ASSOCIATION BETWEEN SERUM LEVEL OF CYTOKERATIN 18 M65ED AS AN INDEPENDENT INDICATORS OF CARDIOMETABOLIC DISORDERS IN WOMEN WITH POLYCYSTIC OVARIAN SYNDROME

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ABSTRACT

PCOS is a hyperandrogenicanovulatory disease with an adverse metabolic and inflammatory profile, hyperandrogenism is linked to a negative metabolic phenotype and a higher risk of cardiovascular disease. The main objective of the study is to prove the CK18 M65ED marker is an indicator of Cardiometabolic Disorders in women with PCOS. 40 PCOS women enrolled in the study and 41 healthy women as control. Serum levels of CK18 M65ED were assessed in both groups. PCOS women had a statistically significant (P<0.05) increase in FBG, HbA1c compared to healthy control women. Furthermore had a highly statistically significant (P<0.01) increase in LH, (LH/FSH) Ratio, Testosterone, Insulin, HOMA-IR, and Ck18 compared to control women. And a highly statistically significant (P<0.01) decrease of FSH. PCOS entails many complications and metabolic diseases such as hyperinsulinemia, hyperglycemia, development of IR, which are cardiometabolic disorders. CK18 plays a role in the monitoring of Cardiometabolic Disorders in women with PCOS.

Keywords: Polycystic ovary syndrome (PCOS), Cytokeratin 18 (CK18), apoptosis, cardiomyopathy, LH, FSH.

I. INTRODUCTION

PCOS is commonly recognized as a hyperandrogenicanovulatory disease with an adverse metabolic and inflammatory profile, with the first two characteristics acting as diagnostic hallmarks. PCOS is the most prevalent endocrine disorder in women of reproductive age with a prevalence of 6–20 percent and one of the main causes of female infertility around the world. Despite the scientific community’s best efforts, the etiology and pathogenesis of PCOS are still uncertain. It may be triggered by a hereditary predisposition affected by the gestational climate, lifestyle influences, or a mixture of the two(1,2). The pathogenesis of polycystic ovarian disease has been imposed by abnormalities of the hypothalamic-pituitary-ovarian or adrenal axis. A disruption in the gonadotrophin-releasing hormone (GnRH) secretion rhythm leads to a relative increase in LH to FSH release (3). LH induces the development of androgens such as testosterone by the ovarian theca cells. Due to a relative deficit of FSH, testosterone is aromatized inefficiently by the granulosa cells, resulting in hyperandrogenemia. Additionally, there appears to be an increase in the activity of steroidogenic enzymes in polycystic ovaries, contributing to the androgen surplus (4). Via complex and multidirectional pathways, hyperandrogenism is linked to a negative metabolic phenotype and a higher risk of cardiovascular disease (5,6).

Cytokeratin 18 is a cytoskeleton intermediate filament protein that is found in simple epithelial cells, hepatocytes, cholangiocytes, the pancreas, and the colon (7–9). Cytokeratin 18 (CK18) is a cell death marker that is active in the cell death pathway. When a cell reacts to aberrant cellular stresses such as endoplasmic reticulum (ER) stress and oxidative stress, which are hallmarks of cardiometabolic disorders, cell death (apoptosis, necrosis, autophagy, etc.) may help to get rid of spoiled cells and safeguard cell integrity(10,11).

Apoptosis was observed in cardiometabolic disorders in reaction to glucose overload(12). Because of the ability of CK18 M65 to identify both native and intact CK18, can be used as a cell death marker in cardiometabolic disorders (13). The CK18 M65 assay employs the grab antibody M6 and the detection antibody M5, which are aimed against two separate epitopes of CK18 and can detect both full-length CK18 and cleaved fragments of CK18, regardless of whether or not they are cleaved by caspases (13). The M65ED assay employs the M6 antibody for the disclosure
and the M5 antibody for capture, resulting in increased binding specificity and lower signal levels in stable controls (8,14). Insulin tolerance and other cardiometabolic risk factors can be influenced by hyperandrogenemia (either prenatally or as an adult) (7,8). Tan et al. established a link between increased CK18 M30 levels and IR in women with the polycystic ovarian syndrome (15). Civera et al. shown that in individuals with extreme obesity, enhanced insulin resistance results in a rise in CK18 M30 levels (16). As has been found that CK18 M65ED is positively correlated with the insulin resistance index of the HOMA-IR [homeostasis model assessment (HOMA)] based on the results of Tan et al. and Civera et al.,(15,16).

II. MATERIALS AND METHODS

Subjects:
The study involved 81 women. Forty-one healthy subjects were enrolled as the control for the study, as well as Forty women were diagnosed with PCOS from Iraq, Karbala. The subjects ages were between (18-40) years old. The laboratory side of the study was performed at the laboratory Al-Hussein Teaching Hospital.

Sample collection:
Five milliliters of venous blood were drawn on the second day of the menstrual cycle from the fasting women (after an overnight fast), with the blood sample being slowly withdrawn via the needle of a syringe to avoid hemolysis. and were divided into two parts:

The first part is 1.5 milliliters of blood retained in EDTA tubes to get plasma for measuring glycated hemoglobin HbA1C.

The second part, 3.5 milliliters of blood were transferred into a gel tube and left for 15 minutes. After coagulation, separated by centrifugation at 3000 rpm. for 10 min. Immediate measurements of serum hormones Testosterone, Follicle Stimulating Hormone(FSH), and Luteinizing hormone(LH), and insulin were measured. Blood glucose level (fasting) was measured.

The rest were stored at -80 Cº in deep freeze, CK18 M65ED was is measured using the ELISA technique test based on the quantitative sandwich principle.

Methods:
The serum level of fasting glucose levels was determined by using the spectrophotometric method. LH, FSH and, testosterone hormones, as well as serum insulin, were determined by using fluorescence Immunoassay (FIA) (Boditech –Korea) based on the quantitative sandwich. And HbA1C level was measured by (Cobas e 411 – Germany). While HOMA-IR was calculated by performing calculations between the fasting serumInsulin and the fasting serumglucose. In addition to CK18 M65ED was determined by using an ELISA kit. BioTech ELISA microplate washer ELX50 and microplate reader ELX800 devises were used. SPSS (Statistical Package for Social Sciences) version 22 was used to do data statistical analysis, independent t-test of mean comparisons, and Person’s correlation coefficient by using SPSS.

Results:
Results are expressed in the form of mean±SD. When comparing patient women with PCOS to control women, as shown in table 1: the results of this study found that the mean FSH was lower (5.39 ± 1.22 vs 7.28 ± 1.99) mIU/ml and this difference was highly statistically significant (P=0.00). In addition, the mean LH level of the patient was greater than the control level (10.05 ± 3.72 vs 5.78 ± 3.49) mIU/ml and this difference were highly statistically significant (P=0.00), as well the mean (LH/FSH) Ratio level of the patient was greater than the control level (1.86 ± 0.51 vs 0.85 ± 0.55) and also this difference was highly statistically significant (P=0.00), as well as the mean Testosterone level in patient was greater than in control level (0.46 ± 0.26 vs 0.25 ± 0.11) pg/ml and this difference were highly statistically significant (P=0.00).

When comparing patient women with PCOS to control women based on insulin dysfunction, it was discovered that the mean FBG was greater (95.60 ± 8.80 vs 89.46 ± 8.02) and this difference was statistically significant (P=0.02). The mean HbA1c % was greater in PCOS patients comparing with control (5.49 ± 0.43 vs 5.29 ± 0.38) and this difference was statistically significant (P=0.03). while the mean Insulin was greater in PCOS patients comparing with control (18.60 ± 2.20 vs 16.84 ± 1.92) and this difference was highly statistically significant (P=0.00). as well
mean HOMA-IR was greater in PCOS patients comparing with control (4.29 ± 0.64 vs 3.71 ± 0.52) and this difference was highly statistically significant (P=0.00).

The results of cardiac marker levels in patients with PCOS shown that the levels (mean± SE) of serum cytokeratin18 in the patient group were significantly higher than the control group (438.21 ± 41.23 vs 242.14 ± 42.41) mIU/mL and this difference was statistically significant (P=0.00).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patient , N=40</th>
<th>Control , N=41</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (mIU/ml)</td>
<td>5.39 ± 1.22 **</td>
<td>7.28 ± 1.99</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>10.05 ± 3.72 **</td>
<td>5.78 ± 3.49</td>
</tr>
<tr>
<td>(LH/FSH) Ratio</td>
<td>1.86 ± 0.51 **</td>
<td>0.85 ± 0.55</td>
</tr>
<tr>
<td>Testosterone (pg/ml)</td>
<td>0.46 ± 0.26 **</td>
<td>0.25 ± 0.11</td>
</tr>
<tr>
<td>FBG (mg/dL)</td>
<td>95.60 ± 8.80 *</td>
<td>89.46 ± 8.02</td>
</tr>
<tr>
<td>HbA1c %</td>
<td>5.49 ± 0.43 *</td>
<td>5.29 ± 0.38</td>
</tr>
<tr>
<td>Insulin(µIU/mL)</td>
<td>16.80 ± 2.20 **</td>
<td>16.84 ± 1.92</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>4.29 ± 0.64 **</td>
<td>3.71 ± 0.52</td>
</tr>
<tr>
<td>Ck18 (mIU/mL)</td>
<td>438.21 ± 41.23 **</td>
<td>242.14 ± 42.41</td>
</tr>
</tbody>
</table>

T.test P value was: * No significant (P> 0.05) , * Significant (P≤ 0.05) , ** Highly significant (P ≤ 0.01) .

III. DISCUSSION:

There was a significant rise in Luteinizing hormone levels (p < 0.01) in PCOS individuals compared to controls. This study corroborates the findings of Yue et al. An increase in the amount of LH in the PCOS group (17). The levels of FSH were significantly decreased (p < 0.01) in PCOS individuals compared to controls. The reduction in blood FSH levels results in atresia in the present cohort's smaller follicles, which lack the necessary sensitivity to FSH to survive. While an increase in LH stimulates the ovarian theca cells to generate more androgens, inadequate follicle-stimulating hormone (FSH) may be a more immediate cause of anovulation in people with PCOS Thus, a high level of LH and FSH insufficiency may result in menstrual cycle disruption, infertility, decreased sexual desire and vaginal dryness, as well as osteoporosis, which may result in a predisposition to bone fractures and eventually lead to Polycystic Ovarian Syndrome (18). Additionally, there was a significant rise (p 0.01) in the LH/FSH ratio in PCOS subjects when compared to controls. This is consistent with the findings of Yue et al. and Malini and Roy George, who observed significant increases in LH and the LH/FSH ratio in the PCOS group. Increased LH/FSH ratios are indicative of hormonal dysregulation in PCOS (17,19). When compared to the non-PCOS control group, there was a significant rise (p < 0.01) in the testosterone levels of the PCOS group. This study corroborates the findings of Bartolone et al. (2000), who discovered an abnormally high amount of testosterone in women with PCOS (20). This demonstrates the critical role of testosterone in identifying PCOS. When comparing patient women with PCOS with control women in this study, Insulin concentrations in PCOS patients demonstrate a highly significant rise (p < 0.01) when compared to controls, and the same in HOMA-IR there was a highly significant rise (P < 0.01) in PCOS individuals compared to controls, while Fasting blood glucose (FBG) levels demonstrate a significant rise (P < 0.05) in PCOS individuals compared to controls. also, there was a significant rise in HbA1c levels (p < 0.05) in PCOS individuals compared to controls. This is consistent with the findings of Meenakumari et al. (2004), who found an elevated insulin level during the luteal phase in women with PCOS (21). Additionally, Meenakumari et al. likewise suggested that elevated insulin levels inhibit the synthesis of progesterone by granulosa cells isolated from women with PCOS but not from normal women (21). Insulin, on the other hand, may operate at the pituitary, ovarian, and/or hepatic levels, increasing androgen production and/or serum concentrations of free testosterone (21). There was a highly statistically significant rise in the mean concentration of cytokeratin 18 M65ED (p<0.001) in our research population. It has been shown according to previous studies that The progression of cardiometabolic disorder starts with insulin tolerance (22). Additionally, it has been discovered that CK18 M65ED is positively correlated with the insulin resistance index of the HOMA-IR (15,16), CK18 exhibited a strong positive correlation with fasting plasma glucose levels in individuals with T2D, regardless of NAFLD status (23). This study concurs with Qian et al., 2020, in that serum levels of CK18 M65ED, a cell death marker, were considerably elevated in patients with cardiometabolic diseases, and these enhanced levels were independently linked with the risk of cardiometabolic disorders parameters and NAFLD (24).In our study population of people with polycystic ovaries. In view of the high CK18 M65ED marker, which, as it has been proven, is found in
metabolic complications associated with heart disease (cardiometabolic) complication, consequently, CK18 M65ED is a good indicator to monitor complications of PCOS patients and it must be included in clinical examination.

IV. CONCLUSION

The PCOS does not only impact reproduction but also implies numerous consequences and metabolic illnesses such as hyperinsulinemia, hyperglycemia, development of IR, which are cardiometabolic disorders. CK18 has a function in the monitoring of Cardiometabolic Disorders in women with PCOS.

REFERENCE: