30% in heterozygous subjects and by 70% in homozygous subject and this polymorphism ,which reduce the activity of the enzyme leads to hyperhomocysteinemia[47]. The MTHFRC677T gene's single-nucleotide polymorphism from alanine to valine lowers enzymatic activity, which in turn lowers the concentration of 5-methyltetrahydrofolate, which controls endothelial cells and nitrite oxides (NO) vasodilator, may contribute to the development of hypertension[48]. This study searched for this genetic variation MTHFRC677T to find out whether it exists among our races, and we found that it exists among the people of neighboring countries, such as the Saudi[49], Jordanian[50] and Iranian people [51]. Research was conducted in Saudi Arabia and the result showed that genetic polymorphisms MTHFRC677T gene are associated with the risk of hypertension like a results of [52]. Similar conclusions have been reached in several other studies such as a study on Turkish Male They discovered that the MTHFRC677T polymorphism in Turkish men may be a risk factor for the development of hypertension. Another study also found the MTHFRC677T genotype were associated with hypertension like a result of [53]. according to the results of this research, it was found that the MTHFRC677T gen polymorphism is significantly present in patients with hypertension, and this polymorphism reduce the activity of the MTHFR enzyme, and this lead to hyperhomocysteinemia, all of these results could be underlying cause of hypertension, according to the mechanisms previously explained for both genetic variation and markers. the results of this research are consistent with the results of other previous studies ,such as results of studies [40],[54],[15],[55],[53],[56].Furthermore, a contrasting study has indicated that there is no association between the presence of genetic polymorphism related to the MTHFRC677T gene polymorphism with the likelihood of developing hypertension in the specific Algerian community being examined [57].

4. Conclusions

Hyperhomocysteinemia , MTHFR enzyme deficiency, and a decrease in vitamin B12 may be one of the underlying causes of hypertension, There is no relationship between the level of folic acid in serum and hypertension , decrease level of MTHFR is associated with increased level of Hcy so that the two parameters are impact on the hypertension ,the strong parameter for associated with hypertension according to the AUC and accuracy is vitamin B12 ,ARBs drugs that use to

treatment hypertension does not affected on the level of Hcy , MTHFR enzyme , Folic acid and vitamin B12. The MTHFRC677T polymorphism is statistically significant in relation to hypertension and is a risk factor for hypertension.

5. References

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