Evaluation of HE4 and IGFBPs as Novel Biomarkers of Systemic Lupus Erythematosus with Lupus Nephritis

NoorAlhuda Kh. Ibrahim[1], Kadhim K. Ghudhaib[2], Ali Abdulmajid Dyab Allawi [3], Faten Abdulghani Hammoudi [4]

[1]Post graduate in Department of Chemistry, College of Science for Women, University of Baghdad, Iraq, [2]Assistant professor in Department of Chemistry, College of Science for Women, University of Baghdad, Iraq, [3]Professor, FRCP London, consultant transplant nephrologist in Baghdad College of Medicine, University of Baghdad- Iraq, [4]Specialist in diagnostic laboratory, Baghdad- Iraq

Abstract

Background: many organs of the body such as kidneys, skin, joints, nervous system, blood cells, blood vessels and serous membranes can be affected by systemic lupus erythematosus (SLE). A wide range of this disease effects may be attributed to its behavior as an autoimmune disease; this means, numerous complications of SLE with many organs can be results. In this study, levels of serum human epdidymis secretory protein4 (HE4) and insulin like growth factor binding protein-2 (IGFBP-2) for Iraqi patients in SLE with and without lupus nephritis (LN) were investigated to knowledge their ability to be useful markers for identification of kidney diseases, like lupus nephritis and chronic kidney disease in patients who suffering from SLE.

Methods: one hundred twenty subjects from both sexes were enrolled in this study. They have been classified into two patients groups together with the control group. Forty patients of SLE with LN (8male-32female) with age range (19-44) years, represent the first patients group, the second patients group includes forty patients suffer from SLE without LN at (16-45) years age range and the control group which consists of 40 healthy subjects with age range (19-62) years. HE4 and IGFBP were estimated by ELISA method.

Results: results show high level of HE4 and IGFBP in both patient groups with SLE in comparison with control group. At the same time, results confirmed a high positive correlation between HE4 and IGFBP ($r = 0.85$, $p<0.01$), ROC analysis data revealed that HE4 is the best parameters for predicting development lupus nephritis as a main complication of SLE disease.

Conclusion: both patient groups revealed a significantly increase in levels of HE4 and IGFBP compared with control group. High positive correlation coefficient between HE4 and IGFBP is obtained. Data of ROC analysis confirmed that HE4 represents a better biomarker for diagnosis of LN in patients with SLE disease.

Keywords: *SLE, LN, HE4, IGFBP-2, CKD*

Introduction

Systemic lupus erythematosus (SLE) complications include many organs; lupus nephritis represents the major one of these complications that consists about 50% of patients with SLE disease [(1)]. Previous studies were referred to the association between hyperglycemia and nephropathy [(2, 3)], neuropathy [(4, 5)], osteoporosis [(6, 7)] and the treatment by plant extracts [(8, 9)]. In spite of the early diagnosis and fast treatment of renal disease course, which attributed to the extensive of treatment, it is very necessary to determine a suitable bio marker to predict lupus nephritis in patients with SLE at early time [(10, 11)].

The identification and characterization of HE4 was conducted in previous study [(12)], it is widely detected in different organs that include salivary glands, kidney, respiratory tract and nasopharynx [(13-15)], while numerous studies were reported that HE4 coordinated

or no coordinated with the tumor marker (cancer antigen 125) to follow the development of ovarian cancer [16-17]. Lately, considerable augmentation of HE4 level was found in different kidney disorders [18-19].

Proteins that binding with insulin growth factor, which are abbreviated as (IGFBPs) form a high affinity secreted proteins group that serves for transport and regulating bioavailability and function of IGFs. Family of IGFBPs consists of six types of IGFBP, specifically IGFBP1 - IGFBP6. On the other hand, some proteins with little binding attraction to IGFs were mistakenly named as IGFBP7, IGFBP8 and IGFBP9 [20-21]. Depending on the protein composition conservation and the rising binding similarity, just the six types of IGFBPs are true binding proteins to the IGFs. Accordingly, in the case of cancer status "through IGF-independent pathways'' and autoimmune diseases "via IGF dependent and IGF-independent pathologies" IGFBPs participate in development of SLE complications disorders [22-23].

This study aimed to estimate of HE4 and IGFBPs levels in sera of patients with SLE disease and its complication of LN, to knowledge their ability, whether be useful as biomarkers that can applied for early predicting for this complicated disease.

Materials and Methods

One hundred twenty persons from both genders were enrolled in this study, who attended to Baghdad Teaching Hospital/Medical City, Iraq, for a period (December 2018 to May 2019). They are classified into patients group and control group. Patients group is subdivided into two groups: first patients group include 40 patients who suffering from SLE with LN (8male-32female) with age scope (19-44) years, second patients group consist of 40 patients in SLE without LN with age variety (16-45) and 40 hygienic subjects as control group with age range (19-62) years. Screening markers that include dsDNA and ANA together with C3 and C4 were used in clinical diagnosis for patients group without LN, whereas patients with renal nephritis analyzed by biopsy. Venous blood (5 ml) was drawn from each subject into a suitable syringe at the period of 8.30-11 AM. An adequate amount (3 ml) was transferred into sterile tubes. Three ml of each sample transferred to sterile plain tubes and permitted to coagulate at room temperature, then centrifuged for 15 min at 3000 RPM. The obtained serum were divided into numerous aliquots and directly frozen at -20 °c until tested. The remaining amount (2 ml) of the blood specimens were transferred into EDTA tubes for hematological analysis. HE4 and IGFBP analyzed by ELISA kit using sandwich method.

Statistical Analysis

Statistical programs include ANOVA, LSD, ROC, and Chi square were applied in this study to distinguish of results differences factors, probability, correlation coefficient and the best biochemical factors that can be represent a better biomarker for early prediction of the studied disorders[24] .

Results

Values of several factors that include age, BMI, duration of disease, urea, creatinine, total protein, Albumin C3, C4, ANA, Anti ds DNA, ESR and h-CRP for all the studied groups were recorded in table (1). The results exhibit a significance differences in age between patients and healthy groups ($p<0.01$), a significant increase was shown in body mass index (BMI), blood urea (BU), creatinine (Cr) , ANA, Anti dsDNA, ESR and h-CRP in patient groups (SLE with LN ,SLE without LN) in comparison with control group. Whereas, a significant decrease was found in total protein, Albumin C3, C4 in patient groups than healthy subjects.

Table (1): Demographic and clinical characteristics

Factors	Mean ± SE			LSD value
	SLE without nephrites	SLE with nephrites	Control	
Age (year)	29.37 ± 1.25 b	31.05 ± 1.33 b	41.02 ± 2.01 a	4.400 **

Cont... **Table (1): Demographic and clinical characteristics**

BMI (kg/m2)	28.54 ± 0.49 a	29.56 ± 0.61 a	26.94 ± 0.40 b	1.438 **
Duration (year)	3.63 ± 0.19 b	6.11 ± 0.26 a	---	0.657 **
Urea (mg/dl)	31.08 ± 0.63 b	57.98 ± 1.06 a	23.47 ± 0.88 c	2.45 **
Total protein (mg/dl)	6.66 ± 0.08 b	6.50 ± 0.08 b	7.05 ± 0.17 a	0.348 **
Creatinine (mg/dl)	0.887 ± 0.03 b	1.785 ± 0.06 a	0.815 ± 0.04 b	0.139 **
Albumin (mg/dl)	3.55 ± 0.07 b	2.96 ± 0.34 b	4.21 ± 0.08 a	0.592 **
C3 (mg/dl)	90.81 ± 1.38 b	80.55 ± 1.33 c	161.56 ±5.13 a	8.86 **
C4 (mg/dl)	23.23 ± 0.46 b	25.03 ± 0.78 b	47.96 ± 3.10 a	5.23 **
AnitdsDNA (IU/ml)	60.23 ± 1.39 b	75.48 ± 2.37 a	10.66 ± 0.90 c	4.68 **
ANA (IU/ml)	7.45 ± 0.34 b	19.01 ± 1.07 a	1.264 ± 0.80 c	2.24 **
h-CRP 9(mg/l)	9.32 ± 0.22 b	15.14 ± 0.60 a	1.800 ± 0.15 c	1.077 **
ESR (mm/h)	47.09 ± 0.85 b	57.84 ± 1.22 a	4.88 ± 0.21 c	2.44 **
** (P<0.01). differed in significant values of the measured factors in this table are represented by different letters in the same row				

Table 2 shows mean± SE values of HE4 and IGFBP-2. The results referred to present a significant increase in these parameters within patient groups (SLE with LN and SLE without LN) than control group.

Table -2: Mean ± SE of HE4 and IGFBP-2 levels in all the studied groups

parameters	**Mean ± SE**			**LSD value**
	SLE without nephritis	**SLE without nephritis**	**control**	
HE4 (pmol/l)	5.34 ± 0.30 b	24.51 ± 2.63 a	3.94 ± 0.87 b	4.52 **
IGFBP-2 (ng/ml)	5.34 ± 0.41 b	39.08 ± 3.22 a	1.17 ± 0.12 b	5.26 **
** (P<0.01). differed in significant values of the measured factors in this table are represented by different letters in the same row				

The correlation between HE4 and IGFBP-2 was conducted. The results revealed very strong correlation coefficient (0.85) between HE4 and IGFBP-2 as shown in figure 1.

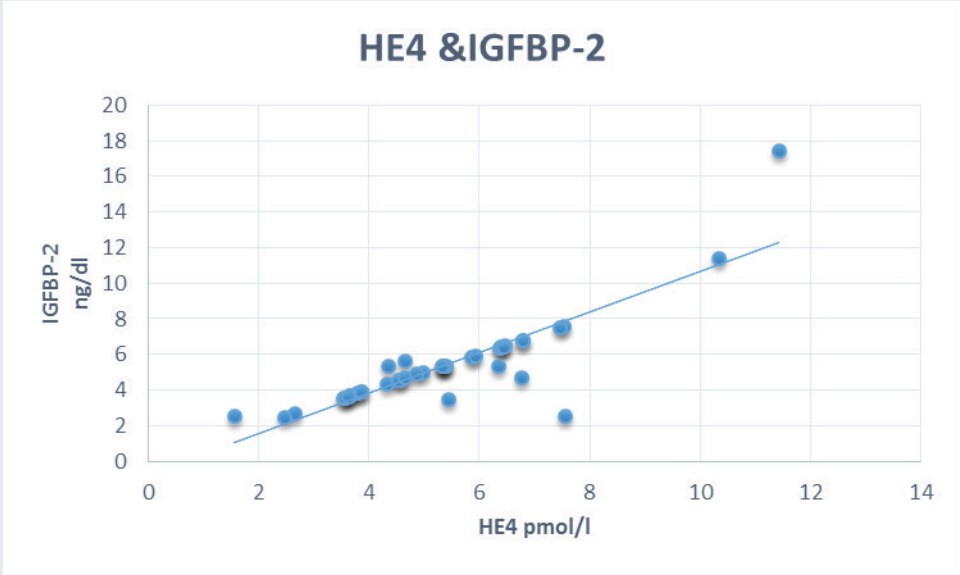

Figure (1): The correlation between HE4and IGFBP-2

Correlation between each of HE4and IGFBP-2 and other parameters in this study was studied. Correlation study was included two cases, SLE with nephritis and without nephritis. The results confirmed that no correlation occurred between these parameters as shown in tables 3.

Table 3 shows correlation between HE4, IGFBP-2 in SLE patients group without nephrites and other relevant biochemical parameters.

parameters	HE4		IGFBP-2	
	r	p	r	p
Age	-0.26	0.103	-0.27	0.086
BMI	0.03	0.810	-0.08	0.585
Creatinine	-0.21	0.188	-0.17	0.283
Total protein	-0.13	0.393	-0.03	0.847
Albumin	0.07	0.661	0.08	0.595
h-CRP	-0.06	0.668	-0.04	0.798
ESR	-0.17	0.283	-0.09	0.547
C3	-0.06	0.681	-0.04	0.771
C4	-0.04	0.780	-0.01	0.968
Anti ds DNA	0.23	0.139	0.14	0.360
ANA	0.05	0.741	-0.01	0.930
* (P: 0.01-0.05), ** (P: less than 0.01), NS (P: More than 0.05).				

ROC analysis has been applied in this study; the analysis reveals that both HE4 and IGFBP-2 are increased significantly. The level of HE4 considers a strong parameter to diagnose SLE (P<0.0001). Also, the results of ROC analysis showed that both sensitivity (98.7%) and specificity (97.5%) recorded at a value of ≤ 2.41(pmol/l).

Consequently, the test value below 2.41 (pmol/l) considers abnormal case (disease condition) while, the value above 2.41(pmol/l) represents the healthy condition. As shown in figure (2).

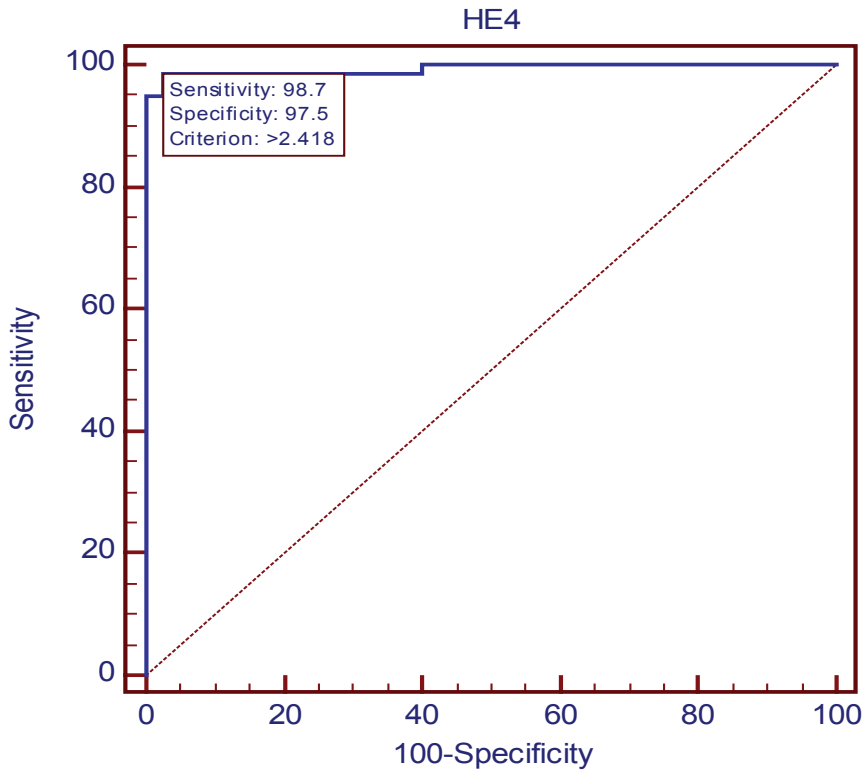

Figure (2) shows the ROC curve data for HE4

ROC analysis for IGFBP-2 also drawn, the AUC was found to be (0.994). The best cut-off point derived from the ROC curve shows a sensitivity of (93.7%) and specificity of (100.0%), it is found to be of≤2.81(ng/ml). That means, the test value less than 2.81(ng/ml) represents the abnormal case (disorders condition), whereas the value more than 2.81 (ng/ml) refers to the healthy case, the significance level is obtained at (P<0.001).

Discussion

The investigation of association of both HE4 and IGFBP-2 with SLE complications like LN patients considers the first attempt that conducted for Iraqi patients. HE4 and IGFBP-2 levels were found to be increased in disease case than healthy condition. On the other hand, all the screen factors that mentioned in this study have been useful to follow the development the complications for this type of diseases, but they represent a modest predictive value [25].

Lupus nephritis identification can be conducted by invasive test. This test is not accepted by patients. Moreover this invasive test considers unreliable test due to the global kidney status. Thus, noninvasive biomarker that can early discriminate LN before kidney injure in the case of SLE disease. Consequently, HE4 level in lupus nephritis is found to be higher than in patients without LN, and higher than control. The mechanism of HE4 elevation is not clear yet. The suggested mechanism in previous study and our study can be summarized on the basis of the dysfunction of kidney may let to escape HE4 out of kidney which leads to elevation of its level in

blood especially in the fibrosis case. Thus, the elevation of HE4 and renal disorder may be an interactive loop [26].

In spite of approved HE4 to follow the growth of ovarian cancer, several attempts were conducted to apply its medical values to in other diseases as reported in previous studies [27].

It was reported that IGF-1 IGFBP4 and IGFBP6 involved in tumor growth and some other disease such as SLE and LN. In other studies IGFBP2 was investigated in SLE disease, the studies referred to that IGFBP2 as a best predictor for kidney histopathology and biological changes in lupus nephritis[28].

Acknowledgment : Our thanks and appreciation to the staff of the Baghdad Teaching Hospital in the Medical City, Baghdad for their supports in this work, and the staff of the Graduate Studies Division in College of Science for Women, University of Baghdad for their facilities in this study.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Seshan, SV, Jennette, JC. Renal disease in systemic lupus erythematosus with emphasis on classification of lupus glomerulonephritis advances and implications. *Arch Pathol Lab Med* 2009; 133: 233– 248.
2. Sabreen A. M., Kadhim K. G. and Ali A. D. Evaluation of pentraxin3 level in Iraqi patients with diabetic nephropathy. Biochemical & Cel. Arch. 2018; 18; 2; 2473-2477.
3. Sabreen A. M., Ali A. D.and Kadhim K. G. Determination of TNF1 level and its association with ACR and eGFR in Iraqi patients with different stages of diabetic nephropathy. J. of Global Pharma Technology| 2018; 10:05:449-454.
4. Kadhim K. G., Firas Y. M., Hind S. Mohammed. Evaluation of Antioxidant Levels in Blood Sera of Iraqi Patients with T2DM. Journal of global pharma technology 2018; 10:3: 895-899.
5. Hind S. M., Kadhim K. G., Firas Y. M. Estimation of enzymatic and non-enzymatic antioxidants in sera of Iraqi patients with sensory neuropathy. Biochemical and Cellular Archives.2018;18: 1, 1011-1016.
6. Kadhim K. G., Kismat M.T. and Sabreen A. Muza**l.** Estimation of Serum Osteocalcin Levels in Osteoporotic Postmenopausal Women with Type 2 Diabetes Mellitus. Baghdad Sci. J. 2014:11 (4):1549-1555.
7. Kadhim K. G., Kismat M. T., Sabreen A. M., Gaze T. A. and Mohammed H. A. Association of Serum Adiponectin with Serum Insulin and Body mass density (BMD) in postmenopausal Osteoporotic women with and without T2DM. IOSR. Journal of Applied Chemistry (IOSR-JAC). ;2014:7; 5 : 119-122
8. Kadhim K. Ghudhaib. Effect of alcoholic catechin extract on hyperglycemia, hyperlipidemia and liver functions in alloxan –diabetic mice. Baghdad Sci. J. 2014;11 :3: 1192-1200.
9. Kadhim K. G. Evaluation of Ginger Rhizomes Extract Effect on Glucose Level, Lipid Profile and Liver Function in Induced Alloxan Diabetic Mice. Research Journal of Pharmaceutical, Biological and Chemical Sciences.2018; 9:3:435-441.
10. Korbet, SM, Lewis EJ, Schwartz, MM, et al. Factors predictive of outcome in severe lupus nephritis. *Am J Kidney Dis* 2000. 35: 904– 14.
11. Esdaile, JM, Joseph, L, Mackenzie, T, et al. The benefit of early treatment with immunosuppressive agents in lupus nephritis. *J Rheumatol* 1994; 21: 2046– 51.
12. Kirchhoff, C, Habben, I, Ivell, R, et al. A major human epididymisspecific cDNA encodes a protein with sequence homology to extracellular proteinase inhibitors. *Biol Reprod* .1991; 45: 350– 357.
13. Wasnaa J. Mohammed1; Basim Sh.; Nizar Ab. Jasim; Mohammed Sh. Jebur. Validity of CD4 enhancer gene polymorphism in susceptibility and severity of some Iraqi rheumatoid arthritis patients. International Journal of Advanced Research, 2016, 3:3:1200-1205.
14. Bingle L, Cross SS, High, AS, et al. WFDC2 (HE4): A potential role in the innate immunity of the oral cavity and respiratory tract and the development of adenocarcinomas of the lung. *Respir Res*. 2006: 7:

61.

15. Moore, RG, McMeekin, DS, Brown, AK, et al. A novel multiple marker bioassay utilizing HE4 and CA125 for the prediction of ovarian cancer in patients with a pelvic mass. *Gynecol Oncol* .2009; 112: 40– 46.

16. Andersen, MR, Goff, BA, Lowe, KA, et al. Use of a symptom index, CA125, and HE4 to predict ovarian cancer. *Gynecol Oncol*(2010); 116: 378–383.

17. Nolen, B, Velikokhatnaya, L, Marrangoni, A, et al. Serum biomarker panels for the discrimination of benign from malignant cases in patients with an adnexal mass. *Gynecol Oncol*. 2010; 117: 440–445.

18. Huhtinen, K, Suvitie, P, Hiissa, J, et al. Serum HE4 concentration differentiates malignant ovarian tumours from ovarian endometriotic cysts. *Br J Cancer* .2009; 100: 1315– 1319

19. Anastasi, E, Marchei, GG, Viggiani, V, et al. HE4: A new potential early biomarker for the recurrence of ovarian cancer. *Tumour Biol* .2010; 31: 113–119.

20. Wang, J, Gao, J, Yao, H, et al. Diagnostic accuracy of serum HE4, CA125 and ROMA in patients with ovarian cancer: a meta-analysis. *Tumour Biol*. 2014; 35: 6127– 6138

21. Romeo, V, Framarino Dei Malatesta, M, Nudo, F, et al. Is HE4 serum level a valid screening test in women candidates for kidney transplant? A case report and a review of literature.*Clin Ter* ,2014; 165: 162– 165.

22. Jr, Nagy B, Krasznai, ZT, Balla, H, et al. Elevated human epididymis protein 4 concentrations in chronic kidney disease. *Ann Clin Biochem* .2012; 49: 377– 380

23. Boer, RA, Cao, Q, Postmus, D, et al. The WAP four□disulfide core domain protein HE4: a novel biomarker for heart failure. *JACC Heart Fail*. 2013; 1: 164– 169.

24. SAS. Statistical Analysis System, User's Guide. Statistical. Version9.1th ed. SAS. Inst. Inc.(2012) Cary. N.C. USA.

25. Kim H-S, Nagalla SR, Oh Y, Wilson E, Roberts CTJ, Rosenfeld RG. Identification of a family of low-affinity insulin-like growth factor binding proteins (IGFBPs): characterization of connective tissue growth factor as a member of the IGFBP superfamily. Proc Natl Acad Sci USA. 1997; 94:12981–6. 10.1073/pnas.94.24.12981

26. Holbourn KP, Acharya KR, Perbal B. The CCN family of proteins: structure-function relationships. Trends Biochem Sci. 2008; 33:461–73.

27. Baxter RC. IGF binding proteins in cancer: mechanistic and clinical insights. Nat Rev Cancer .2014; 14:329–41.

28. Escudero, JM, Auge, JM, Filella, X, et al. Comparison of serum human epididymis protein 4 with cancer antigen 125 as a tumor marker in patients with malignant and nonmalignant diseases. *Clin Chem* .2011: 57: 1534– 1544