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AutoAbs (ANA, ds-DNA) and trace elements (Zn, Cu, AL) and essential element (Ca) serum levels in Iraqi patients with SLE

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Abstract

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease, with a wide range of clinical symptoms. Numerous studies have indicated to the association between autoantibodies (ANA and ds-DNA), trace elements [Zinc (Zn), Copper (Cu), Aluminum (AL)], and essential element Calcium (Ca) serum levels and pathogenesis of SLE. The current study aims to evaluate the role of some autoantibodies (ANA, ds-DNA), Trace elements (Zn, Cu, AL), and essential element (Ca) in pathogenesis of early diagnosed SLE patients and treated patients. The study included 180 females diagnosed with SLE and a healthy individuals as control (60 females as early diagnosed SLE without treatment, 60 females as SLE patients under treatment with (prednisolone, hydroxychloroquine) and 60 females healthy as control group, with ages ranging from 20 to 45 years. The serum levels of autoantibodies (ANA and ds-DNA) were assessed by Enzyme linked immunosorbent assay (ELISA). Trace elements (Zn, Cu, Al) and essential element (Ca) were assessed using atomic absorption spectrophotometer (AAS). The results of ANA and ds-DNA showed that the serum level of ANA was significantly higher in early diagnosed group followed by treated group compared with control group ($p<0.05$). The ds-DNA serum level was significantly higher in both SLE patients groups (early diagnosed and treated) compared with control group ($p<0.05$). The results of trace elements and Ca showed that the serum levels of Zn and Ca were significantly lower in both SLE patients groups (early diagnosed and treated) compared with healthy control group ($p<0.05$). The Cu and AL serum levels were significantly higher in both SLE patients groups (early diagnosed and treated) compared with healthy control group ($p<0.05$). Based on these results, autoantibodies (ANA, ds-DNA), trace elements (Zn, Cu, Al), and essential element (Ca) may plays a role in the pathogenesis of SLE.

Keywords:

SLE, ANA, ds-DNA, zinc, Copper, Aluminum, Calcium

SLE is an autoimmune disease that is diverse in nature and has the potential to affect any organ in the body. It is characterized by elevated levels of circulating anti-nuclear autoantibodies (Dahham, Haddad 2023; Pascoe et al., 2017). SLE is a disease that can be fatal and is linked to a high rate of morbidity and mortality (Abd-Alrasool, Gorial, Hashim 2017; Al-Hasso et al., 2020). Both environmental and genetic factors are implicated in SLE pathogenesis (Al-Rawi et al., 2014). As a result, immune cells, particularly T and B lymphocytes are triggered to produce various immune components (Abbas, Melconian, Ad'hiah 2019), such as antinuclear antibodies (ANA) and anti-double strand DNA (anti-dsDNA) (Olsen, Karp 2014). ANA

is important immunodiagnostic tools in SLE, although their clinical and pathologic significance have not yet been clarified and remain a matter of debate (Al-Mughales 2022). ANA is helpful as an initial screening method, but it is not specific for this disease. Additional serologic testing is typically required in patients with positive ANA test findings to confirm a diagnosis of SLE (Lam, Ghetu, Bieniek 2016). Formation of anti-dsDNA autoantibodies before SLE was clinically diagnosed (Arbuckle 2001). Up to 70% of SLE patients develop antibodies against double-stranded DNA during the course of the disease. These antibodies show 95% specificity in established SLE cohorts and can be discovered using numerous techniques (Craig, Ledue 2011). On the other hand, trace elements have a major influence as a component of numerous enzymes on a variety number of biological processes (Mohammed, Fezea 2017). Trace elements concentrations are frequently considered to be a good predictor for the diagnosis and prognosis of various diseases (Hasan 2017). Certain trace elements fulfill the functions as cofactors for necessary enzymes and antioxidant compounds, and are notably engaged in humoral and cellular immunological responses (Cannas et al., 2020). Deficit in trace elements is a common observation in autoimmune disorders, this deficiency may result from or contribute to the genesis of autoimmune disorders (Sahebari, Rezaieyazdi, Khodashahi 2019). These elements can function as immunological adjuvants or immunosuppressants. In fact, its deficiency or accumulation can encourage a different course that might lead to the start of disease. For instance, low levels of zinc and high levels of copper, manganese, and iron help to trigger inflammatory reactions and responses to oxidative stress induced by the reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Mezzaroba et al., 2019). Research has demonstrated that trace elements such as zinc and copper play a crucial role in supporting the optimal function of both the immune system and metabolism (Pedro et al., 2019). Although peroxidative damage is believed to contribute to autoimmune diseases, there has been limited studies investigating the association between trace elements and disease activity in SLE (Sahebari et al., 2014). Also, the pathophysiology of SLE may potentially be influenced by toxic metals and trace elements, according to some studies (Kamen 2014). On the other hand, a number of biological processes are controlled by the essential element Calcium. Ca signals in the immune system are crucial for a number of cellular processes, including proliferation, differentiation, apoptosis, and various gene transcriptions (Park et al., 2020). Signaling through intracellular Ca^{2+} has been associated in the congenital immunodeficiencies and pathogenesis of autoimmune (Izquierdo et al., 2014). Studies have revealed a negative correlation between the level of serum total calcium and the severity of SLE (Sha et al., 2020). The study aims to evaluate the role of some autoAbs (ANA, ds-DNA), trace elements (Zn, Cu, Al), and essential element (Ca) in pathogenesis of SLE early diagnosed patients and treated patients.

Material and Method

Subject collection

The current study was conducted in the Department of Rheumatology at Al-Yarmouk Teaching Hospital and the Medical City (Baghdad Teaching Hospital) in Baghdad between March 2022 and August 2022. The study comprised a total of 180 females aged between 20 to 45 years. Specifically, it involved 60 females patients as early diagnosed SLE (without treatment) represented as G1, 60 females patients with SLE who under treatment with (hydroxychloroquine, prednisolone,) represented as G2 , and 60 healthy females as control group, represented as G3. The diagnosis of all patients was based on the revised classification criteria for SLE published by the American College of Rheumatology (ACR) (Hochberg 1997), and confirmed through clinical examination by a rheumatologist. Patients with other disease (Hypertension, cardiovascular disease, diabetes types I, II) or any other chronic disease including autoimmune disease were excluded from the study.

Blood collection

5 ml of a sample of intravenous blood was withdrawn from every female participant patients and control, then centrifuged at 4000 r.p.m for about 10 minutes and kept at -20 °C for subsequent analysis. ELISA technique was used to assess serum level of ANA and ds-DNA, ELISA kits purchased from the German company (Demeditec Diagnositic Gmbh). Flame Atomic absorption specterophotometer (AAS;

nova 300, Analytic Jena, Germany) was used to assess serum level of Zn, Cu, Ca. Furnace Atomic absorption specterophotometer (AAS; Model 210 VGP, Buck Scientific, USA) was used to assess AL. Following the methods of Czupryn et al (1993) and Pfeil (1993).

Statistics

The data were analyzed using SPSS Statistical software ((IBM SPSS 26.0). Used least significant difference (LSD) test by probability of less than 0.05 ($p<0.05$).

Results

The ANA and ds-DNA results revealed that the serum level of ANA was significantly higher in both patients groups (G1 and G2) as compared to G3. The results were (3.13±0.26, 2.37±0.24) U/ml respectively, while control recorded (0.504±0.05 U/ml). The ds-DNA serum level recorded a significantly higher in both patients groups (75.85±7.67, 59.72±8.35) U/ml respectively, as compared to G3 (22.12±1.35 U/ml), ($P<0.05$), as shown in Table 1.

Table 1: Serum levels of ANA and ds-DNA.

Groups	ANA (U/ml) Mean ± SE	ds-DNA (U/ml) Mean ± SE
Early diagnosed patients (G1)	3.13 ± 0.26^{A}	75.85 ± 7.67^{A}
Treated patients (G2)	2.37 ± 0.24^{B}	59.72 ± 8.35^{A}
Control (G3)	0.504 ± 0.05^{C}	22.12 ± 1.35^{B}
P-value	0	0
LSD	0.596	18.4

* Different letters denote to the significant difference at $P<0.05$.

In regard to trace elements (Zn, Cu, AL) and essential element (Ca) serum levels the results showed that Zn and Ca recorded a significant decreasing serum level in both patients groups (G1 and G2) as compared to G3. The results were (72.91±1.22, 7.65±0.09) and (72.86±1.31, 7.71±0.12) mg/dl respectively, while G3 recorded (100.2±2.36, 9.22±0.07) mg/dl, ($P<0.05$). while Cu and AL recorded a significantly increasing in both patients groups (146.91±1.93, 10.87±0.3) and (148.5±1.65, 11.15±0.27) mg/dl respectively as compared to G3 (105.51±2.27, 6.97±0.21) mg/dl, ($P<0.05$). as shown in Table 2.

Table 2. Serum levels of Zn, Cu, AL, Ca.

Groups	Zn (mg/dl) Mean ± SE	Cu (mg/dl) Mean ± SE	AL (mg/dl) Mean ± SE	Ca(mg/dl) Mean ± SE
Early diagnosed patients (G1)	72.91 ± 1.22^{B}	146.91 ± 1.93^{A}	10.87 ± 0.3^{A}	7.65 ± 0.09^{B}
Treated patients (G2)	72.86 ± 1.31^{B}	148.5 ± 1.65^{A}	11.15 ± 0.27^{A}	7.71 ± 0.12^{B}
Control (G3)	100.2 ± 2.36^{A}	105.51 ± 2.27^{B}	6.97 ± 0.21^{B}	9.22 ± 0.07^{A}
P-value	0	0	0	0
LSD	4.80	5.52	0.74	0.276

* Different letters denote to the significant difference at $P<0.05$.

Disscusion

Serum levels of ANA and ds-DNA

ANAs, which are produced in response to different nuclear antigens, are a hallmark of the autoimmune disease SLE (Rekvig 2015). According to evidence, ANA responses can decrease over time as a result of the effects of treatment or the natural history of disease (Pisetsky, Lipsky 2020). This explains why it is lower in treated patients compared to early diagnosed patients. Anti-double-stranded DNA (anti-dsDNA) antibodies are among these ANAs, and they play crucial roles in the pathogenesis of lupus nephritis (LN), a significant cause of morbidity and mortality in SLE. They also act as diagnostic and prognostic markers (Rekvig 2015). To initiate autoimmune diseases, ds-DNA must be present in immunological privilege sites to disrupt the tolerance to autoantigen and trigger the generation of autoantibodies (Bai et al., 2018). Therefore, it is extremely uncommon (less than 0.5%) to find them in healthy individuals and other clinical diseases (Cozzani et al., 2014). Also, the discovery of anti-dsDNA in SLE patients several years prior to the beginning of the disease suggests their involvement towards a clinically overt disease (Arbuckle et al., 2003). This explains why ds-DNA increased in SLE patients compared to healthy control group. The results of this study agree with previous studies done by Kamil, Kadr, Alabassi (2022) and ELAMIR et al. (2019) and Gheita et al.

(2018) who found significant increases in the serum levels ANA and ds-DNA in SLE patients compared to control group.

Serum level of zinc

Zn deficiency is linked to immune system deterioration, and chronic inflammation results from this (Foster, Samman 2012). Furthermore, a deficiency in Zinc has been linked to an increased vulnerability to autoimmune disease and infections due to disordered tolerance. Individuals with autoimmune disorders exhibit low levels of Zn in their serum and plasma, indicating a potential etiological or pathogenic role of Zn in such diseases (Gammoh, Rink 2020). Low Zn level in serum has been detected in female patients with breast cancer (Al-Abassi et al., 2018). In diseases characterized by chronic inflammation a constant recruitment of trace element (Zn) within the cells would be established, leading to a reduction of zinc levels in the serum (Sanna et al., 2018). The decreased levels of Zinc in the blood of SLE patients could be attributed to the elevated consumption of Zinc due to oxidative stress, as Zinc is a cofactor of antioxidant enzymes. Inadequate Zinc levels may also contribute to the impairment of T cell function (Sahebari et al., 2014). Notably, severe Zn deficiency may cause T-lymphocyte dysfunction and thymus atrophy (Ala et al., 2013). Furthermore, Zn plays a regulatory role in the immune system, which has implications in pathologies where there is a deficiency of Zn and inflammation. Samples from people with autoimmune diseases had significantly less Zn than did controls (Sanna et al., 2018). It is advised that SLE patients have a balanced diet rich in minerals like zinc, which typically reduces SLE activity through several immunomodulatory processes (Islam et al., 2020). A zinc-restricted diet determines an elevation of corticosteroid levels in the serum, which may aid in the control of SLE (Brown 2000). The results of this study agree with previous study done by Sanna et al. (2018) and Sahebari et al., (2014) who observed lower serum levels of Zn in patients with SLE disease. The current study disagree with previous study done by Nossent et al. (2017) who who found no change in serum Zn levels between SLE patients and healthy individuals. Also, The current study disagree with study done by Refai et al. (2022) who observed increased blood Zn levels in patients with SLE disease compared to healthy individuals.

Serum level of copper

Cu considers a biological and environmental element, plays a major role in the physiology of the cell, is a cofactor or component of enzymes, involved in antioxidative processes, detoxification of oxygen free radicals. Cu is also responsible for proper cartilage mineralization, elastin, and collagen structure formation (Tapiero, Townsend, Tew 2003). Research has demonstrated that Cu may aid in the preservation of optimal immune system function and metabolism (Pedro 2019). Patients with SLE have been found to have high serum copper concentrations, and these levels are closely correlated with the disease's activity and likely inflammatory response. Cu is believed to have therapeutic benefits for the management of chronic disorders, since its storage in the liver is inadequate to meet the requirements of the inflammatory response (Klack, Bonfa, Borba Neto 2012). This finding could be explained by an increase in hepatic ceruloplasmin synthesis, and the subsequent release into the blood, in response to a higher production of some Interleukins increased in SLE disease, such as IL-1 and IL-6 (Sahebari et al., 2014). The current study agrees with previous study done by Strecker, Mierzecki, Radomska (2013) and Sahebari et al. (2014) who recorded an increased in serum levels of Cu in SLE subjects.

Serum level of aluminum

Immunotoxicity is a side effect of aluminum that can effect the immune system. Autoimmune diseases may develop after prolonged aluminum exposure. Consequently, there is an increase in circulating immune complexes and a decrease in red blood cell immune function, which is associated with a decline in the capacity of circulating immune complexes (Zhu et al., 2012). AL is the main type of adjuvant used in human vaccines (Ruiz et al., 2017). AL, which is found in many vaccines has been reported to induce autoimmune phenomena in people who have a genetic propensity for autoimmunity. For example, more than 40 genes are implicated in the development and etiopathogensis of SLE diasease (Relle et al., 2015). Autoantibody production following HPV vaccination

has been observed in adolescent girls diagnosed with SLE (Soybilgic et al., 2013). There have been reports of SLE or lupus-like syndrome post-vaccination symptoms that range from the production of autoantibodies to clinically significant disease (Orbach, Agmon-Levin, Zandman-Goddard 2010). Agmon-Levin and colleagues demonstrated that mice given the hepatitis B vaccination (HBV) had reduced red blood cell counts and greater levels of the antibody against double-stranded DNA (anti-dsDNA) (Agmon-Levin et al., 2014). Additionally, aluminum adjuvants stimulate the immune system by activating both the innate and adaptive immune systems through a variety of different mechanisms. The NLRP3 inflammasome pathway activation is among the most important (Exley, Siesjö, Eriksson 2010). NLPR3 activation has been shown in SLE patients, greater release of inflammatory cytokines, and significant tissue damage (Oliveira et al., 2021).

Serum level of calcium

In SLE, calcium may maintain the immune system's and metabolism's optimal functioning, according to several studies. Research have revealed a negative correlation between serum total calcium levels and SLE disease activity (Sha et al., 2020). As SLE patients are more likely to experience hypocalcaemic, calcium levels may have a larger impact on the SLE disease process than was previously believed (Watad et al., 2017). Low levels of calcium in the blood are referred to as hypocalcemia. It may result from taking diuretics or other drugs, from medical treatments, or from certain disease processes including hypoparathyroidism or renal failure (Pravina, Sayaji, Avinash 2013). Given that SLE patients frequently used various steroid dosages depending on their disease condition during therapy and that long-term use of glucocorticoids will result in calcium reduction (Tedeschi et al., 2019). Corticosteroids have various impacts on calcium and bone metabolism, including an increase in the natural rate of bone resorption, a decrease in bone formation, a reduction in the amount of calcium absorbed by the intestine, and an increase in calcium excretion through the kidneys. Steroids also directly affect bone tissues to enhance resorption and reduce formation. Moreover, their impact on calcium leads to an indirect elevation in destruction, by stimulating the parathyroid glands to release more parathyroid hormone (PTH) (Buckleyet al., 2017; Panday, Gona, Humphrey 2014). In addition, vitamin D is responsible for maintaining and regulating calcium homeostasis (Veldurthy et al., 2016). According to reports, patients with SLE frequently lack sufficient amounts of vitamin D (Ruiz-Irastorza et al., 2008). It suggests that hypocalcemia possibly enhance the disease activity of SLE patient, which warrants clinical attention (Du et al., 2022). This study agree with study done by Sha et al., (2020) who suggested that SLE patients had lower serum Ca level than that of healthy individuals.

Conclusion

Based on these results, autoantibodies (ANA, ds-DNA) and trace elements (Zn, Cu, AL), essential element (Ca) may plays a role in the pathogenesis of SLE.

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