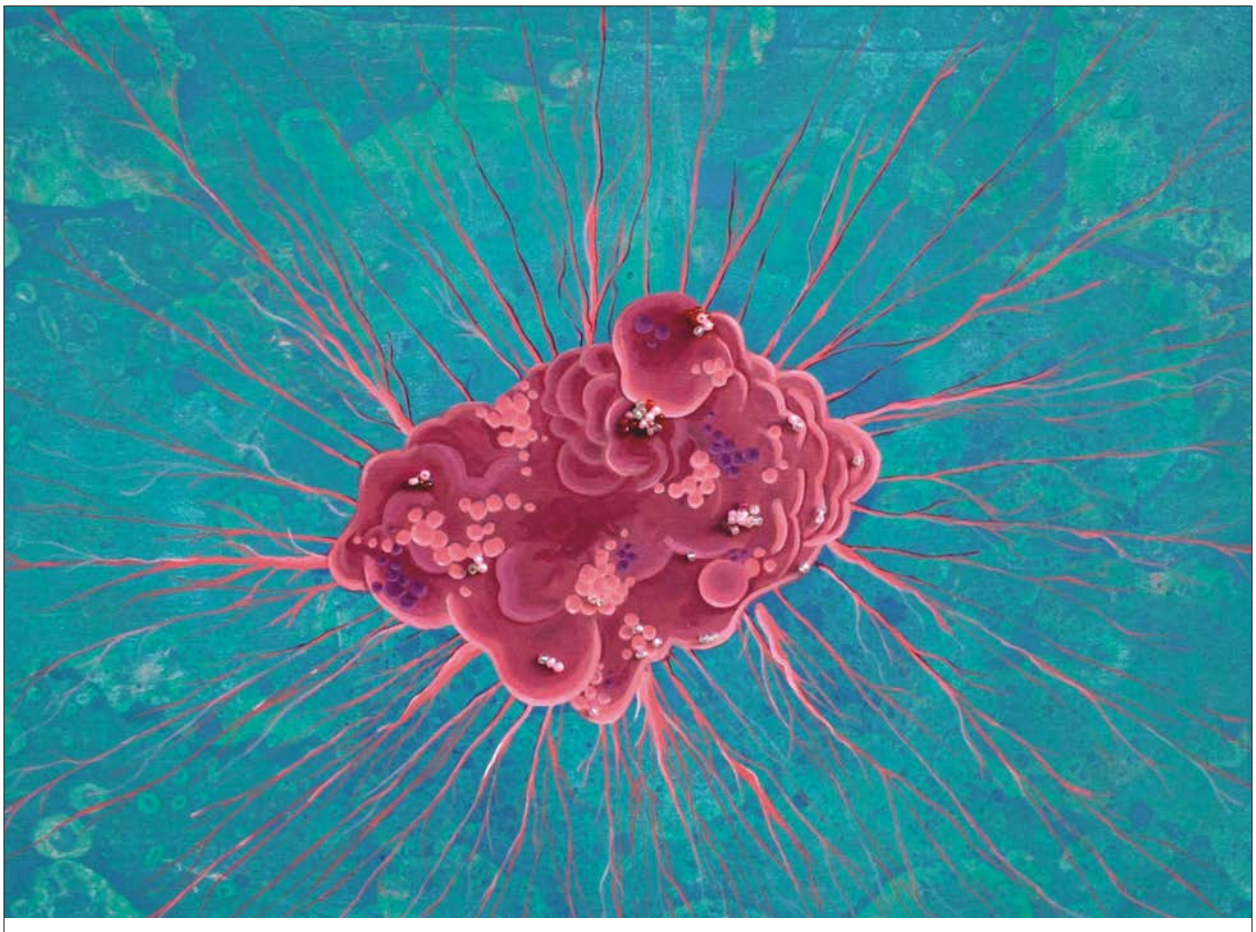

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A Novel Approach to Acute Lymphoblastic Leukemia in Adults: Association Analysis of Polymorphisms in Vascular Endothelial Growth Factor (VEGF) Gene and Clinical Outcome

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ABSTRACT

Angiogenesis has been shown as an important process in hematological malignancies especially leukemia, Vascular Endothelial Growth Factor (VEGF) plays a central role in promoting angiogenesis. Its different genotypes are associated with many human diseases. This study was to investigate the frequency of -460T/C and -1154G/A VEGF gene SNPs in adult ALL patients and their impact on serum concentration of VEGF and on the clinical outcome. Allele specific PCR for -460T/C and -1154G/A VEGF gene SNPs detection were done for 65 adult subjects (40 ALL patients and 25 normal healthy controls) and the serum VEGF was measured. Mean serum VEGF levels is higher in ALL than control subjects ($p = 0.00$) and it decreased markedly after complete remission ($p = 0.00$). There was marked association between TC genotype of -460T/C in ALL with higher association between the C allele and ALL than the T allele ($p = 0.018$) with high frequency of CA haplotype of -460 T/C and -1154 G/A in patients compared to control group ($p = 0.019$). Some VEGF genotypes may have impact on ALL pathogenesis and treatment outcome.

Key words: VEGF, SNP, allele specific PCR, ELISA, genes polymorphism, angiogenesis

INTRODUCTION

Acute Lymphoblastic Leukemia (ALL) is a malignant disorder that originates from one single hematopoietic precursor committed to the B-or the T-cell lineage (Narayanan and Shami, 2012). The ALL cells carry numerous genetic alterations with specific prognostic value. Therefore, their description is an important part of the diagnostic procedure, not only for choice of risk adapted treatment, but also because some of the altered proteins can be subjected to highly efficient targeted therapy (Graux, 2011).

Angiogenesis has been shown as an important process in hematological malignancies (Folkman, 2006). Many studies reported that Leukemias have been associated with angiogenesis, since the acute myeloid leukemia cell line HL60 was first used to clone the VEGF gene (Aguayo *et al*., 1999). Increased bone marrow vascularity has been reported in chronic and acute leukemia in adults (Aguayo *et al*., 2000) and children (Schneider *et al*., 2011).

Vascular Endothelial Growth Factor (VEGF) plays a central role in promoting angiogenesis and it is over-expressed in acute lymphoblastic leukemia (Duffy *et al*., 2000). Its genotypes are associated with many human diseases including hematological malignancies, solid tumors and other diseases (Demacq *et al*., 2010). It plays important role in progression of many types of

hematological malignancies by stimulation of angiogenesis and increasing vascular permeability, which is associated with reduced drug delivery and tumor cell metastasis, VEGF also induces activation of antiapoptotic genes, including bcl-2 which protect tumor cells from apoptosis as well as it works in concert with numerous signaling molecules, such as, angiopoietins, ephrins, hepatocyte growth factor, hypoxia-inducible factor, IL-6 and endostatin to promote tumor cell surviva, VEGF impacts hematopoiesis by blocking the differentiation of multiple hematopoietic lineages and inhibits the maturation of dendritic cells by reducing NF-κB activation (Yang *et al*., 2015).

Gene polymorphism may play an important role in the prognosis of malignant disorders and may explain patient variability in response to treatment (Chien *et al*., 2013). The two splicing variants of VEGF (ENST00000280193 and ENST00000507638) have been reported in the Ensemble database (vers. GRCh37). One encodes the functional VEGF protein (NM-005429, 420 amino acids), but the other only processes transcripts (CF128431, without protein production (Awata *et al*., 2002). A number of single nucleotide polymorphisms (SNPs) in VEGF gene (vers. GRCh37) are of clinical relevance. Those which are localized in the promoter region (-2578C/A, with a reference sequence (rs): 699947; -1154G/A, rs 1570360; -634G/C, rs 2010963 and -460T/C, rs 833061) has been reported in various studies to affect the rate of expression of VEGF gene (Demacq *et al*., 2010).

We examined the association between two VEGF polymorphisms and risk for acute lymphoblastic leukemia in adult, these polymorphisms are those located in the promoter region (-1154G/A, rs 1570360 (gene symbol: VEGFA, gene ID: 7422) and -460T>C, rs 833061) (gene symbol: VEGFC, gene ID: 7424).

Some authors have detected higher plasma concentrations of VEGF in newly diagnosed children with ALL than their levels after complete remission and than those in normal controls (Yang *et al*., 2007). On contrary, Kalra *et al*. (2012) has found lower levels of serum VEGF at the time of diagnosis of ALL as compared to its level after induction of treatment.

This study is designed to investigate the frequency of -460T/C and -1154G/A VEGF gene SNPs in ALL adult patients and their impact on the concentration of VEGF in the serum of ALL patients as well as on the clinical outcome of patients after induction therapy. Furthermore, correlation of our data with clinicopathological factors of patients on ALL was also addressed.

MATERIALS AND METHODS

Subjects: In a case-control study plasma VEGF level was measured and patients mononuclear cells were analyzed for 2 VEGF Single Nucleotide Polymorphisms (SNP) by Allele specific PCR in 65 adult subjects (40 ALL patients and 25 normal healthy controls) recruited from Ain Shams University hospitals, from October 2010-October 2012. All subjects included in this study gave an informed consent. The study was approved by Ethics Committees of the Ain Shams University Hospitals.

Forty patients were newly diagnosed as acute lymphoblastic leukemia attending the clinical Hematology and oncology department. Their ages ranged from 16-57 years old, twenty two patients (22/40) (55%) were females while eighteen (18/40) patients were males (45%). Twenty four patients (24/40) (60%) were less than 35 years old, while sixteen patients (16/40) (40%) aged 35 years or more, on follow up after 28 days, bone marrow aspirate showed that, twenty two patients (22/40) (55%) got complete hematological remission, Seven patients (7/40) (17.5%) died before day 28 and 11 patients (27.5%) show resistance to treatment. The remaining 25 participants were

age and sex matched control subjects. Eleven subjects (11/25) (44%) were females and fourteen subjects (14/25) (56%) were males. Their ages ranged from 20-57 years old.

Collection of samples: Five milliliter of venous blood were withdrawn in Ethylene Diamine Tetra acetic Acid (EDTA), sterile tubes and centrifuged at 1000 rpm for 10 min. Plasma samples were collected and stored at -20°C for subsequent VEGF analysis.

Quantitative detection of VEGF: Plasma level of VEGF were conducted according to manufacturer's protocols of enzyme-linked immunosorbent assay kit (Boster Biological Technology, LTD). Assay sensitivity was 31.2 pg mL^{-1}.

Mononuclear cell separation: Mononuclear cells were separated using Ficoll-hypaque density gradient centrifugation (Gawad *et al*., 2012).

Genotyping: The DNA was isolated from nucleated cells by lysis of the cells with anionic detergent in the presence of DNA stabilizer. DNA had been extracted from Peripheral Blood Mononuclear Cells (PBMC) using the QIAamp® DNA Kits Blood Mini (QIAGEN, USA). DNA was quantified according to a previous study (Yoshikawa *et al*., 2011). We used two allele-specific sense primers and a common antisense primer for each polymorphism. The amplification of the gene coding for VEGF polymorphism required 2 μg of purified DNA and 25 pmol μL^{-1} of each primer, their sequences were as follow: -460 T/C (rs 833061) primers: Zarbock *et al*. (2009) Sense primers (F1): 5'-TGCGTGTGGGGTTGAGGGC-3', (F2): 5'-TGCGTGTGGGGTTGAGGGT-3' and antisense primer: 5'-GGCTCTGCGGACGCTCAGTGA-3',-1154 G/A (rs 1570360) primers: Kawai *et al*. (2007) Sense primers (F1): 5'-GCCCGAGCCGCGTGTGGAA-3', (F2): 5'-GCCCGAGCCGCGTGTGGAG-3' and antisense primer: 5'-CCCCGCTACCAGCCGACTT-3'. Their Accession number was: NT-007592.15. First step of PCR activation was done at 95°C for 5 min, then repeated 40 cycles of (Denaturation at 94°C for 1 min, annealing at 63°C for 1 min (for 460 T/C primers) and at 65°C for 1 min (for 1154 G/A primers), Extension at 72°C for 1 min) then final extension at 72° C for 10 min. The amplified VEGF-460 T/C DNA products were found at 130 bp and VEGF-1154 G/A DNA products were found at 341 bp. The products were separated on 2% agarose and visualized by ethidium bromide staining. In all the runs 1 μL of DEPC treated water was used instead of the DNA sample as negative control.

Statistical analysis: The data were expressed as median 1and the Chi-square analysis (χ^2) of the association of category variables. Hardy-Weinberg Equilibrium (HWE) was assessed using a goodness-of-fit X2-test for biallelic markers (Available at http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). The association between haplotypes in both groups was done at (http://bioinfo.iconcologia.net/SNPstats_web). The threshold value for optimal sensitivity and specificity of VEGF was determined by ROC (receiver operating characteristics curve) (Metz, 1978). The cutoff value that maximized the sum of sensitivity and specificity was chosen for discrimination between VEGF association with ALL pathogenesis or not. All statistical analysis were performed using the software package SPSS for Windows, version 15.0 (SPSS Inc., Chicago, Illinois). The p-value was significant if ≤ 0.05 and highly significant if ≤ 0.01.

Accession numbers:

- VEGFA: GeneID: 7422
- VEGFC: GeneID: 7424
- VEGF primers: Accession number: NT-007592.15

RESULTS

Serum concentration of VEGF is significantly higher in ALL patients at diagnosis compared to healthy controls and to the patients after treatment and follow-up (ATF) for 28 days ($p<0.01$) for both as shown in Table 1.

Regarding clinical outcomes, There is a significant association between the VEGF levels at diagnosis and the clinical outcomes of the patients ($p = 0.05$) with highest levels noticed with died patients as shown in Table 1.

The ROC curve for determination of best cut-off value has been constructed to discriminate between ALL patients and controls. Arrow denotes cut off point at 856.16 pg mL^{-1}, at which the sensitivity is 97.5% and specificity, is 100% as shown in Fig. 1.

VEGF 460T/C and VEGF 1154G/A are genotyped using Allele specific PCR. A PCR for each sample of both groups has been performed twice, using primers with the specific sequence for each allele. The PCR products are 341 and 130 bp, respectively. The sizes of PCR products have been determined relatively to the migration of a 100 bp step ladder (Fig. 2).

There is no significant association between the VEGF serum levels in ALL patients and different genotypes of both.

The -460T/C and -1154G/A genotypes and allele frequencies in ALL patients and healthy controls are shown in Table 2. Hardy-Weinberg Equilibrium (HWE) test of SNP was performed. The genotype frequencies of the two SNPs-460T/C and -1154G/A in controls did not show any

Table 1: Plasma VEGF levels in the control group, patients group (at diagnosis and after treatment and follow-up) and different clinical outcomes of patients group

	Statistics	Plasma VEGF (pg mL^{-1})	χ^2	p-value
ALL patients at diagnosis (n = 40)				
	Median	3055.00	$^{a}\chi^2 = 45.27$	0.000**
			$^{b}\chi^2 = 42.11$	0.000**
	Mean ranks	45.48		
Control (n = 25)	Median	85.75		
	Mean ranks	13.04		
ALL patients ATF (n = 33)				
	Median	877.40		
	Mean ranks	41.36		
Plasma VEGF at diagnosis (clinical outcome)				
Complete remission (n = 22)	Median	3462.20	5.903	0.05*
	Mean ranks	21.73		
Resistant (n = 11)	Median	1879.40		
	Mean ranks	13.91		
Died (n = 7)	Median	3823.00		
	Mean ranks	27.00		
Plasma VEGF in ATF (clinical outcome)				
Complete remission (n = 22)	Median	800.00	1.997	0.158
	Mean ranks	15.32		
Resistant (n = 11)	Median	877.00		
	Mean ranks	20.36		

ATF: After treatment and follow-up, *p<0.05 is considered significant, **p<0.01 is considered highly significant, $^{a}\chi^2$: ALL patients at diagnosis versus control, $^{b}\chi^2$: ALL Patients at diagnosis versus ATF

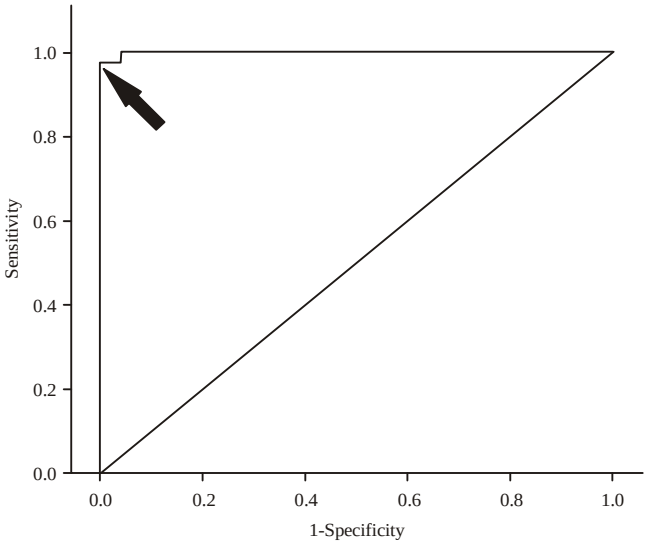

Fig. 1: ROC curve analysis for ELISA technique Plasma VEGF protein in patients group versus control groups at diagnosis to calculate the best cut off value. Area under the curve is 0.999, standard error is 0.002 and confidence limit is (0.996-1.002). Arrow denotes cut off point at 856.16 mg dL^{-1}, at which the sensitivity is 97.5% and specificity is 100%

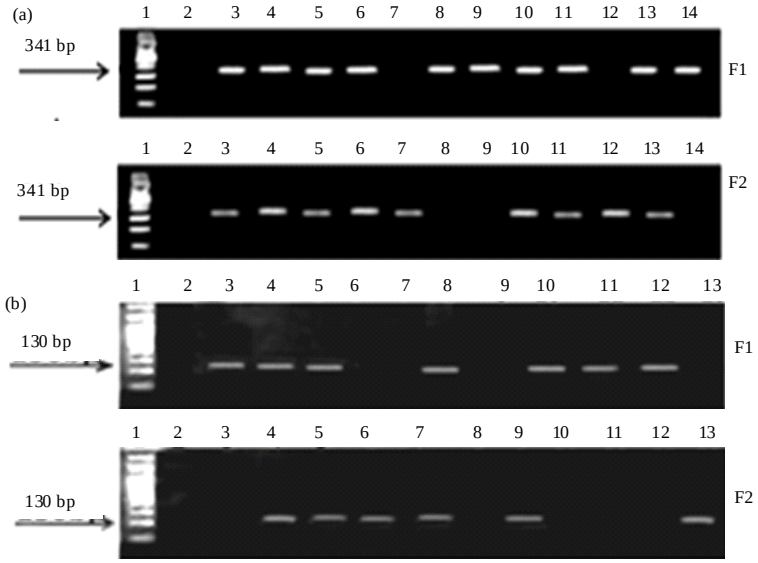

Fig. 2(a-b): Ethidium bromide stained agarose gel electrophoresis showing the Allele specific PCR product analysis of (a) 460T/C polymorphism at 341 bp. Lane 1:ladder, Lane 2: -ve control, Lanes 3, 4, 5, 6, 10, 11: Patient heterozygous TC genotype, Lanes 7, 12: Patient homozygous CC genotype, Lanes 8, 9: Control homozygous TT genotype, Lane 13: Control heterozygous TC genotype, Lane 14: Patient homozygous TT genotype and (b) 1154G/A polymorphism at 130 bp. Lane 1: Ladder, Lane 2: -ve control, Lanes 3, 8: Patient homozygous GG, Lanes 4, 5: Patient heterozygous GA genotype, Lanes 6, 7: Patient homozygous AA genotype, Lanes 9, 13: Control homozygous AA genotype, Lanes 10, 11, 12: Control homozygous GG genotype

Table 2: Genotype distribution and allelic frequency of the VEGF 460 T/C and 1154 G/A in all patients, normal controls and the outcome (http://bioinfo.iconcologia.net/SNPstats-web)

	ALL (n = 40)		Control (n = 25)		
Genotypes	n	%	n	%	p-value
VEGF -460T/C genotype					
T/T	8	20.0	14	56	0.006**
T/C	22	55.0	7	28	0.042*
C/C	10	25.0	4	16	0.539
Allele frequency					
T	38	47.5	35	70	0.018*
C	42	52.5	15	30	0.018*
VEGF -1154 G/A genotype					
G /G	17	42.5	15	60	0.207
G/A	12	30.0	7	28	1.00
A/A	11	27.5	3	12	0.216
Allele frequency					
G	46	57.5	37	74	0.063
A	34	42.5	13	26	0.063

	VEGF -460T/C genotype							VEGF -1154 G/A genotype						
	TT		TC		CC			GG		GA		AA		
Clinical outcome of ALL	n	%	n	%	n	%	p-value	n	%	n	%	n	%	p-value
Complete remission (n = 22)	5	22.7	9	40.9	8	36.4	5.80 (0.21)	10	45.5	6	27.3	6	27.3	1.965 (0.742)
Resistant (n =11)	1	9.1	9	81.8	1	9.1		3	27.3	4	36.4	4	36.4	
Died (n = 7)	2	28.6	4	57.1	1	14.3		4	57.1	2	28.6	1	14.3	

*Significant at ≤0.05, **p≤0.01, respectively

Table 3: Association between VEGF-460 T/C and -1154 G/A Haplotypes in All Groups. (http://bioinfo.iconcologia.net/SNPstats-web)

Haplotype	ALL (n = 40)	Control (n = 25)	p-value	OR (95% CI)
TG	(34.1%)	(50.5%)	---	1 (Reference)
CG	(23.3%)	(23.4%)	0.5	1.43 (0.5-4.12)
CA	(29.1%)	(6.5%)	0.019*	5.07 (1.35-9.02)
TA	(13.3%)	(19.4%)	0.97	0.98 (0.37-2.60)

*Significant at p≤0.05

significant deviation from HWE. Regarding patients, only the SNP -460T/C was consistent with in Hardy-Weinberg equilibrium while the SNP-1154G/A was out of Hardy-Weinberg equilibrium. There is no statistical significant association between clinical outcomes of the patients and different -460T/C and -1154 G/A genotypes (p≥0.05). The dominance of TC genotype has been noted in all the outcomes (Complete remission, resistance and died patients) and in -1154 G/A genotypes the GG genotype was the dominant in both complete remission and died patients as shown in Table 2.

VEGF 460T/C and 1154 G/A have 4 different haplotypes (TG, CG, CA and TA). The frequency and association of VEGF 460T/C and 1154 G/A haplotypes in patients with ALL and healthy controls. The TG was the most frequent haplotype in both patients and controls (34.1 and 50.5%, respectively). There was significant increase in the frequency of CA haplotype in patients compared to control group (p = 0.019) as shown in Table 3.

DISCUSSION

Acute Lymphoblastic Leukemia (ALL) is a heterogeneous group of disorders that result from the clonal proliferation and expansion of malignant lymphoid cells in the bone marrow, blood and other organs. It is the most common type of cancer in children and adolescents accounting for 23-25% of all malignant diseases (Masetti and Pession, 2009).

Recent clinical studies have suggested that local bone marrow angiogenesis with increased blood vessel density is important both for disease development and chemo-sensitivity in acute leukemias. Many studies have shown that patients with leukemia and lymphoma have increased micro-vascularity as well as increased levels of pro-angiogenic vascular growth factors, including VEGF (Sanaat *et al*., 2014; Zeng *et al*., 2014; Yang *et al*., 2015).

There are many evidences highlight the significant biological role of the VEGF-C/VEGF-R3 axis in vascular endothelial cells (Awata *et al*., 2002). The VEGF has an important role in the induction of neovascularization, thereby promoting tumor growth and metastatic potential. Besides that, autocrine and paracrine VEGF/VEGF-related loops were described in hematological malignancies such as acute and chronic leukemia, myelodysplastic syndromes, myeloproliferative neoplasms, lymphomas and multiple myeloma (Karjalainen *et al*., 2011; Sanaat *et al*., 2014).

This study was constructed to assess two main important points: The serum levels of VEGF at diagnosis and after the induction of chemotherapy in ALL patients in correlation to VEGF polymorphisms and the association of these polymorphisms to ALL and the clinical outcome of patients.

Two VEGF polymorphisms in ALL were evaluated. These polymorphisms are those located in the promoter region (-1154G/A, rs 1570360 and -460T/C, rs 833061).

Our results have showed that plasma VEGF levels are significantly higher in newly diagnosed cases of leukemia than controls ($p<0.01$). These results are in agreement with Avramis *et al*. (2006) and Stachel *et al*. (2007) who reported that, significantly elevated levels of VEGF are observed in a variety of hematological malignancies (Avramis *et al*., 2006; Stachel *et al*., 2007; Zeng *et al*., 2014).

Plasma VEGF levels at diagnosis were decreased significantly after complete remission ($p = 0.000$). These may be attributed to the effect of chemotherapy on the bone marrow. These results are in harmony with Yang *et al*. (2007). They found that plasma concentrations of ALL patients before treatment were significantly higher than those in normal controls ($p<0.01$) and the plasma concentrations of VEGF before treatment were significantly higher than those after complete remission ($p<0.05$) (Yang *et al*., 2007; Sanaat *et al*., 2014; Zeng *et al*., 2014).

On contrary, Kalra *et al*. (2012) and Sanaat *et al*. (2014) found lower levels of serum VEGF at the time of diagnosis of ALL as compared to the end of induction therapy, with the attainment of remission, serum VEGF rose to levels almost similar to those of healthy controls. These results confirm the study by Yetgin *et al*. (2001) and Aref *et al*. (2007) that found significantly lower serum level of VEGF at the time of diagnosis than those of the control group and of the patients in remission (Yetgin *et al*., 2001; Aref *et al*., 2007). Considering that VEGF is expressed in normal hematopoietic cells (Kalra *et al*., 2012) hypothesized that patients suffering from ALL, the proportion of hematopoietic cells is decreased in comparison with tumor cells and consequently, the level of VEGF may be lower (Kalra *et al*., 2012). However, during remission, the renewal of normal hematopoiesis may explain the rise of serum VEGF near normal levels, as evidenced in their reports and in this study.

In the current study there is significant association between the VEGF levels and the clinical outcome of the patients ($p = 0.05$) with highest levels noticed with dead patients. The first results concerning the prognostic value of angiogenic factors were reported in AML adult patients, showing that high levels of cellular VEGF were significantly correlated with shorter survival of patients ($p<0.04$) (Molica *et al*., 2007).

There are more than 350 studies examining the association between VEGF genotypes and human diseases including hematological malignancies, solid tumors and other diseases (Rogers and D'Amato, 2012). Only few studies investigated the association between VEGF SNPs and childhood ALL (Demacq *et al*., 2010), but our study is among the rare studies that evaluate VEGF SNPs in relation to adult ALL which is the main novelty of this study.

Analysis of -460T/C SNP has revealed that: The TC genotype is more frequent in ALL patients (55%) ($p = 0.042$) with increase in the frequency of C allele (52.5%) over T allele ($p = 0.018$) while TT genotype is significantly higher in control group (56%) ($p = 0.006$) with increase in the frequency of T allele (70%) over C allele.

On the other hand, analysis of 1154 G/A SNP has revealed no statistical significant change in the distribution of 1154G/A genotypes between ALL patients (42.5% GG, 30% GA and 27.5% AA), with increase in the frequency of the G allele (57.5%) over the A allele. GG genotype is more frequent in control group (60%), with increase in the frequency of G allele (74%) over A allele.

Median serum VEGF level is higher in both 460TT and 1154 AA genotypes (3718 and 3499.65 pg mL^{-1}, respectively). However, the relationship between the VEGF levels in ALL patients and different genotypes of both VEGF 460T/C and 1154 G/A SNPs, does not reach to statistical significance.

Regarding clinical outcome, in the current study, there is no statistical significant association between both 460T/C and 1154G/A different genotypes and the clinical outcome of the patients ($p>0.05$), but the dominance of 460 TC genotype has been noted in resistance to treatment 81.8% but it does not reach to statistical significance. While, 1154 GG genotype was dominant in both complete remission (45.5%) and dead patients (57.1%). The VEGF 460T/C and 1154 G/A., have 4 different haplotypes (TG, CG, CA and TA). The TG was the most frequent haplotype in both patients and controls (34.1 and 50.5%, respectively). There was significant increase in the frequency of CA haplotypes in patients compared to control group ($p = 0.019$).

Present results were in harmony with (Demacq *et al*., 2010), who examined the association of three VEGF SNPs (-2578C/A, -1154G/A and -634G/C) with prognosis of childhood ALL (Demacq *et al*., 2010). On the other hand, another study evaluated the genetic effects of VEGF-460T/C polymorphism on the development of lung cancer. It showed that the TT genotype was associated with increased lung cancer risk than those with the CC genotype. Moreover, it was observed that the TT genotype associated with the advanced stage among lung cancer patients (Sun *et al*., 2013). Agreeing with our results, a retrospective long-term study, in 113 Oral Squamous Cell Carcinoma (OSCC), a significant increased incidence of OSCC in smokers with the VEGF-460 TC genotype was seen ($p<0.0001$). Patients with-1154AA allele had a significant worse survival and a worse disease-free survival ($p<0.04$) (Kammerer *et al*., 2013). Chen *et al*. (2011) observed a marked increase in intra-tumor and circulating VEGF levels in patients with the TC or CC genotypes ($p = 0.01$) (Chen *et al*., 2011).

Association between a disease and VEGF polymorphisms does not necessarily mean that the disease is angiogenesis dependent (Rogers and D'Amato, 2012). For example, Huang *et al*. (2013) and Debrah *et al*. (2007) assessed the role of VEGF genetic polymorphisms in acute macular degeneration and in hydrocele development in a cohort of lymphatic filariasis patients, respectively (Debrah *et al*., 2007; Huang *et al*., 2013). However, because these conditions are characterized by extensive vascular leak, they are likely that the effect is mediated by induction of vascular permeability by VEGF, rather than by angiogenesis. These results were in concordance with our results, in the higher association of the different diseases with C allele than T allele of the

VEGF -460 T/C SNP. However, Debrah *et al*. (2007) were not consistent with our regarding the VEGF levels and different genotypes of VEGF 460T/C SNP, as they found that the -460 CC homozygous individuals had the highest plasma VEGF levels, followed by TC heterozygous individuals, whereas, the homozygous TT individuals had the lowest plasma VEGF levels (Debrah *et al*., 2007). The reason of this discrepancy can be explained by different tissue pathologies that may result in considerable variability in concentrations in different genotypes.

Present results are in harmony with a study that performed a haplotype analysis for the VEGF (-460, +405, -1154 and -2578) in association with endometriosis. It reported that the haplotypes that increased the risk for endometriosis were (460C/-1154A/-2578C) (De Trovo, 2012).

It is clear now that polymorphisms in angiogenesis-regulating genes can affect a large number of phenotypes, including a wide variety related to disease processes in man. The absence of association doesn't imply that the gene/protein in question is not involved in disease. Rather, it may simply mean that the functional polymorphisms in the gene have not been identified or do not exist at sufficient frequency in the study populations (Del Bo *et al*., 2008). It is likely that the sum of angiogenesis-regulating variation plays a major role in determining the length and quality of life (Rogers and D'Amato, 2012).

CONCLUSION

This study provides evidence that some VEGF genotypes may have impact on ALL pathogenesis and treatment outcome and that these effects may possibly be due to altered VEGF expression according to VEGF polymorphisms. These findings may help toward clarifying the mechanisms of ALL development and course of events. As well as, emphasize on the importance of targeting VEGF as an interesting treatment strategy, not only for the inhibition of angiogenesis, but to disrupt the autocrine loop associated with the survival and invasiveness of leukemic cells. Therefore, new approaches for inhibition of VEGF could be a therapeutic target to limit aggressiveness of not only proliferative forms of ALL, but also other hematological malignancies, solid tumors and other human diseases.

However, more prospective multicentric studies enrolling larger number of adult ALL patients with longer follow up periods of acute leukemic patients with variable VEGF gene polymorphisms are mandatory to analyze the influence of angiogenesis in leukemogenesis and the potential interest of angiogenic therapies and to put emphasis on the possible use of VEGF and its gene SNPs as a useful marker for monitoring minimal residual disease.

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