GENETIC POLYMORPHISM OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) ASSOCIATED WITH HYPOTHYROID IN HEMODIALYSIS PATIENTS

Sondos Taleb Faris[a], Adnan Jassim Mohammed Al-Fartosy[b]*, Adil Ali Al-Fregi[c]

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Abstract: Background: A vital role of vascular Endothelial Growth Factor was angiogenesis in kidney, that one of the most heavily vascularized organs. the current study aimed to analyze the genotype frequency of VEGF gene polymorphism (-460, -2578 and +936) between 104 hypothyroidism patients at the dialysis unit in Basrah Hospitals and 60 healthy controls, also studied effect on serum level VEGF-A.

Methods: Amplification Refractory Mutation System Real-Time PCR (ARMS RT-PCR) was used to identify the VEGF -460 C/T, -2578C/A, and +936 C/T single nucleotide polymorphisms (SNP) in all included patients.

Results: In comparison to the patient group, the control group had a larger distribution of the VEGF-460 C/T genotype .The CC genotype of -460 SNP showed a higher frequency with the hypothyroidism patients on hemodialysis. For VEGF – 2578 C/A, VEGF + 936 C/T, for cases and healthy control persons, we did not find any differences in genotypes that were statistically significant (p=0.7 and p=0.5, respectively). The dominant model showed a significant association with the protective effect of subclinical hypothyroidism .However ,no significant association was observed between this polymorphism and subclinical hypothyroidism susceptibility in recessive model and allele frequency for -460 C/T. There was no association between -460 rs 833061 polymorphism with overt hypothyroidism in all genetic models .There was no association between VEGF-2578 C/A and +936 C/T polymorphism with hypothyroidism (subclinical ,overt) in all genetic models .VEGF levels in serum from patients were significantly higher than those in healthy subjects .

Conclusion: The VEGF gene is highly polymorphic, and some variations may be connected to ESRD in hypothyroid patients. The pathophysiology of chronic kidney failure may be significantly influenced by VEGF polymorphisms, Our findings indicated that VEGF polymorphisms decreased the risk of hypothyroidism. Since this the first study ,further studies with different races are required to confirm our results.

Keywords: Single nucleotide; polymorphism, hypothyroidism, hemodialysis, vascular endothelial; growth factor

[a]. Department of Chemistry, College of Science, University of Basrah, Basrah, Iraq
[b]. PhD, Department of Chemistry, College of Science, University of Basrah, Basrah, 61004, Iraq.
[c]. Department of Chemistry, College of Science, University of Basrah, Basrah, Iraq

***Corresponding Author**

E-mail: adnan.jassim@uobasrah.edu.iq

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INTRODUCTION

Chronic kidney disease (CKD) is classifiable as a multifactorial disease because the accumulation and combination of genetic factors and environmental factors influence the onset and development of ESRD. The degree of proteinuria and tubulointerstitial damage rather than the pathogeneses that cause CKD, such as glomerulonephritis, diabetes, hypertension, and urologic diseases, are the most indicative of ESRD progression(J. Zhong et al., 2017). There are probably some common pathways to ESRD, and glomerular and peritubular capillaries seem to have major micro vasculatures that are influenced by both genetic and environmental variables(Pandita et al., 2017; Tripathi et al., 2008). The podocytes, tubular cells and mesangial cells in the kidneys of glomerulonephritis patients have all shown signs of VEGF production(Noguchi et al., 1998), and VEGF receptors have been found in both the endothelium of glomeruli and peritubular capillaries(Faris et al., 2022; Fu et al., 2016). Inflammatory cells including monocytes (Al-Fartosy et al., 2021a; Itaya et al., 2001) and lymphocytes that promote fibrosis in the tubulointerstitial layer of the kidney can express VEGF (Shim et al., 2018). The expression of VEGF is regulated by a number of hormones, growth factors, cytokines, and certain stimuli such as hypoxia. Individual differences in VEGF expression levels also have been reported (Parazzini et al., 2020). The promoter region, 5'-untranslated region (UTR), and 3'-UTR were reported to be particularly polymorphic regions of the VEGF gene(Al-Fartosy et al., 2021b; Renner et al., 2000; Watson et al., 2000). VEGF levels and disorders such as acute renal allograft rejection(Prakash et al., 2018), diabetic retinopathy (Ahuja et al., 2019), breast cancer(Langsenlehner et al., 2015) , and the progression to end-stage renal disease (Adnan et al., 2020) have been

linked to the polymorphisms in these locations, according to reports. A crucial component in regulating blood vessel growth is vascular endothelial growth factor (VEGF). It is essential for promoting endothelial survival and maintaining these micro blood vessels(Badr et al., 2021). VEGF stimulates macrophage chemotaxis and activation, and its administration accelerates the formation of atherosclerotic plaque . Yet, it is still unclear whether VEGF has vascular protective or atherosclerogenic affects(F. A. Chen et al., 2010). These common pathways are regulated by the interstitium in the human kidney through tubule fibrosis. One of the most heavily vascularized organs is the kidney. As previously mentioned, both proteinuria and decreased per itubular capillary are putatively significant predictive factors for ESRD due to rheumatoid arthritis(Paudyal et al., 2020) and sarcoidosis. Injury to the glomerular capillary causes proteinuria, and the decrease in per itubular capillary results in chronic hypoxia (Miao et al., 2022). The current study aimed to elucidate the genetic role of VEGF polymorphisms as a predisposing factor for progression level. The distribution of each genotype in ESRD patients and healthy control subjects was compared after the polymorphism of the VEGF gene was identified. The study also evaluated the associations between VEGF polymorphism and serum VEGF-A levels in identifying the functional polymorphism that may influence VEGF-A levels.

MATERIALS AND METHODS

Study subjects

In this study, 60 healthy individuals served as a control group and 104 patients with ESRD and hypothyroidism 82 with subclinical hypothyroidism and 22 with overt hypothyroidism) who had hemodialysis at two centers in the province of Basrah were included. There was no statistical difference between the patients and the control subjects with respect to age (56.25±3.63 versus 55.10±3.75 yr). All individuals in the control group were healthy and showed no urinary abnormality, renal dysfunction, or hyperglycemia they reported no use of medication. Basrah University gave the study its ethical clearance (no. 7/54/1377), and each participant signed an informed consent form following a thorough description of the methods. The Helsinki deceleration for the year 2000 served as the basis for informed consent and ethical norms.

Genomic DNA isolation

According to the manufacturer's instructions, we used genomic DNA extraction kits (G-spinTM Total DNA extraction kit, Intron biotechnology) to isolate genomic DNA from blood samples which were collected in ethylene di amine tetra-acetic acid (EDTA) tubes.

Detection of VEGF gene polymorphism

(ARMS RT -PCR) method(Ntziora et al., 2013), with specific PCR primer pairs listed in Table 1, was used to identify the three VEGF genotype polymorphisms (-2578 C/A (rs 699947), +936 C/T (rs 3025039), and -460 C/T (rs 833061) using the kit provided by the Alpha DNA company. The PCR mixture (total, 20µl) containing 10µL SYBER-GREEN, 3µL DNA sample, 1µL forward primer, 1µL reverse primer, and 5µL deionized water nuclease-free. The PCR mixture was incubated at 95 °C for 5 minutes, then 1 cycle at 95 °C for 30 seconds to denature, (59 °C, 57 °C, 62 °C) for 40 seconds to anneal the primers (-2578A/C, +936C/T, and -460 C/T) respectively, and 72 °C for 5 minutes to elongate the strand PCR.

Measurement of serum VEGF levels

Serum VEGF-A levels were measured using by ELISA kit (Pharma Genie, Ireland). According to the manufacturer's instructions, standards and samples were added to the appropriate wells coated with anti-human VEGF-A, and the plate was incubated for 2.5 hours at room temperature in order to quantify the VEGF levels. The Biotinylated Detection Antibody was added to each well and left there for 1 hour at room temperature after being washed four times with the appropriate wash solution. The washing step was again repeated, and each well received a 45-minute addition of horseradish peroxidase (HRP)-streptavidin solution at room temperature. 3,3′,5,5′-tetramethylbenzidine (TMB) substrate reagent was added after the further round of washing and was left in the dark for 30 minutes at room temperature. Finally, Stop Solution was added, and a plate reader was used to quickly read the absorbance at 450 nm.

Statistical analysis

Statistical analysis was performed using SPSS software version 24 (IBM Corporation, New York, USA). The data were distributed normally and the comparison between groups was analyzed using the analysis of variance followed by t-test to find the statistical significance. Genotype frequencies were calculated using Fisher exact test. Hardy-Weinberg equilibrium was assessed between outcome groups . Categorical data were expressed as frequency (percentage). The odds ratio (OR) and 95% confidence intervals (CI) were also estimated. Logistic regression analysis was used to assess the independent effect of each risk polymorphism on hypothyroidism in dialysis patients. $P<0.05$ was considered to indicate a statistically significant difference.

RESULTS

Study population

The hemodialysis patients with hypothyroidism consisted of 48 males and 56 females. The subject's clinical information and the outcomes of the biochemical analysis at the time of the study were summarized in Table 2.

VEGF genotype

The distributions of the three VEGF genotypes frequencies of the hemodialysis patients and the control subjects were presented in Table 3. In both the case and control patients, all VEGF polymorphisms were compatible with Hardy-Weinberg equilibrium. RT-PCR was appropriate for determining the polymorphism of the VEGF gene Fig 1. the-460 rs 833061 CT frequency was significantly greater in control compared to subclinical and overt hypothyroidism groups(61.6% vs 36.6% ,36.4% p=0.004). There were no significant differences in genotype subgroups and the frequencies between hemodialysis patients group and healthy controls for the VEGF-2578(rs699947) and+936(rs3025039). The current study's results revealed differences between subclinical hypothyroidism and healthy controls in the genotypes of the VEGF gene, 2578 C/A (OR=1.4 ,95%CI=0.66-2.94) in a dominant model but the difference was not

significant(p=0.37),in recessive model (OR =2.22,95% CI= 0.56-8.73) for +936 C/T. The -460 rs 833061 CT frequency was significantly higher in control compared to subclinical hypothyroidism(p=0.002), (OR=0.29 95%,CI=0.12-0.64) as shown in Table 4.The dominant model showed a significant association with the protective effect of subclinical hypothyroidism .However ,no significant association was observed between this polymorphism and subclinical hypothyroidism susceptibility in recessive model and allele frequency for -460 C/T. There was no association between -460 rs 833061 polymorphism with overt hypothyroidism in all genetic models as shown in Table 5.

ASSOCIATION OF 2578 C/A ,+936 C/T AND 460C/T POLYMORPHISM With Serum VEGF levels

The -2578 CC, +936 CC, and -460 CC individuals had significantly higher serum VEGF levels than the other genotypes . VEGF level in serum from patients were significantly higher, than those in health subjects , as shown in Fig2.

DISCUSSION

To our knowledge, this is the first report of its kind that investigates the various laboratory tests and genetic polymorphisms used in the diagnosis of hypothyroidism conditions in ESRD patients in the Province of Basrah/Iraq. Hence, the current study was to assess the genetic variants of VEGF-A (-2578 C/A, +936 C/T, and -460 C/T) on the risk of hypothyroidism in ESRD and its association with disease progression.

The VEGF -2578C/A,+936 C/T polymorphism were found no a statistically significant association with ESRD while the-460 C/T polymorphism were found have to a statistically significant association with ESRD in the study, which involved 104 ESRD patients overall and 60 healthy control subjects. In fact, our study revealed that hypothyroidism in ESRD patients may be related to the significant ($p < 0.01$) elevation of serum VEGF-A levels and their genotypes, 936CC, 2578CC, and 460CC.

Moreover ,no significant differences were observed between polymorphism of +936 C/T gene of the control and cases (subclinical ,overt hypothyroidism).

However, several scientific studies found significant associations between the 936C/T polymorphism and a number of different diseases, including gastric cancer(Tahara et al., 2009), rheumatoid arthritis (Y. Chen et al., 2012; Mahmoodi et al., 2019) and breast cancer (Langsenlehner et al., 2015) As a potent survival factor for endothelium, VEGF appears to be crucial in maintaining normal kidney vasculature.

In this study ,we analyzed a possible effect of -2578 VEGF polymorphism in promoter of VEGF gene on the hypothyroid in ESRD we found a higher frequency of CC genotype in the group of overt hypothyroidism compared than control group . Safránková et al., (2011) showed risk association against CC genotype of -2578 C/A polymorphism .

Our findings indicated that the frequency of CT genotype of -460C/T gene polymorphism was significantly higher in the control compared to hypothyroid groups, and this genotype probably has a protective effect against hypothyroidism .also our results showed CC genotype of -460 C/T a significant association with the risk of hypothyroidism .

As reported by recent studies VEGF-460 C/T polymorphism were CC genotype and C allele were significantly associated with increased risk of esophageal cancer (Guleria et al., 2022). VEGF polymorphisms have previously been described in solid carcinoma ,containing lung cancer (F. Yang et al., 2018) cervical cancer (W. Zhong et al., 2022) colorectal cancer (Kontham et al., 2022) and renal cell carcinoma ,results showed dramatically high risk for renal cell carcinoma were found regarding most genetic models and alleles of the +936 C/T polymorphism ,in addition significant increased risk with -2578 C/A .However ,no significant association were found between renal cell carcinoma risk and the -460C/T polymorphism (Gong et al., 2017)

Han et al., (2015) they observed CC genotype was risk for coronary heart diseases (OR=2.5, 95%CI =1.1-5.68) and the frequencies of -460 C/T CC genotype (13.6%) was found higher in the case group than that of control group (6.7%).

In this study elevated serum level of VEGF were associated with the -460 C/C, -2578 C/C and +936 C/C. We observed that VEGF levels in serum from patients were significantly higher than those in healthy control ,reflecting the angiogenesis and or chronic inflammation in patients with ESRD.

the serum is the correct medium to measure VEGF ,as platelets represent a large source of stored VEGF (Awata et al., 2002). One possible mechanism in progressive renal disease is that high levels of VEGF may initially increase blood flow and raise the permeability of glomerular endothelial cells . The chemotactic effect of VEGF would amplify the inflammatory process and promote atherosclerosis . Activation of macrophages and monocytes may cause localized immune injury affecting epithelial cells , podocytes and mesangial cells , triggering further VEGF release and exacerbating the process of epithelial cell destruction . Expression of VEGF and its receptors is significantly increased in the PHN and PAN rat models of proteinuria , suggesting a role for VEGF in the disease process (Kanellis et al., 2004).

According to Kang et al., (2002), VEGF protected the endothelium and reduced renal impairment. In addition, VEGF can chemotactically attract monocytes and macrophages, which could intensify inflammatory reactions(Al-Fartosy & Ati, 2021; Mobarrez et al., 2018).

One important mediator or regulator of neovascularization and endothelial dysfunction has been identified as VEGF. Our data may reflect that elevated VEGF levels were linked to vascular inflammation and a higher risk of hypothyroidism ,especially in cases of severe primary myxedema, which is linked to profound abnormalities in renal function. Low cardiac output, a decline in renal blood flow and glomerular filtration rate are among the frequently described abnormalities in primary hypothyroidism. . More research should be done to verify it, though. The results of scientific studies showed that lower blood volume and elevated peripheral resistance occur in hypothyroidism. Additionally, because of myocardial abnormalities, cardiac output is low. On the other hand, thyroid hormone therapy can correct these deficiencies that arise from the lack of inotropic and chronotropic actions of thyroid hormones. Furthermore, myxedema is known to be accompanied by decreased glomerular filtration rate and decreased renal blood flow (proportional to the drop in cardiac output (Chandra, 2016). Therefore, it appears that both

hereditary and environmental variables play a significant role in the progression of CKD to ESRD.

However, lethal or sub-lethal injury to renal cells may cause acute or chronic nephritis as well as new local antigen expression, inflammatory cell infiltration, and activation of pro-inflammatory and chemo-attractant cytokines. It has been shown that damaged kidney cells activate nuclear factor kappa B (NFKB), which is similar to nuclear transcription factors, and release pro-inflammatory cytokines into the interstitium, which may lead to chronic tubulointerstitial inflammation (J. W. Yang et al., 2011).

Our study had some limitations ,First, all hypothyroid were studied from dialysis unites in Basra city therefore thus may not be representative of the target population. Second the sample number in this study was small ,which might affect the accuracy of the present results .Third , just three VEGF SNPs—2578C/A, +936C/T, and -460C/T were examined. The association between VEGF SNPs 2578C/A, 936C/T, and -460 C/T and hypothyroidism susceptibility could not, therefore, comprehensively studies are recommended to support the findings .

In conclusion, the current study is the first to examine the relationship between VEGF polymorphism and overt and subclinical hypothyroidism. The VEGF polymorphisms examined in this investigation, however, were connected to hypothyroid susceptibility It's interesting to note that the VEGF polymorphism genotypes -460CC was probably the new genetic markers for patients with ESRD who have overt and subclinical hypothyroidism. The genotype study has added to the emerging evidence that VEGF plays an important role in progressing in hemodialysis patients .

Finally ,no significant relationships was found between VEGF- 2578 (rs 699947) and+936(rs 3025039) polymorphism and hypothyroidism risk in all genetic models .CT genotype of -460 SNP probably has a protective effect against hypothyroidism ,while CC genotype of -460 SNP associated with the risk of hypothyroidism .

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Conflict of interest: The authors have no conflict of interest.

Consent of Ethics: Administrative approval was taken from all places where samples were collected, as well as written and oral consent was taken for all participants in the research

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Table 1. Specific primers according to three VEGF polymorphism

VEGF-SNP number	Primers
-2578C/A (rs699947)	5́-GTAGGCCAGACCCTGGCG-3́(forward)
	5́-GTAGGCCAGACCCTGGCG-3́(forward)
	5́-GTAGGCCAGACCCTGGCG-3́(forward)
	5́-CACCAAGTTTGTGGAGCTGA-3́(reverse)
+936C/T (rs3025039)	5́-GGCGGGTGACCCAGCGT-3́(forward)
	5́-GGCGGGTGACCCAGCGC-3́(forward)
	5́-CCAGGCTCCTGAATCTTCCA-3́(reverse)
-460C/T (rs833061)	5́-GCGTGTGGGGTTGAGGAT-3́(forward)
	5́-GCGTGTGGGGTTGAGGAG-3́(forward)
	5́-GCGTGTGGGGTTGAGGAC-3́(forward)
	5́-CAGTGATTTGGGGAAGTAGAGCA-3́(reverse)

Table 2. Clinic Patients Characteristic

Characteristics	Subclinical hypothyroidism		Overt hypothyroidism		Healthy Controls		P value
	Mean ± SD		Mean ± SD		Mean ± SD		
	Male(38)	Female(44)	Male(10)	Female(12)	Male(30)	Female(30)	
age(years)	54.26±4.42	56.25±3.63	60.20±2.53	57.33±1.49	56.13±5.37	55.10±3.75	>0.05 a, b , c ,d
GFR (ml/min/1.73m^2)	27.79±1.26**	25.20±1.18**	19.22±1.65**	17.40±0.68**	93.26±12.66	92.80±1.24	<0.001**a ,b ,c ,d
BMI(kg/m^2)	24.69±0.9	24.21±0.9	25.90±1.64	25.30±0.40	26.10±1.19	25.82±1.15	>0.05a,b, c ,d
HD duration(years)	4.8±1.17	6.5±1.21	6.7±1.22	4.6±1.18	0	0	
Creatinine (mg/dL)	9.80±0.99**	10.90±0.95**	11.20±0.69**	11.70±0.63**	0.48±0.15	0.62±0.24	<0.001**a,b,c,d
Urea (mg/dL)	112.37±1.25**	124.50±1.22**	178.80±1.78**	192.30±1.64**	32.70±1.24	28.49±1.24	<0.001**a,b,c,d
Albumin (g/dL)	3.26±0.12**	3.32±0.12**	3.10±0.09**	3.08±0.07**	4.48±0.139	4.56±0.11	<0.001**a,b,c,d
TSH (mLu/L)	7.10±1.19**	7.30±1.18**	10.20±1.79**	10.40±1.71**	2.80±1.24	2.50±1.24	<0.001**a,b,c,d

VEGF (pg/ml)	316.50±8.46**	305.60±11.61**	320.44±4.26**	298.20±6.01**	127.69±9.22	129.80±9.28	<0.001**a,b,c,d

BMI: Body Mass Index, GFR: Glomerular Filtration Rate, HD: Hemodialysis Duration, VEGF: Vascular Endothelial Growth Factor , Data are presented as Mean ± SD, SD: Standard Deviation ; a: comparison between male healthy group and Subclinical hypothyroidism male; b:comparison between female healthy group and Subclinical hypothyroidism female; c: comparison between male healthy group and Overt hypothyroidism male; d: comparison between female healthy group and Overt hypothyroidism female.* significant; **highly significant.

Table 3. Frequencies of Genotypes for VEGF Gene in Hypothyroidism

VEGF SNP	Sub clinical Hypothyroid (N=82)	%	Overt Hypothyroid (N=22)	%	Control N=60	%	P- value
-2578							
CC	**36**	**43.9%**	**11**	**50%**	**21**	**35%**	**0.707**
CA	**33**	**40.2%**	**9**	**40.9%**	**27**	**45%**	
AA	**13**	**15.9%**	**2**	**9.1%**	**12**	**20%**	
+936							
CC	**49**	**59.8%**	**11**	**50%**	**32**	**53.33%**	**0.506**
CT	**25**	**30.5%**	**7**	**31.8%**	**19**	**31.67%**	
TT	**8**	**9.8%**	**4**	**18.2%**	**9**	**15%**	
-460							
CC	**34**	**41.5%**	**9**	**40.9%**	**12**	**20%**	**0.004****
CT	**30**	**36.6%**	**8**	**36.4%**	**37**	**61.67%**	
TT	**18**	**22.0%**	**5**	**22.7%**	**11**	**18.33%**	

**highly significant

Table 4. Allelic and genotypic of VEGF SNPS -2578 C/A ,+936 C/T ,-460C/T in Subclinical Hypothyroidism and healthy control group

SNP 2578 C/A polymorphism	Control N=60 (%)	Sub clinical Hypothyroid (N=82) (%)	OR (95% CI)	P- value
SNP -2578				
Codominant				
AA	12 (35)	13 (15.9)	0.63(0.24-1.63)	0.34
CA	27 (45)	33 (40.2)	1.28(0.80-2.03)	0.29
CC (Reference)	21 (20)	36 (43.9)		
Dominant genetic model				
(CA + AA)	39 (80)	46 (56.1)	1.40(0.66-2.94)	0.37
CC (Reference)	21 (20)	36 (43.9)		
Recessive genetic model				
AA	12 (35)	13 (15.9)	0.68(0.34-1.36)	0.28
CA+CC (Reference)	48 (65)	69 (84.1)		
Allele Frequency				
C (wild allele)	0.57	0.64		
A (mutant allele)	0.43	0.36	0.75(0.31-1.79)	0.52
SNP +936				
Codominant				
TT	9 (15)	8 (89)	1.16(0.55-2.45)	0.69
CT	19 (31.7)	25 (30.5)	0.58(0.20-1.66)	0.31
CC (Reference)	32 (53.3)	49 (59.8)		
Dominant genetic model				
(CT + TT)	28 (46.7)	33 (40.3)	0.77(0.39-1.50)	0.44
CC (Reference)	32 (53.3)	49 (59.8)		
Recessive genetic model				
TT	9 (15)	8 (9.8)	0.61(0.22-1.69)	0.34
(CT + CC) (Reference)	51 (85)	74 (40.3)		
Allele Frequency				
C (wild allele)	0.69	0.75		
T(mutant allele)	0.31	0.25	1.26(0.78-2.04)	0.32
SNP -460				
Codominant				
TT	11 (18.3)	18 (22)	0.58(0.21-1.56)	0.28

CT	37 (61.7)	30 (36.5)	0.29(0.12-0.64)	0.002
CC (Reference)	12 (20)	34 (41.5)		
Dominant genetic model				
(CT + TT)	48 (80)	48 (58.5)	0.35(0.16-0.76)	0.007
CC (Reference)	12(20)	34 (41.5)		
Recessive genetic model				
TT	11 (18.3)	18 (22)	1.25(0.54-2.89)	0.59
(CT + CC) (Reference)	49 (81.7)	64 (78)		
Allele Frequency				
C (wild allele)	0.51	0.59		
T (mutant allele)	0.49	0.41	1.15(0.61-2.18)	0.65

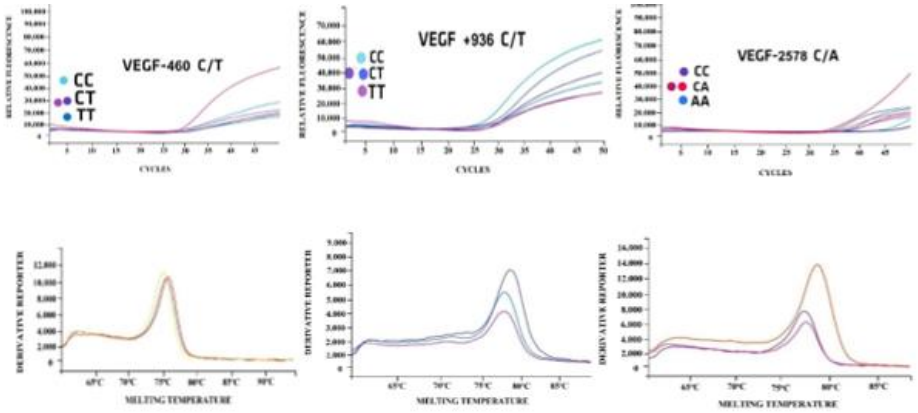
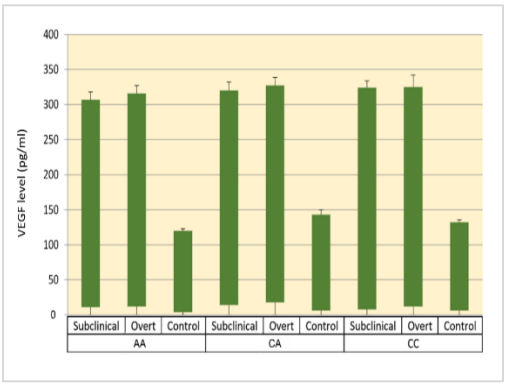
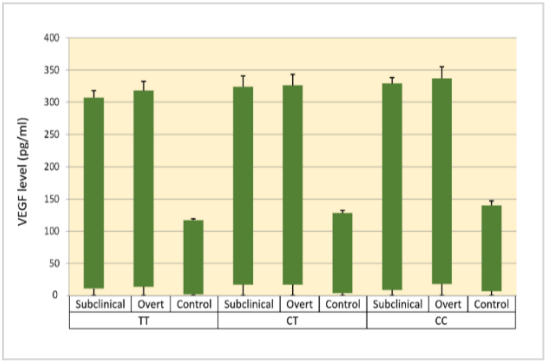


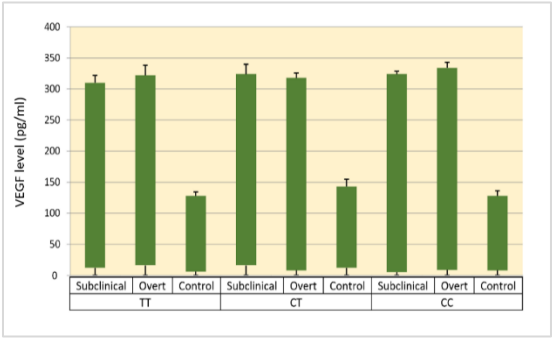
Fig.1. Genotype of VEGF gene by Real-Time PCR assay. (A):amplification curves. (B):melting curves.



A) VEGF-2578 C/A



B) VEGF + 936 C/T



C) VEGF – 460 C/T

Fig.2.Association of the VEGF 2578C/A,+936 C/T and -460 C/T polymorphism with Serum VEGF levels

Table 5. Allelic and genotypic of VEGF SNPS -2578 C/A ,+936 C/T ,-460C/T in Subclinical Hypothyroidism and overt

polymorphism	**Overt Hypothyroid (N=22) (%)**	**Sub clinical Hypothyroid (N=82) (5%)**	**OR (95% CI)**	**P-value**
SNP -2578				
Codominant				
AA	2 (9.1)	13 (15.9)	1.12(0.41-3.04)	0.82
CA	9 (40.9)	33 (40.2)	1.40(0.62-3.19)	0.41
CC (Reference)	11 (50)	36 (43.9)		
Dominant genetic model				
(CA + AA)	11 (50)	46 (56.1)	1.27(0.49-3.28)	0.61
CC (Reference)	11 (50)	36 (43.9)		
Recessive genetic model				
AA	2 (9.1)	13 (15.9)	1.88(0.39-9.05)	0.42
CA+CC (Reference)	20 (90.9)	69 (84.1)		
Allele Frequency				
C (wild allele)	0.70	0.64		
A (mutant allele)	0.30	0.36	1.30(0.65-2.59)	0.45
SNP +936				
Codominant				
TT	4 (18.2)	8 (9.8)	2.05(0.55-7.58)	0.28
CT	7(31.8)	25 (30.4)	0.69(0.36-1.36)	0.27
CC (Reference)	11(50)	49 (59.8)		
Dominant genetic model				
(CT + TT)	11 (50)	33 (40.2)	0.80(0.27-2.32)	0.68
CC (Reference)	11(50)	49 (59.8)		
Recessive genetic model				
TT	4 (18.2)	8 (9.8)	2.22(0.56-8.73)	0.25
(CT + CC) (Reference)	18 (86.8)	74 (90.2)		
Allele Frequency				
C (wild allele)	0.66	0.75		
T(mutant allele)	0.34	0.25	1.48(0.57-3.82)	0.41
SNP -460				
Codominant				
TT	5 (22.7)	18 (22)	1 .04(0.30-3.60)	0.93
CT	8 (36.4)	30 (36.5)	1. 007(0.34-2.8)	0.98
CC (Reference)	9 (40.9)	34 (41.5)		
Dominant genetic model				
(CT + TT)	13 (59.1)	48 (58.5)	1.02(0.39-2.66)	0.96
CC (Reference)	9 (40.9)	34 (41.5)		
Recessive genetic model				
TT	5 (22.7)	18 (22)	1.05(0.33-3.22)	0.57
(CT + CC) (Reference)	17 (77.3)	64 (78)		
Allele Frequency				
C (wild allele)	0.59	0.58		
T (mutant allele)	0.41	0.42	1.03(0.40-2.10)	0.97