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Multiple myeloma in egyptian population and interleukin 10 (IL-10) and its receptor (IL-10R) polymorphism

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***Abstract*---**Background: Multiple myeloma (MM) is a cancer of bone marrow (BM) that is caused by the clonal plasma cells which produce monoclonal immunoglobulin that cause manifestation such as hypercalcemia, pathological bone fracture, renal impairment, BM deficiency, and hyper viscosity. Interleukin 10 (IL-10) has anti-inflammatory effects by binding to IL-10R dependent signals on cell surface; if IL-10 mutation affects cancer immunity so may be cause of many cancers including hematologic malignancies. Objective: To investigate role of IL-10 gene promoter (rs1800872) and its receptor beta (rs2834167) genotypes in (MM) patients on clinical presentation, laboratory findings, treatment response and outcome in a group of MM patients in Egyptian patients. Patients and Methods: The present study included 50 MM patients compared to 50 matched healthy individuals in age and sex with normal laboratory findings as a control group for analysis of single nucleotide polymorphisms (SNP) of IL-10 gene promoter (rs1800872), and IL-10Rβ (rs2834167) genotypes using Real time- Polymerase Chain reaction (RT-PCR) technique. Results: The genotypes frequency distribution of IL-10 SNP (rs1800872), there was statistically significant difference between MM patient and the control groups (P- value 0.003) but not found as regard IL-10 RB (P –

value 0.142). There was statistical significance difference between both genes as regarding median hemoglobin level , calcium level , M protein level, B2 microglobulin and plasma cells as (p- value <0.005). We found a statistical significance difference between both genes as regarding bone lesions and ISS as (P-value <0.001). We didn't find any statistical significance difference between genes and IG subtype, creatinine level or albumin level p-value >0.05. Comparing the frequency of different genotypes of IL10 and IL10RB with clinical and laboratory data after management of MM patients; there was a statistical significant difference between genes and M protein level, plasma cells and bone lesions (P-value=0.05). Regarding follow up of patients after therapy the number of patients achieved complete remission less in the CC (homozygous) than CA(heterozygous) genotypes of IL10 SNP as (P-value =0.046) and less in the (GG –GA)(homo-heterozygous) than AA(wild) of IL 10RB genotypes as (P-value < 0.001). As regard overall survival; there was no statistical significant difference between IL10 SNP genotypes in MM patients (p-value=0.472) but less in the (GG –GA) (homo-heterozygous) than AA (wild) o IL10RB genotypes (p-value < 0.001). The number of patients achieved DFS and EFS was less in the (GG –GA)(homo-heterozygous) than AA(wild)of IL10RB genotypes (p-value < 0.001) but no statistical significant difference between IL10 genotypes MM patients as DES(p-value = 0.713) and EFS (p-value = 0.603). In conclusion, IL-10 and its receptor beta polymorphisms are associated with MM and clinicopathological features as high ISS stage and no CR after chemotherapy. IL-10 has a significant role in the development and pathogenesis of this disease. IL-10RB polymorphisms are associated with bad OS, DFS and EFS. So IL-10 and its receptor B polymorphisms may be a valuable new marker in the diagnosis and prognosis of MM patients. Therefore, serum IL-10 level might be helpful for predicting stage and management of MM.

***Keywords*---**cancer, multiple myloma, gene polymorphism.

Introduction

MM is a hematologic threat gotten from the dangerous clonal multiplication of bone marrow B cells and the emission of monoclonal immunoglobulins that harm the objective organs. MM is the second most analyzed hematological threat, the fundamental clinical attributes are anemic manifestation, hypercalcemia, rehashed contamination, renal harm, and bone crack, high rate in the older population [1]. The monoclonal protein delivered by these plasma cells is an unusual (immunoglobulin G [IgG], IgM, or IgA, or, seldom, IgE or IgD) or light chain protein (kappa or lambda), both of which causes hyperviscosity and end-organ harm. Intrusive bone sores can cause pathologic cracks of bone, osteoporosis, and increase calcium level [2]. Different cytokines in the BM microenvironment advance myeloma and development by means of advancement of neighborhood angiogenesis (upregulation of VEGF), avoidance of cell-intervened

insusceptibility (downregulation of TNF-α and IL-12), advancement of clonal expansion, and avoid apoptosis by upregulation of PDGF and IGF-1 [3].
The total impact of this cytokine condition is to act as anti apoptotic action inside the beginning myeloma cells, preferring unregulated clonal development and dangerous multiplication. These cytokines and dissolvable factors all give expected objective focuses to medicate design [4]. IL-10 has a role during infection as inhibits the host immune response to pathogen, leading to harm to the host and keeping up typical tissue homeostasis; so its dysregulation lead to immunopathology and improvement of numerous immune system diseases [5]. IL-10 is cytokine that bind to transmembrane receptor complex, comprising of dimer of IL-10R alpha (otherwise called IL-10R1) and dimer of IL-10R beta (otherwise called IL-10R2) proteins [6]. The two receptors have a place with the receptor family class II, and comprise of three areas: an intracellular space, a transmembrane area, and an extracellular area .The receptor complex collects consecutively: IL-10Rα, with higher partiality, firstly bind to IL-10 and afterward IL-10Rβ [7].Upon the receptor-ligand commitment, phosphorylation of the receptor-related protein tyrosine kinase JAK1 is selected to the intracellular area by the IL-10R1 chain, while non-receptor TYK2 is enlisted to the receptor complex by IL-10R2 [8]. IL-10 can essentially improve the multiplication of B cells, being associated with their terminal differantiation into plasma cells [9].

Materials and Methods

Patient characteristics

The current study included 50 people with multiple myeloma who had been diagnosed clinically. As a control group, 50 healthy people of the same age and gender with normal laboratory results were used. The Revised Helsinki Declaration of Bioethics was followed to ensure data confidentiality (2008). Prior to treatment, patients' blood samples were taken. A one-year follow-up was performed on the patients.

IL10 and IL10R genotyping

The Real-time Polymerase Chain Reaction (RT-PCR) approach had been used to identify the IL10 promoter −592C/A (rs1800872), and IL10Rβ (rs2834167) genotypes. DNA extraction Gene JET Genomic DNA purification kit (Cat. #K078, Thermo Scientific) had been used to separate DNA from whole blood. Genomic DNA is introduced into a reaction mixture composed of TaqMan Genotyping Master Mix, forward and reverse primers and two TaqMan MGB Probes. The primers 5′ AGGTCACAGTGACGTGG 3′ (upstream primer) and 5′GGTGAGCACTACCTGACTAGG 3′ (downstream primer) were used to analyse IL10 gene promotor −592C/A. We used 5′TACAGTGGGAGTCACCTGCTTTTGTC 3′ (upstream primer) and 5′TAGTCAGACTCCCTTCCTCCTGT 3′ (downstream primer) to analyse IL10RB K47E.

Statistical analysis

The statistical package for the social sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA) was used to code and enter the data. For quantitative

variables, mean, standard deviation, median, minimum, and maximum values were used, while for categorical variables, frequencies (number of cases) and relative frequencies (percentages) were used. Comparisons of quantitative variables were performed using the unpaired T test in normally distributed data and the non-Mann-Whitney test in non-normally distributed data [10]. The Chi square (2) test was used to compare categorical data. When the expected frequency is less than 5, the exact test is used instead. Using chi-square tests, the disease and control groups were compared in terms of genotype and allele frequencies. The odds ratio (OR) with 95% confidence intervals was calculated. P-values less than 0.05 were considered statistically significant. The Kaplan-Meier method was used to analyse event-free and disease-free survival, and the log-rank test was used to compare differences between survival curves [11] .

Results

Clinical characteristics of MM patients

Patients were 32 males (64%) and 18(36%) females, while the control group were 26 male (52%) and 24 female (48%). The patients' ages varied from 35 to 68 years, with a mean value of 53.78 ± 7.80, the control group's age ranged from 30 to 77 years old, with a mean value of 50.98 ± 11.5. In terms of age, there was no statistically significant difference between MM patients and controls (p-value = 0.158). The median Hemoglobin (HB) and serum albumin (Alb) levels were significantly lower in MM patients (HB 9.35 g /dl)and (Albumin 3.80 g /dl) than in controls (HB 12.25g /dl) and (Albumin 4.30 g /dl) (P- value < 0.001). While median of total serum calcium (Ca) level was significantly higher in MM patients (11.45 mg /dl) than controls (8.30 mg/dl) (P- value <0.001). Comparing median serum creatinine (Cr) level in patients and controls didn't show significant difference (P- value =0.890).

X- ray examination of MM patients ;there were 34%(68%) of patients with bone lesions. Renal plasmocytoma (Extra medullary diseases) were found in one patient (2 %). There were 39 (78%) MM patient with IG G subtype and 11(22%) with immunoglobulin A subtype. The M protein of MM patients ranged from 2.7 - 9.5 g/dl with a median value 5.90g/dl. The B2 microglobulin of MM patients ranged from 2.9 - 8 ng/ml with a median value 5.00 ng/ml. The bone marrow plasma cells of MM patients ranged from 20% - 67% with a median value 28.50%. According to International Staging System (ISS) of MM patients there were 16 (32%) stage I, 14 (28%) stage II and 20 (40%) stage III . 24(48%) of MM patients underwent bone marrow transplantation.

Post treatment radiological finding and clinical data in MM patients

Bone lesion detected by X – rays after management of MM patients with comparison to X – rays before management; there were 11(32.3%) MM patients not reduced bone lesion ,2(5.9%) >50% reduction of bone lesion in size and 21(61.8%) with disappearance of bone lesion . The M protein after treatment of MM patients ranged from 0.2 g/dl to 8 g/dl with a median value 0.90. The bone marrow plasma cells after treatment of MM patients ranged from 1% to 20% with

a median value 3.50%. The B2-microglobulin level after treatment of MM patients ranged from 1 ng/ml to7 ng/ml with a median value 2.08.

Follow up of 50 MM patients

Thirty out of fifty MM patients developed complete remission CR (60%), Partial remission 9(18%) and no CR in 11 (22%) of MM patients. From 39 patients 26 still in CR (66.6%), 9 developed Relapse (23.07%) and 4 died (10.25%). At the end of the follow up time (70%) of patients were alive. Fifteen patients out of fifty MM patients died (30%).

3.2 Frequencies of genotypes and alleles in controls and MM patients

The genotype and allele frequency patterns of the IL10−592C/A, and IL10Rβ polymorphisms are summarized in (Table 1). As regards single nucleotide polymorphisms of IL-10 gene promoter (rs1800872) genotypes, there was a statistically significant difference between the groups of MM patients and controls (P-value 0.003). There was statistically significant difference between control and MM group as regards the C allele of IL-10 gene promoter (rs1800872) (P- value 0.013). As regards IL-10Rβ (rs2834167) genotypes, there was no statistically significant difference between the groups of MM patients and controls. In terms of the G allele of the IL-10RB gene promoter (rs2834167), there was no statistically significant difference between the control and MM groups (P- value 0.108).

Table 1
Genotypes frequency of IL-10 SNP (rs1800872) and IL-10 receptor B SNP (rs2834167) results in MM group and Control group

		Cases	Controls	P value	OR	95% CI	
		N 50(%)	N 50 (%)			Lower	Upper
IL10 SNP (rs1800872)	AA (Wild)	0	8(16%)	Reference			
	CA (Heterozygous)	30(60.0%)	31(62%)	0.003	--	--	--
	CC (Homozygous)	20(40%)	11(22%)	0.003	--	--	--
	allele A	30(30%)	47(47%)	Reference			
	allele C	70(70%)	53(53%)	0.013	--	--	--
IL 10 RB SNP (rs2834167)	AA (Wild)	25(50%)	28(56%)	Reference			
	GA (Heteroozygous)	7(14%)	12(24%)	0.437	0.653	0.223	1.918
	GG (Homozygous)	18(36%)	10(20%)	0.142	2.016	0.785	5.174
	allele A	57(57%)	68(68%)	Reference			
	allele G	43(43%)	32(32%)	0.108	1.603	0.900	2.855

Comparison between IL10 SNP with clinical variables and prognosis in MM

IL10 SNP and clinical variables in MM patients are summarized in (Table 2,3). There was a highly statistically significant difference in hemoglobin level between the CC genotype and CA genotype of IL10 SNP with a P-value <0.001. There was a highly statistically significant difference in calcium level between the CC genotype and CA genotype of IL10 SNP with a P-value <0.001. There was a highly statistically significant difference in M protein level between the CC genotype and CA genotype of IL10 SNP with a P-value <0.001.There was a highly statistically significant difference in B2 microglobulin level between the CC genotype and CA genotype of IL10 SNP with a P-value <0.001. There was a highly statistically significant difference in bone marrow plasma cells between the CC genotype and CA genotype of IL10 SNP with a P-value <0.001. Bone lesions were significantly higher among patients with CC genotype 20(100%) than CA genotype 14 (46.7%). There was a highly statistical significant difference in ISS staging between the CC genotype and the CA genotype of IL10 SNP with a P-value <0.001

Table 2
Comparison between IL 10 SNP(rs1800872genotypes in relation to patients age and laboratory data

	IL 10 SNP						
	CC (N=20)			CA (N=30)			P value
	Mean ±SD	Median	Range	Mean ±SD	Median	Range	
Age	53.10 ±7.68	53.00	38.00-64.00	54.23 ±7.97	55.00	35.00-68.00	0.620
HB(g/dl)	7.95 ±0.50	8.10	6.90-8.50	10.40 ±0.86	10.60	8.80 - 11.70	< 0.001
Ca (mg/dl)	12.72 ±0.43	12.80	12.10-13.80	10.81 ±0.56	10.50	10.00 - 11.80	< 0.001
Cr(g/dl)	0.88 ±0.19	0.90	0.60-1.20	0.93 ±0.27	0.90	0.60 - 1.90	0.453
Alb (g/dl)	3.80 ±0.24	3.75	3.50-4.20	3.75 ±0.32	3.80	3.10 - 4.30	0.624
Ig (g/dl) (M protien)	7.43 ±1.22	7.90	5.40-8.50	5.00 ±1.52	4.40	2.70 - 9.50	< 0.001
B2 microglobulin	6.56 ±0.88	6.30	5.60-8.00	3.82 ±0.91	3.25	2.90 - 5.20	< 0.001
BM Plasma cell (%)	44.1 ±11.61	42.00	28.0-67.00	25.23 ±3.41	25.00	20.00 - 33.00	< 0.001

Table 3
Comparison between IL 10 SNP (rs1800872) genotypes in relation to patients' clinical data and staging of MM patients

		IL 10 SNP				P value
		CC (N=20) (Homozygous)		CA (N=30) (Heterozygous)		
		N	%	N	%	
Bone lesions	postive	20	100.0%	14	46.7%	< 0.001
	negative	0	0.0%	16	53.3%	
Plasmocytoma	Renal plasmocytoma	0	0.0%	1	3.3%	1
	negative	20	100.0%	29	96.7%	
ISS stage	I	0	0.0%	16	53.3%	< 0.001
	II	0	0.0%	14	46.7%	
	III	20	100.0%	0	0.0%	

The relationships between IL10 polymorphisms and the clinical data in MM patients after treatment are summarized in (Table 4). Median M protein level after treatment of MM was significantly higher in CC genotype (3.25 g/dl) than CA genotype (0.75g/dl) as P-value =0.006. Median BM plasma cells percentage after treatment of MM were found significantly higher in CC genotype (7.50%) than CA genotype (2%) as P-value =0.011. The median B2-microglobulin level following MM therapy was significantly greater in the CC genotype (5ng/ml) than in the CA genotype (2.35ng/ml) with a P-value =0.016.

Table 4
Post treatment laboratory data in IL 10 SNP (rs1800872)

	IL 10 SNP						P value
	CC (N=20) (Homozygous)			CA (N=30) (Heterozygous)			
	Mean±SD	Median	Range	Mean±SD	Median	Range	
M protein(g/dl)	3.94±3.14	3.25	0.50-8.0	1.43±1.43	0.75	0.20-6.0	0.006
BM Plasma cell (%)	9.15±7.31	7.50	1.00-20.0	4.00±4.37	2.00	1.00-20.0	0.011
B2-microglobulin (ng/ml)	4.34± 2.27	5	1.5-7	2.95±1.66	2.35	1-6	0.016

There was statistically significant difference in complete remission between the CC genotype and the CA genotype of the IL10 SNP, with a P-value =0.046. There was a no statistical significant difference in follow up regarding overall survival in relation to IL10 SNP genotypes MM patients. (P-value= 0.472). There was no statistically significant difference in follow up regarding disease free survival in relation to IL10 SNP genotypes MM patients. (p-value= 0.713).There was no

statistically significant difference in follow up regarding event free survival in relation to IL10 SNP genotypes MM patients (Table 5, Figure 1).

Table 5
Follow up of MM patients regarding IL 10(rs1800872) genotypes

		IL 10 SNP				
		CC (Homozygous)		CA (Heterozygous)		P value
		N	%	Count	%	
Remission	complete	9	45.0%	21	70.0%	0.046
	partial	3	15.0%	6	20.0%	
	no	8	40.0%	3	10.0%	
OS	Alive	12	60.0%	23	76.7%	0.472
	Dead	8	40.0%	7	23.3%	
DFS(no=39)	Complete remission	9	75.0%	17	63%	0.713
	Relapse or Death	3	25.0%	10	37%	
EFS	Complete remission	9	45.0%	17	56.7%	0.603
	No CR, Relapse or Death	11	55.0%	13	43.3%	

CR (Complete remission) PR (Partial remission) No CR(No complete remission)
P < 0.05 Sig P<0.001 HS P>0.05 NS

Comparison between IL-10 Rβ polymorphisms with clinical variables and prognosis in MM

IL10-Rβ SNP and the clinical variables in MM are summarized in (Table 6, 7). There was a highly statistically significant difference in median hemoglobin level between the (GG-GA) genotypes and the AA genotype of IL10RB SNP with a P-value <0.001. There was a highly statistically significant difference in median calcium level between the (GG-GA) genotypes and the AA genotype of IL10RB SNP with a P-value <0.001. There was a highly statistically significant difference in median M protein level between the (GG-GA) genotypes and the AA genotype of IL10RB SNP with a P-value <0.001. There was a highly statistically significant difference in median B2 microglobulin level between the GG-GA genotypes and the AA genotype of IL10RB SNP with a P-value <0.001. There was a statistically significant difference in median bone marrow plasma cells between the GG –GA genotypes and the AA genotype of IL10RB SNP with a P-value <0.001. There was a highly statistically significant difference in bone lesion between the GG-GA genotypes and the AA genotype of IL10RB SNP with a P-value <0.001. There was a highly statistically significant difference in ISS staging between the (GG –GA) genotypes and the AA genotype of IL10BR SNP with a P-value <0.001.

Table 6
Comparison between IL 10RB SNP (rs2834167) genotypes in relation to patients' age and laboratory data

	IL10 receptor B SNP						
	AA (N=25) (Wild)			GG+GA(N=25) (Homo/Heterozygous)			P value
	Mean± SD	Median	Range	Mean±SD	Median	Range	
Age	52.80±8.08	54	35.0-68.0	54.76±7.54	55	41.0-65.0	0.38
HB g/dl	10.17±1.33	10.9	8.10-11.7	8.68±1.08	8.8	6.9-10.30	< 0.001
Ca mg/dl	11.10±1.14	10.5	10.0-13.8	12.05±0.77	11.8	11.0-13.3	0.001
Cr mg/dl	0.92±0.20	0.9	0.60-1.2	0.90±0.28	0.8	0.60-1.90	0.731
Alb g/dl	3.85±0.23	3.8	3.50-4.3	3.69±0.33	3.7	3.10-4.20	0.056
Ig (g/dl) M protein	5.14±1.83	4.4	2.70-8.5	6.80±1.46	6.9	3.7-9.50	0.001
B2 microglobulin	4.02±1.31	3.2	2.90-6.1	5.81±1.40	5.2	3.40-8.0	< 0.001
BM Plasma cell (%)	27.92±6.91	25	21.00-44.0	37.64±14.20	31	20.0-67.0	0.004

Table 7
Comparison between IL 10RB SNP(rs2834167) genotypes in relation to patients' clinical data

		IL10B receptor SNP				
		AA(N=25) (Wild)		GG+GA(N=25) (Homo/Heterozygous		P value
		N	%	N	%	
Bone lesions	postive	9	36%	25	100%	< 0.001
	negative	16	64%	0	0%	
Plasmocytoma	Renal plasmocytoma	0	0.0%	1	4%	1
	negative	25	100.0%	24	96%	
ISS stage	I	16	64%	0	0%	< 0.001
	II	1	4%	13	52%	
	III	8	32%	12	48%	

The relationships between IL10 SNP with the clinical data in MM patients after treatment are summarized in (Table 8, 9). Median M protein level after treatment of MM was significantly higher in (GG –GA) genotype (3.5 g/dl) than AA genotype (0.6g/dl) as P-value < 0.001. Median plasma cells percentage after treatment of

MM was significantly higher in (GG –GA) genotype (7%) than AA genotype (1%) as P-value < 0.001. Median B2-microglobulin level percentage after treatment of MM was significantly higher in (GG –GA) genotype (5ng/ml) than AA genotype (2ng/ml) as P-value < 0.001. Bone lesions detection by X – rays after management of MM patients with comparison to X – rays before management; there was a highly statistical significant difference between the (GG –GA) genotypes and the AA genotype of IL10 RB SNP with a P-value < 0.001.

Table 8
Post treatment laboratory data in IL 10 SNP(rs2834167)

	IL10 receptor B SNP						
	AA(N=25) (Wild)			GG+GA(N=25) (Homo/Heterozygous)			P value
	Mean±SD	Median	Range	Mean±SD	Median	Range	
M protein g/dl	0.83±1.26	0.6	0.20-6.8	4.04±2.54	3.5	0.60-8.0	< 0.001
Plasma cell%	1.60±0.76	1	1.00-4.00	10.52±6.06	7	1.00-20.0	< 0.001
B2 – microglobulin (ng/ml)	1.86±0.67	2	1.00-4.00	5.156±1.51	5	1.00-7.00	< 0.001

Table 9
Comparison between IL 10RB SNP (rs2834167) genotypes in relation to radiological data after treatment of MM patients

		IL10 receptor B SNP				
		AA(N=25) (Wild)		GG+GA(N=25) (Homo/Heterozygous)		P value
		N	%	N	%	
Radiological	Not reduced	0	0.00%	11	44.00%	< 0.001
	>50% reduction	0	0.00%	2	8.00%	
	Negative	25	100.00%	12	48.00%	

There was highly statistically significant difference between the (GG –GA) genotypes and AA genotype of IL10BR SNP regarding complete remission as P-value <0.001. There was highly statistically significant difference in follow up regarding overall survival in relation to IL10BR SNP genotypes MM patients (P-value < 0.001). There was highly statistically significant difference in follow up regarding disease free survival in relation to IL10BR SNP genotypes MM patients (P-value < 0.001). There was highly statistically significant difference in follow up regarding event free survival in relation to IL10 RB SNP genotypes MM patients (p-value < 0.001) (Table 10, Figure 2).

Table 10
Follow up of MM patients regarding IL 10RB SNP (rs2834167)

		IL 10 RB SNP(rs2834167)				
		AA(N=25) (Wild)		GG+GA(N=25) (Homo/Heterozygous)		P value
		N	%	N	%	
Remission	CR	25	100%	5	20%	< 0.001
	PR	0	0%	9	36%	
	No CR	0	0%	11	44%	
OS	Alive	24	96%	11	44%	< 0.001
	Dead	1	4%	14	56%	
DFS(no=39)	CR	24	96%	2	14.30%	< 0.001
	Relapse or Death	1	4%	12	85.70%	
EFS	CR	24	96%	2	8%	< 0.001
	No CR, Relapse or Death	1	4%	23	92%	

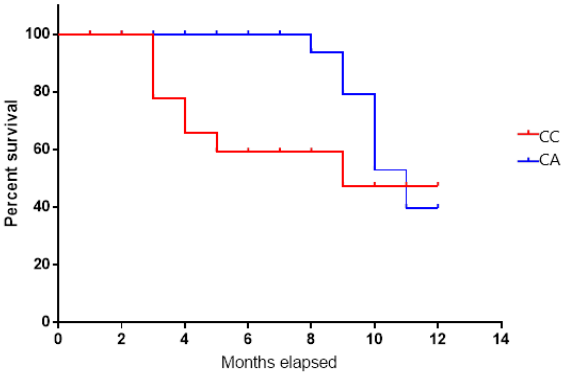

Figure 1. Overall survival in relation to IL 10 SNP in MM patients

Survival at 1 years was alive 12(60%) in CC genotype patients and 23(76.7%) in CA genotype of MM patients and dead 8(40%) in CC genotype patients and 7(23.3%) in CA genotype of MM patients (p-value= 0.472).

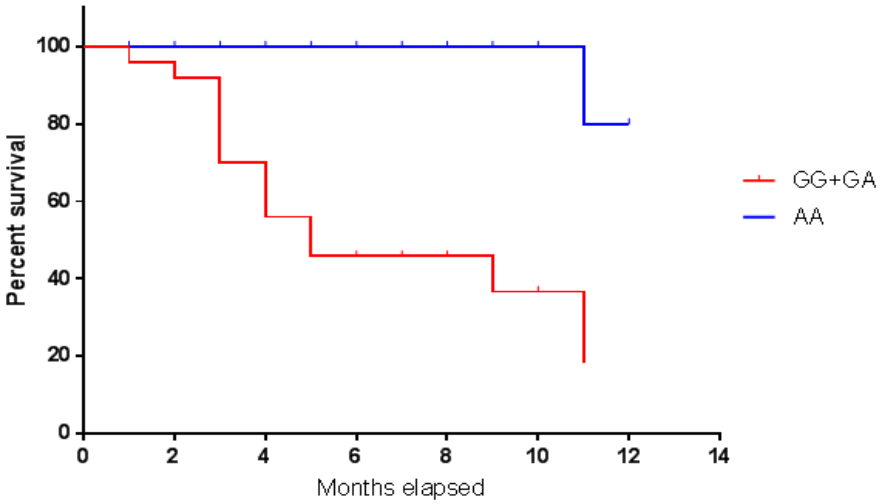

Figure 2. Overall survival in relation to IL 10 BR SNP in MM patients

Survival at 1 years was alive 11(44%) in GG-GA genotypes patients but 24(96.0%) in AA genotype of MM patients and died 14(56%) in GG-GA genotypes patients but only one (4%) in AA genotype of MM patients died (p-value < 0.001).

Discussion

Multiple myeloma (MM) is a hematological danger issue. It is the second most determined hematological threat to have the occurrence pace of ~1.1/100,000 that represents 2.1% of all new disease cases in 2016. Regularly, MM is a serious malignant growth of the plasma cells, which is distinguished by the invasion and clonal multiplication of immune response emitting post-germinal focus plasma cells in the bone marrow that lead to renal deficiency, bone illness and BM disease [12]. The pathology of MM is conjectured to include numerous elements including hereditary, resistant framework and natural causes. Since MM is a heterogeneous infection, hereditary components assume a significant part in its etiology. Quality polymorphisms of inflammatory elements might be engaged with the movement of MM by unbalancing supportive of and calming cytokines profile [12].

IL10 is a cytokine that can affect the proliferation of B cells as it has a role in their terminal differentiation into plasma cells [13].It has a strong immune inhibitory effect, because it inhibits action of Th1 lymphocytes so IL10 SNP may favor tumor growth in vitro by stimulating cell proliferation and inhibiting apoptosis so has role in hematological malignancy as lymphoma,chronic lymphoproliferative disease as CLL and MM [14]. Thus, order to gain a better understanding of the Impact of IL-10 gene promoter(rs1800872) and IL-10RB(rs2834167) genotypes on MM patients; we studied these different genotypes with a specific goal of identifying their relationship to clinical features , laboratory findings , effect of treatment and outcome in a cohort of MM diseased patients from Egyptian population. According to previous results; Kasamatsu and

his colleagues investigated the impact of IL10 promoter (rs1800872) and IL10RB (rs2834167) genotypes on MM incidence and the clinical presentation of MM using (REFLP PCR) restriction fragment length polymorphism method ; there was no statistically significant difference observed in the allele frequencies of IL10 −592C/A and IL10RB between MM patients and healthy controls [15]. According to Shekarriz et al.,2018 and H. Wang et al., 2016 ; levels of IL-10 in serum were measured in MM diseased patients and controls using an enzyme linked immunosorbent assay (ELISA). IL-10 concentrations in serum of patients were significantly higher ($p<0.0001$) than that in healthy controls .[13] [16]According to Kovacs, 2010; explained that MM proliferation affected by IL-6, causes a marked increase production of IL-10 myeloma cell lines. Both cytokines enhanced these cells by activating the JAK-STAT signaling pathway via binding to cell surface receptor specific to them.[17] Marangon and his colleages also found that was less frequency of A (IL10 -592) alleles in DLBCL patients if there was increase IL-10 level, so that IL10 low or intermediate level associated with genotypes and high level of IFN-γ production were associated with disease protection , possibly favoring the immune response of T helper 1 and decrease the capacity of cell proliferation [14]. According to Iwasaki et al., 2012, the IL-10 592 CC genotype was significantly associated with increased β2 microglobulin level (CC vs non CC) (p-value<0. 05) [18].

In our study; we tried to find a relation between different disease variables and the presence of IL-10 and its receptor B genotypes; there was a statistical significance difference between genotypes as regarding bone lesions (P-value <0.001). Bone lesion was higher in CC genotype than of CA genotype of IL10 SNP and higher in GG-GA genotype than of AA carrying genotype patients of IL10 RB SNP. Our findings differed from those of Kasamatsu et al., 2017 and Shekarriz et al.,2018, in that there was no statistically significant difference between IL 10 and its receptor B polymorphisms in bone lesions and plasmacytoma with a P-value >0.05 [15] [16]. Shekarriz et al.,2018 and H. Wang et al., 2016 found that serum IL-10 level measured by ELISA was significantly higher in patients with high stage of ISS and in our study mutated genotypes were significantly associated with high ISS stage [13] [16]. All patients were treated according to protocol therapy consisted of induction with a regimen of Cyclophosphamide, bortezomib and dexamethasone (CyBorD). The primary response endpoint was assessed after four cycles of 28 days each then autologous stem cell transplantation and lastly maintenance therapy. Follow up after treatment of patients for one year was done to evaluate treatment response. Our findings are consistent with those of Kasamatsu et al., 2017; the IL10 the CA and CC genotypes were associated with less survival than AA genotype (median OS, 46.3 vs 74.5 months; P = .047). The IL10RB GG genotype, on the other hand, was significantly associated with bad survival (median OS, 46.3 vs 78.8 months; P = .015) [15]. Patients with the IL10 rs1800872 AA genotype had a significantly higher complete remission (CR) and overall response rate (ORR) significantly. Patients with IL10 rs1800872 AA had a longer progression-free survival (PFS) (P = 0.017) and AA with AC of rs1800872 were associated with longer PFS and EFS in DLBCL patients according to Liu et al 2017 but Kim et al., 2016: found no association with OS (p = 0.06) and PFS (p = 0.08) in B-cell lymphoma patients [19] [20]

Conclusions

IL-10 SNP (rs1800872) has a significant role in the pathogenesis of MM. IL-10(rs1800872) and its receptor B (rs2834167) polymorphisms are associated with MM clinicopathological features. IL-10(rs1800872) and its receptor B (rs2834167) polymorphisms showed high rate of no CR after chemotherapy. Patients with IL-10RB polymorphism showed worse OS, DFS and EFS, high rate of no CR after chemotherapy. IL-10 polymorphisms can be used as a diagnostic marker in MM patients. IL-10 receptor B polymorphisms can be used as a marker to detect patients' response to treatment.

Abbreviations used

MM Multiple Myeloma
IL 10 Interleukin-10
PCR Polymerase chain reaction

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