Association of MTHFR gene C677T Mutation with Diabetic Nephropathy

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Abstract: Introduction: Genetic predisposition has been implicated in diabetic nephropathy (DN). Methylenetetrahydrofolatereductase (MTHFR) is a regulatory enzyme of homocysteine (Hcy) metabolism. The C677T variant of MTHFR gene may be associated with DN. In this study, we examined the distribution of the MTHFR genotypes and the association between the C677T variant and DN. **Methods:** 40 DN patients and 20 controls were recruited in the study. FPG, HbA_{1c}%, lipid profile, eGFR, serum creatinine and urinary microalbumin were measured. SerumHcy level was measured using ELISA method. MTHFR genetic C677T polymorphism was determined using PCR-restriction fragment length polymorphisms (RFLP). **Results:** The distribution of MTHFR C677T polymorphism observed in the current study showed differences from the frequencies predicted by the Hardy–Weinberg equilibrium in DN group. Observed CC homozygous in DN group was 62.5% while expected was 63%. Observed CT heterozygous in DN group was 37.5% while expected was 33%. Observed TT homozygous was 0% while expected was 4%.CT genotype and T allele were significantly associated with cases when compared to control group. **Conclusions:** Our findings suggest that the C677T mutation in the MTHFR gene was associated with DN. The T allele of this mutation presumably acting by elevating Hcy levels and seems to be associated with a faster progression of nephropathy to end-stage renal disease (ESRD).

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1. Introduction:

Diabetes mellitus (DM) is a chronic disease characterized by insulin deficiency or its peripheral resistance resulting in hyperglycemia and non-enzymatic glycation of protein **(Neupane etal., 2016).** The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels **(ADA, 2018)**.

Diabetic nephropathy (DN) is the leading cause of chronic renal disease and a major cause of cardiovascular mortality. Several factors are involved in the pathophysiology of DN, and genetic susceptibility to type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) is of great importance **(Duran-Salgado and Rubio-Guerra, 2014).**

The pathogenesis of DN has an inherent genetic manner as evidenced by familial aggregation and ethnic-specific prevalence rates of microalbuminurea (MA),indicating several environmental and genetic factors plays crucial role in the development of DN **(Rizvi etal., 2014).**

As far as the genetic factors are concerned, it was clearly shown that candidate gene polymorphisms has the major impact associated with the disease progression DN**.** However, studies were conducted in several populations yielded contradictory outcomes in association with progression of DN and genetic polymorphisms **(Zhang etal., 2014).**

Although several factors are involved in the pathophysiology of DN, the MTHFR gene plays an important role in DN susceptibility through regulating the intracellular folate homeostasis and metabolism. MTHFR is an enzyme that catalyzes the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for Hcyremethylation to methionine **(Chen etal., 2015).**

Homozygosity for the C to T substitution at nucleotide 677 of the MTHFR gene leads to a 50% reduction in enzyme activity and is the most common inherited cause of moderate hyperhomocysteinemia (HHcy). This polymorphism is located in the catalytic domain of the enzyme and results in the production of a thermolabile protein. Many studies have investigated MTHFR gene polymorphism effects on susceptibility to T2DM, but the results are inconclusive **(Chen et al., 2015).**

Globally, studies investigated to find the relationship between reduced MTHFR activity and

genetic polymorphisms of MTHFR gene. Furthermore, the single nucleotide polymorphism 677C→T leads to an Ala222Val substitution, which significantly associated with diminished enzyme activity.The individual specific genotype with different levels of enzyme activity was also observed in different populations (**Ramanathanetal.,2017).**This thesis aims to investigate the possible association of MTHFR gene C677T mutation and different genotype frequencies in DN patients.

2. Subject and Method:

The present study was carried out on forty (40) DN patients selected from inpatient ward of internal medicine and endocrinology departments at Al-Zahra University hospital, Al-Azhar University together with twenty (20) apparently healthy control individuals. Hypertensive patients were excluded.

Verbal informed consents were obtained from all participants before enrollment in the study. The study protocol was approved by the Researcher Ethics Committee at faculty of medicine, Al-Azhar University.

All patients and controls are subjected to complete history including Name, age, history of diabetes, duration of diabetes, type of treatment (insulin, oral orinsulin/oral, and other endocrinal diseases, oral contraceptives for females and history of smoking)**.**Full clinical examination for blood pressure using standard sphygmomanometer, presence of micro vascular complications, also complete chest and abdominal examination were done to exclude other illnesses. Laboratory Investigations include serum fasting glucose, serum creatinine, Lipid profile (TC, TGs, LDL-C, and HDL-C), HbA1c%, eGFR, microalbumin, serum Hcy level and MTHFR gene C677T mutation.

Sampling:

Sampling was done under complete aseptic conditions from patients and controls on two occasions. First sample, 5 ml venous blood was collected after 10 hour fasting, and were divided as follows i) 3 ml were collected in tubes with gel, centrifuged, serum was separated and divided into two parts, one used for determination of sugar, creatinine, lipid profile (TC, TG, HDL-c, LDL-c) and the other one stored at -20c until assay of Hcy.ii) 2ml was collected on EDTA tubes for estimation of HbA1c%. Another sample(4 ml) was collected on EDTA tubes for DNA extraction. Isolated DNA was stored as a suspension in ethanol at -20˚C until performance of molecular technique. 24 hours urine samples were collected in sterile urine cups,stored at 4˚C for measurement of urinary microalbumin.

All biochemical tests were done on COBAS 311 Autoanalyzer , HbA1c% using cation exchange resin, urinary microalbuminusing ARCHITECT c8000 automated chemistry analyzer.Hcy was done using ELISA method.MTHFR polymorphism by PCR-REFLP strategy.

Methylenetetrahydrofolatereductase (MTHFR) Mutation analysis:

The DNA was extracted from peripheral blood through digestion with proteinase in alysis solution. The lysate was then mixed with ethanol and loaded onto the purification column, where the DNA binds to the silica membrane. Impurities were removed by washing the column with wash buffers. Genomic DNA was then eluted under low ionic strength conditions with an elution buffer. The following steps were performed:20 µL of proteinase solution was added to 200 µL of whole blood. After mixing, 400 µL of lysis solution were added.The sample was incubated at 56°C for 10 min while vortexing occasionally, until the cells were completely lysed. Then, 200 µL of ethanol (98%) were added and mixed.The mixture was transferred to the spin column and centrifuged for one min at 6000 × g. The collection tube containing the flow-through solution was discarded and the column was placed into a new 2 ml collection tube. Then, 500 µL of wash buffer (with ethanol) was added and centrifuged for one min at 8000 ×g. The flow-through solution was discarded and the column was placed back into the collection tube. Then, 500 µL of wash buffer (with ethanol) was added to the column and centrifuged for 3 min.The collection tube was emptied and the purification column was placed back into the tube. The column was re-spent for 1 min at the maximum speed (≥20000 ×g). The collection tube containing the flow-through solution was discarded and the column was transferred to a sterile 1.5 ml microcentrifuge tube. Then, 200 µL of the elution buffer were added to the center of the column membrane to elute genomic DNA, incubated for 2 min at room temperature and centrifuged for 1 min at 8000×g.The purification column was discarded and the purified DNA was stored at -20°C.

Polymerase chain reaction:

Initial denaturation:

25 µL of the PCR master mix were added to 1.5 µL of the forward primer (5'-TGAAGGAGAAGGTGTCTGCGGGA-3'), 1.5µL of the reverse primer (5'-AGGACGGTGCGGTGAGAGTG-3"), 5 µL of the extracted DNA and 17 µL of a nuclease free

water. The mixture was incubated at 95^{0}C for 11 min.

PCR cycles:

30 cycles were performed consisting of: Denaturation: incubation at 95^{0}C for 30 seconds. **Annealing:** incubation at 55^{0}C for 1 min. **Extension:** incubation at 72^{0}C for 30 seconds. **Final extension:** incubation at 72^{0}C for 10 min. **Digestion:** Digestion was performed by the enzyme HinfI at 37^{0}C for four hours. **Detection:** the digested products were run on 3.5% agarose gels and stained with ethidium bromide and visualized under ultra-violet light **(Elmrghni et al., 2011)**.

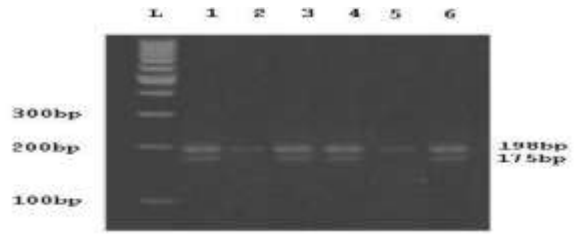

Figure (1): DNA bands in different genotypes by electrophoresis.

Lanes 1, 3, 4 and 6 show two bands.

Lanes 2 and 5 show one band.

Mutation	Restriction enzyme	Wild type	Heterozygous (CT)
MTHFR C677T	Hinf1	198pb one band	198pb and 175pb two bands

3. Results

The studied SNP is comprised of C and T alleles. It is located on short arm of chromosome 1 on MTHFR gene. Codon change of GCC into GTC results in alanine change into valine.

Table (1): Assessment Hardy Weinberg equilibrium in Studied Groups.

	Group I Controls N=20		Group II DN Patients N=40	
	Observed	Expected	Observed	Expected
CC	100%	100%	62.5%	63%
CT	0%	0%	37.5%	33%
TT	0%	0%	0%	4%
HW p	1		0.144	

Applying Hardy Weinberg equilibrium (HWE), revealed that MTHFRrs1801133 genotypes in control as well cases group were in HWE.

Table (2): Comparison of MTHFR Gene Polymorphism between Studied Groups.

		Group I Controls N=20		Group II DN Patients N=40		P
		N	%	N	%	
Genotypes	CC	20	100	25	62.5	0.002
	CT	0	0	15	37.5	
Alleles	C	40	100	65	81.3	0.003
	T	0	0	15	18.8	

Chi square was used.

CT genotype and T allele were significantly associated with DN patients when compared to control group.

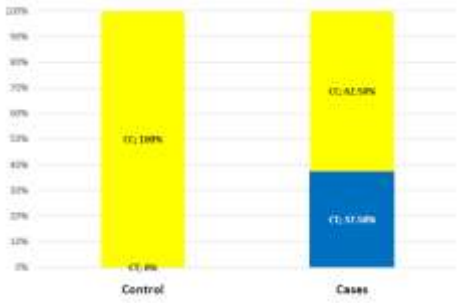

Fig (2): MTHFR Gene Polymorphism between Two Studied Groups.

Table (3): Comparison of FBS, HbA_{1c}% And Some Renal Function Tests Between MTHFR Genotypes in DN Patients.

		CC			CT			P
FBG (mg/dl)	mean±SD	219.2	±	67.3	221.60	±	70	.933
	Min-max	78	-	450	108	-	347	
HbA_{1c}%	mean±SD	10.7	±	2.9	10.1	±	3.3	.581
	Min-max	5.5	-	16	5.1	-	16	
Creatinine (mg/dl)	mean±SD	2	±	0.7	2.6	±	0.8	.110
	Min-max	0.8	-	3.9	1	-	6.3	
eGFR	mean±SD	44.1	±	13.1	38.5	±	14.2	.283
	Min-max	18	-	66	12	-	69	
Urinary Microalbumin (mg/24h)	mean±SD	128.1	±	38.5	188.2	±	61.3	.130
	Min-max	32	-	346	56	-	527	

No significant differences were found in FBG and HbA_{1c}% and some renal function tests between MTHFR genotypes in DN patients.

Table (4): Comparison of Lipid Profile and Hcybetween MTHFR Genotypes in DN Patients.

		CC			CT			P
TG (mg/dl)	**mean±SD**	148.7	±	34.4	122.7	±	61.3	.216
	Min-max	40	-	287	55	-	280	
TC (mg/dl)	**mean±SD**	183.7	±	46.3	174.1	±	52.3	.547
	Min-max	101	-	270	101	-	292	
LDL-C (mg/dl)	**mean±SD**	117.8	±	32.9	109.3	±	33.8	.550
	Min-max	46	-	189	32.6	-	176	
HDL-C (mg/dl)	**mean±SD**	34.8	±	6.9	38.4	±	12.5	.267
	Min-max	20	-	51	27	-	72	
Serum Hcy (µmol/L)	**mean±SD**	8.4	±	2.8	18	±	3.9	<0.001
	Min-max	5	-	16	12	-	26	

No significant differences were found in lipid profile between MTHFR genotypes in DN patients.

Table (5): AUC and Performance Characteristics of Creatinine, eGFR, Urinary MicroalbuminandHcy for Discrimination between Controls and DN Patients.

	Creatinine (mg/dl)	eGFR (mL/min/1.73 m^2)	Urinary Microalbumin(mg/24h)	Serum Hcy (µmol/L)
AUC	.964	1	1	.811
P	<0.001	<0.001	<0.001	<0.001
95% CI	.922-1	1-1	1-1	.705-.918
Cut off	1.3	71.8	30.5	7.85
Sensitivity (%)	87.5	100	100	70
Specificity (%)	100	100	100	90

AUC, area under ROC curve, CI, confidence interval.

ROC curve of creatinine, eGFR, microalbumin and Hcy was conducted for discrimination between controls and DN patients. eGFR and urinary microalbumin showed perfect AUCs, creatinine showed excellent AUC and Hcy showed good AUC for discrimination between normal and DN subjects. Cut off values and performance characteristics are shown (table 5).

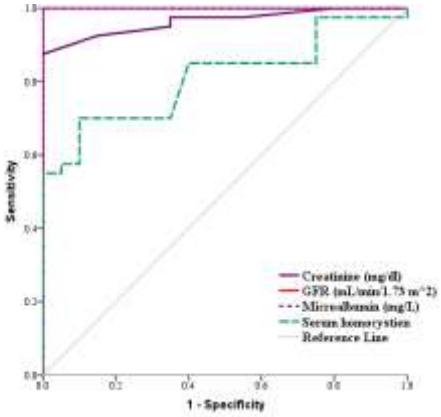

Fig (3):ROC of Creatinine, eGFR, Urinary Microalbumin and Hcy for Discrimination between Controls and DN Patients.

Serum Hcy showed significant positive correlation with urinary microalbumin, significant negative correlation with weight, TG. Otherwise, no significant correlation was found regarding Hcy level with other parameters in all studied cases.

Table (6): Correlations of Hcywith Other Parameters in DN Patients.

	R	P
Age (years)	.064	.695
Gender	-.162	.318
Weight (kg)	-.347	.028
FBG (mg/dl)	.103	.525
HbA_{1c}%	-.214	.184
Creatinine (mg/dL)	.169	.296
eGFR (mL/min/1.73 m^2)	-.263	.101
Urinary Microalbumin (mg/24h)	.315	.047
TG (mg/dl)	-.316	.047
TC (mg/dl)	-.203	.209
LDL-C (mg/dl)	-.172	.287
HDL-C (mg/dl)	.056	.732

r, Pearson correlation coefficient.

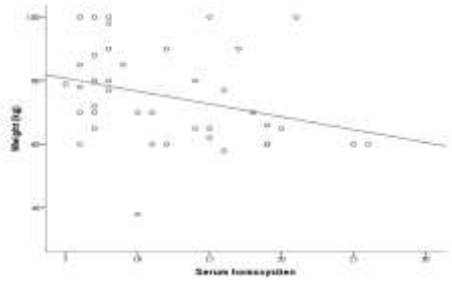

Fig (4): Correlations of Hcywith Weight in DN Patients.

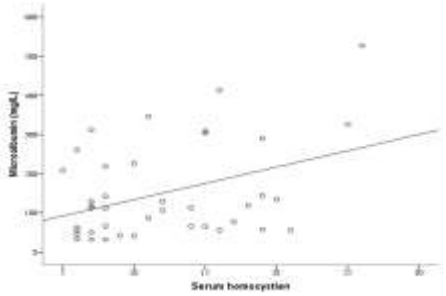

Fig (5): Correlations of Hcywith Urinary Microalbumin in DN Patients.

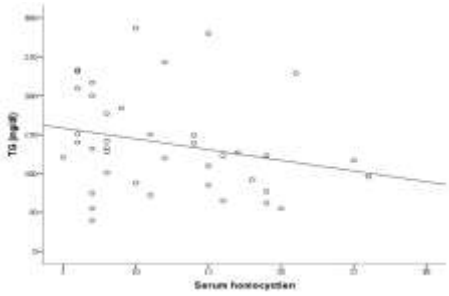

Fig (6): Correlations of Hcy with TG in DN Patients.

Table (7): Regression Analysis for Prediction of Microalbuminuria within DN Patients.

	Univariable				Multivariable			
	p	OR	95% CI		P	OR	95% CI	
Age	.447	1.028	.957	1.105				
Gender	.493	1.842	.321	10.56				
Weight	.155	.957	.902	1.017				
FBG	.475	.996	.986	1.006				
HbA_{1c}%	.014	.627	.431	.911	.132	.707	.450	1.111
Creat	.591	1.186	.637	2.208				
eGFR	.127	.959	.909	1.012				
TG	<0.001	.986	.979	.992	.144	.985	.966	1.005
TC	.293	.990	.973	1.008				
LDL-C	.374	.991	.972	1.011				
HDL-C	.026	1.091	1.011	1.177	.081	1.108	.809	1.215
Hcy	.024	1.177	1.022	1.355	.015	1.20	1.03	1.391
MTHFR	.233	2.667	.532	13.37				

Logistic regression analysis was conducted for prediction of microalbuminuria within DN patients; using age, gender, laboratory data and MTHFR genotypes as covariates. Elevated HbA_{1c}%, TG, HDL-C, Hcy, CT genotype, were associated with microalbuminuria. Multivariable analysis was done using those covariate that were significant in univariable analysis. Only higher Hcy was associated with microalbuminuria risk in multivariable analysis.

4. Discussion:

In our study there were non-significant difference in DN patients as regards as age, gender and weight as compared to controls,

As regard FBS and HbA1c% there was high significant increase, Also serum creatinine and urinarymicroalbumin in DN patient as compared to control group, this finding is in agreement with **Mashitani et al., (2014)**who found that there was significantly higher baseline urinary microalumin in patients with DN. And with **Xiang et al., (2017),** who pointed out that serum creatinine and urinary microalbumin were higher in the DN patients as compared to the healthy individuals.

The DN patients showed significantly higher levels of TC, LDL-c, and TG (although TG level did not reach significant level), significantly lower HDL-c as compared to control. This finding is in agreement with **Xiang et al., (2017)** and **Wang et al., (2017)**, studies they found that TC, TG, LDL-C were higher, and HDL-C was lower in the diabetic nephropathy patients as compared with the healthy individuals.

Significantly higher serum Hcy levels were seen in DN patients when compared to control group. This finding is in agreement with **Wang et al., (2015)** who showed Hcy level was significantly elevated in patients with micro-and macro albuminuria. Elevated level of plasma Hcy might result from disturbed Hcy clearance in the failing kidney. Accordingly, this study hypothesize that changes in plasma Hcy level might be a sensitive marker for alterations observed in the diabetic kidney and predictive of progression of DN at early stage. This finding was also in agreement with **Bakheet et al., (2016)** who noted a significant association between high levels of Hcy and DN. His study was on 100 Egyptian diabetic patients, those were divided into three groups; (38 normoalbuminuria, 33 microalbuminuria and 29 macroalbuminuria). It showed statistical significant positive correlation between Hcy and creatinine.

The distribution of MTHFR C677T polymorphism observed in the current study showed differences from the frequencies predicted by the Hardy–Weinberg equilibrium (HWE) in DN patients.

Observed CC homozygous in DN patients was 62.5% while expected was 63%. Observed CT heterozygous in DN patients was 37.5% while expected was 33%. Observed TT homozygous was 0% while expected was 4%.

This finding was against **Wang et al., (2017),** that showed that the genotype frequencies of MTHFRC677T in DN patients and controls were in agreement with that predicted by HWE. By the chi-square test, a statistically significant difference was observed between the DN patients and controls in regards to the genetic distributions of MTHFRC677T. In such study, the prevalence of CC homozygous, CT heterozygous and TT homozygous were 42.59%, 44.44% and 12.96% respectively. This finding also was against our results.

Also, another study **Russo et al., (2016)** showed that the distribution of MTHFR C677T polymorphism did not differ from the frequencies predicted by the HWE, with a TT homozygous prevalence of 24.3 %. In such study, the prevalence of CC homozygous was 29.7% while prevalence of CT heterozygous was 46%.

Settin et al., (2015) had a case controlled study involving 203 patients with T2DM and 311 healthy controls. Cases were recruited from the Diabetes and Endocrinology Departments, Internal Medicine Specialized Hospital, Mansoura University, Egypt. Testing for genetic equilibrium among controls showed that the distribution of frequencies of polymorphic variants of MTHFR 677 C>T conformed to the Hardy-Weinberg Equilibrium. The prevalence of CC homozygous in patient group, CT heterozygous and TT homozygous were 54.7%, 32% and 13.3% respectively. The prevalence of CC homozygous in control group, CT heterozygous and TT homozygous were 50.2%, 43.4% and 6.4% respectively.

Sharaf et al., (2012) study was done on fifty T2DM patients from the Outpatient Clinic of Zagazig University Hospital. These were (30 males, 20 females); 20 healthy individuals (13males,7 females) served as the control group.the frequencies of homozygous mutated genotype and the mutated allele is higher in diabetic patients than control group. T Allele frequency was 22% in control healthy subjects while it was 37% in diabetic patients and genotype frequency was 65%for CC, 25% for CT and 10% for TT.

In other study done in Tunisia, **Mtiraoui et al., (2007),** T allele frequency was 22% in healthy subjects , and more prevalent among T2DM patients, with allele frequencies of 0.36.Genotypes distribution was 44% for CC, 38%for CT, 18% for TT with none significant difference between two groups ($\chi2 = 2.5$, $P > 0.05$).This results was similar to other study, **Sun et al., (2005),** which showed that the distribution in T2DM patients in which 44.3% were CC, 34.2% were CT and 21.5% were TT. There were no significant differences in genotype distribution between T2DM patients and control group ($\chi2 = 3.67$, $P > 0.05$). T Allele frequency was 20%, 59% in patients with or without nephropathy respectively. These findings indicate that the presence of the C677T polymorphism in the MTHFR gene is of pathophysiological significance.

CT genotype and T allele was significantly associated with DN patients when compared to control group in our study. This finding was in agreement with **Zhou et al., (2015)** who showed that T allele and TT genotype were distinctly associated with DN susceptibility. Also, **Chen et al., (2015)** showed that the MTHFR C677T allele is more likely to increase the risk of DN in West Asian, and Chinese populations, but they did not find this association in East Asian and Japanese populations. In their opinion, this inconsistency may be caused by two reasons: diabetes duration and ethnicity. They noted that the prevalence of 677T/T among Bahrainis (2.0%) was lower than that in Caucasians, and a north-south gradient in its prevalence has been described, supporting the ethnic contribution of 677T/T to DN risk. This finding suggested that MTHFR C677T may play an important role in DN development in the early stages of type 2 DM. With increasing DM duration, other factors may contribute to risk of DN, thus diluting the influence of the MTHFR C677 T mutation.

Non significant differences were found in FPG, HbA_{1c}%, some renal function tests (S.creatinine, eGFR and urinary microalbumin), TG, TC, LDL-c and HDL-c between MTHFR genotypes in DN patients. Patients carrying CT genotype showed significantly higher serum Hcy when compared to those carrying CC genotype. This finding was also in agreement with **Bakheet et al., (2016)** who showed that serum levels of Hcy were associated with C677T mutation. **Russo et al., (2016)**showed that in DN patients, circulating levels of Hcy and MTHFR C677T mutation are not associated with DN, which was predicted by creatinine levels and dyslipidemia.

Although there are many studies analyzing the research results about the MTHFR C677T polymorphism and their associations with DN, definite conclusions cannot be drawn. Some studies conducted on Belgium population **(Hermans et al., 2006)** and Chinese diabetic nephropathy **(Sun et al., 2003)** had demonstrated that the significant association between MTHFR C677T polymorphism and T2DM with vascular complications. On the contrary, other studies in Australian **(Kaye et al., 2002)** and Chinese populations **(Zhang et al., 2007)**

reported there is no relationship between the C677T polymorphism and T2DM with vascular complications. These results suggest that the T2DM with vascular complications and gene polymorphisms of MTHFR was controversial. However, few studies which focused on development of nephropathy among diabetic patients reported no association with MTHFR C677T polymorphism in Turkish populations **(Eroglu et al., 2007)**.

There are many published meta-analyses regarding MTHFR C677T polymorphism and DN risk. Of these, **Yang et al., (2013)** reported that there was significant association between MTHFR C677T polymorphism and DN risk in Caucasian individuals. **Zintzaras et al., (2007)** made a meta-analysis included 15 studies, of which 8 involved Caucasians and 5 East Asians; 11 studies involved subjects with T2DM and 4 with T1DM. The main analysis (all studies) revealed significant heterogeneity between the studies and a marginal association between the 677T allele and the risk of developing DN. **Niu and Oi, (2012**) analyzed a total of 7807 and 1599 subjects from 21 and 8 studies for DN and diabetic retinopathy, respectively. Carriers of 677TT genotype were 1.71 (95% confidence interval [95% CI]: 1.02–2.88; P = 0.042) and 2.89 (95% CI: 1.51–5.53; P = 0.001) times more likely to develop DN separately relative to diabetic patients without nephropathy and non-diabetic controls. Likewise, this association was preserved for diabetic patients with retinopathy referring to those without (odds ratio [OR] = 1.86; 95% CI: 1.21–2.86; P = 0.004). Subgroup analyses showed that ethnicity was a possible confounder, especially in West Asians and Africans, and so were gender and duration of diabetes mellitus in DN studies.

Cui et al. (2012) made another meta-analysis to clarify the relationship between MTHFR C677T and DN in the Chinese population. Such study included 12 studies in a Chinese population published up to 2011 were combined. The 677T allele showed significant association with DN (OR = 1.97, 95% CI [1.71, 2.28], p <0.00001), but no relationship with DM (OR = 1.03, 95% CI [0.89, 1.18], p = 0.70) compared with the 677C allele in a Chinese population.

Xiong et al., (2016) made a meta-analysis involved 15 case-control studies with 1227 DN patients, 586 healthy controls and 1277 DM controls. Their results showed that a significantly elevated risk of DN was associated with all variants of MTHFR C677T when compared with the healthy or DM groups.

C allele frequency in our study was 40 (100%) and 65 (81.3%) in control and patient groups respectively. T allele frequency was 0 (0%) and 15 (18.8%) in control and patient groups respectively. This indicated that DN was associated with MTHFR 677 T allele carriage. This finding was in agreement with **Settin et al., (2015)**that noted that MTHFR 677 T allele carriage was found to be associated with diabetes among Egyptians.

The presence of MTHFR 677T allele increased the risk of macroalbuminuria (OR=2.667). This finding was in agreement with **Bakheet et al., (2016)** that noted that The presence of MTHFR 677T allele increased the risk of macroalbuminuria 3.3 times when compared to normoalbuminuria (OR=3.27). It was found, in the same study, that the mutant allele was associated with increased risk for nephropathy.

Settin et al., (2015) showed that the MTHFR 677 TT genotype was associated with T2DM susceptibility and complications. Also, **Ramanathanet al., (2017)** showed that the MTHFR gene polymorphism C677T contributed significantly with the progression of CKD in DN.

Wang et al., (2017) showed that individuals carrying with the TT genotype of MTHFRC677T were associated with a significant increase in type2 diabetic nephropathy risk compared to the CC genotype. In addition, the T allele of MTHFRC677T significantly elevated DN risk when compared with the C allele. This study was done on 162 patients diagnosed with type 2 diabetic nephropathy and 302 control subjects.

Zhou et al., (2015), reported that the TT genotype and T allele of MTHFRC677T might be a significant genetic molecular marker for the risk of type 2 diabetic nephropathy in patients. **El-Baz et al., (2012),** carried out a study on Egyptian population, and reported that the MTHFRC677T and A1298C were genetic risk factors for type 2 diabetic nephropathy in patients with T2DM.

We concluded that the C677T mutation in the MTHFR gene could be associated with DN patients. Hcy also has a major role so increased intake of folate and vitaminsB6 and B12 can reduce plasma Hcy levels in patients with DN. Further studies in larger number of patients are necessary to establish a role of this interesting polymorphism in the genesis of DN. It will also be important to study prospectively whether folate supplementation reduces the incidence of DN in T2DM in individuals who carry the C677T allele.

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