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Area under the curve of carotid artery Doppler as a sensitive marker of insulin sensitivity among Iraqi women with polycystic ovarian syndrome: a cross-sectional study

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ABSTRACT

Objective. Polycystic ovarian syndrome (PCOS), a common endocrinopathy of reproductive-aged women, harbours increased insulin resistance (IR) and cardiovascular risk. Screening for associated health risks reduces morbidity and mortality. Measurement of intima-media thickness (IMT) by carotid artery Doppler (CCA) was tested as a marker for IR previously; herein, we aimed to verify the value of area under the curve (AUC) of CCA Doppler wave as a possible marker for IR in PCOS.
Patients and Methods. 140 PCOS women were recruited from the ultrasound department of Al-Yarmouk Hospital; from CCA-Doppler, we recorded CCA-IMT, pulsatility index (PI), and resistance index (RI). By Graph software, AUC-CCA was calculated for all. Patients' biochemical (lipid and glucose) and hormonal parameters (FSH, LH, insulin, and testosterone) were checked. Lastly, PCOS cases were split into three subgroups based on IR centile to show the relationship between CCA and AUC.
Results. There are strong inverse correlations between CCA-AUC *versus* HDL and SHBG (-0.98, -0.99), $p < 0.0001$. CCA-AUC correlated strongly and positively with [LDL, Cholesterol, LH/FSH, Serum Insulin, CCA-IMT, and HOMA-IR], $r = (0.99, 0.78, 0.98, 0.97, 0.99, 0.99)$, $p < 0.0001$. Sub-group analysis signified increased CCA-AUC with increasing IR severity (31.83 ± 3.90, 46.54 ± 5.03, 59.96 ± 2.57), $p < 0.001$; likewise, RI and PI of the CCA showed positive correlations ($p = 0.040$, $p = 0.758$) respectively.
Conclusions. A strong correlation of CCA-AUC with all hormonals, biochemical, and biophysical changes among PCOS cases, in addition to its simplicity and accuracy, makes CCA-AUC a recommendable parameter in PCOS women for diagnostic and prognostic values.

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is the most prevalent endocrine condition affecting women, with a 6%-10% incidence. Due to the heterogeneity of the disease, it frequently remains unnoticed. Infertility, hyperandrogenism, ovulatory dysfunction, and polycystic ovaries are all symptoms and signs of PCOS, in addition to a wide range of effects on the body [1, 2]. With the essence of the third decade of the twenty-first century, women with PCOS have become the target of a wide range of researchers from many medical and biological branches. This change was mediated by

the breakthrough in understanding PCOS, as it is one variant of insulin resistance disorder (IR) [3]. Cardiovascular disease is more prevalent in PCOS than in healthy women, which is attributed to chronic inflammation, insulin insensitivity, hyperandrogenism, hyperlipidaemia, and elevated oxidative stress, all of which are implicated in the development of premature atherosclerosis and elevated cardiovascular events [4].

Continuous screening has focused on reducing possible remotely associated complications in the vasculature. Increased intima-media thickness (IMT) of the arterial walls associated with IR [5, 6] is one of the screening methods. The atherogenic milieu among PCOS women causes widespread premature atherosclerosis in virtually every artery in the body [7]. Doppler of the common carotid artery (CCA) was implemented to screen for cardiovascular risk among PCOS cases due to its superficial proximity and clear-cut repeated scans [8]. IR triggers premature atherosclerosis via endothelial injury and increases intima-media thickness and arterial remodelling. The increase in the carotid artery wall thickness consequently causes a reduction in its lumen and increases blood speed in this artery [9]. Based on the physics of fluid flow, fluid speed in any vessel increases inversely with the diameter of the vessel, and since Doppler is a direct measure of the blood speed in the artery both in the systolic and diastolic phases [10], we hypothesized that the measurement of area under the curve (AUC) of carotid artery Doppler scan could give a reliable indicator of IR among women with PCOS [10]. The measurement of the CCA area under the curve (CCA-AUC) is literally the graphical presentation of blood speed in the carotid because the amount of blood passed per unit area in the artery section equals blood speed [11].

CCA-IMT and its predictors were examined in PCOS for a possible correlation of increased cardiovascular morbidity and mortality, including ischemic heart disease, cerebrovascular accidents, and peripheral vascular diseases; however, they present inconsistent results [12-14].

Since measuring AUC is more accurate, as described in earlier work [9, 15, 16], this study was designed to measure the change of blood speed reflected by the area under the curve of CCA Doppler and its correlation with the hormonal, biochemical, and biophysical markers of insulin resistance among women with PCOS.

PATIENTS AND METHODS

Study design and setting

A cross-sectional study recruited 140 participants in Al-Yarmouk Teaching Hospital from January 2019 to December 2021. Referred patients to the ultrasound department for pelvic scans suspected to have PCOS or to check for endometrial thickness were offered to participate in the study after being briefed about the study's aim and methods. Verbal consent was taken for all patients recruited in this study. Helsinki's declaration was followed. The ethics committee issued this study's approval (IRB 180 on 1-1-2019).

Inclusion criteria and exclusion criteria

We enrolled unmarried females aged 18-35 suspected to be PCOS based on Rotterdam diagnostic criteria [17], where 2 out of 3 were considered for the diagnosis: first, history of oligomenorrhea or amenorrhea; second, hyperandrogenism, whether clinical or biochemical; third, signs of polycystic ovaries in the ultrasound (US) scans [2]. We restricted our inclusion to women with a body mass index (BMI) ranging from 18-30 kg/m^2.

Regarding oligomenorrhea, it was defined as a history of fewer than six cycles per year. As for amenorrhea, it was defined as when the cycle was absent for more than one year [14].

Hyperandrogenism was defined on clinical examination; hirsutism was considered with a score equal to or more than eight based on Ferriman-Gallwey score with or without acne or was biochemically determined based on our lab reference values.

Ultrasonic signs of PCOS were at least one ovary having 12 or more ovarian follicles that measures 2-9 mm in diameter and/or increased volume of the ovaries by more than 10 ml [17].

An exclusion was made to women with:

1. BMI < 18 or > 30, they were excluded to eliminate the obesity effect.
2. Those who received insulin-sensitizing agents or any hormonal drug for cycle regulation in the last six months were also excluded.
3. Cases with medical comorbidities such as thyroid diseases, diabetes hyperprolactinemia, renal, cardiovascular diseases, and those with blood diseases and those with incomplete data shown in study flowchart (**Figure 1**).

The pelvic ultrasound scan was trans-abdominal conducted; then, the examination was extended to

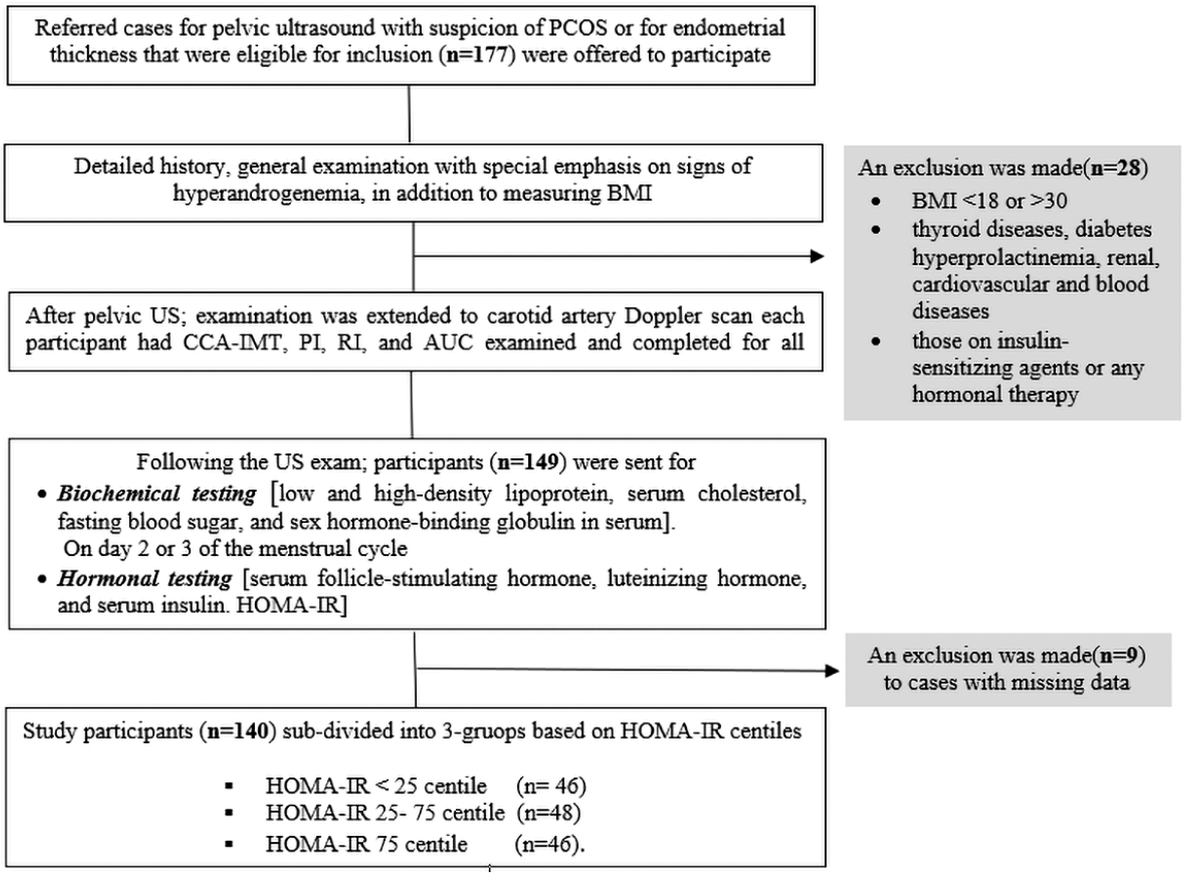

Figure 1. *Study flowchart.*

Doppler of CAA for enrolled cases who satisfied our inclusion criteria. Usually, one side is scanned while the patient lies in a supine position with their head turned to the opposite side of the examined artery. Exploration was done in B-mode utilizing a TOSHIBA Ultrasound Machine, logic p5, linear probe, 7.5-10 MHz. Doppler parameters included the pulsatility index (PI), resistance index (RI), and repeated systolic and diastolic velocity measures were performed in addition to the measurements of IMT. The latter was defined as the distance between the lumen intima and the adventitia media layer line of interference on the far wall in the longitudinal axis. The duration of study for each patient is around 10 minutes. For each participant, we recorded biophysical parameters, CCA-IMT, PI, RI, and AUC. They were stored in excel sheets for further analysis. After the ultrasound and carotid artery Doppler scan was completed for all patients, they were sent for biochemical and hormonal testing in the teaching labs of the hospital.

Biochemical testing

Biochemical testing was done overnight fasting and included the following: low and high-density lipoprotein, serum cholesterol, fasting blood sugar, and sex hormone-binding globulin in serum.

Hormonal testing

Hormonal testing took place on day 2 or 3 of the menstrual cycle, including serum follicle-stimulating hormone, luteinizing hormone, and fasting serum insulin. HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) was calculated from serum insulin and fasting blood sugar.
HOMA-IR = (glucose mmol/L × insulin μU/mL)/22.5 [18].
Enzyme-linked immunoassays (ELISA) were used to evaluate patients' hormonal status. Biophysical testing of the carotid artery scan and Doppler included measuring the carotid intima-media thickness in millimetres IMT, PI, RI, and AUC.

Method of AUC measurement

The explanation of how the area under the curve (AUC) was measured in this paper can be easily explained by giving an example. Below is a normal carotid artery Doppler sample that was taken in

this study (**Figure 2A,C**). Notice that the different heights of the second wave in this photo, which is shown as blue vertical lines, are measured by the same ultra-sonographer taking this photo (**Figure 2A**). Those measurements averaged 8-10, depending on the wave width. The measured heights were stored in an excel table for further analysis by simple free software called GRAPH, downloadable from https://www.padowan.dk/download [19].

Those measurements were fed to the Graph software, which can measure the area under the curve with one click on the icon specified for this function. In addition, the software can also give a visual simulation of the wave in a standard JPG picture format which is shown in **Figure 2A**; as we can see from the result box shown in **Figure 2B** produced by GRAPH software, the AUC for wave 2 in **Figure 2A** is = 60.5 unit^2.

Sample size calculation

Sample size = $(Z1\text{-}\alpha/2)^2 \times SD2/d^2$

$Z1\text{-}\alpha/2$ is standard normal variate = 1.96

SD = standard deviation of the variable. The value of SD can be taken from a previously done study [20].

d = absolute error or precision as determined by the researcher. In our study, we suspect the absolute error is 0.05.

Sample size = $(1.96)^2 \times (0.3)^2/(0.05)^2 = (3.84 \times 0.09)/0.0025 = 138$ *patients.*

So, the sample size is 138 patients; our study involved 140 patients.

Statistical analysis

This study's main variables were checked by Shapiro- Wilk test for normality. The mean and standard deviation was then calculated for each variable. Then, all the main group data were classified into three subgroups according to the centile of the HOMA-IR; the first group included patients with HOMA-IR less than the 25th centile (n = 46). The second group had a HOMA-IR centile between the 25th and 75th centile (n = 48). Finally, the third group included patients with HOMA-IR of more than 75th (n = 46). The statistical differences among the three subgroups were made by one-way ANOVA test. Then, a series of linear regression analyses with calculation coefficient of correlation r between AUC under carotid artery Doppler wave as the main dependent variable with all other insulin-re-

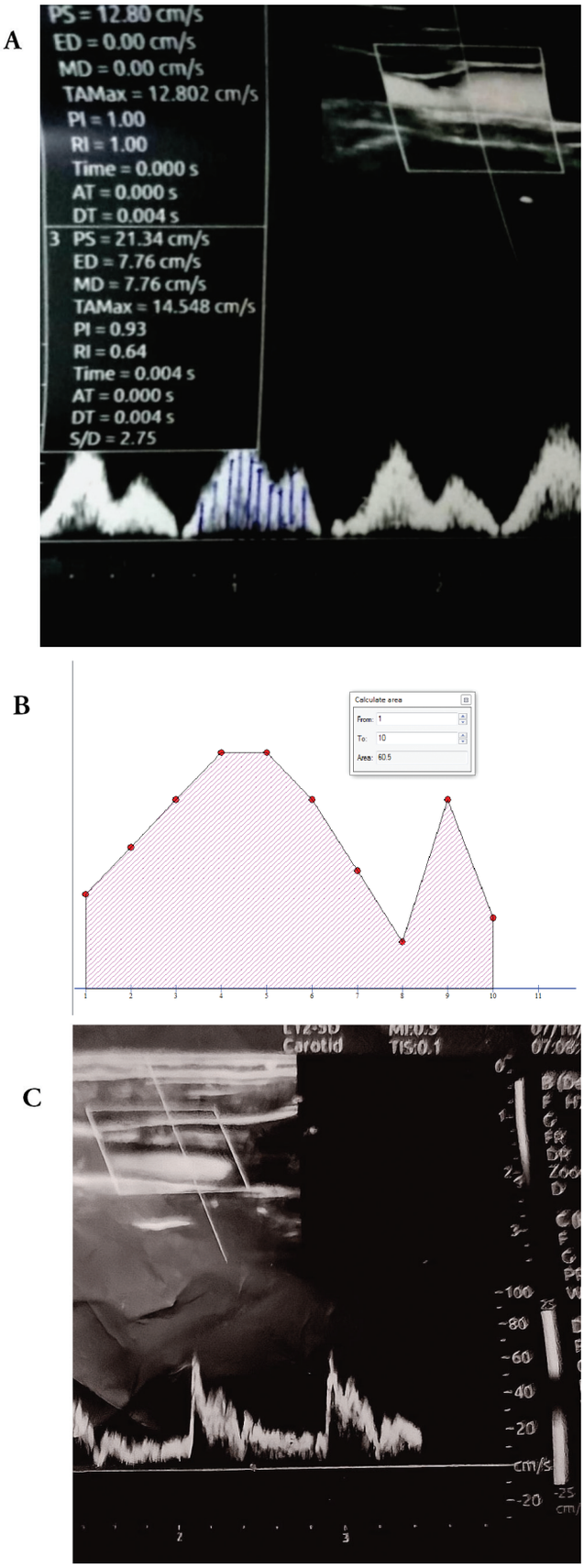

Figure 2. ***(A)*** *A standard carotid artery Doppler image is taken from the patient, note the two peaks that the wave has demarked with blue lines;* ***(B)*** *A simulation of one carotid wave Doppler image made by graph software after ten measurements were taken showing AUC = 60.5 unit^2;* ***(C)*** *Plain standard carotid artery Doppler image.*

sistant parameters for hormonal and biochemical as independent variables with their associated P-values. A P-value less than 0.05 was considered significant. Med calc version 20 was used for statistical analysis. Despite the software GRAPH being mainly used for AUC measurements, the results were further checked by Med calc and NCSS software; they can measure the area under the curve for any given point.

RESULTS

For 140 confirmed PCOS women, we collected hormonal, biochemical, and biophysical profiles in the form of CCA-IMT, PI, RI, and AUC for the carotid artery. **Table 1** shows the main characteristics of those profiles as mean and standard deviation of all variables taken in this study. To shed more light on the data shown in **Table 1** with regard to insulin sensitivity, they were subdivided into three main subgroups according to the centile of HOMA-IR to elucidate the statistical difference among them. Those groups were women who were HOMA-IR centile below 25 in the first group (n = 46), and those with HOMA-IR centile between 25th-75th as the second group (n = 48). Finally, those women whose HOMA-IR centile lies above the 75th centile represented the third group (n = 46). **Table 2** shows those women's main biochemical, hormonal, and biophysical profiles and their associated statistical comparison by one-way ANOVA test. The *post-hoc* remarks analysis is explained below. It is interesting to note in **Table 2** that AUC is different among

Table 1. *The main demographic criteria of the study variables.*

Primary criteria (n = 140)	Mean ± SD
Age (years)	24.61 ± 3.14
HDL (ng/dL)	27.18 ± 4.97
LDL (ng/dL)	164.93 ± 24.41
Serum cholesterol (ng/dL)	312.1 ± 37.31
SHBG (mole/L)	316.5 ± 39.17
FSH (IU/L)	5.45 ± 0.44
LH (IU/L)	12.17 ± 2.43
LH/FSH ratio	2.42 ± 1.74
Serum testosterone (ng/dL)	101.57 ± 11.22
Serum insulin (mIU/L)	35.34 ± 7.48
CCA intima-media thickness IMT (mm)	0.49 ± 0.08
CCA resistance index RI	0.61 ± 0.04
CCA pulsatility index PI	1.19 ± 0.12
CCA Doppler AUC	46.11 ± 12.14

HDL: high-density lipoprotein; LDL: low-density lipoprotein; SHBG: sex hormone binding globulin; FSH: follicular stimulating hormone; LH: luteinizing hormone; RI: resistance index; PI: pulsatility index; IMT: intima-media thickness.CCA: common carotid artery.

Table 2. *The demographic criteria are shown subdivided into three groups based on the HOMA-IR centiles < 25th, 25th-75th, and above 75th centiles with associated P-value by one-way ANOVA test.*

Characteristics	HOMA-IR < 25th centile (n = 46)	HOMA-IR 25th-75th centile (n = 48)	HOMA-IR 75th centile (n = 46)	P-value
HDL mg/dL	33.26 ± 1.29	26.71 ± 1.60	21.61 ± 1.34	0.001*
LDL mg/dL	135.74 ± 7.93	166.71 ± 10.19	192.26 ± 4.7	< 0.001*
Serum cholesterol mg/dL	269.61 ± 12.45	310.58 ± 12.98	356.17 ± 10.68	< 0.001*
SHBG mole/L	362.30 ± 9.81	315.50 ± 15.68	271.74 ± 13.03	< 0.001*
FSH IU/L	5.30 ± 0.36	5.44 ± 0.43	5.619 ± 0.47	< 0.05**
LH IU/L	9.43 ± 0.59	12.08 ± 1.10	15.0 ± 0.85	< 0.001*
LH/FSH ratio	1.14 ± 0.26	1.88 ± 0.19	4.25 ± 0.19	< 0.001*
Serum testosterone	89.35 ± 4.1	100.31 ± 4.02	115.11 ± 3.34	< 0.001*
Serum insulin mIU/L	27.69 ± 2.20	33.79 ± 2.824	44.61 ± 2.92	< 0.001*
CCA - RI	0.59 ± 0.04	0.61 ± 0.04	0.62 ± 0.03	0.040**
CCA - PI	1.18 ± 0.12	1.19 ± 0.13	1.20 ± 0.11	0.758
CCA-IMT mm	0.39 ± 0.03	0.49 ± 0.03	0.58 ± 0.02	< 0.001*
CCA- AUC	31.83 ± 3.90	46.54 ± 5.03	59.96 ± 2.57	< 0.001*

All data are presented as mean ± standerd deviation; **post-hoc* among all the three groups, **only between group 1 *versus* 3; HDL: high-density lipoprotein; LDL: low-density lipoprotein; SHBG:sex hormone binding globulin; FSH: follicular stimulating hormone; LH: luteinizing hormone; RI: resistance index; PI: pulsatility index; IMT: intima-media thickness.

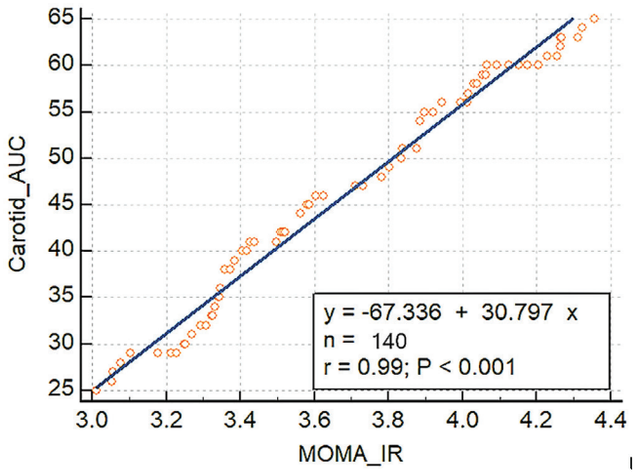

Figure 3. *Regression line showing a highly significant cause-effect relationship between HOMA-IR as an independent variable versus AUC in the carotid artery as the dependent variable.*

the three subgroups, while post hoc comparison also shows highly significant differences among each sub-group compared in pairs. While **Figure 3** shows a highly significant linear correlation between HOMA-IR – as an independent variable – *versus* AUC in the carotid artery – as the dependent variable –, with r equivalent to 0.99 and P-value < 0.001, **Table 3** compares the main biochemical, biophysical, and hormonal variables taken in this study to assess insulin resistance – as independent variables – *versus* AUC in the carotid artery Doppler – as the main dependent variables. All correlations were statistically meaningful, $p < 0.001$, with a strong correlation coefficient. Both HDL and SHBG showed an inverse correlation in contrast to the rest parameters with positive correlations, highlighted in **Table 3**.

DISCUSSION

The current study examined the value of CCA-AUC as a marker of IR and tested its correlation to hormonal and biochemical biomarkers among PCOS women. As far as this study's main finding, we find strong inverse correlations between CCA-AUC *versus* HDL and SHBG, $p < 0.0001$. These results agree with earlier researchers like Osibogun *et al*. and Macut *et al*. [21, 22].

Conversely, CCA-AUC correlated positively with [LDL, cholesterol, LH/FSH, serum insulin, CCA-IMT, and HOMA-IR], $p < 0.0001$, which was in accordance with earlier studies [7, 23, 24].

The above correlation can be explained based on PCOS pathophysiology: affected women have a state of permanent hyperandrogenaemia mediated

Table 3. *A series of correlation coefficients between the common carotid artery- area under the curve (CCA-AUC) and various variables taken in this study with their associated P-values.*

CCA-AUC *versus* variable	Correlation coefficient (r)	P-value
HDL	-0.98	< 0.001*
LDL	0.99	< 0.001*
Serum cholesterol	0.99	< 0.001*
SHBG	-0.99	< 0.001*
LH/FSH ratio	0.78	< 0.001*
Serum testosterone	0.98	< 0.001*
Serum insulin	0.97	< 0.001*
IMT	0.99	< 0.001*
HOMA-IR	0.99	< 0.001*

*Significant at 0.05; HDL: high-density lipoprotein; LDL: low-density lipoprotein; SHBG: sex hormone binding globulin; FSH: follicular stimulating hormone; LH: luteinizing hormone; RI: resistance index; PI: pulsatility index; IMT: intima-media thickness; CCA: common carotid artery; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance.

by an elevated luteinizing hormone from the pituitary gland. LH affects both the ovarian stroma and the adrenal cortex to overproduce androstenedione, which acts as an insulin sensitizer at the cell membrane level. Moreover, increased LH levels reduce sex hormone-binding globulin production from the liver, leading to high serum-free testosterone levels. Testosterone is well known to lower high-density lipoprotein and cause a significant increase in low-density lipoprotein. Those lipid serum changes are associated with significantly increased serum cholesterol levels and lower serum HDL among PCOS women. The permanent rise of IR, manifested by high HOMA-IR, is associated with a wide range of complications. Indeed, PCOS patients had a two-fold greater risk of ischemic heart disease, cerebrovascular accident, and higher coronary artery and aortic calcification incidence [4, 6, 7]. The main finding of this study is the correlation of CCA-AUC with IR among PCOS women. IR is a key player in PCOS patients, and it promotes premature atherosclerosis in more than one way [25]. Insulin resistance increases lipolysis and promotes serum dyslipidaemia; the latter has an apoptosis effect on pancreatic islets. Eventually, IR will result in a vicious circle of glucose intolerance and a state of persistent hyperglycaemia.

Additionally, IR is associated with hypertension because it inhibits the insulin-specific endothelium pathway that generates nitric oxide, diminishing its relaxing impact on the blood vessels. Enhanced vasoconstriction of the blood vessels speeds up blood

flow and increases arterial pressure. Lastly, persistent hyperinsulinemia causes endothelial damage, inflammation, and thickening of the artery wall (through IMT) and contributes to atherosclerosis [26-28]. The other arterial flow parameters, RI and PI of the CCA, further support our results, which showed a trend of positive correlations with increased severity of insulin resistance, manifested by HOMA-IR. However, PI failed to have statistical significance in subgroup analysis, $p = 0.76$, while RI was statistically significant, $p = 0.040$. These results were consistent with the findings of Lakhani [29, 30].

The effect of BMI on the increased IMT was discussed by Talbott *et al*. [31] who enrolled older PCOS cases, and Lakhani *et al*. [30], who recruited younger PCOS cases. They confirmed that increased IMT was independent of the BMI, disturbed lipid profile, and insulin levels. Their result signifies that PCOS is an independent risk factor for premature atherosclerosis. Even after adjusting for body mass index, the affected women had more CCA stiffness than the controls [30-32].

Another study found a strong positive correlation between hyperglycaemia, HOMA-IR *vs* PI, and RI in a group of diabetic patients they examined. They recommended using PI and RI as early IR progression markers among diabetic patients [6].

To this point, our study showed that the Doppler of the artery of one heartbeat waveform equals exactly the amount of fluid or blood passed per one beat or unit time [33]. In other words, measuring the amount of blood passed per heartbeat is an indirect measure of the insulin resistance or HOMA-IR found among women with PCOS, which forms the study's novelty.

Study strength

AUC was easily calculated with free software downloadable from the website. Measuring CCA-IMT is non-invasive, feasible at all centres, and accepted by most patients [24]. At least it does not involve needle pricking. HOMA-IR calculation requires measuring fasting insulin and fasting blood sugar and has reproducibility issues, in addition to the financial feasibility of Doppler compared to biochemical testing [34].

The future implications of CCA-AUC

The future implications of CCA-AUC can identify women at the highest risk for IR and cardiovascular-related comorbidities to benefit from therapeutic or preventive intervention [35-37].

The CCA-IMT may serve as a diagnostic and prognostic marker in PCOS, by estimating the magnitude of IR and simultaneously assessing the usefulness of insulin sensitizer therapies in addition to lifestyle changes, that will help to reduce modifiable risk factors. So CCA-IMT can be diagnosed, evaluated the risk, and used for follow-up and prognostic values.

Many acknowledge that IR and chronic hyperglycaemia make PCOS women more prone to earlier signs of systemic atherosclerosis, which can have long-term effects on the vasculature [26]. Thus, in those at high risk, early vascular screening may be beneficial in reducing disease morbidity [38-40].

Study limitation

The IR and chronic hyperglycaemia make PCOS women more prone to earlier signs of systemic atherosclerosis, which can have long-term effects on the vasculature [26]. Thus, in those at high risk, early vascular screening may be beneficial in reducing the disease morbidity [40, 43].

We recommend further studies, possibly by adding an aged-matched control group, so that observed CCA-IMT changes would be exclusively related to the PCOS pathological process to eliminate other clinical conditions where similar findings of CCA-IMT are seen.

CONCLUSIONS

CCA-AUC was correlated with all the hormonal, biochemical, and biophysical changes seen among women with PCOS by a strong, statistically meaningful correlation. It was linked with adverse glycaemic and cardiovascular risks independent of the body mass index. CCA-AUC simplicity and accuracy make it a recommendable parameter in the follow-up panel of PCOS women for its diagnostic and prognostic values.

COMPLIANCE WITH ETHICAL STANDARDS

Authors contribution

W.N.: Study design, conceptualization, writing – original draft, writing – review & editing. WA:

Methodology. M.A.Z.: Data collection, methodology. W.N., W.A.: Writing – original draft, statistical analysis.

Funding

None.

Study registration

N/A.

Disclosure of interests

The authors declare that they have no conflict of interests.

Ethical approval

The ethics committee of Mustansiriyah University, College of Medicine, approved the study (IRB 180 on 1-1-2019). The study followed the Helsinki declaration, and all study method was done under Helsinki tent and comparable medical standard.

Informed consent

All women gave informed consent before participation.

Data sharing

All data are available under reasonable request to the corresponding author.

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