

Prevalence of Plasma Cell Free DNA and its Prognostic Role in Systemic Lupus Erythematosus Patients

Thesis

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم العظيم

صدق الله العظيم

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List of Contents

Title	Page No.
List of Tables	i
List of Figures.....	iii
List of Abbreviations	v
Introduction	1
Aim of the Work	3
Review of Literature	
▪ Pathogenesis of SLE	4
▪ Circulating (cell free) DNA in diagnosis of several human diseases.....	40
Subjects and Methods.....	49
Results	59
Discussion.....	85
Summary and Conclusion.....	93
References	95
Arabic Summary	

List of Tables

Table No.	Title	Page No.
Table (1):	American College of Rheumatology (ACR) criteria for the classification of systemic lupus erythematosus	30
Table (2):	Systemic Lupus Erythematosus Disease Activity Index (SLEDAI).....	33
Table (3):	British Isles Lupus Assessment Group (BILAG) Index	34
Table (4):	Systemic Lupus Activity Measure (SLAM) Index	35
Table (5):	European Consensus Lupus Activity Measurement (ECLAM).....	37
Table (6):	Systemic Lupus International Collaborating Clinic/American College of Rheumatology (SLICC/ACR) Damage Index	39
Table (7):	The PCR reaction mix (Total volume of 20 μ L) is shown in the following table	56
Table (8):	Thermal cycler programming for amplification shown in the following table.....	57
Table (9):	Descriptive and comparative statistics of the groups in the study	60
Table (10):	Descriptive data of the 3 studied groups.	62
Table (11):	Descriptive Clinical Data in the Studied Patients	63
Table (12):	Comparison between studied groups as regard CBC	64
Table (13):	Post-Hoc test for comparison between studied groups as regard CBC parameters (TLC, Hb and platelets)	65

List of Tables (cont...)

Table No.	Title	Page No.
Table (14):	Comparison between studied groups as regard CRP.....	68
Table (15):	Comparison between studied groups as regard ESR.....	69
Table (16):	Post-Hoc test for comparison between studied groups as regard ESR	70
Table (17):	Comparison between studied groups as regard C3 and C4	70
Table (18):	Post-Hoc test for comparison between studied groups as regard C3 and C4.....	72
Table (19):	Comparison between patient groups (Ia and Ib) as regard Anti-DNA titre.....	73
Table (20):	Comparison between patient groups (Ia and Ib) as regard ANA.....	74
Table (21):	Comparison between studied groups as regard cf- DNA (CT)	76
Table (22):	Post-Hoc test for comparison between studied groups as regard cf- DNA (CT).....	78
Table (23):	Correlation study between cf- DNA (CT) and ANA & Anti-DNA) in group Ia.....	81
Table (24):	Correlation study between cf- DNA (CT) and (ANA & Anti-DNA) in patients of group Ib	83

List of Figures

Fig. No.	Title	Page No.
Figure (1):	Role of neutrophils and lds in the pathogenesis of sle and associated organ damage, upon exposure to micro organisms, damaged ceoo products, immune complexes and other as yet unidentified stimuli, neutrophils and lds undergo Netosis	9
Figure (2):	Schemic presentation of various pathways by which nucleic acids are released into circulation. Other nucleated cells include T-cells, haematopoitic cells, etc.....	43
Figure (3):	Comparison between studied groups as regard age.....	61
Figure (4):	Comparison between studied groups as regard sex.	61
Figure (5):	Comparison between studied groups as regard Total leucocytic count.	66
Figure (6):	Comparison between studied groups as regard lymphocytes.....	66
Figure (7):	Comparison between studied groups as regard Hemoglobin.	67
Figure (8):	Comparison between studied groups as regard Platelets.....	67
Figure (9):	Comparison between studied groups as regard CRP.....	68
Figure (10):	Comparison between studied groups as regard ESR.....	69
Figure (11):	Comparison between studied groups as regard C3.....	71
Figure (12):	Comparison between studied groups as regard C4.....	71
Figure (13):	Comparison between patient groups (Ia and Ib) as regard anti – DNA titre	73

List of Figures (cont...)

Fig. No.	Title	Page No.
Figure (14):	Comparison between patient groups (Ia and Ib) as regard ANA.	75
Figure (15):	Comparison between patient groups (Ia and Ib) as regard ANA.	75
Figure (16):	Comparison between studied groups as regard cf- DNA (CT).....	76
Figure (17):	ROC curve of cf- DNA (CT) between patient and control groups.....	77
Figure (18):	ROC curve between group Ia and group II as regard cf- DNA (CT).....	79
Figure (19):	ROC curve between group II and group Ib as regard cf- DNA (CT).....	80
Figure (20):	Negative correlation between cf- DNA (CT) and ANA in patients of group Ia.....	81
Figure (21):	Negative correlation between cf- DNA (CT) and Anti-DNA in patients of group Ia.	82
Figure (22):	Negative correlation between cf- DNA (CT) and ANA in patients of group Ib.....	84
Figure (23):	Negative correlation between cf- DNA (CT) and Anti-DNA in patients of group Ib.	84

List of Abbreviations

Abb.	Full term
ACR:	<i>American College of Rheumatology.</i>
ADCC:	<i>Antibody dependent cell mediated cytotoxicity.</i>
AICD:	<i>Activation induced cell death.</i>
AIH:	<i>Auto immune hepatitis.</i>
ANA:	<i>Antinuclear antibody.</i>
ANCA	<i>Antibodies to neutrophil cytoplasmic antibodies</i>
Anti p	<i>Antibodies and phosphoprotein antibodies.</i>
anti-ds DNA:	<i>Anti-double stranded DNA</i>
Anti-Sm:	<i>Anti-Smith.</i>
APC	<i>Antigen presenting cell.</i>
ApL:	<i>Antiphospholipids.</i>
BAFF:	<i>B-cell activating factor.</i>
Bcl-2	<i>Anti apoptotic molecule.</i>
BCR:	<i>B cell receptor.</i>
BILAG	<i>British Isles Lupus Assessment Group.</i>
C	<i>Complement protein.</i>
CAD:	<i>Coronary artery disease</i>
CD:	<i>Cluster of differentiation.</i>
cf-DNA:	<i>Cell free deoxyribonucleic acid.</i>
CK:	<i>Creatine kinase.</i>
CLIF	<i>Crithidia luciliae immunofluorescence.</i>
CNA:	<i>Circulating nucleic acids.</i>
CNS	<i>Central Nervous system.</i>
CTLA4	<i>Common T-lymphocyte antigen 4.</i>
DAS28	<i>Disease activity score of 28 joints.</i>
DCs	<i>Dendritic cells</i>
DIL	<i>Drug induced lupus</i>
ds-DNA:	<i>Double stranded DNA.</i>
ds-RNA:	<i>Double stranded RNA.</i>
EBV	<i>Epstein Barr Virus.</i>
ECLAM:	<i>European Consensus Lupus Activity measurement.</i>

List of Abbreviations (cont...)

Abb.	Full term
ELISA:	<i>Enzyme linked immunosorbent assay.</i>
ENA:	<i>Extractable nuclear antigen.</i>
EPCs:	<i>Endothelial progenitor cells.</i>
ER_β	<i>Esterogen receptor β</i>
ER_α	<i>Estrogen receptor α</i>
FARR:	<i>Radio immunoassay for anti – DNA.</i>
FcγRIIa	<i>Low affinity immunoglobulin – γ Fc region receptor.</i>
G CSF:	<i>Granulocytes colony stimulating factor.</i>
GCs:	<i>Germinal centers.</i>
GCSF-R:	<i>G-CSF receptor.</i>
GMCSF:	<i>Granulocytes macrophage colony stimulating factor.</i>
HEP	<i>Human epithelial tissue.</i>
IC	<i>Immune complex</i>
ICOS:	<i>Inducable co-stimulator.</i>
IFN-R	<i>IFN receptor.</i>
IFNs	<i>I interferons</i>
IIF:	<i>Indirect immunofluorescence.</i>
IL37:	<i>Interleukin 37.</i>
INF	<i>Interferon.</i>
ITAMs:	<i>Immunoreceptor based activation motifs.</i>
ITIM	<i>Immunoreceptor tyrosine inhibitory motif.</i>
LDG:	<i>Low density granulocytes.</i>
MBL:	<i>Mannose binding lectin.</i>
mDC	<i>Myeloid dendritic cell.</i>
MI	<i>Myocardial infarction.</i>
MPO	<i>Myeloperoxidase.</i>
MRI:	<i>Magnetic resonance image.</i>
NET:	<i>Neutrophil extracellular trap</i>
NETosis:	<i>Extracellular traps formed by neutrophils.</i>
NETs:	<i>Neutrophil extended traps.</i>

List of Abbreviations (cont...)

Abb.	Full term
NMDA:	<i>N-methyl-D-aspartate.</i>
OxLDL:	<i>Oxidized low density lipoprotein.</i>
PBMCs	<i>Peripheral blood mononuclear cells</i>
PDCs:	<i>Plasmacytoid dendritic cells.</i>
PTPN22	<i>Protein tyrosine phosphatase N22</i>
RA:	<i>Rheumatoid arthritis.</i>
RNA:	<i>Ribonucleic acid.</i>
RNP:	<i>Ribonucleoprotein.</i>
SCLE:	<i>Sub acute cutaneous lupus erythematosus.</i>
SLAM:	<i>Systemic lupus activity measures.</i>
SLE:	<i>Systemic lupus erythematosus.</i>
SLEDAI:	<i>Systemic lupus erythematosus disease activity index.</i>
SLICC:	<i>Systemic lupus international collaborating clinics.</i>
Sn RNP	<i>Small nuclear ribonucleoprotein.</i>
SS	<i>Systemic sclerosis.</i>
SS-DNA:	<i>Single stranded DNA.</i>
TCR	<i>T cell receptor.</i>
Tfh:	<i>T follicular helper.</i>
TGFβ:	<i>Transforming growth factor-β.</i>
TLR	<i>Toll like receptor.</i>
TNFα:	<i>Tumor necrosis factor α.</i>
WB:	<i>Western blot.</i>

INTRODUCTION

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease with a complex pathogenesis involving multiple genetic and environmental factors. The disease is characterized by enhanced autoantibody production, abnormalities in function of immune-inflammatory system and inflammatory manifestations in several organs (*Young et al., 2009*).

The clinical course of lupus disease usually occurs in exacerbation and remission pattern. It may involve virtually any organ system and have a wide range of disease severity. Clinical presentations of SLE may be in the form of fatigue, weight loss or fever in the absence of infection, butterfly rash, photosensitivity rash, musculoskeletal problems (arthritis, myositis or arthralgia), renal problems (proteinuria, haematuria, cellular casts or nephritic syndrome), hematological problems (thrombocytopenia, anemia or leucopenia) (*Uzuelli et al., 2009*).

Cell death has been regarded as an important event in lupus pathogenesis as it leads to release of antigens as nucleic acids for immune complex formation. DNA-antibody complexes in the circulation are one of the hallmarks of SLE that leads to events such as complement activation, immune complex deposition, cytokine release and many other detrimental effects causing manifestations of SLE. Fluctuation in circulating DNA level might be one of the driving factors behind flare-ups of SLE (*Chen, 2010*).

Circulating cell-free deoxyribonucleic acid (cf-DNA), defined as extracellular DNA occurring in blood serum or plasma, present in only limited amounts in healthy individuals, since dying cells and remnants of dead cells are efficiently removed, mainly in the liver. Reactive oxygen species are implicated as a cause of damage to DNA, including breaking of single and double strands, releasing of free nucleobases, chemical changes of nucleobases, and modification of sugar moieties (*Su and Pisetsky, 2009*).

Circulating cf-DNA has been widely studied and is considered as a potential biomarker for the detection and monitoring of various human diseases such as stroke, myocardial infarction, sepsis, acute pancreatitis, as well as cancer. Analyzing and quantitating cell-free plasma DNA could serve as a valuable diagnostic tool (*Rainer et al., 2003*).

AIM OF THE WORK

To estimate the prevalence of plasma cf - DNA in SLE patients and evaluate it as a prognostic marker in comparison to anti double stranded DNA-titre.

Chapter 1

PATHOGENESIS OF SLE

Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disease of unclear etiology that affects multiple organs and afflicts mostly women of childbearing age. The development of SLE is attributed to disruptions in adaptive immunity, triggered by genetic predisposing factors and various environmental insults, which lead to the loss of tolerance of self antigens. Indeed, the development and progression of SLE require T-lymphocytes and B-lymphocytes, which highlights the key role of autoimmune reactivity in this disease (*Dorner et al., 2011*).

a) Role of innate immunity in pathogenesis of SLE

Evidence over the past decade indicates that patients with SLE also have profound disruption in innate immunity that could play a crucial part in the initiation and perpetuation of the disease, as well as in the development of organ damage. Abnormalities in the phenotype and function of monocytes, macrophages, dendritic cells (DCs), and other cellular and humoral components of the innate immune system have been clearly identified in patients with SLE. These defects might be involved in key events in the pathogenesis of SLE, including regulation of cell death, presentation of putative autoantigens and synthesis of type I interferons (IFNs) (*Moulton et al., 2017*).