Vascular endothelial growth factor & basic fibroblast growth factor polymorphism as prognostic factors in Non-Hodgkin Lymphoma

Rania M. Bakry,[1]Ebtesam M. El-Gezawy,[2]Abeer M. Darwish,[3]Eman Nasr Eldin,[4]Noha Gaber,[5] Khalid A. Nasif, [6]Essam Abd El-Mohsen,[7] Salma Mahfouz.[8]

1- **Professor of clinical pathology, South Egypt cancer Institute, AssiutUniversity. E-mail address: rbakry.md@gmail.com**
2- **Professor of Clinical Pathology, Faculty of Medicine, Assiut University. E-mail address: Eelgezawy1@yahoo.com**
3- **Lecturer of clinical pathology, South Egypt cancer Institute, Assiut University. E-mail address: Abeer_mdarwish@yahoo.com**
4- **Associate Professor of Clinical Pathology, faculty of medicine, Assiut University.E-mail address: emannasr2000@yahoo.com**
5- **Lecturer of Oncological Clinical Pathology, South Egypt Cancer Institute, Assiut University. E-mail address:noha.gaber@yahoo.com**
6- **Associate Professor of Biochemistry Department, Faculty of medicine, Minya University. E-mail address: Nasifkhalid@yahoo.com**
7- **Associate Professor of Internal Medicine, Faculty of Medicine, Assiut University. E-mail address: essam_223@yahoo.com**
8- **Demonstrator of clinical pathology, South Egypt cancer Institute, Assiut University. E-mail address: dr.salma108@hotmail.com**

Abstract

Lymphoma growth and progression may be enhanced by angiogenesis. Both vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) play an important role in this process. The present study aimed to investigate the association of VEGF -936C/T and bFGF -921C/G gene polymorphisms with the clinicopathological characteristics of the studied patients and progression of NHL disease.

Patients and methods: Clinico-hematological profiles were done for 50 NHL patients. The genotypes and allelic frequencies of VEGF and bFGF polymorphisms were detected using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). PCR products after adding restriction endonuclease were analyzed using QIAxcel advanced (automated) instrument.

Results: there was a trend of significance of VEGF 936-T allele among NHL patients with advanced clinical staging compared to patients with early stages with ***P*** value 0.082. As regards the progression of the disease determined by international prognostic index (IPI), it was noticed that there was a borderline significance between VEGF CT genotype and IPI score among patients with intermediate high/high IPI compared to patients with low/intermediate low IPI with ***P*** value 0.074. The bFGF 921-G variant was associated frequently with aggressive histological subtype of NHL and showed a borderline significance with NHL patients presented with intermediate high/high IPI score and BM infiltration with ***P*** value 0.098 and 0.054 respectively.

Conclusion: our results demonstrated that VEGF-936C/T and bFGF-921C/G gene polymorphisms may potentially affect the progression of NHL. Further larger scale studies are needed.

Keywords:
Non-Hodgkin's lymphoma, vascular endothelial growth factor, basic fibroblast growth factor.

Introduction

Non-Hodgkin's lymphoma (NHL) is a highly heterogeneous group of lymphoproliferative malignancies. Immune-suppression, genetics, and exposure to chemical agents have contributed to increase the incidence of NHL ***(1).*** The management of NHL is primarily influenced by histology and stage ***(2).*** According to**,** Ferlay et al***, (3),*** the estimated incidence of NHL is 5/100,000 (385,741 new cases), with a mortality rate of 2.5/100,000 (199,630 deaths) worldwide.Genetic susceptibility studies of lymphoma may serve to identify at risk populations and clarify important disease mechanisms ***(4).*** Angiogenesis is a crucial process in the growth, development, and metastasis of many tumor types, including NHL ***(5).*** Both VEGF and bFGF play an important role in this process.VEGF is a prime determinant and regulator of angiogenesis by autocrine stimulation of tumor cells as well as paracrine influences of the proangiogenic tumor microenvironment ***(6).***Over expression of angiogenic factors in particular VEGF in most of hematologic malignancies may explain the increased angiogenesis found in these malignancies and correlate with poor prognosis as well as decreased overall survival ***(7).***Also, bFGF plays an important role in promoting angiogenesis by increasing cell proliferation and stimulating migration. Expression of bFGF has been reported to becorrelated with poor survival and aggressive lymphoma***(8).*** The aim of the present study was to detect VEGF-936C/T and bFGF-921C/G gene polymorphisms in patients with NHL using PCR-RFLP technique and to correlate with the clinicopathological characteristics of our studied patients to determine an association between them and progression of NHL disease.

2. Patients and Methods

2.1. Patients and Controls:- A prospective randomized study was carried on 50 Egyptian patients from 2015 to 2017. They were 27 males versus 23 females and their ages ranged from 28 to 80 years old with mean age of (55.20 ± 13.05). The patients were presented at the outpatient clinic of Medical Oncology Department at South Egypt Cancer Institute, Assiut University and the Department of Internal Medicine, Faculty of Medicine, Assiut University after approval of the medical ethics committee of Faculty of Medicine, Assiut University with IRP number (17100381) and all the participants gave informed consent. In addition, 50 individuals (healthy blood donors) served as control group. Their ages ranged from 25 to 50 years old. They were 32 males and 18 females. Patients were diagnosed as NHL on the basis of lymph node excisional biopsy, bone marrow aspirate/biopsy and immunohistochemical studies ***(9).*** The stage of the disease was assessed using An Arbor staging, the performance status was determined using the Eastern Cooperative Oncology Group (ECOG) criteria ***(10)*** while the prognosis was assessed using IPI ***(11).*** All patients were subjected to full history taking, clinical examination and laboratory investigations. For patients' demographic data and characteristics, (table 1, 2) 2.2 Genotyping of VEGF -936C/T and bFGF -921C/G gene polymorphisms by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay ***(12)***:- DNA was isolated from the peripheral whole blood taken on EDTA using the Qiagen DNA isolation kit (QiAmp Blood Kit, Qiagen GmbH, Hilden, Germany, Lot No: 148026863) following the recommendations of manufacturer. a) For VEGF 936C/T (rs3025039) genotyping, the following primers: 5′-AAG GAA GAG GAG ACT CTG CGC AGA GC-3′(forward) and 5′-TAA ATG TAT GTA TGT GGG TGG GTG TGT CTA CAG G-3′(reverse) were used to get a 211-bp fragment. The PCR reaction was performed in a total volume of 25 µl containing 12.5 µl MyTaqRed Mix, 2x (BIO-25043 : 200 x50µL reactions : 4x 1.25 ml), 0,3 µl forward primer, 0,3 µl reverse primer and 1 µl genomic extracted DNA. The thermocycler (ARKTIC, Thermo scientific, Vantaa, Finland, SN: Akc481205729) program applied was 95 °C for 3 min, followed by 30 cycles of 95 °C for 15 s, 60 °C for 15 s and 72 °C for 10 s , with a final extension step at 72 °C for 7 min. b) For bFGF 921C/G (rs308395), genotyping was performed by using the following primers: 5′-TGA GTT ATC CGA TGT CTG AAA TG-3′ (forward) and 5′-TAAC TTG AAT TAG ACG ACG CAG A-3′ (reverse) to generate a 482-bp fragment. The PCR reaction was performed in a total volume of 25 µl containing 12.5 µl My Taq Red Mix, 1 µl forward primer, 1 µl reverse primer and 1 µl genomic extracted DNA. The PCR cycling conditions were as follows: 95°C for 3 min followed by 30 cycles of 95 °C for 15 s, 60 °C for 15 s and 72 °C for 10 s, with a final elongation step at 72 °C for 7 min. Analysis of PCR products was done using Electrophoresis in a 2% agarose gel with ethidium bromide and visualized under a UV-transilluminator and the results were recorded by Photography.

2.3. The PCR products of VEGF and bFGF were digested with Hin1II (Thermo Scientific Fast Digest enzyme, Third Avenue Waltham, MA168, USA, Lot N:00302448) and BseNI (Thermo Scientific Fast Digest enzyme, Third Avenue Waltham, MA168, USA,Lot N:00301809) restriction endonucleases, respectively and analyzed using equipments and reagents of QIAxcel DNA Screening Kit (QIAGEN GmbH, Hilden, Germany, Lot N:154026133) of QIAxcel advanced instrument (QIAGEN, Hilden, Germany) figure (1,2). For VEGF, the following patterns were observed: 211 bp fragment (individuals homozygous for VEGF C allele), three fragments of 211, 122 and 86 bp fragments (heterozygous individuals) or two fragments of 122 and 86 bp fragments (individuals homozygous for the variant T allele). For bFGF polymorphism, the original 482 bp fragment was detected in individuals homozygous for bFGF C allele, three fragments of 482, 397 and 67 bp fragments were observed in heterozygous individuals or two fragments of 397 and 67 bp fragments in individuals homozygous for the variant G allele.

2.4. Statistical analysis Data entry and data analysis were done using computer program SPSS version 19 (Statistical Package for Social Science). Data were presented as number, percentage, mean± SD. For statistical evaluation, Chi-square test was used to compare between qualitative variables, Independent sample t-test was used to compare quantitative variables and Mann-whitney test was used to determine significance for numerical variables that were non parametric. Hardy-Weinberg equation was applied for determination of allelic frequencies from genotypic frequencies.

Probability value< 0.05 was considered significant while that between 0.05 and 0.1 were considered as indicative of a trend.

3. Results

3.1. Genotypes and allelic frequencies of the studied polymorphisms in NHL patients and controls are presented in (table 3) where the VEGF CC, CT, and TT genotypes were respectively detected in 44 (88%), 6 (12%) and none of the patients. The entire control group presented with homozygous wild genotype (CC) and none of them showed the heterozygous or homozygous mutated genotypes. Regarding bFGF, 48 patients (96%) presented with homozygous wild genotype (CC), 2 (4%) were heterozygous genotype (CG) and none of them presented with homozygous mutated genotype (GG). All the control group presented with homozygous wild genotype (CC). The allelic frequencies were as the following: VEGF for patients—C: 0.94, T: 0.06 and controls-- C: 1.0, no T allele presented in control group while bFGF allelic frequencies were C: 0.98, G: 0.02 for patients and C:1.0 for controls, no G allele presented in control group.

3.2. The relation between VEGF gene polymorphism and the clinical and pathological data of the studied patients revealed that there was a trend of significance of VEGF T allele among NHL patients with advanced clinical stages compared to patients with early stages with ***P*** value 0.082. Also, the correlation between VEGF CT genotype and the progression of the disease could be detected where there was a trend of significance between VEGF CT genotype and IPI score among patients with intermediate high/high IPI compared to patients with low/intermediate low IPI with ***P*** value 0.074. NHL patients presented by IPI (3 or 4) score were more frequently carrying the VEGF T allele, they were (66.7%) (table 4). No other significant relations could be detected as regard gender, response to treatment, histological aggressiveness and bone marrow infiltration.

3.3. Comparison between patients harboring the wild or the polymorphic genotypes of bFGF showed that there was a borderline significance of bFGF polymorphic variant (CG genotype) among patients with intermediate high/high IPI score compared to patients with low/intermediate low IPI and also among patients with BM infiltration compared to those with no involvement with ***P*** value 0.098, 0.054 respectively (table 4).We found that there was no statistical significance between bFGF G variant and histological aggressiveness of NHL patients, but patients presented with bFGF G variant were aggressive in histological subtype. They were 2 out of 21 patients with aggressive NHL compared to none of the patients carried the G variant out of 29 patients with indolent NHL (table 5). No other significant relations could be detected regarding gender, response to treatment andAn Arbor staging.

4. Discussion

NHL is one of the most common malignancies all over the world and its incidence increased significantly during the last years.

Angiogenesis plays an important role in the progression and maintenance of many hematopoietic malignancies including NHL. Both VEGF and bFGF have role in the initiation of angiogenesis In previous study of Wróbel et al, reported that the VEGF CC, CT, and TT genotypes were respectively detected in 61 (78%), 17 (22%) and none of the patients and in 101 (83%), 13 (11%) and 8 (6%) controls ***(12).*** comparing the VEGF (936 C/T) genotypes and allelic frequencies among our patients and controls with this study revealed that there was a discrepancy in the frequency of VEGF genotypes (CC/CT) among our patients and the study of Wróbel et al with uncommonly presence of heterozygous (CT) and homozygous mutated (TT) genotypes in his control group. It may be due to Functional polymorphisms, which have an effect on the regulation of gene expression, can contribute to the differences between individuals in susceptibility and severity of a disease. The effect may be seen by a polymorphism alone, or in combination with other polymorphisms ***(13).*** A trend of significance between VEGF CT genotype and IPI among patients with IPI score 3 or 4 compared to patients with IPI score 1 or 2 was noticed with ***P*** value 0.074. It was in agreement with Wróbel et al, ***(12)*** who reported a strong tendency towards the higher frequency of the VEGF T allele among patients with intermediate high/high IPI (3 or 4) as compared to patients with low or intermediate IPI (1 or 2) (P = 0.077). On the contrary to Wróbel et al, our study showed another different result where there was a trend of significance between VEGF T allele and Ann Arbor staging as the T variant was more frequent in late stages with P value 0.082. Hefler et al, pointed out that metastatic disease frequency was more accentuated among the +936TT carriers ***(14).*** However, Yapijakis et al noted that the low-VEGF-production T allele is strongly associated with increased risk for oral cancer ***(15)***. Also, previous studies concerning this polymorphism yielded that the T allele may confer protection especially against breast cancer and small cell lung carcinoma ***(16, 17, 18).*** The +936CC genotype influences the VEGF plasma levels positively, possibly resulting in the facilitation of angiogenesis with clinical manifestations and to the tumor cells' clonal advantage for further expansion. On the other hand, Polterauer et al presented the VEGF +936C/T gene polymorphisms were not associated with prognosis in patients with ovarian cancer***(19).***The human VEGF gene is highly polymorphic and there is considerable variation between individuals in VEGF expression ***(20).*** In cancer cases, genotype frequencies in individuals are influenced by different genetic background ***(21).*** A balanced explanation for most of the relationships between the SNPs and the clinical behavior of the tumors still remains largely obscure. VEGF gene functional alteration, either towards the activating or the repressing side, possibly in synergy with differential expression or action of co-factor molecules may be a rational explanation ***(22).*** bFGF gene Polymorphism plays an important role in promoting angiogenesis. The presence of the bFGF −921G variant was more frequently detected among NHL patients with aggressive histological subtype of the disease ***(12).***Comparing the frequency of bFGF genotypes in the current study and another study of Wróbel et al, revealed that there was higher frequency of (CG) genotype in his patients with uncommonly presence of the heterozygous and homozygous mutated genotypes in the control group. Wróbel et al, found the bFGF CC and CG genotypes in 57 (73%) and 21 (27%) patients and in 99 (81%) and 22 (18%) controls, respectively. The GG homozygosity was very rare. Only one healthy individual was carrying this genotype ***(12)***. This discrepancy may be explained as different genes in different individuals are thought to cause genetic susceptibility to the disease ***(23).*** To assess whether bFGF genotypes are associated with unfavorable progression of NHL in our study, distributions of bFGF alleles and genotypes were compared among patients with different clinical characteristics and IPI. It was found that there was no statistical significance between bFGF G variant and histological aggressiveness of our NHL patients, but our patients presented with bFGF G variant were aggressive in histological subtype. They were 2 out of 21 patients with aggressive NHL compared to none of the patients was carrying the G variant out of 29 patients with indolent NHL. On the other hand, Wróbel et al, found that aggressive NHL patients were more than twice as frequently presented with the bFGF G variant as those with the indolent histological type. Among 38 patients with aggressive NHL, 14 (37%) were carrying the G variant as compared to 7 out of 40 patients (18%) with indolent disease (***P*** = 0.095) ***(12).***The presence of significance in the previous study may be due to different statistical analysis and different environmental conditions between both studies. In contrast to Wróbel et al, our study showed a trend of significance between bFGF G variant and BM infiltration and IPI score with ***P*** value 0.054 and 0,098 respectively. These results were not

observed by Wróbel et al as a polymorphic variant of a gene can lead to the abnormal expression or to the production of an abnormal form of the protein that may be un-functioning or with different functions. This abnormality may cause or be associated with the disease ***(24).*** The previous results could be confirmed by Ria et al, who reported that soluble bFGF levels that decline after radiotherapy in NHL, suggesting that may have predictive significance for response to treatment and recurrence ***(8)***. Also, in malignant lymphoma, high pretreatment levels of bFGF were a prognostic factor for survival in multivariate analysis, independently of other risk factors, including serum lactate dehydrogenase and number of extranodal sites ***(25).*** In conclusion, it was found that VEGF and bFGF were associated with increased susceptibility and progression of the disease. The VEGF 936T allele showed a trend of significance towards worse prognosis and advanced staging while the bFGF 921G allele was associated frequently with aggressive histological subtype of the disease.

Conflict of interest The authors declare no conflict of interest

References:-

1. Parkin, D.M., Bray, F., Ferlay, J., Pisani, P. Global cancer statistics, (2002). CA Cancer J Clin 2005;55:74-108.
2. Winkfield,k.M., Tsang, R.W., Gospodarowicz. M.K., (2016). Non-Hodgkin's lymphoma in Clinical Radiation Oncology (fourth edition); 77: 1524-1546.
3. Ferlay, J., Soerjomataram, I., Ervik, M., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D.M., Forman, D., Bray, F., GLOBOCAN (2012) v1.0. Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11. Lyon, IARC, 2013.
4. Skibola, C.F., Curry, J.D., Neiters, A., (2007). A genetic susceptibility to lymphoma.Haematologica; 92:960.
5. Hanahan, D., Weinberg, R.A., (2011). Hallmarks of Cancer: The Next Generation. Cell; 144, 646–674.
6. Ho, Q.T., Kuo, C.J.,(2007). Vascular endothelial growth factor: Biology and therapeutic applications. The International Journal of Biochemistry & Cell Biology 39, 1349–1357.
7. Dong, X., Han, Z.C., Yang, R., (2007).Angiogenesis and antiangiogenic therapy in hematologic malignancies.Crit Rev OncolHematol; 62:105–11.
8. Ria, R., Cirulli, T., Giannini, T., Bambace, S., Serio, G., Portaluri, M., Ribatti, D., Vacca, A., Dammacco, F., (2008). Serum levels of angiogenic cytokines decrease after radiotherapy in non-Hodgkin lymphomas. ClinExp Med; 8:141–145.
9. Ravichandran, S., Nair, R., (2015). Approach to lymphadenopathy: A focussed discussion on non-Hodgkin lymphoma.
10. Liu, M.A., Hshieh, T., Condron, N., Wadleigh, M., Abel, G.A., Driver, J.A., (2016). Relationship between physician and patient assessment of performance status and survival in a large cohort of patients with haematologic malignancies. British Journal Of Cancer; 115, 858.
11. Zhou, Z., Sehn, L.H., Rademaker, A.W., Gordon, L.I., LaCasce, A.S., Crosby-Thompson, A., Vanderplas, A., Zelenetz, A.D., Abel, G.A., Rodriguez, M.A., Nademanee, A., Kaminski, M.S., Czuczman, M.S., Millenson, M., Niland, J., Gascoyne, R.D., Connors, J.M., Friedberg, J.W., Winter, J.N., (2014). An enhanced International Prognostic Index (NCCN-IPI) for patients with diffuse large B-cell lymphoma treated in the rituximab era. Blood; 123: 837–842.
12. Wróbel, T., Mazur, G., Dzietczenia, J., Gębura, K., Kuliczkowski, K., Bogunia-Kubik, K.,(2013). VEGF and bFGF Gene Polymorphisms in Patients with Non-Hodgkin's Lymphoma. BioMed Research International; 1–6.
13. Jin, Q., Hemminki, K., Enquist, K., Lenner, P., Grzybowska, E., Klaes, R., Henriksson, R., Chen, B., Pamula, J., Pekala, W., Zientek, H., Rogozinska-Szczepka, J., Utracka-Hutka, B., Hallmans, G., Försti, A., (2005).Vascular endothelial growth factor polymorphisms in relation to breast cancer development and prognosis.Clin Cancer Res; 11:3647–365.
14. Hefler, L.A., Mustea, A., Könsgen, D., Concin, N., Tanner, B., Strick, R., Heinze, G., Grimm, C., Schuster, E., Tempfer, C., Reinthaller, A., Zeillinger, R., (2007). Vascular endothelial growth factor gene polymorphisms are associated with prognosis in ovarian cancer. Clin Cancer Res; 13:898–901.
15. Yapijakis, C., Vairaktaris, E., Vassiliou, S., Vylliotis, A., Nkenke, E., Nixon, A.M., Derka, S., Spyridonidou, S., Vorris, E., Neukam, F., Patsouris, E., (2007). The low

VEGF production allele of the +936C/T polymorphism is strongly associated with increased risk for oral cancer. J Cancer Res ClinOncol; 133:787–791.

16. Krippl, P., Langsenlehner, U., Renner, W., Yazdani-Biuki, B., Wolf, G., Wascher, T.C., Paulweber, B., Haas, J., Samonigg, H., (2003). A common 936 C/T gene polymorphism of vascular endothelial growth factor is associated with decreased breast cancer risk. Int J Cancer; 106:468–471.

17. Lee, S.J., Lee, S.Y., Jeon, H.S., Park, S.H., Jang, J.S., Lee, G.Y., Son, J.W., Kim, C.H., Lee, W.K., Kam, S., Park, R.W., Park, T.I., Kang, Y.M., Kim, I.S., Jung, T.H., Park, J.Y., (2005). Vascular endothelial growth factor gene polymorphisms and risk of primary lung cancer. Cancer Epidemiol Biomarkers Prev; 14:571–575.

18. Kataoka, N., Cai, Q., Wen, W., Shu, X.O., Jin, F., Gao, Y.T., Zheng, W., (2006). Population-based case-control study of VEGF gene polymorphisms and breast cancer risk among Chinese women. Cancer Epidemiol Biomarkers Prev; 15:1148–1152.

19. Polterauer, S., Grimm, C., Mustea, A., Concin, N., Tanner, B., Thiel, F., Heinze, G., Reinthaller, A., Zeillinger, R., Hefler, L.A., (2007). Vascular endothelial growth factor gene polymorphisms in ovarian cancer. GynecolOncol; 105:385–389.

20. Stevens, A., Soden, J., Brenchley, P.E., Ralph, S., Ray, D.W., (2003). Haplotype analysis of the polymorphic human vascular endothelial growth factor gene promoter. Cancer Res; 68:812–816.

21. Diao, L.P., Yu, X.M., Gao, Y.H., Li, Y., Liu, H.S., Liu, L.H., Zhou, R.M., Wang, Na., Wu, L.L., Wang, S.J., (2009).Association of VEGF genetic polymorphisms with the clinical characteristics of non-Hodgkin's lymphoma.J Cancer Res ClinOncol; 135: 1473-1481.

22. Kim, E.J., Jeong, P., Quan, C., Kim, J., Bae, S.C., Yoon, S.J., Kang, J.W., Lee, S.C., JunWee, J., Kim, W.J., (2005). Genotypes of TNF-a, VEGF, hOGG1, GSTM1, and GSTT1: useful determinants for clinical outcome of bladder cancer. Urology; 65:70–75.

23. Mathew, C., (2001). Science medicine and the future—postgenomic technologies: Hunting the genes for common disorders. British Medical Journal; 322(7293):1031–1034.

24. Wu, C.C., Gupta, T., Garcia, V., Ding, Y., Schwartzman, M.L., (2014). 20-HETE and blood pressure regulation: clinical implications. Cardiology in Review; 22 (1): 1–12.

25. Ribatti, D., Nico, B., Ranieri, G., Specchia, G. &Vacca, A., (2013). The Role of Angiogenesis in Human Non-Hodgkin Lymphomas. Neoplasia; 15, 231–238

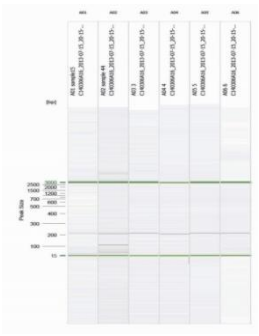

Figure (1)

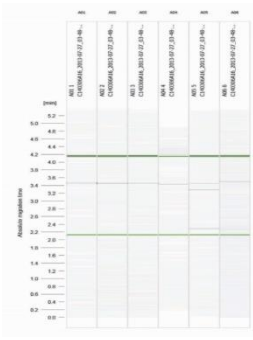

Figure (2)Figure (1): Analysis by QIAxcel instrument after digestion of PCR products with Hin1II RE. All columns show one band at 211 bp (homozygous wild type, CC) while the second column shows 3 bands at 211, 122, 86bp (heterozygous genotype, CT)

Figure (2): Analysis by QIAxcel instrument after digestion of PCR products with BseNIRE.All columns show one band at 482 bp (homozygous wild type, CC) while the fifth column from the left side shows 3 bands at482, 397, 67bp (heterozygous genotype, CG).

Table (1):The demographic data of NHL patients and the controls group

	Patients (n= 50)		**Controls (n= 50)**		P-value
	No.	**%**	**No.**	**%**	
Sex:					0.309
Male	27	54.0	32	64.0	
Female	23	46.0	18	36.0	
Age: (years)					0.001 *
Mean ± SD	55.20 ± 13.05		36.24 ± 7.48		
Range	28.0 – 80.0		25.0 – 50.0		

Chi-square ***test and Independent samples t-test***
Significant P value < 0.05
N: number
SD: standard deviation

Table (2): Clinical and pathological characteristics of the studied NHL patients

	No. (n= 50)	**%**
Ann Arbor staging:		
Grade I	20	40.0
Grade II	7	14.0
Grade III	8	16.0
Grade IV	15	30.0
Performance status:		
< 2	39	78.0
≥ 2	11	22.0
IPI risk group:		
Low risk	26	52.0
Intermediate low	8	16.0
Intermediate high	12	24.0
High risk	4	8.0
Extra-nodal sites:		
Yes	17	34.0
No	33	66.0
Histological		
Indolent	**29**	**58.0**
FL	5	10.0
SLL	4	8.0
MZL	3	6.0
Other B-cell lymphoma	17	34.0
Aggressive	**21**	**42.0**
DLBCL	20	40.0
Peripheral T-cell	1	2.0
BM		
No infiltration	38	76.0
Infiltration	12	24.0

IPI:*International prognostic index,****BM****:Bone Marrow , ****N****:number, ****FL:****Follicular lymphoma, ****SLL****:Small lymphocytic lymphoma,****MZL****:Marginal zone lymphoma&****DLBCl****:Diffuse large Bcell lymphoma*

Table (3): The frequency of VEGF and bFGF genotypes and alleles in NHL patients and controls

	Patients (n= 50)		Controls (n= 50)		P-value
	No.	%	No.	%	
VEGF:					
CC	44	88.0	50	100.0	0.027*
CT	6	12.0	0	0.0	
C	0.94	-	1	-	
T	0.06	-	-	-	
BFGF:					
CC	48	96.0	50	100.0	0.910
CG	2	4.0	0	0.0	
C	0.98	-	1	-	
G	0.02	-	-	-	

***VEGF** :Vascular endothelial growth factor, **bFGF**:Basic fibroblast growth factor.*
Chi-square test for calculation of genotypic frequencies between the study groups
Hardy-Weinberg equation for calculation of allelic frequencies between the study groups
**Significant P value < 0.05*

Table (4): The relation between VEGF-936 gene polymorphism and clinical and pathological data of the studied patients

Clinical and pathological data	VEGF				P-value
	CC (n= 44)		CT (n= 6)		
	No.	%	No.	%	
Response to treatment:					
Complete remission	23	52.3	4	66.7	0.674
Partial remission	14	31.8	1	16.7	0.654
No response	7	15.9	1	16.7	1.000
An arbor staging:					
Grade I/ Grade II	26	59.1	1	16.7	0.082
Grade III/ Grade IV	18	40.9	5	83.3	
IPI risk group:					
Low risk/ Intermediate low	32	72.7	2	33.3	0.074
Intermediate high/ High risk	12	27.3	4	66.7	
Histological aggressiveness:					0.223
Indolent	27	61.4	2	33.3	
Aggressive	17	38.6	4	66.7	
BM:					
No infiltration	35	79.5	3	50.0	0.141
Infiltration	9	20.5	3	50.0	

***VEGF**: Vascular endothelial growth factor, **IPI:** International prognostic index, **BM**:Bone marrow*
Chi-square test
Significant P value < 0.05

Table (5): The relation between bFGF -921 gene polymorphism and clinical and pathological data of the studied patients

Clinical and pathological data	bFGF				P-value
	CC (n= 48)		CG (n= 2)		
	No.	%	No.	%	
Response to treatment:					
Complete remission	27	56.3	0	0.0	0.207
Partial remission	14	29.2	1	50.0	0.514
No response	7	14.6	1	50.0	0.297
An arbor staging:					0.207
Grade I/ Grade II	27	56.3	0	0.0	
Grade III/ Grade IV	21	43.8	2	100.0	
IPI risk group:					0.098
Low risk/ Intermediate low	34	70.8	0	0.0	
Intermediate high/ High risk	14	29.2	2	100.0	
Histological aggressiveness:					0.171
Indolent	29	60.4	0	0.0	
Aggressive	19	39.6	2	100.0	
BM:					0.054
No infiltration	38	79.2	0	0.0	
Infiltration	10	20.8	2	100.0	

***bFGF**: basic fibroblast growth factor, **IPI:**International prognostic index,**BM**:Bone marrow*
Chi-square test
Significant P value < 0.05