



Fibroblast Growth Factor Receptor 4 Gly388Arg Gene Polymorphism and Non-Hodgkin Lymphoma Susceptibility and Prognosis in Egyptian population: Case-control Study

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Abstract

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BACKGROUND: Angiogenesis is a multistep process having an essential role in the growth and progression of various tumors including hematolymphoid malignancies. Basic fibroblast growth factor (bFGF) is one of angiogenic growth factors which level is considered as prognostic factor in lymphoma and leukemia. It mediates its action by binding to high affinity cell surface receptors-fibroblast growth factor receptor 1-4 (FGFR4) with receptor kinase activity. Therefore, upregulation of BFGF-FGFR system may cause increased risk of non-Hodgkin lymphomas (NHLs).

AIM: Our study aimed to determine the association between the FGFR4 Gly388Arg (rs351855G/A) polymorphism and NHL disease susceptibility and prognosis.

MATERIALS AND METHODS: The present study included 75 NHL patients and 100 healthy controls. Genotyping of FGFR4 was done by Polymerase Chain Reaction-Restriction Fragment Length polymorphism (PCR-RFLP). As after the amplification of the target gene, the PCR products were digested with BstNI restriction endonuclease enzyme.

RESULTS: Analysis of FGFR4 Gly388Arg polymorphism revealed that the frequency of heterozygous (GA) mutation as well as the mutant allele (A) was significantly higher in cases compared to control subjects with $p < 0.001$ and 0.002 , respectively. The mutant genotypes were more prevalent at older age, aggressive clinical stage, bone marrow involvement, anemia, and thrombocytopenia at presentation. The mean of overall survival and the event free survival of our NHL patients were shorter in the mutant genotypes with $p = 0.049$ and 0.017 , respectively.

CONCLUSION: This study provides evidence that FGFR4 Gly388Arg polymorphism confers a genetic susceptibility to NHL among Egyptians and has a poor prognostic impact.

Introduction

Non-Hodgkin lymphomas (NHLs) are a heterogenous group of lymphoproliferative malignancies with different presenting features, clinical course, and a response to the treatment. B lymphocytes represent 86% of origin of NHL, while 14% arises from T lymphocytes or natural killer (NK) cells [1]. Among the many subtypes of NHL, diffuse large B cell lymphoma (DLBCL) is the major subtype. It accounts for 24% of NHL new cases [2], [3] Follicular lymphoma (FL) is the second most common [4] while small lymphocytic leukemia (SLL) represents 7% of the NHL new cases [5].

Worldwide incidence of NHL revealed to be the most common malignancy; the tenth in males and the twelfth in females [6], with a high mortality as estimated deaths were 248,724 in 2018 [7]. Lymphoma in Egypt is the fourth most frequent tumor in adults. NHL assembly 76.6% with incidence rates are 16.3/100,000

person [8]. In 2018, NHL is the third most common cancer in Egyptian males (8.2%) as well as among females (7.2%) with 7.3% deaths for both [9].

The etiologies of most lymphomas remain understood; however, altered immunological function and genetic susceptibility are assumed to play a crucial role in pathogenesis of lymphomas [10]. There is a high evidence that angiogenesis plays a key role in lymphoid malignancies. Expression of angiogenic factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) corresponds with the clinical features in lymphoma, and their level may act as prognostic markers for assessing the response to the treatment and recurrence [11].

Fibroblast growth factor receptors (FGFRs) are trans-membrane receptors; consist of four structurally related proteins (FGFR1-4). They belong to the family of tyrosine kinase receptors (RTKs). FGFR1-4 expressed differentially in various cell types involved in signaling through interactions with FGFs [11], [12].

FGFRs are incorporated in the regulation of several important biological activities, including cell metabolism, migration, angiogenesis, proliferation, differentiation, and survival [13].

FGFs stimulate angiogenesis through mitogen activated protein kinase (MAPK) activation and protein kinase C (PKC) activation by stimulating endothelial cell proliferation, driving extracellular matrix degradation, changing intercellular adhesion by affecting cadherins, and altering integrin expression. Consequently, several pharmaceutical companies are developing highly potent FGFR tyrosine kinase inhibitors (TKIs), which are selective over VEGF receptors (VEGFRs) [14].

FGFR4 is present on the lymphocytes. Mutations of FGFR4 may lead to the imbalance of the FGF–FGFR system with subsequent angiogenesis [11]. FGFR4 Gly388Arg single nucleotide polymorphism (SNP) (rs351855) leads to change in a glycine residue to an arginine within the trans-membrane domain associated with disease progression and poor prognosis in many tumor types, as colon, breast, prostate, head and neck, soft tissue sarcomas, melanoma, and lung cancer [15], [16]. FGFR4 Gly388Arg is found to be correlated with increased susceptibility and poor prognosis of NHL in Chinese population [11].

The aim of this work was to investigate the relationship of FGFR4 Gly388Arg to susceptibility and prognosis of B-NHL in the Egyptian population to identify some issues of genetic basis in relation to one of the most common cancer in our population to use it as a target point in the treatment in the future researches.

Patients and Methods

The present study was conducted on 75 adult Egyptian patients with newly diagnosed B-NHL with exclusion treatment received or double malignancies cases. Patients were selected from the department of Medical Oncology, National Cancer institute (NCI) as well Haematology Outpatient Clinic, Faculty of Medicine, Cairo University in the period from year 2014 to 2015 with 5 years follow-up. Diagnosis was done by histopathology and immunohistochemistry according to the WHO classification of hematological malignancies. One hundred age-, gender-, and race-matched healthy volunteers were included in the study as a control group.

Patients were subjected to full history taking, clinical examination, performance status assessment, routine laboratory work up of lymphoma including; complete blood picture, liver and kidney function tests, serum lactate dehydrogenase (LDH), cytological examination of any effusion, cerebrospinal fluid (CSF)

cytology (if needed), and bone marrow examination (aspirate/biopsy) for staging.

The study was approved by the Faculty of Medicine, Cairo University ethical committee on April 2014 with approval number (I-420414). It was conducted in concordance with Declaration of Helsinki (ethical principles for medical research involving human subjects). A written informed consent was obtained from each participant.

Sample collection

Three to five millimeters of venous blood were collected on ethylene diamine tetra-acetic acid (EDTA) by sterile venepuncture using a sterile vacutainer tube. Samples were either stored in the same vacutainer at -20°C or used directly within 24 h for DNA extraction.

Identification of FGFR4 polymorphism Gly388 Arg genotypes

DNA extraction was performed using QIAamp DNA Blood Mini Kit (Cat. No. 51104), Qiagen, Germany. Primers were provided by Thermo scientific. The forward primer sequence was: 5'-GACCGCAGCAGCGCCCGAGGCCAG-3', and the reverse was: 5'-AGAGGGAAGAGGGAGAGCTTCTG-3'. Polymerase Chain Reaction (PCR) was performed in a total volume of 25 μl containing 12.5 μl of DreamTaq PCR Master Mix (2X) (Cat. No. K1071- Thermo scientific), 5.5 μl Nuclease-free water, 1 μl of each primer, and 5 μl of Genomic extracted DNA.

PCR performed as the thermo-cycler program was conducted by initial denaturation at 95°C for 5 min, followed by 35 cycles at 94°C for 30 s, 68°C for 30 s, 72°C for 45 s, and final elongation at 72°C for 5 min.

After amplification, the PCR products (168 bp) were digested with Fast Digest BstNI restriction endonuclease enzyme (Cat. No.: FD0554, Thermo Scientific): According to the manufacturer's protocols. Visualization of the restricted fragments by ethidium bromide stained agarose gel electrophoresis. Two fragments of 82 and 27 bp characterized the mutant A allele, whereas a single visible band of 109 bp was observed for the wild G allele with additional fragments of 22 and 37 bp presents in both genotypes (Figure 1).

Statistical methods

Data were analyzed using IBM SPSS advanced statistics version 22 (SPSS Inc., Chicago, IL). Numerical data were expressed as a mean and standard deviation or a median and range as appropriate, qualitative data were expressed by frequency and percentage. Chi-square test or Fisher's exact test was used to examine the relation between qualitative variables. For quantitative data, a comparison between two groups was done using either

Student's t-test or Mann–Whitney test (non-parametric t-test) for not normally distributed data. Odds ratio (OR) with its 95% confidence interval (CI) was used for risk estimation. All tests were two-tailed. Survival rates of NHL patients were analyzed using Kaplan–Meier method and Log Rank Test. $p \leq 0.05$ was considered significant.

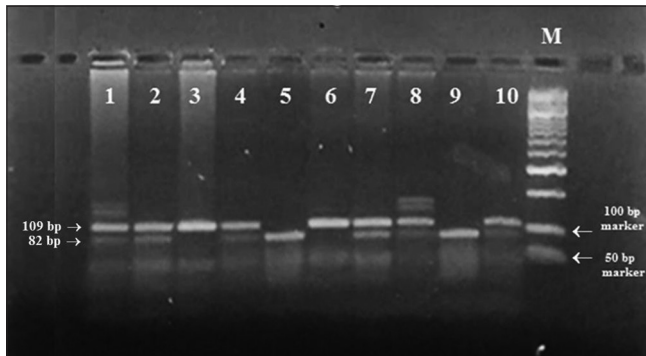


Figure 1: Identification of fibroblast growth factor receptor 4 polymorphism Gly388Arg genotypes by gel

Results

Demographic data

This study included 36 (48%) male and 39 (52%) female Egyptian patients. Their ages ranged between 17 and 79 years with a mean of 47.3 ± 16.2 years. Fifty-five (73.3%) patients were <60 years and 20 (26.7%) were ≥ 60 years. The control group included 56 males (56%) and 44 females (44%). Their ages ranged between 18 and 77 years with a mean of 43.8 ± 13.6 years. The patients and controls were age and gender matched ($p = 0.133, 0.294$, respectively) as well race matched.

According to histological classification of our NHL patients, they were classified into 53 (70.7%) DLBCL, 13 (17.3%) FL, and 9 (12%) SLL cases.

Regarding DLBCL patients, they were 26 (49.1%) males and 27 (50.9%) females. Their ages ranged between 18 and 76 years with a mean of 48.1 ± 15 years, 39 (73.6%) were <60 years, and 14 (26.4%) were ≥ 60 years. FL cases were 6 (46.2%) males and seven (53.8%) females, 11 (84.6%) were <60 years, and 2 (15.4%) were >60 years alongside the age ranged between 17 and 79 years with a mean of 39 ± 19.6 years. SLL participants were 4 (44.4%) males and 5 (55.6%) females. Their age range was between 38 and 79 years with a mean \pm SD of 54.7 ± 14.5 years to be divided into 5 (55.6%) patients <60 years and 4 (44.4%) cases >60 years old.

Clinical criteria and treatment regimen

In our NHL patients, 28 (37.3%) patients presented with B-symptoms in the form of fever and/

or night sweats and/or weight loss. Lymphoma origin was nodal in 53 (70.7%) patients and extra-nodal in 22 (29.3%) patients. Extra-nodal involvement was present in 43 (57.3%) patients to be <2 sites in 64 (85.3%) patients while ≥ 2 sites in 11 (14.7%) patients. Bone marrow involvement was present in 20 (26.7%) patients. Splenomegaly, hepatomegaly, mediastinal disease, and CNS involvement were noticed in 34 (45.3%), 31 (41.3%), 16 (21.3%), and 2 (2.7%) patients, respectively.

Nineteen (25.3%) patients were of Stages I and II according to Ann Arbor scoring system [17] while 56 (74.7%) ones were Stages III and IV. Forty-five (60%) cases were score <2 of Eastern Cooperative Oncology Group (ECOG) performance status [18] while 30 (40%) patients were ≥ 2 . Regarding the International Prognostic Index (IPI) scoring system [19]; 44 (58.7%) patients were low/intermediate low risk (1 and 2) and 31 (41.3%) of them were intermediate high/high risk (3 and 4). Treatment regimen [20] was chemotherapy, radiotherapy, combined chemotherapy and radiotherapy or chemotherapy with antiCD20 monoclonal antibody (mAb) drugs in 32 (42.1%), 2 (2.6%), 4 (5.3%), and 37 (48.7%), respectively.

Regarding DLBCL patients; B-symptoms were conferred in 21 (39.6%) patients. Nodal lymphoma origin was detected in 33 (62.3%) patients while extra-nodal origin was found in 20 (37.7%) patients. Extra-nodal involvement was observed in 32 (60.4%) patients classified into <2 sites in 43 (81.1%) patients and ≥ 2 in 10 (18.9%) patients. Bone marrow infiltration was in 12 (22.6%) patients. Splenomegaly and hepatomegaly were present in 22 (41.5%), 22 (41.5%), while mediastinal disease and CNS involvement were emerged in 13 (24.5%) and 2 (3.8%) patients independently.

On the other hand, 12 (22.6%) patients were designated as Ann Arbor Stages I and II together with 41 (77.4%) cases were rated as Stages III and IV. Performance status was found to be <2 in 29 (54.7%) patients as well ≥ 2 in 24 (45.3%) patients. IPI revealed that 27 (50.9%) patients were low/intermediate low risk (1 and 2) while 26 (49.1%) were intermediate high/high risk (3 and 4). Treatment regimen [20] was chemotherapy in 17 (32.1%), radiotherapy in 1 (1.9%), and combined chemotherapy and radiotherapy in 2 (3.8%) while antiCD20 mAb drugs were added to chemotherapy in 33 (62.3%) of DLBCL patients.

FL cases presented with B-symptoms in 3 (23.1%) patients. All patients were of nodal lymphoma origin. Extra-nodal involvement of <2 sites was recognized in 5 (38.5%) patients with no involvement of ≥ 2 sites. Bone marrow affection, splenomegaly, hepatomegaly, mediastinal disease, and CNS involvement were observed in 4 (30.8%), 6 (46.2%), 4 (30.8%), and 3 (23.1%) patients, respectively.

Four (30.8%) patients were Ann Arbor Stages I and II and 9 (69.2%) cases were of Stages

III and IV with 9 (69.2%) patients were of score <2 of performance status and 4 (30.8%) were of score ≥ 2 . Low/intermediate low risk (1 and 2) of IPI was found in 12 (92.3%) patients while intermediate high/high risk (3 and 4) was in only one (7.7%) case. Nine (69.2%) patients received chemotherapy alone while 2 (15.4%) received combined chemotherapy and radiotherapy as well 2 (15.4%) received chemotherapy with antiCD20 mAb drugs [20].

In SLL, B-symptoms were present in 4 (44.4%) patients. Nodal lymphoma origin was detected in 7 (77.8%) patients while extra-nodal origin was remarked in 2 (22.2%) patients. Extra-nodal involvement was observed in 6 (66.7%) patients which categorized into <2 sites in 8 (88.9%) and ≥ 2 sites in 1 (11.1%) patient only. On the other hand, 4 (44.4%) patients showed bone marrow infiltration. Splenomegaly was present in 6 (66.7%) patients while hepatomegaly in 5 (55.6%) of patients. Mediastinal disease and CNS involvement were absent in SLL patients group.

Three (33.3%) patients were of Ann Arbor Stages I and II while 6 (66.7%) were of Stages III and IV. Performance status of score <2 and ≥ 2 was discerned in 7 (77.8%) and 2 (22.2%) patients, respectively. IPI was calculated to be low/intermediate low risk (1 and 2) in 5 (55.6%) patients and intermediate high/high risk (3 and 4) was in the other 4 (44.4%) patients. Treatment protocol [20] was declared to be chemotherapy, radiotherapy, combined chemotherapy, and radiotherapy or chemotherapy with antiCD20 mAb drugs in 6 (66.7%), 1 (11.1%), 0 (0%), and 2 (22.2%), respectively.

Laboratory parameters of routine investigations

In our NHL cases, the hemoglobin level (Hb) ranged between 7.1 and 15.7 g/dL with a median value of 11.8 g/dL. The median value and the range of the total leukocyte count (TLC) were $7.9 \times 10^3/\mu\text{L}$ and ($1.4\text{--}151 \times 10^3/\mu\text{L}$) while for the platelets count (PLTs) were $213 \times 10^3/\mu\text{L}$ and ($29\text{--}678 \times 10^3/\mu\text{L}$), respectively. The range and median of LDH level were (105–2219 IU/L) and 258 IU/L independently. Thirty-eight (50.7%) patients had high LDH levels (≥ 247 IU/L) while 37 (49.3%) cases had normal LDH levels (≤ 247 IU/L).

For DLBCL patients, the ranges of Hb, TLC, and PLTs were (7.6–15.5 g/dL), ($1.80\text{--}14.8 \times 10^3/\mu\text{L}$), and ($29\text{--}678 \times 10^3/\mu\text{L}$) with a median value of 11.7 g/dL, $7.8 \times 10^3/\mu\text{L}$, and $235 \times 10^3/\mu\text{L}$, respectively. LDH level ranged between 105 and 2219 IU/L with a median value of 298 IU/L. High LDH levels were found in 23 (43.4%) patients while normal LDH levels were present in 30 (56.6%) cases.

Regarding FL participants, the median value of Hb, TLC, and PLTs was 12.9 g/dL, $6.60 \times 10^3/\mu\text{L}$, and $221 \times 10^3/\mu\text{L}$ independently. Each of them was

ranged between (8.7–15.7 g/dL), ($2.5\text{--}13.7 \times 10^3/\mu\text{L}$), and ($150\text{--}346 \times 10^3/\mu\text{L}$), respectively. LDH level was ranged between 119 and 645 IU/L with a median value of 205 IU/L. High and normal LDH levels were found in 3 (23.1%) and 10 (76.9%) patients, respectively.

SLL group showed the median value and range of LDH as 262 IU/L and (120–1099 IU/L) independently with normal LDH in 4 (44.4%) patients while high LDH was found in 5 (55.6%) ones. The median values of Hb, TLC, and PLTs were 11.4 g/dL, $18.2 \times 10^3/\mu\text{L}$, and $128 \times 10^3/\mu\text{L}$ and their ranges were (7.1–12.9 g/dL), ($1.4\text{--}151 \times 10^3/\mu\text{L}$), and ($38\text{--}166 \times 10^3/\mu\text{L}$), respectively.

Genotyping of FGFR4 Gly388Arg polymorphism

The mutant genotypes (GA+AA) conferred an increased risk to NHL as well as to their subtypes; SLL, FL, and DLBL (OR and 95% CI: 3.317 [1.700–6.472]; 2.839 [1.363–5.914]; 3.429 [1.037–11.332]; and 8.000 [1.839–34.793]), respectively. Similarly, the mutant allele A was found to be a risk factor for NHL, DLBCL, and SLL (OR [95% CI]: 2.401 [1.364–4.227], 2.263 [1.219–4.203], and 3.667 [1.259–10.676]), respectively (Tables 1 and 2).

Table 1: Genotyping of FGFR4 polymorphism Gly388Arg among NHL cases and controls

Genotypes	Controls (n=100) (%)	NHL patients (n=75) n (%)	p value*	OR	95% CI	
GG	80 (80)	41 (54.7)	---	Ref.	---	---
GA	16 (16)	31 (41.3)	<0.001*	3.780	1.857	7.698
AA	4 (4)	3 (4)	0.629	1.463	0.313	6.851
GA and AA	20 (20)	34 (45.3)	<0.001*	3.317	1.700	6.472
G allele	88.0	75.3	0.002*	2.401	1.364	4.227
A allele	12.0	24.7				

*p value is significant if ≤ 0.05 . CI: Confidence interval, NHL: Non-Hodgkin lymphoma, OR: Odds ratio.

Association between genotypes of FGFR4 Gly388Arg polymorphism, demographic, clinical, and laboratory data of NHL patients

In NHL cases, the mutant genotypes (GA + AA) were more frequently encountered than the wild genotype in cases ≥ 60 years old compared to cases <60 years old ($p = 0.039$). The mutant genotypes were more frequently encountered in cases with aggressive clinical stage (III and IV) and with bone marrow involvement ($p = 0.054$ and 0.039 , respectively).

No statistically significant difference was noticed between NHL patients with wild and mutant genotypes regarding their gender, the presence of B-symptoms at presentation, lymphoma origin, extranodal involvement, number of extranodal sites, splenomegaly, hepatomegaly, clinical staging, performance status, international prognostic index, mediastinal disease, CNS involvement, or histopathological types (Table 3).

The Hb and PLTs were significantly lower in the mutant genotypes compared to the wild genotype ($p = 0.010$ and 0.007 , respectively). No statistically significant difference was noticed between NHL patients

Table 2: Genotyping of FGFR4 polymorphism Gly388Arg among subtypes of NHL and controls

Genotypes	DLBCL patients (n=53) (%)	OR	95% CI	p value*	FL patients (n=13) n (%)	OR	95% CI	p value*	SLL patients (n=9) n (%)	OR	95% CI	p value*
GG	31 (58.5)	Ref.	---	---	7 (53.8)	Ref.	---	---	3/9 (33.3)	Ref.	---	---
GA	19 (35.8)	3.065	1.400 6.710	0.005*	6 (46.2)	---	---	---	6/9 (66.7)	---	---	---
AA	3 (5.7)	1.935	0.409 9.149	0.405	0 (0)	---	---	---	0/9 (0)	---	---	---
GA and AA	22 (41.5)	2.839	1.363 5.914	0.005*	6 (46.2)	3.429	1.037 11.332	0.035*	6/9 (66.7)	8.000	1.839 34.793	0.002*
G allele	76.4	2.263	1.219 4.203	0.009*	76.9	2.200	0.804 6.023	0.117	66.7	3.667	1.259 10.676	0.012*
A allele	23.6				23.1				33.3			

*p value is significant if ≤0.05. CI: Confidence interval, DLBCL: Diffuse large B-cell lymphoma, FL: Follicular lymphoma, OR: Odds ratio, SLL: Small lymphocytic lymphoma.

with wild and mutant genotypes regarding their serum LDH and TLC (Table 3).

Table 3: Association between genotypes of FGFR4 polymorphism Gly388Arg, demographic, clinical, and laboratory data of NHL patients

NHL cases characters	Wild (n=41) %	Mutant (n=34) %	p value*
Age			
≤60 years	34 (61.8)	21 (38.2)	0.039*
≥60 years	7 (35)	13 (65)	
Gender			
Male	23 (63.9)	13 (36.1)	0.123
Female	18 (46.2)	21 (53.8)	
B-symptoms	14 (50)	14 (50)	0.531
Lymphoma origin			
Nodal	28 (52.8)	25 (47.2)	0.620
Extra-nodal	13 (59.1)	9 (40.9)	
Extra-nodal involvement	22 (51.2)	21 (48.8)	0.480
Number of extra-nodal sites			
≤2	36 (56.3)	28 (43.8)	0.506
≥2	5 (45.5)	6 (54.5)	
Bone marrow involvement	7 (35)	13 (65)	0.039*
Splenomegaly	18 (52.9)	16 (47.1)	0.785
Hepatomegaly	14 (45.2)	17 (54.8)	0.165
Mediastinal disease	9 (56.3)	7 (43.8)	0.886
CNS involvement	2 (100)	0 (0)	---
Clinical stage			
I and II	14 (73.7)	5 (26.3)	0.054*
III and IV	27 (48.2)	29 (51.8)	
PS			
Score≤2	27 (60)	18 (40)	0.256
Score≥2	14 (46.7)	16 (53.3)	
IPI risk groups			
Low/Intermediate low (1 and 2)	27 (61.4)	17 (38.6)	0.165
Intermediate high/high (3 and 4)	14 (45.2)	17 (54.8)	
Treatment			
Chemotherapy	14 (34.1)	18 (52.9)	0.240
Radiotherapy	2 (4.9)	0 (0)	
Chemotherapy+radiotherapy	3 (7.3)	1 (2.9)	
Chemotherapy+antiCD20 mAb	22 (53.7)	15 (44.1)	
Hb g/dL			
Median	12.6	11.0	0.010*
Range	8.0–15.7	7.1–15.0	
TLC×10 ³ /uL			
Median	8.0	7.6	0.682
Range	2.4–18.2	1.4–151.0	
PLTs×10 ³ /uL			
Median	242	180	0.007*
Range	97–678	29–564	
LDH IU/L			
Median	238	284.5	0.380
Range	115–980	105–2219	

*p value is significant if ≤0.05, CNS: Central Nervous System, Hb: Hemoglobin, IPI: International Prognostic Index, LDH: Lactate dehydrogenase, mAb: Monoclonal antibody, NHL: Non-Hodgkin lymphoma, PLTs: Platelets, PS: Performance status, TLC: Total leukocyte count.

FGFR4 genotyping and prognosis of NHL

Over a follow-up period of 5 years; the number of deaths within GG, GA, and AA genotypes was 16 (39%), 19 (61.3%), and 2 (66.7%), respectively. The mean of overall survival (OS) was significantly lower in mutant genotypes (GA + AA): 30.806 months compared to be 42.674 months in the wild GG genotype with (p = 0.049) (Figure 2).

The mean of the event free survival (EFS) in patients with GG, GA, and AA genotypes was 38.6, 23.6, and 29.97 months, respectively, which was a statistically significant difference (p = 0.047). The mean EFS was lower in mutant (GA + AA) genotypes

(24.2 months) compared to 38.6 months in wild (GG) genotype (p = 0.017) (Figure 3).

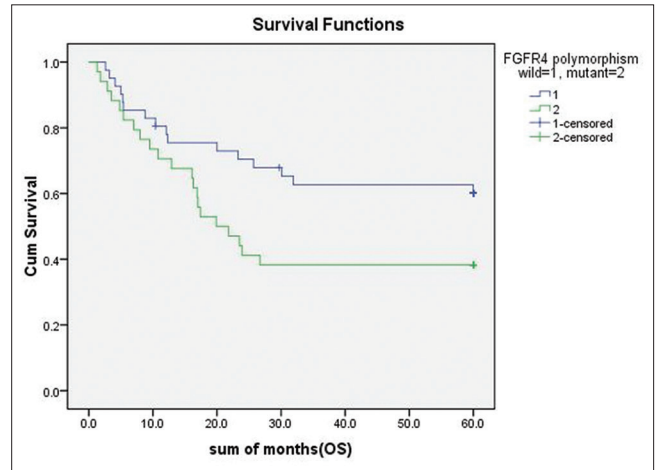


Figure 2: Overall survival of Non-Hodgkin lymphomas patients according to genotypes of fibroblast growth factor receptor 4 rs351855G/A polymorphism

Discussion

NHLs include different subtypes of lymphoproliferative disorders with a variety of presentations and prognosis [21]. NHL is considered one of the most common types of all cancers in world as well as Egypt [6], [8]. The underlying mechanisms of NHL are still questionable with many contributing factors [10].

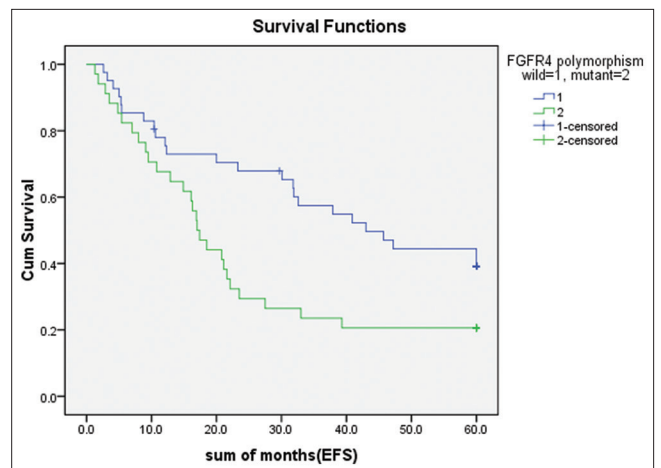


Figure 3: Event free survival of Non-Hodgkin lymphomas patients according to genotypes of fibroblast growth factor receptor 4 rs351855G/A polymorphism

Angiogenesis including FGF–FGFR system disturbance is considered an important key in development and progression of lymphomas. The FGFR4 rs351855G/A (Gly388Arg) was detected in various tumors as prostate, pancreatic, renal cell carcinomas as well as NHL as risk and poor prognostic factor [16]. Our study confirmed at that this SNP is risk factor and a poor prognostic indicator in NHL Egyptian patients.

The present work revealed that the presence of heterozygous mutation GA conferred a 3.7 fold increased risk for NHL susceptibility (OR = 3.780, 95% CI = 1.857–7.698), the presence of the mutant allele (A) carried a higher risk for NHL incidence (OR = 2.401, 95% CI = 1.364–4.227).

In consistence with our results, Cha *et al.* (2014) [22] investigated 412 patients with NHL and 476 healthy controls for FGFR4 (Gly388Arg) polymorphism by PCR-Restriction Fragment Length polymorphism (PCR-RFLP). To confirm the genotyping results, more than 10% of PCR-amplified DNA samples were examined by DNA sequencing. Results between PCR and DNA sequencing analysis were 100% concordant. They reported that the frequencies of FGFR4 (Gly388Arg) in patients group for the heterozygous mutant genotype (GA) and homozygous mutant genotype (AA) were 45.9% and 28.1%, respectively, compared to those of controls 50.4% and 15.8%, respectively. These results showed that the frequencies of homozygous mutant genotype (AA) were significantly higher in patients than in controls ($p < 0.0001$, OR = 2.12, 95% CI = 1.99–3.48).

Similarly, Gao *et al.* (2014) [11] investigated 421 patients with NHL and 486 healthy controls for FGFR4 (Gly388Arg) polymorphism by PCR-RFLP. More than 5% of PCR-amplified DNA samples were examined by DNA sequencing. Results between PCR and DNA sequencing analysis were 100% concordant. They found that the frequencies of FGFR4 (Gly388Arg) in patients group for the heterozygous mutant genotype (GA) and homozygous mutant genotype (AA) were 44.9% and 27.3%, respectively, compared to those of controls that were 49.4% and 15.4%, respectively. These results showed that the frequencies of homozygous mutant genotype (AA) were significantly higher in patients than in controls ($p < 0.001$, OR = 2.02, 95% CI = 1.91–3.23).

In the present study, the allelic frequencies for G and A alleles in NHL patients were 75.3% and 24.7%, respectively, compared to those of controls 88% and 12%, respectively. The presence of the mutant allele (A) conferred a 2.4 fold increased risk for NHL susceptibility ($P = 0.002$, OR = 2.401, 95% CI = 1.364–4.227).

In agreement with our findings, Cha *et al.* (2014) [22] found that the allelic frequencies for G and A alleles in patients were 48.9% and 51.1%, respectively, compared to those of controls 59% and 41%, respectively. Thus, the frequency of the mutant

(A) allele was higher in patients than in controls. This was statistically significant ($p < 0.0001$, OR = 1.45, 95% CI = 1.21–1.88).

Our results are also consistent with Gao *et al.* (2014) [11] regarding the allelic frequencies, as they found that frequencies for G and A alleles in patients were 50.2% and 49.8%, respectively, compared to those of controls 59.9% and 40.1%, respectively. Thus, the mutant (A) allele was significantly higher in patients than in controls ($p < 0.001$, OR = 1.33, 95% CI = 1.16–1.75).

Furthermore, we analyzed the frequencies of FGFR4 (Gly388Arg) polymorphism genotypes in different histopathological subtypes compared to controls. We are first to demonstrate these relations.

The heterozygous mutant genotype (GA) was overrepresented compared to the wild genotype (GG), ($P = 0.005$, OR = 3.065, 95% CI = 1.400–6.710). The mutant genotypes (GA and AA) frequency was significantly higher in DLBCL subjects ($P = 0.005$, OR = 2.839, 95% CI = 1.363–5.914, respectively).

Similarly, the mutant allele (A) frequency was significantly higher in DLBCL patients compared to controls ($p = 0.009$, OR = 2.263, 95% CI = 1.219–4.203). The mutant genotypes (GA + AA) frequency in FL cases were significantly higher compared to controls ($p = 0.035$, OR = 3.429, 95% CI = 1.037–11.332).

In SLL, the genotype distribution could not be assessed statistically due to the small group numbers; however, the frequency of the mutant allele (A) was significantly higher in SLL patients compared to controls ($P = 0.012$, OR = 3.667, 95% CI = 1.259–10.676).

Our result demonstrated the prevalence of mutant genotypes in patients older than 60 years ($p = 0.039$), advanced clinical stage ($p = 0.054$), with bone marrow involvement ($p = 0.039$), lower hemoglobin ($p = 0.01$), and platelet count at diagnosis ($p = 0.007$). These findings point to a possible association of that SNP with poor prognosis. No statistically significant difference regarding lymphoma origin could be detected as found in Gao *et al.* (2014) [11].

FGFR4 is expressed on different cell types including lymphoid cells. It is one of receptor tyrosine kinases (RTK). RTKs activation institutes a series of internal reactions through kinase activation involved in cellular proliferation and survival [22]. FGFR4 Arg388 allele decreases receptor stability with persistent receptor stimulation. Therefore, it may aggravate carcinogenesis by augmenting its growth, mobilization, and angiogenesis [23].

Ulaganathan and Ullrich (2016) demonstrated that FGFR4 Gly388Arg disrupts transmembrane portion of the receptor with subsequent exposure of membrane-proximal binding site of cytoplasmic STAT3 Y390-(P)XXQ393. This stimulates phosphorylation of STAT3 with increasing cell proliferation [24].

However, the constitutional behavior of FGFR4 rs351855G/A is uncertain. Many proofs indicate its implication in cancer susceptibility. *In vitro*, a study in breast cancer demonstrated that the cells which carry the variant of rs351855G/A showed an increase motility than those of wild type. In prostatic cancer, transfection of cDNA carrying the variant form was found to increase the violation features of prostatic epithelial cell line [16].

Our results demonstrated shorter OS and EFS in mutant genotypes (GA+AA) compared to wild genotype. This was consistent with Cha *et al.* (2014) [22], who found shorter OS in AA genotype compared to GA + GG genotypes with $p = 0.002$ as well as in the study performed by Gao *et al.* (2014) [11] with $p < 0.001$. This suggests the impact of FGFR4 Gly388Arg on prognosis of NHL.

Tateno *et al.* (2011) [25] showed that the effect on FGFR4 Gly388Arg constituted mainly disruption of receptor/ ligand affinity, decaying, and interactions. In another issue, FGFR4 Gly388Arg showed acceleration in the phosphorylation of STAT in mitochondria in pituitary carcinogenesis with a subsequent enhancing in the cell growth [16].

FGFR4 Arg (388) allele was found to be higher in germ cell lines of many tumors of cancer patients of poor prognosis [26]. It affects cancer severity; lymph nodes affection, deterioration of its clinical stage, and survival of various tumor such as prostatic cancer and lung adenocarcinoma [27]. This SNP appeared to play an important role in carcinogenesis as well as in prognosis and may act in tumors as a marker of screening and a target of therapy by future further researches. As the exact process by which that variant enhanced NHL probability is still unexplained, so FGFR4 Gly388Arg is our focus of research.

The authors faced some obstacles due to the lack of a financial support to do the confirmatory sequencing of the results although it was done in the previous studies in different ethnic groups. This study still contributes an important issue in the understanding of the association between FGFR4 polymorphisms and NHL in our population.

Conclusion

Our study suggests that FGFR4 polymorphism rs351855 (Gly388Arg) plays a significant role in NHL risk in our population. The patients with mutant genotypes are at higher risk to have an advanced clinical stage, bone marrow involvement, anemia, and thrombocytopenia at presentation as well as shorter OS and EFS.

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