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Relation between Vitamin D Receptors Gene Polymorphism and Lupus Nephritis

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Abstract:

The goal of this study is to Correlate between Vitamin D receptor polymorphism (FOKI) and lupus nephritis. Eighty subjects were included in this study. They were divided into two groups as follows: Control group: consisted of twenty apparently healthy volunteers of comparable age and socioeconomic status to the patients group. Patients group: consisted of sixty patients with SLE. They were selected from the Internal Medicine departement and Rheumatology out patient clinic of Beni-suef University hospital. In this study, Regarding CBC changes ,The prevalence of thrombocytopenia and lymphopenia was significantly higher among SLE patients compared to our controls, However the prevalence of lymphopenia and leucopenia was significantly higher among SLE patients with GG genotype compared to SLE patients with AG and AA mutants ,Regarding liver function tests, The mean serum Albumin level was significantly lower among SLE patients compared to controls .Antinuclear antibody titre was significantly higher among SLE patients than controls ,Also Anti Ds DNA is represented in SLE patients in ahigh titre than healthy controls ,Furthermore, All SLE patients with GG genotypes had positive Anti Ds DNA(100%) compared to SLE patients with AG (0,0) and AA (0,0) mutants. It is recommended to carry out further studies on FOKI gene polymorphisms in larger groups of patients and controls.

Keywords: Vitamin, D Receptor, Lupus Nephritis.

1. Introduction:

Lupus nephritis is an inflammation of the kidneys caused by systemic lupus erythematosus , an autoimmune disease, Lupus nephritis (LN) is highly prevalent, and a significant portion of LN will develop into end stage renal disease in systemic lupus erythematosus (1)

The risk of ESRD in class IV-LN has been as high as 44% over the past 15 years despite the current available treatment regimens including steroid and immunosuppressants as well as various monoclonal antibodies **(**2) . Renal fibrosis is a common pathological feature of ESRD that requires renal replacement therapy and is a huge economic burden worldwide **(**3) .

It is a type of glomerulonephritis in which the glomeruli become inflamed. As the result of SLE, the cause of glomerulonephritis is said to be secondary and has a different pattern and outcome from conditions with a primary cause originating in the kidney *(*4).

Vitamin D is a nutrient long reflected as vital for skeletal health. However it is now attracting attention from medical and nutritional communities as knowledge develops of its biological functions and its association with lessened risk of numerous chronic diseases (5). There are three recognized sources for VD in human specifically; sunlight, dietary intake and VD supplementation (6).

2. Patients and Methods

This was a randomized study performed in in Beni-Suef university hospital Eighty subjects were included in this study. They were divided into two groups as follows: Control group: consisted of twenty apparently healthy volunteers of comparable age and socioeconomic status to the patients group.

Patients group: consisted of sixty patients with SLE. They were selected from the Internal Medicine departement and Rheumatology out patient clinic of Beni-suef University hospital.

For all subjects :

- **Detailed history taking**
- **Physical examination**
- **Preliminary laboratory investigations including:**
- - Serum levels of urea, creatinine ,albumin.
- -ESR ,CRP ,ANA ,Anti Ds DNA , 25-hydroxy VD ,CBC ,C3 ,C4 ,SGPT.
- **Molecular studies were done and included the following:** Genomic DNA extraction and analysis for FOKI gene polymorphism using by Real time-Polymerase Chain reaction (RT- PCR) technique.

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.

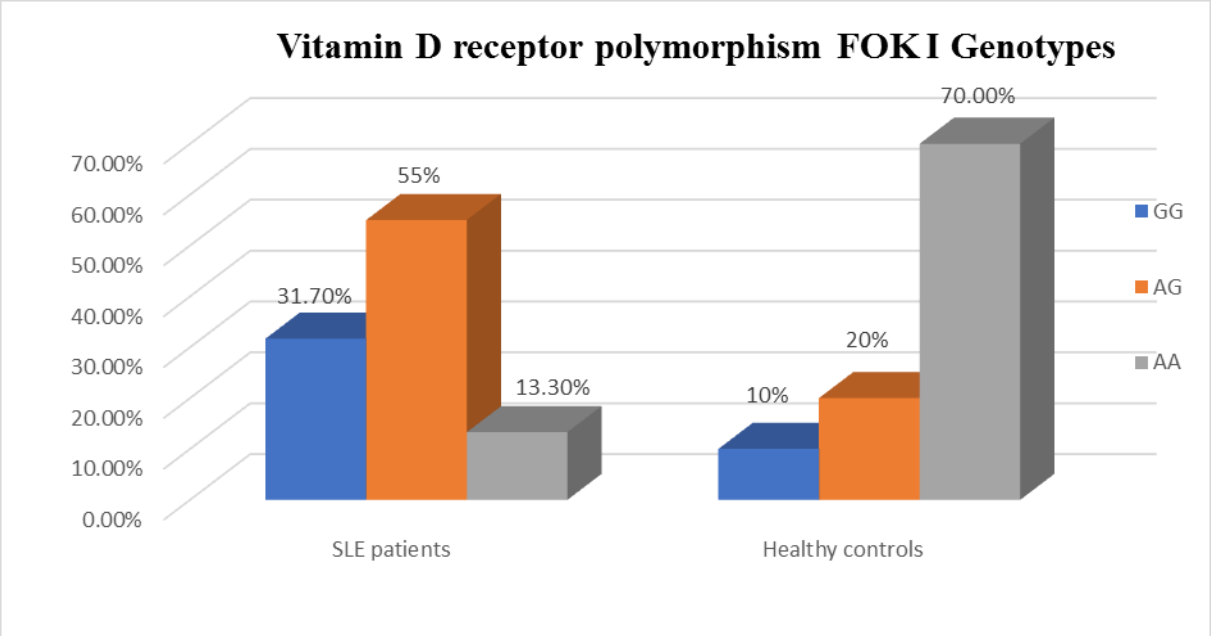
- Unpaired t-test was used to compare quantitative variables, in parametric data (SD < 50 % mean)

• P value > 0.05 insignificant

• P < 0.05 significant

• P < 0.01 highly significant [20].

3. Results



It shows Distribution of Vitamin D receptor polymorphism FOK I Genotypes in the Studied SLE patients and Controls.

It investigated the possible association between VDR Fok I polymorphisms and SLE disease. The prevalence of homozygous (GG) and heterozygous (AG) mutants were significantly higher in SLE patients 31.7% and 55.0% compared to healthy controls 10.0 and 20.0%, respectively. Also, the prevalence of homozygous mutant AA was significantly higher in healthy controls 70.0% compared to SLE patients 13.3%, at p value = 0.001*.

Vitamin D receptor polymorphism FOK I Genotypes	Serum 25 (OH) Vitamin D Level		P value
	Mean	SD	
GG	17.78	±5.03	0.001*
AG	27.87	±6.15	
AA	26.25	±4.83	
Total	24.46	±7.23	

It shows Association between Vitamin D Receptor Polymorphism FOK I Genotypes and 25(OH) Vitamin D Level in the Studied SLE patients

It demonstrated that SLE patients with GG genotypes had lower serum level of 25 (OH) Vitamin D (17.78±5.03) compared to patients with AG and AA mutants 27.87±6.15 and 26.25±4.83, respectively at p value=0.001.

Renal Biopsy	Vit D receptor polymorphism FOK I						P value
	GG		AG		AA		
	No.	%	No.	%	No.	%	
Class 3,4 (active, early chronic)	14	73.7	0	0.0	0	0.0	0.001*
Class 3,4 (in active, chronic)	0	0.0	7	43.8	2	40.0	
Class 5 (active, early chronic)	5	26.3	0	0.0	0	0.0	
Class 5 (in active, chronic)	0	0.0	5	31.2	2	40.0	
Class 6	0	0.0	4	25.0	1	20.0	

Distribution of lupus nephritis patients as regards Vit D Receptor polymorphism FOK I Genotypes and Renal Biopsy

Findings; no=40

Almost all lupus nephritis patients with GG genotypes had active stage in renal biopsy either Class 3,4 (active, early chronic) 73.7% or class 5 (active, early chronic) 26.3% compared to SLE patients with AG and AA mutants were inactive stages either class 3&4 or class 5 at p value (0.001).

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	Lupus nephritis (No.=40)		Lupus non-nephritis (No.= 20)		P value
	No.	%	No.	%	
Vitamin D receptor polymorphism FOK I Genotypes					
GG	19	47.5	0	0.0	0.001*
AG	16	40.0	17	85.0	
AA	5	12.5	3	15.0	
Serum 25 (OH)Vitamin D Level ng/ml					
Mean± SD	23.45± 7.16		26.50± 7.11		125.0

It shows Distribution of the Studied SLE patients with and without Renal Involvement regarding Vitamin D receptor polymorphism FOK I Genotypes and Serum 25(OH)Vitamin D Level

SLE Patients were categorized into two groups (lupus nephritis and non‐nephritis) based on kidney involvement phenotype. The prevalence of homozygous (GG) was significantly higher among Lupus nephritis patients (47.5%) compared to lupus non-nephritis (0.0%), p value=0.001. while there was no statistically significant difference between them as regards 25‐OH vitamin D serum level.

CBC Changes		Vit D receptor polymorphism FOK I						P value
		GG		AG		AA		
		No.	%	No.	%	No.	%	
RBC	Normal	16	84.2	27	81.8	7	87.5	0.921
	Anemia	3	15.8	6	18.2	1	12.5	
Lymphocyte	Normal	0	0.0	33	100.0	8	100.0	0.001*
	Lymphopenia	19	100.0	0	0.0	0	0.0	
Thrombocytes	Normal	13	68.4	25	75.7	5	62.5	0.704
	Thrombocytopenia	6	31.6	8	24.3	3	37.5	
Leucocytes	Normal	10	52.6	29	87.8	7	87.5	0.024*
	Leucopenia	8	42.1	2	6.1	1	12.5	
	Leukocytosis	1	5.3	2	6.1	0	0.0	

It shows Association between Vitamin D receptor polymorphism FOK I Genotypes and CBC changes in SLE patients; no.=60

it demonstrated that the prevalence of lymphopenia and leucopenia was significantly higher among SLE patients with GG genotypes (100.0 % & 42.1%) compared to SLE patients with AG (0.0% & 6.1%) and AA (0.0 & 12.5%) mutants at p value (0.001& 0.024), respectively.

Demographic and Clinical Characteristics	SLE patients (NO.=60)		Controls (NO.=20)	
	NO.	%	No.	%
Age	Mean±SD= 32± 6.5	Range=16-60	Mean±SD= 30±4.3	Range=16-55
Sex				
Male	5	12.0	2	10.0
Female	55	88.0	18	90.0
Arthritis	42	70.0	0	0.0
Nephritis	40	66.7	0	0.0
Oral ulcer	12	20.0	0	0.0
Malar rash	43	71.6	0	0.0
Discoid rash	5	8.3	0	0.0
Serositis	8	13.3	0	0.0
Seizures	2	3.3	0	0.0
Psychosis	3	5.0	0	0.0
Alopecia	13	21.7	0	0.0
Photosensitivity	39	65.0	0	0.0

It shows Distribution of the Studied SLE Patients and Healthy Controls according to their Demographic and Clinical Findings:

it demonstrated that a total of 60 SLE patients and 20 healthy subjects were included in the present study. In SLE patients, age ranged from 16 -60 years old. Age of healthy controls ranged from 16-55 years old. Clinical profiles of the patients included arthritis (70.0%), nephritis (66.7%), oral ulcer (20.0%), malar rash (71.6%), Discoid rash (8.3%), serositis (13.3%), seizures (3.3%), psychosis (5.0%), alopecia (21.7%) and photosensitivity (65.0%).

4. Discussion:

Vitamin D is a nutrient long reflected as vital for skeletal health. However it is now attracting attention from medical and nutritional communities as knowledge develops of its biological functions and its association with lessened risk of numerous chronic diseases (5). There are three

recognized sources for VD in human specifically; sunlight, dietary intake and VD supplementation (6).

Lupus nephritis is an inflammation of the kidneys caused by systemic lupus erythematosus , an autoimmune disease, Lupus nephritis (LN) is highly prevalent, and a significant portion of LN will develop into end stage renal disease in systemic lupus erythematosus (1)

The risk of ESRD in class IV-LN has been as high as 44% over the past 15 years despite the current available treatment regimens including steroid and immunosuppressants as well as various monoclonal antibodies (2) . Renal fibrosis is a common pathological feature of ESRD that requires renal replacement therapy and is a huge economic burden worldwide (3) .

In the current study, we found a significant difference in genotype distribution between patients with SLE and healthy controls. The homozygous GG genotype and heterozygous AG mutants were overpresented in patients with SLE compared with our controls, Moreover, we observed a significant negative association between the VDR allele A and susceptibility to SLE suggesting that allele A was in some fashion protective against SLE. Our data shows that the GG genotype constituted a risk factor for the development of lupus nephritis (OR: 4.8) and was associated with higher disease activity index score among studied patients with SLE.

In this study, regarding CBC changes, the prevalence of thrombocytopenia and lymphopenia was significantly higher among SLE patients compared to our controls, However the prevalence of lymphopenia and leucopenia was significantly higher among SLE patients with GG genotype compared to SLE patients with AG and AA mutants ,Regarding liver function tests, The mean serum Albumin level was significantly lower among SLE patients compared to controls .

Kidney function tests, SLE patients had ahigh levels of urea, Creatinine and 24h urinary protein compared to controls .

Antinuclear antibody titre was significantly higher among SLE patients than controls ,Also Anti Ds DNA is represented in SLE patients in ahigh titre than healthy controls ,Furthermore, All SLE patients with GG genotypes had positive Anti Ds DNA (100%)compared to SLE patients with AG (0,0) and AAn (0,0) mutants.

Prevalence of 25(OH) vit D status among controls and SLE patients was quantified , The prevalence of Vit D deficiency and insufficiency was significantly higher among SLE patients(

20%, 43,3%) compared to healthy controls (25%,10%) ,Furthermore, We found that SLE patients with GG genotype had lower serum level of 25 (OH) Vit D compared to SLE patients with AG and AA mutants.

Regarding both ESR , CRP, the level of ESR and CRP were significantly higher among SLE patients with GG genotypes compared to SLE patients with AG and AA mutants.

Regarding complement activity tests, the mean level of C3,C4 were significantly lower in SLE patients with GG genotype compared to SLE patients with genotypes AA and AG mutants .

Renal Biopsy , All lupus nephritis patients with GG genotype had active stage in renal biopsy either C3,4 (active, early chronic) or C5 (active, early chronic) compared to SLE patients with AG and AA mutants were in an inactive stage either C3,4 or C5

5. Conclusion and Recommendations:

1. It is recommended to carry out further studies on FOKI gene polymorphisms in larger groups of patients and controls.
2. Further studies are needed on other VDR polymorphisms and their associations with SLE and its complications.

6. References:

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