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Evaluation of Testosterone, TRH, Oxidative Stress enzymes, and some biochemical variables in women with polycystic ovary syndrome

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Abstract:

Polycystic ovarian syndrome is a common endocrine disorder affecting 8–13% of women of reproductive age. Objectives: This study aimed to assess hormone levels and oxidative stress enzymes in women with PCOS. Materials and Methods: The study included 90 women (30 healthy controls and 60 PCOS patients aged 18–48 years on metformin). Blood samples (5–8 ml) were collected, and serum was analyzed for hormonal levels, oxidative stress enzyme activity, and random blood sugar (RBS).Results: Compared to controls, PCOS patients showed a non-significant decrease in testosterone (0.46±0.22 vs. 0.36±0.2, $p = 0.0505$), and a significant decrease in thyroid-stimulating hormone (TSH) (2.31±0.82 vs. 1.63±0.87, $p < 0.0001$). Glutathione peroxidase levels were significantly reduced (57.59±36.72 vs. 28.24±22.49, $p < 0.0001$), while decreases in glutathione S-transferase and catalase were non-significant. RBS showed a slight, non-significant increase (106.1±0.22 vs. 102.68±29.79, $p = 0.0284$). ROC analysis revealed an AUC of 0.751 for glutathione peroxidase and 0.74 for TSH, suggesting both markers have a strong association with PCOS.

Keywords: Polycystic ovary syndrome, Testosterone, Thyroid stimulating hormone, Random blood sugar, oxidative stress enzymes.

تقييم هرمون التستوستيرون، وهرمون تحرير الغدة الدرقية، وأنزيمات الإجهاد التأكسدي، وبعض المتغيرات الكيميائية الحيوية لدى النساء المصابات بمتلازمة تكيس المبايض

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مستخلص:

متلازمة المبيض المتعدد الكيسات هي اضطراب شائع في الغدد الصماء يصيب 13-8٪ من النساء في سن الإنجاب. الأهداف: هدفت هذه الدراسة إلى تقييم مستويات الهرمونات وإنزيمات الإجهاد التأكسدي لدى النساء المصابات بمتلازمة تكيس المبايض. المواد والطرق: شملت الدراسة 90 امرأة (30 مريضا صحيا و60 مريضا بمتلازمة تكيس المبايض تتراوح أعمارهم بين 18 و 48 عاما يتناولون الميتفورمين). تم جمع عينات الدم (8-5 مل) ، وتم تحليل المصل للمستويات الهرمونية ونشاط إنزيم الإجهاد التأكسدي وسكر الدم العشوائي (RBS). النتائج: بالمقارنة مع الضوابط ، أظهر مرضى متلازمة تكيس المبايض انخفاضا غير معتد به في هرمون التستوستيرون (0.22±0.46 مقابل 0.2±0.36 ، ص = 0.0505) ، وانخفاض كبير في الهرمون المنبه للغدة الدرقية (0.82±2.31) (TSH مقابل 0.87±1.63 ، ص < 0.0001). انخفضت مستويات الجلوتاثيون بيروكسيداز بشكل ملحوظ (36.72±57.59 مقابل 22.49±28.24 ، ص < 0.0001) ، في حين أن الانخفاض في الجلوتاثيون S-transferase والكاتالاز لم يكن كبيرا. أظهر RBS زيادة طفيفة وغير معتد بها (0.22±106.1 مقابل 29.79±102.68 ، ص = 0.0284). كشف تحليل ROC عن AUC قدره 0.751 للجلوتاثيون بيروكسيداز و 0.74 ل TSH ، مما يشير إلى أن كلتا العلامتين لهما ارتباط قوي بمتلازمة تكيس المبايض .

الكلمات المفتاحية: متلازمة المبيض المتعدد الكيسات ، التستوستيرون ، هرمون تحفيز الغدة الدرقية ، سكر الدم العشوائي ، إنزيمات الإجهاد التأكسدي.

Introduction

Polycystic ovarian syndrome (PCOS) is the predominant endocrinological disorder in women, impacting 8% to 13% of those of reproductive age (1). Polycystic ovarian syndrome was initially identified in females in 1935 by Stein and Leventhal. Hyperandrogenism, a variety of male-like traits, is a symptom of polycystic ovarian syndrome, which is why the condition is sometimes called Stein and Leventhal syndrome. Obesity, subcutaneous and abdominal fat, hirsutism, alopecia, clitromegaly, a deep voice, seborrhea, acne, and other physical indications of hyperandrogenism are many. Alterations to the metabolic profile occur apart from these physical indicators. Hyperandrogenism individuals, especially those with polycystic ovary syndrome (PCOS), often have low levels of serum sex hormone-binding globulin (SHBG) (2). Women who suffer from polycystic ovary syndrome (PCOS) may experience oxidative stress due to certain symptoms such as elevated androgen hormones, insulin resistance, belly fat, and obesity. Yet, elevated oxidative stress and impaired antioxidant capacity may contribute to insulin resistance, central obesity, hypertension, cardiovascular disease, and hyperlipidemia (3). The imbalance between oxidants and antioxidants, along with the excessive creation of reactive oxygen species (ROS), is known as oxidative stress. Many studies have shown that oxidative circulating indicators are much higher in PCOS patients than in the general population, suggesting that they may play a role in the development of PCOS (4).

There are two main types of these processes: enzymatic and nonenzymatic. Some examples of enzymes that can neutralize free radicals are catalase, superoxide dismutase (SOD), glutathione reductase (GR), and glutathione oxidase (GPX). Enzymatic antioxidants such as superoxide dismutase (SOD), catalase (GPX), and GP are essential for eliminating oxygen compounds that are damaging to cells (5). The most prominent example of the nonenzymatic antioxidants found in embryos and oocytes is glutathione (GSH) (6). The following are examples of antioxidants that do not involve enzymes: carotene, beta-carotene, selenium, zinc, vitamin C, and vitamin E. (7). For ROS me-

tabolism to work, catalase is an essential enzyme. This is why catalase has been the subject of so much research as of late. On the other hand, there is scant data on the role of catalase in regulating follicular differentiation and growth. Peroxisomes are the main locations for catalase. In 1975, immunohistochemistry was the first method to identify catalase in ovarian tissue (8). Enzymes belonging to the GPx family prevent oxidative stress by converting hydroperoxides of lipids to their alcohol counterparts and hydrogen peroxide to water. Various studies documented the examination of GPx activity with the purpose of assessing antioxidant defense in PCOS. Sabuncuet al. assessed the antioxidant and oxidant levels of polycystic ovary syndrome (PCOS) patients. It was shown that GPx was similar in the PCOS group compared to the healthy control group (4). An essential class of enzymes known as glutathione S-transferases (GSTs) removes toxins from the body and from outside sources, including drugs and pollution (9)First described in 1961 by Booth et al., the Glutathione-S-transferase (GST) is (10). Xenobiotics that are electrophilic can be neutralized by glutathione-s-transferase. Additionally, the body's excretion is provided by the dimeric enzymes (11,12). The GST family is able to destroy reactive oxygen derivatives that are hazardous when oxidative stress occurs because of its antioxidant actions. Many disorders, including polycystic ovary syndrome (PCOS), in women may be caused by mutations in GST enzymes or other structural or genetic abnormalities (13).

One of the most important anabolic steroids is testosterone. The body cannot function properly without this sex hormone. In humans, it controls parameters such as libido, fat distribution, hair growth, bone density, muscular mass, and RBC count. Additionally, it is essential for the maturation of the testes and prostate, two male reproductive organs, and for the creation of sperm. Estradiol, a kind of estrogen, is produced from a little quantity of circulating testosterone through a biosynthetic process that begins with cholesterol (14). Identifying patients with aberrant androgen constellations is crucial since PCOS patients with androgen excess are at a greater risk for metabolic repercussions including insulin

resistance and liver disorders (15). The anterior pituitary gland's thyrotrophs (basophile cells) produce and emit thyroid stimulating hormone (TSH), a glycoprotein. As with follicle-stimulating hormone (FSH), luteinizing hormone (LH), and hCG, it is a glycoprotein hormone. To far, glycoprotein hormones have been identified as one of the most complex and massive endocrine ligands. These hormones are biologically distinct because they are heterodimeric cystine-knot glycoproteins with a common -subunit and a unique -subunit for each hormone (16). Some thyroid illnesses share clinical symptoms with polycystic ovary syndrome. Recent studies have shown that the incidence of hypothyroidism is higher in people diagnosed with PCOS (11–14%) compared with control participants (1-2%), adding to the growing body of data linking PCOS to an increased incidence rate of thyroid illnesses (17).

Among carbohydrates, glucose, or random blood sugar (RBS), is the most vital fuel for the body. Because it cannot be further broken down by hydrolysis, glucose is categorized as monosaccharide. In the fed state, diet is the primary source of circulating glucose. Due to its six carbon atoms and the presence of an aldehyde group on carbon 1, it is further classed as a hexose and an aldose, respectively. Glucose levels in the blood typically remain largely constant between 80 and 120 mg/dl (18). Metabolic diseases include obesity, insulin resistance, and hyperglycemia are associated with polycystic ovary syndrome (PCOS). Type 2 diabetes mellitus (T2DM) was four times more common in PCOS patients compared to normal controls in the Danish national registration study, and patients with PCOS were diagnosed at a younger age (19).

Materials and Methods

This study was conducted at the Chemistry Department, College of Science, University of Tikrit, from September 2023 to January 2024. Blood samples were collected from women diagnosed with polycystic ovary syndrome (PCOS) who attended private clinics in Tikrit. The study included 60 women aged between 18 and 48 years. Blood samples were collected under aseptic conditions using sterile disposable syringes. Serum was separated by centrifugation at 3000 rpm for 10 min-

utes and stored at –20°C until analysis. Biochemical parameters were measured using ELISA kits for hormones (LH, FSH, insulin, testosterone) and spectrophotometric methods for glucose and lipid profile. All reagents and kits were obtained from reliable commercial sources and used according to the manufacturers' instructions. Statistical analysis was performed using SPSS software version 25.

Biochemical tests: Hormones include Testosterone, thyroid stimulating hormone (TSH), and Random blood sugar (RBS) were measured by using (chemiluminescence immunoassay Mindray CL-900i (Mindray Bio-Medical Electronics, Shenzhen, China) (20). The activity of oxidation enzymes was measured using a device UV-Vis Spectra Photometer (UV-752, Shanghai Yoke Instrument Company, Shanghai, China) (21).

Statistical analysis: The researchers used SPSS and MedCalc to run an F-test with a significance level of $P < 0.001$ to find out if the biochemical parameters being evaluated were different in the PCOS patient group compared to the control groups. Through the use of receiver operating characteristic (ROC) curve analysis, the diagnostic accuracy of serum biochemical parameter levels in distinguishing between patients and control groups was assessed. This method was also used to find the optimal cut-off levels for the biochemical parameters and Testosterone that were being studied. Additionally, the AUC was calculated.

Results and discussions

The below table shows there was a significant decrease in the levels of thyroid- stimulating hormone ($P<0.0001$) in the blood of the group of patients also in Glutathione peroxidase enzyme . in other hand there is no significant in Glutathione-S-transferase, Catalase, RBS and Testo.

Table: (1) Serum biochemical parameters levels patients with PCOS

Parameters	Controls (mean ± SD)	PCOS Patients (mean ± SD)	p-value
Testo(ng/ml)	0.46 ± 0.22	0.36 ± 0.2	0.0505
RBS (mg/dl)	106.1 ± 14.56	102.68 ± 29.79	0.0284
TSH(µIU/ml)	2.31 ± 0.82	1.63 ± 0.87	<0.0001
Glutathione peroxidase	57.59 ± 36.72	28.24 ± 22.49	<0.0001
Glutathione-S-transferase	0.34 ± 0.371	0.373 ± 0.315	0.2059
Catalase	101.51 ± 71.04	93.94 ± 94.108	0.2657

It was noted that there was a significant decrease in the levels of thyroid- stimulating hormone (P<0.0001) in the blood of the group of patients taking drugs (metformin) according to the results The results of this study are consistent with many other studies that showed a decrease in thyroid-directing hormone levels in patients with PCOS (22–24). While thyroid-stimulating hormone levels have been found to have increased in patients with polycystic ovaries in other studies (25). When treating type 2 diabetes, metformin is often prescribed as a first-line medication. Women with polycystic ovary syndrome (PCOS), type 2 diabetes (T2DM), or impaired glucose tolerance (IGT) who do not respond to lifestyle changes, or who experience menstrual irregularities and are unable to use or tolerate hormonal contraceptives, should be considered for metformin as a second-line treatment, according to the Endocrine Society. The production of androgens by the ovaries may be directly reduced by metformin. When coupled with clomiphene citrate, metformin increases the rate of ovulation in women who are resistant to it alone. When compared to Caucasians and Europeans of the same body mass index, South Asians exhibit far lower insulin sensitivity and insulin resistance. Within four months of starting 6-month metformin monotherapy, menstrual periods returned to normal, and within three months of stopping the drug, the patient's condition had returned to its pre-treatment state. Some recent studies have shown that metformin can affect thyroid function tests, specifically

by lowering serum thyrotropin (TSH) levels. On the other hand, euthyroid patients who did not have any underlying thyroid malfunction did not see any changes in TSH levels when taking metformin. Complex and multifaceted is the mechanism of metformin's impact on the thyroid axis. Metformin has the potential to alter either the quantity or the affinity of thyroid hormone receptors. It might have an immediate effect on TSH control or raise central dopaminergic tone.

Because of this, the pituitary gland is able to receive thyroid hormones more effectively [16]. The hypothalamic AMPK activity is inhibited by metformin, and the inhibitory control of thyroid hormones on TSH secretion is enhanced. The study's findings are supported by the observation that the patient group's blood testosterone levels decreased, though not significantly, to 0.36 ±0.2, which is slightly lower than the healthy group's (0.46 ±0.22) levels 26,27)). Although histologic studies of ovaries from older PCOS women are not yet available, it is believed that the number of theca cells decreases and the rate of primary and antral follicle loss accelerates in normal women starting around the ages of 37 to 38. In PCOS, the androgens come from hyperplastic theca cells. The decline in testosterone levels in middle-aged PCOS women may be explained by a decrease in the number of theca cells in their bodies. Another possible cause of decreased androgen production could be an age-related decline in an enzyme involved in testosterone biosynthesis, like P450c17, which has been linked to polycystic ovary syndrome (PCOS). Metformin, an insulin sensitizing drug, can help reduce serum testosterone levels by acting on serum insulin and enhancing tissue insulin sensitivity in polycystic ovary syndrome (PCOS). It also has a slight positive effect on menstrual cycle regularity and other clinical outcomes. Additionally, it might help with dyslipidemia, hypertension, and obesity, which are all symptoms of syndrome X. But if the follow-up research continues for longer, we can see the clear clinical improvement, which is marked by less hirsutism, acne, and higher fertility. Consistent with the findings of the last study, it was observed that patients' blood sugar levels (102.68 ±29.79) were somewhat higher than healthy people's (106.1 ±14.56)

(28). Patients with polycystic ovary syndrome often have insulin resistance, which causes hyperinsulinemia and eventually hyperglycemia and type 2 diabetes mellitus. Elevated basal insulin production and reduced hepatic insulin clearance are likely the causes of hyperinsulinemia. Insulin production is abnormally low compared to insulin resistance in PCOS women, regardless of obesity status, indicating pancreatic P-cell malfunction. showed that PCOS women had impaired P-cell function, as indicated by reduced postprandial insulin secretory responses and irregularities in entrainment of insulin secretion to an oscillatory glucose infusion. In polycystic ovary syndrome, P-cell dysfunction may coexist with glucose intolerance. To lower the risk of cardiovascular disease, women with polycystic ovary syndrome (PCOS) should undergo glucose intolerance testing because they are at increased risk for developing type 2 diabetes mellitus and glucose intolerance at an earlier age. Age, body mass index (BMI), and waist circumference (WC)—all of which raise risk—determine the testing frequency. Additionally, it was observed that patients with polycystic ovary syndrome (PCOS) have significantly lower levels of the glutathione peroxidase enzyme ($P<0.0001$), and these findings align with those of earlier research29,30)). The GPx is a selenium-containing enzymatic antioxidant. To produce glutathione disulfide, it oxidizes glutathione and successfully decreases lipid peroxides to lipid alcohols and water, respectively.

Inadequate glutathione levels or GPx activity prevent the detoxification of hydrogen peroxide and lipid peroxides, leading to their conversion to hydroxyl and lipid peroxyl radicals, respectively. The levels of the enzyme catalase in the serum of patients were found to be lower (93.94 ±94.108) compared to healthy individuals (101.51 ±71.04), which is in line with the findings of the prior study (31). Catalase is an antioxidant enzyme found mostly in cell peroxisomes and to a lesser extent in the cytoplasm. It catalyzes the reduction of hydrogen peroxide to water and molecular oxygen. The levels of the enzyme glutathione-S-transferase in the serum of patients were found to be somewhat lower (0.373 ±0.315) compared to healthy individu-

als (0.34 ±0.371), which is in line with the findings of the prior research (32). The glutathione-S-transferase (GST) family of proteins is an electrophilic chemical family that may detoxify the blood of toxic hydrophilic substances. In addition to its role in xenobiotic and free radical detoxification, glutathione transferase (GST) catalyzes the conjugation of many endogenous substrates, such as steroids and prostaglandins. Further roles for GST include binding to bile acids, neurotransmitters, and steroid hormones. Hyperandrogenemia is thought to contribute to polycystic ovary syndrome (PCOS) via increasing free radical generation. The egg follicles of PCOS patients are impacted by the increased oxidative stress. Some research has linked polycystic ovary syndrome (PCOS) to oxidative stress indicators. An increase in dangerous compounds termed reactive oxygen species (ROS) happens when the amounts of oxidant and antioxidant molecules are different.

Table: (2) AUC, cut off values, sensitivity, and specificity for biochemical parameters by ROC analysis in patients and control groups

Parameters	Cut off	Specificity	Sensitivity	+PV	-PV	ACCURACY	AUC
RBS (mg/dl)	≤97.6	66.67	58.33	N/A	N/A	0.3333	0.627
Testo(ng/ml)	≤0.37	60	62.07	N/A	N/A	0.2207	0.625
TSH(µIU/ml)	≤1.9	73.33	73.33	N/A	N/A	0.4833	0.74
GPX	≤38.67	66.67	83.33	83.3	66.7	0.5	0.751
Glutathione S	>0.259	53.33	56.67	N/A	N/A	0.3	0.590
Catalase	≤69.98	56.67	55	N/A	N/A	0.2333	0.574

When comparing the patient group with the control group, the analysis of the characteristics of the receiver trigger shows that the enzyme glutathione peroxidase is signifying a strong association with the disease (P<0.0001 : AUC 0.751) as well as for thyroid-stimulating hormone also signifying a strong association with the disease (P<0.0001 : AUC 0.74) While catalase (P=0.2657 : AUC 0.574)and glutathione-S-transferase (P=0.2059 :AUC 0.590), testosterone (P=0.0505 : AUC 0.625) and blood sugar (P=0.0284 : AUC 0.3333)

show no significant difference respectively. The parameters with the highest reported sensitivity values were: GPX 83.33%, BMI 80%, TSH 73.33%, Testerone 62.07%, Blood Sugar 58.33%, Glutathione - S-Transferase 56.67% and Catalase 55% according to Table 2 and Figure 1-6.

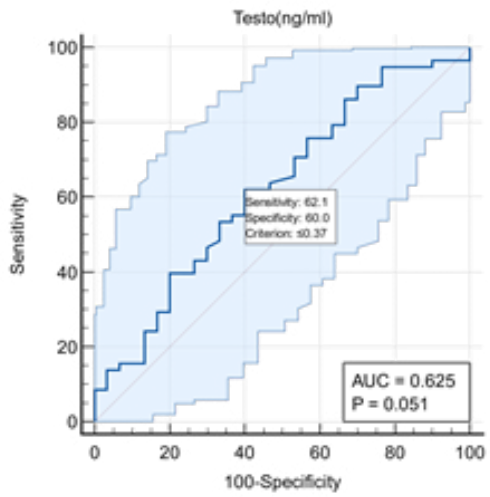

Fig. 1. ROC analysis of Testo in patient and control groups

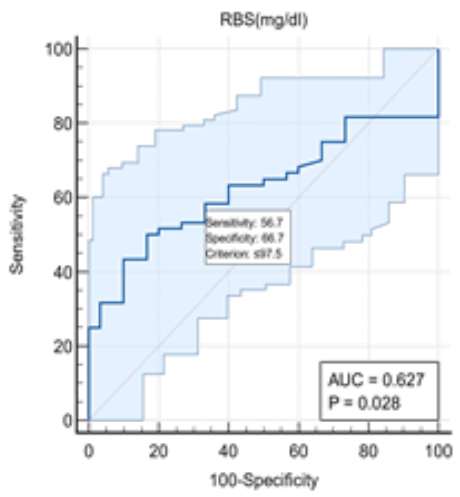

Fig. 2. ROC analysis of RBS in patient and control groups

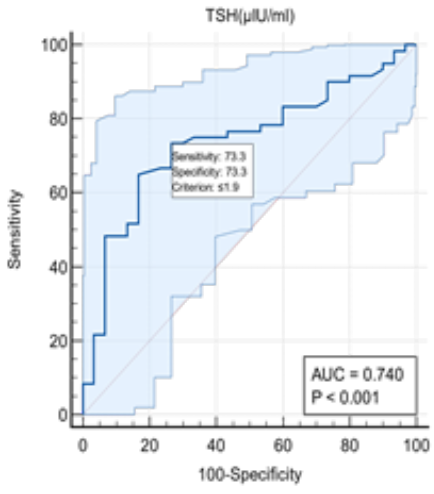

Fig. 3. ROC analysis of TSH in patient and control groups

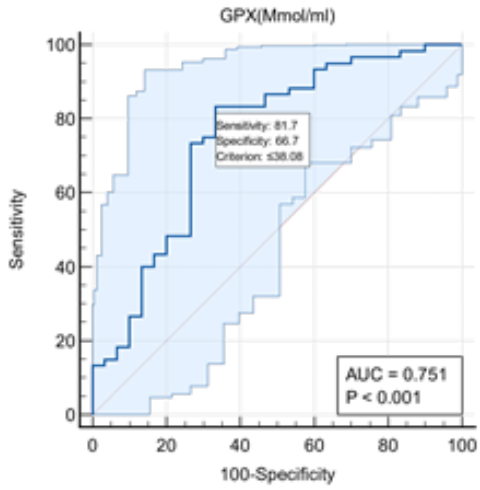

Fig. 4. ROC analysis of GPX in patient and control groups

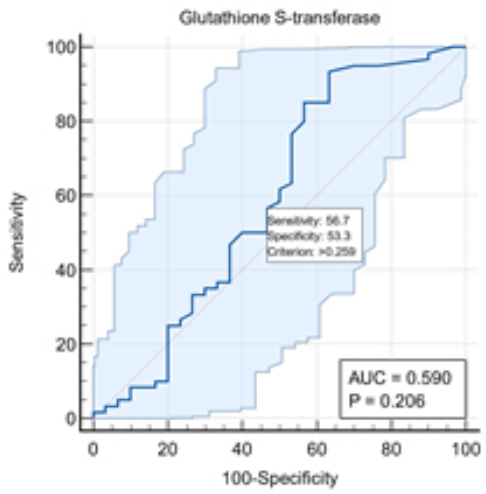

Fig. 5. ROC analysis of Glutathione in patient and control groups

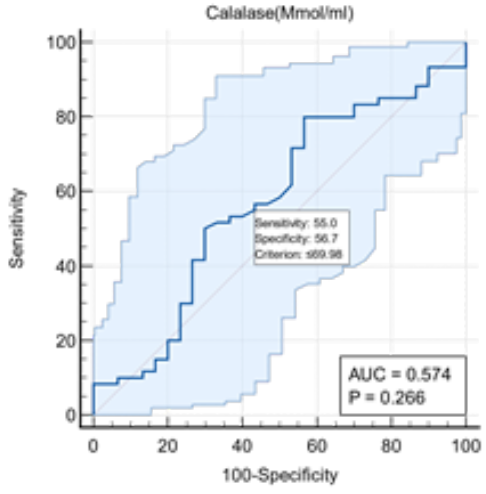

Fig. 6. ROC analysis of Catalase in patient and control group

Conclusion:

PCOS is characterized by abnormal reductions in oxidative stress enzymes and thyroid-stimulating hormone levels. It is recommended that patients with PCOS who experience menstrual cycle irregularities and ovulatory disturbances undergo monitoring of TSH and testosterone levels during treatment, as these may serve as useful biomarkers.

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