Assessment of Serum Concentration of Growth Arrest Specific Protein 6 (GAS6) and The Soluble Form of Its Tyrosine Kinase Receptors (sAXL) in Patients with Systemic Lupus Erythematosus

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Abstract: Background: Generally it is known that systemic lupus erythematosus (SLE) is an autoimmune disorder known by over production of different autoantibodies and immune complex development. SLE is affecting many systems, connective-tissue disorder with a broad range of clinical presentations. Therapeutic decisions in SLE are depend on the disease action and nature of organ involved. There are different clinical and laboratory approaches to evaluate the lupus flares. **Aim of the work:** The aim of the present study is to assess serum concentration of growth arrest specific protein 6 (GAS 6) and the soluble form of its tyrosine kinase receptors (sAXL) in SLE patients and whether these biomarkers are enough for assessment of SLE disease activity. **Patients and methods:** This prospective study was performed on 25 SLE patients with active disease, 25 SLE patients with inactive disease and other 25 healthy individuals were included as control. The active SLE patients were subdivided into active SLE patients with nephritis and without nephritis. All candidates were subjected to thorough full history taking, complete clinical examination, and laboratory *investigations* including complement (C3, C4), antinuclear antibody (ANA), anti – double stranded DNA (anti-dsDNA), GAS 6, sAXL and systemic lupus erythematosus disease activity index (SLEDAI) were estimated. The means of serological measurements were then interrelated with SLEDAI score. **Result:** Detailed analysis of the results revealed serum level of GAS 6 and sAXL were elevated significantly in individuals with activity as matched with those without activity and normal ones. Also, The serum level of GAS 6 and sAXL were significantly higher in patients with lupus nephritis. Also, significant positive correlation was founded between both GAS 6 and sAXL with SLEDAI score. Moreover, a negative correlation was observed with C3and C4 levels in our patients. Also, We found that the sensitivity of proportions of GAS6 was 84% and 80% specificity while those of sAXL was 88% sensitivity and 94 % specificity in active patients. So, sAXL is more sensitive than GAS6 in predicting lupus flares. More ever, their values for prediction of lupus nephritis were 86.7% sensitivity and 97.1 % specificity of GAS6 and 80% sensitivity and 91.4 % specificity of sAXL. So, GAS6 is more sensitive than sAXL in predicting lupus nephritis. **Conclusion:** Comparative analysis of serum level of GAS 6 and sAXL in SLE patients revealed a significant association with SLE activity and with active nephritis. Therefore, estimation of serum GAS 6 and sAXL could be an important tool for assessment of SLE activity and prediction of lupus nephritis.

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Keywords: Growth arrest specific protein 6 (GAS 6), Systemic lupus erythematosus (SLE), tyrosine kinase receptors.

1. Introduction

It is known that systemic lupus erythematosus (SLE) is a chronic autoimmune illness and characterized by complex clinical symptoms which affecting vital tissues and organs like the kidney, the brain and blood, in utmost patients, the massive bulk of patients are females of motherhood age, requires labors to advance analytic methods and efficient drugs. (1)

SLE is regarded as a deficiency of the control of the immune system and growth of auto-antibodies leading to the establishment of immune multiplexes. (2) These immune precipitates in the tissues stimulate an immune response by triggering the complement force and inflammatory cells.(3)Even though the actual etiology is not identified, one of the conceivable prompts of this autoimmune response is a deficit in the process of efferocytosis, the clearance of apoptotic cells. (4,5) In case of SLE disease, the ability of macrophage to clear the apoptotic cells from the circulation is abolished, which subsequently may permit the apoptotic cells to attend as immunogens for the generation of autoreactive T and B cells and initiative the manufacture of auto-antibodies. (6)

Macrophages distinguish apoptotic cells via a grouping of surface receptors. (7) Between them, the

Axl, Tyro3 and MerTK (TAM) tyrosine kinases show an exclusively significant part in the phagocytosis of apoptotic cells by dendritic cells and macrophages. [8] It is observed that mice deficient in the three TAM receptors quickly grow lupus-like signs [9-11]. The chief ligands that combine to and stimulate the TAM family of receptors are protein S. and growth arrest-specific 6 (GAS6) [12]

TAM receptors undertake a proteolyticaction in the cell membrane, which consequently increasing the levels of circulating extracellular fragments of the receptors in the plasma (soluble receptors). Therefore, the soluble Axl can be detached from the cell membrane due to proteolytic processes. These soluble formulas have been presented to cooperate with the ligands, changing their regulatory functions. [13,14] Soluble formulas of Axl (sAxl) which composed of the extracellular part of the protein and the ligand Gas6 are found in plasma at little levels. They augment the reaction to acute phase responses. [15]

GAS6 is vitamin K-dependent glycoproteins (VKDPs), It is structurally related to the anticoagulant protein S. [16,17] It is expressed in several tissues, such as vascular smooth muscle cells, bone marrow cells and capillary endothelial cells [18]. Plasma GAS6 level was found to be increased in individuals suffering from septic shock and severe sepsis, severe acute pancreatitis, in response to acute phase reactions and inflammatory diseases without infectious agents. [19,20]

Programmed death cells (Apoptosis) release phosphatidylserine (PS) on the surface of cell, which combined directly to phagocytes through receptors like many soluble proteins, such as the protein S. and TAM receptor ligands Gas6 [21]. In this procedure, both protein S and Gas6 union with the TAM family of tyrosine kinase receptors. The binding of Gas6 to Axl convinces Axl phosphorylation and stimulation of the Phosphoinositide 3-kinase (PI3k), which has anti-apoptotic and pro-survival impact. sAxl and Gas6 have also been demonstrated to be vital for the processes of phagocytosis of apoptotic cells.[22]

Furthermore, the Gas6/TAM system controls an important assortment of procedures, such as cell adhesion and migration, cell survival and blood clot stabilization and proliferation. Gas6 can regulate the inflammatory response by downregulating interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α) and interferon secretion in dendritic cells, Axl and Gas6 are included in stimulating the endothelium as a result to reply to inflammation, rising the leucocyte extravasation and elimination of transplants. [23,24]

For that reason, it is realistic to postulate that one of the TAM receptor families (Axl) and one of its ligands, Gas6 might have a vital role in the pathogenesis of SLE. Therefore, the goal from the current work is to measure levels of Gas6 and its soluble tyrosine kinase receptor sAxl in SLE in the serum of patients compared with normal control and to like these concentrations with SLE disease activity index (SLEDAI) and renal contribution.

2. Patients and Methods

This study was conducted in Rheumatology Unit of Internal Medicine Department of Tanta University Hospitals from December 2014 to December 2015.

The study was carried out on three groups Group I:25 active SLE patients, group II:25 inactive SLE patients and group III: 25 healthy volunteers, age and sex matched as a control group. Moreover, group I patients was be grouped into two additional subgroups: IA - SLE patients with nephritis (lupus nephritis). IB - SLE patients without lupus nephritis. All candidates were subjected to thorough full history taking, complete clinical examination, and laboratory *investigations* including complement (C3, C4), antinuclear antibody (ANA), anti – double stranded DNA (anti-dsDNA), GAS 6, sAXL, systemic lupus erythematosus disease activity index (SLEDAI) were estimated and percutaneous renal biopsy (if indicated). An informed written consent was obtained from all participants in this research after explanation of the benefits and possible risks of the study and how we will overcome these risks. **The study was approved by the Ethics Board of Tanta University.**

3. Results

The demographic data of the twenty five patients of group I showed that 20 (80 %) were female and 5 (20 %) males with a mean (±SD) age of 25.9± 8.3 years. (Table 1) (Figure 18 and figure19) The twenty five patients of group II showed that 22 (88 %) were female and 3 (12%) male with a mean (± SD) age of 31.2± 8.3years and the twenty five patients of group III showed that,17 (68 %) were female and 8 (32%) were male with a mean (± SD) age of 29.3± 8.0 years. (Figure 1 & 2)

Comparison between all the studied groups as regard age and sex showed non-significant values in all analysis.

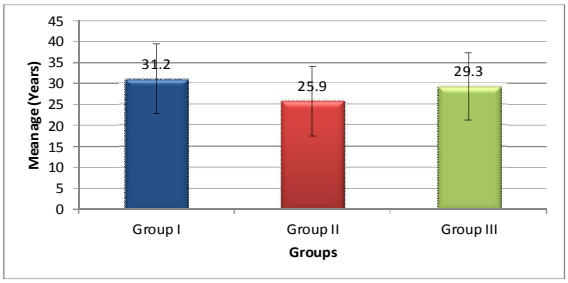

Figure (1): Comparison between the studied groups regarding *age in years*.

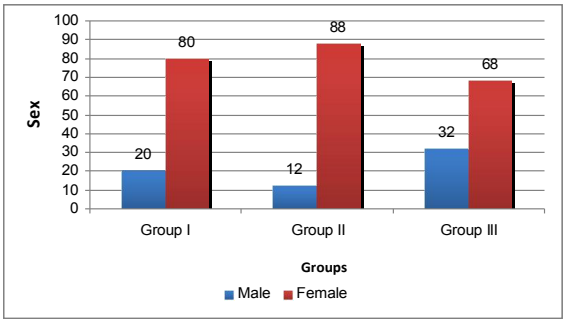

Figure (2): Comparison between the studied groups regarding gender.

In our study we have observed that in group I, the mean of SLEDAI score (± SD) is 21.300 ± 6.416 but in group II, the mean of SLEDAI (± SD) is 2.600 ± 2.836. Comparison between the studied groups showed statistically significant values as SLEDAI in all analysis. (Table 1) (Figure 3)

Table (1): Comparison between studied SLE patients as regard *SLEDAI score.*

		Group			Tests of significance	
		Total (N = 75)	I (N = 25)	II (N = 25)	Statistic	p
SLEDAI Score	Min - Max	0.0 - 96.0	21.0 - 96.0	0.0 - 6.0	Z_{MW}= -6.10	<0.001*
	Median	4.0	43.0	4.0		
	IQR	0.0 - 27.0	27.0 - 63.0	0.0 - 6.0		
	Mean ranks		38.0	13.0		

Z_{KW}: Kruskal-Wallis test; Z_{MW}: Mann-Whitney test;
* P value < 0.05 (significant)

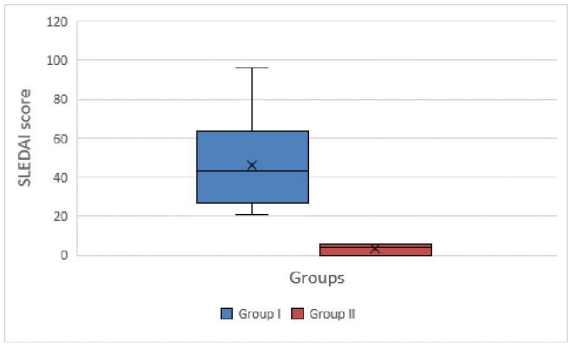

Figure (3): Comparison between studied SLE patients as regard *SLEDAI score.*

There was no statistical difference in ANA level between the studied SLE patients groups (group I & II) with p value 0.200. (table2) (figure4)

The statistical analysis of laboratory findings between the studied SLE patients groups (group I & II) showed significant increase in serum level of Anti ds DNA in group I than group II with p value 0.002 and significant decrease serum levels of C3 & C4 in group I than group II with p value<0.001 & 0.003respectively. (table 2) (figures 5-7)

Table (2): Comparison between the studied SLE patients (group I & II) as regard *serum level of ANA, C3, C4 & Anti ds DNA.*

		Group			Tests of significance	
		Total (N = 50)	I (N = 25)	II (N = 25)	Statistic	p
ANA (U/ml)	Min - Max	2.8 - 89.0	2.8 - 89.0	2.8 - 80.0	Z_{MW}=1.28	0.200
	Median	18.9	8.7	21.0		
	IQR	6.9 - 58.5	5.5 - 60.0	16.0 - 58.5		
	Mean ranks		22.3	27.6		
Anti ds DNA (U/ml)	Min - Max	5.3 - 1549.0	5.3 - 1549.0	17.4 - 512.0	Z_{MW}= -3.11	0.002*
	Median	110.0	146.5	55.0		
	IQR	32.0 - 173.0	104.0 - 415.5	32.0 - 110.0		
	Mean ranks		31.5	18.8		
C3 (mg/dl)	Min - Max	4.0 - 174.0	4.0 - 98.0	81.0 - 174.0	t= -8.17	<0.001*
	Mean	91.9	58.6	122.6		
	SD	42.0	27.5	26.8		
C4 (mg/dl)	Min - Max	3.0 - 78.0	3.0 - 78.0	12.0 - 60.0	Z_{MW}= 2.95	0.003*
	Median	18.0	11.5	22.0		
	IQR	12.0 - 27.1	8.0 - 21.5	16.0 - 32.0		
	Mean ranks		18.9	30.9		

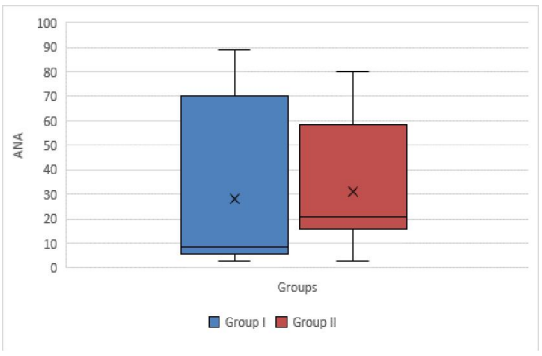

Figure (4): Comparison between the studied SLE patients (group I & II) as regard *serum level of ANA*.

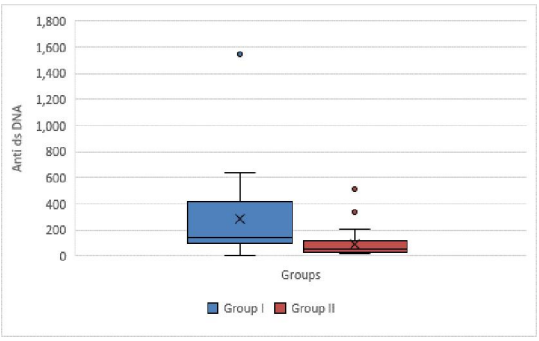

Figure (5): Comparison between the studied SLE patients (group I & II) as regard *serum level of Anti ds DNA*.

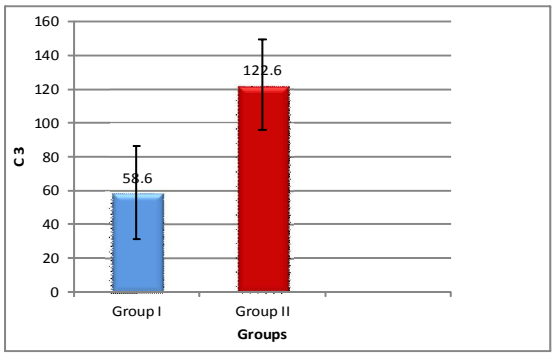

Figure (6): Comparison between the studied SLE patients (group I & II) as regard *serum level of C3*

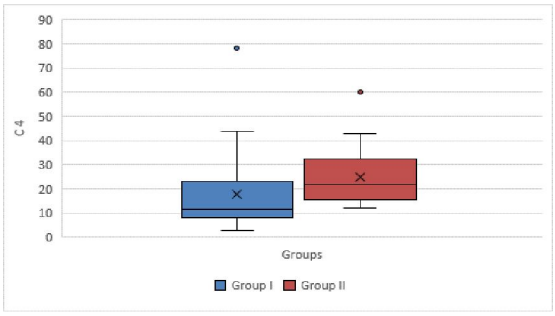

Figure (7): Comparison between the studied SLE patients (group I & II) as regard *serum level of C4*.

The serum levels of GAS6 & sAXL were significantly higher in group I (active patients) than group II & control group with p value <0.001 & <0.001 respectively. (table 3) (Figures 8 & 9).

Table (3): Comparison between the studied groups (I, II & III) as regard *serum level of GAS6 & sAXL*.

		Group				Tests of significance	
		Total (N = 75)	**I (N = 25)**	**II (N = 25)**	**III (N = 25)**	**Statistic**	**p**
S AXL (ng/ml)	**Min - Max**	0.1 - 39.7	4.7 - 39.7	2.3 - 8.0	0.1 - 5.8	Z_{KW}= 52.37	**<0.001***
	Median	4.7	10.4	4.6	2.8		
	IQR	3.2 - 8.0	7.9 - 16.1	3.7 - 6.0	2.1 - 3.6		
	Mean ranks		61.4	35.7	17.0		
GAS 6 (ng/ml)	**Min - Max**	3.6 - 177.1	13.9 - 177.1	8.1 - 26.4	3.6 - 43.0	Z_{KW}= 39.08	**<0.001***
	Median	16.3	39.9	17.5	8.4		
	IQR	10.1 - 24.8	19.3 - 78.9	12.6 - 19.1	6.6 - 13.6		
	Mean ranks		57.5	37.5	19.0		

Z_{KW}: Kruskal-Wallis test; Z_{MW}: Mann-Whitney test.
* significant at p<0.05.

As shown in table (4). There was strong positive correlation between the serum level of GAS6 with serum level of sAXL ($r_{s=}$ 0.545), Anti ds DNA ($r_{s=}$ 0.311) & SLADAI ($r_{s=}$ 0.781) score with p value of <0.001, <0.001 & <0.001 respectively in SLE patients (group I & II) (table 9) (figure 45-47). Also there was negative correlation between the level of GAS6 & serum levels C3($r_{s=}$ -0.625) & C4($r_{s=}$ -0.252) with p value of 0.02 and 0.03 respectively in group I. There was strong positive correlation between the serum level of sAXL with GAS6 ($r_{s=}$ 0.545), Anti ds DNA ($r_{s=}$ 0.228) & SLADAI ($r_{s=}$ 0.774) score with p value of <0.001, <0.001 & <0.001 respectively in SLE patients (group I & II). (table 9) (figure 45,50 & 51). Also there was negative correlation between the serum level of sAXL & serum levels of C3($r_{s=}$ -0.485) & C4($r_{s=}$ -0.305) with p value of 0.02 and 0.03 respectively in group I.

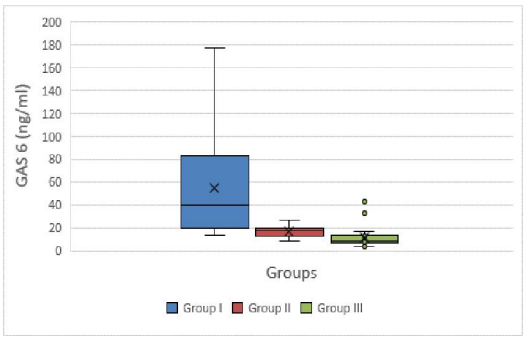

Figure (8): Comparison between the studied groups (I, II & III) as regard *serum level of GAS6.*

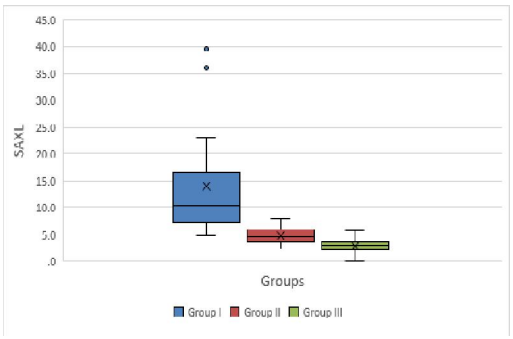

Figure (9): Comparison between the studied groups (I, II & III) as regard serum level of serum sAXL

Table (4): Correlations in the studied SLE patients (group I & II)

(N=50)		S AXL (ng/ml)	GAS 6(ng/ml)
S AXL (ng/ml)	rs	1.000	0.545
	p		**<0.001***
GAS 6 (ng/ml)	rs	0.545	1.000
	p	**<0.001***	
SLEDAI Score	rs	0.781	0.774
	p	**<0.001***	**<0.001***
Anti ds DNA (u/ml)	rs	0.311	0.228
	p	**0.030***	0.116
C3 (mg/dl)	rs	-0.625	-0.485
	p	**<0.001***	**<0.001***
C4 (mg/dl)	rs	-0.252	-0.305
	p	0.080	0.033*

Receiver-operating characteristic (ROC) curve was realistic to attain the utmost sensitive and specific cut off concentration for Gas6 & sAXL with the purpose of distinguish active from inactive SLE patients.

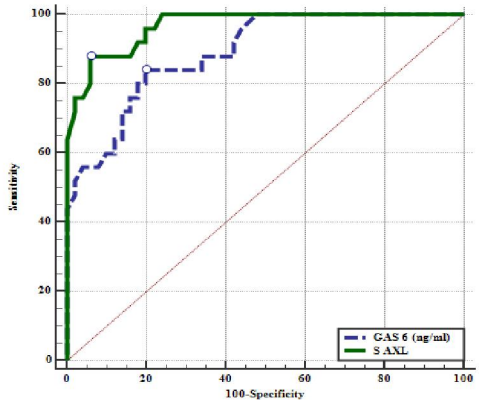

Figure (10): ROC of GAS6 & sAXL for prediction of active SLE

The diagnostic performance of active SLE using ROC curve analysis showing that the best cutoff value of GAS6 was >18.4 with 84%sensitivity and 80% specificity with area under the curve (AUC) was 0.890 while sAXL cutoff value of >6.2 with 88% sensitivity and 94 % specificity with area under the curve (AUC) was 0.968 (. So, sAXL is more sensitive than GAS6 in predicting lupus flares. (table5) (figure10)

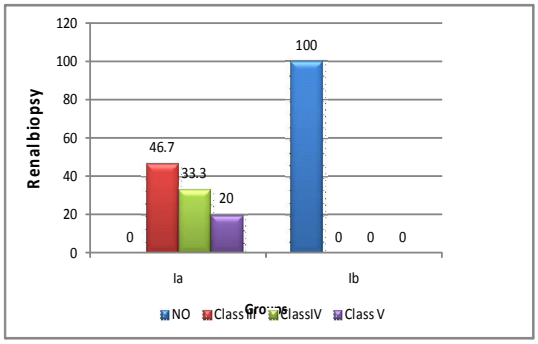

Figure (11): Renal biopsy among the active patients with Lupus Nephritis (Subgroup IA) and without nephritis (subgroup IB).

As showen in figure (11), Renal biopsy was done for SLE patients with nephritis (subgroup IA), and findings were classified according to WHO ISN /RPS

classification showing that the majority of the patients had class III nephritis (7/15) representing 46.7%, 5 patients had class VI nephritis representing 33.3%, and only 3 patients had class V nephritis representing 20%.

Comparison between patients with LN and patients without lupus nephritis regarding the other studied parameters (ANA, Anti DNA, C3 & C4) demonstrating non-significant values in all analysis as shown in table (6).

Table (5): ROC curve show the diagnostic performance of *active disease*.

Variable	AUC	Cut off value	Sensitivity	Specificity
GAS6	0.890	> 18.4	84.0	80.0
SAXL	0.968	>6.2	88.0	94.0

Table (6): Comparison between subgroup IA & subgroup IB as regard *serum level of ANA, AntiDNA, C3 & C4.*

		Subgroups		Tests of significance	
		Ia (no nephritis) (N = 10)	**Ib (nephritis) (N = 15)**	**Statistic**	**p**
ANA (U/ml)	**Min - Max**	3.0 - 89.0	2.8 - 80.0	Z_{MW}= -1.206	0.228
	Median	27.1	8.2		
	IQR	5.5 - 80.0	5.5 - 12.5		
	Mean ranks	14.6	11.0		
Anti ds DNA (U/ml)	**Min - Max**	13.5 - 640.0	5.3 - 1549.0	Z_{MW}= -1.581	0.114
	Median	330.0	122.5		
	IQR	138.0 - 603.0	98.0 - 155.0		
	Mean ranks	15.2	10.6		
C3 (mg/dl)	**Min - Max**	26.0 - 98.0	4.0 - 85.0	t= 1.40	0.177
	Mean	67.5	51.7		
	SD	26.1	27.5		
C4 (mg/dl)	**Min - Max**	3.0 - 44.0	5.0 - 78.0	Z_{MW}= -0.469	0.639
	Median	15.0	10.0		
	IQR	8.1 - 18.0	6.1 - 25.0		
	Mean ranks	13.3	11.9		

The serum level of GAS6, sAXL & SLEDAI score were significantly higher in subgroup **I A** (with lupus nephritis) than subgroup **I B** (without lupus nephritis) with p value <0.001, 0.010 & 0.010 respectively. (table 7) (figures 12-14).

Table (7): Comparison between subgroup IA & subgroup IB as regard *serum level of GAS6, sAXL* & SLEDAI

		Subgroups		Tests of significance	
		Ia (with nephritis) ***(N = 15)***	**Ib** (without nephritis) ***(N = 10)***	**Statistic**	**P**
S AXL (ng/ml)	**Min - Max**	4.7 - 39.7	5.1 - 13.9	Z_{MW}= 2.58	**0.010***
	Median	14.5	7.9		
	IQR	9.6 - 23.2	6.6 - 9.7		
	Mean ranks	16.1	8.4		
GAS 6 (ng/ml)	**Min - Max**	22.3 - 177.1	13.9 - 78.9	Z_{MW}= 3.55	**<0.001***
	Median	59.2	18.8		
	IQR	39.9 - 97.1	14.5 - 19.9		
	Mean ranks	17.3	6.6		
SLEDAI Score	**Min - Max**	33.0 - 96.0	21.0 - 38.0	Z_{MW}= 4.00	**<0.001***
	Median	57.0	26.0		
	IQR	43.0 - 67.0	23.0 - 33.0		
	Mean ranks	17.8	5.8		

Z_{MW}: Mann -Whitney test; * significant at p<0.05.

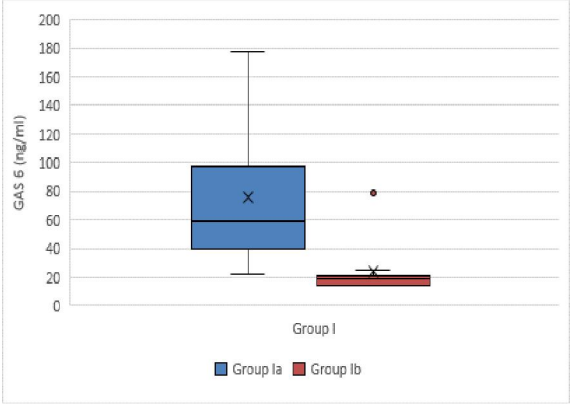

Figure (12): Comparison between subgroup IA & subgroup IB as regard *serum level of GAS6*

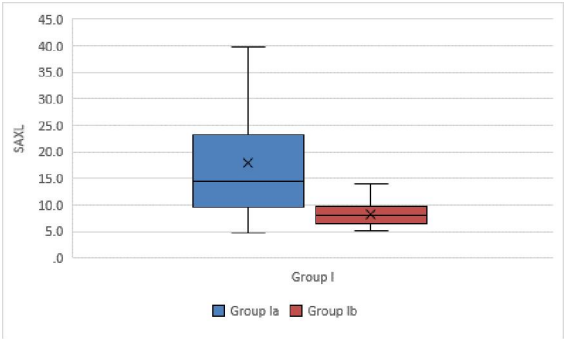

Figure (13): Comparison between subgroup IA & subgroup IB as regard *serum level of sAXL*

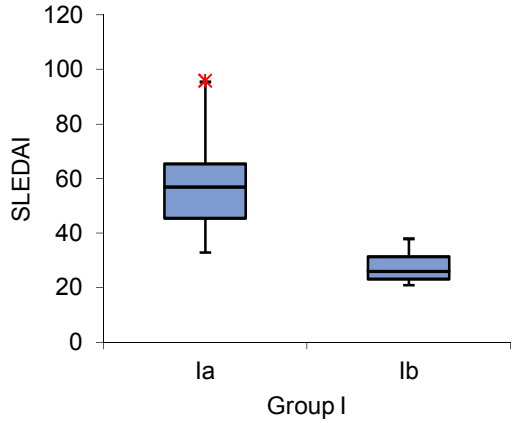

Figure (14): Comparison between subgroup IA & subgroup IB *as regard serum Level of SLEDAI*

As showen in table (8), there was positive correlation between the serum level of GAS6 with serum level of sAXL (r_s=0.235) with p value of 0.258 in subgroup **I A** (with lupus nephritis) (table 17) (figure 74). There was strong positive correlation between the serum level of GAS6 and serum level of sAXL with SLADAI score with p value of <0.001 & 0.048 respectively in subgroup **I A** (with lupus nephritis). There was positive correlation between the serum level of GAS6 and serum level of sAXL with Anti DNA with p value of 0.454 & 0.829 respectively in subgroup **I A** (with lupus nephritis). Also there was negative correlation between the level of GAS6 & sAXL with serum levels of C3 & C4in subgroup **I A** (with lupus nephritis).

Table (8): Correlations in subgroup IA (with lupus nephritis)

		GAS 6	S **AXL**
GAS 6 (ng/ml)	**r_s**		0.235
	p		0.258
	N		15
S AXL (ng/ml)	**r_s**	0.235	
	p	0.258	
	N	15	
SLEDAI Score	**r_s**	0.818	0.400
	p	**<0.001***	**0.048***
	N	15	15
Anti ds DNA (U/ml)	r_s	0.218	0.079
	p	0.454	0.829
	N	15	15
C3 (mg/dl)	**r_s**	- 0.252	- 0.041
	p	0.245	0.894
	N	15	15
C4 (mg/dl)	**r_s**	- 0.165	0.033
	p	0.572	0.880
	N	15	15

The diagnostic performance of GAS6 in subgroup **I A** (with lupus nephritis) using ROC curve analysis showing that the best cutoff value was > 26.4 for discriminating patients with 86.7%sensitivity and 97.1 % specificity with area under the curve (AUC) was 0.962 (95 % CI 0.866 to 0.996) while sAXL cutoff value of > 8.5 with 80% sensitivity and 91.4 % specificity with area under the curve (AUC) was 910 (95 % CI 0.795 to 0.973). So, GAS6 is more sensitive than sAXL in predicting lupus nephritis. (table 9) (figure15)

Table (9): ROC curve show the diagnostic performance of GAS6 & sAXL for *lupus nephritis*.

Variable	AUC	Cut off value	Sensitivity	Specificity
GAS6	**0.962**	**> 26.4**	**86.7**	**97.1**
SAXL	**910**	**> 8.5**	**80.0**	**91.4**

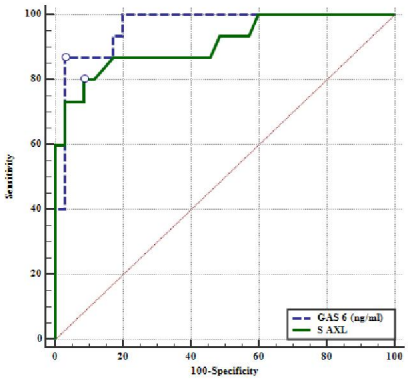

Figure (15): ROC curve show the diagnostic performance of GAS6 & sAXL for lupus nephritis.

4. Discussion

Generally, systemic lupus erythematosus (SLE) is known as an autoimmune disease described by the occurrence of auto-reactive B and T-cells, which are accountable for the abnormal release of a extensive and various group of autoantibodies. In case of SLE disease, the ability of macrophage to clear the apoptotic cells from the circulation is abolished, which subsequently may permit the apoptotic cells to attend as immunogens for the generation of autoreactive T and B cells and initiative the manufacture of auto-antibodies. [25]

The Mer (TAM), Axl and Tyro3 kinases are main controllers of phagocytosis of apoptotic cells and innate immunity. They show an exclusively significant role in the phagocytosis of apoptotic cells by dendritic cells and macrophages. The two ligands, protein S and Gas6, union with TAM, and regulate cell migration and adhesion, cell proliferation and survival, and inflammatory cytokine release. [26, 27]

Furthermore, the Gas6/TAM system controls an important assortment of procedures, such as cell adhesion and migration, cell survival and blood clot stabilization and proliferation. Gas6 can regulate the inflammatory response by downregulating interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α) and interferon secretion in dendritic cells, Axl and Gas6 are included in stimulating the endothelium as a result to reply to inflammation, rising the leucocyte extravasation and elimination of transplants [23].

So, The main goal of this work was to measure serum concentrations of Gas6 and its soluble tyrosine kinase receptor sAxl in SLE patients compared with normal control, as reliable biomarkers for monitoring SLE activity and, prediction of lupus nephritis and correlation of these biomarkers with SLEDAI.

In this study, our results showed that the serum level of GAS6and sAXL were higher significantly in individuals with SLE activity as in comparison with those without activity and normally ones. Also, Their serum levels were elevated significantly in cases with lupus nephritis compared with those not complaining from nephritis. Also, Significant positive correlation was noticed between GAS6 & sAXL with SLEDAI score but a negative correlation was observed with GAS6 & sAXL with C3 & C4 levels in our patients.

In our study, The musculoskeletal signs were more frequent 56%. On the other hand the CNS manifestation were less frequent 10%. The renal affection was about 28%. This data was more or less comparable with that shown previously according to **Alarcón GS et al (2002)** reported in their European cohort study that active nephropathy was 27.9% and was 40% in an American series. [28]

Our study showed that 84% of the total of 50 SLE were women and their age between 18.0 - 49.0 years with mean 25.9 years. Also in agreement with these findings, **CHI CHIU MOK et al (2016,)** reported in their cross-sectional study that a total of 94 SLE patients (98% women, mean age was 28.76 ±9.4 years, mean duration of disease was 5.46 ±5.0 years) and 49 healthy controls were matched. [29]

The relationship between the GAS 6 and SLE disease activity remains controversial. Many studies have been done and some reported that GAS6 reacted with clinical activity and subsequent exacerbations of the disease. Nonetheless, others suggested that such correlations were weak. For some patients, the serum level of GAS6 was a good measurement of disease activity. The current data revealed that both serum Gas6 and sAxl were increased significantly in SLE patients than normally control. Also, we have

observed that the levels of serum GAS6 & sAXL were higher significantly in SLE individuals with activity **(group I)** than those without activity **(group II)**. GAS6 and sAXL levels were correlated positively with antidsDNA antibody and SLEDAI score. In addition, serum GAS6 and sAXL levels were correlated negatively with C3 and C4. Also, the diagnostic performance of active SLE using ROC curve analysis showing that the best cutoff value of GAS6 was >18.4 with 84%sensitivity and 80% specificity with area under the curve (AUC) was 0.890 (95 % CI 0.797 to 0.951) while sAXL cutoff value of >6.2 with 88% sensitivity and 94 % specificity with area under the curve (AUC) was 0.968 (95 % CI 0.898 to 0.995). So, sAXL is more sensitive than GAS6 in predicting lupus flares.

Ekman et al (2011), demonstrated that both sAxl and Gas6 concentrations associated positively with SLEDAI. Additionally, levels of sAxl and Gas6 associated positively with ESR and CRP but inversely with hemoglobin concentration. Likewise, both sAxl and Gas6 levels were higher significantly in subjects with glomerulonephritis anti-DNA antibodies and leucopenia. All previous results proposed that sAxl and Gas6 may carry an important role in lupus pathogenesis. [20]

In agreement with our study, **Hyoun-Ah Kim et al. (2013),** concluded that serum Gas6 levels of SLE patients were significantly higher ($p<0.001$) than those of NC (43.01±28.02 vs. 20.15±9.23 ng/mL,). When estimated sensitivity and specificity of the Gas6 as a diagnostic tool for SLE plotting in ROC curves, the sensitivity and specificity were 72.7 % and 84 %, respectively, with a cut-off value of 25.3 ng/mL. Serum Gas6 concentration was higher significantly in the subjects complaining from renal disorder (65.66 ±32.28 ng/mL) and serositis (70.04±30.85 ng/mL) matched to those free. Gas6 concentrations were connected positively with ESR (r00.204, p0.013), SLEDAI (r00.512, $p<0.001$) and antidsDNA antibody (r00.199, p0.015). Moreover, serum Gas6 concentration was correlated negatively with lymphocyte count (r0−0.165, p00.043), hemoglobin (r0−0.165, p00.043). Additionally, variation in serum Gas6 concentration was interrelated with variation in SLEDAI concentration in the SLE subjects that were monitored up (r00.524, $p<0.001$). The Gas6 concentration was higher in SLE subjects with high disease activity compared with those with inactive disease. It is not known the cause of elevated Gas6 conc. in complex disease like SLE. It may be it considered secondary elevation which may be attributed to inflammatory situations or a causative factor to the pathogenesis of the illness. The present data proposed that serum Gas6 can be a consistent clinical indicator for observing disease activity and response to the therapy in SLE. [30]

CHI CHIU MOK et al. **(2016)** found that the levels of these 4 protein markers, insulin-like growth factor binding protein-2 (IGFBP-2), tumor necrosis factor-alpha receptor (TNFRII) and Axl, ferritin, were increased in active SLE than inactive disease and normal controls, and interrelated with disease activity scores and conventional indicators of disorder, like complement C3 levels and anti-dsDNA titer. These new markers had commonly higher specificity and positive predictive assessment, but somewhat lower in sensitivity, than conventional markers in discovering coexisting SLE activity. Especially, IGFBP-2 and Axl were favorable biomarkers for lupus nephritis, because they had higher specificity in distinguishing active renal disease from active non-renal or inactive SLE disease, than complement C3 and conventional anti-dsDNA. Merging Axl and IGFBP-2 moreim proves the specificity for SLE renal activity without negotiating sensitivity. [31]

Zhu H et al. (2014), noticed a significant increase in plasma sAxl in SLE subjects than normal control. Increased sAxl concentrations were positively interrelated with increased levels of 24-hour proteinuria elimination, anti-dsDNA antibodies and SLEDAI. These results indicated that elevate of sAxl concentrations were accompanying with exacerbation of SLE. [32]

Also in agreement with our findings, **Recarte-Pelz et al. (2013)** demonstrated that plasma Gas6 and all 3 soluble receptor concentrations were elevated in lupus but free protein S was declined. Those factors inter related with SLEDAI scores, and Gas6 was elevated in the greatest severe cases, while free and total protein S were dropped but this relationship is influenced by common polymorphisms in the genes of the system. These studies support the possibility that TAM kinases and Gas6 could be promising biomarkers. [5]

In contrast with our study, other studies have indicated that Gas6 and Axl have no value in predicting lupus flares. The authors did not recorded a significant variation concerning Gas6 concentration between active and inactive disease groups. [32,33] **in addition, Suh CH et al (2010),** did not show the association among Gas6 and activity of disease in SLE cases. [19]

Gheita TA et al (2012) reported in their studies serum level of Gas6 and Axl did not significantly correlate with the SLEDAI. Regarding SLE, the Gas6 was obviously dropped in those with neuropsychiatric manifestations and class 1 lupus nephritis. This reduction in the Gas6 level may implicate its involvement in the apoptotic processes in SLE. The plasma concentrations of Axl and Gas6 were

significantly different in SLE proposing that the Axl receptor detaching is an active process influenced by Gas6-mediated Axl signaling in SLE. Some authors found that the level of Gas6 was significantly decreased and its sAxl receptor elevated than the control group. This drop in Gas6 level was elucidated by its depletion in the apoptotic processes in SLE whereas, the elevation of Axl was elucidated by the incessant prompt of the macrophage pool by apoptotic fragments that may elevate its shedding and expression. [34]

Also, in contrast with our study, other studies further more demonstrated no significant variation in the serum sAxl concentrations among active and inactive SLE individuals. Also, there was no significant association among sAxl and SLEDAI. **Zhu et al. (2014),** The present work demonstrated that Gas6 concentrations in plasma were slightly diminished in SLE subjects than in normal control without significance variation. Moreover, our results exhibited that Gas6 concentrations in plasma were somewhat decreased in SLE cases than in normally subjects without recording a significant variation. [31]

Some researchers showed that Gas6 plasma levels were nearly similar among SLE subjects and harmonized control group. **Suh CH et al. (2010)** showed that Gas6plasma levels were not varied significantly among SLE subjects and healthy control. Conversely, the Gas6 concentrations were risen in individuals suffering from neurologic disease. The obtained data may be influenced by the patients with other concomitant diseases like neurologic disorder. This was illustrated by that practically all Gas6 found in normal individuals is destined by soluble Axl. This may elucidate why there is really little free Gas6 found in both normal control or SLE in the serum of patients in spite of the degree to which their ELISA can identify Axl-bound Gas6 has not been verified. [19]

Bellan et al. **(2016),** demonstrated that normal individuals had only somewhat increase in the level of Gas6 in the plasma compared with diseased subjects (22,8ng/ml [IQR 19,2–24,7] vs 14,7 ng/ml [11,8–20,5]. [275]

In addition, Glomerulonephritis is considered one of the utmost severe expressions of systemic lupus erythematosus, with substantial morbidity and mortality. Mer and Axl are considered from TAM family receptor tyrosine kinases which play an essential role in the conservation of immune homeostasis in the kidney. [35]

The significance of the Gas6/Axl pathway has also been involved in several kinds of kidney disorders. Suppression of Gas6 showed to be valuable in nephritis. [36,37] Two foremost tasks of Axl may impact SLE. 1. Gas6-stimulated Axl in the kidney is essential for renal mesangial cell proliferation, which donates to nephritis.2. Axl signaling stops the manifestation of inflammatory cytokines via the initiation of a transcriptional inhibitor, Twist, in macrophages. [28,37]

The current data illustrated that Gas6 and sAxl levels in the serum were elevated significantly in lupus nephritis (LN) patients (subgroup **I A**) than non LN patients (subgroup **IB)**. Also, Significant positive correlation was noticed between GAS6 & sAXL with SLEDAI score but a negative correlation was observed with GAS6 & sAXL with C3 & C4 levels in LN patients. We also observed, the diagnostic performance of GAS6 in subgroup **IA** (with lupus nephritis) using ROC curve analysis showing that the best cutoff value was > 26.4 for discriminating patients with 86.7%sensitivity and 97.1 % specificity with area under the curve (AUC) was 0.962 (95 % CI 0.866 to 0.996) while sAXL cutoff value of > 8.5 with 80% sensitivity and 91.4 % specificity with area under the curve (AUC) was 910 (95 % CI 0.795 to 0.973). So, GAS6 is more sensitive than sAXL in predicting lupus nephritis

Zizzo G et al (2013), reported in their study that soluble Axl (sAxl) and sMer were concomitant with antiphos-pholipid and antichromatin antibodies, and with renal and hematologic involvement. On the other hand, strong associations with SLEDAI, complement decrease and anti-dsDNA antibody titer were present for only sMer, not for sAxl. [38]

Furthermore, Fiebeler et al. (2004) found that Axl and Gas6 are present in patients complaining from renal disordered and act as signaling molecules and may be probable therapeutic goals. [255]**,** also some authors reported that in cases of lupus nephritis the levels of both Gas6 and sAXL were elevated [20].

Yanagita et al (2002) postulated that Gas6 could be imperative in some inflammations such as glomerulonephritis and nephrotoxic nephritis. They also added that, Gas6 may play a special role in renal pathophysiology particularly in case of SLE. The mode of action of Gas6 in vascular biology may be via activation of receptor tyrosine kinases leading to proliferation in vascular smooth muscle cells, inhibition of leukocytes adhesion to endothelial cells in the presence of chemo-attractants and adhesion and migration which may put in logical evidence for this hypothesis**.** [37]

Fiebeler et al. (2004), demonstrated that Axl and GAS6 signaling is implicated in human renal disorders. The Ang II–induced Axl and Gas6 expression may be dependent on NADPH-oxidase. Therefore, both Axl and Gas6 are considered an important biomarkers in cases of kidney disorders and can be used as a diagnostic tool. [36]

Wu CS et al. (2014). showed that the concentration of Gas6 was elevated in SLE, nephritis

and cutaneous vasculitis patients than to control individuals. [39]

Li Set al (2017), showed that the concentrations of AxlTK and MerTK expression were elevated in LN patients, than to primary nephrotic syndrome (NS), and linked to pathological alteration to some ranges. [40]

Wu T et al (2016), reported that serum AXL level was elevated in subjects with active LN. in spite of it corresponded with renal flares in some SLE observed patients, it possessed only moderest association with concomitantly documented eGFR, serum creatinine, SLEDAI, in addition to renal pathology criteria. [41]

Some authors reported recently that the expression of Axl and Gas6 was up synchronized in rat glomerulonephritis. The suppression of the Axl and Gas6 action by the extracellular domain of Axl or warfarin can supresses the development of diseases. Also in vitro study, Gas6 was observed to endorse mesangial cell proliferation. [37]

Many factors are found to play a significant role in the variations between different studies, which may be one of the following, an intrinsic heterogeneity of the populations in each case, the effect of specific genetic factors on plasma levels of sAxl and Gas6, different populations are seemed to found variations in the genetic allocation of the alleles accountable for Gas6 and sAxl expression. Therefore, this could be reflected in the levels of the proteins under investigation. Definitely, definite Gas6 variants illustrated extensive variations among inhabitants from Asia and Europe. [42,43]

In the current work, there are many possible illustrations for the inconsistency between these researches:

1-The difference of various studied patients like age, race, clinical symptoms and laboratory testes, and further complete multicenter studies would help to resolve this crisis.

2-The dissimilar treatment programs may persuade the circulating level of Gas6.

3- The intense heterogeneity of SLE.

4- The size of patient sample, may influence the precision, where the small sample volume may not exactly symbolize the larger number of population, and additional studies by using large sample volume are thus required to prove the accurate roles of Gas6/TAM system in SLE

5- The GAS6/ProS-TAM system can be subjective by common polymorphisms in the genes of the system Encouragingly, many advanced researches in human being assumed that the genetic relationship may found among the Gas6/TAM system and SLE in diverse populations. [44]

Conclusion

Obvious elevation in the rate of CD4+CD25-Foxp3+ T cells in individuals with renal diseases and in patients with more proliferative lupus nephritis hold the suggestion that extent of CD4+CD25-Foxp3+ T cells may have a pathogenic role linked with this exacting organ symptoms and may be used as a method to pass up non-required kidney biopsies and/or intensive therapy.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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2/4/2019