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Assessment of Total Serum Prostate Specific Antigen (TPSA), Anti Mullerian Hormone (AMH) Level among Sudanese Women with Polycystic Ovarian Syndrome

(A study in Khartoum State)

التقييم المصلي الكلي لمستضد البروستاتا المحددة ومستوى مضاد هرمون مولريان
لدي النساء السودانيات المصابات بمتلازمة تكيس المبايض
في ولاية الخرطوم

A dissertations submitted in partial fulfillment for the requirement of master Degree in clinical chemistry

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DEDICATION

To My Princess Mam, Dad, My Brothers and My Friends……..With Love

Safaa …

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Thanks first to **ALLAH** who enabled me conduct this study by the grace of him and give me strengths and patience, my respect to my supervisor **Dr.Ghada** for her patience, wisdom, invaluable sound advice and careful guidance.

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Words can never help to express my feeling towards every one stand beside me to carry this work. So I would like to thanks all those offered me and helped me to complete this work.

Thanks all…

Abstract

Back ground: Polycystic ovarian syndrome (PCOS) is one of the commonest endocrine disorders in women of reproductive age group, cause of androgen excess in women. (PSA) may be a new one diagnostic tool for PCOS. Serum AMH is synthesized by small antral follicles, which are precisely those seen on ultrasound and could help us to diagnose PCOS. The aim of this study to evaluate the levels of serum TPSA, AMH among Sudanese women with polycystic ovary syndrome and to determine the performance of PSA in diagnosis of PCOS.

Material and Method: In a cross sectional case control study, 50 women newly diagnosed with polycystic ovary syndrome and 50 apparently healthy controls were enrolled. Sample were collected during the period between February to May 2017 from Elsir Abo Alhassen Center for Infertility in Khartoum State, serum TPSA , AMH levels were measured using an ultrasensitive method (Electrochemiluminesent (ECL) immunoassay method) . Results were analyzed using statistical package for social science (SPSS) computer program.

Result: Serum levels of TPSA, AMH were significant higher in polycystic ovary syndrome group versus control group with mean ± SD for TPSA (0.019 ±0.009 ng/ml) versus control group (0.001 ± 0.001 ng/ml) and a p.value=0.00. AMH was (14.11±9.2 ng/ml) versus control group (2.404 ± 1.00ng/ml) p.value 0.00. There was a slight decrease in level of TPSA in females who had regular cycle in contrast to females that had irregular cycle with p.value = 0.04, where there was no difference in AMH p.value = 0.6. Also, this study showed that there was positive correlation between TPSA and BMI (P.value=0.00, r=.893) and negative correlation between AMH and BMI (P.value=0.025, r = -.317). The majority of females had irregular cycle were obese 36%, the overweight were 24% and those with normal weight were12%. Also there was significant increase in TPSA and AMH in PCO patients who had family history, TPSA (mean+ SD 0.022 ± 0.021

ng/mL versus 0.0165±0.006 ng/ml) pvalue 0.03, and AMH (mean+ SD 21.3±10.0 ng/ml versus 10.6± 5.6 ng/ml) p value 0.00.

Conclusion: The study concluded that the serum levels of TPSA, AMH are increased in PCOS. TPSA and AMH were significantly increased in patients who had family history.

المستخلص

المقدمة: متلازمة تكيس المبايض هي واحدة من اضطرابات الغدد الصماء الأكثر شيوعا في النساء في الفئة العمرية الإنجابية، سبب زيادة الاندروجين في النساء. البروستاتاك اسبيسفك انتيجين، لم يعد أن يكون علامة محددة لورم انسجة البروستاتا فقط، قد يكون أداة تشخيصية جديدة ل تكيس المبايض.يتم تصنيع أل انتي مليرين هرمون من قبل بصيلات أنترال صغيرة، والتي هي على وجه التحديد تلك التي رأيت على الموجات فوق الصوتية ويمكن أن تساعدنا على تشخيص تكيس المبايض، والهدف من هذه الدراسة لتقييم مستوى البروستاتاك اسبيسفك انتيجين، أنتي مليرين هرمون بين النساء السودانية مع متلازمة المبيض المتعدد الكيسات.

المواد والطريقة: في دراسة المقطع العرضي للحالة تم تسجيل 50 امرأة مصابة حديثا بمتلازمة المبيض المتعدد الكيسات و 50 امرأة على ما يبدوبحالة صحية جيدة، وتم جمع العينات في الفترة ما بين شباط / فبراير إلى أيار / مايو 2017 من مركز السيد أبو الحسن للعقم في ولاية الخرطوم، تم قياس مستوى انتي مليرين هرمون, بروستاتاك اسبيسفك انتيجين باستخدام طريقة أولتراسنزيتيف (طريقة إلكتروشيميلومينيسنت المناعية)، وتم تحليل النتيجة باستخدام حزمة إحصائية للعلوم الاجتماعية برنامج الكمبيوتر.

النتيجة: كان مستوى مصل بروستاتاك اسبيسفك انتيجين، أنتي مليرين هرمون أعلى معنويا في مجموعة متلازمة المبيض المتعدد الكيسات مقابل مجموعة مجموعة السيطرةالمتوسط ± الانحراف المعياري ل بروستاتاك اسبيسفك انتيجين (0.019 ± 0.009نانو غرام / مل) مقابل مجموعة السيطرة (0.001 ± 0.001 نانوغرام / مل) القيمة الاحتمالية= 0.00 ، أنتي مليرين هرمون (14.11 ± 9.2نانوغرام / مل) مقابل مجموعة السيطرة (2.404 ± 1.00نانوغرام / مل) القيمة الاحتمالية=0.00. كان هناك انخفاض طفيف في مستوى بروستاتاك اسبيسفك انتيجين لدى الإناث لديهن دورة منتظمة على النقيض من الإناث اللاتي كانت لديهن دورة غير منتظمة القيمة الاحتمالية=0.04 ، حيث لم يكن هناك اختلاف في مستوي انتي مليرين هرمون القيمة الاحتمالية=0.6 ، كما أظهرت هذه الدراسة وجود علاقة ارتباط إيجابية بين بروستاتاك اسبيسفك انتيجين و مؤشر كتلة الجسم (القيمة الاحتمالية=0.00 ، r = .893) و الارتباط السلبي بين أنتي مليرين هرمون و مؤشر كتلة الجسم (القيمة الاحتمالية=0.025 ، r = -.317). كانت غالبية الإناث غير منتظمة دورة من البدناء= 36٪، أكثر من الوزن الطبيعي= 24٪ والوزن الطبيعي =12٪. كان هناك زيادة معنوية في مستوى مصل بروستاتاك اسبيسفك انتيجين و أنتي مليرين هرمون في المرضى اللذين لديهم تاريخ عائلي للمرض, بروستاتاك اسبيسفك انتيجين (متوسط ± الإنحراف المعياري 0.022±0.021 نانوغرام/مل

مقابل 0.016±0.006 نانوغرام/مل) القيمة الإحتمالية(0.03)أنتي مليرين هرمون (متوسط ± الإنحراف المعياري 21.3± 10.0 نانوغرام/مل مقابل 10.6± 5.6 نانوغرام/مل) القيمة الإحتمالية(0.00).

الاستنتاج: خلصت الدراسة إلى أن مستويات مصل بروستاتك اسبيسفك انتيجين و أنتي مليرين هرمون تزداد في متلازمة تكيس المبايض. زيادة معنوية في مستوى توتال اسبيسفك انتيجين وانتي مليرين هرمون في المرضى اللذين لديهم تاريخ عائلي للمرض.

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Introduction, Objectives and Rationale

1.1 Introduction

Polycystic ovarian syndrome (PCOS) is the most common endocrinopathy of women, is combination of chronic anovulation or oligomenorrhoea and clinical or biochemical hyperandrogenism and ovarian polycystic changes are observed by ultrasound (Diamanti., 2008), affect about 6–8% of the population (Rudnicka et al., 2016). In recent years, acceptance of the concept that PCOS is a heterogeneous disorder (thatis, capable of having somewhat different manifestations in different people) and the exact cause not known until now, and it is a very common problem among patients attending infertility clinics (Rudnicka et al., 2016). Recent study conducted to assess Serum Anti-Müllerian hormone (AMH) as laboratory predictor in infertile women with PCOS (Tayrab et al., 2014). Prostate specific antigen (PSA) is a serine protease of human glandular kallikrein family and has historically been used as the most specific and sensitive marker for prostatic cancer in males (Nagar., and Msalati., 2013), PSA production in the prostate is under the control of steroid hormones. Androgens up-regulate the expression of the PSA gene through the androgen receptor, PSA has been detected in some female tissues (including breast, ovarian, and endometrial tissues) and body fluids (amniotic fluid, milk, and breast cyst fluid) (Melegos et al., 1997), The presence of PSA in these female tissues seems to be associated closely with steroid hormone regulation, especially androgens, glucocorticoids, and progestin but not estrogen (Mashkoor et al., 2013). Previous study found that serum PSA levels were increased significantly in women with

hirsutism ((Melegos et al., 1997), other study demonstrated that women with PCOS had significantly higher serum concentration of TPSA than healthy women and showed that TPSA positively correlated with testosterone (Rudnicka et al., 2016).

1.2 Rationale

Recently PCOS in become one of the most common problem among women in reproductive age that visit fertility centers in Sudan , the diagnosis and differential diagnosis of PCOS remains confused to many clinicians because have different criteria and different phenotypes and have different manifestations in different people due to genetic and environmental background and until now have no separately lab test and clinicians depend on rule out all other cause of heperandrogenism and ultrasound so these may lead to miss diagnosis, PSA may be a new biomarker helping in diagnosis of PCOS, so may reduce infertility and other complication.

The strong involvement of AMH in the pathophysiology of PCOS has opened a wide discussion about whether AMH could be involved in facilitating the diagnosis of PCOS, as a more sensitive and specific marker than follicle count in ultrasonographic examination.

1.3 Objectives

1.3.1 General Objective

To assess the levels of serum total Prostate specific antigen (TPSA) and Anti mullerian Hormone (AMH) among Sudanese women with polycystic ovarian syndrome.

1.3.2 Specific objectives

1. To estimate the level of serum TPSA, AMH in polycystic ovarian syndrome patients compared to control group.
2. To compare between TPSA, AMH with menstrual cycle (regular, irregular) in case group.
3. To calculate the body mass index (BMI) based on the weight and height of study group.
4. To correlate between TPSA, AMH and BMI.

Chapter Two

Literature Review

2.1Polycystic Ovary Syndrome

Polycystic Ovary Syndrome (PCOS) is a set of symptoms that result from a hormonal imbalance affecting women and girls of childbearing age. Women with PCOS usually have at least two of the following conditions,absence of ovulation, leading to irregular menstrual periods or no periods at all ,high levels of androgens (a type of hormone) or signs of high androgens, such as havingexcess bodyor facial hair ,cysts (fluid-filled sacs) on one or both ovaries—"polycystic" literally means "having manycysts"Some women diagnosed with PCOS have the first two conditions listed above as well as other symptoms of PCOS but do not have cysts on their ovaries(Azziz., 2006).

First description of the syndrome by Stein &Leventhal , who in their original report described seven women with variable clinical characteristics (i.e. obesity, hirsutism, acne and amenorrhoea) associated with enlarged bilateral polycystic ovaries(Conway et al., 2014). At the time, these signs were strictly adhered to in the diagnosis of what was then known as Stein-Leventhalsyndrome . These investigators also reported the results of bilateral wedge resection of the ovaries, in which at least half of each ovary was removed as a therapy for PCOS. Most of their patients resumed menses and achieved pregnancy after ovarian wedge resection. They postulated that removal of the thickened capsule of the ovary would restore normal ovulation by allowing the follicles to reach the surface of the ovary (Tayrab et al., 2014).

The exact mechanism responsible for the therapeutic effect of removal or destruction of part of the ovarian tissue is still not well understood. On the basis of Stein and Leventhal‘s work, a primary Ovarian defect was inferred, and the disorder was commonly referred to as polycystic ovarian disease. Subsequent clinical, morphologic, hormonal, and metabolic studies uncovered multiple underlying pathologies, and the term polycystic ovary syndrome was introduced to reflect the heterogeneity of this disorder. One of the most significant discoveries regarding the pathophysiology of PCOS was the demonstration of a unique form of insulin resistance and associated hyperinsulinemia (Franks., 2006).

2.1.1 Etiology of Polycystic Ovary Syndrome(PCOS)

The etiology of this syndrome is still speculative while its pathophysiology appears to be both multifactorial and polygenic.There are no certainties about the origin of PCOS, and a variety of hypotheses about either the genetic or the environmental origins of PCOS have been postulated. PCOS phenotype can be found from early infancy to puberty, based on predisposing environmental influences and genetic factors. There is some evidence that PCOS may partly depend on genetic factors. However, it is unlikely that PCOS represents a single gene defect and it is more likely to be polygenic or oligogenic. One hypothesis suggested that the clinical features of PCOS may develop as a consequence of genetically determined hypersecretion of androgens by the ovary starting at puberty or very likely long before puberty, so that typical clinical and biochemical characteristics of PCOS may become expressed as a consequence of exposure to androgen excess at or before. (Rosenfield., and Ehrman., 2016) .

2.1.1.1 Genetic factor forPolycystic Ovary Syndrome (PCOS):

familial aggregation of PCOS phenotypes and of associated metabolic and reproductive abnormalities has been long noted, While clustering of cases in families strongly support the role of genetic factors in the development of PCOS, heterogeneity of phenotypic features in different families and even within the same family underscores the importance of the environmental contribution. PCOS appears to be a common and complex trait and the exact pattern of inheritance is yet to be fully explained.The model of inheritance of PCOS has not yet been defined. Some researchers have postulated autosomal dominant transmission linked to a single genetic defect, but most authors define PCOS as a polygenic pathology. It is also possible that a particular gene in a given family may have a predominant effect, influencing the phenotypic manifestations of the syndrome. The main candidate genes are those encoding for factors involved in the synthesis, transport, regulation and effects of androgens (Leo.V et al., 2016). in addition biochemical parameters, including fasting insulin levels or hyperandrogenemia, seem to be highly heritable parameters, suggesting that some clinical signs, symptoms, or biochemical parameters of PCOS could be transmitted as mendelian autosomal dominant , or X-linked traits , but the genetic studies have not as yet concluded the pattern of heredity , While studies, so far, are unable to exclude an autosomal or X-linked dominant mode of inheritance, the heritability of PCOS is probably more complex, similar to that of type 2 diabetes mellitus or cardiovascular disease (Rosenfield., and Ehrman., 2016) .

2.1.2 Pathophysiology of Polycystic Ovary Syndrome(PCOS)

The exact pathophysiology of PCOS is complex and remains largely unclear (Teede et al., 2010), Theories regarding the pathophysiology of PCOS implicate primary defects in the hypothalamic–pituitary axis, ovarian function, and insulin secretion and action (Azziz., 2016).

2.1.2.1Hyperandrogenism

The clinical and/or biochemical signs of androgen excess in PCOS result from increased synthesis and release of ovarian androgens. Elevated luteinising hormone and insulin synergistically increase androgen production. Insulin resistance leads to hyperinsulinaemia, reduces sex hormone binding globulin(SHBG) and raises free circulating testosterone and together, hyperandrogenism and hyperinsulinaemia impairs ovarian follicle development. Clinical hyperandrogenism primarily includes hirsutism, acne and male pattern alopecia (Azziz., 2016).

PCOS is a common cause of hirsutism occurring in approximately 60% of cases, however this varies with race and degree of obesity (Franks., 2006), Hirsutism, Acne affects one third of cases and is not particularly specific for PCOS. Male pattern hair loss (androgenic alopecia) is less frequently seen in PCOS cases, as it generally requires a familial predisposition. Other features of hyperandrogenism include virilisation, which, especially if presenting with clitoromegaly and rapid onset, requires exclusion of other causes including adrenal or ovarian androgen-secreting tumours (Legro et al., 2013).

Biochemical hyperandrogenism is present in most patients with PCOS. Measurement of biochemical androgens in PCOS is limited by poor accuracy and reproducibility of assays, which are designed for significantly higher male androgen levels. Free androgen index

measurements are generally recommended, derived in the lab from SHBG and total testosterone measurements.Dehydroepiandrosterone sulfate (DHEAS) and androstenedione are not routinely recommended in PCOS (Franks., 2016).

2.1.2.2Hirsutism

Hirsutism is defined as the excessive growth of terminal hair in women and child in distribution similar to that occurring in post pubertal men (Brassard et al., 2008) Clinical features of hyperandrogenism frequently seen in PCOS include hirsutism, acne, and androgenic alopecia. Hirsutism is the presence of terminal hairs on the face and/or body in a female in a male-type pattern. The most common method of determining the presence of hirsutism uses a visual score. (Azziz et al., 2009). Various methods have been proposed. The most commonly used method is a modification of a method originally reported by Ferriman and Gallwey. Nine body areas, including the upper lip, chin, chest, upper back, lower back, upper and lower abdomen, upper arm, and thigh, are assigned a score of 0–4 based on the density of terminal hairs. A score of 0 represented the absence of terminal hairs, a score of 1 minimally evident terminal hair growth, and a score of 4 extensive terminal hair growths. The cutoff value should be established after the study of a large population of unselected women. Using this approach, cutoff values for defining hirsutism have been variously reported to be a score of 6 or greater, 7 or more, and 8 or more. (Azziz et al., 2009). However, we should note that the prevalence of hirsutism in PCOS will vary according to the race and ethnicity of the population being studied. These data suggest that the degree of body and terminal hair growth and the prevalence of hirsutism are not significantly different between unselected White and Black women. Consequently, it is likely that there will be little difference in the prevalence of hirsutism between Black and White PCOS women. Although this remains to be confirmed. Consistent with the lower population

prevalence of hirsutism observed in East Asian women, a comparative study of patients with PCOS from the United States (primarily Mexican Americans), Italy, and Japan noted that Japanese women had a significantly lower mean hirsutism score than their non-Asian counterparts. However, the lesser prevalence of hirsutism among East Asian PCOS patients may not extend to all groups in theregion. For example wijeyaratne and colleagues observed that hirsutism was more prevalent and more severe among PCOS patients of Southern Asian extraction (Pakistani, Bengali, Gujarati, or Dravidian Indian) than Whites. Likewise, among women of Indian descent in New Zealand, about two thirds of women with PCOS presented with clinical evidence of hirsutism, similar to the prevalence found in women of European, Maori, and Pacific Island descent. Although it is clear that there is racial variation in hair growth patterns, race-specific normative ranges have not been well established, which is required to determine whether a particular woman has excessive amounts of body of facial hair. Overall, hirsutism is an important feature of PCOS, affecting approximately 65% to 75% of patients with PCOS, including women of White, Black, and Southeast Asian race. The prevalence of hirsutism in PCOS is likely to be less among women of East Asian extraction (Azziz et al., 2009).

2.1.2.3Acne

Acne affects approximately 12% to 14% of white (PCOS) patients although the prevalence of this dermatologic abnormality varies with ethnicity: it is reportedly higher in Asian Indians and lower in Pacific Islanders. In a study of 248 women with (PCOS) in Italy, acne alone in the absence of other pilosebaceous features was present in 23.4%. Among 716 patients with (PCOS), 14.5% presented with acne, either alone or in combination with hirsutism. In a prospective study of women presenting for blood donation, Asuncion and colleagues noted that of the 10 women

diagnosed with PCOS, four (40%) had acne, three without associated hirsutism. However, various surveys have noted a relatively high prevalence of acne in the general population, particularly among younger women. Approximately 20% of individuals in their midteens and 15% of those in their early 20s complain of acne; even 10% of women in their 30s and 5% of women 40 to 60 years old will complaint of, albeit mild, acne. Consequently, the degree to which PCOS increases the risk of acne above the general population prevalence is unclear. The variability in the prevalence of acne is compounded by the fact that there is no single scoring system used. Overall, although acne affects 15% to 25% of PCOS patients, it is unclear whether the prevalence of acne is significantly increased in these patients over that observed in the general population (Azziz et al., 2009).

2.1.2.4 Androgenic alopecia

Scalp hair loss in women is a distressing complaint with significant psychologic morbidity. It usually represents the pilosebaceous unit response to endogenous androgens and may be associated with acne and hirsutism. Androgen sensitivity of the pilosebaceous unit varies, and there is poor correlation between clinical features and evidence of biochemical hyperandrogenism. The presence of DHT, formed from the 5a-reduction of tin the dermal papilla, is associated with a higher 5a-reductase activity in the hairs plucked from a scalp presenting with androgenic alopecia. In addition to androgen excess, other potential etiologies of alopecia or diffuse scalp hair loss in any woman may be genetic (i.e., familial premature scalp follicular loss), environmental (e.g., damage following the use or abuse of hair cosmetics), and nutritional (e.g., poor protein intake, zinc deficiency, iron-deficient anemia). Androgenic alopecia is a

recognized sign of (PCOS). However, the prevalence of this abnormality in PCOS is unclear. Although we previously noted that PCOS patients may account for _10% to 40% of all women with alopecia, literature defining the incidence of alopecia in either normal women or women with PCOS is sparse. The pattern of hair loss in PCOS generally involves thinning of the crown with preservation of the anterior hairline. Androgenic related alopecia in women with PCOS tends to be seen in the anterior midvertex area extending to the crown. The anterior hairline remains intact in women with PCOS and significant a bit emporal scalp hair recession is unusual except in virilizing syndromes. Unfortunately, a loss of at least 25% of scalp hair is needed before a woman becomes aware of thinning of her scalp hair. The sole presence of alopecia or diffuse scalp hair loss in women may be the sole dermatologic sign of PCOS (Azziz et al., 2009).

2.1.3 Specific steroidogenic enzyme defects in (PCOS)

In vitro studies suggest that the enhanced steroidogenic potential of PCOS theca cells resides in the increased enzyme activities of 17a-hydroxylase/17,20- lyase (CYP17a1), 3-beta-hydroxysteroid dehydrogenase type II (HSD3B2) and side-chain cleavage enzyme (CYP11A1); the enzyme activities remain elevated after many passages in culture (Nelson., 2001). These three enzymes act at various steps of the pathway of androgen synthesis. CYP11A1 performs the first step of steroid biosynthesis: the conversion of cholesterol to pregnenolone. CYP17a1 (cytochrome P450c17; 17a-hydroxylase/17,20-lyase) has dual functions: the hydroxylase activity catalyses the 17ahydroxylation of both pregnenolone and progesterone; and the 17,20-lyase activity cleaves the C17–C20 bond of 17a-hydroxypregnenolon and 17a-hydroxyprogesterone to form dehydroepiandrosterone (DHEA) and androstenedione, respectively. HSD3B2

transforms D5-steroids (pregnenolone, 17ahydroxypregnenolone and DHEA) into their D4- congenors. The increased enzyme activity of CYP17A1 is reflected at the transcriptional level. (Rosenfield .; and Ehrmann.; 2016).

2.1.4 Gonadotropins Hormones

Impaired gonadotropin dynamics may contribute to excessive androgen production in (PCOS), Increased LH pulse frequency and amplitude leading to persistently increased LH levels may directly enhance theca androgen synthesis. However, it has been suggested that elevated LH levels result from an impaired negative feedback on LH secretion, due to excessive androgen action on the hypothalamic–pituitary axis. The relatively reduced FSH levels (in relation to LH) may have an indirect role. The decreased stimulation of aromatase by FSH results in the decreased conversion of androgen to oestrogen and aggravates the ovarian androgen excess (Diamanti., 2008).

2.1.5IntraovarianFactors

Intraovarian factors of granulosa cell origin, such as anti Mullerian hormone (AMH) and inhibins, may contribute to the steroidogenic activity of theca cells. AMH is a dimeric glycoprotein of the transforming growth factor b (TGF-b) superfamily involved in follicular dynamics (Knight., and Glister.,2006). AMH type II receptors (AMHRII) have recently been detected on theca cell membranes of maturing follicles and could mediate a paracrine effect of AMH on androgen production. Additionally, AMH may indirectly contribute to the ovarian androgen excess by inhibiting FSH action or by suppressing aromatase activity (Diamant., 2008). The significant positive relationship between AMH and testosterone levels is compatible with the putative role of AMH in perpetuating ovarian

androgen excess. Similarly, theca cells have been shown to express inhibin receptors on their membrane, supporting the notion of a paracrine action of inhibins on theca steroidogenesis (Wang et al., 2016).

2.1.6 Adrenal Hyperandrogenism

There is a body of evidence to suggest that adrenal hyperandrogenism by putative dysregulation of CYP17A1 is a genetically determined trait in PCOS. Increased peripheral metabolism of cortisol has also been proposed to contribute to the functional adrenal hyperandrogenism. In particular, the enhanced inactivation of cortisol by 5a-reductase or the impaired reactivation of cortisone by 11-beta-hydroxysteroidogenase1 could lead to decreased feedback suppression of adrenocorticotropic hormone (ACTH) secretion. Notably, insulin resistance may in part account for the enhanced 5a-reduction of cortisol without affecting cortisol production. In this setting, the hypothalamic–pituitary–adrenal axis may be stimulated, leading to increased adrenal androgen production in PCOS. Nevertheless, aberrations related to adrenal function appear to contribute to a limited extent to the hyperandrogenism of PCOS (Diamanti., 2008).

2.1.7 Poly cystic ovary syndrome and ovarian dysfunction

Ovarian dysfunction usually manifests as oligomenorrhoea/amenorrhoea resulting from chronic oligo-ovulation/anovulation (Brassard et al., 2008). However, prolonged anovulation can lead to dysfunctional uterine bleeding which may mimic more regular menstrual cycles. The majority of PCOS patients have ovarian dysfunction, with 70% to 80% of women with PCOS presenting with oligomenorrhoea or amenorrhoea. Among those with oligomenorrhoea, 80% to 90% will be diagnosed with PCOS (Brassard et al., 2008). Among those with amenorrhoea, only 40% will be diagnosed with PCOS as hypothalamic dysfunction

is a more common cause (Azziz et al., 2009). Oligomenorrhoea occurs usually in adolescence, with onset later in life often associated with weight gain. Menstrual irregularity is then often masked by the oral contraceptive pill (OCP), until cessation, when the underlying irregular cycles recur. Menorrhagia can occur with unopposed estrogen and endometrial hyperplasia, further exacerbated by elevated estrogen levels in obesity. Whilst inadequate research exists, it is generally recommended that greater than four cycles per year may protect the endometrium. Women with regular menstrual cycles can also now be diagnosed with PCOS based on newer diagnostic criteria (Azziz., 2006). In those with PCOS and infertility, 90% are overweight. Obesity independently exacerbates infertility, reduces efficacy of infertility treatment and induces a greater risk of miscarriage (Brassard et al., 2008). There is currently an active debate about the appropriate limit for body mass index for assisted reproduction therapies, given the reduced success rates and the demonstrated risks of pregnancy in overweight women ideally, weight should be optimized prior to pregnancy. Age-related infertility also exacerbates infertility and timely planning of families may warrant discussion (Yuan et al., 2016).

2.1.7.1 Amenorrhoea

Amenorrhoea can be primary (menstruation has never occurred) or secondary. Oligomenorrhoea is sparse or infrequent menstruation; it can be due to less severe forms of some of the causes of amenorrhoea. Primary amenorrhoea can occur as part of the syndrome of female hypogonadism, but can also be present in normally feminized women. The commonest cause of amenorrhoea in women of child-bearing age is pregnancy, and this possibility, however unlikely, must always be excluded. The finding of an apparently high plasma LH concentration may suggest pregnancy before a pregnancy test is performed: chorionic gonadotrophins cross-reacts in some assays for LH. Pregnancy apart, amenorrhoea in normally feminized

women is most frequently due to a hormonal disturbance that results in a failure of ovulation (Marshall et al., 2014). Causes include: disordered hypothalamo-pituitary function, related to weight loss (30–35% of cases in most series) or hyperprolactinemia (10–12%), but idiopathic in some 10% of cases, ovarian dysfunction (e.g. autoimmune disease leading to premature menopause) (10–12%), increased androgen production (particularly polycystic ovary syndrome (PCOS) and late-onset congenital adrenal hyperplasia) (30–35%). Weight loss can lead to a decrease in the frequency of the pulsatility of GnRH secretion and thus decreased secretion of LH and FSH. Menstruation almost always ceases if weight falls below 75% of the ideal, and may do so with smaller losses. Regular menstruation returns if weight is regained. Severe stress and intensive exercise regimens, such as are adopted by elite long-distance runners, ballet dancers and gymnasts, can also lead to amenorrhoea, probably for complex neuroendocrinological reasons in addition to any effect of decreased body weight. Amenorrhoea due to excessive androgen secretion is often associated with hirsutism or even virilism. Uterine dysfunction is an uncommon cause of amenorrhoea. It can be excluded, if necessary, by the progestogen challenge test. If medroxyprogesterone acetate is given orally (10 mg daily for 5 days), the occurrence of vaginal bleeding 5–7 days later signifies that the uterus was adequately oestrogenized. If bleeding does not occur, the test is repeated, giving estrogen (ethinyloestradiol, 50 mg daily for 21 days, with progestogen on the last 5 days). Absence of bleeding indicates uterine disease. If bleeding occurs, oestrogen deficiency is present. The diagnosis of hormonal causes of amenorrhoea requires basal measurements of plasma FSH, LH and prolactin concentrations. A high FSH concentration is indicative of ovarian failure (and is more sensitive in this respect than LH). If LH, but not FSH, is elevated, and the patient is not pregnant, the most likely diagnosis is PCOS, and pelvic ultrasonography should be performed. If LH and FSH concentrations are normal or low, a pituitary or hypothalamic disorder should be sought, by anatomical studies

and dynamic testing of the hypothalamo-pituitary axis in a manner similar to that described for male hypogonadism. As in males, however, the results of such tests do not always distinguish between pituitary and hypothalamic disorders. The management of amenorrhoea depends on the cause, and whether fertility is required. In hyperprolactinemia, the treatment is directed to the underlying cause wherever possible (e.g. withdrawal of drugs, treatment of hypothyroidism). In ovarian, pituitary or hypothalamic disease, when fertility is not required, cyclical estrogen and (if the patient has a uterus) progestogen replacement is given. In established ovarian failure, pregnancy is only possible using donated ova. If fertility is required in pituitary failure, treatment is with human FSH and LH; HCG may be required to mimic the mid-cycle LH peak and stimulate ovulation. . Careful monitoring of plasma oestradiol concentrations is necessary to detect hyper stimulation, which carries a risk of multiple pregnancies and the production of ovarian cysts. Patients with hypothalamic disease may respond to clomiphene. This substance blocks oestradiol receptors in the hypothalamus and may stimulate GnRH (and thus LH and FSH) secretion (Azziz et al., 2009) (Coffler et al., 2003). Nonresponders are treated with pulsatile GnRH. Clomiphene is also useful in inducing ovulation in patients with PCOS. When it has not been possible to distinguish between hypothalamic and pituitary disease, a failure to respond to pulsatile GnRHsug gests that amenorrhoea is due to pituitary dysfunction.

Amenorrhea due to androgen excess can be due to adult onset CAH, corticotropin-dependent Cushing syndrome, or polycystic ovary syndrome (PCOS). Some individuals with 21-hydroxylase deficiency do not manifest any developmental abnormalities or salt wasting, but they present with signs of androgen excess. This clinical syndrome, referred to as non classic, adult-onset, or late-onset CAH, may be clinically indistinguishable from PCOS. Serum androstenedione and testosterone concentrations (total and free concentrations) are elevated, with mean concentrations 50 to 150% higher than normal. Abnormal bleeding patterns seen in

PCOS are due to chronic anovulation and lack of progesterone stimulation and withdrawal. Chronic estrogen exposure without progesterone may predispose patients to endometrial cancer. Some attempt has been made to link PCOS to leptin, a hormone that is secreted by adipocytes and is thought to play a role in regulating food intake and metabolism. Animals that lack leptin are infertile; leptin injection increases gonadotropin secretion and restores fertility. For women with PCOS who wish to conceive, treatment is aimed at ovulation induction. Weight reduction should be attempted first in those women who are overweight, as it often helps to promote ovulation. If ovulation does not occur, then medications such as clomiphene citrate, metformin, and aromatase inhibitors may be useful. Ovarian hyperthecosis, a non-neoplastic lesion of the ovary characterized by the presence of islands of luteinized thecal cells in the ovarian stroma, is sometimes confused with PCOS (yuan et al., 2016).

2.1.7.2 Intraovarian Androgens

Intraovarianhyperandrogenism may be causatively linked with anovulation in PCOS. Intraovarian androgen excess could impair folliculogenesis in a dual fashion, in part by stimulating the growth of small follicles (Jonard., and Dewailly ., 2004),and in part by hindering follicular maturation towards the dominant stage . Androgens appear to have diverse actions on ovarian granulosa cells according to the stage of the oocyte developmental program and the concurrent environmental milieu. Early follicles acquire the androgen receptor before other receptors (FSH receptor, AMH receptor). Therefore, androgens could affect folliculogenesis at the initial FSH independent phase (recruitment) and, in concert with other growth factors, contribute to the exaggerated early follicular growth in PCOS (Diamanti., 2008). And enhance FSH-induced granulosa cell differentiation, as demonstrated in primate models. These effects are at variance with evidence

showing inhibition of granulosa cell proliferation and maturation by androgens (Diamanti., 2008).

The androgen-induced inhibition of aromatase activity ,may contribute to the distortion of the later stages of folliculogenesis. This abnormality may lead to the failure of the selection of the dominant follicle and the demise of the subordinate cohort of follicles, which are required for mono-ovulation (Jonard., and Dewailly., 2004).

2.2 AMH

2.2.1 AMH physiology

In the male foetus, the Sertoli cells in developing testes produce AMH, which through AMH receptor-type II (AMHR-II) activation induces regression of the paramesonephric ducts. Strong AMH expression is involved in testicular differentiation during foetal development up to puberty. In the female foetus, the absence of AMH results in the development of the paramesonephric ducts into the oviduct, uterus, and the upper 2/3 of the vagina. Production of AMH starts from about the 36th week of gestation and lasts until menopause (Rey et al., 2013).

AMH is mainly expressed in granulosa cells of growing antral and pre-antral follicles in the gonadotropin-independent phase. Serum AMH reflects the secretion of AMH only from the follicles that are vascularised. AMH regulates follicle growth by the inhibition of their sensitivity to follicle-stimulating hormone (FSH). In the physiological menstrual cycle, when the follicle matures and reaches a diameter of around 6 mm, it enters the FSH-dependent growth phase, resulting in follicle selection and ovulation. It should be highlighted that selection of the follicle is based on its own sensitivity to FSH, and it requires FSH to continue its growth. After that time, there is a gradual reduction of intra follicular AMH concentration. Oocyte, depending on the stage in preantral, antral, and

preovulatory follicles, can regulate AMH expression in granulosa cells (Pellatt et al., 2011). Additionally, elevated oestrogen production and FSH can down-regulate AMH activity (Pellatt et al., 2011).

2.2.2 AMH in PCOS

Recent study demonstrate use of AMH in diagnosis of PCOS, AMH concentration correlates with the number of antral follicles as well as age of the women .AMH peaks at age 24.5 years, followed by a decline to the menopause . AMH values decrease steadily in a manner highly correlated with advancing age. The average yearly decrease is higher through age 35. On the other hand, a lower AMH level predicts poor ovarian response especially in invitro fertilization process (Tayrab et al., 2014), AMH is a promising biochemical marker for the prediction of ovarian response and that a cut-off point indicating the value of 2.97 ng/ml can be adopted for this prediction (Kunt et al., 2011). Systematic review and meta-analysis suggest that AMH is a useful first-line investigation in the identification of women with PCOS (Iliodromiti et al., 2013). In Indonesian women, AMH can be used as an alternative diagnostic criteria for PCOS patients with a cut-off value of 4.45 ng/ml. AMH value rise when hyperandrogenism is present therefore serum AMH levels also reflect the phenotype of PCOS (Wiweko et al., 2014). Serum AMH measurement is very valuable in diagnosis of PCOS patients. The serum AMH level in women with hyperandrogenism, or oligo-anovulation could indicate the diagnosis of PCOS when reliable ultrasonography data are not available or when typical clinical and laboratory findings do not exists. The serum AMH level is a new and useful diagnostic tool in PCOS diagnosis (Sahmay et al., 2013).

AMH serum levels seem to be related to severity of PCOS — women with classic phenotype have the highest serum AMH levels (Piouka et al., 2009) Supporting these findings, reported that 97% of women with AMH > 10 ng/mL presented PCOS and that AMH correlated positively with LH, total testosterone, and

dehydroepiandrosteronesulphate. Additionally, AMH showed high predictive ability for the presence of menstrual disorders (Cassar et al., 2014).

2.2.3 AMH and its relation to obesity and insulin resistance in PCOS

There are contradictory results concerning the association of AMH and obesity in women with PCOS. Recently, Olszanecka-Glinianowicz et al. reported that AMH levels were lower in obese women with PCOS than in normal weight PCOS women (Olszanecka et al., 2015) Conversely, Cassar et al found no differences in AMH levels between lean and obese women with PCOS (Cassar et al., 2014).In previously published studies, it has been proposed that insulin resistance, related to obesity, influences the function of granulosa cells and decreases AMH production. Furthermore, obesity could have an inhibitory effect on gonadotropin release, suppressing LH and follicle development, leading to elevated AMH concentrations (Grossman et al., 2008). Taking into account that PCOS women are commonly insulin resistant, the association between AMH and insulin resistance (IR) has been studied. Indirect measurements of IR have been associated with AMH either positively or negatively, or no association has been found (Olszanecka et al., 2015).

2. 3Prostate specific antigen

2.3.1 Biochemistry of PSA

Prostate specific antigen is a 28 400 Da glycol- protein 40 comprising 237 amino acid residues , with five inter-chain disulphide bonds and approximately 8% carbohydrate in the form of a N-linked oligosaccharide sidechain. In seminal plasma PSA can be shown to exist in five isoforms, two biologically active and differing in the degree of glycosylation, and three biologically inactive or `nicked‘ forms. Prostate specific antigen exhibits serine protease activity (EC3.4.21.77) similar to chymotrypsin (Ward et al., 2001). Prostate specific antigen is a member of the human kallikrein family, with which it shares considerable structural and

functional homology and a gene location on the long arm of chromosome 19 (19q13.2±q13.4). Prostate specific antigen, designated as hK3 in conformity with the unified nomenclature of the kallikreins, has 80% sequence homology with hK2 (glandular kallikrein) and 63% with hK1 (pancreatic±renalkallikrein) (Ward et al., 2001).

Prostate specific antigen is synthesized in the ductal and acinar epithelium of the prostate gland, whence it is secreted into the seminal plasma at a concentration of 0´5±2´0 g/L; secretion has been identified in the Para urethral and perianal glands as well as in apocrine sweat glands and the mammary glands. Synthesis of PSA has also been demonstrated in a number of tumor cell-lines, notably neuroblastoma(Ward et al., 2001). The function of PSA is to liquefy the seminal coagulum by proteolysis, with release of the entrapped spermatozoa, and may have a bioactive role in fertilization. In relation to carcinoma of the prostate the literature is confusing and even contradictory, with evidence promoting both its stimulatory and inhibitory role. It has been shown that PSA can cleave insulin-like growth factor binding protein-3, [liberating bioactive insulin-like growth factor (IGF-1)], activate the transforming growth factor (TGF-b) and regulate the bioactivity of parathyroid hormone-related protein. (Ward et al., 2001). Recent experimental evidence suggests that PSA may also have antiangiogenic activity by inhibition of endothelial cell proliferation induced by fibroblast growth factor (FGF-2) and vascular endothelial growth factor (VEGF). PSA has also been shown to release angiostatin-like fragments from plasminogen (Ward et al., 2001) (Franks., 2006).

2.3.2 Molecular Forms of PSA

PSA circulates in serum in complexed forms (bound to protease inhibitors) or in uncomplexed (free or unbound) forms. fPSA represents 10±30% of TPSA. Approximately 70±90% of the TPSA is bound to the serine protease inhibitor

ACT. Trace amounts of PSA are also bound to API (old nomenclature: a 1 - antitrypsin), ITI, pregnancy zone protein (an A2M- analogue), or PCI, whereby the PSA-PCI complex could only be detected in seminal plasma and not in serum.(Stephan et al.; 200040) Another fraction of the PSA is bound to the 25-fold-larger A2M molecule. Because of its large size, the A2M completely encapsulates the smaller PSA molecule, and no free epitope sites of PSA remain for its detection. The PSA-A2M can only be measured after the complex has been opened (Stephan et al., 2000).

2.3.3 Non Prostatic-PSA

The organ specificityof PSA has recently been challenged and PSA is no longer said to be confined to males and prostate gland. With the availability of highly sensitive immunoassays it has become apparent that PSA is expressed in non-prostatic tissues and most noteworthy is that it is now quantifiable in females. Several investigators have reported the presence of PSA in other tissues and biological fluids including breast cyst fluid, amniotic fluid, breast milk, nipple aspirate fluid pituitary, endometrium, ascetic fluid and CSF (Nagar., and Msalati., 2014, Dash., 2015).

PSA in females (Diamandis., and YU., 1995) first revealed that low levels of PSA are released into the female serum, the level of which is about 1000-fold less than those in male serum. Nipple aspirate fluid has the second highest PSA concentration after seminal plasma while the third highest level is found in breast milk of lactating women. Females lack prostate gland hence the exact source of PSA in females is still contentious. Researchers demonstrated that PSA level varies with the phase of menstrual cycle observing a rise in serum PSA level with the progesterone peak with a 10–12 day lag period, suggesting that PSA in females is regulated by corpus luteum steroids (Nagar., and Msalati., 2013). It was also reported that women with elevated androgen levels, consequently exhibiting

hirsutism also had elevated levels of PSA with the bound form PSA–ACT as the major molecular form present in them (Dash., 2015). Tumor extract analysis revealed the presence of both estrogen and progesterone receptors and high quantities of PSA which was predominantly in the free form. Their data support the contention that PSA immune reactivity in intra cystic fluid of breast carcinoma is partly the result of secretory activity by the neoplastic cells and that the steroid receptors modulates its expression (Dash., 2015).

CHAPTER THREE

Materials and Methods

3.1 Study design

Cross sectional case control study.

3.2 Study area and duration

The study was conducted in Khartoum state; Patients enrolled in this study were from (Dr.Alsir Abu alhassan Fertility Center) during February 2017 - May 2017.

3.3 Study population

Fifty Sudanese women with PCOS which came to fertility center, which will diagnosis as PCOS (diagnosed according to hormonal profile and ultrasound and confirm the diagnosis by Anti-mullrien Hormone above 4ng/ml consider PCOS were be recruited as case and other50 apparently healthy women volunteers were involved as a control group. Both groups were age matched.

3.4 Selection criteria

(a) Inclusion criteria:

Sudanese women that newly diagnosed as PCOS (age between 18-35), agree to participate in this study was enrolled.

(b) Exclusion criteria:

Women who (use ovulatory agents, and glucocorticoids) and have any endocrine disease, was excluded.

3.5 Ethical consideration of the study

The study was revised and ethically approved by the ethical committee of the Faculty of Medical Laboratory sciences, Sudan University for science and technology and the Permission of this study was obtained from the medical lab directors of (Dr.Alsir Abu alhassan Fertility Center).the objectives of the study were explained to all participant and verbal Consent was taken regarding

acceptance to participate in the study and re-assurance of confidentiality. Before the specimen was collected.

3.6 Sampling

3.6.1 Data collection

A questionnaire was used to obtain the clinical data for each participant in this study (appendix I).

3.6.2 Sample collection

Blood sample was collected from peripheral Vein from each subject ,In sterile condition and using a local antiseptic for skin, 5mls of venous blood was collected in plain blood container and centrifuged for 5 minutes at 3500rpm; and serum was separated and stored in -20 until used.

3.7 Biochemical measurements

3.7.1 Total prostatic specific Antigen (TPSA) measurement:

Total PSA Was measured using an ultrasensitive method by Electrochemiluminesent (ECL) immunoassay method using Roche‘s e411 technology.

3.7.1.1 Principle of the method

The Roche Elecsys Total PSA method is a sandwich electrochemiluminescent immunoassay that employs a biotinylated monoclonal PSA-specific antibody and a monoclonal PSA-specific antibody labeled with ruthenium complex. PSA in the specimen reacts with both the biotinylated monoclonal PSA-specific antibody (mouse) and the monoclonal PSA-specific antibody (mouse) labeled with a ruthenium, forming a sandwich complex. Streptavidin-coated microparticles are added and the mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of voltage to the electrode induces the

chemiluminescent emission, which is then measured against a calibration curve to determine the amount of PSA in the patient specimen. (Henttu and Vihko.;1994)

3.7.1.2 Procedure of TPSA measurement (appendix II).

3.7.2Anti Mullerian Hormone AMH Measurement

AMH was measured using ultrasensitive method by Electrochemiluminesent(ECL) immunoassay method using Roche‘s e411 technology.

3.7.2.1 Principle of the method:

The Roche Elecsys Total PSA method is a sandwich electrochemiluminescent immunoassay that employs a biotinylated monoclonal AMH-specific antibody and a monoclonal AMH-specific antibody labeled with ruthenium complex. AMH in the specimen reacts with both the biotinylated monoclonal AMH-specific antibody (mouse) and the monoclonal AMH-specific antibody (mouse) labeled with a ruthenium, forming a sandwich complex. Streptavidin-coated microparticles are added and the mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of voltage to the electrode induces the chemiluminescent emission, which is then measured against a calibration curve to determine the amount of AMH in the patient specimen. (Wilson *et al*.;1993)

3.7.2.2 Procedure of AMH measurement (appendix III).

3.8 Quality control

The Roche e411 analyzer was calibrated at first step, before running of sample, control sera used to be sure that results obtained were accurate and precise. (Appendix IV)

3.9 Statistical analysis:

Data was analyzed to obtain means standard deviation and correlation of the sampling using statistical package for social science (SPSS) computer Programmed version 15, t test and person correlation were used for comparison and correlation

Chapter Four

Results

This is an analytical cross-sectional , hospital based study , which was carried out during the period from February to May in Elsir abu Alhassan Center for fertility in Khartoum State .The current study included 50 patients who were newly diagnosed as polycystic ovary syndrome and 50 apparently healthy Sudanese women ,age was match in the study groups as in table (4.1).

Table (4.1) BMI (mean± SD 29.26±7.30 kg/m2 versus 27.82+5.32 kg/m2) and Age (mean ± SD 26.58±4.59 versus 26.42±4.58) years.

Table (4.2) comparison between means of TPSA, AMH among Sudanese patients with PCO and control group p.value≤ 0.05 consider significant AMH (mean ± SD 14.11± 9.2 ng/ml versus 2.404 ± 1.00 ng/ml p.value0.00) TPSA (mean ± SD 0.019 ± 0.009 ng/ml versus 0.00104 ± 0.001ng/ml p.value 0.00)

Table (4.3) comparison between the level of TPSA,AMH in PCOS female with regular and irregular cycle p.value≤0.05 consider significant TPSA (mean+sd0.01500±0.00633ng/ml in 13 female with regular cycle versus 0.019 ± 0.009 ng/ml in 37 female had irregular cycle p value 0.04) AMH (mean ± SD 015.56 ± 13.40 ng/ml in 13 female with regular cycle versus 13.60 ± 7.3ng/ml in those had irregular cycle p value 0.62)

Table (4.4) shows association between PCO patients with (normal weight and irregular cycle = 14%, normal weight regular cycle =6%, obese), (over weight irregular cycle =24%, over weight regular cycle=12%) and (obese and irregular cycle= 36% , obese regular cycle= 8%).

Table (4.5) shows comparison between means of serum AMH, TPSA and body mass index (BMI) sub groups among patients with PCOS**,** TPSA (mean ± SD 0.011+ 0.003 ng/mL in normal weight patients versus 0.014 ± 0.004 ng/mL in overweight p value 0.05), (mean ± SD 0.025±0.009 ng/ml in obese p value

0.00).AMH (mean ± SD 20.1±14.2 ng/ml in normal weight versus 14.2 ±7.4 ng/ml in overweight patient p value 0.1), (mean ± SD 11.1 ± 6.3 ng/ml in obese p value 0.01).

Table (4.6)comparison between means of serum AMH, among PCOS patients with and without family history, TPSA (mean± SD 0.0222 ± 0.021 ng/mL in 18 patients with family history versus 0.0165 ± 0.006 ng/ml in 32 patients without family history p value 0.03) .AMH (mean± SD 21.3 ± 10.0 ng/ml versus 10.6 ± 5.6 ng/ml p value 0.00).

Figure (4.1) Show correlation between the level of TPSA and BMI in PCO patients (p value=0.00, r=.893) (positive correlation).

Figure (4.2) show correlation btw the level of AMH and BMI in PCO patient (p value=0.025, r = -.317) (negative correlation).

Table (4.1) comparison between means of age and body mass index in study group:

Variable		Mean ±SD	P value
Age /year	Case	26.0 ±3.9	0.7
	control	25.8±3.9	
BMI Kg/m2	Case	29.5±6.1	0.06
	control	27.4±4.7	

-Independent t test was used.

Result express as (mean ± SD), P value ≤ 0.05 considered significant.

Table (4.2) comparison between means of serum AMH, TPSA among patient with PCOS and control group:

Variable	Mean ± SD	P.Value
AMH(ng/ml) Case	14.11 ± 9.2	
Control	2.404 ± 1.00	0.00*
PSA(ng/ml) Case	0.019 ± 0.009	
Control	0.001± 0.001	0.00*

-Independent t test was used.

-P value ≤ 0.05 considered significant.

Table (4.3) comparison between means of serum AMH, TPSA with menstrual cycle (regular, irregular) among patients with(PCOS):

Variable	N	Mean ±SD	P.Value
PSA regular cycle	13	0.015 ± 0.006	0.04*
(ng/ml) irregular cycle	37	0.019 ± 0.009	
AMH regular cycle	13	15.56 ± 13.40	0.62
(ng/ml) irregular cycle	37	13.60 ± 7.3	

- Independent t test was used.

-P value≤ 0.05 considered significant

Table (4.4) illustrates cross tabulation between BMI groups(normal weight, obese and overweight) and menstrual cycle among patients with PCO

	Menstrual cycle		total
	Irregular	Regular	
Normal weight	14%(n=7)	6%(n=3)	10
Over weight	24%(n=12)	12%(n=6)	18
Obese	36%(n=18)	8% (n=4)	22
total	37	13	50

Table (4.5) comparison between means of serum AMH, TPSA and body mass index (BMI) sub groups among patients with (PCOS):

	Variable	Mean + SD	P.Value
TPSA (ng/ml)	Normal weight	0.011 ± 0.003	0.05*
	Over weight	0.014 ± 0.004	
	Normal weight	0.011 ± 0.003	0.00*
	Obese	0.025 ± 0.009	
AMH (ng/ml)	Normal weight	20.1 ± 14.2	0.1
	Over weight	14.2 ± 7.4	
	Normal weight	20.1 ± 14.2	0.01*
	Obese	11.1±6.3	

- Independent t test was used.

-P value ≤ 0.05 considered significant.

Table (4.6) comparison between means of serum AMH, among (PCOS) patients with and without family history:

Variable		No	Mean + SD	P.Value
TPSA (ng/ml)	Had family history	18	0.022 ± 0.021	0.03*
	Had no family history	32	0.016 ± 0.006	
AMH (ng/ml)	Had family history	18	21.3 ± 10.0	0.00*
	Had no family history	32	10.6 ± 5.6	

- Independent t test was used.

-P value ≤ 0.05 considered significant.

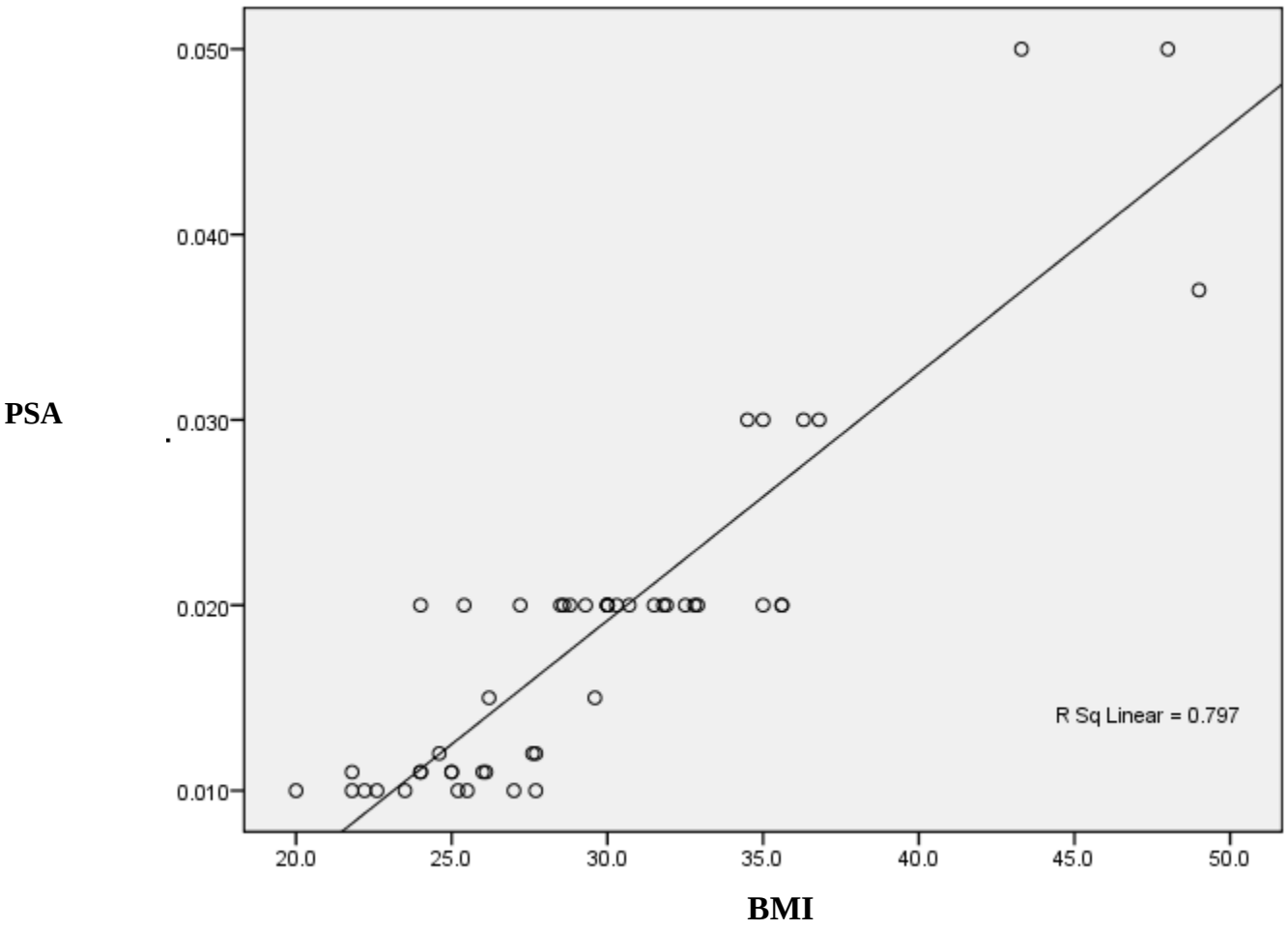

Figure 4.1: Shows the Correlation between TPSA and BMI Level In Case Group, Significant Positive Correlation (P Value =0.00, r =.893)

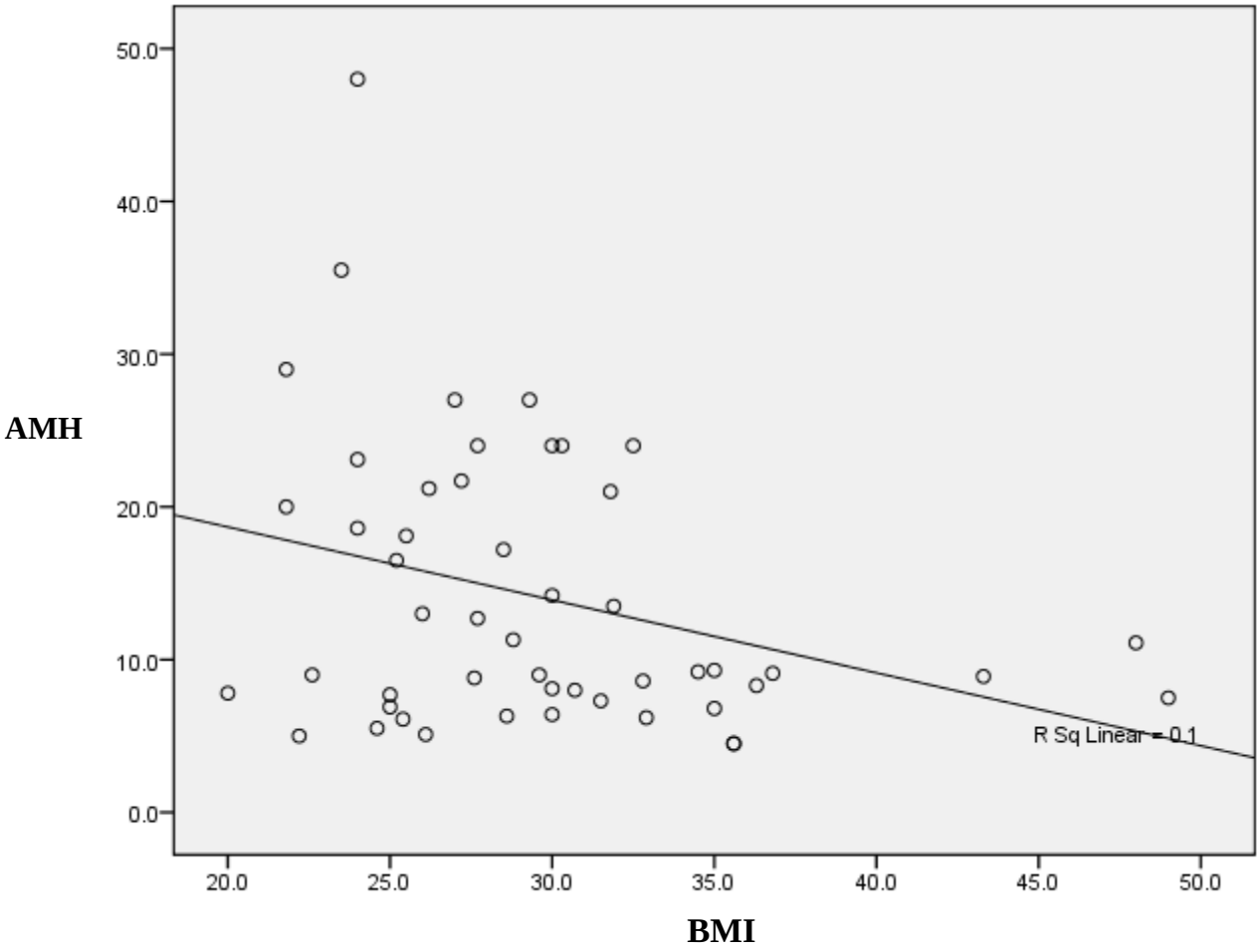

Figure (4.2): Shows Correlation between AMH and BMI Level In Case Group, Significant Negative Correlation (P =0.025, r = -.317)

Chapter Five

Discussion, Conclusion, Recommendations

5.1 Discussion

Polycystic ovarian syndrome is associated with increased ovarian and adrenal androgen secretions and hyperandrogenism and resultant hirsutism, hyperinsulinemia and central obesity (Azizz et al., 2009).

Prostatic specific antigen (PSA) is the most specific prostatic tumor marker in men , PSA can no longer be regarded as a tissue specific or tumor-specific marker for only prostatic tissue, due to recent finding in different female tissues in small amount, because the control is under androgen regulation (Vural et al., 2007).

The strong involvement of AMH in the pathophysiology of PCOS has opened a wide discussion about whether AMH could be involved in facilitating the diagnosis of PCOS. Increased serum AMH level of > 35 pmol/L (or > 5 ng/mL) has been proposed in the diagnosis of PCOS, as a more sensitive and specific marker than follicle count in ultrasonography examination (Piouka et al., 2009, Roe., and Dokaras., 2011).

The current study showed a significant increase in the means of total prostate specific antigen (TPSA) in PCOS cases than normal control (p-value 0.00), also showed AMH was significant higher in cases with PCOS than in control (p-value 0.00). Similar results were found in other study by (Rudnicka et al., 2016),which report statistically significant difference in level of TPSA in PCOS than in control (p-value 0.003), also, this study agree with studies carried by (Fleming et al., 2005) and (Sahmay et al ., 2013). They found that AMH levels were significantly higher in PCOS patients, while it agrees with other study by (AlBayatti., 2004), that found significant difference in level of TPSA in PCOS group compared to control group

(p-value <0.001). This difference is mainly may be due to hyperandogenism among PCOS (ovarian androgen over production that stimulate the target tissues capable of producing PSA like (breast and periurethral glands).

The current study showed that there was significant difference between the means of total prostate specific antigen (TPSA) with regular and irregular cycle p.value (0.04) which agree with that ovarian dysfunction usually manifests as oligomenorrhoea /amenorrhoea (Brassard et al., 2008) , but there was insignificant difference between the means of (AMH) in PCOS case with regular and irregular cycle p.value(0.62) which disagree with other study showed AMH had predictive ability for the presence of menstrual disorders carried by (Cassar et al., 2014) .

The result showed there were significant positive correlation between BMI and the TPSA (r =0.893 p.value =0.00), and there were negative significant correlation between AMH and BMI (r = -0.317 p.value =0.25), this finding agree with another result carried by (SLim et al., 2012) and (Vural et al., 2007). The study showed that there was significant variation on TPSA level among BMI sub groups, where the higher level in obese than normal and overweight p.values were significant, where we observed the level of AMH decrease with the increase of BMI, the highest level in normal weight, but the p.value was insignificant between normal and overweight this may due to small sample size.

Also we found that the majority of patients had irregular cycle were obese 36%, overweight 24% and normal weight 14% , this agree with study of (yuan et al., 2016) weight reduction is the first step to promote ovulation.

In this study shown significant increase in mean of TPSA, AMH among PCOS patients had family history of disease p.value (0.03) (0.00) respectively, this strong the evidence that particular gene in some families had predominant effect (Rosenfield., and Ehrman., 2016) .

5.2 Conclusions

The study concludes that the serum levels of TPSA, AMH were increased in PCOS, there was weak significant relation between TPSA and menstrual cycle where was insignificant correlation between AMH and menstrual cycle, the majority of females had irregular cycle were obese. Significant positive relation between TPSA and BMI, the higher concentration of TPSA found in obese patients than overweight and those with normal weight. Where there was significant negative correlation between AMH and BMI, the highest concentration found in patients with normal weight. Serum levels of TPSA and AMH were significant increase in patients who had family history.

5.2 Recommendations

From the results of this study, it is recommended that:

1. TPSA, AMH can be used as markers for PCOS within age (17-32year).
2. Further studies are needed to measure PSA, AMH in different age above 32year, and to measure the levels of testosterone and SHBG among PCO patients, large number of sample size will be necessary
3. Monitoring PSA level before, during and after treatment.
4. Women should have healthy life style to avoid complication of polycystic ovary syndrome by minimizing BMI and impair insulin response. .
5. Genetic studies should be done.

References

Al Bayatti, A.A.; Al-Samak, S.H; and Al Bahar, A.J.; (2004). Can serum prostate-specific antigen be a promising marker for patients with polycystic ovary syndrome and hirsutism.*Middle East Fertility Society Journal*, *9*(3), 227-231.

Azziz, R., (2006). Diagnosis of polycystic ovarian syndrome: the Rotterdam criteria are premature. *The Journal of Clinical Endocrinology & Metabolism*, *91*(3), 781-785.

Azziz, R.; (2016). PCOS in 2015: New insights into the genetics of polycystic *ovary syndrome. Nature Reviews Endocrinology, 12(2), 74-75.*

Azziz, R.; Carmina, E.; Dewailly, D.; Diamanti-Kandarakis, E.; Escobar-Morreale, H.F.; Futterweit, W.; Janssen, O.E.; Legro, R.S.; Norman, R.J.; Taylor, A.E.; and Witchel, S.F.; (2009). The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertility and sterility, 91(2), 456-488.

Brassard, M.; AinMelk, Y.; and Baillargeon, J.P.; (2008). Basic infertility including polycystic ovary syndrome. Medical Clinics of North America, 92(5), 1163-1192.

Cassar, S.; Teede, H.J.; Moran, L.J.;(2014) Polycystic ovary syndrome and anti-Müllerian hormone: role of insulin resistance, androgens, obesity and gonadotrophins. ClinEndocrinol; 81(6): 899–906 .

Coffler, M.S.; Patel, K.; Dahan, M.H.; Yoo, R.Y.; Malcom, P.J.; and Chang, R.J.; (2003). Enhanced granulosa cell responsiveness to follicle-stimulating hormone during insulin infusion in women with polycystic ovary syndrome treated

with pioglitazone. *The Journal of Clinical Endocrinology & Metabolism*, *88*(12), 5624-5631.

Conway, G.; Dewailly, D.; Diamanti-Kandarakis, E.; Escobar-Morreale, H.F.; Franks, S.; Gambineri, A.; Kelestimur, F.; Macut, D.; Micic, D.; Pasquali, R.; and Pfeifer, M.; (2014). The polycystic ovary syndrome: a position statement from the European Society of Endocrinology. *European journal of endocrinology*, *171*(4), P1-P29.

Dash, P.; (2015). Reconnoitring the status of prostate specific antigen and its role in women. *Indian Journal of Clinical Biochemistry*, *30*(2), 124-133.

Diamandis, E.P.; and Yu, H.; (1995). New biological functions of prostate-specific antigen?.*The Journal of Clinical Endocrinology & Metabolism*, *80*(5), 1515-1517.

Diamanti-Kandarakis, E.;(2008). Polycystic ovarian syndrome: pathophysiology, molecular aspects and clinical implications. Expert Reviews in molecular medicine, (30) 10, 115-127.

Franks, S.;(2006). Diagnosis of polycystic ovarian syndrome: in defense of the Rotterdam criteria. The Journal of Clinical Endocrinology & Metabolism, 91(3), 786-789.

Grossman, M.P.; Nakajima, S.T.; Fallat, M.E.; and Siow, Y.; (2008). Müllerian-inhibiting substance inhibits cytochrome P450 aromatase activity in human granulosa lutein cell culture. *Fertility and sterility*, *89*(5), 1364-1370

Iliodromiti, S.; Kelsey, T.W.; Anderson, R.A.; and Nelson, S.M.;(2013). Can anti-Müllerian hormone predict the diagnosis of polycystic ovary syndrome? A systematic review and meta-analysis of extracted data. The Journal of Clinical Endocrinology & Metabolism, 98(8), 3332-3340.

Jonard, S.; and Dewailly, D.; (2004). The follicular excess in polycystic ovaries, due to intraovarian hyperandrogenism, may be the main culprit for the follicular arrest. Human reproduction update, 10(2), 107-117.

Knight, P.G.; and Glister, C.;(2006). TGF-β superfamily members and ovarian follicle development. Reproduction, 132(2), 191-206.

Kunt, C.; Ozaksit, G.; Kurt, R.K.; Gungor, A.N.C.; Kanat-Pektas, M.; Kilic, S.; and Dede, A.; (2011). Anti-Mullerian hormone is a better marker than inhibin B, follicle stimulating hormone, estradiol or antral follicle count in predicting the outcome of in vitro fertilization. Archives of gynecology and obstetrics, 283(6), 1415-1421.

Legro, R.S.; Arslanian, S.A.; Ehrmann, D.A.; Hoeger, K.M.; Murad, M.H.; Pasquali, R.; and Welt, C.K.; (2013). Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. *The Journal of Clinical Endocrinology & Metabolism*, *98*(12), 4565-4592.

De Leo, V.; Musacchio, M.C.; Cappelli, V.;Massaro, M.G.; Morgante, G.; and Petraglia, F.; (2016). Genetic, hormonal and metabolic aspects of PCOS: an update. *Reproductive Biology and Endocrinology*, *14*(1), p.38.

Mashkoor, F.C.; Al-Asadi, J.N.; and Al-Naama, L.M.; (2013). Serum level of prostate-specific antigen (PSA) in women with breast cancer. *Cancer epidemiology*, *37*(5), 613-618.

Melegos, D.N.; Yu, H.; Ashok, M.; Wang, C.; Stanczyk, F.; and Diamandis, E.P.; (1997). Prostate-specific antigen in female serum, a potential new marker of androgen excess. *The Journal of Clinical Endocrinology & Metabolism*, *82*(3), 777-780.

Nagar, R.; and Msalati, A.A.; (2013). Changes in Serum PSA during normal menstrual cycle. *Indian Journal of Clinical Biochemistry*, *28*(1), 84-89.

Olszanecka-Glinianowicz, M.; Madej, P.; Owczarek, A.; Chudek, J.; and Skałba, P.; (2015). Circulating anti-Müllerian hormone levels in relation to

nutritional status and selected adipokines levels in polycystic ovary syndrome. *Clinical endocrinology*, *83*(1), 98-104.

Pellatt, L.; Rice, S.; Dilaver, N.;(2011) Anti-Müllerian hormone reduces follicle sensitivity to follicle-stimulating hormone in human granulosa cells. FertilSteril; 96(5): 1246–12451

Piouka, A.; Farmakiotis, D.; Katsikis,;(2009) Anti-Mullerian hormone levels reflect severity of PCOS but are negatively influenced by obesity: relationship with increased luteinizing hormone levels 296(2): 238–243

Rey, RA.; Grinspon ,RP.; Gottlieb, S.;(2013) Male hypogonadism: an extended classification based on a developmental, endocrine physiology-based approach. Andrology; 1(1): 3–16.

Roe, A.H.; and Dokras, A.; (2011). The diagnosis of polycystic ovary syndrome in adolescents. *Reviews in Obstetrics and Gynecology*, *4*(2), 45.

Rosenfield, R.L., Barnes, R.B., Jose'F, C. and Lucky, A.W., 1990. Dysregulation of cytochrome P450c17α as the cause of polycystic ovarian syndrome. *Fertility and sterility*, *53*(5), 785-791.

Rudnicka, E.; Radowicki, S.; and Suchta, K.;(2016). Prostate specific antigen (PSA) in diagnosis of polycystic ovarian syndrome–a new insight. *Gynecological Endocrinology*, *32*(11), 931-935.

Sahmay, S.; Atakul, N.; Aydogan, B.; Aydın, Y.; Imamoglu, M.; and Seyisoglu, H.;(2013). Elevated serum levels of anti-Müllerian hormone can be introduced as a new diagnostic marker for polycystic ovary syndrome. *ActaobstetriciaetgynecologicaScandinavica*, *92*(12), 1369-1374.

Stephan, C.; Jung, K.; Lein, M.; Sinha, P.; Schnorr, D.; and Loening, S.A.;(2000). Molecular forms of prostate-specific antigen and human kallikrein 2 as promising tools for early diagnosis of prostate cancer. *Cancer Epidemiology and Prevention Biomarkers*, *9*(11), 1133-1147.

Tayrab, E.; Ali, M.; Modawe, G.A.; Naway, L;. and Abdrabo, A.A.;(2014). Serum Anti-Müllerian hormone as laboratory predictor in infertile women with and without polycystic ovary syndrome. *American Journal of Research Communication*, *2*(3), 61-66.

Teede, H.; Deeks, A.; and Moran, L.; (2010). Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. *BMC medicine*, *8*(1), p.41.

Vural, B.; Özkan, S.; and Bodur, H.; (2007). Is prostate-specific antigen a potential new marker of androgen excess in polycystic ovary syndrome?.*Journal of Obstetrics and Gynaecology Research*, *33*(2), 166-173.

Wang, F.; Niu, W.B.; Kong, H.J.; Guo, Y.H.; and Sun, Y.P.;(2017). The role of AMH and its receptor SNP in the pathogenesis of PCOS. *Molecular and cellular endocrinology*, *439*, 363-368.

Ward, A.M.; Catto, J.W.F.; and Hamdy, F.C.; (2001). Prostate specific antigen: biology, biochemistry and available commercial assays. *Annals of clinical biochemistry*, *38*(6), 633-651.

Wiweko, B.; Maidarti, M.; Priangga, M.D.; Shafira, N.; Fernando, D.; Sumapraja, K.; Natadisastra, M.; and Hestiantoro, A.;(2014). Anti-mullerian hormone as a diagnostic and prognostic tool for PCOS patients. *Journal of assisted reproduction and genetics*, *31*(10), 1311-1316.

Yuan, X.; Hu, T.; Zhao, H.; Huang, Y.; Ye, R.; Lin, J.; Zhang, C.; Zhang, H.;Wei, G.; Zhou, H.; and Dong, M.;(2016). Brown adipose tissue transplantation ameliorates polycystic ovary syndrome. *Proceedings of the National Academy of Sciences*, *113*(10), 2708-2727.

Appendix (I)

Sudan University of Science and Technology

College of Graduate studies

Questionnaire

Master Degree

Assessment of serum Prostate specific antigen (PSA), Anti mullerian Hormone (AMH) level among Sudanese women with polycystic ovarian syndrome In Khartoum state

A- General information:

Sex: female ( )

Age: ……..Years

BMI= weight …… Kg. Height ……..m

B- Clinical information:

Duration of disease: …….. (Better new cases)

Menstrual cycle: regular ( ) irregular ( )

Number of cycle / years

Family history: ……..

1st relative ---------- 2ed relative degree----------

Number of child: ……

Lab investigation:

Serum AMH ----------ng/ml

Serum Total PSA ----------ng/ml

Appendix (II)

ms_04641655190V12.0

total PSA

cobas®

total (free + complexed) PSA - Prostate-specific antigen (tPSA)

REF	Σ	SYSTEM
		Elecsys 2010
		MODULAR ANALYTICS E170
04641655 190	100	**cobas e** 411
		cobas e 601
		cobas e 602

English

Please note

The measured tPSA value of a patient's sample can vary depending on the testing procedure used. The laboratory finding must therefore always contain a statement on the tPSA assay method used. tPSA values determined on patient samples by different testing procedures cannot be directly compared with one another and could be the cause of erroneous medical interpretations. If there is a change in the tPSA assay procedure used while monitoring therapy, then the tPSA values obtained upon changing over to the new procedure must be confirmed by parallel measurements with both methods.

Intended use

This assay, a quantitative in vitro diagnostic test for total (free + complexed) prostate-specific antigen (tPSA) in human serum and plasma, is indicated for the measurement of total PSA in conjunction with digital rectal examination (DRE) as an aid in the detection of prostate cancer in men aged 50 years or older. Prostate biopsy is required for diagnosis of prostate cancer. The test is further indicated for serial measurement of tPSA to aid in the management of cancer patients.

The **electrochemiluminescence immunoassay** "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Prostate-specific antigen (PSA) is a glycoprotein (molecular weight 30000-34000 daltons) having a close structural relationship to the glandular kallikreins. It has the function of a serine proteinase.[1]

The proteolytic activity of PSA in blood is inhibited by the irreversible formation of complexes with protease inhibitors such as alpha-1-antichymotrypsin, alpha-2-macroglobulin, and other acute phase proteins.[2] Beside these complexes, about 30 % of the PSA present in blood occurs in the free form, but is proteolytically inactive.[3,4,5]

Elevated concentrations of PSA in serum are generally indicative of a pathologic condition of the prostate (prostatitis, benign hyperplasia or carcinoma).[6,7]

As PSA is also present in para-urethral and anal glands, as well as in breast tissue or with breast cancer, low levels of PSA can also be detected in sera from women. PSA may still be detectable even after radical prostatectomy.

The main areas in which PSA determinations are employed are the monitoring of progress and efficiency of therapy in patients with prostate carcinoma or receiving hormonal therapy.

The steepness of the rate of fall in PSA down to no-longer detectable levels following radiotherapy, hormonal therapy or radical surgical removal of the prostate provides information on the success of therapy.[8]

An inflammation or trauma of the prostate (e.g. in cases of urinary retention or following rectal examination, cystoscopy, coloscopy, transurethral biopsy, laser treatment or ergometry) can lead to PSA elevations of varying duration and magnitude.

The two monoclonal antibodies used in the Elecsys total PSA assay recognize PSA and PSA-ACT on an equimolar basis in the range of 10-50 % free PSA/total PSA which are the free PSA-ratios as seen in clinical practice.[9]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample, a biotinylated monoclonal PSA-specific antibody, and a monoclonal PSA-specific antibody labeled with a ruthenium complex[a)] react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack is labeled as TPSA.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-PSA-Ab~biotin (gray cap), 1 bottle, 10 mL:
Biotinylated monoclonal anti-PSA antibody (mouse) 1.5 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

R2 Anti-PSA-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 10 mL:
Monoclonal anti-PSA antibody (mouse) labeled with ruthenium complex 1.0 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on Elecsys 2010, MODULAR ANALYTICS E170, **cobas e** 411 and **cobas e** 601	8 weeks
on **cobas e** 602	4 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

total PSA

total (free + complexed) PSA - Prostate-specific antigen (tPSA)

cobas®

Serum collected using standard sampling tubes or tubes containing separating gel.

Lithium heparin, K_3-EDTA and sodium citrate plasma. When sodium citrate is used, the results must be corrected by + 10 %.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 5 days at 2-8 °C, 6 months at -20 °C. Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 04485220190, total PSA CalSet II, for 4 x 1 mL
- REF 11776452122, PreciControl Tumor Marker, for 2 x 3 mL each of PreciControl Tumor Marker 1 and 2 or REF 11731416190, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: This method has been standardized against the Stanford Reference Standard/WHO 96/670 (90 % PSA-ACT + 10 % free PSA).[10,11,12]

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Tumor Marker or PreciControl Universal.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in ng/mL or µg/L).

Limitations - interference

The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), hemolysis (Hb < 1.4 mmol/L or < 2.2 g/dL), lipemia (Intralipid < 1500 mg/dL) and biotin (< 246 nmol/L or < 60 ng/mL).

Criterion: Recovery within ± 10 % of initial value.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.

There is no high-dose hook effect at tPSA concentrations up to 17000 ng/mL.

In vitro tests were performed on 28 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

It is known that in rare cases PSA isoforms do exist which may be measured differently by different PSA tests. Findings of this kind have occasionally been reported for PSA tests from various manufacturers.[13,14,15]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

total PSA

total (free + complexed) PSA - Prostate-specific antigen (tPSA)

cobas®

Limits and ranges

Measuring range

0.002-100 ng/mL (Elecsys 2010 and **cobas e** 411 analyzers) or 0.003-100 ng/mL (MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers) (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.002 ng/mL or < 0.003 ng/mL. Values above the measuring range are reported as > 100 ng/mL (or up to 5000 ng/mL for 50-fold diluted samples).

Lower limits of measurement

Lower detection limit (LDL)

	Elecsys 2010 and **cobas e** 411 analyzers	MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers
LDL	0.002 ng/mL	0.003 ng/mL

The lower detection limit (LDL) is calculated as the concentration lying two signal standard deviations away from an analyte-free sample or from the lowest standard (repeatability, n = 21).

Limit of Blank (LoB) and Limit of Detection (LoD)

	Elecsys 2010 and **cobas e** 411 analyzers	MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers
LoB	0.007 ng/mL	0.006 ng/mL
LoD	0.011 ng/mL	0.014 ng/mL

Both Limit of Blank and Limit of Detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

Dilution

Samples with tPSA concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:50 (either automatically by the MODULAR ANALYTICS E170, Elecsys 2010 or **cobas e** analyzers or manually). The concentration of the diluted sample must be > 2 ng/mL.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** software automatically takes the dilution into account when calculating the sample concentration.

Expected values

Expected values in normal healthy males

a) Studies in two clinical centers in the Netherlands and Germany with the Elecsys total PSA assay on sera from 244 healthy men of various age groups yielded the following results:

		tPSA (ng/mL)	
Age (years)	N	Median	95th percentile
< 40	45	0.57	1.4
40-49	42	0.59	2.0
50-59	107	0.75	3.1
60-69	41	1.65	4.1
≥ 70	9	1.73	4.4

b) The distribution of tPSA results was measured in a cohort of 395 normal healthy males aged 50-94 years (results of a study in the USA).

The subsequent table presents the tPSA values as measured on the Elecsys 2010 immunoassay analyzer.

		tPSA (ng/mL)	
Age (years)	N	Median	95th percentile
50-59	154	0.81	3.89
60-69	131	0.95	5.40
≥ 70	110	1.11	6.22

tPSA values in detection of prostate cancer

A multicenter cohort study was performed to demonstrate the effectiveness of the Elecsys total PSA assay when used in conjunction with digital rectal examination (DRE) as an aid in the detection of prostate cancer in men 50 years of age or older.

A total of 1121 serially accrued men 50 years of age or older participated in the study. The mean age of the cohort was 66.4 years (95 % confidence interval = 65.9 to 66.8 years).

Distribution of tPSA values by biopsy result and digital rectal examination result

Prostate biopsy result: benign

		tPSA (ng/mL)		
DRE result	N	Median	Minimum	Maximum
Normal	375	5.8	0.4	75.8
Pathological	355	4.9	0.3	29.6
Total	730	5.4	0.3	75.8

Prostate biopsy result: malignant

		tPSA (ng/mL)		
DRE result	N	Median	Minimum	Maximum
Normal	146	7.2	2.5	122.1
Pathological	245	7.8	0.5	778.5
Total	391	7.4	0.5	778.5

Utility of tPSA in detection of prostate cancer

As shown in the table below, within this cohort of 1121 males, 391 (34.9 %) prostate cancers were detected by biopsy. Abnormal digital rectal examination (DRE) results were reported for 245 (62.7 %) of the 391 prostate cancers while tPSA results above 4 ng/mL were reported for 336 (85.9 %) cancers for the Elecsys 2010 analyzer. Of the 391 men diagnosed with cancer, 379 (96.9 %) had either an abnormal DRE result or a tPSA value above 4.0 ng/mL.

The positive predictive value for the Elecsys total PSA assay on the Elecsys 2010 analyzer was 0.390 using 4.0 ng/mL as a cutoff (malign prostate biopsy + tPSA > 4.0 ng/mL: n = 336 / tPSA > 4.0 ng/mL: n = 862).

Results for digital rectal examination and tPSA as referred to prostate cancers detected by biopsy in a cohort of:

1121 males 50 years or older referred to an urologist for prostate evaluation.

	Total	DRE+b)	PSA+c)	PSA+ or DRE+	PSA+ and DRE+	PSA+ and DRE-d)	PSA- and DRE+e)
Total number	1121	600	862	1037	425	437	175
No. of malignant prostate biopsies	391	245	336	379	202	134	43
% positive biopsies	34.9	40.8	39.0	36.5	47.5	30.7	24.6

b) abnormal DRE

c) tPSA value > 4 ng/mL

d) normal DRE

e) tPSA value < 4 ng/mL

Analysis of tPSA values was performed with Elecsys 2010 analyzers.

Appendix (III)

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AMH

Anti-Mullerian hormone

cobas®

REF	Σ	SYSTEM
		Elecsys 2010
		MODULAR ANALYTICS E170
06331076 190	100	**cobas e** 411
		cobas e 601
		cobas e 602

English

Intended use

Immunoassay for the in vitro quantitative determination of anti-Mullerian hormone (AMH) in human serum and plasma. The determination of AMH is used for the assessment of the ovarian reserve in conjunction with other clinical and laboratory findings.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

The anti-Mullerian hormone is a homodimeric glycoprotein belonging to the transforming growth factor β (TGF β) family. All members of this superfamily are involved in the regulation of tissue growth and differentiation. Prior to secretion, the hormone undergoes glycosylation and dimerization to produce an approximately 140 kDa precursor of two identical disulfide-linked 70 kDa subunits. Each monomer contains a large N-terminal pro-region and a much smaller C-terminal mature domain. In contrast to other TGF β family members, AMH is thought to require the N-terminal domain to potentiate activity of the C-terminal domain to attain full bioactivity.[1,2]

A part of AMH is then cleaved at a specific site between the pro-region and the mature region during cytoplasmic transit to generate biologically active 110 kDa N-terminal and 25 kDa C-terminal homodimers which remain associated in a non-covalent complex. The AMH type II receptor (AMH RII) has the capacity of binding only the biologically active form of AMH.[2]

In males, AMH is secreted by the Sertoli cells of the testes. During embryonic development in males, secretion of AMH from testicular Sertoli cells is responsible for the regression of the Mullerian duct and the normal development of the male reproductive tract. The secretion of AMH by the Sertoli cells starts during the embryogenesis and continues throughout life. AMH is continuously produced by the testicles until puberty and then decreases slowly to post-puberty values.[3]

In females AMH plays an important role in the ovarian folliculogenesis.[4] Follicle development in the ovaries comprises two distinct stages: initial recruitment, by which primordial follicles start to mature, and cyclic recruitment, which leads to the growth of a cohort of small antral follicles, among which the dominant follicle (destined to ovulate) is subsequently selected. FSH directs the cyclic recruitment. AMH expression in granulosa cells starts in primary follicles and is maximal in granulosa cells of preantral and small antral follicles up to approximately 6 mm in diameter. When follicle growth becomes FSH-dependent, AMH expression diminishes and becomes undetectable. This pattern of AMH expression supports the inhibitory role of AMH at two distinct stages of folliculogensis. First, AMH inhibits the transition of follicles from primordial into maturation stages and thereby has an important role in regulating the number of follicles remaining in the primordial pool. Second, AMH has inhibitory effects on follicular sensitivity to FSH and therefore has a role in the process of follicular selection.[5,6]

Serum levels of AMH are barely detectable at birth in females, reach their highest levels after puberty, decrease progressively thereafter with age, and become undetectable at menopause.[7,8] Serum AMH levels have been shown to be relatively stable during the menstrual cycle with substantial fluctuations being observed in younger women.[9,10,11] AMH levels further demonstrate lower intra- and inter-cyclic variation than baseline FSH.[10] Serum AMH levels decrease significantly during the use of combined contraceptives.[12] Clinical applications of AMH measurements have been proposed for a variety of indications.[13,14,15] Measurement of serum AMH is clinically mainly used for assessment of ovarian reserve reflecting the number of antral and pre-antral follicles, the so-called antral follicle count (AFC), and for the prediction of response to controlled ovarian stimulation.[13,15,16] Further clinical applications of AMH are diagnosis of disorders of sex development (DSD) in children[17,18] and monitoring of granulosa cell tumors to detect residual or recurrent disease.[19,20] AMH has been suggested as a surrogate biomarker for AFC in the diagnosis of polycystic ovary syndrome (PCOS)[21,22] and for the prediction of time to menopause.[23,24]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 50 µL of sample, a biotinylated monoclonal AMH-specific antibody, and a monoclonal AMH-specific antibody labeled with a ruthenium complex[a)] form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack is labeled as AMH.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-AMH-Ab~biotin (gray cap), 1 bottle, 8 mL:
Biotinylated monoclonal anti-AMH antibody (mouse) 1.0 mg/L, phosphate buffer 50 mmol/L, pH 7.5; preservative.

R2 Anti-AMH-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 8 mL:
Monoclonal anti-AMH antibody (mouse) labeled with ruthenium complex 1.0 mg/L, phosphate buffer 50 mmol/L, pH 7.5; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on the analyzers	8 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

AMH

Anti-Mullerian hormone

cobas®

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin plasma. Do not use EDTA plasma.

Criterion: Recovery within ± 30 % of serum value ≥ 0.5 ng/mL; recovery within ± 0.2 ng/mL for serum value < 0.5 ng/mL and slope of 0.9-1.1 + intercept within ± 0.1 ng/mL + coefficient of correlation ≥ 0.95.

Stable for 3 days at 20-25 °C, 5 days at 2-8 °C, 6 months at -20 °C. Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 06331084190, AMH CalSet, for 4 x 1 mL
- REF 06709966190, PreciControl AMH, for 2 x 2 mL each of PreciControl AMH 1 and 2
- REF 05192943190, Diluent Universal 2, 2 x 36 mL sample diluent or REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: This method has been standardized against the Beckman Coulter AMH Gen II ELISA (unmodified version without predilution) assay.

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl AMH.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in ng/mL or in pmol/L).

Conversion factors:	pmol/L x 0.14 = ng/mL
	ng/mL x 7.14 = pmol/L

Limitations - interference

The assay is unaffected by icterus (bilirubin ≤ 1129 µmol/L or ≤ 66 mg/dL), hemolysis (Hb ≤ 0.621 mmol/L or ≤ 1.0 g/dL), lipemia (Intralipid ≤ 1000 mg/dL), biotin (≤ 143 nmol/L or ≤ 30 ng/mL), IgG ≤ 2.5 g/dL, IgA ≤ 1.8 g/dL and IgM ≤ 0.5 g/dL.

Criterion: Recovery within ± 10 % of initial value.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1000 IU/mL.

There is no high-dose hook effect at AMH concentrations up to 1400 ng/mL.

In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

AMH

Anti-Mullerian hormone

cobas®

Limits and ranges

Measuring range

0.01-23 ng/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.01 ng/mL. Values above the measuring range are reported as > 23 ng/mL (or up to 46 ng/mL for 2-fold diluted samples).

Lower limits of measurement

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)

Limit of Blank = 0.007 ng/mL

Limit of Detection = 0.010 ng/mL

Limit of Quantitation = 0.030 ng/mL with a total allowable error of ≤ 20 %

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable relative error of ≤ 20 %.

Dilution

Samples with AMH concentrations above the measuring range can be diluted automatically with Diluent Universal 2. Manual dilution can be performed with Diluent Universal 2 or Diluent Universal. The recommended dilution is 1:2 (either automatically by the MODULAR ANALYTICS E170, Elecsys 2010 or **cobas e** analyzers or manually). The concentration of the diluted sample must be > 10 ng/mL.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** software automatically takes the dilution into account when calculating the sample concentration.

Expected values

A study in a Caucasian population with the Elecsys AMH assay on samples from apparently healthy adults (148 males, 493 females not taking contraceptives) and 149 women with Polycystic Ovary Syndrome yielded the following results (Roche study No. RD001727):

	N	5th perc. ng/mL (95 % CI[b])	10th perc. ng/mL (95 % CI)	Median ng/mL (95 % CI)	90th perc. ng/mL (95 % CI)	95th perc. ng/mL (95 % CI)
Healthy men						
	148	1.43 (0.256-1.97)	2.15 (1.35-2.43)	4.79 (4.35-5.35)	10.1 (9.14-11.6)	11.6 (10.3-17.0)
Healthy women (years)						
• 20-24	115	1.66 (0.862-1.85)	1.88 (1.49-2.28)	3.97 (3.55-4.33)	7.29 (6.82-10.1)	9.49 (7.38-11.5)
• 25-29	142	1.18 (0.853-1.81)	1.83 (1.18-2.07)	3.34 (3.03-3.87)	7.53 (6.74-9.16)	9.16 (7.63-10.1)
• 30-34	110	0.672 (0.473-0.932)	0.946 (0.602-1.19)	2.76 (2.34-3.55)	6.70 (5.57-7.64)	7.55 (6.76-9.34)
• 35-39	57*	-	0.777 (0.159-0.932)	2.05 (1.78-3.24)	5.24 (4.83-7.34)	-
• 40-44	41*	-	0.097 (0.021-0.247)	1.06 (0.734-2.13)	2.96 (2.59-5.70)	-
• 45-50	28*	-	0.046 (0.018-4.16)	0.223 (0.125-0.498)	2.06 (0.018-4.16)	-
PCOS women**						
	149	2.41 (1.67-3.01)	3.12 (2.29-3.77)	6.81 (6.30-7.42)	12.6 (11.5-17.1)	17.1 (13.3-20.3)

b) CI = confidence interval

* Due to the lower numbers of patients in these age groups the extreme percentiles were not calculated.

** According to the revised diagnostic criteria of PCOS defined by the Rotterdam ESHRE/ASRM-sponsored (ESHRE = European Society of Human Reproduction and Embryology; ASRM = American Society of Reproductive Medicine) PCOS consensus workshop group.[25]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Use of AMH for the assessment of ovarian reserve

The use of AMH for the assessment of ovarian reserve was investigated in a prospective study with n = 451 women between 18-44 years old, where AMH values were correlated to the antral follicle count (AFC) of the women (Roche study No. RD001542). AFC was determined by transvaginal sonography measuring follicles of 2-10 mm diameter in size. Both AFC and AMH were determined on days 2-4 of the same menstrual cycle. Between 17 to 115 women were recruited per site at 6 different European sites and 1 Australian site.

No significant differences in mean AMH values were observed between the sites (pval = 0.301). The mean age values between the sites were significantly different, and also AMH and age showed a significant negative correlation (Spearman correlation coefficient -0.47). The age adjusted site effect of AMH showed no significance (pval = 0.193). The determined AFC values showed significant differences between the sites, with and without age adjustment. The overall correlation of AMH with AFC was 0.68 (Spearman's rank coefficient).

The figure below shows the scatterplot of AMH versus AFC, as well as the site-specific AMH and AFC distributions.

Appendix (IV)

05403073001V2

PreciControl Universal

cobas®

REF 11731416 190
REF 11731416 922 (QCS)

→ 4 x 3.0 mL

11731416190 / 11731416922 V 9
http://e-labdoc.roche.com

English

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV.

The initial thyroid glandular tissue extract containing the human thyroglobulin has shown to be free from HBsAg and antibodies to HCV and HIV.

The testing methods applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.

The controls may not be used after the expiration date.

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Method Sheet download

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Deutsch

Vorsichtsmaßnahmen und Warnhinweise

In-vitro-Diagnostikum.
Die beim Umgang mit Laborreagenzien üblichen Vorsichtsmaßnahmen beachten.
Die Entsorgung aller Abfälle ist gemäß den lokalen Richtlinien durchzuführen.
Sicherheitsdatenblatt auf Anfrage für berufsmäßige Benutzer erhältlich.

Humanmaterial gilt als potentiell infektiös. Für alle aus Humanblut hergestellten Produkte wird nur Blut von einzeln getesteten Spendern verwendet, bei denen weder Antikörper gegen HCV und HIV noch HBsAg nachzuweisen sind.

Bei dem ursprünglich verwendeten glandulären Gewebeextrakt, welches humanes Thyreoglobulin enthält, waren kein HBsAg und keine Antikörper gegen HCV und HIV nachzuweisen.

Die angewendeten Testmethoden sind von der US-Gesundheitsbehörde (FDA) genehmigt bzw. erfüllen die Anforderungen der Europäischen Direktive 98/79/EG, Anhang II, Liste A.

Da keine Testmethode mit absoluter Sicherheit eine potentielle Infektionsgefahr ausschließen kann, sollte das Material mit der gleichen Sorgfalt behandelt werden wie eine Patientenprobe. Im Falle einer Exposition ist entsprechend den Anweisungen der zuständigen Gesundheitsbehörden vorzugehen.

Nach Ablauf des Verfallsdatums dürfen die Kontrollen nicht mehr verwendet werden.

Schaumbildung bei allen Reagenzien und Probenarten (Proben, Kalibratoren und Kontrollen) vermeiden.

Download von Methodenblättern

Der eingerahmte Text am Beginn des Dokumentes zeigt den Produkt-Code sowie die dazugehörige Version des Dokumentes.
Dieses Dokument kann von der folgenden Webseite heruntergeladen werden: http://e-labdoc.roche.com.
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Français

Précautions d'emploi et mises en garde

Pour diagnostic in vitro.
Observer les précautions habituelles de manipulation en laboratoire.
L'élimination de tous les déchets doit être effectuée conformément aux dispositions légales.
Fiche de données de sécurité disponible sur demande pour les professionnels.

Tous les matériaux d'origine humaine doivent être considérés comme potentiellement infectieux. Tous les dérivés de sang humain utilisés ont été préparés uniquement à partir de sang de donneurs où la recherche de l'antigène HBs et des anticorps anti-VHC et anti-VIH a conduit à un résultat négatif.

L'extrait de tissu glandulaire thyroïdien initial contenant de la thyroglobuline humaine a été testé négatif pour l'antigène HBs et les anticorps anti-VHC et anti-VIH.

Les méthodes utilisées pour le dépistage étaient approuvées par la FDA ou conformes à la directive européenne 98/79/CE, Annexe II, liste A.

Cependant, comme le risque d'infection ne peut être exclu avec certitude par aucune méthode, ce produit doit être traité avec le même soin que les échantillons de patients. En cas d'exposition, suivre les directives de l'autorité compétente en matière de santé.

Les contrôles ne doivent pas être utilisés au-delà de la date de péremption indiquée.

Éviter la formation de mousse dans les réactifs et les échantillons de tous types (échantillons de patients, calibrateurs et contrôles).

Téléchargement de fiches techniques

Le cadre en haut de la page montre le code du produit et la version du document.
Ce document est téléchargeable sur http://e-labdoc.roche.com.
Le site affiche la version indiquée ci-dessus ou toute autre version ultérieure disponible correspondant au code du produit. Si vous n'avez pas accès à Internet, veuillez contacter le représentant Roche de votre pays. Vous obtiendrez ce document gratuitement.

Español

Medidas de precaución y advertencias

Producto sanitario para diagnóstico in vitro.
Observe las medidas de precaución habituales para la manipulación de reactivos.
Elimine los residuos según las normas locales vigentes.
Ficha de datos de seguridad a la disposición del usuario profesional que la solicite.

El material de origen humano debe considerarse como potencialmente infeccioso. Los hemoderivados han sido preparados exclusivamente con sangre de donantes analizada individualmente y libre de HBsAg o de anticuerpos anti-HCV y anti-HIV.

El extracto de tejido de glándula tiroidea inicial conteniendo la tiroglobulina humana ha demostrado estar exento de HBsAg y de anticuerpos anti-HCV y anti-HIV.

Los métodos analíticos aplicados fueron aprobados por la FDA o se encuentran en comprobada conformidad con la Directiva Europea 98/79/CE, Anexo II, Lista A.

Sin embargo, dado que nunca puede excluirse con total seguridad el riesgo de infección, se recomienda tratar este producto con el mismo cuidado que una muestra de paciente. En caso de exposición, proceda según las instrucciones de las autoridades sanitarias competentes.

No utilice los controles pasada la fecha de caducidad.

Evite la formación de espuma en reactivos y muestras de todo tipo (especímenes, calibradores y controles).

Descargar la metódica

El recuadro en la parte superior de la página indica el código del producto junto con la versión del documento.
El documento puede descargarse a través de http://e-labdoc.roche.com.
La página web muestra la versión aquí indicada o, en su caso, una versión

PreciControl Universal

cobas®

használtuk fel, akiket egyénileg leteszteltek, és HBsAg-re valamint HCV- és HIV-vírus elleni antitestekre negatívnak bizonyultak.

A reagens előállításához felhasznált, humán tireoglobulint tartalmazó pajzsmirigyszövet-kivonat HBsAg-től illetve HIV- és HCV-elleni antitestektől mentesnek bizonyult.

Az alkalmazott vizsgálati eljárások egy részét az FDA hagyta jóvá, míg a többiek megfelelnek a 98/79/EK Európai Irányelv II. melléklete A listájának.

Mivel azonban nem létezik olyan vizsgálati eljárás, amely teljes mértékben képes lenne kizárni a fertőzés lehetőségét, ezért ezeket az anyagokat ugyanolyan elővigyázatosan kell kezelni, mint a betegmintákat. Ha valaki mégis érintkezésbe kerül az anyaggal, akkor a felelős egészségügyi hatóságok egészségvédelmi irányelvei szerint kell eljárni.

A kontrollokat tilos a feltüntetett lejárati dátum után felhasználni.

Az összes reagens- és mintatípus (betegminták, kalibrátorok és kontrollok) esetén kerülni kell a habképződést.

A módszerleírás letöltése

A termékkódot és a vonatkozó dokumentum verziószámát a jelen dokumentum elején található keret tartalmazza.
A dokumentumot a http://e-labdoc.roche.com honlapról lehet letölteni.
A honlapról a megadott verziószámú dokumentumot vagy - ha van ilyen - a megadott kódú termékre is érvényes valamelyik későbbi dokumentum-verziót lehet letölteni. Ha nincs internet-hozzáférésük, akkor kérjük, hogy forduljanak a helyi Roche képviselethez. Ők ingyenesen megküldik Önöknek a dokumentumot.

Ελληνικά

Προφυλάξεις και προειδοποιήσεις

Για in vitro διαγνωστική χρήση.
Να τηρούνται οι συνήθεις προφυλάξεις οι οποίες απαιτούνται κατά τον χειρισμό όλων των εργαστηριακών αντιδραστηρίων.
Η απόρριψη όλων των αποβλήτων θα πρέπει να πραγματοποιείται σύμφωνα με τις τοπικές κατευθυντήριες οδηγίες.
Διατίθεται, κατόπιν αιτήσεως, φύλλο δεδομένων ασφάλειας για επαγγελματίες χειριστές.

Όλα τα υλικά ανθρώπινης προέλευσης θα πρέπει να θεωρούνται δυνητικώς μολυσματικά. Όλα τα προϊόντα που προέρχονται από αίμα ανθρώπου έχουν παρασκευαστεί αποκλειστικά από αίμα δοτών που έχουν εξεταστεί χωριστά και έχουν βρεθεί ελεύθεροι αντιγόνου HBsAg και αντισωμάτων έναντι των ιών HCV και HIV.

Το αρχικό εκχύλισμα ιστού θυρεοειδούς αδένα που περιείχε την ανθρώπινη θυρεοσφαιρίνη βρέθηκε ελεύθερο αντιγόνου HBsAg και αντισωμάτων έναντι των ιών HCV και HIV.

Οι μέθοδοι εξέτασης που εφαρμόστηκαν ήταν εγκεκριμένες από τον FDA ή σε συμφωνία με την Ευρωπαϊκή Οδηγία 98/79/ΕΚ, Παράρτημα II, Κατάλογος Α.

Ωστόσο, καθώς καμία μέθοδος ελέγχου δεν μπορεί να αποκλείσει το δυνητικό κίνδυνο μόλυνσης με απόλυτη βεβαιότητα, ο χειρισμός των υλικών θα πρέπει να γίνεται με την ίδια προσοχή που δίνεται κατά το χειρισμό των δειγμάτων ασθενών. Σε περίπτωση έκθεσης, θα πρέπει να ακολουθούνται οι οδηγίες των αρμόδιων υγειονομικών αρχών.

Οι οροί ελέγχου δεν πρέπει να χρησιμοποιούνται μετά την παρέλευση της ημερομηνίας λήξης.

Αποφύγετε το σχηματισμό αφρού σε όλα τα αντιδραστήρια και τους τύπους δειγμάτων (δείγματα, βαθμονομητές και διαλύματα ελέγχου).

Λήψη φύλλων μεθόδου

Το πλαίσιο στο επάνω μέρος αυτού του εγγράφου υποδεικνύει τον κωδικό προϊόντος μαζί με την αντίστοιχη έκδοση του εγγράφου.
Για τη λήψη αυτού του εγγράφου μεταβείτε στην ιστοσελίδα: http://e-labdoc.roche.com.
Η ιστοσελίδα εμφανίζει είτε τον αριθμό έκδοσης που παρατίθεται εδώ είτε, εάν είναι διαθέσιμη, οποιαδήποτε μεταγενέστερη έκδοση που ισχύει επίσης για το συγκεκριμένο κωδικό προϊόντος. Σε περίπτωση που δεν διαθέτετε πρόσβαση στο Διαδίκτυο, επικοινωνήστε με την τοπική θυγατρική εταιρεία της Roche για να λάβετε το έγγραφο χωρίς χρέωση.

Türkçe

Önlemler ve uyarılar

İn vitro diagnostik kullanım içindir.
Tüm laboratuvar reaktiflerinin kullanılmasında gerekli olan normal önlemleri uygulayın.

Tüm atık malzemelerin atılması yerel yönetmeliklere göre olmalıdır.
Talep edildiği takdirde profesyonel kullanıcılara güvenlik veri formu verilebilir.

İnsanlardan elde edilmiş tüm maddeler potansiyel olarak bulaşıcı kabul edilmelidir. İnsan kanından elde edilen tüm ürünler sadece ayrı ayrı test edilmiş donörlerden alınan ve içinde HBsAg'nin ve HCV ile HIV'e karşı oluşan antikorların bulunmadığı gösterilmiş kandan hazırlanmıştır.

İnsan tiroglobulini içeren ilk tiroid glandüler doku ekstresinde HBsAg'nin ve HCV ile HIV'e karşı oluşan antikorların bulunmadığı gösterilmiştir.

Uygulanan test yöntemleri FDA onaylıdır veya Avrupa Direktifi 98/79/EC, Ek II, Liste A'ya uygun şekilde izinlidir.

Ancak, hiçbir test yöntemi potansiyel enfeksiyon riskini kesin olarak dışlayamayacağı için, materyal, hasta örneğine gösterilen düzeyde dikkatle kullanılmalıdır. Maruz kalma durumunda, sorumlu sağlık yetkililerinin direktiflerine uyulmalıdır.

Kontroller son kullanma tarihinden sonra kullanılamaz.

Tüm reaktifler ve numune tiplerinde (örnek, kalibratör ve kontrol) köpük oluşumunu engelleyin.

Yöntem Sayfası yüklemesi

Bu belgenin üst tarafındaki kutuda ilgili belge sürümü ile birlikte ürün kodu gösterilir.
Bu belgeyi yüklemek için lütfen http://e-labdoc.roche.com adresine gidin.
Web sitesinde burada listelenen sürüm görüntülenir veya varsa, yine bu ürün kodu için geçerli sonraki başka bir sürüm görüntülenir. İnternet erişiminiz yoksa, belgeyi ücretsiz temin etmek için lütfen Roche Diagnostik Sis. Tic. A.Ş.'ye başvurun.

Български

Предпазни мерки и предупреждения

За ин витро диагностично приложение.
Спазвайте нормалните предпазни мерки, необходими при работа с всички лабораторни реактиви.
Изхвърлянето на всички отпадни материали трябва да се извършва в съответствие с местните разпоредби.
Налични са листовки с данни за безопасност за професионалните потребители при поискване.

Всички материали от човешки произход трябва да се считат за потенциално заразни. Всички продукти, получени от човешка кръв, се изготвят изключително от кръвта на индивидуално тествани донори, които нямат HBsAg и антитела за HCV и HIV.

Първоначалният екстракт от тъкан на тироидната жлеза, съдържащ човешки тироглобулин, няма HBsAg и антитела към HCV и HIV.

Приложените методи на тестване са одобрени от FDA или отговарят на изискванията, посочени в Европейска Директива 98/79/EC, Анекс II, Списък А.

Но тъй като никой метод на тестване не може да изключи с абсолютна сигурност потенциалния риск от заразяване, материалът трябва да се третира така внимателно, както пациентските проби. В случай на пряк контакт, спазвайте директивите на отговорните здравни власти.

Контролите не могат да се използват след изтичането на посочения срок на годност.

Да се избягва образуването на пяна във всички реактиви и типове проби (проби, калибратори и контроли).

Сваляне на листовката

Кутията в горната част на този документ показва кода на продукта, заедно със съответната версия на документа.
Моля, отидете на http://e-labdoc.roche.com, за да свалите този документ.
В уебсайта ще видите посочената тук версия или, ако е налична, всяка следваща версия, която също е валидна за кода на този продукт. Ако нямате интернет достъп, моля свържете се с местния клон на Roche, за да получите документа безплатно.

Eesti keel

Ettevaatusabinõud ja hoiatused

Kasutamiseks in vitro diagnostikas.
Kõigi laborireaktiivide käsitsemisel tuleb rakendada tavapäraseid ettevaatusabinõusid.
Kõikide jäätmematerjalide käitlemisel tuleb järgida kohalikke suuniseid.
Professionaalsetele kasutajatele on vajadusel kättesaadavad ohutussertifikaadid.

PreciControl Universal

cobas®

REF 11731416 190 LOT 160281 2018-10

			PreciControl U1 LOT 147130			PreciControl U2 LOT 147140			
Components	Method		Value	Range	1SD	Value	Range	1SD	Units
FT3 III	Elecsys FT3 III 06437206	e 411	5.75	4.72 - 6.79	0.35	24.5	20.1 - 28.9	1.47	pmol/L
			0.37	0.30 - 0.44	0.02	1.59	1.30 - 1.88	0.10	ng/dL
			3.74	3.07 - 4.41	0.22	15.9	13.0 - 18.8	0.95	pg/mL
		E170/e 601/e 602	5.75	4.72 - 6.79	0.35	24.5	20.1 - 28.9	1.47	pmol/L
			0.37	0.30 - 0.44	0.02	1.59	1.30 - 1.88	0.10	ng/dL
			3.74	3.07 - 4.41	0.22	15.9	13.0 - 18.8	0.95	pg/mL
FT4	⚠ Elecsys FT4 11731297	e 411	15.2*	12.9 - 17.5	0.76	39.0*	33.2 - 44.9	1.95	pmol/L
			11.8*	10.0 - 13.6	0.59	30.3*	25.8 - 34.8	1.51	ng/L
			1.18*	1.00 - 1.36	0.06	3.03*	2.58 - 3.48	0.15	ng/dL
		E170/e 601	15.2*	12.9 - 17.5	0.76	39.0*	33.2 - 44.9	1.95	pmol/L
			11.8*	10.0 - 13.6	0.59	30.3*	25.8 - 34.8	1.51	ng/L
			1.18*	1.00 - 1.36	0.06	3.03*	2.58 - 3.48	0.15	ng/dL
FT4	Elecsys FT4 11731297	e 602	15.2	12.9 - 17.5	0.76	39.0	33.2 - 44.9	1.95	pmol/L
			11.8	10.0 - 13.6	0.59	30.3	25.8 - 34.8	1.51	ng/L
			1.18	1.00 - 1.36	0.06	3.03	2.58 - 3.48	0.15	ng/dL
FT4 II	Elecsys FT4 II 06437281	e 411	15.5	13.2 - 17.8	0.78	41.3	35.1 - 47.5	2.07	pmol/L
			12.0	10.2 - 13.8	0.60	32.1	27.3 - 36.9	1.61	ng/L
			1.20	1.02 - 1.38	0.06	3.21	2.73 - 3.69	0.16	ng/dL
		E170/e 601/e 602	15.5	13.2 - 17.8	0.78	41.3	35.1 - 47.5	2.07	pmol/L
			12.0	10.2 - 13.8	0.60	32.1	27.3 - 36.9	1.61	ng/L
			1.20	1.02 - 1.38	0.06	3.21	2.73 - 3.69	0.16	ng/dL
HCGSTAT	Elecsys HCG STAT 03300811	e 411	5.68	3.81 - 7.55	0.62	43.8	29,3 - 58.3	4.82	mIU/mL=IU/L
		e 601/e 602	5.02	3.36 - 6.68	0.55	41.5	27.8 - 55.2	4.57	mIU/mL=IU/L
HCG-BETA	Elecsys HCG+β 03271749	e 411	5.40	3.78 - 7.02	0.54	43.1	30.2 - 56.0	4.31	mIU/mL=IU/L
		E170/e 601/e 602	5.13	3.59 - 6.67	0.51	43.1	30.2 - 56.0	4.31	mIU/mL=IU/L
IGE	Elecsys IgE II 04827031	e 411	122	96.4 - 148	8.54	313	247 - 379	21.9	IU/mL
			293	231 - 355	20.5	751	593 - 909	52.6	ng/mL
		E170/e 601/e 602	122	96.4 - 148	8.54	313	247 - 379	21.9	IU/mL
			293	231 - 355	20.5	751	593 - 909	52.6	ng/mL

PreciControl Universal

Roche/Hitachi MODULAR ANALYTICS E170

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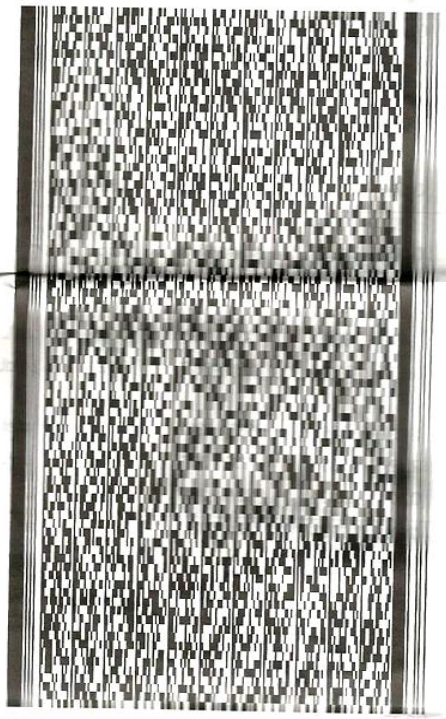
11731416 190

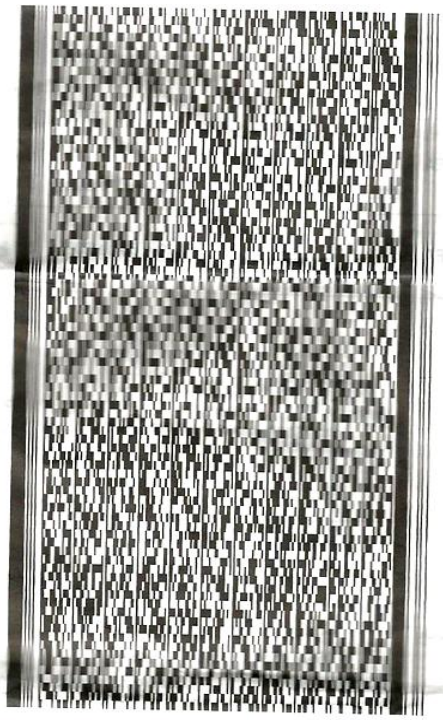
Control Barcodes

LOT 160281 2018-10

PC U1 Code: 147130

PC U2 Code: 147140





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