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The Association between Autoimmune Regulator *(AIRE)-2075876 G/A* Single Nucleotide Polymorphism in Rheumatoid Disease

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Abstract:

Background: Rhuematoid Arthritis influences all aspects of life as it causes co-morbiditis in vascular,metabolic and articular aspects.There are many factors that contribute to development of RA as genetic and environmental factors. **Objectives**: to evaluate the association between single nucleotide polymorphism of *AIRE* gene (SNP rs2075876) and risk of RA in Egyptian patients. **Methods:** Acase control study conducted on 50 participants collected:30 previously diagnosed rheumatoid arthritis according to ACR/EULAR critria (25 females and 5 males) and 20 healthy group age and sex matched controls. Participants were screened for routine laboratory investigations & immunological investigation as RF and anti-ccp and *AIRE* gene polymorphism by using real time polymerase chain reaction. On comparing the 2 groups (controls and cases) **Results:** the results showed that there is statistically significant difference between cases and controls regarding SNP rs2075876 for the distribution of genotype and alleles. Therefore,we conclude that there is strong association between *AIRE* gene SNP rs2075876 and risk of rheumatoid arthritis.

Keywords: Rheumatoid arthritis , *AIRE* gene, SNP rs2075876 polymorphism , polymerase chain reaction.

1. Introduction:

Rheumatoid arthritis is a chronic autoimmune disease that causes progressive articular destruction and progresses from distal joints to proximal. It is also associated with comorbidities in vascular, metabolic, bone and psychological aspects [1].

The etiology of RA is very complex. It has a wide spectrum of clinical manifestations, variability in disease severity, progression and differences in therapeutic response. These heterogeneous phenotypes of RA may suggest that variety of factors can contribute in the development of this complex trait, which includes genetic , environmental factors and other factors [2]. There are many epigenetic and environmental risk factors for RA such as silica dust, alchol and cigarette smoking by enhancing post-translational citrullination forming new epitope [3].

RA is strongly associated with genes of the inherited tissue type major histocompatibility complex (MHC) antigen ,HLA-DR4 is the major genetic factor implicated. Genome-wide association studies examining single-nucleotide polymorphisms have found around one hundred genes associated with RA risk, with most of them involving the HLA system (particularly HLA-DRB1) [4].The cumulative risk was 1% before age 50 years reflecting the small incidence of RA at those ages women are approximately 3 times more prone than men and the onset is more frequent when the person is in their 40s or 50s [5]. Many researches suggested that an increase in BMI could contribute to higher risk of developing RA [6].

RA primarily starts as a state of persistent cellular activation leading to autoimmunity and immune complexes in joints and other organs where it manifests. The initial site of disease is the synovial membrane, where swelling and congestion lead to infiltration by immune cells. Three phases of progression of RA are an initiation phase (due to non-specific inflammation), an amplification phase (due to T cell activation), and chronic inflammatory phase, with tissue injury resulting from the cytokines, IL–1, TNF-alpha and IL–6 [7].

The autoimmune regulator gene *(AIRE)* is a transcription factor expressed in the thymus and in secondary lymphoid organs, it was first identified in 1997 and named autoimmune regulator *(AIRE*) [8]. *AIRE* plays a key role in shaping central immunological tolerance by facilitating negative selection of T cells in the thymus, building the thymic microarchitecture, and inducing a specific subset of regulatory T-cells [9].

AIRE is located in 21q22.3 and contains 14 exons encoding a 545-amino-acid protein with a molecular weight of 57.5

kDa .Over 100 mutations in the *AIRE* gene have been reported in the Human Gene Mutation

Database varying from single nucleotide substitutions to large deletions spread out across the coding sequence[10]. *AIRE* plays an important role in negative selection by upregulating the expression of TSAs. Decreased TSA expression in the setting of *AIRE*-deficiency results in multi-organ autoimmune disease by allowing self-reactive T cells to escape from the thymus and enter the periphery where they can provoke autoimmunity [9].

AIRE has apro inflammatory role in keratinocyte that undergoes tumerogenoesis unlike its immunoregulatory role in mTEC [11]. During embryogenesis,*AIRE* transcription becomes highly restricted to particular cell types,including mTECs, extrathymic *AIRE*-expressing cells and thymic B cells translated in mature mTECs.In addition, the transcription of *AIRE* seems to undergo strict spatiotemporal regulation[8].

Expression of *AIRE*, as well as *AIRE*-dependent genes, declines with age in thymic B cells and that indicates that aging may diminish the ability of thymic B cells to tolerize T cells revealing apotential mechanistic link between aging and autoimmunity [12].

AIRE promotes TSA expression through the release of stalled RNA polymerase to elongate RNA transcripts. *AIRE* interacts with positive transcription elongation factor b (P-TEFb), a protein that controls the release of stalled RNA polymerase [13]..The role of *AIRE* in preventing autoimmunity is evident by the spontaneous development of autoimmunity in humans with *AIRE* mutations. Recently, the roles of *AIRE* in modulating other diseases have been delineated For example , the function of *AIRE* in preventing effective antitumour immunity has recently been clarified [14].

Although the first European GWAS was unable to find any association between *AIRE* and RA, following studies identified single nucleotide polymorphisms (SNPs) that appears strictly related to the disease. In particular, a study by Terao et al. identified two SNPs, rs2075876 and rs760426, within the intron of the *AIRE* gene that showed a strong association with RA development [15].

2. Patients and Methods:

This study is a case control study performed

on samples which were collected from donors recruited from rheumatology department at the Faculty of Medicine,Beni-Suef University.

The present work included 50 subjects; the subjects were divided into 2 groups.

patient group:

30 rheumatoid patient, they were 25 females (83.3%) and 5 males (16.7%) diagnosed according to ACR/EULAR 2010

Rhuematoid Arthritis classification criteria[16].

Control group:

Twenty healthy volunteers who are age and sex matched with the patients group. They were 13 females (65%) and 7 males (35%).

2.1 Inclusion criteria:

Rheumatoid patients with polyarticular arthritis with postive RF,or postive ANA and elevated ESR.

Exclusion criteria:-

- Chronic liver Disease.
- Chronic Kidney disease.
- Diabetic
- Obesity
- Patient under treatment

.2 All patients were subjected to

- Full History taking (according to age,sex,family history).
- Thorough clinical examination.
- Laboratory investigation:-

Routine laboratory investigations:

- CBC,ANA,AST,ALT,RF,ESR,CRP, Anti-ccp
- Renal function (urea and creatinine).
- Lipid profile
- Blood glucose

Genetic analysis :

Genomic DNA extraction and analysis for rs2075876 G/A gene polymorphism using Real time- Polymerase Chain reaction (RT-PCR) technique. Averbal consent of the study was taken from each patient before the starting.

Sample collection and storag

9 ml venous blood were withdrawn from all subjects after fasting 8-12 hours and divided into:

2 ml were collected in a sterile vacutainer containing ethylene diamine tetra acetate "EDTA" which are used for DNA extraction. DNA extracts were stored at -70^{o} C to be used for performing real time-PCR for rs2075876 G/A gene polymorphism.

5 ml were collected in plain tubes, left for 10 minutes to clot and centrifuged to separate the serum. Serum was used for routine laboratory investigations.

Statistical methodology

• Analysis of data was done by IBM computer using SPSS (statistical program for social science) as follows;

- Description of quantitative variables as mean, SD and range.

- Description of qualitative variables as number and percentage.

- Unpaired t-test was used to compare quantitative variables, in parametric data (SD < 50 % mean)

• P value > 0.05 insignificant

• P < 0.05 significant

• P < 0.01 highly significant.

3. Results:

This study is a case control study performed on samples which were collected from donors recruited from rheumatology department at the Faculty of Medicine, Beni-Suef University to evaluate the association between SNP rs2075876 (G> A) polymorphism in *AIRE* gene with RA.

The present work included 50 subjects; the subjects were divided into 2 groups **:-**

patient group:

30 rheumatoid patient, they were 25 females and 5 males .They were selected from Beni-suef university rheumatoid clinics, their age ranged from 26 - 87 years

Control group:

Twenty healthy volunteers who are age and sex matched with the patients group. They were 13 females and 7 males with their age ranged from 29-87 years

Table (1) Baseline characteristics of the two groups under the study:

Criteria	Cases N=30(100%)	Controls N=20(100%)	P-value
Age			
Mean±SD	48.1±15	48.7 ±17.3	
Range(min-max)	(26-87)	(29-87)	0.858
Median	45	46	
Sex N(%):			
Males	5(16.7)	7(35)	
Females	25(83.3)	13(65)	0.137
Family history:			
Negative	20(66.7)	20(100)	**0.004***
Positive	10(33.3)	0(0)	

P-value is significant at <0.05.

Table (1): show no statistically significant difference between both groups according age and sex , but there was a statistically significant difference as regards family history.

Liver and kidney functions	Cases N=30(100%)	Controls N=20(100%)	P-value
Urea mg/dl			

Mean±SD	27.7±12.9	14.3±4.2	**<0.001****
Range(min-max)	(15-70)	(5-19)	
Median	**24**	15	
Creat mg/dl			
Mean±SD	0.97±0.36	0.95±0.22	0.810
Range(min-max)	(0.5-2.5)	(0.5-1.3)	
Median	0.9	0.95	
ALT U/L			
Mean±SD	27.7±10.7	19.4±3.3	**0.001***
Range(min-max)	(8-54)	(13-25)	
Median	**25.5**	20	
AST U/L			
Mean±SD	31.3±9.1	23.9±4.7	**0.02***
Range(min-max)	(17-56)	(17-56)	
Median	**29.5**	23	
Cholesterol mg/dl			
Mean±SD	**248.3±82.9**	142.3±31.2	**<0.001****
Range(min-max)	(110-400)	(106-198)	
Median	263	132	
TG mg/dl			
Mean±SD	**194.5±80**	103.1±26	**<0.001****
Range(min-max)	(67-356)	(50-150)	
Median	195	107	
Fasting blood glucose mg/dl	**150.3±50.2**	89.2±13.1	**<0.001****
Mean±SD	(70-210)	(70-109	
Range(min-max)	170	87	
Median			

Scale data was presented as mean±SD & categorical data was presented as number (%) *P-value is significant at <0.05

Table (2) demonstrated that there was a statistical significant difference between cases and controls regarding urea, ALT, AST (P-value<0.05) but, there was no statistical significant difference between cases and controls regarding the creatinine (P-value>0.05).

ESR	Cases N=30(100%)	Controls N=20(100%)	P-value
ESR 1st hour			
Mean±SD	47.4±34.3	13.1±4	**<0.001****
Range(min-max)	(10-140)	(5-18)	
Median	**32.5**	13	
ESR 2nd hour			
Mean±SD	76.4±40.3	19.1±4.6	**<0.001****
Range(min-max)	(14-170)	(10-26)	
Median	**69.5**	20	

Table (3):show there was a statistical significant difference between cases and controls regarding ESR at 1st and 2nd hours (P-value<0.001).

Table (4) Anti-CCP, anti-nuclear antibody and C reactive protein in cases and controls:

Criteria	Cases N=30(100%)	Controls N=20(100%)	P-value
Anti CCP			
Negative	27(90)	20(100)	0.145
Positive	10(10)	0(0)	
Anti NA			
Negative	22(73.3)	20(100)	**0.012***
Positive	**8(26.7)**	0(0)	
CRP mg/L			
Negative	1(3.3)	20(100)	**<0.001****
Positive	**29(96.7)**	0(0)	

Data was presented as number (%) *P-value is significant at <0.05 **P-value is highly significant

Table (4) : there was a statistical significant difference between cases and controls regarding the ANA and CRP distribution P-value was 0.012 and <0.001; respectively.

Table (5) Genotype and allelotype in cases and controls:

Criteria	Cases N=30(100%)	Controls N=20(100%)	P-value
Genotype			
GG	19(63.3)	0(0)	**<0.001****
GA	7(23.3)	4(20)	
AA	4(13.4)	16(80)	
Allele			
G	45(75)	4(10)	**<0.001****
A	15(25)	36(90)	

Data was presented as number (%) *P-value is significant at <0.05 **P-value is highly significant

Table (5) & figure (1): there was a statistical significant difference between cases and controls regarding the genotype and allelotype (P-value<0.001) as there were 19(63.3%) of cases had GG Vs no one in controls, 7(23.3%) of cases had GA Vs 4(20%) in controls and 4(13.4%) of cases had AA Vs 16(80%) in controls. Also for the allelotype there were 45(75%) cases had G and 15(25%) had A Vs 4(10%) had G and 36(90) had A in cases.

Table (6) show correlation between gene pcr and DAS28:

			DAS28			Total
			Mild	Moderate	Severe	
gene pcr	GG	Count	12	3	4	19
		% within DAS28	75.0%	37.5%	66.7%	63.3%
	GA	Count	3	2	2	7
		% within DAS28	18.8%	25.0%	33.3%	23.3%
	AA	Count	1	3	0	4
		% within DAS28	6.3%	37.5%	0.0%	13.3%
Total		Count	16	8	6	30
		% within DAS28	100.0%	100.0%	100.0%	100.0%

P-value=0.164

Table (6): there was no statistically significant difference between the DAS 28 score grades regarding the distribution of different genotypes (P-value=0.164).

Table(7) show disease duration of cases:

groups		Disease duration
Cases	N	30
	Mean	5.4917
	Std. Deviation	10.27146
	Minimum	.25
	Maximum	57.00
	Median	3.0000

Figure (1) genotype of cases and controls under the study:

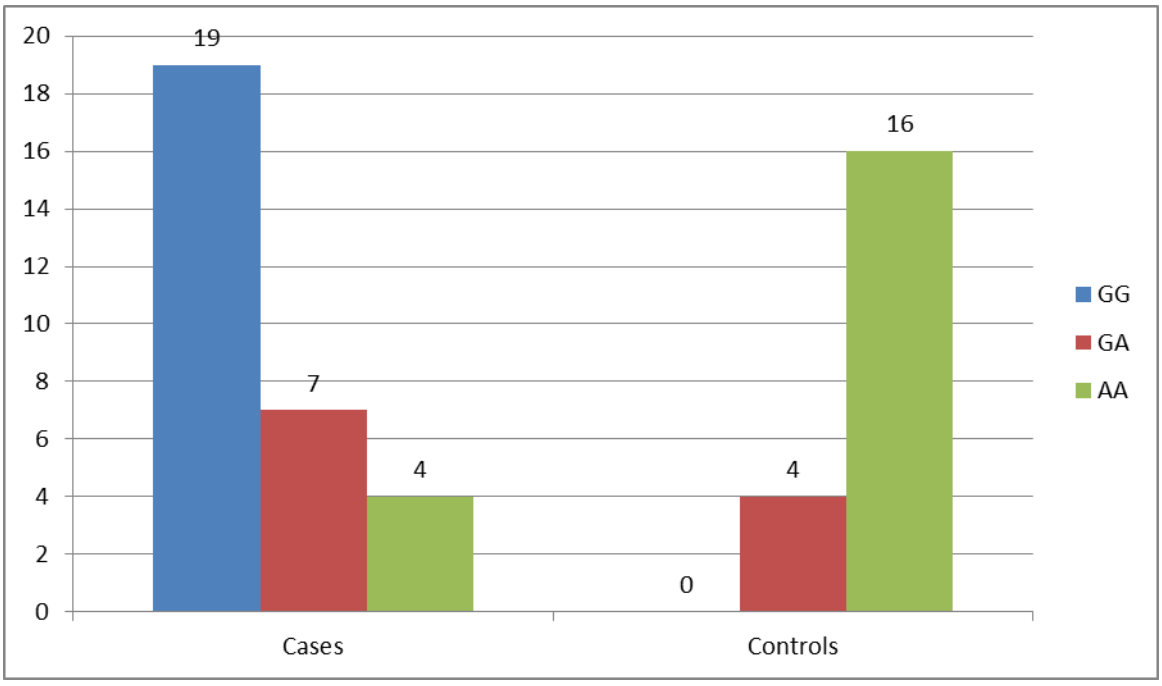

Figure (2) allelotype between cases and controls:

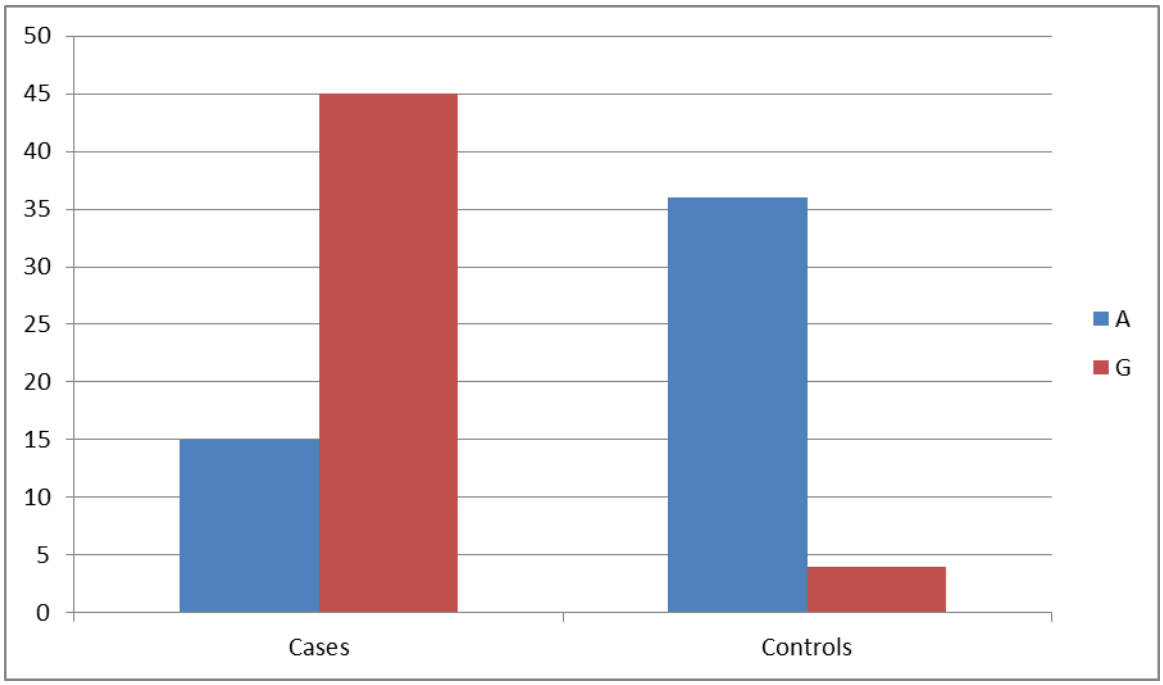

Table (8) Estimate Risk of mutant gene on developing rheumatoid arthritis:

Criteria	Cases N=30(100%)	Controls N=20(100%)	P-value	Odds ratio	95% CI for OR
Genotype					
Mutant(GG-GA)	26(86.7)	4(20)	**<0.001****	26	(5.7-118.8)
Wild(AA)	4(13.3)	16(80)			

Data was presented as number (%) *P-value is significant at <0.05 **P-value is highly significant

Table (8) :shows mutant gene was detected in 26(86.7%) of cases and 4 (20%) of controls but the wild gene (AA) was detected in 4 (13.3%) of cases and 16(80%) of controls and this difference was statistically significant (P-value<0.001). Presence of mutant gene (GG or GA) increases the risk of the individual 26 times to get the disease compared to presence of wild gene (AA) (OR=26).

Table (9) correlation between disease activity and different patient parameters:

DAS28	ALT	AST	ESR 1st hour	ESR 2nd hour	urea	WBCs
r value	0.178	0.185	**0.889****	**0.880****	-0.102	-0.079
P-value	0.346	0.329	**0.001**	**<0.001**	0.598	0.677

***Correlation is significant at the 0.01 level (2-tailed).**

Table (9) : there was no significant linear correlation between disease activity and ALT, AST, Urea, WBCs (P-value>0.05) but, there was a highly significant strong positive linear correlation between disease activity and ESR after 1st and 2nd hours (P-value<0.001).

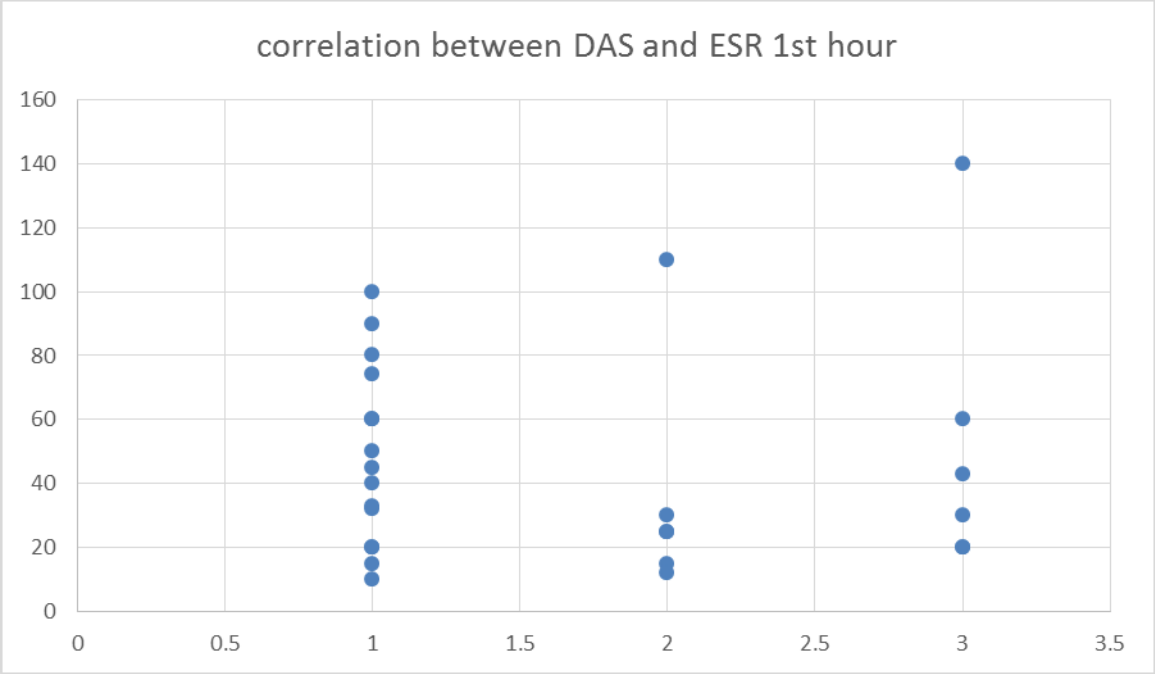

Figure (3) :correlation between DAS18 and ESR 1st hour

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3. Discussion:

Rheumatoid arthritis (RA) is an inflammatory autoimmune disease of unknown etiology and characterized by systemic involvement. Genetic predisposition and environmental factors contribute to the pathogenesis and diversity of clinical findings [17]. Although the disease is observed more frequently in women compared to men, its frequency is 0.5–1% in the general population

RA is characterized by symmetric joint involvement as it causes erosion and deformity in the joints as a result of synovial inflammation The etiology of the disease is complicated as there are many risk factors contribute to the disease including genetic factors as Major histocomptability antigen (MHC) ,HLA-DR4,environmental factors as alchol and smoking ,age and obesity [4].

The autoimmune regulator *(AIRE)* is 12.5 kb in length, and contains 14 exons that encode a 545 amino acid protein of 58 kD .*AIRE* plays a key role in shaping central immunological tolerance by facilitating negative selection of T cells in the thymus, building the thymic microarchitecture, and inducing a specific subset of regulatory T cells [18].

This study was conducted to study the association between SNP rs 2075876(G/A) polymorphism in *AIRE* gene with rheumatoid arthritis in some Egyptian patients.

Thirty patients complaining of rheumatoid arthritis (25 females and 5 males) were included in this study and twenty healthy age and sex matched volunteers were selected as control group As regards *AIRE* gene SNP rs 2075876 the current study showed that there was a significant difference between cases and controls regarding the genotype(P-value<0.001) as there were 19(63.3%) of cases had GG Vs no one in controls, 7(23.3%) of cases had GA Vs 4(20%) in controls and 4(13.4%) of cases had AA Vs 16(80%) in controls.

According to allelotype ,there was a significant difference between cases and controls as (p-value<0.001) .Also, there were 45(75%) cases had G and 15(25%) had A Vs 4(10%) had G and 36(90) had A in controls.

In addition ,the mutant gene was detected in 26(86.7%) of cases and 4 (20%) of controls but the wild gene (AA) was detected in 4 (13.3%) of cases and 16(80%) of controls and this difference was statistically significant (P-value<0.001).

Presence of mutant gene (GG or GA) increases the risk of the individual 26 times to get the disease compared to presence of wild gene (AA) (OR=26).

These results are in agreement with study done by [19] that demonstrated that the SNP rs2075876 showed significant association with the risk of RA. The A allele of rs2075876 increased the risk of RA (pa = 0.008, OR = 1.991).

Also it is in agreement with study conducted by[15] demonstrated ,on Japanese

patients ,that there is a statistically significant correlation between *AIRE* SNP2075876 with rheumatoid arthritis risk as the association p-value reached P-value<0.001.

Furthermore,the results are in agreement with[20] that found a significant difference in the allele frequency of *AIRE* rs2075876 polymorphism between cases and controls was detected (A versus G, OR 1.33,95 %CI 1.04–1.69, P=0.02.

On the contrary , the current study is against [21] that demonstrated that there is no significant correlation between rheumatoid arthritis risk and *AIRE* SNP rs2075876 as p-value >0.05 due to ethnicity effects .

Also this study is against the study of [20] that demonstrated that the frequency of the minor allele G of *AIRE* rs760426 polymorphism was higher in patients compared with controls (47.8 % versus 42.1 %), and this deviation showed a trend towards significant level (P=0.06).

In addition,this study is against[9] that showed that Mutations in *AIRE* were originally linked to the autosomal recessive disease autoimmune polyglandular syndrome type 1 (APS1; also known as APECED).

There is not a significant difference between cases and patients regarding age and gender(p- value>0.05)and these results are in agreement with [19] who found that there is not a signicant difference between rheumatoid patients and control group according age and gender (p-value>0.05).

The results of the current study showed that in comparing with control and rheumatoid groups, there was a significant difference between cases and controls regarding the rheumatoid parameters (ESR 1st hour ,ESR 2nd hour and CRP ,RF, anti-ccp , p- value <.001) As they are significantly higher in cases than controls.

5. Conclusion and Recommendations:

In comparison the two groups (patients of RA and controls) as regards SNP rs 2075876 G/A genotypes frequencies and allele frequencies in the current study, there was a statistically significant difference between both groups. A larger scale for a similar study is recommended in order to attain more statistically significant results. Encouragement for studies of interaction between *AIRE* gene polymorphism and developing autoimmune diseases and neoplastic diseases.

Further researches may be recommended on rheumatoid patients for detection of *AIRE* gene polymorphism. Patients with G/G and G/A genotype can be enrolled in researches for early intervention and follow up as a severity marker, aiming to prevent rheumatoid disease manifestations and complications.

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