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The Role of Collagen IV and Neopterin in Iraqi Patients with Cushing's Disease

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ABSTRACT

Objective: The aim of the present study is to investigate the relationship between the biochemical parameters (collagen IV and neopterin) and the etiology of Cushing's disease in Iraqi patients and to examine the biochemical action of the treatment (prednisolone and hydrocortisone) on the biochemical parameters (cortisol, ACTH, collagen IV, and neopterin).

Methods: The patients were classified into four groups: G3 composed of (25) newly diagnosed males with Cushing's disease / without treatment, G4 composed of (25) newly diagnosed females with Cushing's disease / without treatment, G5 composed of (25) males with Cushing's disease / under treatment with prednisolone and hydrocortisone and G6 composed of (25) females with Cushing's disease / under treatment with prednisolone, Cushing's patient were compared with healthy subjects with approximately the same range of age-matched with the patients and regarded as two control group: G1 composed of (25) healthy males and (G2) composed of (25) healthy females. The levels of all parameters were determined in sera of both patients and control groups.

Results: Statistically, the level of cortisol and neopterin were highly significantly increased, ACTH level was significantly increased and collagen IV was highly significantly decreased in G_3 and G_4 compared with G_1 and G_2 respectively, the levels of cortisol and neopterin were decreased (non-significantly for males and significantly for females regarding cortisol, highly significant for males and significantly for females regarding neopterin) in G_5 and G_6 compared with G_3 and G_4 respectively while the same treatment caused a side effect on both ACTH and collagen IV. Cortisol level was a highly significant increase in newly diagnosed females compared with males while the difference was non-significant regarding ACTH, Collagen IV, and neopterin.

Conclusion: The current study submits novel findings by elucidating that collagen IV and neopterin show promise as novel biochemical markers for CD. Also, it highlights the reactive action of prednisolone and hydrocortisone in shifting cortisol and neopterin into the balance and the side effects and non-significant effects of these drugs on ACTH and collagen IV respectively.

Keywords: Cushing's disease, cortisol, ACTH, collagen IV, neopterin

INTRODUCTION

Cushing's syndrome (CS) is a physiological defect characterized by higher levels of cortisol in the blood due to not only exogenous steroids as a treatment but also endogenous overproduction of cortisol, this complicated disorder ranges between 2-8 million people yearly[(1)]. A higher level of cortisol is regarded as a risk factor for several diseases such as Cardiovascular Disease, diabetes mellitus, and high BP [(2)]. Cushing's disease (CD) is a specific and exclusive type of CS reflected by ACTH overproduction. Hence ACTH dependent CS is caused by excessive production of ACTH by a corticotropic cell of the Pituitary gland, this physiological and biochemical

condition is regarded as Cushing's disease (CD). On the other hand, other types of Cushing's syndrome are caused by non-pituitary tumors which in turn lead to excessive cortisol production[3]. Cortisol is a steroid hormone produced by the adrenal gland[4,5]. Higher levels of cortisol in human blood can be used as a biochemical marker for acute[6] and chronic stress-related mental and physiological disorders, neurodegenerative diseases, and mood disorders [1,7]. ACTH is the pituitary hormone essential for the normal and vital functions of the adrenal glands, cortisol biosynthesis, and physiological state. Interestingly, it is highlighted in a number of endocrine disorders such as Cushing's disease because of its diagnostic power[8]. Several laboratory abnormalities are included to predict Cushing's disease such as decreased lymphocytes, eosinophils, monocytes and basophils, hypokalemia, and elevated liver enzymes [1]. Collagen is a fibrous protein making up approximately 25 percent of total human proteins[9]. In particular, Collagen IV is the key component of basement membranes, a specific form of extracellular matrix[10] collagen that enables the genesis and evolution of multicellular epithelial tissues[11]. Neopterin (Np) is a biochemical marker of cell-mediated immune response. It has a systematic name 2-amino-4-hydroxy-6-(D-erythro-1,2,3-trihydroxypropyl)-pteridine, this marker belongs to the class of pteridines. It has been commonly studied in several inflammatory diseases, including viral, bacterial, and parasite infections, cardiovascular diseases, autoimmune diseases, and malignant tumors[12]. Remarkably, neopterin is an inflammation biochemical marker and is regarded as an early and key indicator marker of the cellular immune response. Neopterin has a low molecular weight and is known as a reactive metabolite of guanosine triphosphates synthesized by triggered dendritic cells and macrophages after activation by gamma interferons, consequently, it is regarded as a biochemical parameter to monitor diseases linked with immunological defects[13,14]. Prednisolone and hydrocortisone are synthetic glucocorticoids used widely and commonly to treat Cushing's disease [15,16]. Collagen IV and Neopterin were selected in the present study to emphasize their potential clinical relevance beyond their immune or CNS functions.

MATERIALS AND METHODS

Study design and patients' selection

One hundred 100 Cushing's disease patients were enrolled in the present study; each one attended the abdominal division of either Baghdad Teaching Hospital (the medical city) or Al-Yarmouk Teaching Hospital in Baghdad. The ethical approval number is No. 33 on January 1st, 2024, and the body name is Baghdad Medical City Center of training for verification is they were diagnosed by both C.T scan and MRI as Cushing's disease patients (CT or MRI scans can take pictures of the pituitary and adrenal glands to see if anything shows up, such as tumors. Remarkably, those patients (30-64) years were classified into four groups: G3 composed of (25) newly diagnosed males with Cushing's disease / without treatment, G4 composed of (25) newly diagnosed females with Cushing's disease / without treatment, G5 composed of (25) males with Cushing's disease / under treatment with prednisolone and hydrocortisone and G6 composed of (25) females with Cushing's disease / under treatment with prednisolone, (0.5) mg film-coated Table of prednisolone and (10) mg of hydrocortisone/one does daily were given to G5 subjects while only prednisolone with the same dose was given to G6 subjects. The duration of the treatment with both prednisolone and hydrocortisone ranged between five months to two years (Approximately 25% of the patients were treated for less than one year). Cushing's patients were compared with healthy subjects with approximately the same range of age-matched with the patients (without any chronic disease which may affect the accuracy of the biochemical measurement) and regarded as two control groups: G1 composed of (25) healthy males and (G2) composed of (25) healthy females. Interestingly, the females enrolled in the present study were not menopausal. Honestly, G5 and G6 cases were in the same range of age and the same physiological state compared with G3 and G4 respectively, the only difference is that G3 and G4 cases are newly diagnosed / without treatment while G5 and G6 are under treatment. Practically, G5 and G6 cases were enrolled to examine treatment action via comparison with both healthy control and newly diagnosed subjects. Remarkably, patients' selection was not randomly applied; they were selected according to their history taken from the physicians.

Exclusion Criteria

Cushing's disease patients who have chronic diseases such as complications like diabetes, fibrosis, heart diseases, and kidney failure were excluded from the present study depending on their diagnostic reports such as laboratory tests. Honestly, this study was designed to highlight the definite biochemical status of Cushing's syndrome because any change in the balance may be due to another chronic disease rather than Cushing's if the exclusion was not performed. Moreover, all menopausal women were also excluded from both the control and patient groups to avoid any error when the females of G2, G4, and G6 were compared with each other.

Blood sample collection

Five (5) ml of venous blood were sampled and put in a gel tube from each person who participated in the present study, blood sera were separated from the whole blood by the centrifuge for 5-7 min at 4000 r.p.m the resulting sera were divided into four portions (for each parameter), each division put within Eppendorf and kept frozen at (-20 °C) until the beginning with the biochemical laboratory tests.

Biochemical determination

According to cortisol, ACTH, collagen IV, and neopterin, the quantitative sandwich enzyme-linked immunosorbent assay ELISA technique was applied, using kits (cortisol, ACTH, collagen IV, neopterin). Kits manufacturers (company), catalog numbers, and the origin are explained in Table 1. A definite antibody for each cortisol, ACTH, collagen IV, and neopterin was pre-coated on a microplate, standards and the samples (containing the antigen) were pipetted into the wells. Avidin-conjugated horse radish peroxidase was applied as a second antibody. The optical density (OD) was detected at 450 nm and the concentrations of cortisol, ACTH, collagen IV, and neopterin were subsequently determined. The absorbance was recorded at 578nm.

Table 1. Kits manufacture (company), catalog numbers, and the origin of each kit

Kit	Company	Catalog number	Origin
Cortisol	Cloud –Clone Corp	CEA462Ge 96	Spain
ACTH	Elabscience	E-EL-H0137	France
Collagen IV	Elabscience	IS007	Korea
Neopterin	Elabscience	EH3413	USA

STATISTICAL ANALYSIS

The data were quantified and documented by the student T-test reflected by the probability (P) by comparing the difference between the data for each parameter, when $p \leq 0.05$, the difference is regarded as significant while when $p \leq 0.001$ the difference is known as highly significant. On the other hand, when $p>0.05$ the difference is considered as non-significant.

RESULTS AND DISCUSSION

Table 2. The levels of Cortisol (ng/mL) in blood sera of Cushing's and control groups.

Group	Mean±S.D	Group	Mean±S.D
G_1	102.323±5.286	G_2	107.092±50.841
G_3	165.576±80.904	G_4	865.100±696.474
G_5	150.956±89.346	G_6	108.4821±86.308
p G_3 vs G_1: 0.000790752 (H.S)		p G_4 vs G_2: 1.85484E-06 (H.S)	
p G_5 vs G_3 : 0.5470 (N.S)		p G_6 vs G_4: 0.003533188 (S)	
p G_5 vs G_1: 0.014686985 (S)		p G_6 vs G_2: 0.944970784 (N.S)	
p G_4 vs G_3: 8.38765E-06 (H.S) sample size: 25 for each group			

S.D: standard deviation.

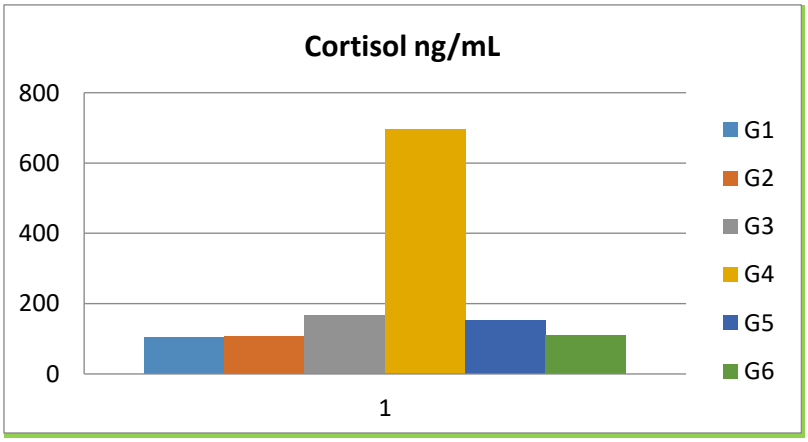

Figure 1. Cortisol levels ng/mL in sera of the studied groups.

Sample size: 25 for each group

G_1, G_2: healthy subjects (males and females respectively)

G_3, G_4: newly diagnosed CD patients (males and females respectively)

G_5, G_6: under treatment patients (males and females respectively)

Cortisol is the key diagnostic biochemical marker for CD. [1,3] As reported by Table 2, cortisol level was a highly significant increase in sera of G_3 (165.576±80.904) ng/ mL and G_4 (865.100±696.474) ng/ mL compared with G_1 (102.323±5.286) ng/ mL and G_2 (107.092±50.841) respectively. A recent study has reported that Cushing's disease is caused by either overexposure to glucocorticoids or by endogenous overproduction of cortisol [1]. The result of the two ways is higher levels of cortisol [17]. Regarding glucocorticoids, their effects are mediated via receptors, glucocorticoid receptors (GRs) are expressed in several brain regions and cell types. In this regard, Cushing's patients express psychiatric complications, cognitive impairments, and extensive volumetric changes in the brain [18]. According to the cortisol structure which is characterized by strong lipophilicity, it is allowed to penetrate the central nervous system through its receptors. Generally, two receptors have an affinity for cortisol, glucocorticoid receptors (GR) located in the hippocampus and prefrontal cortex and mineralocorticoid receptors (MR) located throughout the central nervous system [19]. As a result, cortisol binding with its receptors expresses its higher level and function in Cushing's disease, this is why its level was increased in G_3 and G_4 compared with G_1 and G_2 respectively. In contrast, cortisol level was decreased in G_5 (150.956±89.346) ng/ mL and G_6 (108.4821±86.308) ng/ mL compared with G_3 (165.576±80.904) ng/ mL and G_4 (865.100±696.474) respectively, this decrease reflects the reactive role of the treatment. Regarding the immune system, A recent study has reported that the higher level of glucocorticoids (in particular cortisol) in Cushing's patients has an impact on the immune cells and the result is overall immune suppression which is reflected by cell death, disordered immune regulation, and defective immune responses [20,21]. Similarly, a previous study has revealed that circulating levels of pro-inflammatory cytokines are promoted in patients with Cushing's disease [22]. Hence, Corticosteroids are part of the treatment of many disorders in which inflammation [23]. Prednisolone has anti-inflammatory action mediated by inhibition of the biosynthesis of prostaglandin in two ways: firstly prednisolone inhibits the transcription factors AP-1 and NF-kβ included in the regulation of pro-inflammatory proteins involving cyclo-oxygenase2 secondly, prednisolone is an activator for the biosynthesis of anti-inflammatory proteins annexin-1 which in turn has an inhibitory effect on phospholipase A_2 (the rate limiting enzyme included in the biosynthesis of arachidonic acid) . On the other hand, prednisolone as a synthetic glucocorticoids can transport across the cell membrane and the bind with a specific glucocorticoids receptors (in the cytoplasm of the target Cells) to produce glucocorticoid-receptor (GR) complexes, these complexes actively up-regulates the expression of anti-inflammatory Proteins and down-regulates the pro-inflammatory proteins [24] . Remarkably, the results of Table 2 have suggested a significant increase in G_5 (150.956±89.346) ng/mL compared with G_1 (102.323±5.286) ng/mL and non-significant increase in G_6 (108.4821±86.308) ng/mL compared with G_2 (107.092±50.841) ng/mL , these results indicates the biochemical role of the treatment in shifting the cortisol level towards the balance particularly in females. Interestingly, a highly significant increase of cortisol level was recorded in G_4 (865.100±696.474) ng/mL compared with G_3 (165.576±80.904) ng/mL . Hence, the females involved in the present study were more exposed to the severity of this disease

Table 3. The levels of ACTH (pg/mL) in blood sera of Cushing's and control groups

Group	Mean±S.D	Group	Mean±S.D
G_1	48.412±10.079	G_2	48.604±10.054
G_3	63.997±27.485	G_4	67.693±28.832
G_5	74.720±57.821	G_6	8.8024±1.600
p G_3 vs G_1: 0.01054 (S)		p G_4 vs G_2 : 0.003007819 (S)	
p G_5 vs G_3: 0.406478 (N.S)		p G_6 vs G_4: 1.33235E-13 (H.S)	
p G_5 vs G_1:0.029675(S)		p G_6 vs G_2: 1.68527E-24(H.S)	
p G_4 vs G_3: 0.644796(N.S) Sample size: 25 for each group			

S.D: standard deviation.

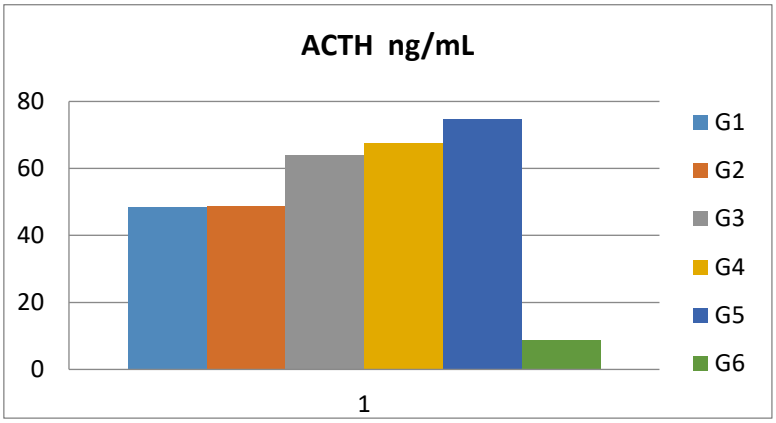

Figure 2. ACTH pg/mL levels in blood sera of the studied groups.

Sample size: 25 for each group

G_1, G_2: healthy subjects (males and females respectively)

G_3, G_4: newly diagnosed CD patients (males and females respectively)

G_5, G_6: under treatment patients (males and females respectively)

As shown in Table 3, ACTH level was significantly increased in blood sera of G_3 (63.997±27.485) Pg/mL and G_4 (67.693±28.832) pg/mL compared with G_1 (48.412±10.079) Pg/mL and G_2 (48.604±10.054) Pg/mL respectively, these results typically reported that G_3 and G_4 groups are Cushing's ACTH dependent cases, because Cushing's syndrome either depend or not depend on ACTH but Cushing's disease particularly is due to excessive ACTH production from pituitary adenoma and is responsible for (75-80)% of endogenous Cushing's syndrome[25]. Remarkably, ACTH level was non significantly increased in G_5 (74.720±57.821) pg/mL compared with G_3 (63.997±27.485) Pg/mL and highly significantly decreased in G_6 (8.8024±1.600) pg/mL compared with G_4 (67.693±28.832) pg/mL, Table 3. These results have revealed the strong impact of the treatment on females. Nevertheless, the increase of ACTH for males after treatment is minimal. Since Cushing's disease is characterized by a disordered immune system[21] and inflammatory responses,[22] prednisolone as a synthetic glucocorticoid is commonly used to treat many auto-immune and inflammatory conditions[26]. Moreover, the results of Table 3 have reported a significant increase in G_5 (74.720±57.821) pg/mL compared with G_1 (48.412±10.079) pg/mL revealing that the treatment couldn't modulate ACTH in males towards the balance while a highly significant decrease has been reported in G_6 (8.8024±1.600) pg/mL compared with G_2 (48.604±10.054) pg/mL, suggesting a side effect of the treatment on ACTH for females. In this regard, a previous study has elucidated that many problems are associated with prednisolone use explained as suppression of the adrenal gland due to the continuous treatment for crushing patients[24]. Hence, a previous study has reported that adverse effects from prolonged oral corticosteroids such as prednisolone are more frequent in women [27].

Interestingly, a recent study has confirmed that prednisolone as any drug must deliver an optimal concentration of its reactive pharmaceutical ingredient at the targeted site of its action to do the perfect pharmacological effect [28]. As revealed in Table 2, The difference between G_3 (63.997±27.485) pg/mL and G_4 (67.693±28.832) pg/mL was non-significant reflecting that ACTH level in Cushing's patient is not influenced by gender characteristics.

Table 4. The levels of Collagen IV(ng/mL) in blood sera of Cushing's and control groups

Group	Mean±S.D	Group	Mean±S.D
G_1	17.475±1.617	G_2	16.996±1.687
G_3	6.208±0.766	G_4	6.043±0.913
G_5	6.098±0.531	G_6	6.035±0.794
p G_3 vs G_1 : 1.03096E-33 (H.S)		p G_4 vs G_2 : 9.08294E-32 (H.S)	
p G_5 vs G_3 : 0.558742284 (N.S)		p G_6 vs G_4 : 0.972583315 (N.S)	
p G_5 vs G_1 : 6.62365E-35 (H.S)		p G_6 vs G_2 : 2.39447E-32 (H.S)	
p G_4 vs G_3: 0.491940668 (N.S) Sample size: 25 for each group			

S.D: standard deviation.

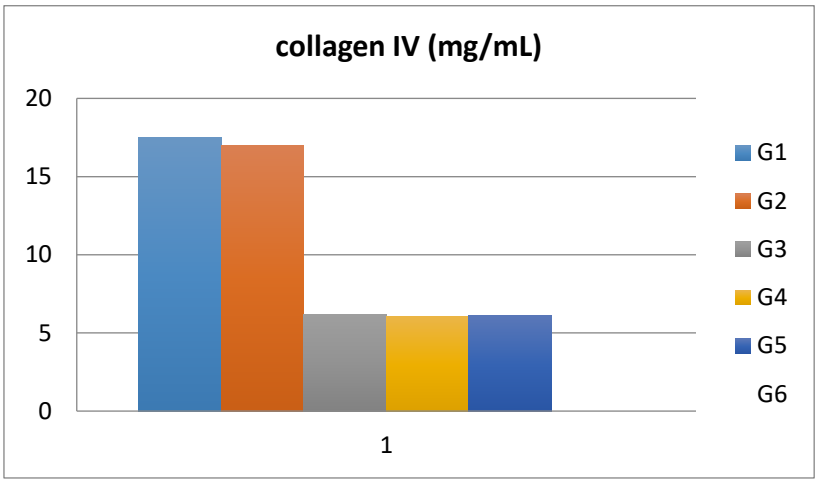

Figure 3. Collagen IV levels (ng/mL) in sera of the studied groups.
Sample size: 25 for each group

G_1, G_2: healthy subjects (males and females respectively)

G_3, G_4: newly diagnosed CD patients (males and females respectively)

G_5, G_6: under treatment patients (males and females respectively)

As reported by Table 4, Collagen IV level was highly significantly decreased in sera of G_3 (6.208±0.766) ng/mL and G_4 (6.043±0.913) ng/mL compared with G_1 (17.475±1.617) ng/ mL and G_2 (16.996±1.687) respectively, these data infer an inverse relationship between collagen IV levels and Cushing's syndrome. In this regard, collagen IV deficiency is linked with muscle weakness, osteopenia, and fragility fractures. Hence a recent study has reported a relationship between macrophages and bone metabolism [(29)].On the other hand, Collagen IV has a relationship with the central nervous system [(30)]. Interestingly, Collagen is the key fibrous protein constituting the higher ratio of the extracellular matrix[(31)]. Moreover, collagen is produced by the CNS mostly astrocytes, neurons, and vascular cells [(30)]. At this point, the defect in the CNS may regarded as a complication of Cushing's disease[(1)]. Hence, a previous study has revealed that collagen IV immune reactivity was detected on centrifugal and centripetal nerve pathways and could be involved in a dynamic process of cell-matrix interactions during the establishment of initial axons pathways and neurite outgrowth in vivo[(32)].
Remarkably, Collagen has a remarkable influence on the immunological responses due to its detection in peripheral blood after ingestion. Moreover, collagen-derived peptides are implicated in inflammatory disorders. Collagen-derived oligopeptides such as polyhydroxy or proxy hydroxyl proline have been reported to do chemotactic actions on fibroblasts, neutrophils, and monocytes, all of these play key roles in inflammatory diseases[(33)]. By highlighting the defects related to both the CNS and the immune system with the collagen side and Cushing's disease on the other side, the current study is the first to reveal that collagen IV is a novel biochemical marker in Iraqi patients with Cushing's disease. This is why collagen IV levels decreased in G_3 and G_4 compared with G_1 and G_2 respectively. As reported in Table 4. Collagen IV level was non significantly decreased in sera of G_5 (6.098±0.531) ng/ mL and G_6 (6.035±0.794) ng/ mL compared to G_3 (6.208±0.766) ng/ mL and G_4 (6.043±0.913) ng/ mL respectively. Although the pivotal role of the Prednisone and hydrocortisone as Glucocorticoids in inflammation suppression, its immunosuppressive effects on Collagen IV are minimal. Hydrocortisone is typically used to treat several autoimmune diseases[(16)]. The highly significant decreased level of Collagen IV in G_5 (6.098±0.531) compared with G_1 (17.475±1.617) and G_6 (6.035±0.794) compared with G_2 (16.996±1.687) revealed that the treatment couldn't shift collagen IV in males and female into the balance confirming the minimal effect of the treatment on collagen IV. The non-significant difference between G3 (6.208±0.766) and G4 (6.043±0.913) indicates that collagen IV level in untreated patients is not influenced by gender.

Table 5. The levels of neopterin ng/ mL in blood sera of Cushing's and control groups.

Group	Mean±S.D	Group	Mean±S.D
G_1	1.959±0.454	G_2	1.947±0.402
G_3	6.701±1.336	G_4	6.385±1.966
G_5	5.037±0.810	G_6	4.902±1.182
p G3 vs G1 : 9.59558E-22 (H.S)		p G4 vs G2 : 8.58663E-15 (H.S)	
p G5 vsG3 : 2.6495E-06 (H.S)		p G6 vs G4 : 0.00223111 (S)	
p G5 vs G1 : 1.7021E-21 (H.S)		p G6 vs G2 : 7.63317E-16 (H.S)	
p G4 vs G3 : 0.508619386 (N.S) Sample size: 25 for each group			

S.D: standard deviation.

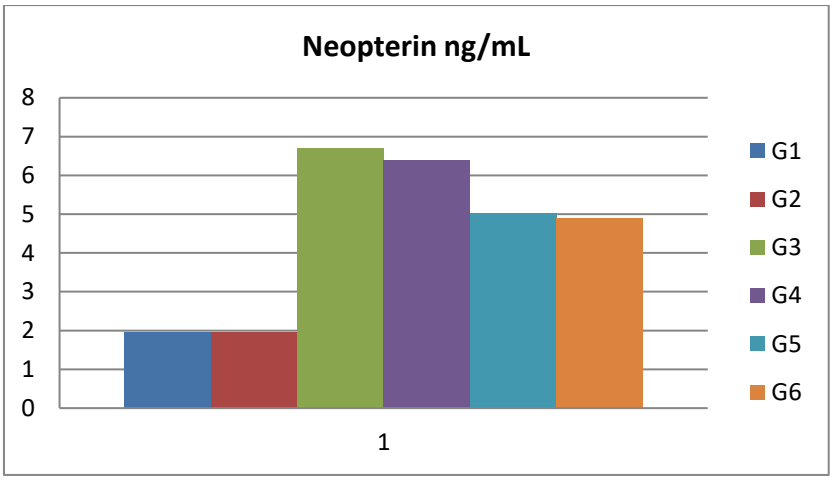

Figure 4. Neopterin levels(ng/mL) in sera of the study groups.
Sample size: 25 for each group

G_1, G_2: healthy subjects (males and females respectively)

G_3, G_4: newly diagnosed CD patients (males and females respectively)

G_5, G_6: under treatment patients (males and females respectively)

As shown in Table 5, neopterin level was highly significantly increased in sera of G_3 (6.701±1.336) ng/mL and G_4 (6.385±1.966) ng/mL compared with G_1 (1.959±0.454) ng/mL and G_2 (1.947±0.402) ng/mL respectively. Accordingly, Cushing's disease is characterized by higher levels of macrophages [33]. In this regard, higher levels of neopterin have a link with macrophage[35]. Hence, Cushing's syndrome has a relationship with the immune system [21]. On the other hand, neopterin has a relationship with cell-mediated immunity[14,36]. This is why neopterin level was increased in G_3 (6.701±1.336) ng/ mL and G_4 (6.385±1.966) ng/ mL compared with G_1 and G_2 respectively. These findings indicate the role of activated macrophages and cellular immunity in CD by highlighting neopterin. In contrast, neopterin level was decreased in G_5 (5.037±0.810) ng/ mL and G_6 (4.902±1.182) ng/ mL compared with G_3 (6.701±1.336) ng/ mL and G_4 (6.385±1.966) respectively, this decrease reflects the pivotal role of the treatment. Regarding the immune system, Cushing's syndrome has a relationship with immune system [21]. Chronic inflammation results from a disorder of the immune system [37]. Hence, recent work has revealed that prednisolone decreases inflammation via inhibition of the migration of polymorph nuclear leukocytes and reversing increased capillary permeability. Also inhibits immunological cells by reducing the activity and the volume of the immunological cells and prostaglandin inhibition [38]. Additionally, corticosteroids are used to treat inflammation[39]. Hydrocortisone as a type of glucocorticoid is used for inflammation suppression, it is typically used to treat several autoimmune diseases [16]. The high significantly increase in level in neopterin level in G_5 (5.037±0.810) compared with G_1 (1.959±0.454) and in G_6 (4.902±1.182) compared with G_2 (1.947±0.402) reveals that neopterin level couldn't reach the normal status although its potential action. The non-

significant difference between G_3 (6.701±1.336) and G_4 (6.385±1.966) indicated that neopterin level in untreated patients is not influenced the gender. Anyway, the limitation of the study may be due to gender imbalance.

Table 6. A summary of group p-value

Parameter	p-value
Cortisol	p G_3 vs G_1 : 0.000790752 (H.S)
	p G_4 vs G_2 : 0.003007819 (S)
	p G_5 vs G_3 : 0.5470 (N.S)
	p G_6 vs G_4 : 0.00223111 (S)
	p G_5 vs G_1 : 1.7021E-21 (H.S)
	p G_6 vs G_2 : 7.63317E-16 (H.S)
	p G_4 vs G_3 : 0.508619386 (N.S)
ACTH	p G_3 vs G_1 : 0.01054 (S)
	p G_4 vs G_2 : 0.003007819 (S)
	p G_5 vs G_3 : 0.406478 (N.S)
	p G_6 vs G_4 : 1.33235E-13 (H.S)
	p G_5 vs G_1 : 0.029675(S)
	p G_6 vs G_2 : 1.68527E-24(H.S)
	p G_4 vs G_3 : 0.644796(N.S)
Collagen IV	p G_3 vs G_1 : 1.03096E-33 (H.S)
	p G_4 vs G_2 : 9.08294E-32 (H.S)
	p G_5 vs G_3 : 0.558742284 (N.S)
	p G_6 vs G_4 : 0.972583315 (N.S)
	p G_5 vs G_1 : 6.62365E-35 (H.S)
	p G_6 vs G_2 : 2.39447E-32 (H.S)
	p G_4 vs G_3 : 0.491940668 (N.S)
Neopterin	p G_3 vs G_1 : 9.59558E-22 (H.S)
	p G_4 vs G_2 : 8.58663E-15 (H.S)
	p G_5 vs G_3 : 2.6495E-06 (H.S)
	p G_6 vs G_4 : 0.00223111 (S)
	p G_5 vs G_1 : 1.7021E-21 (H.S)
	p G_6 vs G_2 : 7.63317E-16 (H.S)
	p G_4 vs G_3 : 0.508619386 (N.S)

CONCLUSION

This is the first study in Iraq to examine collagen IV and neopterin levels in Cushing's disease patients, while these biochemical markers have been studied elsewhere, our findings provide new data specific to Iraqi populations by highlighting the key roles of the immune responses and the CNS. Although a lot of previous and recent studies dealt with cortisol as the major biochemical marker for Cushing's disease, the present study submits a detailed clarification of cortisol status in both newly diagnosed and under-treatment cases by highlighting the reactive role of the immune system and inflammatory responses, the importance of cortisol receptors and the sexual effect. The present study has confirmed that the selected Cushing's syndrome cases are Cushing's disease patients due to the higher level of ACTH compared with the healthy subjects. Additionally, the present study has revealed a key role of glucocorticoid treatment in shifting both cortisol and neopterin into the balance (for the two genders, these markers can guide treatment) and a side effect of the same treatment on ACTH (for females) and a non-significant effect on collagen IV (for the two genders).

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CONFLICTS OF INTEREST

none

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We declare that our research article didn't receive any financial support from my university or other institute. Please declare if the research received financial support from an Institution**.**

ETHICS STATEMENTS

We (as authors) indicate that this research article was performed based on ethical considerations and the ethical approval was received from our university and the Institute in which We did the blood sampling before recruiting patients. All clinical trials were conducted according to the research integrity rules in Baghdad University which follow the Helsinki Declaration.

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دور الكولاجين الرابع و النيوبترين لمرضى عراقيين بمتلازمة كوشينغ

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الخلاصة

الهدف : تهدف الدراسة الحالية إلى تفصيل العلاقة بين الباراميترات الكيموحيوية (الكولاجين الرابع والنيوبترين) ومسببات مرض كوشينغ لدى المرضى العراقيين وأيضا فحص التأثير الكيميائي الحيوي للعلاج (بريدنيزولون وهيدروكورتيزون) على المتغيرات الكيموحيوية (الكورتيزول ، هرمون الأدرينوكورتيكوتروبك ، الكولاجين الرابع والنيوبترين) لمرضى كوشينغ. **طرق العمل :** تم إشتراك مائة مريض في هذه الدراسة وتم تصنيفهم إلى أربع مجموعات: G3 تتكون من (25) ذكرا تم تشخيصهم حديثا بالإصابة ب (CD)/ بدون علاج ، G4 تتكون من (25) أنثى مشخصات حديثا ب (CD)/بدون علاج ، G5 تتكون من (25) ذكر مصاب ب (CD) تحت العلاج ببريدنيزولون وهيدروكورتيزون و G6 تتكون من (25) أنثى مصابة ب (CD) تحت العلاج ببريدنيزولون ، تمت مقارنة المرضى بأشخاص اصحاء لديهم نفس النطاق العمري واعتبروا مجموعتين ضابطتين : G1 تتكون من (25) ذكرا و G2تتكون من (25) أنثى ، تم تحديد مستويات جميع الباراميترات في الامصال لكل من المرضى والمجموعات الضابطة. **النتائج:** كان مستوى الكورتيزول والنيوبترين مرتفع بشكل معنوي عالي وارتفع مستوى هرمون الأدرينوكورتيكوتروبك بشكل معنوي و إنخفض مستوى الكولاجين بشكل معنوي كبيرفي G3 و G4 مقارنة ب G1و G2 على التوالي. وانخفضت مستويات الكورتيزول والنيوبترين (غير معنوي للذكور وبشكل معنوي للإناث فيما يتعلق بالكورتيزول ومعنوي عالي للذكور ومعنوي للإناث فيما يتعلق بالنيوبتيرين) في G5وG6 مقارنة ب G3و G4 على التوالي ، بينما كان لنفس العلاج تأثيرا جانبيا على مستويات كل من هرمون الأدرينوكورتيكوتروبك و الكولاجين الرابع . كان مستوى الكورتيزول مرتفعا بشكل معنوي للغاية في الإناث المشخصات حديثا مقارنة بالذكور بينما كان الفرق غير معنوي فيما يتعلق بهرمون الأدرينوكورتيكوتروبك والكولاجين الرابع والنيوبترين **الإستنتاج :** تقدم هذه الدراسة الحالية نتائج جديدة من خلال توضيح أن الكولاجين الرابع والنيوبترين أثبتت أهميتها كمتغيرات كيموحيوية جديدة لمرض كوشينغ كما انه يسلط الضوء على الدور الفعال للبريدنيزولون والهيدروكورتيزون في أزاحة الكورتيزول و النيوبتيرين نحو التوازن بينما كان لنفس العلاج تأثيرا جانبيا و تأثيرا طفيفا على هرمون الأدرينوكورتيكوتروبك و الكولاجين الرابع على التوالي.

الكلمات المفتاحية : مرض كوشينغ ، الكورتيزول ، هرمون الأدرينوكورتيكوتروبك ، الكولاجين الرابع ، النيوبترين