Study of the Relation between Serum Total Homocysteine and Methylenetetrahydrofolate Reductase Gene Polymorphism In Renal Transplant Recipients

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ABSTRACT

The main cause of reduced long-term graft survival is chronic allograft injury. Cardiovascular risk factors such as hyperhomocysteinemia seem to play an important role. As atherosclerotic lesions in chronic allograft injury may be due to hyperhomocysteinemia, we examined the hypothesis that the C677T variant of the methylenetetrahydrofolate reductase (MTHFR) gene, which is linked to elevated plasma homocysteine levels in patients with renal failure, determines renal allograft dysfunction. Endothelial dysfunction probably has a role in this process. The aim of the present work was to study the influence of the C677T MTHFR gene polymorphism on plasma levels of homocysteine and folate in renal graft recipients, and their impact on chronic graft dysfunction, as well as studying the relation between chronic allograft injury and endothelial dysfunction by estimating von Willebrand factor (vWF) and measuring endothelial dependent dilatation of the brachial artery (EDD). The subjects included in this study were 32 renal allograft recipients (Group I) and 30 normal subjects as a control group (Group II). MTHFR genotype was determined by PCR, subsequently the patients were further classified into three subgroups according to the MTHFR genotypes: Group I (a): 6 allograft recipients with homozygous- TT type. Group I (b): 8 allograft recipients with heterozygous- CT type. Group I (c): 18 allograft recipients with wild- CC type. Estimation of total plasma homocysteine concentration, plasma folic acid, plasma and von Willebrand factor (vWF) were determined. Vascular responses of the brachial artery were performed by high resolution ultrasound imaging. This study showed significantly higher levels of both homocysteine and von Willebrand factor (vWF) were found in renal allograft recipients as compared to the control group. A negative correlation was found between homocysteine levels and creatinine clearance suggesting hyperhomocysteinemia contributes to the renal allograft dysfunction. No significant difference was found as regards the plasma folic acid levels between the patients and controls. Allograft recipients with MTHFR homozygous-TT type showed significantly higher levels of homocysteine and vWF as compared to allograft recipients with heterozygous-CT type and those with wild- CC type. Also allograft recipients with homozygous- TT type showed lower levels of plasma folic acid and creatinine

clearance as compared to the other two subgroups. Lower endothelial dependent dilatation of the brachial artery (EDD) was observed in renal allograft recipients as compared to the control group. The EDD was significantly less in allograft recipients with MTHFR homozygous- TT type than those with MTHFR heterozygous- CT type or wild- CC type. ***CONCLUSION****: The present study supports the hypothesis that the C677T variant of the MTHFR gene is an important determinant of renal-transplant survival, and that certain genotypes of MTHFR gene are associated with chronic allograft injury. Hyperhomocysteinemia, elevated vWF, lower folic acid levels and endothelial dysfunction together with certain genotypes of MTHFR gene increases the risk of development of chronic allograft injury in renal transplant patients.*

Key words: *renal transplant, MTHF gene polymorphism, homocysteine, folic acid, von Willebrand factor, endothelial dysfunction.*

INTRODUCTION

The leading cause of premature mortality in renal allograft recipients is cardiovascular mortality[(1)]. Also, the most important cause of chronic allograft rejection in kidney transplant has been progressive loss of function and sclerotic vascular lesions in the transplant biopsy[(2)]. Interestingly, fibromuscular thickening of small arterial vessels that resemble those found in chronic allograft injury was characteristic in patients suffering from homocysteinuria or other conditions with elevated plasma homocysteine (Hcy) [(3,4)].

In patients with chronic kidney disease, patients on maintenance hemodialysis, and in renal transplant recipients elevated homocysteine levels are commonly found[(5,6,7)]. Hyperhomocysteinemia is considered an independent risk factor for the development of atherosclerotic lesions in patients with impaired renal function. Thus, the elevated plasma Hcy could promote vascular sclerosis in the kidney allograft which could influence long-term renal graft survival[(8)]. Impairment of endothelial dysfunction in renal transplant recipients could be the link between hyperhomocysteinemia and vascular lesions which may cause the development of arteriosclerotic cardiovascular disease in these patients [(9)].

The role of the kidney in plasma Hcy handling is an area of ongoing research. Current data suggest that the healthy kidney plays a major role in Hcy clearance and metabolism, as it does with other amino acids. The underlying cause of hyperhomocysteinemia in renal disease is not yet fully understood, although reduced plasma Hcy clearance is the most proximate cause. Data extrapolated from the normal state and other indirect evidence suggest, but do not prove, that hyperhomocysteinemia is primarily attributable to decreases in Hcy plasma clearance and metabolism by decreased functioning renal mass. An alternative hypothesis involving unidentified uremic inhibitory substances that block normal extrarenal Hcy metabolism cannot be fully discounted at this time and may also contribute [(10)].

The involvement of the kidney in the homocysteine metabolism was

demonstrated by **Bostam et al.**[11] who found lower homocysteine content in blood of the renal vein of rats compared with blood obtained from the renal artery, whereas urinary excretion was negligible. Beside the homocysteine-converting enzymes cystathionine B-synthase, betaine-homocysteine methyltransferase and methionine synthetase, activity of 5,10-methylenetetrahydrofolate reductase (MTHFR) has also been detected in human kidneys. MTHFR provides 5 methyltetrahydrofolate, the active form of folate, which is necessary as a methyl donor for remethylation of homocysteine to methionine.

Recently, a polymorphism C677T in the gene coding for the enzyme MTHFR was identified. This variant, consisting of a cytosine (C) to thymine (T) at nucleotide position 677 leading to exchange of highly conserved alanine to valine in the mature protein, has been associated with reduced activity and increased thermolability of the enzyme[12]. This polymorphism may result in low active folate on the basis of decreased enzyme activity and can cause an increase of total homocysteine plasma levels. Furthermore, homozygosity for the mutant allele (TT) can confer an increased risk for vascular disease[13,14]. The aim of the present work was to study the influence of the C677T MTHFR gene polymorphism on total homocysteine and folate plasma levels in renal graft recipients, and its impact on chronic graft dysfunction and the associated endothelial dysfunction found in those patients.

SUBJECTS & METHODS

The subjects included in this study were thirty two renal allograft recipients (Group I) and thirty normal subjects as a control group (Group II) at transplantation clinic of the Main Alexandria University Hospital. Renal allograft recipients were further classified into three subgroups according to the MTHFR genotypes:

Group I (a): including 6 allograft recipients patients with homozygous-TT type.

Group I (b): including 8 allograft recipients patients with heterozygous-CT type.

Group I (c): including 18 allograft recipients patients with wild- CC type.

The study protocol was approved by the Research Review Committee and Ethics committee of the Faculty of Medicine, Alexandria University and conformed to the 1975 **Declaration of Helsinki and Egyptian law on gene technology**. Informed consent was obtained from each subject.

All subjects were subjected to the following:

- Full history taking including age, sex, cause of renal failure, duration of transplantation.
- Complete clinical examination.
- Routine laboratory investigations include the following: blood urea, serum creatinine, creatinine clearance, serum cholesterol, serum triglycerides, HDL, LDL cholesterol.
- Estimation of total plasma homocysteine concentration was determined using Axis R Homocysteine Enzyme Immunoassay (EIA) [15].

Assay Principle:

Axis R Homocysteine EIA is designed for quantitative determination of total homocysteine in plasma or serum and is based on an enzyme -linked immunosorbent assay (ELISA). Homocysteine, mixed - disulphide and protein bound forms in the sample was reduced by the use of dithiothreitol to free homocysteine which is then enzymatically converted to s-adenosyl l- homocysteine in a separate procedure prior to the immunoassay. The concentration of homocysteine was calculated as (μmol/L)

- Quantitative determination of plasma folic acid was done by a radioimmunoassay (RIA). Plasma folic acid concentration was estimated using the stimuli TRAC-SNB by radioassay Kit which was supplied by ICN pharmaceuticals Inc. Costa Mesa USA. Plasma folic acid concentration was determined as ng/ml [(16)].
- Quantitative determination of plasma von Willebrand factor (vWF) was done by an enzyme linked immunosorbent assay (ELISA). The values of vWF were expressed as (%) of normal[(17)].
- Measurement of endothelial – dependent and independent vascular responses of the brachial artery will be done by high resolution ultrasound imaging with the use of 7.5 MHz phased-array transducer attached to Hewlett Packard 1500 system[(18)].

During the test, vessel images were taken at rest, then during reactive hyperemia (flow mediated dilation, FMD) which is endothelial dependent dilatation (EDD), and finally after sublingual administration of isosorbide dinitrate (nitroglycerin mediated dilation, NMD) which is endothelial independent dilation (EID).

Subjects were studied in the supine position resting for 10 minutes before the test. A single investigator performed all imaging and analysis. A B-mode scan was obtained of the brachial artery in longitudinal section 5-12 cm proximal to antecubital crease, ensuring optimal visualization of anterior and posterior wall-lumen interfaces and a constant artery diameter. The diameter was calculated as the average of measurements made during 4 cardiac cycles at end diastole. All measurements were recorded on super–VHS video–tape for subsequent off–line analysis.

FMD tests were performed by selecting, at rest, three images of the brachial artery at end diastole (Bo, B1, B2 respectively). Four images were recorded during reactive hyperaemia, produced by inflation of pneumatic tourniquet to a pressure of 200 mmHg for 4.5 min. Measurements we made 30, 90, 150, 210 seconds after cuff deflation (T30, T90, T150, T210 respectively).

FMD was calculated by maximum diameter between T30, T90, T150, T210 –mean (Bo, B1, B2) *100/ mean (Bo, B1, B2).

NMD test was performed after at least a 10 min rest. The brachial artery was identified under basal conditions in the same arm position as the FMD test (three images, B3, B4, B5). Sublingual isosorbide dinitrate was then administered and three vessel images were taken 4-6 min later (N1, N2, N3). NMD was calculated by

maximum diameter between N1, N2, N3, -mean (B3, B4, B5) *100/ mean (B3, B4, B5).

- MTHFR genotype was determined. Genomic DNA was prepared from whole blood by an established method for extraction of DNA [19].
- - MTHFR polymorphisms were determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques [20]. A pair of primers were used:
- Forward primer: 5`-TGAAGGAGAAGGTGTCTGCGGGA - 3` and
- Reverse primer: 5`-AGGACGGTGCGGTGAGAGTG – 3`
- PCR amplification products were obtained using 25 µl reactions [0.5 µg genomic DNA, 200 p mol of each primer, 0.5 mM each of deoxy- ATP, GTP, CTP AND TPP nucleotides, 3 mM Mg Cl_2, 1 unit of Taq DNA polymerase and 2.5 µl 10 x PCR buffer (50 mmol/l KCl, 0.001% gelatin and 10 mmol/ l Tris-HCl, pH 8.3)]. The amplification was carried out using thermal cycler according to the following protocol: 5 min denaturation at 95ºC (one cycle), followed by 35 cycles of denaturation at 94ºC for 50 sec, primer annealing at 55ºC for 50 sec, and extension at 72ºC for 30 sec. The reaction was terminated at 72ºC for 7 min (one cycle). PCR products were detected on a 2% agarose-gel containing ethidium bromide. The PCR product is a 198 bp fragment. The MTHFR polymorphism, a C to T substitution at bp 677, creates a *Hin*f1 recognition sequence. If the mutation is present, *Hin*f1 digests the 198 bp fragment into a 175 bp and a 23 bp fragment.

Statistical Analysis:

Statistical analysis was carried out using SPSS version 12 for windows. Qualitative variables were expressed as number and percentage while quantitative variables were expressed as mean (X) ± standard deviation (SD). The following statistical tests were used as appropriate: Chi square test, Student's t-test, ANOVA, Mann-Whitney test, or Kruskal-Wallis test. Correlations between variables were done using Spearman's rank correlation coefficient (r). A 5% level is chosen as a level of significance in all statistical significance tests used.

RESULTS

The causes of renal failure among the transplant patients varied between chronic glomerulonephritis in 12 patients (37.5%), chronic tubulointerstitial nephritis in 6 patients (18.75%), obstructive uropathy in 5 patients (15.6%), benign nephrosclerosis in 4 patients (12.5%), or unknown etiology in 5 patients (15.6%).

The renal allograft recipients were sable, with no acute rejection attacks in the last 6 month. They were on triple conventional immunosuppression protocol of cyclosporine, azathioprine, and corticosteroids.

The main socio-demographic and clinical characteristics, laboratory parameters, and brachial artery vascular response parameters among the two studied groups were shown in table I, II and III respectively.

Table I: The main socio-demographic and clinical characteristics among the studied groups.

	Group (I)	Group (II)	P value
Age (years)	29.87 ± 8.09	32.4±2.84	0.11
Gender (M/F)	1/1	3/2	0.42
BMI (Kg/m^2)	23.9±3.1	22.4±1.7	0.81
Disease duration (years)	4.93±2.1	00±00	
Systolic blood pressure,	134.62 ±7.31	120.1 ±7.18	0.000
Diastolic blood pressure	83.93 ± 8.32	74.10 ±3.67	0.000
Mean blood pressure	100.83 ±7.95	89.43 ±4.97	0.000

Table II: Different laboratory results of the 2 studied groups.

Lab parameters	Group I	Group II	P value
Blood urea (mg/dl)	49.43± 19.09	38.5 ± 3.19	0.003
S. creatinine (mg/dl)	1.66± 0.64	0.99± 0.15	0.000
Creatinine clearance (ml/min)	51.61 ± 16.77	113.30 ± 16.43	0.000
S. cholesterol (mg/dl)	221.11± 31.86	127.10 ±16.87	0.000
LDL (mg/dl)	145.00± 32.15	68.10 ± 10.10	0.000
HDL (mg/dl)	43.88 ± 4.48	60.50 ± 7.16	0.000
Triglycerides (mg/dl)	158.33± 46.12	109.00 ± 15.72	0.001
Homocysteine (µmol/L)	44.42 ± 32.08	11.62 ± 2.57	0.000
Folic acid (ng/ml)	7.41 ± 1.05	7.39 ± 1.17	0.492
vWF (%)	119.71 ±17.71	67.60 ± 28.65	0.000

Table III: Different brachial artery vascular response parameters of the 2 studied groups.

Brachial avascular response parameters	Group I	Group II	P value
EDD (%)	7.84 ±1.08	12.68 ± 0.96	0.000
NMD (%)	10.88 ± 0.50	10.86 ± 0.47	0.828

Univariate Analysis:

We found, among the transplant group, statistically significant correlations between plasma homocysteine and the following parameters: duration of transplantation (r = 0.789, p= 0.00), blood urea (r = 0.530, p= 0.002), serum creatinine (r = 0.398, p= 0.024), creatinine clearance (r = -0.550, p= 0.001), plasma folic acid (r = -0.870, p= 0.00), vWF (r = 0.996, p= 0.00), EDD (r = -0.980, p= 0.00). Homocysteine was not related to systolic, diastolic blood pressure, mean blood pressure, serum triglycerides, serum cholesterol and NMD.

On classifying the transplant group according to their MTHFR genotypes:

Group I (a): including 6 allograft recipients with homozygous- TT type.

Group I (b): including 8 allograft recipients with heterozygous- CT type.

Group I (c): including 18 allograft recipients with wild- CC type.

The different clinical, laboratory and vascular parameters were shown in table IV.

Table IV: Different parameters of the studied transplant subgroups.

Parameters	Group Ia	Group Ib	Group Ic	P value
SBP(mmHg)	134.33± 8.31	137.00± 9.07	133.66± 6.28	0.574
DBP (mmHg)	84.33±8.31	85.75±11.04	83.00±7.29	0.745
MBP	101.00±8.31	102.83±10.36	99.88±6.93	0.697
Blood urea (mg/dl)	69.00±19.49	38.50±14.51	47.77±16.50	0.007a,b
S. creatinine (mg/dl)	2.03± 0.36	1.37± 0.20	1.67± 0.78	0.171
Creatinine clearance (ml/min)	39.60±2.02	57.82±14.84	54.77±15.81	0.05 a,b
S. cholesterol (mg/dl)	178.50±20.20	246.33±25.55	223.50±15.35	0.00 a,b
LDL (mg/dl)	100.00±30.02	169.00±21.48	149.50±10.96	0.00 a,b
HDL (mg/dl)	42.00±3.46	47.33±4.22	42.25±3.95	0.06
Triglycerides (mg/dl)	180.00±30.02	147.66±30.72	155.50±61.08	0.56
Homocysteine (µmol/L)	94.93± 16.29	38.50± 24.68	30.22± 20.43	0.000a,b
Folic acid (ng/ml)	6.16± 0.49	7.45± 0.94	7.82± 0.93	0.002a,b
VWF (%)	149.00± 8.62	115.32±12.15	111.91±10.62	0.000a,b
EDD (%)	6.20± 0.17	8.05± 0.90	8.30± 0.77	0.000a,b
NDD (%)	10.76± 0.28	11.00± 0.45	11.04± 0.45	0.40

a: There is significant difference between Ia, Ib.
b: There is significant difference between Ia, Ic.
c: There is significant difference between Ib, Ic.

DISCUSSION

Homocysteine (Hcy), as a cardiovascular risk factor, was studied over 30 years ago, through the observation of extensive atherosclerotic lesions during autopsies of patients affected by certain genetic variants of homocystinuria. There on, Hcy has been investigated as a factor in the genesis of atherosclerosis. Today, hyperhomocysteinemia is a well-established cardiovascular risk factor in the general population, and some studies suggest that this association is also present among renal transplant recipients [21,22]. In the present study, there was intermediate hyperhomocysteinemia among the renal allograft recipients in comparison to healthy control subjects.

Recent studies have suggested mechanisms through which hyperhomocysteinemia may be an additional factor for the development of atherosclerosis and cardiovascular disease in patients with other risk factors, such as dyslipidemia [23]. **Ducloux et al.** [24] showed a positive correlation between serum Hcy and LDL-cholesterol in clinically stable renal transplant recipients. In this context, endothelial damage occurs due to the predominance of oxidized forms of Hcy in plasma, thus generating reactive oxygen species and tissue toxicity [25]. In the current study, there were significant higher levels of cholesterol, LDL-cholesterol and triglycerides and lower HDL-cholesterol among the hyperhomocysteinemic transplant

patients but we did not show any correlations between the homocysteine and serum cholesterol and LDL-cholesterol levels in those patients.

On the other hand, there was a significant positive correlation between plasma homocysteine and vWF activity and significant negative correlation between plasma homocysteine and the endothelial dependent dilatation of the brachial artery in our transplant patients. These results may point to the endothelial dysfunction found in the transplant patients, and this endothelial dysfunction which is one of the early vascular changes in atherosclerosis may be the result of the high homocysteine encountered in those patients.

The association between hyperhomocysteinemia and renal function was evidence by the significant negative correlation between plasma homocysteine and creatinine clearance in our renal allograft recipients. This association demonstrates the possible role of the hyperhomocysteinemia as a factor for chronic graft dysfunction

Factors associated with hyperhomocysteinemia are age, smoking, systemic arterial hypertension, folate and vitamin B12 levels, elevated cholesterol, sedentary lifestyle and, especially, renal function [(26)]. The present study has shown that there was a strong significant negative correlation between folic acid and Hcy levels and hence, the possible role of superdoses of folate in treatment of this hyperhomocysteinemia [(27)].

A polymorphism C677T in the gene coding for the enzyme MTHFR was identified among transplant patients. Homozygous variant was found in 6 out of 32 patients, the heterozygous variant in 8 out of 32, and the rest of patients were 18 in number of the wild type. As MTHFR plays a key role in Hcy metabolism, the effect of different MTHFR genotypes on Hcy levels were studied in this work among our renal allograft recipients. The homozygous subgroup of patients exhibit significantly higher Hcy levels and lower folic acid in comparison to the other 2 subgroups. Several studies have identified the effects of different MTHFR genotypes on Hcy metabolism in renal transplant recipients [(28- 30)].

In addition, the endothelial derangement was more pronounced in the homozygous group in comparison with the other groups of renal allograft recipients in the present study. This endothelial dysfunction could be the link through which the hyperhomocysteinemia may exert its deleterious effects on the vascular tree. It has been shown that high levels of homocysteine induce sustained injury of arterial endothelial cells and accelerate the development of thrombosis and atherosclerosis. The mechanism by which homocysteine might cause vascular damage is unclear. Experimental evidence suggests that homocysteine promotes atherogenesis by facilitating oxidative arterial injury, damaging the vascular matrix, and augmenting the proliferation of vascular smooth muscle cells[(31)]. Homocysteine has the potential to damage endothelium and accelerate atherosclerosis. Genetic

factors such as the MTHFR C677T polymorphism, and other polymorphisms in folate-related genes associated with high homocysteine levels, may contribute to increasing this vascular risk [32] .

In the current study, the renal impairment was more manifest among the homozygous group in comparison to both the heterozygous and the wild types. These findings agree with the data of **Alvarenga et al.** [33] who showed elevated levels of homocysteine in 80.6% of patients with chronic allograft rejection , and found an effective risk factor when the polymorphisms of the ACE and MTHFR genes and hyperhomocysteinemia were associated (odds ratio 2.51; 95% confidence interval 1.19–5.28).

However, **Artifoni et al.** [34] did not support the hypothesis that the C677T variant of the MTHFR gene is an important determinant of renal-transplant survival. Furthermore, **Liangos et al.** [35] life-table analysis revealed a similar allograft survival over 36 months between the genotype groups (CC 74%, CT 69%, TT 75%). The difference between our results and **Liangos** results may be related to the longer duration of follow up in our group of allograft recipients. Others [36] showed no statistically significant differences between the allelic and genotypic distribution of the MTHFR polymorphism in renal transplant recipients. This might be explained by the small sample sizes of renal allograft recipients.

In Conclusion: The present study supports the hypothesis that the C677T variant of the MTHFR gene is an important determinant of renal-transplant survival, and that certain genotypes of MTHFR gene are associated with chronic allograft injury. Hyperhomocysteinemia, elevated vWB factor, lower folic acid levels and endothelial dysfunction together with certain genotypes of MTHFR gene increase the risk of development of chronic allograft injury in renal transplant patients.

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دراسة العلاقة بين الهوموستاتين الكلى و النمط الجينى المسئول عن مختزل رابع هيدروفولات المثيلين فى مرضى زرع الكلى

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ان السبب الرئيسى لخفض بقاء الكلى الزروعة على المدى الطويل هو اصابة الكلية المزروعة المزمن. يبدو أن مخاطر القلب و الشرايين، مثل زيادة مستوى الهوموسستايين، تلعب دورا مهما فى هذه الأصابة. حيث أن اصابة الكلية الزروعة المزمن ممكن أن ينتج من زيادة مستوى الهوموسستايين فى المصل، أدى هذا الى فرضية ان هذه الزيادة ممكن أن تنتج من تعدد أشكال الجين (سى ٦٧٧ تى) المسئول عن انزيم مختزل رابع هيدروفولات المثيلين. و قد يؤدى هذا الى اصابة الكلى المزمن عن طريق الخلل الوظيفى البطانى. يهدف هذا البحث الى دراسة تأثير تعدد أشكال الجين سى ٦٧٧ تى المسئول عن مختزل رابع هيدروفولات المثيلين على مستوى الهوموسستايين بالمصل و كذلك مستوى الفولات فى المصل فى مستقبلى الكلى المزروعة و كذلك دراسة العلاقة بين اصابة الكلى المزروعة المزمن و اصابة الغشاء البطانى عن طريق قياس نشاط عامل الفون ويليبراند وقياس توسع الشريان العضدى. اشتملت الدراسة على اثنين و ثلاثين مستقبل للكلية المزروعة كمجموعة أولى و تم مقارنتهم بثلاثين شخص مماثلين فى السن و الجنس للمجموعة الأولى كمجموعة ضابطة (مجموعة ثانية) . تم تعيين النمط الجينى المسئول عن مختزل رابع هيدروفولات المثيلين فى كل المرضى و تم تقسيم مجموعة المرضى الى ٣ مجموعات: مجموعة أ (نوع متماثل الزيجوت) و يشمل ٦ مرضى، مجموعة ب (نوع متغاير الزيجوت) و يشمل ٨ مرضى و مجموعة ج (نمط برى) و يشمل ١٨ مرضى. تم قياس مستوى الهوموسستايين بالمصل، حمض الفوليك فى المصل، نشاط الفون ويليبراند بالمصل و استجابة الشريان العضدى المعتمد وغير المعتمد على البطانى. أثبتت النتائج أن مستوى الهوموسستايين فى مستقبلى الكلية المزروعة أعلى من مستواه فى المجموعة الضابطة و هذا الفرق كان له دلالة احصائية. كما أظهرت النتائج أن هناك خلل وظيفى بطانى فى مجموعة مستقبلى الكلية المزروعة دل على ذلك وجود مستوى أعلى لنشاط عامل الفون ويليبراند و نقص توسع الشريان العضدى المعتمد على البطانى بالمقارنة بالمجموعة الضابطة و كانت هذه الفروق ذات دلالة احصائية. كذلك كلن هناك عاتقة عكسية ذات دلالة احصائية بين مستوى الهوموسستايين فى المصل و كفاءة الكرياتينين و هذا يوحى بالتأثير الضار لارتفاع الهوموسستايين على وظيفة الكلية المزروعة. كما أظهرت النتائج أن مستوى الهوموسستايين أعلى ومستوى حمض الفوليك أقل و كفاءة الكرياتنين أقل و توسع الشريان العضدى المعتمد على البطانى أقل فى مجموعة المرضى ذو النمط الجينى المتماثل الزيجوت بالمقارنة بالمجموعتين الأخريين ذات النمط الجينى المتغاير الزيجوت و النمط البرى. استنتج من هذه الدراسة أن أرتفاع مستوى الهوموسستايين بالأضافة الى الأنماط الجينية للجين المسئول عن انزيم مختزل رابع هيدروفولات المثيلين قد يشارك فى زيادة احتمال الخطر من تطور الخلل الوظيفى المزمن للكلية المزروعة.