

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

شَهِدَ اللَّهُ أَنَّهُ لَا إِلَهَ إِلَّا

هُوَ وَالْمَلَائِكَةُ وَأُولُو الْعِلْمِ قَائِمًا بِالْقِسْطِ

لَا إِلَهَ إِلَّا هُوَ الْعَزِيزُ الْحَكِيمُ (١٨)

صدق الله العظيم

آية (18) من سورة آل عمران



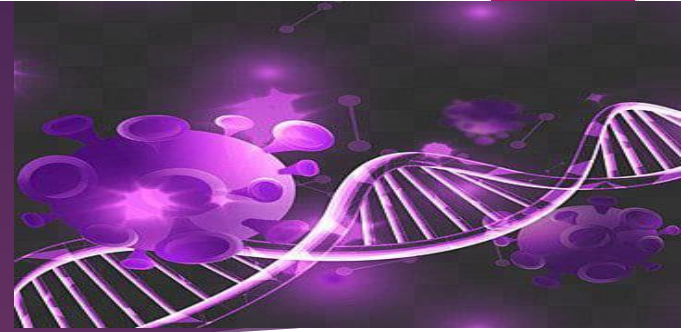
Detection of Toll like Receptor 9 Gene Polymorphisms in Leukemic Cancer of Iraqi Patients

Maryam Qasim Mohammed

Supervised by

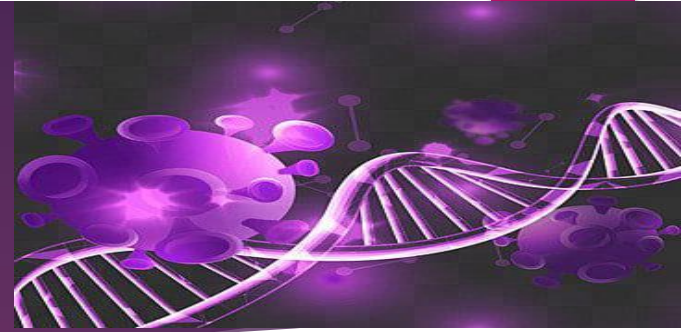
Prof. Dr. Ali Hussein Alwan

Introduction



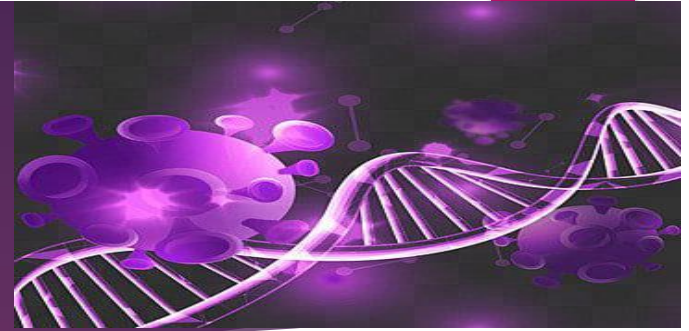
- ▶ **Leukemia:** is one of the types of blood cancer, which results from a dysfunction of the bone marrow, and causes the abnormal proliferation or propagation of white or red blood cells and may occur either acutely or chronically.
- ▶ **Classification:**
- ▶ Acute Myeloid Leukemia , acute lymphocytic leukemia, chronic myeloid leukemia and, chronic lymphocytic leukemia.
- ▶ **Acute Myeloid Leukemia (AML):** is a life-threatening disease that is a stem cell tumor, characterized by the presence of blasts that infiltrate from the bone marrow and press on the normal blood components causing bleeding, fatal infection, the presence or absence of an increase in the number of white blood cells in addition to the infiltration of organs.

Introduction



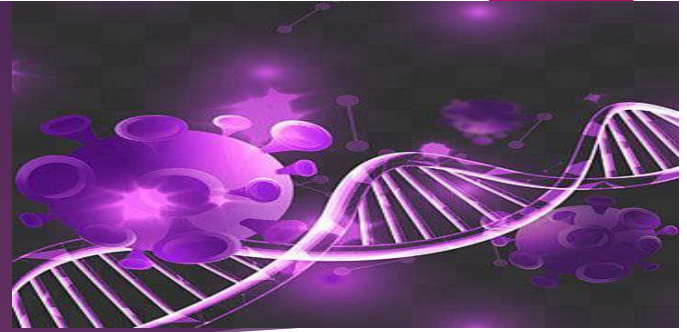
- ❖ Cancer is a major cause of death globally and has caused the death of **10** million people according to the World Health Organization report in 2020.
- ❖ The total number of leukemia cases between 2005 to 2015 has increased by 26%.
- ❖ Leukemia is one of the most common types of cancer in Iraq, as it ranks third and fourth rating for both male and female respectively, among the top ten common cancers, with a rate of **7.79** and **4.68** respectively according to the Iraq Cancer Council Report in 2018.

Introduction



- ▶ **Toll Like Receptors** belong to pattern recognition receptors (PRRs) family of innate immune system that consisting of 10 members which expressed by an antigen-presenting cells (APCs).
- ▶ ***TLR-9*** in humans is located on chromosome 3p21.2, contains two exons and the cells that express it is the plasmacytoid dendritic cell (pDC) and the B cell.
- ▶ Until this time, there is an opposite progression of TLRs towards cancer:
 - On the one hand, stimuli produced by these receptors can enhance the survival of malignant cells and increase their resistance to chemotherapy.
 - On the other hand, the tumor can be suppressed by binding to TLR-ligands

Introduction



❖ Single nucleotide polymorphisms

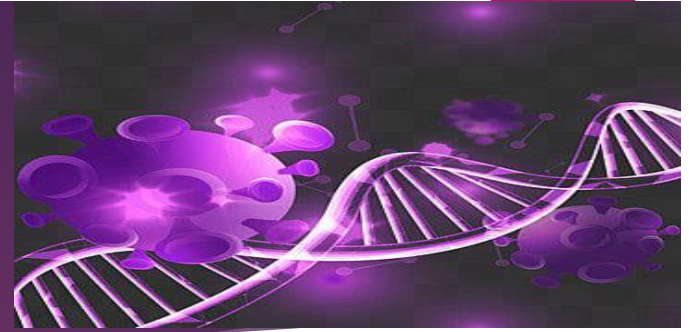
- ▶ **SNPs** which is defined as a simple change in the sequence of the nitrogenous bases constituting DNA that may cause a change in the code for the resulting amino acid and, may not cause a change in amino acid because it may occur in non-coding regions.
- ▶ several studies on the polymorphism of immune-related genes have a role in the prognosis of AML as well as can be used as a target for the treatment of the disease.

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Aims of the study



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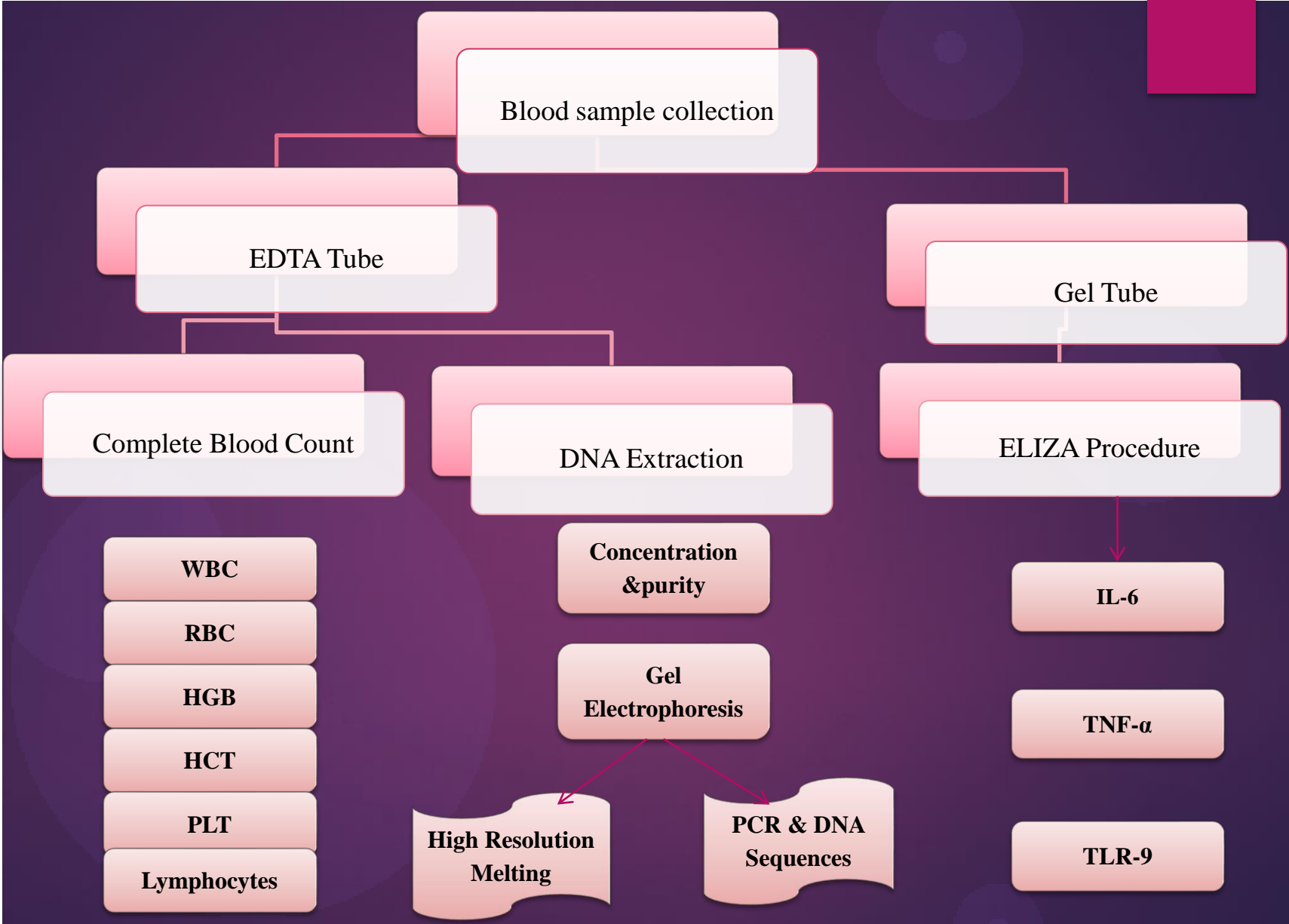


- ❖ Our study aimed :
- ❖ Evaluating single nucleotide polymorphisms of *TLR-9 gene* including (**rs187084**, **rs5743836** and **rs352140**) which may be used as prognostic factors in patients with AML.
- ❖ Evaluating some hematological parameters for comparing patients with healthy controls (**HGB**, **WBC**, **Lymphocytes**, **RBC**, and **HCT**).
- ❖ Evaluating the serum level of some immunological parameters like (**TLR-9**, **TNF- α** , and **IL-6**) using ELIZA technique.

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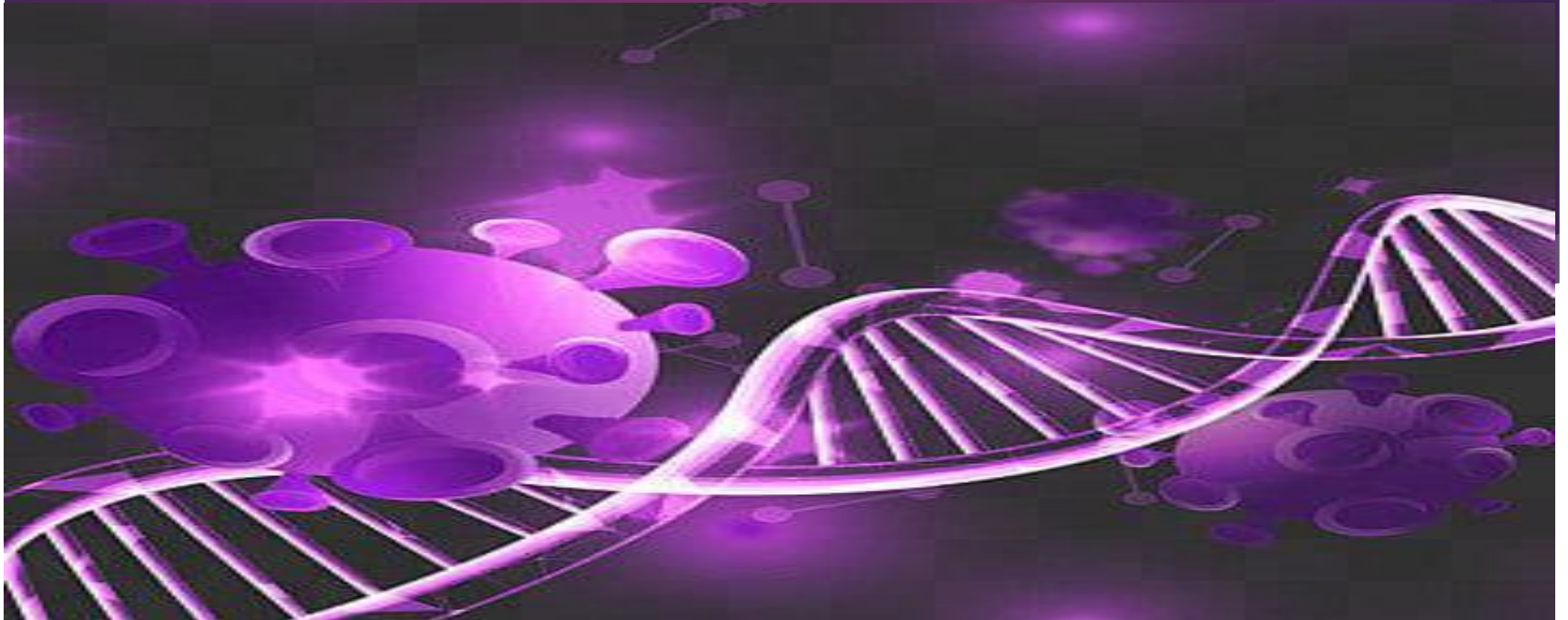
Methods



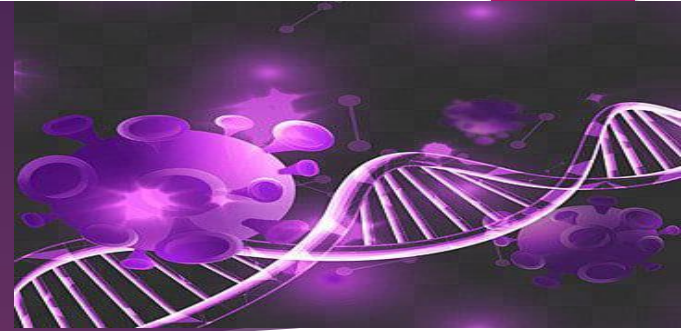


“

Results



Results



Comparison between patients and control groups in Age

Group	No	Mean \pm SE of Age (year)
Patients	60	43.15 \pm 2.47
Control	60	32.15 \pm 1.79
T-test		3.599 **
P-value		0.0001
** (P\leq0.01).		

Results

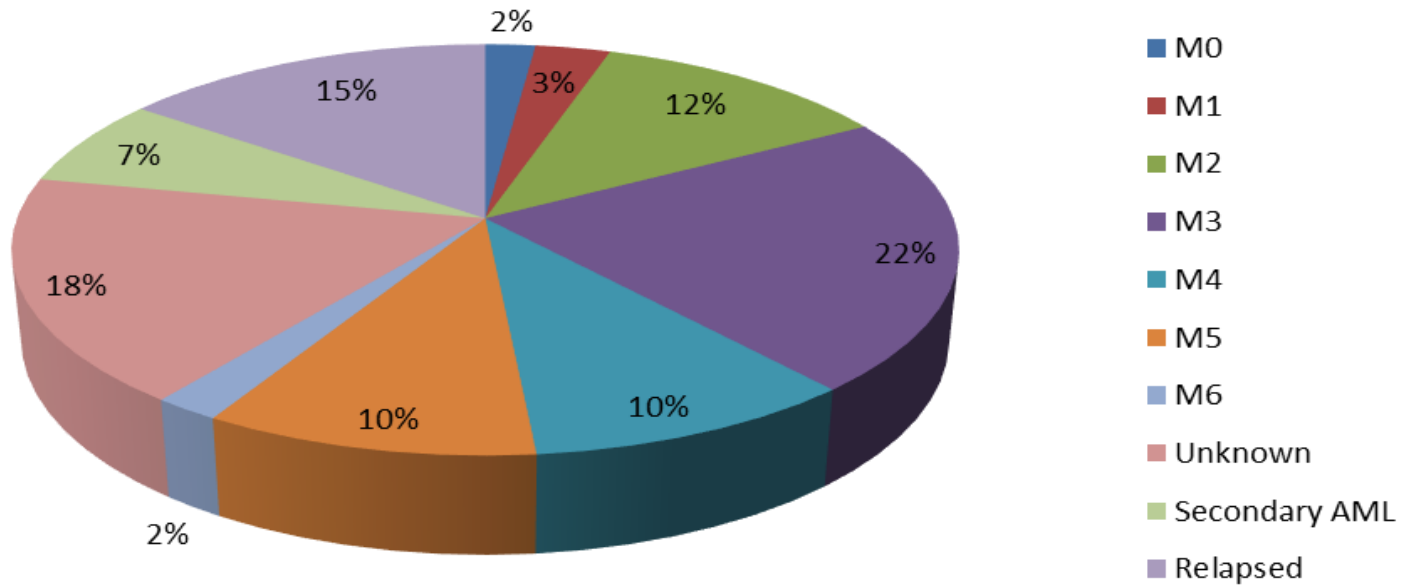


Distribution of sample study according to Gender in different Groups.

Group	No.	Gender		P-value
		Male No. (%)	Female No. (%)	
Control	60	26 (43.33%)	34 (56.67%)	0.0394 *
Patients	60	33 (55.00%)	27 (45.00%)	0.085 NS
P-value	--	0.045 *		---

* ($P \leq 0.05$), NS: Non-Significant.

Results



AML subtypes according to the current study

Results



Comparison between control and patients group in CBC parameters study

Group	Mean \pm SE					
	HGB g/dl	WBC $\times 10^3\mu\text{l}$	PLT $\times 10^3\mu\text{l}$	Lymphocytes $\times 10^3\mu\text{l}$	RBC $\times 10^6\mu\text{l}$	HCT %
Control	14.10 \pm 0.30	7.67 \pm 0.29	253.26 \pm 9.01	3.44 \pm 0.69	4.84 \pm 0.09	41.93 \pm 0.83
Patients	8.34 \pm 0.26	7.99 \pm 1.76	64.00 \pm 8.70	3.19 \pm 1.28	2.86 \pm 0.09	24.97 \pm 0.80
T-test	0.801 **	3.551 NS	24.807 **	2.765 NS	0.264 **	2.291 **
P-value	0.0001	0.756	0.0001	0.896	0.0001	0.0001
** (P\leq0.01), NS: Non-Significant.						

Results



Comparison between different subgroups in CBC parameters study

Sub-group	Mean \pm SE					
	HGB g/dl	WBC $\times 10^3\mu\text{l}$	PLT $\times 10^3\mu\text{l}$	Lymphocytes $\times 10^3\mu\text{l}$	RBC $\times 10^6\mu\text{l}$	HCT %
Control	14.10 ± 0.30 a	7.67 ± 0.29	253.26 ± 9.01 a	3.44 ± 0.69	4.84 ± 0.09 a	41.93 ± 0.83 a
FAB(M3)	8.26 ± 0.36 b	6.88 ± 3.42	80.22 ± 26.29 b	2.34 ± 1.42	2.76 ± 0.13 b	24.65 ± 1.02 b
FAB (Non M3)	8.43 ± 0.36 b	8.99 ± 2.57	66.76 ± 10.40 b	4.03 ± 2.30	2.90 ± 0.13 b	25.48 ± 1.12 b
Relapsed	8.26 ± 0.83 b	8.42 ± 4.73	26.89 ± 9.89 b	2.74 ± 1.75	2.84 ± 0.30 b	24.06 ± 2.46 b
Secondary-AML	7.88 ± 1.56 b	2.14 ± 0.82	71.27 ± 35.84 b	0.935 ± 0.37	2.91 ± 0.58 b	23.97 ± 4.63 b
LSD	1.955 **	8.601 NS	59.67 **	4.051 NS	0.641**	5.547 **
P-value	0.0001	0.742	0.0001	0.917	0.0001	0.0001

The means having the different letters in the same column differed significantly.

** ($P \leq 0.01$), NS: Non-Significant.

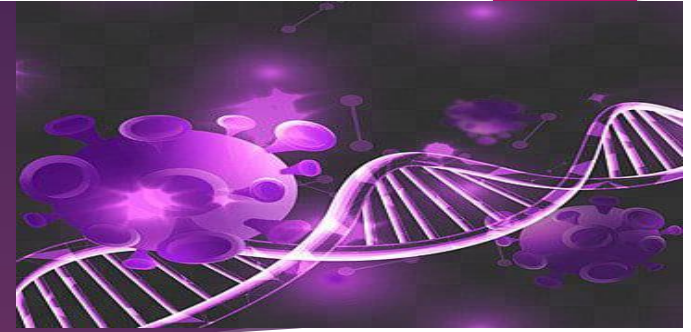
Results



Comparison between control and patients group in TLR-9, TNF- α , and IL-6.

Group	Mean \pm SE		
	TLR-9 (ng /ml)	TNF- α (pg /ml)	IL-6 (pg /ml)
Control	2.219 \pm 0.06	107.98 \pm 3.25	22.82 \pm 0.72
Patients	2.728 \pm 0.04	174.22 \pm 4.07	36.24 \pm 0.86
T-test	0.156 **	10.367 **	2.242 **
P-value	0.0001	0.0001	0.0001
** (P\leq0.01).			

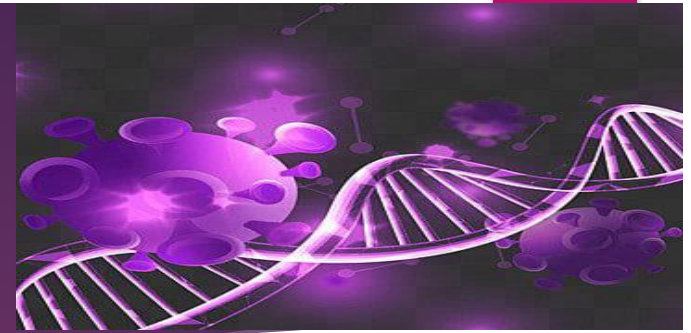
Results



Comparison between different Subgroups in TLR-9, TNF- α , and IL-6.

Sub-group	Mean \pm SE		
	TLR-9 (ng /ml)	TNF- α (pg /ml)	IL-6 (pg /ml)
Control	2.21 \pm 0.06 b	107.98 \pm 3.25 b	22.82 \pm 0.72 b
FAB(M3)	2.71 \pm 0.10 a	178.46 \pm 4.29 a	38.68 \pm 1.02 a
FAB (Non-M3)	2.76 \pm 0.06 a	173.69 \pm 6.21 a	36.28 \pm 1.19 a
Relapsed	2.64 \pm 0.07 a	168.36 \pm 10.33 a	33.39 \pm 1.95 a
Secondary-AML	2.69 \pm 0.16 a	172.11 \pm 26.84 a	34.34 \pm 6.41 a
LSD	0.298 **	27.602 **	4.912 **
P-value	0.0278	0.0084	0.0037

Results



Correlation between TLR-9, TNF- α , and IL-6 in patients.

Parameters	Pearson Correlation	TLR-9	IL-6	TNF- α
TLR-9	r	1	0.182	0.112
	P-Value		0.23	0.47
IL-6	r	0.182	1	0.788**
	P-Value	0.23		0.0001
TNF- α	r	0.112	0.788**	1
	P-Value	0.47	0.0001	

Results



Correlation between TLR-9, TNF- α , and IL-6 in control.

Parameters	Pearson Correlation	TLR-9	IL-6	TNF- α
TLR-9	r	1	0.404**	0.613**
	P-Value		0.006	0.0001
IL-6	r	0.404	1	0.445**
	P-Value	0.006		0.002
TNF- α	r	0.613**	0.445**	1
	P-Value	0.0001	0.002	

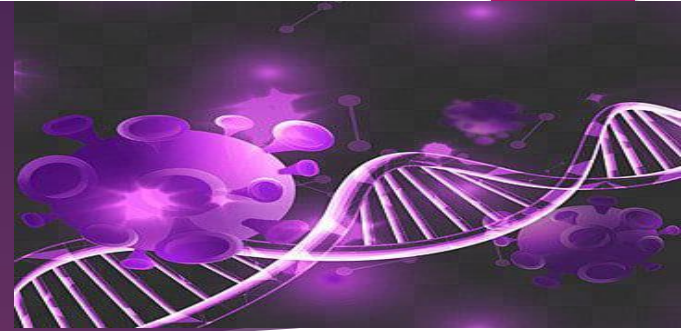
Results



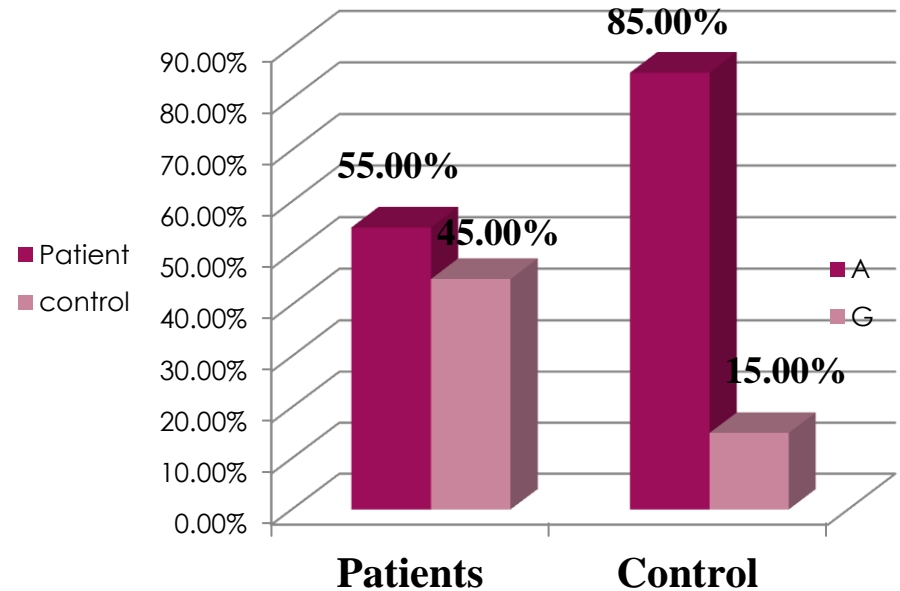
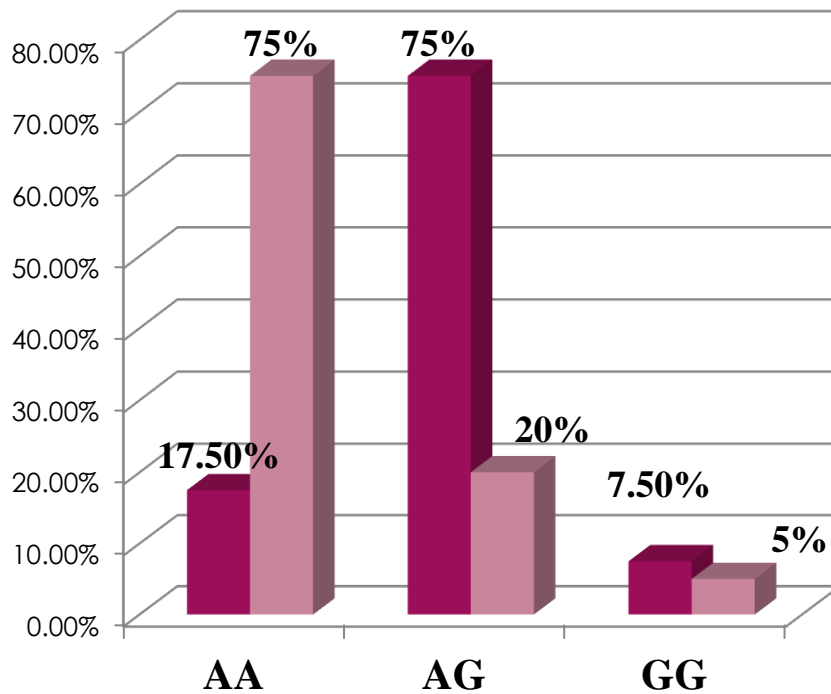
Receiver Operating Characteristic curve data of the immunological biomarker of the current study

Parameters	AUC	Explanation	P value	The best Cut off	Sensitivity %	Specificity%
TLR-9	0.807	Excellent	0.001	2.28	98%	71%
TNF-α	0.950	Excellent	0.001	135.43	88%	91%
IL-6	0.937	Excellent	0.001	30.72	89%	95%

Results



Genotypes and allele frequency of rs187084 in patients and control.



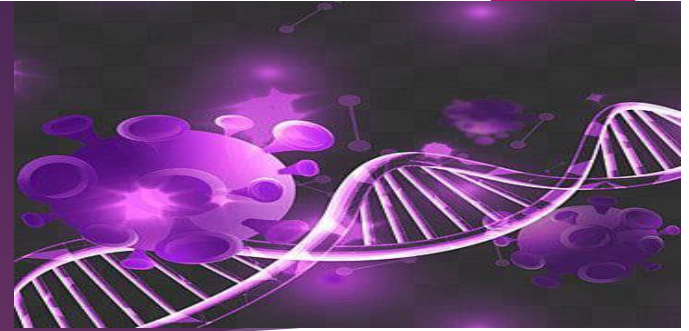
Results



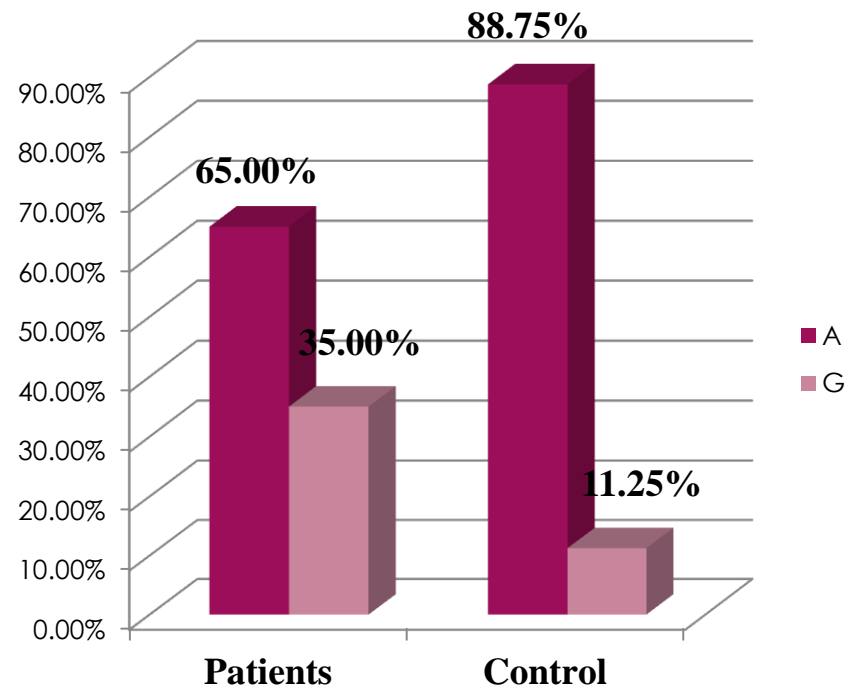
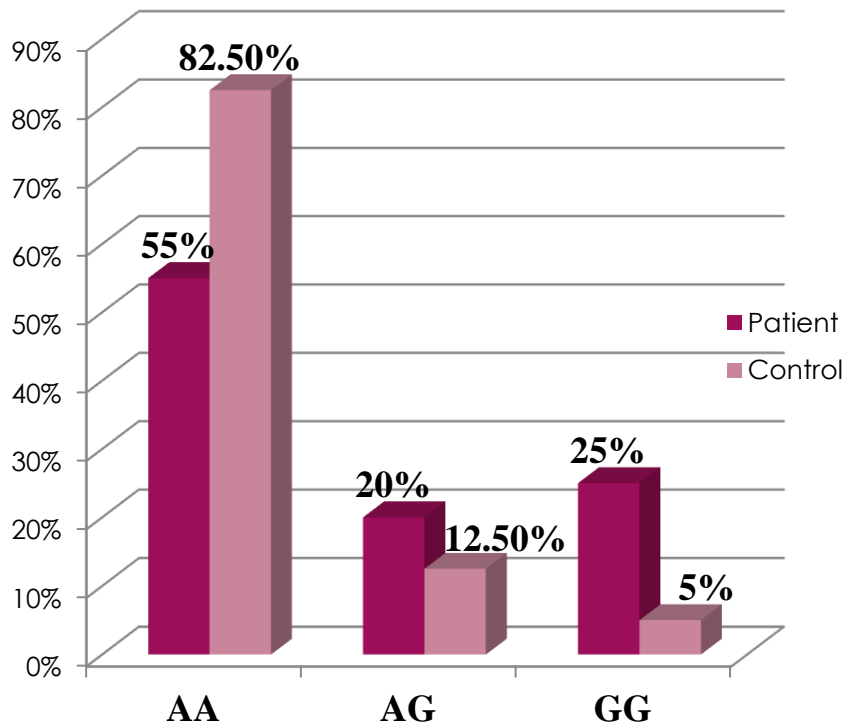
Correlation between the rs187084 SNP in patients and control group and TLR-9.

TLR-9 & rs187084 in Patients Group				
Parameters	Mean± SE			LSD Value
TLR-9	AA(7)	AG(30)	GG(3)	0.356 NS
	2.63 ±0.08	2.76 ±0.05	2.75 ±0.17	
TLR-9 & rs187084 in Control Group				
TLR-9	AA(30)	AG (8)	GG(2)	0.575 NS
	2.15 ±0.08	2.23 ±0.12	2.14 ±0.49	
NS: Non-Significant				

Results



Genotypes and allele frequency of rs5743836 in patients and control.



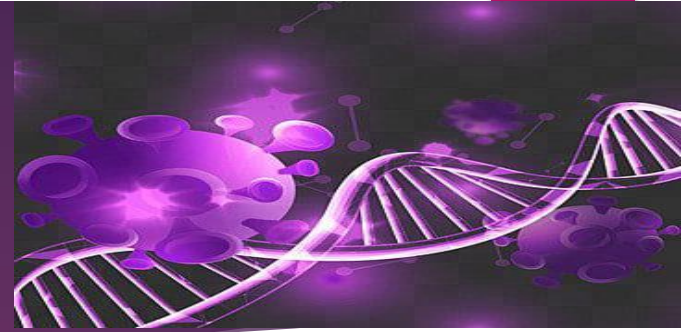
Results



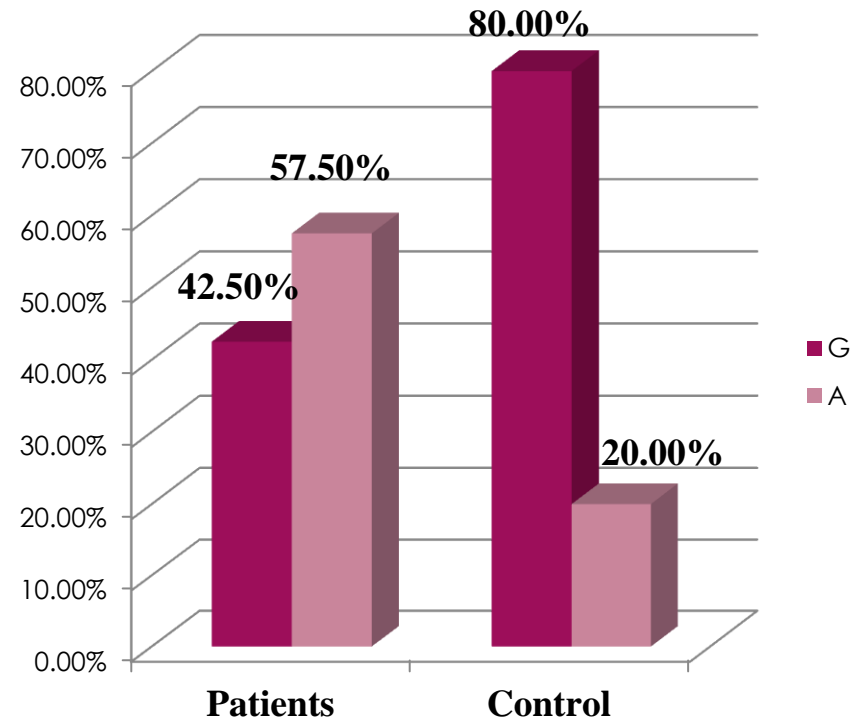
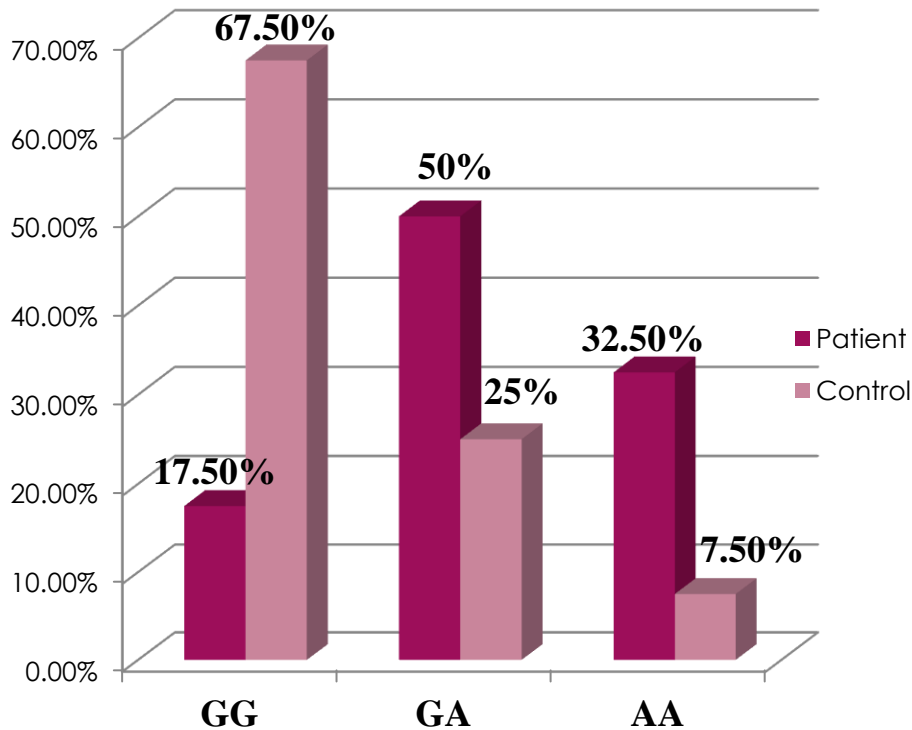
Correlation between rs5743836 SNP in patients and, control group and TLR-9.

TLR-9 & rs5743836 in Patients Group				
Parameters	Mean± SE			LSD Value
TLR-9	AA(22)	AG(8)	GG(10)	0.259 NS
	2.75 ±0.06	2.64 ±0.08	2.78 ±0.09	
TLR-9 & rs5743836 in Control Group				
TLR-9	AA(33)	AG (5)	GG(2)	0.606 NS
	2.12 ±0.07	2.34 ±0.28	2.53 ±0.04	
NS: Non-Significant				

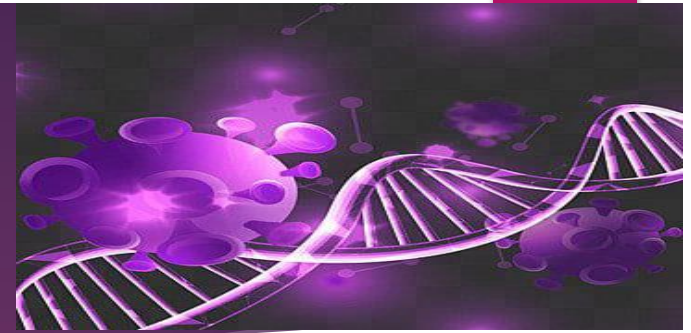
Results



Genotypes and allele frequency of rs352140 in patients and control.



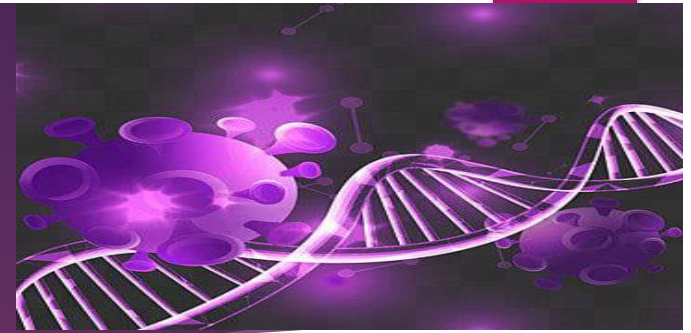
Results



Correlation between rs352140 SNP in patients & control group and TLR-9

TLR-9 & rs352140 in Patients Group				
Parameters	Mean \pm SE			LSD Value
TLR-9	GG(7)	GA(20)	AA(13)	0.260 NS
	2.74 \pm 0.10	2.75 \pm 0.07	2.73 \pm 0.06	
TLR-9 & rs352140 in Control Group				
TLR-9	GG(27)	GA(10)	AA(3)	0.486 NS
	2.11 \pm 0.08	2.19 \pm 0.11	2.59 \pm 0.08	
NS: Non-Significant				

Results



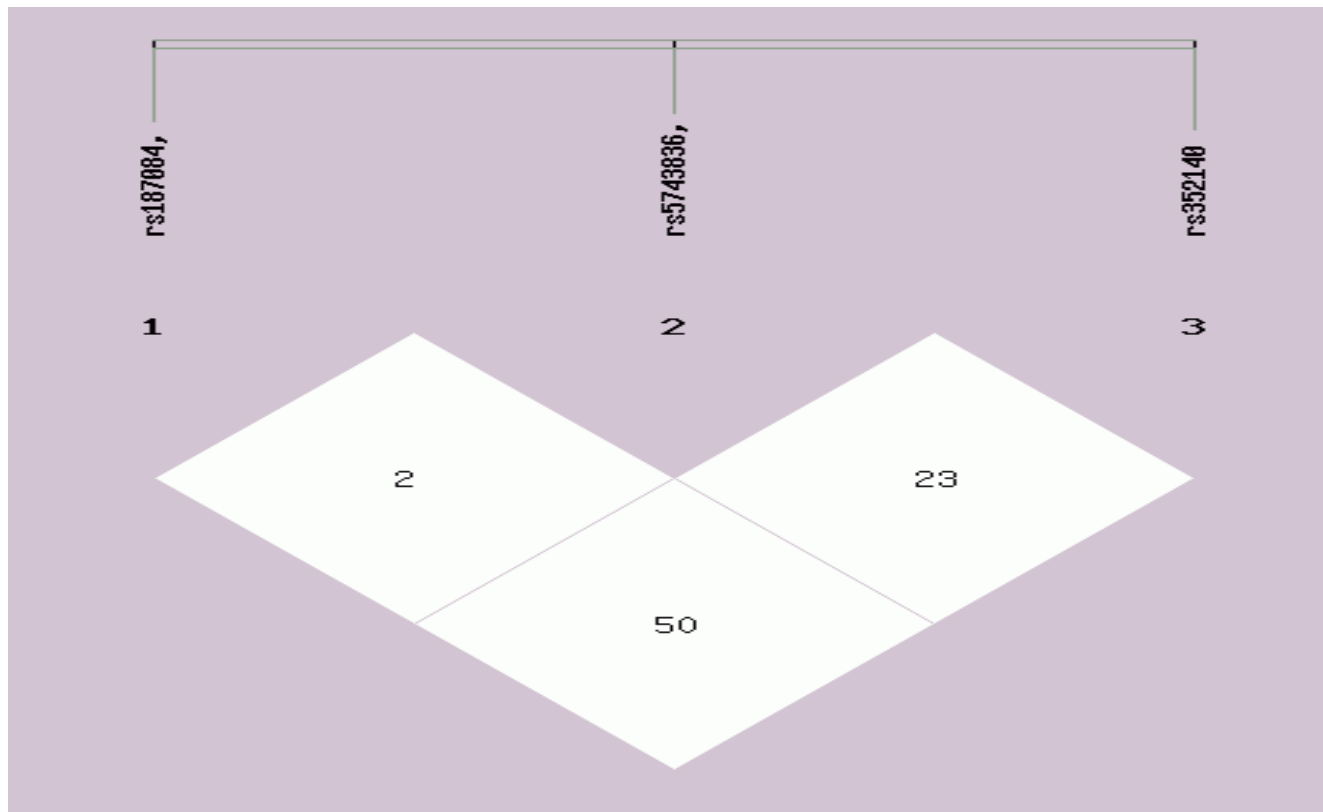
The haplotype analysis of TLR-9 polymorphisms, which fall within the promotor and second exon regions of the gene.

Haplotypes	Frequency		χ^2	Odds Ratio	CI 95%
	Patients	Control			
A A A*	0.19	0.11	2.07	1.92	0.781- 4.730
A A G*	0.10	0.66	53.49	0.06	0.24- 0.134
A G A*	0.04	0.37	0.00	0.99	0.192- 5.081
A G G*	0.22	0.42	11.42	6.53	1.949- 21.886
G A A*	0.33	0.04	22.57	11.73	3.546- 38.832
G A G*	0.03	0.08	1.99	0.33	0.068- 1.641
G G A	0.02	0.01	---	---	---
G G G*	0.07	0.02	2.63	3.93	0.664- 23.082

Results



The linkage disequilibrium of three genetic polymorphisms of the TLR-9 gene (rs187084, rs5743836, rs352140).



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Conclusions



Conclusions



- ▶ **An elevated serum level of TLR-9, TNF- α and, IL-6 were evaluated in AML patients compared to healthy people so it may affect the prognosis of the disease and, increase the risk of the disease progression.**
- ▶ **The AG genotype of rs187084 in the promoter is the common genotype in the population of the study. The allele G is an etiological factor associated with the disease while allele A is the protective agent.**
- ▶ **The AA is the common genotype in the population of study at rs5743836 polymorphism in the Promoter region . The GG may be associated with increase susceptibility to AML.**

Conclusions



- ▶ **Exon 2 region revealed that the GG genotype in rs352140 is common in the Iraqi population and the AA genotype and A allele revealed a highly significant positive association with disease.**
- ▶ **A A G haplotype represents common haplotype and alleles that included were non-mutant and may have a protective role in disease.**
- ▶ **G A A and A G G haplotypes were associated with increased disease risk about eleven-fold and six-fold respectively.**

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Recommendations

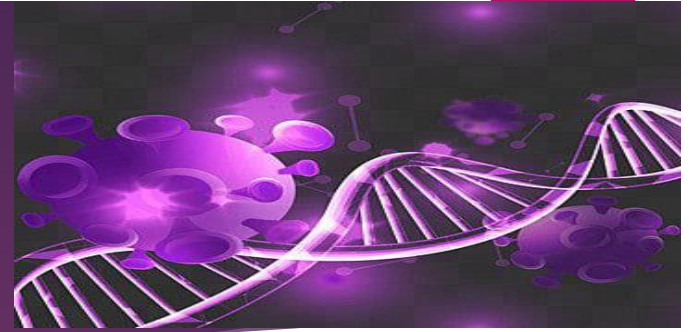


Recommendations



- ❖ **Study of Toll-like Receptor-9 Gene Expression.**
- ❖ **Study of Genetic Variations of TNF- α in AML Patients.**
- ❖ **Study of genetic variations in intron regions of TLR-9.**
- ❖ **More studies on designing potential therapeutic strategies for targeting TLR-9 to control disease progression (SNP Profile of TLR-9).**
- ❖ **Study of DNA methylation for the SNPs located in the promoter region of TLR-9.**

NCBI



Isolate added as,
LC720580: MQM1
LC720581: MQM2
LC720582: MQM3



*Thank You
for
Listening*

