



CXCL12 rs1801157 polymorphism is associated with risk and poor prognosis of non-Hodgkin's Lymphoma

Abdel Aziz RS¹ , Rabea A² , Abd El Dayem OY³

¹ Clinical Pathology Department, National Cancer Institute, Cairo University, Cairo, Egypt.

² Medical Oncology Department, National Cancer Institute, Cairo University, Cairo, Egypt.

³ Clinical Pathology Department, Faculty of Medicine, Cairo University, Cairo, Egypt.

Abstract:

Background: CXCL12 and its receptor CXCR4 belong to the CXC family of chemokines that regulate the migration of immune cells during their development and in inflammatory responses. They also regulate the migration, proliferation and survival signals in multiple cell types, therefore deregulation of their expression or activity have been implicated in the pathogenesis, growth and metastasis of solid as well as haemato-lymphoid malignancies. A single nucleotide polymorphism (SNP) (rs1801157; G>A) have been recognized in CXCL12 gene and was linked to risk, severity and outcome of many tumors; however, its significance in NHL has not been thoroughly studied.

Aim of the study: To investigate the impact of CXCL12 (rs1801157; G>A) genetic polymorphism on risk to, clinicopathologic characteristics, response to treatment and survival in Egyptian NHL patients.

Material and methods: DNA from 80 NHL patients and 150 healthy controls were analyzed using the PCR-RFLP method for identification of the genotypes.

Results: The frequency of CXCL12 (rs1801157; G>A) GA genotype was significantly higher in NHL patients than in the control group (38.8% versus 18.7%, P=0.001) and it could be associated with increased risk of developing NHL (OR=2.8, 95% CI: 1.528-5.240). When the mutant genotypes GA and AA were considered together, they significantly associated with more frequent initial advanced clinical staging III,IV (P=0.019), IPI risk (3,4) (P=0.031), ≥ 2 extranodal involvement (P=0.03), higher initial serum LDH (P=0.02) when compared to GG genotype. Patients carrying the A allele (GA+AA) genotypes had significantly worse overall survival (OS) (P=0.037) and progression free survival (PFS) (P=0.047) than those with the GG genotype.

Conclusion: Our study revealed that CXCL12 (rs1801157; G>A) SNP could be considered as a risk factor for developing de novo NHL in Egyptian population and it may have a predictive and prognostic value in NHL.

Key words: Chemokines, CXCL12, NHL, SNP, PCR-RFLP.

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Authors Information:

Rania Salah Abdel Aziz
Clinical Pathology Department,
National Cancer Institute, Cairo
University, Cairo, Egypt.
email: rania_smansy@yahoo.com

Ahmed Rabea
Medical Oncology Department,
National Cancer Institute, Cairo
University, Cairo, Egypt.
email: ahmed.rabea@live.com

Omnia Yahia Abd El Dayem
Clinical Pathology Department, Faculty
of Medicine, Cairo University, Cairo,
Egypt.
email: omniah.yahia@kasralainy.edu.eg

Corresponding Author:

Rania Salah Abdel Aziz
Clinical Pathology Department,
National Cancer Institute, Cairo
University, Kasr Al Eini Street, Fom El
Khalig, Cairo, Egypt.
email: rania_smansy@yahoo.com

Introduction:

Non-Hodgkin lymphoma (NHL) represents a wide variety of lymphoid neoplasms developed from clonal proliferation of B cells, T cells or NK cells with varying clinical, pathological, genetic features and treatment outcome. The incidence of NHL is the most prevalent among hematologic malignancies [1, 2].

Several risk factors have been linked to the development of lymphomas, the most important of which are altered host cytokine environment, together with genetic susceptibility and gene-environment

interaction although no definite gene has been identified [1, 3].

Chemokines are a family of chemotactic cytokines and their receptors involved in the regulation of leukocytes trafficking in both inflammatory and homeostatic states. De-regulated expression or activity of chemokine ligands or receptors in cancer may modify the tumor microenvironment and affect the migration of cancer cells [4].

CXCL12 known also as stromal cell derived factor-1 (SDF-1), is a member of the CXC family of

chemokines. It is constitutively produced and expressed in many organs such as brain, heart, lung, lymph nodes, and liver. In the bone marrow, CXCL12 is constantly produced by the endothelial cells, stromal cells, fibroblasts and osteoblasts. It executes its biological functions through binding to and activating CXC chemokine receptor CXCR4 and a recently identified CXCR4 co-receptor CXCR7 [5, 6].

Upon binding, the CXCL12/CXCR4 axis triggers many signaling pathways involved in cell proliferation, differentiation, survival, chemotaxis and apoptosis. It plays a key role in the regulation of physiological processes such as stem cell motility, hematopoiesis, trafficking of immune cells, neovascularization and cardiac and neuronal development [5, 6].

In cancer, the CXCL12/CXCR4 axis has been implicated in carcinogenesis, angiogenesis and tumor metastasis, since increased expressions of CXCL12 and CXCR4 were reported in various solid and hematological cancers such as thyroid, kidney, brain, AML, ALL, CLL and NHL [4,7]. Also, increased circulating levels of CXCL12 were detected in plasma and sera from patients with colorectal cancer, biliary tract cancer, acute leukemias and CLL suggesting that they can be used as detective markers for solid and hematological malignancies [8-10]. Owing to its pivotal role in maintaining and promoting cancer stem cells, the CXCL12/CXCR4 axis has been proposed as a target for chemokine antagonists [11].

Single nucleotide polymorphism (SNP) is the most common form of variation in the human genome that can modify gene expression and protein function thus altering the susceptibility to diseases among individuals [12].

A common SNP in the 3'-untranslated region of *CXCL12* gene termed rs1801157 that may regulate *CXCL12* mRNA and protein expression was identified [13], and its significance was investigated in different pathological conditions. It was reported to be associated with the development of dementia [14], risk and severity of SLE [15], and with the outcome of patients with cardiovascular disease [16].

Moreover, recent studies correlated *CXCL12* (rs1801157; G>A) SNP with susceptibility, progression, or response to therapy in various solid and hematological malignancies such as breast [6], renal [17], oropharyngeal carcinoma [18], myeloid leukemias [19], CLL [20],

However, its role in NHL has not been fully elucidated [21].

The aim of the present work was to study the relation of *CXCL12* (rs1801157; G>A) SNP with the susceptibility to NHL in Egypt and its possible impact on NHL prognosis and outcome.

Patients and Methods:

The present study included 80 patients with de novo Non-Hodgkin lymphoma who were diagnosed and treated in the National Cancer Institute (NCI), Cairo University in the period from June 2014 to December 2016 in addition to a control group of 150 age and sex

matched apparently healthy volunteers. The sample size was according to de Oliveira et al. [5]. The patients were 41 males (51.2%) and 39 females (48.8 %), their ages ranged between 18 and 79 years with a mean age \pm SD of 47.2 ± 17.43 and a median of 50 years, while the control group consisted of 78 males (52%) and 72 females (48%), their ages ranged between 18 and 72 years with a mean age \pm SD of 45 ± 16.6 and a median of 47 years (Table 1). Diagnosis of different NHL subtypes were performed by pathological examination and immunohistochemical staining of the involved lymphomatous tissue according to WHO classification, 2008. All cases were thoroughly examined, and investigated by laboratory and radiological work up. All the procedures in the study were in accordance with the ethical standards of the local ethical committee (IRB) of the NCI, Cairo University, and with the Helsinki Declaration of 1975 (revised in 2008). Informed consent was taken from all the participants (patients and controls). Clinical and laboratory data of the patients are presented in tables (2 and 3).

As regards the treatment regimen, the patients received the standard protocol treatment for NHL at the NCI of Cairo University. For Indolent lymphomas (Follicular lymphoma grade I-II, small lymphocytic lymphoma, marginal zone lymphoma) R-CVP (Rituximab, Cyclophosphamide and Vincristine) was given. For patients with diffuse large B-cell lymphoma, Mantle, Follicular lymphoma GIII, and T-cell lymphoma, R-CHOP (Rituximab, Cyclophosphamide, Doxorubicin, Vincristine and Prednisolone) for 6 cycles was given. Burkitt's lymphoma patients were given CODOX-M/IVAC (Cyclophosphamide, Vincristine, Doxorubicin, Methotrexate, Cytarabine, Ifosfamide, Etoposide). Follow up of the patients for their response to treatment was done by CT chest, abdomen and pelvis with contrast and comparison to the initial CT. Response was reported according to RECIST criteria version 1.1 [22]

Table (1): Comparison of age and sex of the non-Hodgkin's lymphoma (NHL) patients and the controls

Characteristics	Patients (no=80)	Controls (no=150)	P value
Sex (no %)			
Male	41 (51.2%)	78 (52%)	0.91
Females	39 (48.8%)	72 (48%)	
Age			
Range	18-79 years	18-72 years	0.46
Mean \pm SD	47.2 ± 17.43	45 ± 16.6	

SD: Standard deviation, qualitative variables were compared by Chi-square test, quantitative data were compared by Student t-test for normally distributed data, significance defined by (p<0.05)

Table (2): Clinicopathologic data of 80 non-Hodgkin's lymphoma (NHL) patients at diagnosis

Parameter	Patient group (n= 80)	
	Number	Percentage
Gender		
- Male	41/ 80	51.2%
- Female	39/ 80	48.8%
Age		
- ≤60 years	57/80	71.2%
- >60 years	23/80	28.8%
B- symptoms:		
- Fever, night sweats, weight loss	33/80	41.3%
Lymphadenopathy	65/80	81.3%
Groups of lymph nodes involved		
- Cervical	40/80	50%
- Axillary	26/80	32.5%
- Inguinal	34/80	42.5%
- Submandibular	15/80	18.8%
- Abdominal	30/80	37.5%
- Para-aortic	13/80	16.3%
Extranodal involvement		
- <2	43/80	53.8%
- ≥2	37/80	46.2%
Splenomegaly	37/80	46.3%
Hepatomegaly	35/80	43.8%
Bone marrow involvement	24/80	30%
LDH level		
- Normal	35/80	43.7%
- Elevated	45/80	56.3%
Clinical stage		
- I&II	37/80	46.2%
- III&IV	43/80	53.8%
P.S		
- Score <2	44/80	55%
- Score ≥2	36/80	45%
IPI risk groups		
- Low/ Intermediate low (1,2)	36/80	45%
- Intermediate high/ High (3,4)	44/80	55%
NHL Histological subtypes		
Indolent	17/80	21.3%
- Follicular lymphoma Grade I&II	6/17	35.3%
- Small lymphocytic lymphoma	5/17	29.4%
- Marginal zone lymphoma	6/17	35.3%
Aggressive	63/80	78.8%
- Diffuse large B-cell lymphoma	57/63	90.4%
- Follicular lymphoma Grade III	1/63	1.6%
- T-cell lymphoma	2/63	3.2%
- Mantle cell lymphoma	1/63	1.6%
- Burkitt lymphoma	2/63	3.2%
Response to therapy		
- Responsive (CR+PR)	48/68	70.6%
- Unresponsive (PD+SD)	20/68	29.4%
- Unavailable	12/80	15%

P.S: Performance status, IPI: International prognostic index, CR: Complete remission, PR: Partial remission, PD: Progressive disease, SD: Stable disease

Table (3): Laboratory data of 80 NHL patients at diagnosis

Parameter	Mean ±SD	Median (Range)
Hb: gm/dL	10.75±1.9	10.9 (7-15.3)
TLC: x10 ⁹ /L	14.8±14.6	10.6 (23-87)
Plts: x10 ⁹ /L	197.38±92.9	178.5 (34-512)
LDH: IU/L	416.83±337.1	285 (86-1200)

Hb: Hemoglobin, TLC: Total leucocytic count, Plts: Platelets, LDH: lactate dehydrogenase

CXCL12 genotyping:

CXCL12 (rs1801157; G>A) SNP was detected using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. DNA was extracted using Gene JET Whole Blood Genomic DNA purification Mini kit (Fermentas Life Sciences, Canada) from peripheral blood leucocytes collected on EDTA according to manufacturer's instructions. The primers for *CXCL12* (rs1801157; G>A) were provided by Thermo scientific™-Lithuania. Primer sequences were as follows:

Forward, 5'-CAG TCA ACC TGG GCA AAG CC-3'
Reverse: 5'-CCT GAG AGT CCT TTT GCG GG-3'

The PCR reaction was performed in a total volume of 25 µL containing 12.5 µL 2X Dream Taq Green PCR Master Mix, 3 µL genomic DNA, 1 µL corresponding to 10 pmol of each of the forward and reverse primers, and 7.5 µL nuclease-free water. DNA amplification was performed in thermal cycler Perkin Elmer No: 9700. The amplification program was done according to de Oliveira et al. [5] as follows: initial denaturation at 94°C for 5min, followed by 35 cycles of denaturation at 94°C for 1minute, annealing at 60°C for 1minute, extension at 72°C for 1minute, and final extension step at 72°C for 10 minutes. The presence of the PCR amplicon was checked by 2% agarose gel electrophoresis under UV. *CXCL12* gave an amplicon of 293 bp that was digested with specific restriction enzyme *HpaII* (Fermentas, USA, Cat. No. FD0514) according to manufacturer's instructions. The digestion products were separated by 2 % agarose gel electrophoresis and stained with ethidium bromide.

Recognition site the enzyme *HpaII*:

5' C ↓ C G G 3'
3' G G C ↑ C 5'

After digestion with the enzyme *HpaII*, the wild genotype produced two bands of 193 and 100 bp, the homozygous variant produced a single band of 293 bp, while the heterozygous variant produced 3 bands of 100, 193, and 293 bp.

Statistical analysis:

Statistical calculations were done using IBM SPSS advanced statistics version 22 (SPSS Inc., Chicago, IL). Quantitative data were presented as mean and standard deviation or median and range as appropriate. Qualitative data were presented as frequency and percentage. For quantitative data, comparison between two groups was done using either Student t-test for normally distributed data or Mann-Whitney test if not normally distributed. Qualitative variables were compared by Chi-square test or Fisher's exact test. Odds ratio (OR) with 95% confidence interval (CI) were used for risk estimation. Estimates for both overall survival (OS) and progression free survival (PFS) were calculated using the Kaplan-Meier method. Differences in OS and PFS across genotypes were compared with the log-rank test. P-values (p<0.05) were considered significant. OS is defined as the time from cancer diagnosis to the date of last follow up or death from any cause. PFS is defined as the time from cancer diagnosis to the date of progression, relapse, last follow up or

death. Hazard ratios (HR) and 95% CI were assessed using multivariate Cox proportional hazard regression.

Results:

Genotypes and allele frequencies of CXCL12 (rs1801157; G>A) genetic polymorphism in both NHL patients and controls are summarized in table 4 showing significantly higher prevalence of *CXCL12* (rs1801157; G>A) GA genotype in NHL patients than in controls, (38.8% versus 18.7%, OR=2.8, 95% CI: 1.528-5.240, P=0.001).

Similarly, the prevalence of (GA+AA) mutant genotypes was significantly higher in NHL patients than

in controls (43.8% vs 23.3%, OR=2.5, 95% CI: 1.429-4.572, P=0.001), while prevalence of the wild *CXCL12* GG genotype was lower in the patients than in the controls (56.2% vs 76.7%) and the mutant homozygous (AA) genotype was the least frequent being encountered in (5%) NHL patient and (4.6%) of controls with no significant difference (P=0.56).

On the other hand, the frequency of the wild G allele was lower in the patients than in the controls (76% vs 86%), while the frequency of the mutant A allele was higher in the patients than in the controls (24% vs 14%) however with no significant difference (P=0.063).

Table (4): Distribution of *CXCL12* (rs1801157; G>A) genotypes in 80 NHL patients and 150 controls

Genotypes	Controls No (%)	NHL No (%)	OR	95% CI		P
GG	115 (76.7%)	45 (56.2%)	(1) Ref.	---	---	---
GA	28 (18.7%)	31 (38.8%)	2.83	1.528	5.240	0.001
AA	7 (4.6%)	4 (5%)	1.46	0.408	5.231	0.559
GA&AA	35(23.3%)	35 (43.8%)	2.5	1.429	4.572	0.001
G allele	0.86	0.76	1.91	0.958	3.820	0.063
A allele	0.14	0.24				

OR: Odds ratio, 95% CI: 95% Confidence interval, Ref: Reference, significance defined by (p<0.05)

Distribution of CXCL12 (rs1801157; G>A) genotypes in relation to Clinicopathologic characteristics and laboratory data of NHL patients: (Tables 5-6)

Statistical evaluation of the association between *CXCL12* (rs1801157; G>A) polymorphism and Clinicopathologic parameters and laboratory data in NHL patients at diagnosis revealed that *CXCL12* (GA+AA) mutant genotypes were significantly more frequent in patients with advanced clinical staging III,IV than in patients with clinical staging I,II (P=0.019), in patients with IPI risk (3,4) than in patients with IPI risk (1,2) (P=0.031), in patients with ≥ 2 extranodal involvement than in patients with < 2 extranodal involvement (P=0.03), and had higher initial serum LDH (P=0.02) when compared to GG genotype.

No significant association was observed between the genotypes of *CXCL12* (rs1801157; G>A) with any of the other clinical or laboratory parameters including age (P=0.335), gender (P=0.382), the presence of B-symptoms at presentation (P=0.241), nodal involvement (P=0.367), splenomegaly (P=0.085) or hepatomegaly (P=0.094), bone marrow involvement (P=0.806),

performance status (P=0.571), or histological aggressiveness subtypes (P= 0.81) as well as TLC (P=0.67), hemoglobin (P=0.06), or platelets count (P=0.29).

Impact of CXCL12 (rs1801157; G>A) genotypes on response to therapy in NHL:

Follow up of the patients for their response to treatment revealed that 33/68 (48.5%) patients showed complete remission (CR), 15/68 (22%) showed partial remission (PR), 16/ 68 (23.5%) showed progressive disease, 4/68 (6%) showed stable disease and 12/80 (15%) patients lost follow up.

Statistical analysis revealed that there was no significant difference in the distribution of *CXCL12* (rs1801157; G>A) genotypes among NHL patients in CR, PR or those with no response (p= 0.4).

Out of 28 patients with mutant *CXCL12*, 20 (71%) responded to treatment (CR + PR) compared to 28/40 (70%) with wild genotype (P= 0.2).

Table (5): Comparison of clinicopathological parameters between *CXCL12* (rs1801157; G>A) mutant and wild genotypes in NHL patients

Parameter	CXCL12 mutant genotypes (n=35)	CXCL12 wild genotype (n=45)	P
Gender			
- Male	16 (45.7%) *	25 (55%)	0.382
- Female	19 (54.3%)	20 (45%)	
Age			
- ≤60 years	23 (65.7%)	34 (75.5%)	0.335
- >60 years	12 (34.3%)	11 (24.5%)	
B-symptoms	17 (48.6%)	16 (35.6%)	0.241
Lymphadenopathy	30 (85.7%)	35 (77.8%)	0.367
Groups of lymph nodes involved			
- Cervical	19 (54.3%)	21 (46.7%)	0.499
- Axillary	11 (31.4%)	15 (33.3%)	0.857
- Inguinal	16 (45.7%)	18 (40%)	0.608
- Submandibular	5 (14.3%)	10 (22.2%)	0.367
- Abdominal	13 (37.1%)	17 (37.8%)	0.954
- Para-aortic	4 (11.4%)	9 (20%)	0.303
Extranodal involvement			
- <2	14 (40%)	29 (64.4%)	0.03
- ≥2	21 (60%)	16 (35.6%)	
Splenomegaly	20 (57.1%)	17 (37.8%)	0.085
Hepatomegaly	19 (54.3%)	16 (35.6%)	0.094
Bone marrow involvement	11 (31.4%)	13 (28.9%)	0.806
LDH level			
- Normal	10 (28.6%)	25 (55.5%)	0.016
- Elevated	25 (71.4%)	20 (45.5%)	
Clinical stage			
- I/II	11 (31.4%)	26 (57.8%)	0.019
- III/IV	24 (68.6%)	19 (42.2%)	
P.S			
- Score <2	18 (47.4%)	26 (57.8%)	0.571
- Score ≥2	17 (48.6%)	19 (42.2%)	
IPI risk group			
- Low/ Intermediate low (1,2)	11 (31.4%)	25 (55.5%)	0.031
- Intermediate high/high (3,4)	24 (68.6%)	20 (45.5%)	
Histological aggressiveness			
- Indolent	7 (20%)	10 (22.2%)	0.81
- Aggressive	28 (80%)	35 (77.8%)	

* No (%), qualitative variables were compared by Chi-square test or Fisher's exact test. P-values (p<0.05) were considered significant, significance defined by (p<0.05)

Table (6): Comparison of laboratory parameters between mutant and wild genotypes of *CXCL12* (rs1801157; G>A) in NHL patients

Parameter	Mutant genotypes (n=35)		Wild genotype (n=45)		P
	Mean ± SD	Median (Range)	Mean ± SD	Median (Range)	
Hb: gm/dL	10.1±1.74	10 (7-14)	11.25±1.86	11.5 (7.8-15.3)	0.06
TLC: x10 ⁹ /L	14±14.37	10 (7-87)	15.4±14.95	8.7 (2.3-63)	0.67
Plts: x10 ⁹ /L	184.97±86.69	161 (34-512)	207±97.48	210 (52-452)	0.29
LDH: IU/L	516.31± 358.17	415 (98-1200)	339.44±301.54	200 (86-1130)	0.02

Hb: Hemoglobin, TLC: Total leucocytic count, Plts: Platelets, LDH: lactate dehydrogenase quantitative data were compared by either Student t-test for normally distributed data or Mann-Whitney test if not normally distributed, significance defined by (p<0.05)

CXCL12 (rs1801157; G>A) SNP and prognosis in NHL

1- Impact of *CXCL12* (rs1801157; G>A) genotypes on overall survival (OS) and progression free survival (PFS) in NHL (Figures 1-2) (Table 7)

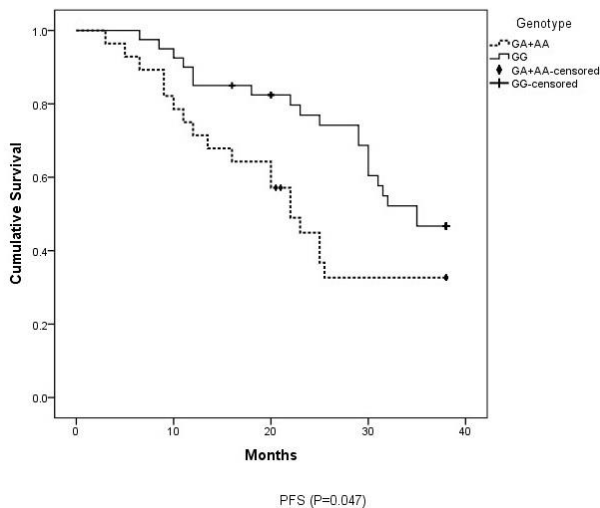


Figure (1): 3-year progression free survival of NHL patients according to *CXCL12* (rs1801157; G>A) genotypes (P=0.047 by log-rank test).

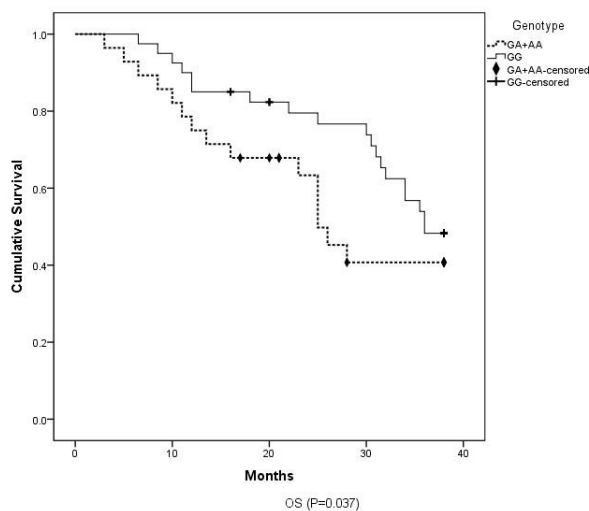


Figure (2): 3-year overall survival of NHL patients according to *CXCL12* (rs1801157; G>A) genotypes (P=0.037 by log-rank test).

Follow up information regarding survival was available for 68 of the 80 patients. The median duration of follow up was 29 months (range 1-38 months). The median OS and PFS for all patients were 34 months (95% CI: 29.565-39.435) and 30 months (95% CI: 24.835-35.165) respectively. Patients with *CXCL12* (GA+AA) genotypes had significantly worse OS and PFS than those with the GG genotype.

The (GA+AA) variant genotypes of *CXCL12* (rs1801157; G>A) had a median OS of 25.5 months (95% CI: 22.441-28.559) compared to 36 months (95% CI: 34.114-37.886) for the wild genotype GG (P=0.037). On the other hand, the median PFS of (GA+AA) genotypes was 22 months (95% CI: 17.374-26.626) compared to 35 months (95% CI: 32.123-37.415) for the wild genotype GG (P=0.047).

2- *CXCL12* (rs1801157; G>A) SNP as an independent predictor of overall survival (OS) and progression free survival (PFS) in NHL (Table 8, 9)

Univariate and multivariate COX regression analyses were used to determine whether the *CXCL12* (rs1801157; G>A) was an independent prognostic factor. We also analyzed some previously established prognostic parameters (IPI, B symptoms, clinical stage, and Response to first line therapy) in NHL.

For OS, results of COX univariate analysis in NHL patients showed significantly increased relative risk of death associated with *CXCL12* (rs1801157; G>A) (GA+AA) genotypes compared to NHL patients with GG genotype (HR 2.1, 95% CI: 1.024-4.173, P=0.043). Other poor prognostic parameters in the univariate analysis were the presence of high IPI score, advanced Ann-Arbor stage, B symptoms, and unresponsiveness to first-line therapy.

As regards PFS, COX univariate analysis revealed that the relative risk of death was also increased (HR 1.9, 95% CI: 0.989-3.576, P=0.054) associated with (GA+AA) genotypes compared to NHL patients with GG genotype however, without a significant value. Significant poor prognostic parameters in the univariate analysis were the presence of high IPI score, advanced Ann-Arbor stage, and unresponsiveness to first-line.

COX multivariate analysis revealed that only initial high IPI score was an independent poor prognostic factor for both OS (P=0.024) and PFS (P=0.01).

Table (7): Comparison of median (95% CI) survival duration between mutant and wild genotypes of *CXCL12* (rs1801157; G>A) in NHL patients

Survival	CXCL12 (rs1801157; G>A) (GA+AA) genotypes	CXCL12 (rs1801157; G>A) wild genotype GG	P
<u>(OS)</u>			
Median (95% CI)	25.5 months (95% CI: 22.441-28.559)	36 months (95% CI: 34.114-37.886)	0.037
<u>(PFS)</u>			
Median (95% CI)	22 months (95% CI: 17.374-26.626)	35 months (95% CI: 32.123-37.415)	0.047

(OS): Overall survival, (PFS): progression free survival, 95% CI: 95% Confidence interval

OS is defined as the time from cancer diagnosis to the date of last follow up or death from any cause. PFS is defined as the time from cancer diagnosis to the date of progression, relapse, last follow up or death.

(Kaplan-Meier Estimation), differences in OS and PFS across genotypes were compared with the log-rank test), significance defined by (p<0.05)

Table (8): prognostic parameters which affect overall survival (OS) in NHL patients in univariate and multivariate analyses

Parameter	Univariate analysis			Multivariate analysis		
	HR	95%CI	P	HR	95%CI	P
rs1801157(GA+AA) vs (GG)	2.1	1.024-4.173	0.043	1.9	0.899-4.155	0.09
IPI (3,4) vs (1,2)	2.7	1.343-5.241	0.005	2.4	1.122-5.015	0.024
Stage (III&IV) vs (I&II)	0.322	0.162-0.640	0.001	0.623	0.211-1.844	0.4
B symptoms (present vs absent)	0.431	0.219-0.819	0.015	0.605	0.203-1.806	0.4
Response to (first line therapy response vs refractory)	0.433	0.201-0.932	0.032	0.485	0.217-1.086	0.08

(OS): Overall survival, HR: Hazard Ratio, 95% CI: 95% Confidence interval, IPI: International prognostic index, OS is defined as the time from cancer diagnosis to the date of last follow up or death from any cause. Hazard ratios (HR) and 95% CI were assessed using multivariate Cox proportional hazard regression, significance defined by (p<0.05).

Table (9): prognostic parameters which affect progression free (PFS) survival in NHL patients in univariate and multivariate analyses

Parameter	Univariate analysis			Multivariate analysis		
	HR	95%CI	P	HR	95%CI	P
rs1801157(GA+AA) vs (GG)	1.9	0.989-3.576	0.054	1.4	0.879-2.965	0.16
IPI (3,4) vs (1,2)	2.6	1.446-4.873	0.002	2.3	1.287-4.133	0.01
Stage (III&IV) vs (I&II)	0.912	1.975-3.988	0.01	0.798	0.983-3.137	0.055
B symptoms (present vs absent)	0.492	0.345-1.089	0.072	0.346	0.149-0.651	0.12
Response to (first line therapy response vs refractory)	0.626	1.047-4.920	0.04	0.721	0.895-3.327	0.08

(PFS): progression free survival, HR: Hazard Ratio, 95% CI: 95% Confidence interval, IPI: International prognostic index, PFS is defined as the time from cancer diagnosis to the date of progression, relapse, last follow up or death. Hazard ratios (HR) and 95% CI were assessed using multivariate Cox proportional hazard regression, significance defined by (p<0.05).

Discussion:

Cancer is a worldwide major health problem and a leading cause of death. Hematological malignancies are in the top ten causes of cancer death, their incidence has been rising in the last decades with NHL the most frequently encountered [2, 13].

Studies have shown that chemokines and their receptors play a critical role in tumor growth, progression and immune evasion through several

mechanisms. It is also clear that host genetic variation can alter the level of cytokine gene expression and cytokine secretion [4, 12].

There is increasing evidence that *CXCL12/CXCR4* axis is implicated in the pathogenesis and metastasis of NHL. Increased expression of *CXCL12* mRNA was detected in lymph nodes stromal cells from patients with malignant lymphoma, and *CXCL12* expression was detected in tissues from DLBL [23]. In addition,

Studies on primary central nervous system lymphoma showed that *CXCL12* helped in providing a suitable microenvironment for NHL survival, and the elevated levels of *CXCL12* in the patients CSF decreased obviously in the group who responded to treatment [24].

On the other hand, *CXCR4* is widely expressed on B lineage cells and its expression was higher in B cell tumors with multiple nodal metastasis such as CLL and MCL than in tumors with restricted nodal invasion [7]. Binding of *CXCL12* to *CXCR4* in mantle cell lymphoma enhanced the migration of the cells and was associated with increased expression of *CXCL12* mRNA by stromal cells of the invaded bone marrow [25] and in vitro migration of follicular lymphoma cells in response to *CXCL12* was also demonstrated [26].

The *CXCL12* gene may contain a single nucleotide polymorphism (SNP) rs1801157 also known as G801A where guanine substitutes adenine at base pair 801 of the 3'-untranslated region of *CXCL12* gene. It was reported to possibly increase the expression of *CXCL12* and the availability of *CXCL12* for binding to *CXCR4* [13]. Previous studies investigated the potential role of *CXCL12* (rs1801157; G>A) polymorphism in many cancers such as cervical carcinoma, colorectal carcinoma [27], as well as in hematological malignancies such as myeloid leukemias [19], ALL [28], and MM [29]. Their results showed that *CXCL12* (rs1801157; G>A) polymorphism associated with cancer susceptibility, severity, metastasis or response to therapy.

In NHL, although the association of *CXCL12* (rs1801157; G>A) polymorphism with risk of NHL was reported [5, 21] however, only one Turkish study evaluated its association with clinical characteristics and patient outcome [21]. To our knowledge this is the second study describing the influence of *CXCL12* (rs1801157; G>A) polymorphism on clinical characteristics and prognosis in NHL.

The present work revealed that the prevalence of *CXCL12* (rs1801157; G>A) GA genotype was significantly higher in NHL patients than in controls ($P<0.001$), while the A allele tended to be more frequent in NHL patients than in controls (24% vs 14%), therefore, *CXCL12* (rs1801157; G>A) polymorphism could be considered as a genetic risk factor for this disease in the Egyptian population.

This is in agreement with Pamuk et al. [21] and de Oliveira et al. [5] who reported that *CXCL12* (rs1801157; G>A) polymorphism may be implicated in NHL pathogenesis which they explained in the light of previous studies [30- 31] that reported the AA genotype associated with more *CXCL12* production compared to other genotypes thus it might have enhanced its interaction with *CXCR4*. Also *CXCL12* (rs1801157; G>A) polymorphism may regulate the expression of *CXCL12* mRNA since studies on AIDS patients reported that both homozygous and heterozygous rs1801157 variants were associated with an increased risk of NHL [32], and increased levels of *CXCL12* mRNA was detected in circulating mononuclear cells from pediatric patients with AIDS related NHL [33].

Moreover, the rs1801157 A variant increased the level and stability of *CXCL12* mRNA in vitro [20].

On the contrary, Zhang et al. [34] reported that the rs1801157 A variant associated with decreased level of plasma *CXCL12* compared to the GG genotype. Also, de Olivera et al. [35] reported the presence of the rs1801157 A variant associated with decreased level of expression of *CXCL12* mRNA and increased level of *CXCR4* mRNA depending on $IFN\gamma$ levels, suggesting that mutual interactions between *CXCL12* chemokine and *CXCR4* are complicated and may involve other molecules other than sole chemokine-ligand interaction [20]. Moreover, a favorable role of *CXCL12*-3'A allele was reported in patients with MM and CLL. In CLL it associated with susceptibility to CLL but with less advanced stages of the disease at diagnosis [20]. In MM it associated with less advanced stages of the disease at diagnosis and with better survival, but since *CXCL12* facilitates MM cell adhesion to endothelial cells and induce MM cells' migration [29]. They explained these associations by increased *CXCR4* expression or other molecular features resulting from the decrease of *CXCL12* levels rather than their increase [20].

The present study also revealed a significant association of *CXCL12* (GA+ AA) variant genotypes with increased frequency of invasion and metastasis in NHL. Patients with *CXCL12*(GA+ AA) genotypes had significantly more frequent advanced clinical staging III, IV ($P=0.019$) and ≥ 2 extranodal involvement ($P=0.03$) when compared to patients with the GG genotype.

This could be attributed to the attraction of the cancer cells by *CXCL12* to sites where the microenvironment is suitable for their survival via activation of *CXCR4* on NHL cells [20]. Since *CXCL12* is expressed in high levels in organs commonly targeted by tumor metastasis such as lymph nodes, bone marrow, lung, and liver [5], it seems that *CXCR4* on NHL cells guides their migration to these organs where its ligand is present in large amounts where they interact causing the cancer cells to form metastases [7].

It could be also explained by the promotion of pathological angiogenesis in tumors by the *CXCL12*/*CXCR4* axis as previous studies reported significant association between *CXCR4* expression and VEGF production, and inhibition of angiogenesis and tumor metastasis via blockade of the *CXCL12*/*CXCR4* axis suggesting it could be a target in cancer therapy [4, 11].

In our study, *CXCL12* (GA+ AA) genotypes also significantly associated with high initial serum LDH ($P=0.02$), and it was more frequent in patients with the IPI risk (3,4) at diagnosis than those with IPI (1or 2) ($P=0.031$). Similar to our results Pamuk et al. [21] reported *CXCL12* mutant genotypes (GA+ AA) significantly associated with high initial serum LDH ($P=0.01$), and tended to have more frequent extranodal involvement ($P=0.09$) than GG genotype. They suggested that *CXCL12* (rs1801157; G>A) polymorphism might be a marker of poor prognosis in NHL patients with GA and AA genotypes.

Moreover, we found that patients with *CXCL12* (GA+AA) genotypes had significantly worse OS (P=0.037) and PFS (P=0.047) than those with the GG genotype. In univariate analysis, the presence of A allele increased significantly the relative risk of death nearly twice for OS (HR 2.1, 95% CI: 1.024-4.173, P=0.043), and for PFS (HR 1.9, 95% CI: 0.989-3.576, P=0.054), though insignificant. Other predictors of poor survival in univariate analysis included the presence of high IPI score, advanced Ann-Arbor stage, B symptoms, and unresponsiveness to first-line therapy for OS, and high IPI score, advanced Ann-Arbor stage, and unresponsiveness to first-line therapy for PFS. However, in multivariate analysis the effect of the polymorphism was not evident, and the presence of high initial IPI score was the only independent factor. Our results are similar to Pamuk et al. [21] Who reported significantly worse OS of NHL patients with *CXCL12* (GA+AA) genotypes than those with the GG genotype (P=0.047) in univariate analysis however, in multivariate analysis the only independent poor prognostic factors were high initial IPI score (HR 4.3, 95% CI: 1.01-18.9, P=0.049), and recurrence within the first 6 months after treatment (HR 19.6, 95% CI: 5.9-62.5, P <0.001). This may be due to small sample size as the effects of previously established prognostic parameters were not also evident in the multivariate analysis, we suggest larger studies should be conducted for better evaluation the effect of *CXCL12* (rs1801157; G>A) polymorphism on the outcome of NHL.

Conclusion:

This study revealed that *CXCL12* (rs1801157; G>A) SNP could be a risk factor for developing de novo NHL in Egyptian population and it may be a predictor of poor prognosis of NHL. We recommend this work should be extended on a larger population of patients, and to correlate the results with the circulating *CXCL12* proteins levels for thorough investigating the role of this polymorphism on the prognosis of NHL.

List of abbreviations:

NHL, Non-Hodgkin lymphoma
 SCDF-1, Stromal cell derived factor-1
 ALL, Acute lymphoblastic leukemia
 CLL, Chronic lymphocytic leukemia
 MM, Multiple myeloma
 DLBL, Diffuse large B cell lymphoma
 CSF, Cerebrospinal fluid
 SNP, Single nucleotide polymorphism
 PCR-RFLP, Polymerase chain reaction-restriction fragment length polymorphism
 P.S, Performance status
 IPI, International prognostic index
 OR, Odds ratio
 Hb, Hemoglobin
 TLC, Total leucocytic count
 Plts, Platelets
 CR, Complete remission
 PR, Partial remission
 95% CI, 95% Confidence interval, Ref: Reference

HR, Hazard Ratio
 OS, Overall survival
 PFS, Progression free survival
 IFN γ , Interferon Gamma

Conflict of interest:

The authors of this study confirm there are no conflicts of interest regarding its publishing.

Authors' contributions:

All authors worked in collaboration to execute this work. Author RSA designed, performed the molecular study and wrote the manuscript, author OYA helped in performing the lab techniques and recruitment of the controls and author AR reviewed the records of the patients, collected and interpreted the data in a comprehensive sheet for the statistical analysis. All authors read and approved the final manuscript.

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