Study of Iron profile Level in Lupus Nephritis

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ABSTRACT
Background: Systemic lupus erythematosus (SLE) is a multi-factorial, chronic autoimmune disorder, characterized by dysfunction of T and B lymphocytes. It affects various vital organ systems, and 70% to 90% of SLE patients are females. Lupus nephritis (LN) is one of the common complications in patients with SLE and influences overall outcome of these patients. About two-thirds of patients with SLE have renal disease at some stage which is a leading cause of mortality in these patient .Iron is critical in nearly all cell functions and the ability of a cell, tissue and organism to procure this metal is obligatory for survival. Iron is necessary for normal immune function, and relative iron deficiency is associated with mild immunosuppression. Concentrations of this metal in excess of those required for function can present both an oxidative stress and elevate risks for infection. As a result, the human has evolved to have a complex mechanism of regulating iron and limiting its availability. Ferritin levels correlate with disease activity in patients   with SLE and developing of lupus nephritis**. Objective:** To correlate between Iron profile and SLE activity and developing lupus nephritis. **Materials and Methods:** A prospective study was conducted on 75 adult persons: 25 Patients with SLE with proteinuria, patients with SLE without proteinuria, 25person have no SLE (control group).These person were Admitted at internal medicine department and outpatient clinic of Al-Hussein university hospital, Cairo, Egypt. SLE patients were diagnosed according to the American College of Rheumatology (ACR) criteria' Lupus Activity assessment by C3&C4, Lupus nephritis assessment using Albumin /creatinine ratio. Iron profile was measured and included: serum iron, serum TIBC, transferrin saturation and Serum ferritin levels were tested by ELISA. **Results:** There were no significant statistical difference between groups as regard age or sex however, There were significant difference between groups as regard S.iron, S. ferritin, TIBC and TSAT,   Between group analysis results showed significantly lower s.iron and TSAT level of SLE patients with and without proteinuria in comparison with control group. While S. ferritin is significantly high in SLE patients with proteinuria in comparison with SLE patients without proteinuria and control group. And this data go with activity markers of SLE. **Conclusions**: 1) Hyperferritinemia is a useful marker in assessment of disease activity and severity of Albuminuria in SLE patients complicated by lupus nephritis, treatment of hyperferritinemia can result in decreased Albuminuria and delayed renal damage. 2) Iron homoeostasis is important in normal immune function and Iron disturbance can result in mild immunosuppression.
Keywords: Iron,Inflammation ,Systemic lupus erythematosus.

Introduction

Systemic lupus erythematosus (SLE) is a multi-factorial, chronic autoimmune disorder characterized by dysfunction of T and B lymphocytes. It affects various vital organ systems, and 70% to 90% of SLE patients are females. It is believed that environmental, host genetic and hormonal factors play crucial roles in the pathogenesis of SLE, but the etiology of the disease has not been clearly understood [(1)].

SLE is characterized by an unpredictable disease course, interspersed with periods of remission and flares. Conventional serological markers of SLE such as anti-dsDNA and complement levels are not ideal as they are not sufficiently sensitive and specific for monitoring of disease activity, particularly in certain systems like the central nervous system and the gastrointestinal tract. Even for more common manifestations such as lupus nephritis, these conventional markers also lack sensitivity and specificity in gauging residual inflammation and predicting flares of renal disease. Thus, novel biomarkers for SLE activity have to be developed. Ideal biomarkers for SLE should have high specificity for the disease (or specific end-organ involvement) to aid early diagnosis have good correlation with disease activity and be sensitive to change in disease status to allow for serial monitoring, and be able to detect flares

early so that treatment strategies can be instituted to minimize organ damage [2] **.**

Iron homoeostasis and the immune system: Iron is critically important in normal immune function. This metal contributes to cell differentiation and growth and is a component of numerous enzymes essential for the proper enzymatic functioning of immune cells (e.g. peroxides, nitric oxide synthase and those involved in cytokine production).While iron deficiency can be associated with an increased risk of infection [3]. There is little evidence to suggest that iron deficiency causes a major, clinically relevant, defect in immune function [4]. Studies of the clinical effect of supplementation among patients with iron deficiency are also lacking.

Supplementation in iron-deficient children in Africa did improve the absolute numbers of T and Th cells but did not affect the incidence of malaria [5]. Iron overload can also be associated with changes in the immune system. Increased levels of iron enhance suppressor T-cell numbers and activity, decrease the proliferative capacity, numbers and activity of Th cells, impair the generation of cytotoxic T cells and alter immunoglobulin secretion [6]. Immune dysregulation associated with iron overload in haemochromatosis could be confounded by an underlying immune defect causing both. For example, recent data have suggested that T cells may play a role in regulating iron absorption from the gut [7]. Cytokines such as TNF-, IFN- and IL-1 have each been demonstrated to change iron movement by reducing the concentration of transferrin receptor on the cell surface, increasing the synthesis of ferritin for metal storage and activating nitric oxide systems to reduce intracellular iron [8]**.**

Patients receiving blood transfusions after either acute myocardial infarction or cardiac surgery have an increased mortality even after adjustment for other predictive factors [9]**.**

Similarly, markers of inflammation are increased in patients with chronic kidney disease who receive intravenous iron [10]**.** These few observations suggest an association between metal availability and the initiation and maintenance of an inflammatory response contributing to the poor outcomes in patients receiving an iron load. Iron availability may not only be important in the defense against infection but may also be relevant to the control of inflammatory damage following other inciting factors. The oxidative stress associated with changes in iron availability may also be a trigger for an inflammatory response to both infectious and non-infectious exposures. This may be important in several forms of arthritis [11]**.**

Limited clinical data suggest a benefit in decreasing iron stores. While iron therapy for those with severe or moderate iron deficiency is likely safe and should not be avoided [8].

The aim of this study was to correlate between Iron profile and SLE activity and developing lupus nephritis.

Subjects and Methods
This prospective study was Included 75 adult persons: 25 Patients with SLE with proteinuria, patients with SLE without proteinuria, person has no SLE (control group).Who were Admitted at internal medicine department and outpatient clinic of Al-Hussein university hospital, Cairo, Egypt.

Inclusion criteria: Age of at least 18 years old, SLE patients were diagnosed according to the American College of Rheumatology (ACR) criteria [12] ,Lupus Activity assessment by C3&C4, Lupus nephritis assessment using Albumin /creatinine ratio, Iron profile was measured and included: serum iron , serum TIBC, transferrin saturation and Serum ferritin levels were tested by ELISA.

Exclusion criteria: Any patients with proteinuria due to other causes rather than SLE, Estimated glomerular filtration rate less than 60ml /min ,Chronic liver diseases, persons who received Iron Supplementation during the last 3month .

All persons enrolled in the study were subjected to full medical history, thorough clinical examination.

Laboratory investigation was included:
Complete blood picture, Liver profile which includes: liver enzymes, Renal profile which includes: serum creatinine, urea, Pelvi Abdominal ultrasound (U/S), Antinuclear antibody (A.N.A by immunofluorescence), Anti Double stranded DNA (Anti ds DNA), Iron profile : serum ferritin level, serum iron level, TIBC, transferrin saturation, Albumin/creatinine

ratio, C3, C4 levels, Erythrocytes sedimentation rate (ESR).

Written informed consent was obtained from all the persons. The study was approved by the Ethics Board of Al-Azhar University.

Results

This study included 75 adult persons: 25 Patients with SLE with proteinuria, patients with SLE without proteinuria, 25 patients have no SLE (control group) and this study revealed that, There was no significant difference between SLE patients and controls as regard age as shown in table(1)

Table (1)

	SLE patients without proteinuria			SLE patients with proteinuria			Control			P value
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	
Age	32.52	8.242	18-45	28.44	7.539	18-42	28.64	5.438	19-38	.083

As regard sex. There was no significant difference between groups as shown in table (2).

Table (2)

Parameter			GROUPS			
			SLE patients without proteinuria	SLE patients with proteinuria	Control	P value
Sex	1	Male N= 7(9.3%)	4 (16.0%)	3 (12.0%)	0 (0.0%)	.129
	2	Female N= 68 (90.7%)	21 (84.0%)	22 (88.0%)	25 (100.0%)	

And CBC shows significant difference between groups as regard HB concentration while no significant difference was found between groups as regard WBCs , platelets as shown in table (3) and In between group analysis showed HB of SLE patients without proteinuria significantly different in comparison with SLE patients with proteinuria .and control persons compared with SLE patients with proteinuria.

Table (3)

	Group 1		Group 2		Group 3		P value
	Mean	SD	Mean	SD	Mean	SD	
HB	10.688	1.1545	9.416	1.4571	12.536	1.2942	< 0.0001
WBCs	7.236	1.9996	5.492	2.7710	6.260	1.8590	.027
PLT	292.60	125.019	248.88	185.152	236.00	53.559	.292

AS regards renal function there was significant difference between groups as regard S.creat and urea as shown in table (4) and In between group analysis showed significant lower in S.creat and urea level of control group in comparison with SLE patients with and without proteinuria.

Table (4)

	Group 1		Group 2		Group 3		P value
	Mean	SD	Mean	SD	Mean	SD	
S.creat	.932	.1069	.916	.1795	.760	.2345	.002
Urea	22.76	3.643	33.56	12.087	26.36	4.999	< 0.0001

As regards inflammatory marker there was significant difference between groups as regard ESR as showen in table (5),In between group analysis ESR level was significantly high in SLE patients with proteinuria in comparison with SLE patients without proteinuria and control group.

Table (5)

	Group 1		Group 2		Group 3		P value
	Mean	SD	Mean	SD	Mean	SD	
ESR	64.96	25.800	94.72	29.563	18.48	7.349	< 0.0001

As regards activity marker there was significant difference between groups as regard C3, C4 and ALB/Creat as showen in table (6), In between group analysis showed significant lower in C3 and C4 level of SLE patients with proteinuria in comparison to SLE patients without proteinuria and control persons. While Alb/Creat. level was significantly high in SLE patients with proteinuria in comparison with SLE patients without proteinuria and control persons.

Table (6)

	Group 1		Group 2		Group 3		P value
	Mean	SD	Mean	SD	Mean	SD	
C3	117.16	34.584	61.12	13.676	123.32	19.566	< 0.0001
C4	22.48	9.522	8.00	5.560	25.04	7.695	< 0.0001
Alb/creat.	16.52	5.245	2131.04	562.825	18.64	4.527	< 0.0001

AS regards iron profile there was significant difference between groups as regard S.iron, S. ferritin, TIBC and TSAT as shown in table (7), In between group analysis showed significant lower in S. iron and TSAT level of SLE patients with and without proteinuria in comparison to

Control persons. While S. ferritin is significantly high in SLE patients with proteinuria in comparison with SLE patients without proteinuria and control persons.

Table(7)

	SLE patients without proteinuria		SLE patients with proteinuria		Control		P VALUE
	Mean	SD	Mean	SD	Mean	SD	
S.iron	35.16	15.491	28.00	5.123	61.92	28.108	< 0.0001
S.ferritin	153.24	74.697	919.48	496.956	108.04	82.511	< 0.0001
TIBC	271.40	89.055	349.56	80.288	266.08	86.019	.001
TSAT	16.7646	15.65267	8.4982	2.73387	25.0349	12.57474	< 0.0001

DISCUSSION:

Systemic lupus erythematosus (SLE) is a common autoimmune disorder that may affect many organ systems and produces severe conditions. Early diagnosis, evaluation of activity and severity of the disease, and proper treatments are essential. Clinical evaluation and paraclinical tests, in particular the detection of some autoantibodies and serologic parameters can help early diagnosis of the disease [13]. Lupus nephritis (LN) is one of the common complications in patients with SLE and influences overall outcome of these patients. About two-thirds of patients with SLE have renal disease at some stage which is a leading cause of mortality in these patients. [14] Anemia is a common hematological abnormality in SLE and can easily be categorised with simple laboratory tests .The importance of good biomarkers depends on their ability to predict disease severity reliably.

Ferritin an acute phase reactant is not only a main protein of iron storage, but a regulator of immune system and may play a special role in autoimmune diseases [13].

In this study, the age of our SLE patients without proteinuria ranged from 18– 45 years with the mean age of 32.52± 8.242 while SLE patients with proteinuria ranged from 18– 42 years with the mean age of 28.44 ± 7.539 in contrast to control group age ranged from 19 – 38 with the mean age 28.64 ± 5.438.There was no significant difference between SLE patients and controls as regard age.

These results agree with the study of **Boddaert et al.** who found that 65% of patients with SLE have disease onset between ages 16 and 55[15]. However, another study done by **Danchenkol et al.** demonstrated that SLE cases

are ranging from children as young as two years old to adults 80 years of age and older [16]. and in the study of **Zandman-Goddard et al** the median age was 37 years old [17]. Most of our patients were females 43 (86%) while male 7(14%). This agrees with the study of **Vanarsa et al., Abbasi et al.** and **Zandman-Goddard et al.** in their studies most of their patients were females (89.3% , 93.3%, and 89% respectively [18,19].

As regard complete blood count in our SLE patients there was significant difference between SLE patients and controls as regard hemoglobin concentration (HB), while there was no significant difference between SLE patients and controls as regard WBCs, platelets count.

SLE patients without proteinuria mean HB 10.688 ± 1.1545 and decreased to 9.416 ± 1.457 in SLE patients with proteinuria while increased in control to 12.536 ± 1.294 with significant difference between groups. The HB of SLE patients with proteinuria significantly decreased in contrast to SLE patients without proteinuria and control groups. This agrees with the study of **Xu XM et al.** and **Aleem et al.** who studied 624 SLE patients for hematological abnormalities of which hematological abnormalities were present in 516 (82.7 %) patients at the time of diagnosis[20,21] . Most common hematological abnormality was anemia (77.5%). Various studies have shown a similar findings [22]**.** And more anaemia in SLE patients with proteinuria come in agreement with that found by **Mohammad-Reza Ardalan** [23].

In our study there was no significant difference between groups as regard WBCs count and. This finding disagree with that indicated by **Schulze-Koops H.** who concluded that Leukopenia is common in SLE and usually is secondary to lymphopenia, neutropenia or combination of both [24].

The main platelets count in our study groups remained within normal without significant difference between groups. This data come against some authors who show thrombocytopenia with disease activity as **Mohammad-Reza Ardalan** [23] .and others . The conflict can be explained by low prevalence which may be 7% in many reports [25].

There was significant increased ESR 1ST h between our patients groups compared to controls. Also **Zein et al** stated that serum level of ESR is elevated in active phase of many inflammatory and autoimmune diseases [26].

The present study showed significant difference between SLE patients and controls as regard C3, C4 and urine albumin/creatinine ratio. All patients were 100% positive ANA and Anti-dsDNA and all controls were 100% negative ANAand Anti-dsDNA.

This agrees with the study of **Wichainun et al** who found that the sensitivity of ANA in diagnosis of SLE at a titer of $\geq 1{:}80$ and $\geq 1{:}160$ was 98% and 90%, respectively, with specificity of 92% and 96%, respectively. The specificity decreased to 88% and 94%, respectively, when using sera from patients with multiple medical problems (MMP). The specificity of anti-dsDNA was 100% and 97%, when using sera from healthy controls (HC) and MMP patients, respectively and concluded that ANA and anti-dsDNA gave high sensitivity and high specificity in patients with SLE, even when using MMP patient's sera as controls [27].

Our results agree with **Walport who** documented that complement is implicated in the pathogenesis of systemic lupus erythematosus (SLE) in several ways[28] . Homozygous deficiency of any of the proteins of the classical pathway is causally associated with susceptibility to the development of SLE, especially deficiency of the earliest proteins of the activation pathway.

The present study showed there was significant difference between groups as regard S.iron, S.ferritin, T.I.B.C and T.SAT. In between group analysis show significant lower in S.iron and T.SAT level of SLE patients with and without proteinuria in comparison to control patients. While S.ferretin and T.I.B.C level is significantly high in SLE patients with proteinuria in comparison with SLE patients without proteinuria and control group.

Abbasi et al. showed a mean increase of 2.7 times the normal serum ferritin titre in their SLE patients[13] . Also, **Orbach et al.** after their study on ferritin levels in a cohort of autoimmune disease patients stated that hyperferritinemia is more frequently encountered in SLE patients compared to DM,MS, and RA[29]. SLE patients described in **Vanarsa et al .** report exhibited 'anemia of chronic disease' rather than iron

deficiency anemia. Whereas anemia of chronic disease is marked by elevated ferritin but reduced transferrin and total iron-binding capacity (TIBC) levels, iron deficiency anemia exhibits the reverse profile [18].

The anemia of chronic disease is still not fully understood. Different causes contribute to anemia in chronic diseases, including diversion of iron, reduced erythropoiesis and reduced response to erythropoietin. Interleukin-6 appears to be the central mediator of anemia of chronic disease in a range of inflammatory diseases, including end stage renal disease and rheumatoid arthritis. Interleukin-6 induces the expression of hepcidin, which suppresses the expression of the iron transporter, ferroportin-1, so inhibiting the absorption of iron from the duodenum and the release of iron from the reticulo endothelial system [30].

Inflammation is also well established to up-regulate hepcidin mediated degradation of the iron transport channel, ferroportin, thereby sequestering iron in the form of ferritin in the cells of reticulo endothelial system. This results in the unavailability of iron for HB production, hence explaining the anemia [18].

Conclusions

1. Hyperferritinemia is a useful marker in assessment of disease activity and severity of Albuminuria in SLE patients complicated by lupus nephritis, Treatment of hyperferritinemia can result in decreased Albuminuria and delayed renal damage.

2. Iron homoeostasis is important in normal immune function and Iron disturbance can result in mild immunosuppression.

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