Possible Role of Anti-Inflammatory cytokine Gene Polymorphism in AML Susceptibility, Egypt

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Abstract: Background*:** Acute myeloid leukemia (AML) is a cytogenetically and molecularly heterogeneous diseases and characterization of transforming genetic events is becoming increasingly important. Interleukins (ILs) are a diverse set of small cell signaling protein molecules. Single nucleotide polymorphisms (SNPs) of ILs alter their function, increasing susceptibility to different diseases. ***Methods: We investigated the association between polymorphism in interleukin-10 (IL-10) -819T/C (rs 1800871) and the risk of AML in the Egyptian population. DNA was isolated from bone marrow of 80 newly diagnosed adult AML patients, and 85 age and sex matched controls. Genetic analysis of IL-10 SNPs at -819T/C was assayed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). ***Results*:** Genetic analysis of IL-10 revealed that the Egyptians have high -819 T allele frequencies in apparently healthy controls while -819CC genotype and the -819C allele frequencies in the AML group were higher than in the controls (P=0.000086). The study suggested that subjects carrying the rs 1800871 CC genotype and C allele had a significantly increased risk for AML. ***Conclusion*:** IL-10 SNP at -819 was associated with enhanced AML risk, suggesting that rs1800871 provides clue for future studies and early detection of AML.

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1. Introduction:

Acute myeloid leukemia (AML) is a life-threatening hematopoietic stem cell neoplasms, it is a cytogenetically and molecularly heterogeneous diseases characterized by clonal proliferation of myeloid precursors and maturation arrest, with accumulation of acquired genetic alterations in hematopoietic progenitor cells that disturb normal mechanisms of cell growth, proliferation and differentiation. **[1]**

The precise molecular origins of AML are unknown. The pathophysiologic mechanisms are multiple, act in concert, and probably are distinct in different types of AML. Inherited genetic predisposition and environmental mutagens such as radiation, drugs and other toxins all play a role in the development of AML.**[2]**

Characterization of transforming genetic events is becoming increasingly important in establishing diagnosis, defining prognosis, and planning therapy in AML. Advances in chemotherapy and supportive management have improved the survival of younger patients with AML. The current challenge is to improve understanding of molecular mechanisms of AML and design leukemia-specific treatments that would be applicable to older patients.**[3]**

Interleukins (ILs) are a diverse set of small cell signaling protein molecules, or cytokines, that function to alter the immune system in humans.**[4, 5]**

Single nucleotide polymorphisms (SNPs) of ILs may alter their function, thus changing cytokine function and dysregulating their expression. **[6]**

IL10 is a pleiotropic cytokine, produced by a variety of cells including T-cells, macrophages, Lyl positive B-cells, EBV positive B-cells, mast cells and keratinocytes.**[7]** IL10 has many inhibitory, **[8], [9]**and stimulatory functions. **[10-12]**

The gene encoding IL-10 is located on chromosome 1 (1q31-1q32) and displays a few polymorphisms in the promoter region of the transcriptional start site, including IL-10 -1082G/A (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872). Very few studies have been published reporting that the polymorphisms in IL-10 were associated with AML. **[13, 14].** To the best of our knowledge there are no published studies its association in Caucasians or African AML cases.

The aim of this study was to investigate the frequency and association between -819 T/C (rs 1800871), one of the polymorphisms of IL -10 and the risk of AML in Egyptian patients. Taking into consideration, numbers of reports on the Egyptian population have documented differences in the frequencies of various SNPs of a number of genes when compared to populations in other parts of the world. **[15-17]**

2. Patients and methods

This study was carried out on 80 newly diagnosed adult AML patients with 85 Age and sex matched apparently healthy individuals as controls over a period of two years. Clinical, morphological, cytochemical, flow cytometric and cytogenetic analysis were done to confirm diagnosis. All the cases met the AML diagnosis standards.**[18]**

A Written informed consent was approved by the Institutional Review board (IRB) ethical committee of the NCI which follows the rules of Helsinki IRB and was obtained from each patient before starting the data collection. For the sake of patients' privacy, they were given code numbers.

Collection and preservation of the sample:

Peripheral blood and bone marrow samples were collected in EDTA 2-5 ml tubes from the participants and treated with erythrocyte lysis solution. Leukocytes were then separated with centrifugation (2000 rpm, 2 min) at 4 °C and stored in Trizol (10^7 leukocytes/ml) at -80 °C for DNA extraction.

Genetic polymorphism analysis:

Total genomic DNA was extracted from bone marrow cells of AML patients using the Mini QIAamp DNA isolation kit (QIAGEN) following the standard procedures according to the manufacturer's instructions. Genetic analysis of IL-10 SNPs at -819 was identified by polymerase chain reaction amplification and restriction fragment length polymorphism analysis (PCR-RFLP). The SNPs sequence of -819T/C (rs 1800871) of human IL-10 was searched through the Gene Bank from the National Center for Biotechnology Information (NCBI). The primer sequence of the desired SNP was as follows: (5′-TCATTCTATGTGCTGGAGATGG-3′) as a forward primer and (5′-TGGGGGAAGTGGGTAAGAGT-3′) as a reverse primer. The genomic DNA of AML patients and healthy controls were used as template and each PCR was performed in a Gene Amp. PCR System 9700 Thermo-cycler (applied Bio-Systems, USA).

PCR was conducted with 150 ng of genomic DNA, 10p/mol. of each of the primer, and 12.5ul of Master Mix (TAQ. PCR Master Mix) (applied Bio-Systems, USA). The reaction was carried out using the following thermal cycle reaction: 95°C for 5 min., followed by denaturation at 94°C for 45 sec., annealing at 59°C for 45 sec., and extension at 72°C for 45 sec., with a final extension at 72°C for 7 min. This resulted in one band at 209bp. Digestion of the PCR product was done by MslI Restriction enzyme (Sib Enzyme, Russia). The polymorphism was detected on a 2.5% agarose gel electrophoresis.

Patients were assessed for response to therapy by bone marrow assessment on days 15 and 28. Patients were considered in remission if the blast count is less than 5 %.

Statistical methods:

Data management and analysis was performed using SPSS, version 20. Categorical data were summarized as percentages; numerical data were summarized using means and standard deviation or medians and ranges. Relation between IL-10 and other variables was assessed using Chi-square test. Overall survival (OS) was defined as the time from diagnosis to the time of death from any cause. Patients who were alive on the date of last follow-up were censored on that date. Progression free survival (PFS) was defined as the time from starting therapy until documented progression or death. For patients without disease progression (DP) at the time of analysis, the date of last follow-up was considered right-censored. OS and PFS were estimated using the Kaplan-Meier analysis. Log rank test was used to compare survival curves. All tests of hypotheses were conducted at the alpha of 0.05 Level, with a 95% confidence interval.**[19]**

3. Results

According to the PC Relectrophoregram, allele analysis revealed 3 genotypes for SNPs at -819:Wild type genotype (TT), homozygotes (CC) and heterozygotes (TC).After digestion of the PCR product at -819site with MslI restriction enzyme, TT produced one band at 209 bp, CC produced two bands at 93bp+116bp and TC produced three bands at 209bp+116bp+93bp respectively. [**Figure1]**

The genotype and allele frequencies for both patients and controls are listed in **Table 1,**showing a high statistical significant difference in the genotype distribution between cases and controls. CC genotype and C allele prevalence were significantly higher in cases than in controls (31, 38.8% versus 9, 10.6%, *P*=0.00014) and (85, 53.1% versus 54, 31.76%, *P*=0.000086). On the other hand, TT genotype and T allele prevalence were higher among controls (40, 47.1% and 116, 68.24%).

Tables 2 summarizes the Demographic characteristics, clinical, hematological, FAB classification and karyotyping of the AML patients. Their mean agewas 34.1 ±1.42 and males represented 49 (61.25%). Stratification of the patients according to FAB Classification showed that the most common subtype was M4 (26, 32.5%), and the least was M0 (1, 1.3%).

FLT mutation was assessed in 77 patients only while the Karyotype analysis was achieved successfully in all patients. Abnormal karyotype was found in 17 patients (21.3%), and the most common chromosomal abnormality was t (8:21) in (8, 10.0%) patients.

Genotype distribution of IL-10 in relation to demographic characteristics, FAB classification and karyotype is shown in **Table 3**, with no statistical

significant association found between any of IL-10 genotypes and age, TLC, FAB subtypes, karyotyping or FLT mutation (*P*= 0.93, 0.61, 0.76, 0.94 and 0.16 respectively).

During follow up of the patients and assessment of the response to therapy, 49 patients (61.3%) achieved complete remission, 16 were resistant to treatment (20.0%), and 15 (18.7%) died before time of evaluation.

Median Progression Free Survival (PFS) and overall survival (OS) for all patients were 12.2 months (95% CI 6.9-17.14) and 6.12 months (95% CI 0.027-12.21) respectively. There was no effect of the Il-10 genotype on PFS (*P*= 0.93) or overall survival (*P*=0.92).[**Figures2,3]**

Table 1. Genotype and Allele Distribution in AML Cases and Controls.

Group	Cases number	Genotype						Allele number	Allele frequency			
		TT		TC		CC			T allele		C allele	
		N	(%)	N	(%)	N	(%)		N	%	N	%
AML	80	26	(32.5)	23	(28.8)	31	(38.8)	160	75	(46.9)	85	(53.1)
Controls	85	40	(47.1)	36	(42.35)	9	(10.6)	170	116	(68.24)	54	(31.76)
P VALUE						**0.00014***						**0.000086***

*Highly significant

Table 2. Demographic characteristics, clinical, hematological, FAB classification and karyotyping of AML patients.

Patients characteristics	**N=80 Mean**	**(%) ±SD**
Age	34.1	±1.42
Sex		
Male	49.0	(61.25)
Female	31.0	(38.75)
Splenomegaly	34.0	(42.5)
Hepatomegaly	34.0	(42.5)
Lymphadenopathy	11.0	(13.75)
Laboratory finding	**Mean**	**+/- SD**
TLC	61.64	+/-7.53
HB	7.8	+/-0.20
Platelet	74.07	+/-18.7
Peripheral blood blasts%	57.1	+/-2.55
Bone marrow blasts%	68.29	+/-2.11
Bone marrow cellularity	**N**	**(%)**
Normo-cellular	9.0	(11.3)
Hyper-cellular	71.0	(88.8)
FLT status	**N=77**	**(%)**
Wild	49.0	(63.6)
Mutant	28.0	(36.4)
FAB		
M0	1	(1.3)
M1	20	(25.0)
M2	22	(27.5)
M3	6	(7.5)
M4	26	(32.5)
M5	5	(6.3)
Cytogenetics		
Normal karyotype	63	(78.8)
t (8:21)	8	(10.0)
t (15:17)	6	(7.5)
Inv. 16	2	(2.5)
t (9:22)	1	(1.3)

N= number, TLC (Total leucocyte count), HB (Hemoglobin), FLT (FMS-related tyrosine kinase 3) FAB (French American British)

Table 3: Genotype distribution of IL-10 according to demographic characteristics, FAB and karyotype

Patients characteristics	*TT N=26*		*TC N=23*		*CC N=31*		*P value*
	N	*(%)*	*N*	*(%)*	*N*	*(%)*	
Gender							*0.35*
Male	*13*	*(50.0)*	*15*	*(65.2)*	*21*	*(67.7)*	
Female	*13*	*(50.0)*	*8*	*(34.8)*	*10*	*(32.3)*	
Age							*0.93*
< 40	*19*	*(73.1)*	*16*	*(69.6)*	*23*	*(74.2)*	
≥ 41	*7*	*(26.9)*	*7*	*(30.4)*	*8*	*(25.8)*	
TLC							*0.61*
<12	*19*	*(73.1)*	*18*	*(78.3)*	*26*	*(83.9)*	
≥12	*7*	*(26.9)*	*5*	*(21.7)*	*5*	*(16.1)*	
FAB							*0.76*
M0	*0*	*(0.0)*	*0*	*(0.0)*	*1*	*(3.2)*	
M1	*6*	*(23.1)*	*6*	*(26.1)*	*8*	*(25.8)*	
M2	*8*	*(30.8)*	*8*	*(34.8)*	*6*	*(19.4)*	
M3	*2*	*(7.7)*	*1*	*(4.3)*	*3*	*(9.7)*	
M4	*7*	*(26.9)*	*8*	*(34.8)*	*11*	*(35.5)*	
M5	*3*	*11.5*	*0*	*(0.0)*	*2*	*(6.5)*	
Cytogenetics							*0.94*
Normal karyotype	*21*	*(80.8)*	*18*	*(78.3)*	*24*	*(77.4)*	
Abnormal karyotype	*5*	*(19.2)*	*5*	*(21.7)*	*7*	*(22.6)*	
FLT status							*0.16*
Wild	*15*	*(57.7)*	*16*	*(69.6)*	*18*	*(58.1)*	
Mutant	*10*	*(38.5)*	*6*	*(26.1)*	*12*	*(38.7)*	

***Significant P<0.05**

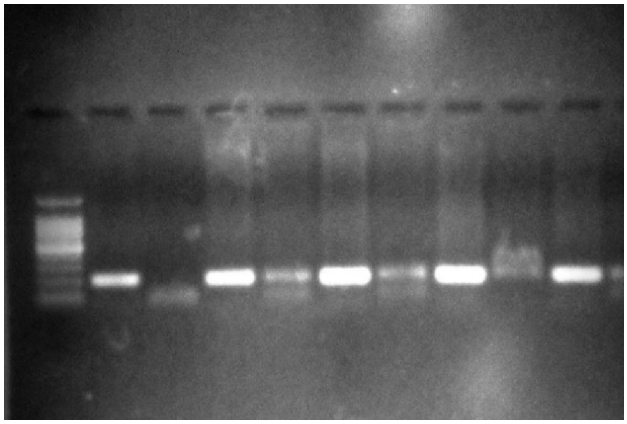

Figure 1: Run for PCR Detection of IL-10 SNPs at -819T/C
Lane 2, 4, 6, 8, 10: PCR product for IL-10 (209bp).
Lane 3: C/C genotype (116bp+93bp).
Lane 5, 7, 11: T/C genotype (209bp+116bp+93bp).
Lane 9: T/T genotype (209bp).

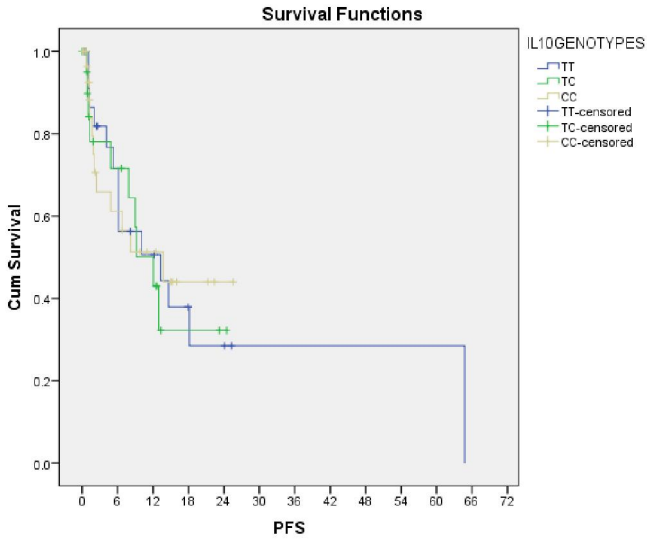

Figure 2: PFS of patients according to IL-10 phenotype

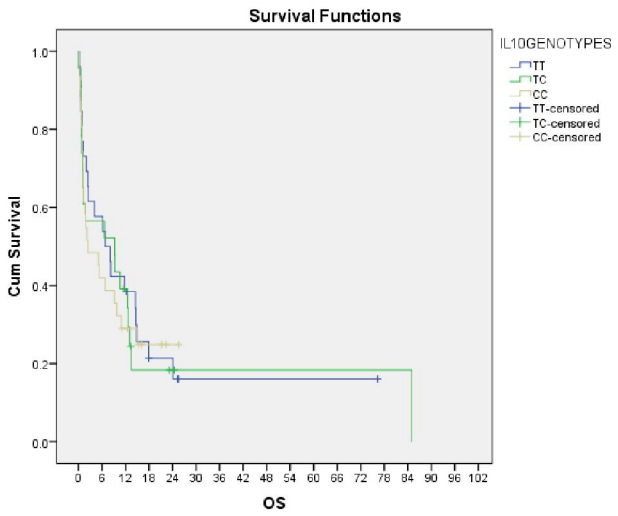

Figure 3: OS of patients according to IL-10 phenotype

4. Discussion:

IL-10 is an important immune-regulatory cytokine which is produced by activated T cells, monocytes, B cells, and thymocytes. IL-10 has immune-stimulating and immuno-suppressive functions and may regulate tumor susceptibility and development.**[20, 21]**

Polymorphisms in the promoter region ofIL-10 gene was associated with cancer susceptibility, affected the severity, progress of the disease and the IL-10 expression level.**[6, 22,23]**

SNPs at-819 T/CUpstream of the transcription start site has been identified and become the research focus in genetic susceptibility of cancer in the recent 10 years.**[24]**

Previous studies revealed that IL-10 polymorphisms are associated with many cancers such as gastric cancer, lung cancer, head and neck squamous cell carcinoma, oral cancer, cervical cancer, and prostate cancer.**[5, 25 - 27]** Others found that IL-10 SNP was associated with the non-Hodgkin's lymphoma.**[24, 28]**

For AML, only 2 studies reported an association between the rs1800871 gene polymorphisms and risk of AML. These published studies were done on Asian populations and showed that polymorphisms in rs1800871 may be associated with increased risk of AML.**[13, 14]**

A study on the Chinese population showed that rs1800871 CC genotype and C allele showed a significantly increased risk for AML (*P*=0.04 and 0.03).**[13]** A result which is consistent with ours.

On the contrary, our results are not consistent with that encountered by Chen *et al.,***[14]** who found TT and T allele prevalence and frequencies were higher in cases than controls (*P*= 0.014 and 0.004) among Han population.

Besides our results, another Egyptian study showed that the apparent healthy controls had the prevalence of TT and T allele in -819 SNPs for IL-10 rs1800872.**[29]**

When compared with other populations in different parts of the world, the Egyptian population has a documented difference in the Genotype and allele frequencies of a number of genes e.g. TPMT, NAT2, GST, SULT1A1 and MDR-1in general population, **[15]** as well as in different diseases e.g. type 1 diabetes mellitus, acute lymphoblastic leukemia and hepatitis C.**[16, 17, 30]**

Such results denote that polymorphisms can influence predisposition to various diseases depending on ethnic genetic makeup not the SNPs per se.

Moreover, it should be taken into consideration, differences in response to therapy between different populations although using the same protocols might be attributed to such genetic variabilities. Egyptian AML patients in spite of receiving the same therapy protocols recommended worldwide have worse outcome than reported elsewhere. **[31]**

In conclusion, our preliminary results for the analysis of the IL-10 SNP at -819 among Egyptian population, was associated with increased risk of AML. Our results together with others, suggested that rs1800871 is a susceptible gene for AML, which provides important clues for future studies and early detection of AML. Further large-scale studies with large sample sizes from different ethnic populations are required to confirm our results. So future studies, will help to further validate the diagnostic and prognostic importance of such polymorphism and once this is clear, it may be possible to appropriately tailor the aggressiveness of therapy needed among different population according to their genetic makeup especially after the high differences in their response to therapy and high relapse rates among them.

Conflict of Interest

None of the authors of this paper has any financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

References

1. Dohner H. Implication of the molecular characterization of acute myeloid leukemia. Hematology. Am. Soc. Hematol. Educ. Program. 2007:412-419.
2. Solomon S, Malkovska V. Acute myeloid leukemia. In: Rodgers GP, Young NS, eds. Bethesda Handbook of Clinical Hematology.

Philadelphia, Pa: Lippincott Williams and Wilkins 2005: pp135-150.
3. Suhag V, Solomon S, Malkovska V. Acute myelogenous leukemia. In: Rodgers P, Young N, eds. Bethesda Handbook of Clinical Hematology. New York: Lippincott Williams and Wilkins 2005: pp 131-147.
4. Lippitz B E. Cytokine patterns in patients with cancer: a systematic review. Lancet. Oncol. 2013; 14: 218-228.
5. Tsai CW, Tsai MH, Shih LC, Chang WS, Lin CC, Bau DT. Association of interleukin-10 (IL10) promoter genotypes with nasopharyngeal carcinoma risk in Taiwan. Anticancer. Res. 2013; 33:3391-3396.
6. Zhou Y, Li N, Zhuang W, Liu GJ, Wu TX, Yao X, *et al.* Interleukin-10 -1082 promoter polymorphism associated with gastric cancer among Asians. Eur. J. Cancer. 2008; 44: 2648-2654.
7. Moore KW, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann TR. Interleukin 10. Annu. Rev. Immunol. 1993; 11:165-190.
8. Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O'Garra A. IL-10 inhibits cytokine production by activated macrophages. J. Immunol. 1991; 147: 3815-3822.
9. De Waal Malefyt R1, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: An autoregulatory role of IL-10 produced by monocytes. J. Exp. Med. 1991; 174:1209-1220.
10. Rousset F, Garcia E, Defrance T, Péronne C, Vezzio N, Hsu DH, *et al.*Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc. Natl. Acad. Sci. USA. 1992; 89: 1890-1893.
11. Mosmann TR, Moore KW. The role of IL-10 in crossregulation of TH1 and TH2 responses. Immunol. Today. 1991; 12: 49-53.
12. Rennick D, Hunte B, Dang W, Thompson-Snipes L, Hudak S. Interleukin-10 promotes the growth of megakaryocyte, mast cell, and multilineage colonies: Analysis with committed progenitors and Thyllo Scal+ stem cells. Exp. Hematol. 1994; 22: 136-141.
13. Yao CJ, Du W, Chen HB, Xiao S, Wang CH, Fan ZL. Associations of IL-10 gene polymorphisms with acute myeloid leukemia in Human, China. Asian Pac. J. Cancer Prev. 2013; 14: 2439-2442.
14. Chenjiao Y, Zili F, Hai BC, Ying L, Sheng X, Lihua H, *et al.*IL-10 promoter polymorphisms affect IL-10 production and associate with susceptibility to acute myeloid leukemia. Pharmazie. 2013; 68: 201-206.
15. Hamdy SI, Hiratsuka M, Narahara K, Endo N, EI-Enany M, Moursi N, *et al.* Genotype and allele frequencies of TPMT, NAT2, GST, SULT1A1 and MDR-1 in the Egyptian population. Br. J. Clin. Pharmacol. 2003; 55: 560–569.
16. El-Kafoury AA, Haroun M, EmbabyAM, Dawoods AS. The association of polymorphic sites in some genes with type 1 diabetes mellitus in a sample of Egyptian children. Egyptian Journal of Medical Human Genetics. 2014; 15: 265–272.
17. Kamel AM, Ebid GTA, Moussa HS. N-Acetyltransferase 2 (NAT2) polymorphism as a risk modifier of susceptibility to pediatric acute lymphoblastic leukemia. Tumor Biol. 2015; 36: 6341-6348.
18. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, *et al.* The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009; 114: 937-951.
19. Dawson-Saunders B, Trapp GT (editors). Basic and Clinical Biostatistics. Norwalk, Connecticut, Lange Medical Books, Appleton & Lange, 1994, p. 123.
20. Villalta SA, Rinaldi C, Deng B, Liu G, Fedor B, Tidball JG. Interleukin-10 reduces the pathology of mdx muscular dystrophy by deactivating M1 macrophages and modulating macrophage phenotype. Hum. Mol. Genet. 2011; 20: 790-805.
21. Helminen M, Lahdenpohja N, Hurme M. Polymorphism of the interleukin-10 gene is associated with susceptibility to Epstein-Barr virus infection. J. Infect. Dis.1999; 180: 496-499.
22. Chen KF, Li B, Wei YG, Peng CJ. Interleukin-10 -819 promoter polymorphism associated with gastric cancer among Asians. J. Int. Med. Res. 2010; 38: 1-8.
23. Zhuang W, Wu XT, Zhou Y, Liu L, Liu GJ, Wu TX, *et al.*Interleukin10 -592 promoter polymorphism associated with gastric cancer among Asians: a meta-analysis of epidemiologic studies. Dig. Dis. Sci. 2010; 55: 1525-1532.
24. Cunningham LM, Chapman C, Dunstan R, Bell MC, Joske DJ. Polymorphisms in the interleukin 10 gene promoter are associated with susceptibility to aggressive non-Hodgkin's lymphoma. Leuk. Lymphoma. 2003; 44: 251-255.
25. Howell WM, Rose-Zerilli MJ. Cytokine gene polymorphisms, cancer susceptibility, and prognosis. J. Nutr. 2007; 137: 194-199.
26. Pan XF, Yang SJ, Loh M, Xie Y, Wen YY, Tian Z, *et al.* Interleukin-10 gene promoter

polymorphisms and risk of gastric cancer in a Chinese population: single nucleotide and haplotype analyses. Asian Pac. J. Cancer Prev. 2013; 14: 2577-2582.
27. Sun JM, Li Q, Gu HY, Chen YJ, Wei JS, Zhu Q, *et al.* Interleukin 10 rs1800872 T/ G polymorphism was associated with an increased risk of esophageal cancer in a Chinese population. Asian Pac. J. Cancer Prev. 2013; 14: 3443-3447.
28. Lan Q, Zheng T, Rthoman N, Zhang Y, Wang SS, Shen M, *et al.* Cytokine polymorphisms in the Th1/Th2 pathway and susceptibility to non-Hodgkin lymphoma. Blood. 2006; 107: 4101-4108.
29. Shaker OG, Sadik NAH. Polymorphisms in interleukin-10 and interleukin-28B genes in Egyptian patients with chronic hepatitis C virus genotype 4 and their effect on the response to pegylated interferon/ribavirin-therapy. Journal of Gastroenterology and Hepatology. 2012; 27: 1842–1849.
30. Pedergnana V, Abdel-Hamid M, Guergnon J, Mohsen A, Le Fouler L, Theodorou I, *et al.* Analysis of IL28B variants in an Egyptian population defines the 20 kilobases minimal region involved in spontaneous clearance of hepatitis C virus. PLoS. One. 2012; 7.
31. El-zawahry HM, Zeeneldine AA, Samira MA, Mattar MM, El-Gammal MM, Abd El-Samee A, *et al.*Cost and Outcome of Treatment of Adults with Acute Myeloid Leukemia at the National Cancer Institute-Egypt. Journal of the Egyptian Nat. Cancer Inst. 2007; 19: 106-113.

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