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Endocrine Identification of Menopausal Status of Sudanese Women

By

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DEDICATION

To my family for all their support to give me a chance to develop my self and my career. I am deeply grateful for their patience during my study and their care for my daughter.

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To all of them I dedicate this work with love and a lot of thanks

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Abstract

This study was conducted in order to identify the menopausal statues of Sudanese women which is critically important in determining the treatment strategy for infertile patients. In this study samples were collected from two hundred Sudanese women, aged between 35 and 62. They were from different social classes and are not suffering any systemic or endocrine disease. They were not exposed to any surgical intervention by complete hysterectomy or partial removal of ovaries or thyroidectomy.

Reproductive hormones were determined for these women. Immunoradiometric Assay (IRMA) was adopted for the measurement of serum prolactin, follicle stimulating hormone, and lutienizing hormone. Enzyme linked immunosorbent assay (ELISA) was used for the determination of estradiol and esterone, whereas, enzyme immunoassay (tube format) was used for the determination of serum testosterone.

Average menopausal age for Sudanese women was determined in this study and found to be 43.0 ±4.2 which is lower than that of the neighboring countries.

A new classification system was developed during this study which uses a combination of symptoms together with hormonal profile in order to identify the menopausal status of women. The three key tools of this system are FSH and LH levels together with the absence of menstrual cycle during the last three months.

The new classification scheme had successfully differentiated the early peri-menopausal women from pre-menopausal ones. The early peri-menopausal women according to the new classification scheme are suffering cycle irregularities and amenorrhoea but with normal hormonal levels. The new classification scheme is now; clearly indicating that amenorrhoea with normal hormonal levels may be an indication to the beginning of the peri-menopausal life.

The big challenge faced during this study had been the sub-classification of the peri-menopausal stage as it is not a single homogeneous stage but a wide heterogonous and transitional stage extending from the early peri-menopause with mild signs and symptoms to the late peri-menopausal stage which almost resembles the post-menopause.

The new classification system using its three “key” tools had successfully classified the heterogeneous peri-menopausal stage into three distinct sub-classes with characteristic hormonal and symptomatic manifestations for each. The three subclasses are early peri-menopausal women who are characterized by presence of normal hormonal levels (FSH and LH) but who suffer amenorrhoea. FSH level of this subclass although within the normal range, it is higher than that of the preceding class “pre-menopausal stage”, but the difference is not statistically significant. Therefore, women who suffer irregular cycles and amenorrhoea due to other endocrine causes (hyperprolactinaemia, hypo- and hyperthyroidism, etc) can be misclassified as early peri-menopausal. Therefore, the other endocrine causes should be ruled out before including them in this subclass by measurement of the respective hormones, namely, prolactin and TSH levels.

The second subclass is the med peri-menopausal women who are characterized by elevated FSH levels but unlike the preceding subclass they do not suffer amenorrhoea, the reason for this is not clear, but it seems the ovary is still secreting sufficient oestrogen levels to prime the uterus and maintains regular menstrual cycles. However, further research with a larger sample size is needed to clarify this situation.

The third subclass is the late peri-menopausal women who are having elevated FSH levels, but normal LH levels and they suffer amenorrhoea, indicating the beginning of cessation menstruation.

The new classification scheme indicated that LH levels increase sharply immediately after menopause signaling the end of reproductive life and the beginning of the post menopausal life. The new classification scheme indicated that early post-menopausal women are those who have elevated FSH and LH, but who experience a menstrual cycle during the last three months. The Late post-menopausal women are those who have elevated FSH and LH and who do not experience a menstrual cycle during the last year.

Findings of this study have also shown that the most common symptoms among the peri-menopausal Sudanese women were irregular menses followed by bladder control problems, Joint and muscles pain, and fatigue. In the post menopausal women the most common symptom was hot flashes, irregular menses , Joint and muscles pain, fatigue, vaginal dryness, numbness in arms or hands, and others different symptoms.

المستخلص

التعرف على حالة سن اليأس لدى النساء السودانيات بواسطة قياس الهرمونات

أجريت هذه الدراسة من أجل التعرف على حالة سن اليأس لدي المرأة السودانية التي تعتبر ذات أهمية حاسمة في تحديد استراتيجية العلاج للمرضى اللاتى يعانين من العقم. في هذه الدراسة تم جمع عينات من مئتي امرأة سودانية ، تتراوح أعمارهن بين 35 و 62 سنة. كن من مختلف الطبقات الاجتماعية ، ولا يعانين من أي أمراض عضوية او غددية. ولم يتعرضن إلى تدخل جراحي عن طريق الاستئصال الكلى او الجزئي للرحم او المبيض أو استئصال الدرقية.

تم قياس هرمونات الخصوبة (هرمون اللبن و هرمونى التبويض) باستخدام طريقة المقايسة المناعية الاشعاعية و طريقة القياس المناعى الخميرى كانت تستخدم لتحديد الاستروجين 1و2 و التستوستيرون. حددت هذه الدراسة متوسط سن الياس لدى النساء السودانيات ب 4.2±43 عاما وهو عمر اقل مقارنة مع الدول العربية و الافريقية المجاورة.

وقد تم تطوير نظام جديد للتصنيف في هذه الدراسة يستخدم مجموعة من الاعراض مع الملف الهرموني لتحديد حالة المرأة بعد انقطاع الطمث. الأدوات الرئيسية الثلاثة من هذا النظام هي الهرمون المحفز للجريبات والهرمون المنبه للجسم الاصفر مع غياب الدورة الشهرية خلال الأشهر الثلاثة الماضية.

وقد نجح نظام التصنيف الجديد في التمييزبين النساء ما قبل سن اليأس والنساء في شبه سن اليأس المبكر. النساء في شبه سن اليأس المبكر وفقا لنظام التصنيف الجديد يعانين عدم انتظام الدورة وانقطاع الطمث ولكن فى وجود المستويات الهرمونية العادية. نظام

التصنيف الجديد الحالى يشير بوضوح إلى أن انقطاع الطمث فى وجود المستويات العادية للهرمونات قد يكون مؤشرا لبداية حياة شبه سن اليأس.

التحدي الكبير الذي واجه هذه الدراسة هو التصنيف الفرعي لمرحلة شبه سن اليأس اذ أنها ليست مرحلة واحدة متجانسة ولكن مرحلة واسعه غير متجانسة تمتد من شبه سن اليأس المبكر- مع وجود علامات وأعراض خفيفة -إلى مرحلة شبه سن اليأس المتأخر التي تشبه تقريبا مرحلة ما بعد سن اليأس.

نجح نظام التصنيف الجديد باستخدام أدوات تصنيفه الثلاثه في تمييزمرحلة شبه سن اليأس المتجانسة إلى ثلاث فئات متمايزة من الباطن مع الأعراض الظاهرة و الهرمونية المميزة لكل منها والفئات الفرعية الثلاث هي فئة شبه سن اليأس المبكرة اللاتي يتميزن بافراز المستويات الهرمونية العادية (الهرمون المحفز للجريبات والهرمون المنبه للجسم الاصفر) و لكنهن يعانين انقطاع الطمث. مستوى الهرمون المحفز للجريبات لهذه الفئة الفرعية على الرغم من انه ضمن المعدل الطبيعي ، هو أعلى من مثيله في "مرحلة ما قبل سن اليأس السابقة ، ولكن الفرق ليس محسوسا من الناحية الاحصائية. لذلك ، يمكن أن يخطأ تصنيف النساء اللواتي يعانين دورات غير منتظمة وانقطاع الطمث نتيجة لأسباب الغدد الصماء الأخرى (ارتفاع هرمون اللبن ، ونقص وفرط نشاط الدرق ، الخ) بأنهن في مرحلة شبه سن اليأس المبكر . ولذلك يجب استبعاد النساء المصابات بأمراض الغدد الصماء عن طريق قياس هرمونات الخصوبة ، وهي مستويات هرمون اللبن والهرمون المنبه للغدة الدرقية.

الفئة الفرعية الثانية هي نساء شبه سن اليأس الوسيطة و تتميز بارتفاع الهرمون المحفز للجريبات ولكن خلافا للفئة فرعية السابقة أنهن لا يعانين من انقطاع الطمث ، والسبب في ذلك ليس واضحا ، ولكن يبدو أن المبيض ما زال يفرز مستويات كافية من

هرمون الاستروجين للرحم ويحافظ بذلك على انتظام الدورة. هناك حاجة إلى مزيد من البحث و مزيد من عدد العينات لتوضيح هذا الوضع.

الفئة الفرعية الثالثة هى النساء عند شبه سن اليأس المتأخرة و هي التي لها مستويات مرتفعة من الهرمون المحفز للجريبات ، بينما مستوي الهرمون المنبه للجسم الاصفر طبيعي وأنهن يعانين انقطاع الطمث ، مما يدل على بداية وقف الحيض.

أشار نظام التصنيف الجديد الي ان مستوي الهرمون المنبه للجسم الاصفر يزيد بشكل حاد مباشرة بعد انقطاع الطمث مما يشير الى نهاية الحياة الإنجابية وبداية حياة بعد سن اليأس. كما أشار نظام التصنيف الجديد ان النساء في مرحلة ما بعد سن اليأس المبكر لديهن ارتفاع في الهرمون المحفز للجريبات والهرمون المنبه للجسم الاصفر، ولكنهن حضن خلال الأشهر الثلاثة الماضية. النساء في مرحلة ما بعد سن اليأس المتأخرة هن اللاتى لديهن ارتفاع في الهرمون المحفز للجريبات والهرمون المنبه للجسم الاصفر ولم يحضن خلال الأشهر الثلاثة الماضية.

وقد أظهرت نتائج هذه الدراسة أن أكثر الأعراض شيوعا بين النساء السودانيات في مرحلة شبه سن اليأس هى عدم انتظام الحيض تليها مشاكل السيطرة على المثانة ، وآلام العضلات والاعياء. اما عند النساء بعد سن اليأس كان أكثر الأعراض شيوعا الهبات الساخنة ، وعدم انتظام الحيض ، وآلام العضلات والاعياء وجفاف المهبل ، وخدر في اليدين أو الأزرع ، وغيرها من أعراض مختلفة.

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List of abbreviations

Abbreviation	Name
WHO	World health organization
FMP	Final menstrual period
MRS	Menopause Rating Scale
BMI	Body mass index
HRT	Hormone replacement therapy
LH	Luteinizing Hormone
FSH	Follicle stimulating hormone
PRL	Prolactin
E2	Estradiol
E1	Esterone
TESTO	Testosterone
PMS	premenstrual syndrome
Hcg	human chorionic gonadotropin
POF	premature ovarian failure
SHBG	sex hormone-binding globulin
TSH	Thyroid stimulating hormone
GnRH	gonadotropin-releasing hormone

CHAPTER ONE

Introduction

1.1. What is menopause?

Menopause is the cessation of menstruation as a result of normal decline in ovarian function. It is a period of time when a woman's hormone levels begin to change and it complete, when menstrual periods have ceased for one continuous year. This stage is often referred to as the "change of life," because it means the end of reproductive years. It is also called menopausal transition, a period when the endocrine, biological and clinical features of approaching menopause begins (Burger *et al.,* 2002). Menopause has become increasingly medicalized which mean it is viewed as some thing that requires intervention and treatment rather than as a natural life transition that may need support. The important part of menopause is its association with aging.

The World Health Organization (WHO) has defined the menopause as the permanent cessation of menstruation resulting from loss of ovarian follicular activity (WHO, 1996). The term 'peri-menopause' includes the period immediately before the menopause (when the endocrinologic, biologic, and clinical features of approaching menopause commence) and the first year after menopause. The term 'menopausal transition' include the period before the final menstrual period when variability in the menstrual cycle is usually increased and has been applied to the part of the perimenopause that ends with the final menstrual period (FMP). The incidence of dysfunctional uterine bleeding and hysterectomy are maximal during the menopausal transition and the incidence of symptoms is similar to that in early postmenopausal women (McKinlay *et al.,* 1992; Dennerstein *et al.,* 1993).

The climacteric is the phase in the aging process of women marking the transition from the reproductive phase to the non-reproductive state. Premenopause is often used either to refer to the 1 or 2 years immediately before the menopause or to refer to the whole of the reproductive period before the menopause. Induced menopause is defined as the cessation of menstruation that follows either surgical removal of both ovaries (with or without hysterectomy) or iatrogenic ablation of ovarian function (e.g., by chemotherapy or radiation). The term 'post-menopause' defines the phase after the final menstrual period (WHO, 1996).

1.2 Rational of the study:

In the Sudan there are no previous studies and established data that determine the average menopausal age in Sudanese women. In infertility clinics, gynecologists are facing difficulties in determining the menopausal status of women and consequently in selecting the suitable treatment strategy. Availability of average menopausal age for Sudanese women might help by provision of reference for comparison and consequently provision of a rough estimate, however, even such information is lacking.

The focus of this study is to determine menopausal status of women using changes in reproductive hormones as women approach menopause together with accompanying changes in signs and symptoms. It was also stated in the literature that the relationship between E2 and E1 inverses at about the time of menopause. This study tends to investigate the relationship between the two hormones and the possibility of using this inversion or inflection point as a cut-off point to determine the onset time of menopause.

1.3 Literature Review

1.3.1 Menopausal age:

While the average menopausal age is 51 for most women in the western world (Peter and Zedler, 2003), natural menopause can actually occurs at any time between the ages of 40 to 55 years. The median age of onset has been reported as between 45.5 and 47.5 years; the average duration is 4 years (Treloar, 1981; McKinlay *et al.*, 1992). Less than 1% of women experience menopause before the age of 40 years (Derek, 1990). In rare cases menopause can occur as early as 30 years of age or as late as 60 years. It is considered premature if it occurs before 40 years, or artificial if it occurs due to radiation exposure, chemotherapeutic drugs or surgical interference (Barrett and Bush., 2001). Other factors that may contribute to the early onset of menopause include the history of smoking, poor nutrition, a co-existing medical condition or even a traumatic experience.

1.3.2 Determinants of the age of natural menopause:

The most consistent findings are that early age at menopause is associated with smoking (Jick *et al.,* 1972; Willett *et al.,* 1983; McKinlay *et al.*,1985; Adena and Gallagher.**,**1982; Daniell .,1978*)* and underweight (Sherman *et al.*,1981). Less clear is the role of menstrual and reproductive factors. Some studies have suggested that early menarche is associated with early menopause (Cramer *et al*, 1995), but the role of the number of births is still controversial (Whelan *et al.,* 1990; Stanford *et al.,* 1987; Soberon *et al.,* 1966). Likewise, the effect of use of oral contraceptives on the age at spontaneous menopause is unclear (Brambilla *et al.,* 1989; Cramer *et al.,* 1995).

The determinants of age at menopause may vary from one population to another according to social, ethnic and cultural factors. Since most of the available evidence comes from United States and North Europe (Parazzini and Progetto, 2007). In a cross-sectional nation-wide study of a random sample of 1000 women drawn from the entire population of Singaporean females (Chinese, Malay, and Indian), the mean age of natural menopause was 49.0 years. There are little differences between the three ethnic groups (Hoe Loh *et al*., 2005)

The average age of menopause varies according to geographic location. In the western world, the average age of menopause is 51 year in united status. In some developing countries, such as India and the Philippines, the median age of natural menopause is considerably earlier, at 44 years. In a study made in Papua New Guinea, series of poorly and better nourished women had median ages of 43.6 and 47.3 years, respectively, which reflect the effect of nutrition on age of menopause (Scragg, 1973). Women underweight may also tend to experience an earlier menopause, while women who are overweight often experience a later menopause. There is study mention that a woman tends to experience menopause at about the same age as her mother did.

1.3.3. Menopausal age in different countries:

The determinants of age at spontaneous menopause have been investigated in several studies (Parazzini and Progetto, 2007). The mean age of menopause in black women in South Africa in the rural and urban women was, 49.5 and 48.9 years, this can be compared with values reported for black women in Durban in 1960; mean and median ages were 47.7 and 48.1 years (Abramson *et al.,* 1960) and in Johannesburg in 1971 (Frere, 1971), where the mean was found to be 50.7 years. In the WHO Report (WHO

Scientific Group, 1981), means or medians for white women ranged from 49.5 to 51.2 years. In a study conducted in Papua New Guinea, series of poorly and better nourished women had median times of 43.6 and 47.3 years, respectively (Walker *et al.*, 1984). In study in Erzurum, an eastern city of Turkey The mean age of menopause was found to be 45.3 years and ranged from 35 to 54 (Seckin, 1998).

The median age at menopause reported from the West (50.3 years) (Rizk *et al.,* 1998) is higher as compared to the range of 45–47 years in developing countries. (Singh, Kaur *et al*., 2002). The mean age at menopause has been found to be 50.7 years in Malaysia (Ismael, 1994), while in southern Thailand it is 48.7 years (range 40–57 years). (Peeyananjarassri *et al.,* 2006). In USA, it is 50.6 years, (Anderson *et al.,*1987), in France it is 52 years, (Salat, 1980) while in the United Kingdom, it is 50 years and 9 months.(Rymer and Morris , 2000). However in UAE, the mean age at menopause is 47.3 years (range 40 – 59 years) which is significantly lower like other developing countries.

In Pakistan, the mean age at menopause has been found to be 49 ± 3.6 years in rural women of Lahore (Yahya and Rehan, 2002) and 47 years in three socioeconomic urban groups in Karachi (Wasti *et al.,* 1993).

In Cross-sectional nation-wide study of a random sample of 1000 women drawn from the entire population of Singaporean females (Chinese, Malay, and Indian) the mean age of natural menopause was found to be 49.0 years (Hoe Loh *et al*., 2005). The average age at menopause amongst the north- Indian women was found to be 48.7 ± 2.3 years (46.4 – 51.0 years). Earlier studies have indicated mean age of menopause in the north-Indian population to be 46.7 years (Gold *et al.,* 2001). The onset of menopause in Turkish women living in rural region is earlier on average compared to women living in western or industrialized countries**.** Age at menopause of post-menopausal women was 44.4 ± 5.3 years and median age was 45.0 years**.** The mean age at natural menopause among Saudi Arabian women was 48.06 years and the median age was 49 years (Maha, 2005).

In Indian populations, different ages of menopause have been reported by various investigators. In Brahmin females of Maharashtra, Rakshit (1962) reported the mean age at menopause as 45.60 years. In Brahmin women of West Bengal, the age at menopause is 47.10 years (Poddar, 1972). Gogoi in 1972 studied Ahom women of

Assam and reported the onset of menopause at 48.44 years. Ghosh and Kumari in 1973 reported menopause at 44.60 years in Sidhu and 46.35 years in Khatris of Delhi. Sharda and his co-workers in 2005 reported that the mean /median age at menopause varies substantially even between the Asian women. Median age at menopause in the Thai women was 49.30 years; (Kono *et al.,* 1990).

1.3.4. Symptoms

The menopausal transition is known to play a major role in the etiology of symptoms due to change in levels of estrogen and progesterone. Various symptoms are reported frequently as being part of a menopausal syndrome. These include hot flashes which are the most common symptom of the climacteric and occur in about 75% of perimenopausal and postmenopausal women in the United States (Freedman, 2005) (Campbell and Whitehead, 1977; Vanessa *et al*., 2002). It begins with a sudden feeling of heat in the upper part or all of the body then face and neck become flushed. Red blotches may appear on the chest, back, and arms. Heavy sweating and cold shivering can follow. Flashes can be as mild as a light blush or severe enough to wake women from a sound sleep (called night sweats). Most flashes last 30 seconds to 5 minutes .The frequency of hot flashes can range from 5 per year to 50 per day, with great variations among individuals or even within an individual. They generally persist for 1 to 5 years, but in some women they can continue for as long as 44 years. There is no accepted metric system for measuring severity of hot flashes (Freedman and Robert , 2005). Kritz and his co- workers in 2000 reported that the most frequently experienced somatic symptoms in the menopausal period are vasomotor symptoms (Kritz *et al.,* 2000) including hot flushes and night sweats, vaginal dryness, and irregular menstruation with changes in sexual behaviour. Evidence indicates that these symptoms are present in higher percentages (up to 88%) of perimenopausal women (Dennerstein *et al*., 2000; Dennerstein *et al.,* 2005). Menstrual irregularity is one of the first signs of change in the periods, they may become less regular and they could be lighter. Some women have short times of heavy bleeding and these are all fairly common. Very heavy bleeding for many days, periods less than 3 weeks apart, periods that last longer than 10 days, or spotting between periods may also happen until women have gone 1 year without a period.

One of the symptoms is that, body tissues in the genital area become drier and thinner as estrogen levels change. Sexual intercourse might become painful because of this dryness. It might also be more likely to have an infection in the vagina and urinary tract problems such as holding urine when there is a feel by need to go to the bathroom (urge incontinence), or problems holding urine when there is sneeze, cough, laugh, run, or step down (stress incontinence).

The most common sexual complains are diminished sexual responsiveness, loss of desire, decreased frequency of sexual activity, the onset of dyspareunia (related to vaginal atrophy and vaginitis), painful intercourse, and sexual dysfunctions of the male partner (Sarrel, 1990 ; Dennerstein *et al.,* 1994 ; Laumann *et al.*,1999). Gonzales and his co-workers in 2004 found some disturbances in one or more of the sexual response domains for 50.3% of sexually active pre-menopausal and postmenopausal women.(Gonzales *et al* .,2004)

Other symptoms such as depression or mood changes, nervous tension, and irritability were found to be associated with menopause, rural residence, low education, high body mass index BMI, and age (Malacara *et al*., 2002). Depression and anxiety symptoms such as irritability, insomnia, poor memory and lack of energy are more common in postmenopausal women. Some authors have suggested that symptoms forming a syndrome are experienced by most women owing to declining levels of estrogen as they transit through menopause. The question of whether, and how, symptoms occur together is important for women who want to know which symptoms can be attributed to menopause and which to aging generally or to other physical or psychosocial factors (Nancy *et al.,* 2005). Other symptoms (visible changes in the body) include a thickening at the waist, loss of muscle mass and increase in fat tissue, or thinning and loss of stretchiness in the skin. Other possible signs include palpitations, headaches, insomnia, lack of energy, difficulty concentrating, dizzy spells and joint and muscle pain.

To study the prevalence of menopausal symptoms the menopause rating scale (MRS) scale is sub-divided into three domains. Three independent dimensions (psychological domain: which includes depressive mood, irritability, anxiety, physical and mental exhaustion, somato-vegetative domain: including hot flushes, heart palpitations, sleep problems, joint and muscular pains and finally urogenital domain: which includes sexual problems, dryness of vagina and bladder problems) have been identified out of

the total list of 11 symptoms enlisted in MRS (Hauser, 1997). Hunter in 1990 using the Women's Health Questionnaire found significant increases only in vasomotor symptoms, sleep problems and in depressed mood when her sample became peri- or post-menopausal, but neither somatic symptoms nor anxiety or sexual behavior changed significantly (Hunter, 1990).

Hunter in 1990 using the Women's Health Questionnaire found significant increases only in vasomotor symptoms, sleep problems and in depressed mood when her sample became peri- or post-menopausal, but neither somatic symptoms nor anxiety or sexual behaviour changed significantly (Hunter, 1990).

Although for many women at mid-life menopause is a time of unexpected new symptoms, (Nedstrand *et al.,* 1998) for others symptoms are minor and menopause is not considered stressful. In order to know what factors are related to stress associated with menopause, numerous studies have investigated women's medical and psychosocial complaints as a correlate of menopausal status. Although the results of these studies are not always consistent, most reveal an increase in psychological complaints during the perimenopausal period; a period characterized by increased occurrence of menopausal symptoms that may lead to insomnia and depressed mood (Hunter, 1990; Palinkas and Barrett, 1992). Understanding the relationship between perceived stress and menopause is important because there may be individual differences in the degree to which women view menopause as stressful.

The risk of onset of menopausal symptoms reached a peak during the two years surrounding natural menopause. It also found that the risk of onset of menopausal symptoms in the year following menopause was higher in women who had an artificial menopause than in women having undergone natural menopause. In other periods, the risks did not differ significantly according to type of menopause.

In those who experienced their first menopausal symptoms in the 1–5 year period following menopause were more likely to have reached their menopause before 51 years. Oppositely, those who experienced their first menopausal symptoms in the 1–5 year period prior to menopause were more likely to reach their menopause at 51 years or later.

There is conflicting data on duration of menopausal symptoms. In some women, menopausal symptoms persist for many years after the cessation of menses, but the

frequency and severity of such symptoms in older women have not been well documented (Vanessa *et al.,* 2002) or it may persist for more than 30years(Kronenberg, 1990). It has been reported that Australian women's vasomotor, psychological and somatic symptoms diminish after menopause while they remain high in Japanese women (Anderson *et al.,* 2004). Conflicting data also exists with regard to educational status and prevalence of vasomotor symptoms (Haines *et al.,* 2005). In study done to evaluate age at menopause, climacteric symptoms and related factors in women living in west Anatolian rural region of Turkey, showed no any relationship between education and vasomotor symptoms. Urogenital symptoms are more common in postmenopausal women, including decreased sexual function, vaginal dryness and loss of sexual desire (Dhillon *et al.,* 2005; Dennerstein *et al.,* 2003). These symptoms are related to the duration of menopause and explained as being more clear during peri-menopausal and first years of post-menopausal period (Discigil, 2006). The same study has shown that urine leakage and decreased libido were reported to be significantly higher in post-menopausal women. Menopause, increasing age and higher body mass index (BMI) values are reported as associated with decrease in libido. High prevalence of urogenital symptoms especially vaginal dryness – has been reported to be related to ethnic factors such as hispanic ethnicity and diabetes (Dennerstein *et al.,* 2003; Pastore *et al*., 2004; Malacara *et al.,* 2002). Obesity was associated with dyspareniua, which could be related to vaginal dryness and cause decrease in libido. However the outcomes of the study did not support urogenital symptoms and diabetes association. In the literature urogenital symptoms such as dysuria and dyspareunia have been reported to be relieved by hormone replacement therapy (HRT) (Haines *et al.,* 2005). Lower prevalence of urogenital symptoms was reported among HRT users.

The presentation of all these various symptoms was unsuitable for establishing cut points in a staging system. Although the frequency of symptoms such as hot flashes and night sweats increases markedly during the menopausal transition, they do not correlate well with changing endocrine profiles or bleeding patterns and they are not universally experienced. Other symptoms, such as vaginal dryness, urine leakage, and stiffness or soreness, may be associated with the menopausal transition, but, like vasomotor symptoms, may vary significantly by race/ethnicity. Body mass index, and

lifestyles and behaviors are otherwise not sufficiently sensitive and specific to demarcate entry into the menopausal transition or progression to subsequent stages (Sherman, 2005).

The clinical turning point of the menopause transition is the late transition, during which a woman goes from relatively estrogen replete to estrogen depleted. Symptoms related to low estrogen, such as hot flashes and vaginal dryness, increase, and bone loss accelerates. Following estrogen status helps predict the course of these processes. There is another set of processes that appear more likely to be chronological and less directly related to hormonal status. These include blood pressure changes, insulin resistance, and weight gain. Finally, some processes are modified by hormonal status, with the changing hormonal balance at menopause changing the course of the process. For example, the acquisition of metabolic syndrome may be related more to the balance between testosterone and estradiol (the T/E molar ratio) than to the level of either hormone alone.

Since this ratio increases with progress through the menopausal transition, hormone related changes develop (Mitchell and Nanette, 2008)

1.3.5. The causes of menopause

The menopausal process occurs concurrently with aging. Changes that happen to women in midlife are often attributed to the menopause, due to the fact it is a significant physiologic and psychologic event for most women. In turn, menopause-related changes are often attributed to the loss of estrogen production by the ovaries. It is helpful to remember, however, that reproductive aging is a parallel process to 'somatic' aging, and can be conceptualized as consisting of multiple stages.

1.3.6 Menopausal Change:

In any study involving the menopausal change, the normal stage of menstruation worth mentioning. The menstrual cycle is a cycle of physiological changes that occurs in fertile females. The menstrual cycle under the control of the endocrine system, is necessary for reproduction. It may be divided into three distinct phases: menstruation, the follicular phase and the luteal phase. Ovulation defines the transition from the follicular phase to the luteal phase. The length of each phase varies from woman to woman and cycle to cycle, though the average menstrual cycle is 28 days (Losos *et al*., 2002). Hormonal contraception interferes with the normal hormonal changes with

the aim of preventing reproduction. Stimulated by gradually increasing amounts of estrogen in the follicular phase, menses slow then stop, and the lining of the uterus thickens.

Follicles in the ovary begin developing under the influence of a complex interplay of hormones, and after several days one or occasionally two become dominant (non-dominant follicles atrophy and die). Approximately at mid-cycle, 24-36 hours after the Luteinizing Hormone (LH) surges, the dominant follicle releases an ovum, or egg in an event called ovulation. After ovulation, the egg only lives for 24 hours or less without fertilization while the remains of the dominant follicle in the ovary become a corpus luteum; this body has a primary function of producing large amounts of progesterone. Under the influence of progesterone, the endometrium (uterine lining) changes to prepare for potential implantation of an embryo to establish a pregnancy. If implantation does not occur within approximately two weeks, the corpus luteum will involute, causing sharp drops in levels of both progesterone and estrogen. These hormone drops cause the uterus to shed its lining in a process termed menstruation.

During the menstrual cycle, changes occur in the female reproductive system as well as other systems (which lead to breast tenderness or mood changes, for example).

1.3.6.1. Menstruation

Menstruation is also called menstrual bleeding, menses, catamenia or a period. The flow of menses normally serves as a sign that a woman has not become pregnant. However, this cannot be taken as certainty, as a number of factors can cause bleeding during pregnancy; some factors are specific to early pregnancy, and some can cause heavy flow (Greenfield, Marjorie, 2001; Anderson, Ann *et al.*, 2007; Patrick and Young, 2007). During the reproductive years, failure to menstruate may provide the first indication to a woman that she may have become pregnant. The symptoms of cramping in the abdomen, back, or upper thighs are common during the first few days of menstruation,when menstruation begins, symptoms such as breast tenderness and irritability generally decrease (John, 2007).

1.3.6.2. Follicular phase:

This phase is also called the proliferative phase because a hormone causes the follicles to grow, or proliferate, during this time. Through the influence of a rise in follicle stimulating hormone (FSH) during the first days of the cycle, a few ovarian

follicles are stimulated. These follicles which present at birth and have been developing in a process known as folliculogenesis and compete with each other for dominance. Under the influence of several hormones, all these follicles will stop growing, while one dominant follicle in the ovary will continue to maturity. The follicle that reaches maturity is called a tertiary, or Graafian follicle, and it forms the ovum (Losos *et al*., 2002).

As they mature, the follicles secrete increasing amounts of estradiol, an estrogen. The estrogens initiate the formation of a new layer of endometrium in the uterus, histologically identified as the proliferative endometrium. The estrogen also stimulates crypts in the cervix to produce fertile cervical mucus, which may be noticed by women practicing fertility awareness (Weschler and Toni, 2002).

1.3.6.3. Ovulation:

When the egg has nearly matured, the level of estradiol in the body has increased enough to trigger a sudden release of luteinizing hormone (LH) from the anterior pituitary gland. In the average cycle this LH surge starts around cycle day 12 and may last 48 hours. The release of LH matures the egg and weakens the wall of the follicle in the ovary, causing the fully developed follicle to release its secandry oocyte (Losos, *et al*., 2002). The secondary oocyte promptly matures into an ootid and then becomes a mature ovum. The mature ovum has a diameter of about 0.2 mm. (Gray and Henry, 2000).

A few studies have reported hormone changes in relation to changes in menstrual cycle characteristics, such as the first self reported change in the amount of menstrual flow, in the frequency of menstruation, or in the combination of changes in flow and frequency, an approach that has been adopted in the Melbourne Women's Midlife Health Project (Burger *et al*., 1995).

The most consistent endocrine finding in perimenopausal women is an elevation of early follicular-phase FSH concentrations, which is not always accompanied by a rise in LH (Sherman *et al*., 1976; Lee *et al*., 1988). The variations in circulating FSH levels with increasing age are most probably, due to changes in ovarian physiology affecting the secretory pattern of the gonadotrope. The ovary becomes increasingly resistant to stimulation by gonadotropins, probably due to the decreased number of follicles, which lead to a decline in the production of both estrogens and inhibins.

After a number of years of erratic functioning, the ovaries almost completely stop producing progesterone and two out of the three estrogen hormones: estradiol and estriol. The third estrogen is estrone which is the only estrogen that is still produced in reasonable amounts in post-menopausal women. Testosterone levels decrease; however, a decrease in testosterone levels begins gradually in young adulthood. Testosterone levels are thought not to drop significantly during the menopause transition because the stroma of the postmenopausal ovary and the adrenal gland still continue to secrete small amounts of testosterone, even during post-menopause.

1.3.7. Premature menopause:

Rarely, the ovaries stop working at a very early age, anywhere from the age of puberty to age 20, and this is known as premature ovarian failure (POF). This is commonly referred to as "premature menopause". 1% of women experience POF and it is not considered to be due to the normal effects of aging. Some known causes of premature menopause include autoimmune disorders, thyroid disease, diabetes mellitus, chemotherapy, eating disorders, and radiotherapy. However, in the majority of spontaneous cases of premature menopause the cause is unknown. Premature menopause is diagnosed or confirmed by measuring the levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH). The levels of these hormones will be abnormally high if menopause has occurred. Rates of premature menopause have been found to be significantly higher in fraternal and identical twins; approximately 5% of twins reach menopause before the age of 40. The reasons for this are not completely understood. Transplants of ovarian tissue between identical twins have been successful in restoring fertility.

1.3.8 Perimenopause.

perimenopause is the term describing the menopause transition years. This probably begins about 3 to 5 years before the last menstrual period. It lasts until 12 months after the final period. Some signs or symptoms of menopause may appear during this time. Perimenopause describes the years both before and after the final period (although it is difficult to determine the final period). During perimenopause, the production of most of the reproductive hormones, including the estrogens, progesterone and testosterone, diminish and become more irregular, often with wide and unpredictable fluctuations in levels. During this period, fertility diminishes, but is

not considered to reach zero until the official date of menopause is determined retroactively 12 months after the last menstrual cycle. Signs and effects of the menopause transition can begin as early as age 35, although most women who become aware of the transition do so about 10 years later, often in their mid to late 40s. The duration of perimenopause with noticeable bodily effects can be a few years, ten years or even longer. The actual duration and severity of perimenopause in any individual woman cannot be predicted in advance or during the process. In the perimenopause years, many women undergo significant bodily changes resulting from hormonal fluctuations.

Most women will gain weight, especially in the lower abdomen, buttocks, and thighs. a bodily adaptation in humans over time to both retain what little estrogen is left in the body longer (estrogens are fat-soluble) and to protect the long bones with padding as estrogen levels decrease and the risk of osteoporosis increases. The most well-known symptom of menopause, though, is the "hot flash", a sudden increase in body temperature caused by declining estrogen levels; the "flash" sensation in a "hot flash" occurs as the body temperature peaks and begins a rapid return to normal. Hot flashes can become so strong that they can raise the body temperature multiple degrees in a very short period of time and cause the sufferer to feel weak and break out in heavy sweating. Despite the discomfort to the woman, hot flashes are not considered harmful by physicians and can be treated to ease discomfort in a variety of ways, such as hormone replacement therapy and plant-based estrogens.

Other common symptoms during the perimenopausal period include mood changes, insomnia, fatigue, and memory problems. Some of these complaints may not be related to the actual hormonal fluctuations involved in menopause, but not enough research has been done to determine why women in menopause suffer from so many of these non-hormonally triggered problems. Even women who are free of any troublesome physical effects of perimenopause may find themselves suffering from psychological issues related to societal perceptions of aging; these issues also lend themselves to medical treatment to lessen their overall impact on a woman's life. A recent research shows that melatonin supplementation in perimenopausal women can produce a highly significant improvement in thyroid function and gonadotropin

levels, as well as restoring fertility and menstruation and preventing the depression associated with the menopause.

1.3.9. Premenopause:

is a word used to describe the years leading up to the last period ever, when the levels of reproductive hormones are already becoming lower and more erratic, and the effects of hormone withdrawal may be present**.**

1.3.10. Post menopause:

Postmenopause is all of the time in a woman's life that take place after her last period ever, or more accurately, all of the time that follows the point when her ovaries become inactive. Awoman who still has her uterus can be declared to be in postmenopause once she has gone 12 full months with no flow at all, not even any spotting. When she reaches that point, she is one year into postmenopause. In women who have no uterus, and therefore have no periods, post-menopause can be determined by a blood test which can reveal the very high levels of Follicle Stimulating Hormone (FSH) that are typical of post-menopausal women. A woman's reproductive hormone levels continue to drop and fluctuate for some time into post-menopause, so any hormone withdrawal symptoms that a woman may be experiencing do not necessarily stop right away, but may take quite some time, even several years, to disappear completely. Any period-like flow that might occur during postmenopause, even just spotting, must be reported to a doctor. The cause may in fact be minor, but the possibility of endometrial cancer can not be excluded.

1.3.11. Hormonal changes leading to menopause:

The transition from reproductive to non reproductive life is characterized by a progressive loss of the ovarian function and consequently decline of ovarian hormones. Decline in ovarian hormones in turn is accompanied by elevation in gonadotrophins levels due to the loss of the feed-back inhibition. This process of hormonal changes take place gradually in a process known as menopausal transition, wherein, hormonal changes are accompanied by signs and symptoms characteristic of this transition.

1.3.12. Estrogen:

Estrogen is probably the most widely known and discussed of all hormones. The term "estrogen" actually refers to any of a group of chemically similar hormones; estrogenic hormones are sometimes mistakenly referred to as exclusively female hormones when in fact both men and women produce them. However, the role estrogen plays in men is not entirely clear. Generally hormones are vital chemical substances in humans and animals. Often referred to as "chemical messengers," hormones carry information and instructions from one group of cells to another. In the human body, hormones influence almost every cell, organ and function. They regulate our growth, development, tissue function, sexual function, the way our bodies use food, the reaction of our bodies to emergencies, and even our moods.

The estrogenic hormones are uniquely responsible for the growth and development of female sexual characteristics and reproduction in both humans and animals. The term "estrogen" includes a group of chemically similar hormones: esterone which is produced by the fatty tissues and it is less potent than estradiol, but takes more importance after the menopause, when estradiol is reduced. Estradiol is the most abundant oestrogen produced by the ovaries and it is the primary circulating estrogen before menopause. It is also the strongest estrogen and responsible to the monthly ovulation and normal menstrual cycles. Estriol is an estrogen that is prominent mostly during pregnancy. It is made from a combination of components from the placenta, fetus, and mother. Overall, estrogen is produced in the ovaries, adrenal glands and fat tissues. More specifically, the estradiol and estrone forms are produced in the ovaries, while estriol is produced by the placenta during pregnancy.

In women, estrogen circulates in the bloodstream and binds to estrogen receptors on cells in targeted tissues, affecting not only the breast and uterus, but also the brain, bone, liver, heart and other tissues. Estrogen controls growth of the uterine lining during the first part of the menstrual cycle, causes changes in the breasts during adolescence and pregnancy, and regulates various other metabolic processes, including bone growth and cholesterol levels. During the reproductive years, the pituitary gland in the brain generates hormones that cause a new egg to be released from its follicle each month. As the follicle develops, it produces estrogen, which causes the lining of the uterus to thicken. Progesterone production increases after

ovulation in the middle of a woman's cycle to prepare the lining to receive and nourish a fertilized egg so it can develop into a fetus. If fertilization does not occur, estrogen and progesterone levels drop sharply, the lining of the uterus breaks down and menstruation occurs. If fertilization does occur, estrogen and progesterone will work together to prevent additional ovulation during pregnancy. Birth control pills (oral contraceptives) take advantage of this effect by regulating hormone levels. Also estrogen helps prevent bone loss and works together with calcium and other hormones and minerals to build bones. But once estrogen levels start to decline, after menopause the body breaks down more bone and osteoporosis occurs.

The majority (over 90 percent) of the estrogen present in pre- menopausal woman's body is made in the ovaries. A smaller additional quantity of estrogen is produced by the adrenal glands and peripheral tissues such as fat, liver, and kidneys by converting androgens to estrogens. Estrogen hormones are also formed in the placenta during pregnancy. At menopause the ovaries stop producing estrogen. However, the other sources continue to produce estrogen. After the menopause quantitatively the most important circulating esterogen is esterone formed by extra glandular conversion of adrenal androgen particularly androstenedione (Sitteri and Macdonald, 1973) Irrespective of that the total quantity available to the body is much smaller as the major contributor to the estrogen production, viz., ovaries, are not producing anymore. The important point to remember is that due to these other sources, the body does not just stop producing estrogen at menopause. Because of the androgen conversion to estrogen in fat cells, the over- weight women may suffer less from menopause-related problems, such as hot flashes and osteoporosis, which are related to estrogen depletion. Yet they can be more at risk for diseases that have been linked to estrogen output, such as endometrial and breast cancer. Researchers have shown that because the overweight woman have less of the protein SHBG (sex hormone-binding globulin) which serves as a carrier for estrogen, less estrogen is bound to SHBG. Therefore, more estrogen remains in the free form which is the active form or the potent form within their systems.

1.3.13. Gonadotropins:

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are called gonadotropins because they stimulate the gonads - testes in males, and ovaries in females. They are not necessary for life, but are essential for reproduction. These two hormones are secreted from cells in the anterior pituitary called gonadotrophs. Most gonadotrophs secrete only LH or FSH, but some appear to secrete both hormones. LH and FSH and pituitary TSH are large glycoproteins composed of alpha and beta subunits. The alpha subunit is identical in all three of these anterior pituitary hormones, while the beta subunit is unique and endows each hormone with the ability to bind its own receptor. Diminished secretion of LH or FSH can result in failure of gonadal function (hypogonadism). This condition is typically manifest in males as failure in production of normal numbers of sperms. In females, amenorrhea is commonly observed. The serum patterns of pituitary-ovarian hormones will change when the women approach menopause, the increase in FSH is attributed to early decline in the ovarian hormone inhibinB, which is negatively, regulates follicle-stimulating hormone secretion. The later rise in serum luteinizing hormone (LH) during the menopause transition is due to a cessation of ovarian follicle development.

1.3.14. Prolactin:

Prolactin is a single-chain protein hormone closely related to growth hormone. It is secreted by so-called lactotrophs in the anterior pituitary. It is also synthesized and secreted by a broad range of other cells in the body, most prominently various immune cells, the brain and the decidua of the pregnant uterus. Prolactin is synthesized as a prohormone. Following cleavage of the signal peptide, the length of the mature hormone is between 194 and 199 amino acids, depending on species. Hormone structure is stabilized by three intramolecular disulfide bonds.

1.3.15. Testosterone:

Testosterone is a steroid hormone from the androgen group. Like other steroid hormones, testosterone is derived from cholesterol. The largest amounts of testosterone are produced by the testes in men. It is also synthesized in far smaller quantities in women by the thecal cells of the ovaries, by the placenta, as well as by the zona reticularis of the adrenal cortex in both sexes. Testosterone is supplied to

target tissues in the blood where much of it is transported bound to a specific plasma protein, sex hormone binding globulin (SHBG). The aging reduces testosterone release .After the menopause testosterone decline by about 20% (Hughes *et al.,* 1991)

1.3.16. Objectives of the study:

1.3.16.1. Justification of over-all problem:

- To study hormonal changes during menopausal transition and consequently menopause to achieve the following:
- Identification of a reliable endocrine marker for detection of menopausal onset time (E2/E1 ratio).
- Identification of reliable hormonal indicators for detection of the different menopausal stages of women.
- Determination of the average menopausal age in Sudanese women.

1.3.16.2. Specific objectives:

- To measure the reproductive hormones and their variation during the menopausal transition so as to be used for the identification of the different menopausal stages.
- To measure E1 and E2 levels in order to arrive at an E2/E1 ratio that may be used as a “cut-off point” for the determination or prediction of the menopausal onset time.

CHAPTER TWO

Subjects and Methods

2.1. Subjects:

The subjects of this study were a group of two hundred Sudanese women covering the age range of 35-60 years. They were divided into three groups including premenopause, perimenopause, and postmenopause. They were of different social classes and are not suffering any systemic or endocrine disease and they were not exposed to any surgical intervention by complete hysterectomy or partial removal of ovaries or thyroidectomy. They were randomly selected from the general public and the different health care centers and other governmental institutes allover Khartoum state. These centers and institutes include Reproductive Health Care Centre (Khartoum), Omdurman Islamic University, Khartoum University and Khartoum University Clinic. Processing and analysis of samples were performed at Sudan Atomic Energy Commission (SAEC) radioimmunoassay laboratory, Reproductive Health Care Centre and the WHO Collaborative Centre for Research in Human Reproduction.

Extensive questionnaires containing full information about marital status, height and weight, menstrual history, hormonal treatment, personal and family history and signs and symptoms of menopause, were filled. Five ml of venous blood were collected from day two to day seven in case of regular cycles and at any time for irregular cycles. Samples were allowed to clot, and then sera were separated and stored at- 20°C until analyzed.

2.2. Materials:

2.2.1. Equipment and disposables:

1. Sterile syringes of 5 ml capacity.

2. Serum storage containers (Eppendorf tubes) for storage of separated serum samples.

3. Round bottom polystyrene assay tubes (10X12X75 ml) with a capacity of 5 ml code NO (5001).

3. Angled rotor small centrifuge from Germany (EBA 20-D- 7853L).

4. Fixed and adjustable micropipettes capable of dispensing 50µl, 200µl, 500µl and 1ml. from Eppendorf Gmbt Itamurge 65, Federal Republic of Germany.

5. Magnetic separator, Multimix Major, Luckham, serial No. 424, Model No MF.

6. Vortex mixture and multi shaker plate.

7. Disposable pipette tips (blue and yellow) from Eppendorf.

8. Adjustable repeating syringes (multidose pipettes), from Eppendorf GMbt, Itamurge 65, Federal Republic of Germany.

9. Aluminum foil.

11. Multi-channel gamma counter. PG-RIA. MAS, Stratec Biochemical Systems AG, SN2486000052.

12. Statfax microplate reader with 450 nm filter, from Awareness Technologies (USA).

13. Corning colorimeter 253. Germany: scientific technical supplies. Capable of measuring absorbance at (500 – 550) nm

14. IRMA kits for PRL, FSH, and LH, from China institute of nuclear sciences.

15. ELISA kits for E1 and E2, from Czech Republic, Biovendor research and diagnostic products.

16. Enzyme immunoassay kit (tube format) for testosterone.

17. 37°C water-bath

2.2.2. Reagents and ready to use kits:

Both radioimmunoassay (RIA) and enzyme linked immunosorbent assay (ELISA) techniques were used in this study.

2.2.2.1. Radioimmunoassay kits:

The most sensitive format of radioimmunoassay which contains two antibodies (immunoradiometric assay) is used in this study.

2.2.2.2. ELISA kits:

It is a competition reaction that occurs between an unlabeled antigen and an enzyme-labelled antigen for a limited number of antibody binding sites on the microwell plate.

2.3. Reagents for Immunoradiometric Assay:-

2.3.1. Follicle Stimulating Hormone (FSH):

FSH kits come in package sizes of 100 tubes

1. The tracer bottle contain (10.5 ml/ vial) of a ready to use ^{125}I anti-FSH-Ab solution (coloured red).

2. FSH – Ab coated tube in package sizes of 25 tubes, four packages per kit.

3. A set of ready to use standards in 6 vials containing (S0 – S5) in the following concentrations (0, 1.5, 5, 15, 40, 100 mIU/mL of FSH respectively). There is a 3ml/ vial for standard zero (S0) and there are1ml/vials for the rest of standards (S1 – S5).

4. Two vials of quality control (QC) samples were included in each kit. These QC samples must be reconstituted, each with 0.5 ml of distilled water prior to use.

2.3.2. Luteinizing Hormone (LH):

LH kits come in package sizes of 100 tubes

1. The tracer bottle contain (10.5 ml/ vial) of a ready to use ^{125}I anti-LH-Ab solution (coloured red).

2. LH – Ab coated tube in package sizes of 25 tubes, four packages per kit.

3. A set of ready to use standards in 6 vials containing (S0 – S6) in the following concentrations (0, 1.5, 5, 15, 45, 100 and 200 mIU/mL of LH respectively). There is a 5ml/ vial of standard zero (S0) and there are 1ml/vials for the rest of standards (S1 – S6).

4. Two vials of quality control (QC) samples were included in each kit. These QC samples must be reconstituted, each with 1ml of distilled water prior to use.

2.3.3. Prolactin(PRL):

PRL kits come in package sizes of 100 tubes

1. The tracer bottle contain (20.5 ML/ vial) of a ready to use ^{125}I anti-PRL-Ab solution (coloured red).

2. PRL – Ab coated tube in package sizes of 25 tubes, four packages per kit.

3. A set of ready to use standards in 7 vials containing (S0 – S6) in the following concentrations (0, 50, 125, 300, 800, 2000 and 4000 mIU/mL of PRL respectively). There is a 5ml/ vial of standard zero (S0) and six vials for the rest of the standards (S1 S6). Lyophilized standards were reconstituted with 0.5 ml distilled water. The standards can be used in 1 week if stored at 2-8°C. For longer storage, the reconstituted standards were frozen at -20°C. Multiple freezing and thawing cycles were avoided by aliquating standards once reconstituted.

4. Two vials of quality control (QC) samples were included in each kit. These QC samples were reconstituted, each with 0.5 ml of distilled water prior to use.

2.4. Reagents for Enzyme Linked Immunosorbent Assay (ELISA):

2.4.1. Esterone (E1) Kit:

The human oesterone kits contain the following:

1. One, ready to use microwell plate, coated with rabbit anti-esterone antibody.

2. A bottle containing Estrone-biotin conjugate concentrate*100 (200 µL/vial) solution.

3. A bottle containing Avidin-horse radish peroxidase (HRP) conjugate concentrate *100 (200 µL/vial) solution.

Both the esterone-biotin and avidin concentrates were diluted (1:100) into the same solution of assay buffer and mixed thoroughly.

4. A set of ready to use standards in six vials containing estrone in protein – based buffer with non mercury preservative, labeled as (SA- SF) in the following concentrations 0 , 15 , 50 , 200,800 and 2000 pg/ml of E1 respectively).

5. One vial of ready to use quality control (QC) sample (0.5ml/vial), was included in each kits.

6. A bottle of wash buffer concentrate *10 (50ml/bottle). Wash buffer was prepared by diluting (1:10) in de-ionized water (50ml of wash buffer in 450 ml of water).

7. A bottle of ready to use assay buffer solution (15 ml/vial).

8. A bottle of ready to use TMB substrate solution (16ml/bottle).

9. A bottle of ready to use stopping solution (6ml/vial).

2.4.2. Estradiol (E2) Kit:

1. Twelve, ready to use micro strips (in strip holder), coated with rabbit anti-estradiol antibody.

2. A set of ready to use standards in seven (1.0 ml) vials, labeled as (SA- SG) in the following concentrations (0, 25, 100, 250, 500, 1000 and 2000 pg/ml. of E2 respectively).

3. A bottle of ready to use enzyme conjugate solution, (25 ml), colored red (covered with a white cap).

4. A bottle of Wash Solution, (30 ml) concentrate for ca.1200 ml, pH 7.3± 0.1 (black cap) containing; Tween20 0.2% and TRIS buffered saline 3.0 nmol/l. The working wash solution was prepared by diluting the wash solution to 1200 ml with fresh, de-ionized water in a suitable container. The bottle was rinsed several times. The stability was up to 2 weeks if stored at 15-25°C.

5. A bottle of ready to use substrate solution (yellow cap) containing (14ml) of pH 3.5 - 4.0 of 3,3,5,5 Tetramethylbenzidin (TMB), 0.26g/l of hydrogen peroxide 0.015% sodium acetate buffer 0.05mol/l and dimethyl formamide (DMSO)

6. A bottle of ready to use stop solution (14ml) covered with a red cap, containing sulphuric acid 0.5 mol/l, and preservatives.

2.5. Reagents for enzyme immunoassay method (tube format):-

2.5.1. Testosterone Kit:

Testosterone kit was obtained from immunometrics (UK) ltd in London.

1. Testosterone standards come in lyophilized form which can be reconstituted, each by addition of 1 ml of distilled water.

2. A bottle of ready to use testosterone blocking reagent is provided in a liquid form (11 ml).

3. A bottle of ready to use testosterone antiserum is provided in a liquid form (11 ml).

4. A bottle of testosterone label enzyme was provided as a 25* concentrate. (0.75 ml).

5. A bottle of testosterone separation reagent (10.5ml). This is prepared by washing with assay buffer immediately before use.

6. A bottle of Assay buffer provided as a 5* concentrate (10 ml), this is prepared by diluting before use.

7. A bottle of wash buffer provided as 5* concentrate (25 ml), this is prepared by diluting before use.

8. A bottle of ready to use substrate buffer provided as liquid (55 ml).

9. Two bottles of ready to use stop buffer provided in liquid form (60 ml each).

10. One vial quality control (QC) sample provided as I ml lyophilized serum was included in each kit.

2.6. Methodology and Principals

2.6.1. Principles of Immunoradiometric Assay (IRMA):

IRMA is a radio labeled antibody immunoradio metric assay. It is the best example of excess reagent method (Edwards, 1996). It uses antibodies at an excess concentration, in which solid phase antibody (Ab) was allowed to react with antigen (Ag) to form the first Ab-Ag complex. The complex formed is washed using wash buffer. Labeled antibody is then added to the medium to form a sandwich complex of Ab-Ag-Ab*. In this complex the antigen is sandwiched between the capture antibody and the reporter antibody. After attaining equilibrium, the complex is washed twice to remove excess labeled antibody and read in a gamma counter for 60 seconds. The concentration of Ag reacted with the antibodies is determined by extrapolation from the standard curve.

2.6.1.1. Determination of Follicle Stimulating Hormone (FSH):

All reagents and serum samples were allowed to come to room temperature prior to use in the assay. The assay tubes were labeled in duplicate and arranged in assay rack, then 100µL from standards, samples and QCs were pipetted into the pre-labeled tubes and 100 µL of (^{125} I – anti-FSH-Ab) solution were added to all tubes. Then all tubes were mixed thoroughly, and incubated at 37 °C for 1 hour. The test tubes rack was then removed from the water bath and placed on a magnetic separator. The supernatant liquid was decanted (after 5 minutes). Tubes were placed an absorbent pad paper (for 5 minutes so as to drain), then tubes were washed three times with 0.5 ml distilled water (except the total). Finally all tubes were counted on a gamma counter for 60 seconds and the results were calculated.

2.6.1.2. Determination of Luteinizing Hormone (LH):

The assay tubes were labeled in duplicate and arranged in assay rack, then 100µL from standards, samples and QCs were pipetted into the pre-labeled tubes, and then 100µL (^{125}I – anti-LH-Ab) solution were added into all tubes. All tubes were mixed thoroughly and incubated at 37 °C for 2 hour, then the test tubes rack was removed from the water path and placed on a magnetic separator. Supernatant liquid was decanted. Then the tubes were placed on an absorbent pad and allowed to drain for

5min then the tubes were washed two times with 1mL distilled water (except the total). Finally all tubes were counted in a gamma counter for 60 seconds and the results were calculated.

2.6.1.3. Determination of Prolactin Hormone (PRL):

The assay tubes were labeled in duplicate and arranged in assay rack, then 50µL from standards, samples and QCs were pipetted into the pre-labeled tubes and then 200µL (^{125}I–anti-PRL-Ab) solution were added into all tubes. All tubes were mixed thoroughly and incubated at 37 °C for 2 hours, then the test tubes rack was placed on magnetic separator for 5 minutes. Then the supernatant liquid was decanted, and the rack of tubes placed on an absorbent pad and allowed to drain for 5 min. Then the tubes were washed two times with 2 mL distilled water (except the total). Finally all tubes were counted in gamma counter for 60 seconds and the results were calculated.

2.6.2. Principles of ELISA assay:-

In enzyme linked immunosorbent assay for small molecules competition reaction occurs between an unlabeled antigen (present in standards, control and patients' samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate was added. The enzymatic reaction is terminated by addition of stopping solution. The absorbance is measured on microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of hormone in patients' samples and control can be directly read.

2.6.2.1. Determination of Estradiol Hormone (E2):

The estradiol ELISA is based on competitive interaction of estradiol and hormone-enzyme conjugate for a limited number of immobilized anti-estradiol antibodies (rabbit). Thus the amount of bound hormone – enzyme conjugate is inversely proportional to the concentration of estradiol in the specimen. After incubation of specimen and hormone- enzyme conjugate in the well, unbound conjugate is removed by washing. When the substrate solution is added, a blue colour develops which changes into yellow after addition of the stop buffer. The intensity of the colour developed is inversely proportional to the amount of estradiol in the specimen. The

absorbance of calibrators and specimens is determined by using an ELISA microplate reader. Concentration of unknown specimens is interpolated from a dose response curve generated by utilizing serum calibrators of known estradiol concentrations.

All reagents and serum samples were allowed to come to room temperature prior to use in the assay. 25µL of standards, samples and QCs were pipetted into the microwell coated with anti-estradiol antibodies and then 200 µL estradiol – enzyme conjugate were add to all microwells. Then microtiter plate was incubated in a plate shaker for 60 min at room temperature. then the microtiter strips were washed three times with working wash solution using automatic washer. Then 100µL of substrate solution were added to all microtiter strips, after that incubated for 1 5min at room temperature in the dark. Then 100µL of stop solution were add to all microwell and mixed carefully. Finally the optical density was measured at 450nm wavelength and the results calculated.

2.6.2.2. Determination of Esterone Hormone (E1):

50µL of each calibrator, control and specimen sample were pipetted into the labeled wells then100µL of the conjugate working solution were added into each well. After that the microtiter strips were incubated on a plate shaker (approximately 200 rpm) for 1 hour at room temperature. Then the wells were washed three times with 300 µL of diluted wash buffer per well, immediately emptied and the plate was firmly tapped against absorbent paper. 150 µL of TMP substrate solution were added into each well, after that the plate was incubated on a plate shaker for 10 minutes at room temperature. Then 50 µL of stopping solution were added into each well and finally the plate was read on a microwell plate reader at 405nm.

2.6.3. Principles of Enzyme Immune Assay (EIA):-

2.6.3.1. Determination of Testosterone Hormone:

The testosterone EIA is a direct assay of a limited "competitive" type. Specific agent (testosterone blocking agent) was used to displace testosterone from the binding protein, thus making it available for antibody binding. The testosterone in samples controls or standards equilibrates with fixed amount of alkaline phosphatase labeled testosterone (testosterone labeled) in binding to a limited amount of monoclonal anti-testosterone antibody. An anti-mouse IgG antibody bound to magnetic particles is

used to separate the testosterone/testosterone label-antibody complex from unbound components by magnetic sedimentation and a double wash step. The magnetic particles were incubated with enzyme substrate solution for a fixed time and the reaction ended by addition of stop buffer. The amount of colour produced is inversely proportional to the amount of testosterone present in the sample. The testosterone concentration of test samples is interpolated from a calibration curve.

2.6.3.2. Assay Performance:

The assay tubes were labeled in duplicate and arranged in assay rack, then 50µL from standards, samples and QCs were pipetted into the pre-labeled tubes. Then 100µL of testosterone blocking reagent were added to all tubes, followed by 100µL of diluted testosterone labeled enzyme. 100µL of testosterone antiserum were added, then all tubes were mixed thoroughly after that incubated at 37 ºC for 30 minutes. Then 100µL of testosterone separation reagent were added to all tubes and briefly mixed and also incubated at 37°C for 30 minutes. Then the assay tubes were removed from the water bath and the rack of tubes was placed onto a magnetic base for 5 minutes. Then the supernatant was decanted and the separator was returned to an upright position and the magnetic base was removed. Then 500µL of testosterone wash buffer were added to all tubes and briefly mixed, then the rack of tubes was placed onto a magnetic base for 5 minutes, then the supernatant was decanted and the separator was returned to an upright position. Then this step was repeated. Then 500µL of substrate solution were added to all tubes and a new tube labeled as blank, and then the tubes were briefly mixed and incubated at 37°C for 60 minutes. Then the tubes were removed from the water path and 1 ml of the stop buffer were added to all tubes and briefly mixed, then the rack of tubes was placed onto a magnetic base for at least 10 minutes. Finally the optical densities of the tubes were read by colorimeter using a 550nm wavelength.

2.7. Statistical analyses:

Data were entered into the computer using the Statistical Package for Social Sciences (SPSS) software version (16.0; SPSS Inc., Chicago, IL). The mean and the standard deviation were calculated for all parameters. The data were presented as mean ± standard deviations (SD) of the mean for all hormones. One-sample Kolmogorov-Smirnov test was used to check the normality of data. If the data were not normally

distributed then the Mann-Whitney U test was used to determine the significance of difference between the variables and one way ANOVA Parameters with p-value less than or equal to 0.05 were considered to be significant. Spearman's rho correlation was used to correlate between hormones in specific groups. The ratio for some hormones and the percentage for symptoms occurrence were performed.

CHAPTER THREE

RESULTS

3.1. Introduction:

In the literature women around menopausal age are classified into pre-, peri-, and post menopausal according to certain signs and symptoms. Pre-menopausal women are those who are in their reproductive age with regular menstruation and normal hormonal levels. Peri-menopausal women are those who experience absence of menstruation for more than three months and post-menopausal women are those who experience absence of menstrual cycle for more than one year.

3.2. New classification and identification scheme:

In this study we adopted a combination of signs and symptoms together with hormonal profile in order to identify the menopausal status of women.

The most challenging task in order to identify the menopausal status of women had been the identification of the peri-menopausal stage as it is a wide transitional stage extending from the early peri-menopause with mild signs and symptoms to the late peri-menopausal stage which almost resembles the post-menopause. Actually there is no clear demarcation between the three stages of menopause and there is an overlap between them.

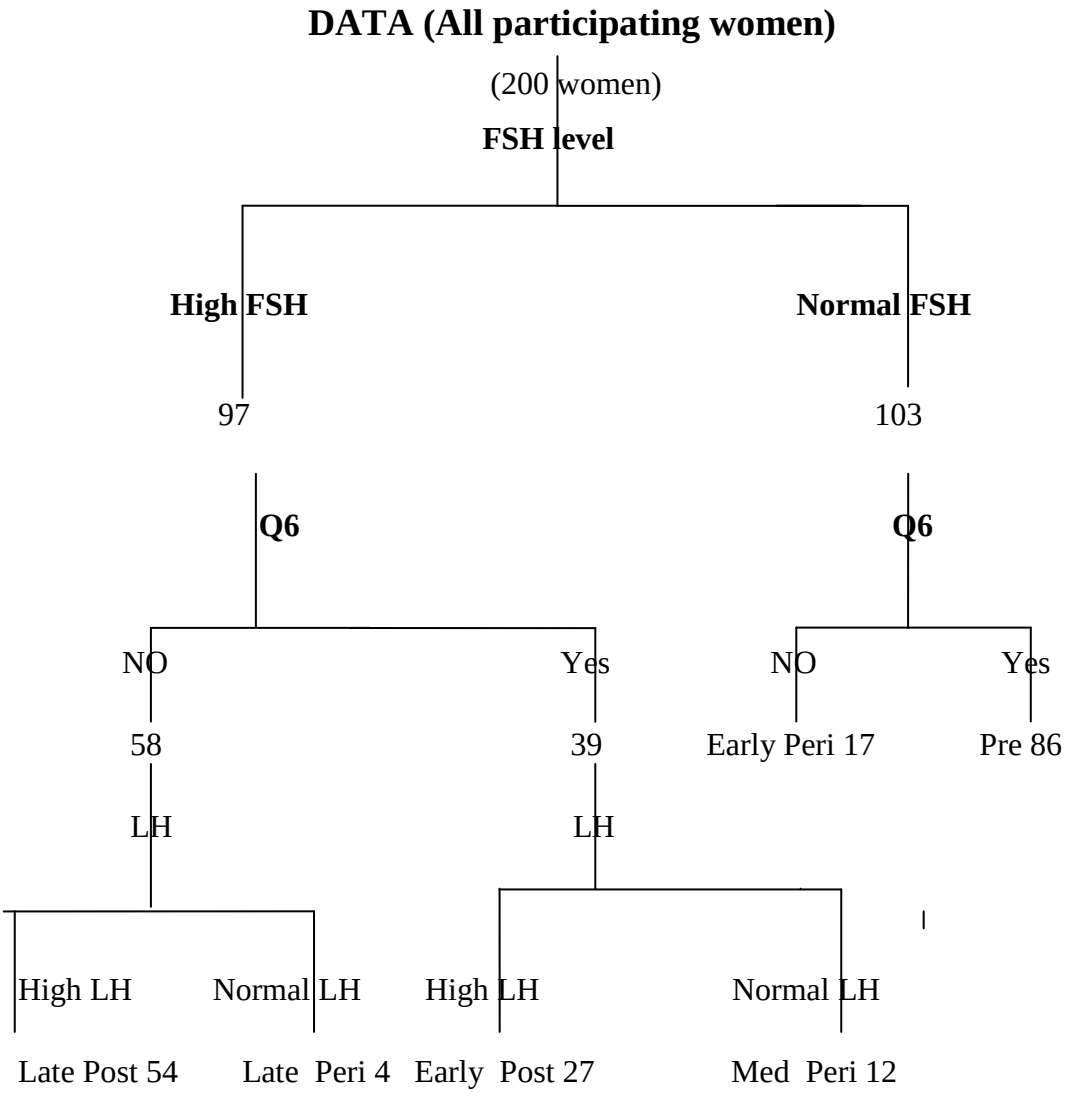
In order to achieve the above mentioned task a combination of hormonal and clinical manifestations (signs and symptoms) was adopted as shown below.

(1) The follicle stimulating hormone being the most sensitive and the most informative among the reproductive hormones (David *et al*., 2002) was taken as a key tool for classification.

(2) The most important among clinical manifestations that is characteristic of peri-menopausal stage is the absence of menstruation for more than three months and this is taken as a key symptom for identification of peri-menopausal women.

(3) Another clinically important hormone that follows FSH (the key hormone) in its variation is LH. This hormone was also used for further filtration and identification of peri-menopausal women at the late Peri-menopausal stage (i.e., those with high FSH but their LH is still within normal).

The three parameters above (two hormonal and one major symptom) were adopted to classify women according to menopausal status and the following classification structure (Fig. 3.1) was obtained:



- **Q6 is do you experience menstruation during the last three months**

Fig. 3.1: A diagram showing classification of women according to menopausal status.

Using FSH as a key hormone for classification of women, the two hundred women were divided into two subgroups, namely, those with high FSH (97) and those with normal FSH (103) as shown on the Fig (3.1)

Those with normal FSH were further sub-classified into two subclasses using the major characteristic symptom for peri-menopausal women (i.e., absence of menstruation during the last three months, which is question 6 (Q6) of the questionnaire shown as annex 1). Using Q6 women with normal FSH can be further sub-divided into two subgroups. Those with regular cycles (those who answered Q6 with Yes), and those who experience absence of the cycle during the last three month (those who answered Q6 with No).

The subgroup that answered Q6 with Yes (86) and are classified to be in their pre-menopausal status according to hormonal and clinical manifestations. The other subgroup with normal FSH but who answered Q6 with No (17) are classified to be in their early peri-menopausal status.

Those with high FSH (97) are also sub-divided into two subgroups using Q6. Those with regular cycles (those who answered Q6 with Yes) and those who experience absence of the cycle during the last three month (those who answered Q6 with No).

The subgroup that answered Q6 with Yes (39) were further sub-classified into two subclasses using the major characteristic hormone for post-menopausal women (LH) into two subgroups, those with high LH and those with normal LH as shown on the figure. The subclass that answered Q6 with Yes and who have normal LH (12) are classified to be in their med peri-menopausal status. The other subclasses that also answered Q6 with Yes but who have high LH levels (27) are classified to be in their early post-menopausal status shown on Fig 3.1.

The subgroup that answered Q6 with No (58) was further sub-classified also according to LH into two subclasses (those with normal and those with elevated LH levels). The subclasses that answered Q6 with No and who have normal LH (4) are classified to be in their late peri-menopausal status. The other subclass that also answered Q6 with No but with high LH (54) are classified to be in their late post-menopausal status as shown on Fig 3.1.

Using FSH and LH levels and absence of menstrual cycle during the last three months women were classified to the following six categories as shown on Fig (3.1) and summarized in table (3.1):-

1- Pre menopausal women are those who have normal FSH and LH levels and regular menstrual cycles.

2- Early peri-menopausal women are those who have normal FSH and LH levels, but experience absence of cycle during the last three months.

3- Med-peri-menopausal women are those who have elevated FSH, normal LH but do not experience absence of menstrual cycle during the last three months.

4- Late peri-menopausal women are those who have elevated FSH, normal LH and who experience absence of cycle during the last three months (Amenorrhoea)

5- Early post-menopausal women are those who have elevated FSH and LH, but who experience a menstrual period during the last three months.

6- Late post-menopausal women are those who have elevated FSH and LH and do not experience a menstrual cycle during the last three months.

Table 3.1: Summary of menopausal categories and classification characteristics of the 200 women.

Pre- (N=86)	**Normal FSH**	**Q6 Yes**	**Normal LH**
Early Peri- (N=17)	**Normal FSH**	**Q6 No**	**Normal LH**
Med-Peri- (N=12)	**High FSH**	**Q6 Yes**	**Normal LH**
Late-Peri- (N= 4)	**High FSH**	**Q6 No**	**Normal LH**
Early post (N= 27)	**High FSH**	**Q6 Yes**	**High LH**
Late Post (N = 54)	**High FSH**	**Q6 No**	**High LH**

3.3. Hormonal changes during the menopausal transition:

Descriptive statistical analysis of each parameter was performed using SPSS programme. The normality for each parameter was checked using the One-sample Kolmogorov-Smirnov test. All data was not normally distributed so analysis of

variance between groups (ANOVA) was used for descriptive statistical analysis for hormones in each group and for multiple comparisons of hormones in subclasses of the groups. Spearman's rho was used to correlate between hormones.

3.3.1. Variation of Follicle Stimulating Hormone Levels:

Mean serum FSH level for pre-menopausal women (n=86) was found to be 8.7mIU/ml, for late peri-menopause (n= 17) was found to be 29.7 mIU/ml and there is a sudden jump to 52.5 mIU/ml during early post-menopause (n=24). Serum FSH level was observed to start rising at early peri-menopause but the difference from the premenopausal stage was not statistically significant ($p \leq 0.99$). However, the hormone continue rising during med- and late peri-menopause where the difference between early peri- and late peri- becomes highly significant ($p \leq 0.000$) showing the sharp increase of serum FSH levels during menopausal transition. FSH levels continue rising during the transition from the late peri-menopausal stage to the immediate early post menopause. The difference between FSH levels during late peri-menopause and early post-menopausal stages is statistically highly significant ($P \leq 0.000$) as shown on table (3.4) indicating that these two groups are actually two distinct groups.

Table 3.2: Variation of FSH during menopause transition

Menopausal subclass	**Mean**	**SD**
Pre-menopause	8.7	4.4
Early-peri	9.4	4.2
Med-peri	26.5	4.4
Late-peri	29.7	10.3
Early-post	52.5	14.9
Late-post	57.4	14.2

3.3.2 Variation of Luteinizing Hormone levels:

Mean serum LH for pre-menopasual women (n=86) was found to be 5.8 mIU/ml for early peri-menopausal women (n= 17) was found to be 8.0 mIU/ml for med-peri (n=12) was found to be 6.8 mIU/ml and for the late peri- (n=4) was found to be 7.5 mIU/ml and for the early post (n=24) was found to be 30.8 mIU/ml as shown on table (3.3) The first significant difference in LH levels between classes appears for the first time between pre- and early post-menopausal stages this difference is statistically highly significant ($p \leq 0.000$) indicating that the LH levels start to increase just with the beginning of post menopausal stage. Unlike serum FSH, serum LH was observed to stay normal during the menopausal transition and start rising very late after menopause (during early post menopausal stage) as shown on Fig (3.2) and table (3.3). Both FSH and LH increase sharply after menopause and the difference between the levels of the two hormones in late peri and early post becomes highly significant as shown on table (3.4).

Table 3.3: Variation of LH during menopause transition:

Menopausal subclass	**Mean**	**SD**
Pre-menopause	5.8	5.7
Early-peri	8.0	6.7
Med-peri	6.8	2.2
Late-peri	7.5	3.0
Early-post	30.8	13.9
Late-post	30.5	15.3

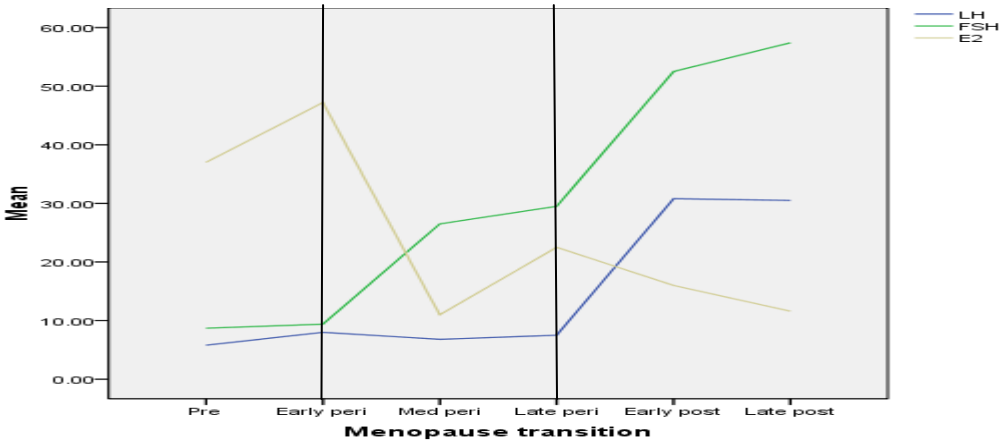


Fig. 3. 2: Hormonal changes during menopausal transition.

Table 3.4: Differences in reproductive hormone levels between late peri and early post-menopause women

Hormone	Late Peri- (17)	Early Post- (24)	Significance Level
Mean FSH (IU/ML)	29.7	52.5	0.000
Mean LH (IU/ML)	7.5	30.9	0.000
Mean PRL (mIU/L	299	174.6	0.41

3.3.3 Prolactin:

Mean serum PRL level vary slightly between the different classes and subclasses, but there is no statistically different variation in its levels during menopausal transition or after menopause.

3.3.4. Variation of Oestradiol levels:

Mean serum E2 level for pre-menopausal women was 37pmol/L. The hormone was observed to increase at the beginning of the peri-menopausal life where it was 47.2 pmol/L for early peri-menopausal women, then it decreased to 22.5 pmol/L for med-peri and eventually droped to 13.4 pmol/L for late peri-menopausal women. The hormone stayed low during the post menopausal life where it was observed to be 16.0 pmol/L for early post menopausal women and 11.6 pmol/L the late post as shown on table (3.5). However, no significant variation in the E2 levels was observed except between pre and late post-menopausal women ($p \leq .001$) and early peri and late post ($p \leq 0.006$).

Table 3.5: Variation of E2 during menopause transition:

Menopausal subclass	Mean	SD
Pre-menopause	37	43.3
Early-peri	47.2	71.4
Med-peri	22.5	5.2
Late-peri	13.4	7.3
Early-post	16	19.6
Late-post	11.6	14.9

Table 3.6: Differences in Sex Hormone Levels between late peri and early post-menopause women

Hormone	Late Peri- (17)	Early Post- (24)	Significance Level
Mean E2 (pmol/L)	13.4	16	0.99
Mean E1 (pmol/L)	90	122	0.81
Mean testo (nmole/L)	1.4	3.0	.005

3.3.5. Variation of Oesterone levels:

Mean serum E1 level for pre-menopausal women was 142.3 pmol/L. The hormone was observed to follow the same behavior as E2 where it decreases gradually during the peri-menopausal life. The hormone was observed to increase at the beginning of the perimenopausal life where it becomes 151 pmol/L for early peri-, then it drops to 95.6 pmol/L during the med-peri and it drops further to 90.2 pmol/L for the late peri-menopausal women. The hormone continues its gradual drop till it finally become 88.5 nmole/L for the late peri-menopausal women The same as for E2 there was no significant variation between all groups in E1 levels except between pre and late post ($p \leq .009$).

3.3.6. The molar ratio between E2 and E1 and the cut-off point for menopausal onset:

It was proposed at the beginning of this study that E2/E1 molar ratio could be used to arrive at a cut-off point for the menopausal onset time. This proposal was based on the fact that as E2 decreases during menopause, E1 predominates. This inverse relationship of decreasing E2 and increasing E1 might lead to an inflection point which could be used as a cut-off pint for menopausal onset time. However, analysis of E2 and E1 results and their molar ratio did not show such an inflection point as shown on table (3.7).

Table 3.7: E2 and E1 molar ratio during the different menopausal stages:

Stage	E1	E2	E1/E2
Pre	142	37	3.8
Early Peri-	151	47.2	3.2
Med-Peri-	95.6	22.5	4.2
Late-Peri-	99.7	13.4	7.4
Early post	122	16	7.6
Late Post	88.5	11.6	7.6

3.3.7. The molar ratio between E2 and Testosterone during the different menopausal stages:

The molar ratio between E2 and testosterone during the different menopausal stages was investigated but no general or specific trend was observed through the groups as shown in table (3.8).

Table 3.8: E2 and Testosterone molar ratio during the different menopausal stages:

Stage	Testosterone	E2	Testo/E2
Pre	2.0	37	0.05
Early Peri-	2.0	47	0.04
Med-Peri-	1.7	22.5	0.07
Late-Peri-	1.4	13.4	0.1
Early post	3.0	16	0.2
Late Post	2.4	11.6	0.2

3.3.8. The molar ratio between E1 and Testosterone during the different menopausal stages:

The molar ratio between E1 and testosterone during the different menopausal stages was investigated but no general or specific trend was observed through the groups as shown in table (3.9).

Table 3.9: E1 and Testosterone molar ratio during the different menopausal stages:

Stage	Testosterone	E1	Testo/E1
Pre	2.0	142	0.01
Early Peri-	2.0	151	0.01
Med-Peri-	1.7	95.6	0.02
Late-Peri-	1.4	99.7	0.01
Early post	3.0	122	0.02
Late Post	2.4	88.5	0.03

3.6. Relationship between age and FSH levels:

There was a significant proportional relationship between the age and the FSH level as shown in (figure 3.3), which mean that FSH level increases with increasing age.

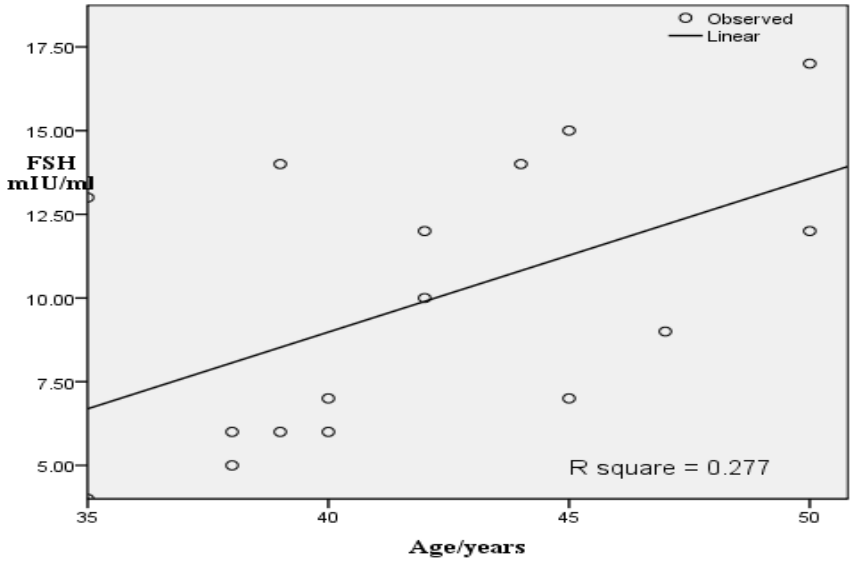


Figure 3.3: Relationship between Age - FSH in peri-menopausal women

3.7. Relationship between age and E1 levels:

There was a significant inverse relationship between the age and the E1 level as shown in (figure 3.4), indicating that E1 level decreases with increasing age.

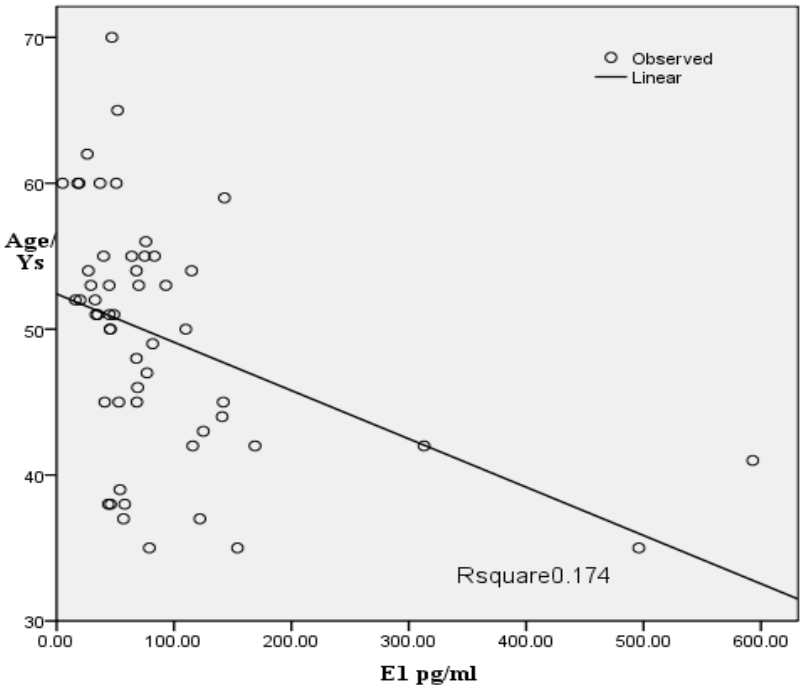


Figure 3.4: Relationship between Age and Esterone (E1) levels

3.4. Variation of hormonal levels with aging:

Another approach tested in this study is to investigate the relationship between aging and hormonal changes. In order to investigate this relationship, women under study were classified to age groups of increasing 5 years periods (i.e., 35-40, 41-45, 46-50, and 51 years and above). Histograms of age groups versus the hormone level were plotted. FSH and LH levels showed gradually increasing levels with increasing age (figures 3:6, 3:7). E2 level showed sudden decrease in the last age group (51years and above) as shown in figure 3.8. E1 showed gradually decreasing levels through the groups until it reached the lowest level in the last group. (Figure 3:9).

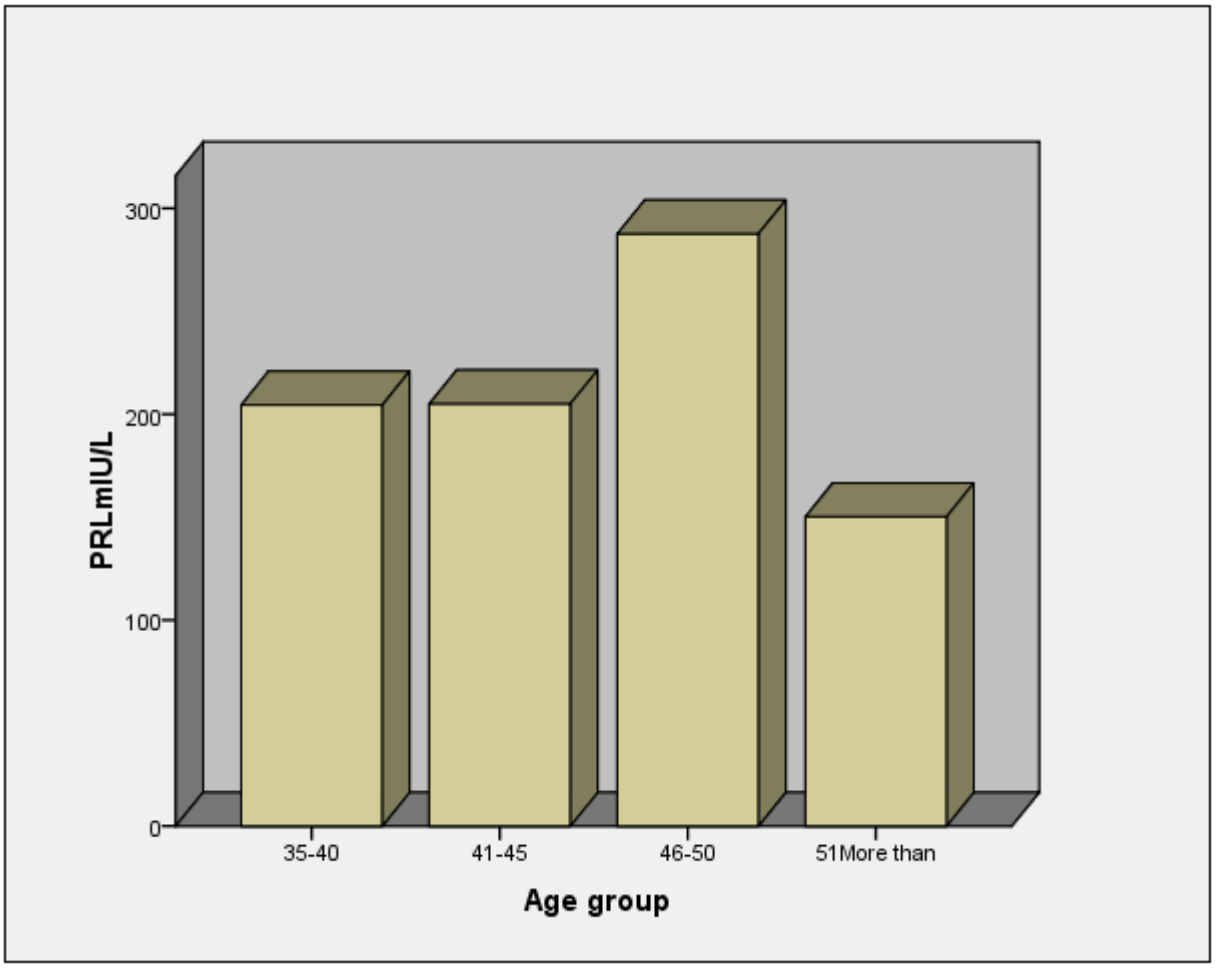


Figure 3.5: PRL status through the age groups.

This figure showed the PRL level statues among the age groups classification and indicated no stable and significant variation between all groups.

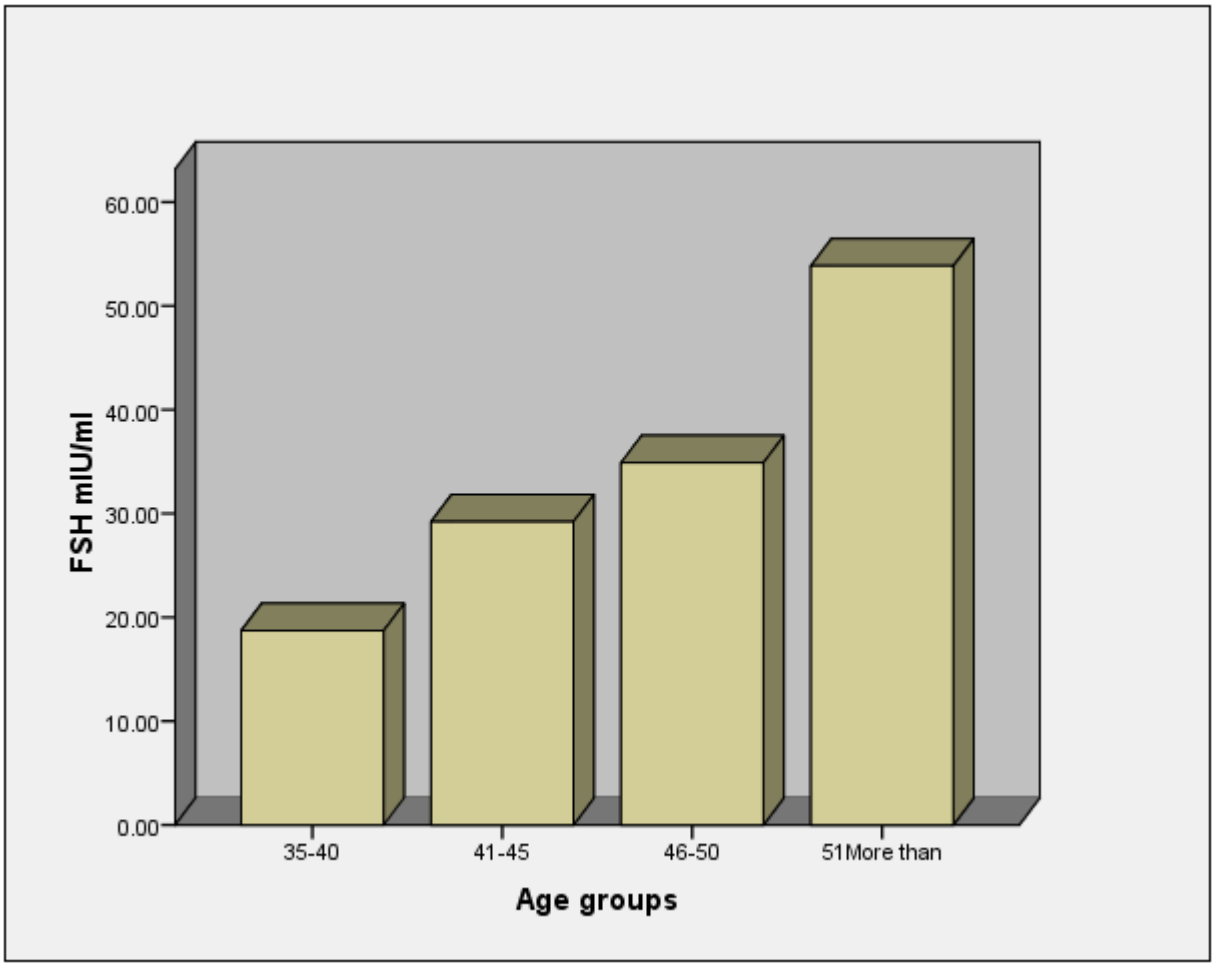


Figure 3.6: FSH status through age groups

This figure showed the FSH levels among the different age groups and indicated that there is a gradual increase in FSH levels starting from the second group (aged 41-45 years), till it reaches the peak in the last group (aged 51 years and more). The reference range of FSH (2 – 18 mIU/ml). This indicates that FSH levels increase with increasing age.

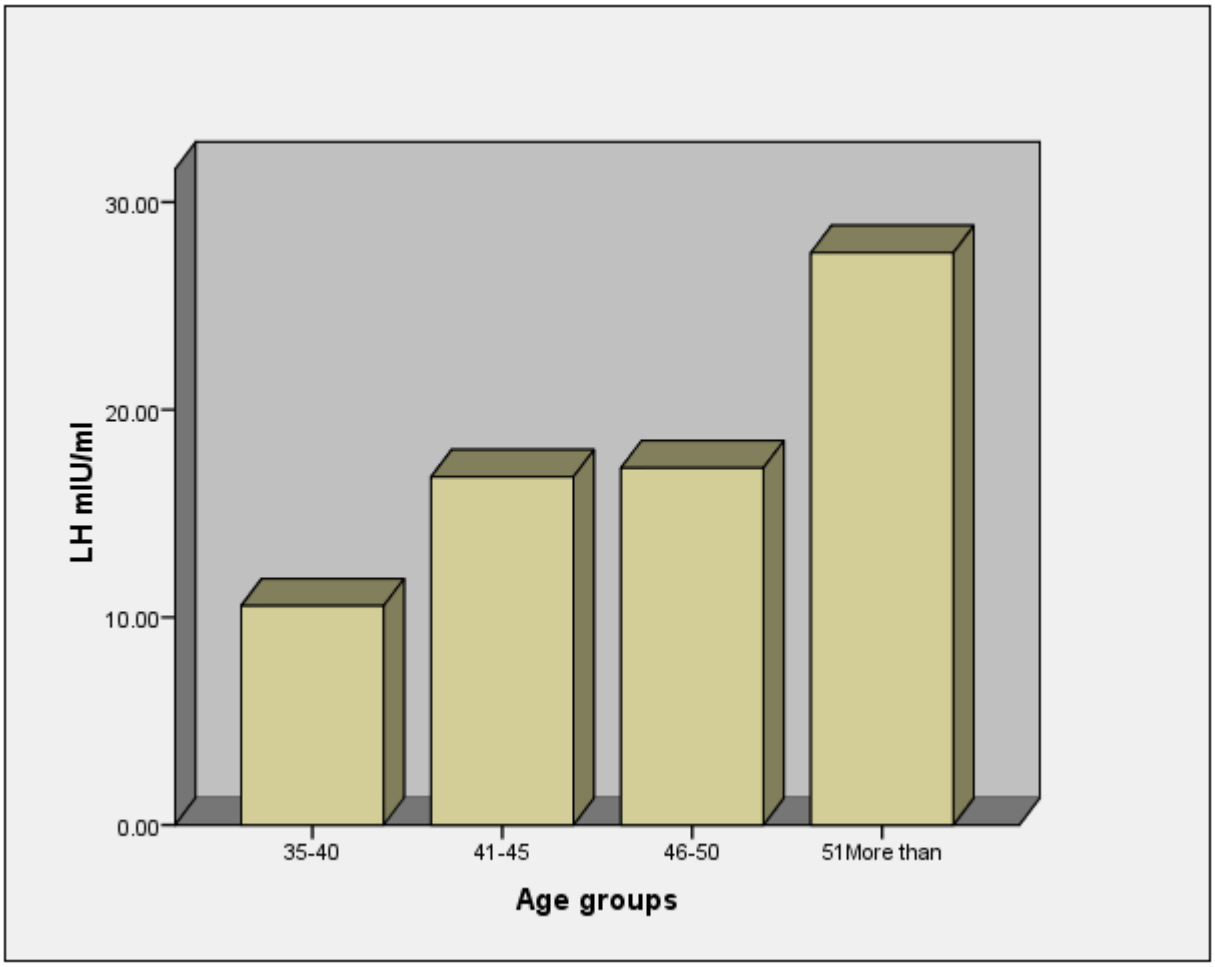


Figure 3.7: LH status through the age groups.

This figure shows that LH levels among the age groups started increasing from the second group (aged 41 – 45 years), continued its increase through the third group (aged 46 – 50 years), till it eventually become very high in the last group. The reference range of LH (1.5– 12 mIU/ml). This indicates that LH levels increase with increasing age.

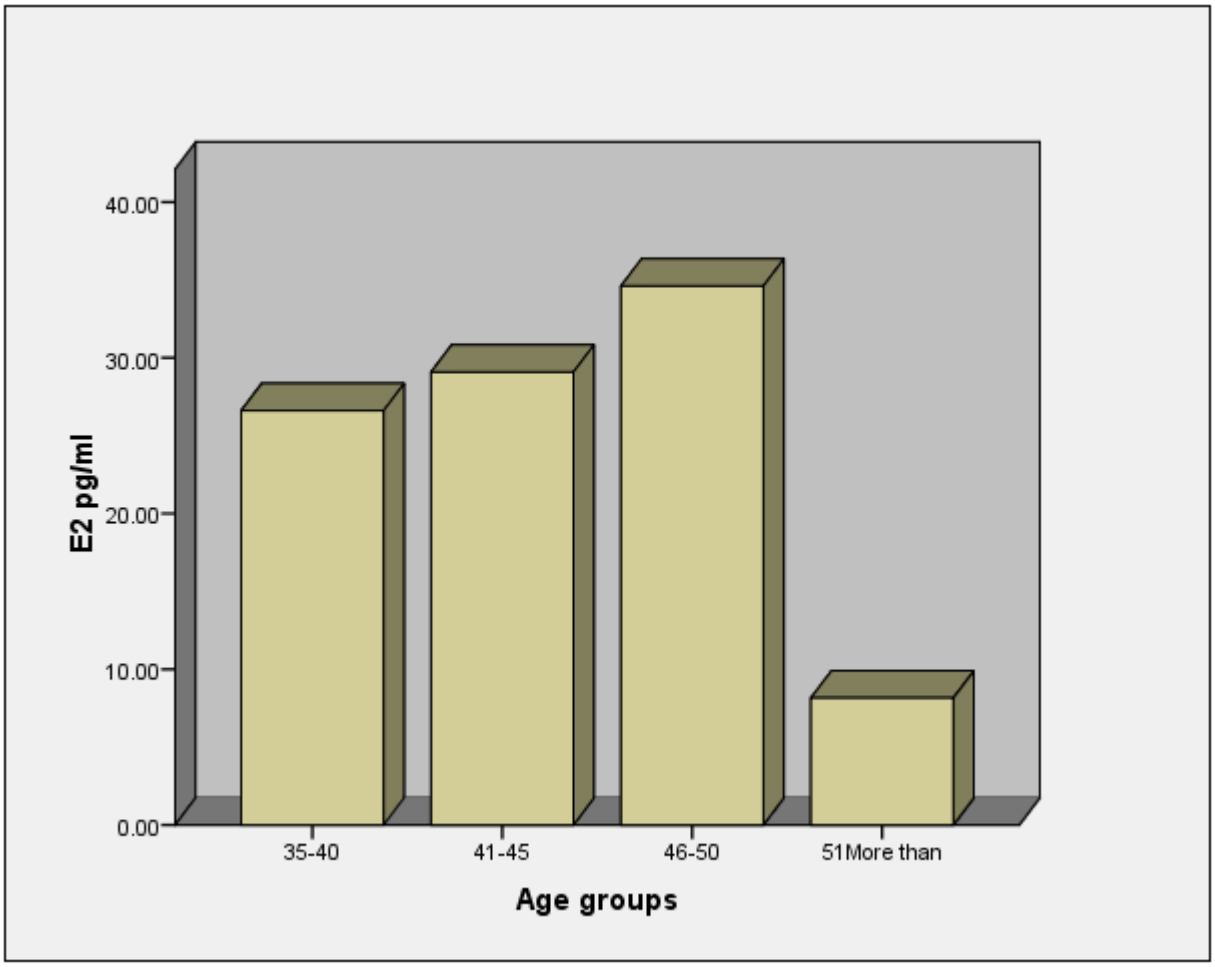


Figure 3.8: E2 status through the age groups.

This figure showed E2 levels among the different age groups. This classification showed slight stability in E2 levels in the first three groups, which vary within the normal range (15 – 60 pg /ml). Then it suddenly drops in the last group (51 years and more). This indicates decrease in the E2 levels with increasing age.

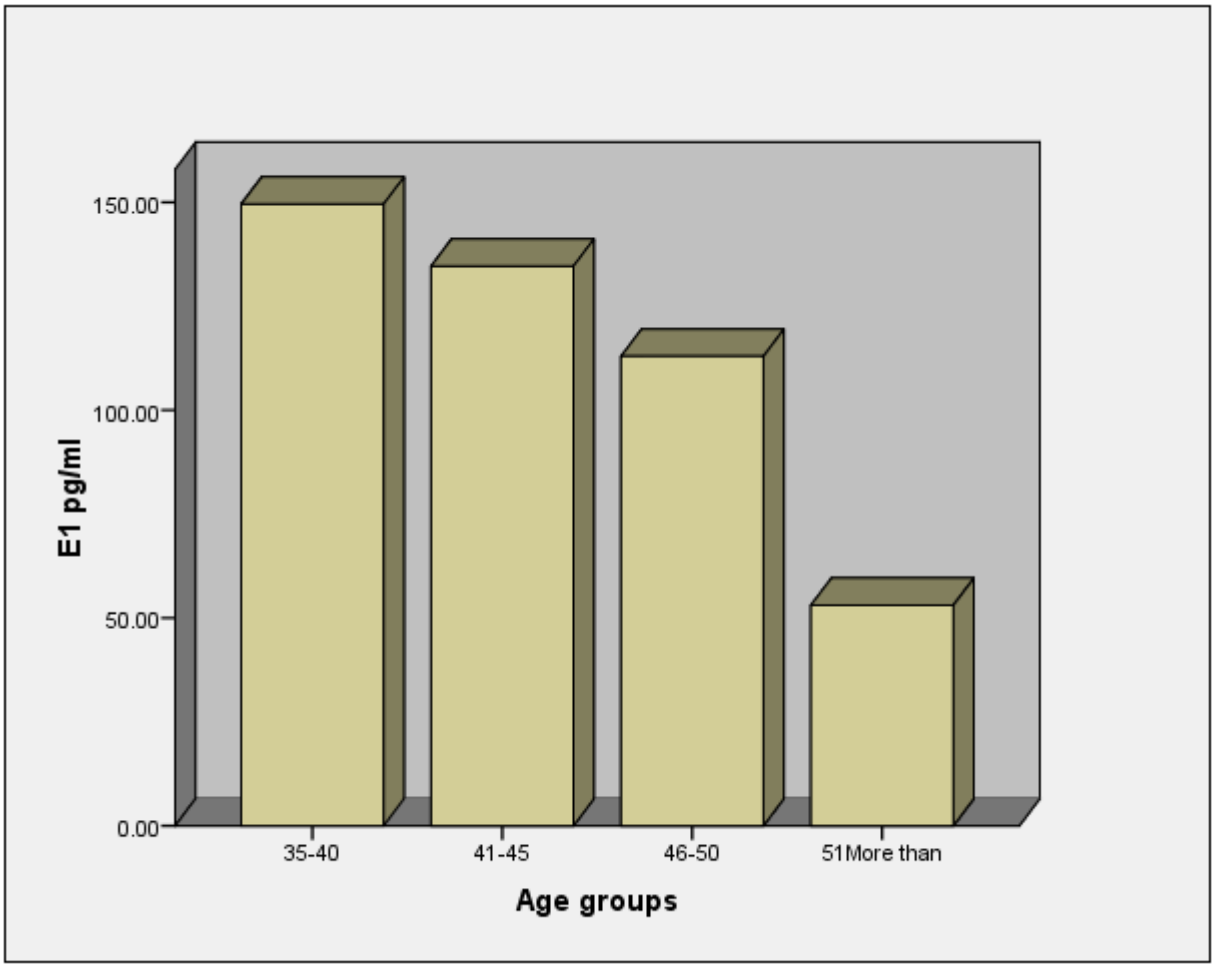


Figure 3.9: E1 status through the age groups.

This figure shows E1 levels among the different age groups. It is clear that there is a gradual decrease in E1 levels through the age groups till it reaches the lowest range in the last group (51 years and more). Normal range (25 –350 pg /ml). This indicates that there is a gradual decrease in E1 levels with increasing age.

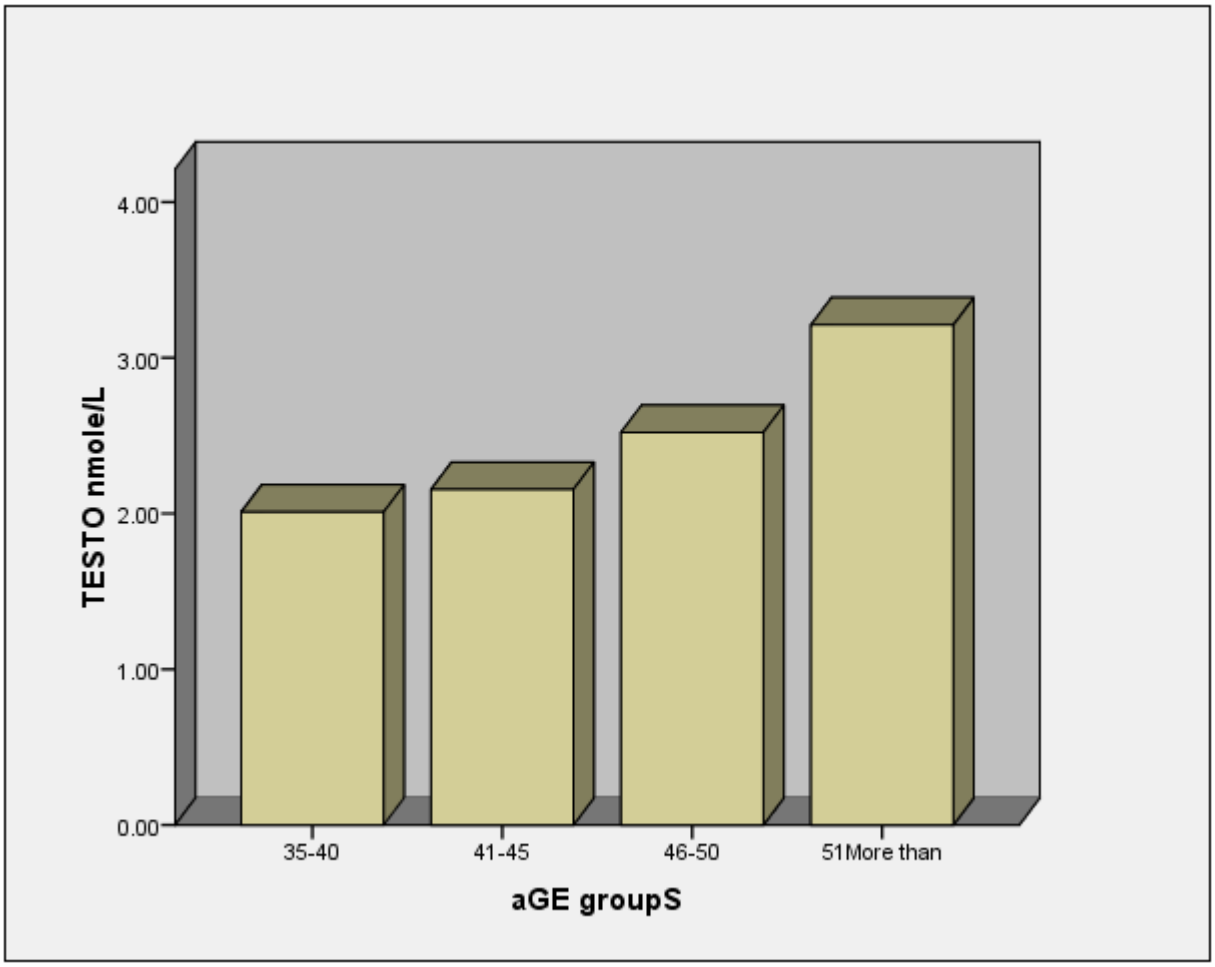


Figure 3.10: Testosterone status through the age groups.

This figure shows the testosterone level status among the age groups classification which indicating that the hormone level in all groups vary within the normal range (0.35-3.5nmole/L). This indicates that there is no clear relationship between testosterone level and different age.

3.5. The menopausal symptoms in Sudanese women:

According to observations obtained from the questionnaire of this study, irregular menses was listed as the most common symptom among the peri menopausal women, followed by Bladder control problems, Joint and muscles problems, fatigue, Numbness in arms or hand, weight gain, headache, depression, changes in sex drive, nausea, hot flashes, Palpitations, difficulty concentrating, shortening in cycle length, vaginal dryness, and finally insomnia as shown in table (3.10).

In post menopause the most common symptom was hot flashes (65%), irregular menses , joint and muscles problems, fatigue, vaginal dryness, numbness in arms or hands, headache, bladder control problems, shortening in cycle length, difficulty concentrating, night sweats, weight gain, palpitations, changes in sex drive, insomnia, and depression as shown in table (3.11).

TABLE 3.10: The most common symptoms of menopause among perimenopausal women according to percentage occurrence.

Symptoms	**Percentage**
Irregular menses	56
Bladder control problems	43.8
Joint and muscles	37.5
Fatigue	37.5
Numbness in arms or hand	37.5
Weight gain	37.5
Headache	31.2
Depression	25
Changes in sex drive	25
Nausea	25
Hot flashes	18.8
Palpitation	18.8
Difficulty concentrating	18.1
Shortening in cycle length	12.5
Vaginal dryness	12.5
Insomnia	12.5

The above table shows the common symptoms of the peri- menopausal stage among Sudanese women as observed and recorded from the questionnaire.

TABLE 3.11: The most common symptoms of post - menopausal women according to percentage occurrence.

Symptoms	Percentage
hot flashes	65.4
Irregular menses	57.7
Joint and muscles	57.7
Fatigue	51.9
Vaginal dryness	50
Numbness in arms or hand	50
Headache	46.2
Bladder control problems	44.2
Shortening in cycle length	44.2
Difficulty concentrating	40.4
Night sweats	38.5
Weight gain	34.6
Palpitations	30.8
Changes in sex drive	30
Insomnia	28.8
Depression	23.1

This table shows the common symptoms of the post- menopausal stage among Sudanese women as observed and recorded from the questionnaire.

TABLE 3.12: International Variability of Ages at Menarche and Menopause

Countries	Age at menarche	Reference	Age at menopause	Reference
Sudan	13.75	Attallah *et al*., (1983)		
Pakistan			44.5± 0.8	Mehreen *et al*., 2007
Sudi Arabia			48.06	Maha , 2005
Egypt	13.2	Attallah (1978)	46.7	Sallam *et al* 2006
Turkey	13.28	Vicdan *et al*., (1996)	47.8± 4.0	Seckin *et al*., (1998)
USA	12.8	Malina and Bouchard (1991)	51.3	Kato *et al*., (1998)
France	13.05	Crognier and Tavares Da Rocha (1979)	52	Salat-Baroux J.1980
Mexico	12.4	Garcia-Baltazar *et al*., (1993)	46. 5	Garrido-Latorre *et al*., (1996)
India (Punjab)	14.31	Singh and Ahuja (1980)	44.6	Singh L *et al* .,1980
Somalia	14.78	Gallo (1975)	—	—
South Africa (black women)	—	—	49.2	Walker *et al*., (1984)

This table shows International Variability of ages at menarche and menopause in the different countries.

CHAPTER FOUR

DISCUSSION

4.1. Determination of menopausal age in previous studies:

All the previous studies in the determination of menopausal age in different countries depend on questionnaires to extract the average menopausal age for women of the target population ((Scragg *et al*., 1973). In this study, the menopausal age can not be extracted from questionnaires as women do not tend to report their exact age and a different methodology was adopted.

4.1.1 Problems faced in determination of menopausal age of Sudanese women:

Women in the Sudan do not tell their true age, either because most women at the age of menopause are illiterate and they may not even have a certificate of birth or they do not tend to tell their true age and their true menopausal age because of cultural believes. This fact had been one of the major obstacles for this study. Different techniques were adopted to extract the fact, sometimes by requesting help from their husbands and in other times by asking about the date of marriage and their age at that time. All the trials were helpless as women do not tend to tell the truth in all cases.

As far as we know there are no previous studies showing the average menopausal age of Sudanese women mainly because of what is mentioned above. In this study the average menopausal age was found to be 43 ± 4.2 years which is much lower than what is mentioned in the literature in the neighboring countries and the nearby Arab countries. This average menopausal age is low because women usually tend to report ages younger than their real ages. Although the average menopausal age is low, however, it is comparable to those of some Asian countries like Pakistan, where the average menopausal age is 44.5 ± 0.8 years (Mehreen *et al.,* 2007), India (Punjab) where the average menopausal age is 44.6 (Singh L et al 1980), Egypt where it is 46.7 years (Sallam *et al.,* 2006), and Turkey where it is 47.8 ± 4.0 years (Seckin *et al.,* 1998).

4.1.1. Determination of the average menopausal age in this study:

In all the previous studies the average menopausal age was determined from extensive epidemiological studies and questionnaires (Scragg *et al*., 1973). Unlike the previous studies the average menopausal age in the present study depended on determining the menopausal status of women first. Then the average menopausal age can be determined by calculating the mean age of the menopausal subclasses that immediately precede the menopause (i.e., the late peri-) and the menopausal subclass that immediately follows (i.e, the early post). The mean age of these two subclasses would be a very good approximation of the average menopausal age which alternatively could only be obtained if a longitudinal study is performed or if an extensive questionnaire is done.

4.1.3. Factors affecting menopausal age:

The most consistent findings in several studies are that early age at menopause is associated with smoking (Jick *et al*., 1972, Willett *et al.,* 1983, McKinlay *et al.,* 1985; Adena and Gallagher, 1982; Daniell, 1978) and underweight (Sherman *et al.,* 1981). However, smoking is not a common practice among Sudanese women and this factor was excluded as contributing to menopausal age. Less clear is the role of menstrual and reproductive factors. Some studies have suggested that early menarche is associated with early menopause (Cramer *et al.,* 1995), but the role of the number of births is still controversial (Whelan *et al.,* 1990; Stanford *et al.,* 1987, Soberon *et al.,* 1966, Progetto, 2001). The basic drawback in this study is that women do not tend to tell the truth about any matters related to age and consequently the age at menarche although available from the questionnaire it can not be used reliably to study its effect on the menopausal age. The other problem faced in this study is that women do not tend to tell the truth about the number of children and they do not accept the question in the first place.

4.2. Advantages of the new classification scheme:

The new classification scheme had successfully differentiated the early peri-menopausal women from pre-menopausal ones. The early peri-menopausal women according to the new classification scheme are suffering cycle irregularities and amenorrhoea but with normal hormonal levels. In infertility clinics women presenting with amenporrhoea but normal reference hormonal levels are suspected to suffer

endometrial problems (Yahya *et al*, 2002). The new classification scheme is now; clearly indicating that amenorrhoea with normal hormonal levels may be an indication to the beginning of the peri-menopausal life.

The big challenge faced during this study had been the sub-classification of the peri-menopausal stage as it is not a single homogeneous stage but a wide heterogonous and transitional stage extending from the early peri-menopause with mild signs and symptoms to the late peri-menopausal stage which almost resembles the post-menopause.

The new classification system using its three "key" tools had successfully classified the heterogeneous peri-menopausal stage into three distinct sub-classes with characteristic hormonal and symptomatic manifestations for each. The three subclasses are early peri-menopausal women who are characterized by presence of normal hormonal levels (FSH and LH) but who suffer amenorrhoea. FSH level of this subclass although within the normal range, it is higher than that of the preceding class "pre-menopausal stage", but the difference is not statistically significant, this may be due to the small sample size. Therefore, women who suffer irregular cycles and amenorrhoea due to other endocrine causes (hyperprolactinaemia, hypo- and hyperthyroidism, etc) can be misclassified as early peri-menopausal. Therefore, the other endocrine causes should be ruled out before including them in this subclass by measurement of the respective hormones, namely, prolactin and TSH levels.

The second subclass is the med peri-menopausal women are characterized by elevated FSH levels but unlike the preceding subclass they do not suffer amenorrhoea, the reason for this is not clear, but it seems the ovary is still secreting sufficient oestrogen levels to prime the uterus and maintains regular menstrual cycles. However, further research with a larger sample size is needed to clarify this situation.

The third subclass is the late peri-menopausal women are those with elevated FSH levels, but normal LH levels and they suffer amenorrhoea, indicating the beginning of cessation menstruation.

The new classification scheme indicated that LH levels increase sharply immediately after menopause signaling the end of reproductive life and the beginning of the post menopausal life. The new classification scheme indicated that early post-menopausal women are those who have elevated FSH and LH, but who experience a menstrual cycle during the last three months. The Late post-menopausal women are those who

have elevated FSH and LH and who do not experience a menstrual cycle during the last year.

4.3. Hormonal changes during the different menopausal stages:

There is no overlap, neither clinically nor hormonally between the pre- and post menopausal women and their classification is not a concern. The pre-menopaual women enjoy regular cycles with normal FSH and LH, whereas the post menopausal women suffer absence of menstrual cycles and extremely elevated gonadotrophin levels.

4.3.1. Pre-menopause:

This stage covers the reproductive age of the women which implies that all hormones are steady and fluctuate within the normal range.

4.3.2. Peri-menopause:

The new classification scheme successfully separated the early peri-menopausal from pre-menopausal one. The perimenopause is a time of markedly fluctuating hormone levels.The findings of this study have shown that there is a rise in FSH levels which starts at the beginning of peri-monopause and continues in med-peri till it reaches high levels at the late peri-menopausal stage. This finding is combatable with Burger and his co-workers (Burger *et al*., 1998) findings who considered elevated FSH levels to be an outstanding characteristic of the menopausal transition and consequentlty the peri-menopausal stage. It also goes on lines with the findings of the same author and his co-workers in 1995, which have shown that serum FSH levels were higher in early peri-menopausal women than in premenopausal women, (Burger *et al*., 1995). The finding in this study shows that the mean concentration FSH in the late prei was elevated, while the mean concentration of LH was normal to the same stage. This combatable with (Sherman *et al*., 1976; Lee *et al*., 1988) (Burger *et al*., 1998) finding they considered the most consistent endocrine finding in peri-menopausal women is an elevation of early follicular-phase FSH concentrations, which is not always accompanied by a rise in LH. The raise in FSH also associated with a decrease in serum estradiol (E2) (Burger *et al*., 1999).

The findings of the present study have shown that there no significant variation in the testosterone mean concentration between the groups during menopausal transition. This finding differs from Henry who considered concentrations of testosterone have been reported to fall by about 50% during reproductive life, between the ages of 20 and 40 years. It changes a little during the transition and, after menopause, and may even rise (Henry *et al*, 2002).

4.3.3. Post-Menopause:

Previous literature reports have shown that oestradiol levels gradually decrease in the post-menopausal women and the oestrone become the most abundant female sex hormone. In agreement with the previous literature reports our findings have shown that oesterone is the most prevailing sex hormone during this stage of menopause as its levels are almost ten times those of the estradiol levels.

4.3.3.1. E2/E1 ratio “an indix that shows menopausal on-set time”:

The gradually decreasing oestradiol levels and the predominance of oestrone during this stage lead us to put forth our proposal that the ratio of the two hormones can generate an index which might be used as a cut-off point for the identification of the menopausal onset time and which can tell whether the woman is already post-menopausal or not.

However, no such a clear cut-off point was obtained from our data, this is simply because our study is a cross-sectional study and to arrive to such an index require a longitudinal study and follow up of these two hormones in each individual woman separately.

4.4. Identification of menopausal status of women:

4.4.1. Importance of identification of menopausal status of women:

One of the big challenges that face the gynecologists in infertility clinics is the menopausal status of women under investigation. Identification of menopausal status is critically important in determining the treatment strategy. Average menopausal age of a population can help, but even such basic data is lacking in Sudan.

4.5. Symptoms related to menopause among Sudanese women:

The most common menopausal symptom among Sudanese women is the irregularity of menstruation with changes in length of bleeding. This constitutes 56% of the study population. And this also agree with the most common menopausal symptoms among Saudi women experienced during the menopausal transition include irregular menstruation (40.5%) (Maha, 2005). This menstraul irregularity is a good indicator that the lady had approached her peri-menopause. Most of Sudanese women in this study did not experienced psychological symptoms such as depression, anger, or irritability. This agrees with study among Saudi women (Maha, 2005).

In the literature , The reporting from different location of USA , that the hot flashes or night sweats is higher among women in early perimenopause (38%) compared with premenopausal women (21%), another study reported that the hot flashes or night sweats increases considerably from early perimenopause to late perimenopause (38% to 55%) (Nancy *et al*., 2005). In the United Arab Emirates, hot flashes were considered as the most common menopausal symptomatology that occurred in 45% of the women studied, followed by urinary incontinence (30%) (Rizk *et al*., 1998).

Compared to the previous studies which considered that the vasomotor symptoms which include the hot flashes, very significantly in peri-menopause transition, it did not appear to be significant among Sudanese women in which the hot flashes and night sweats in peri-menopausal women was (18.8% , 38.5% respectively). While these symptoms were 65.4% among the post-menopausal women. Hot flashes may continue for several years into postmenopausal life. The present study findings show that vaginal dryness shows a significant change from the peri (12.5%) to post menopause (50%). This shows the gradual decline in sexual capabilities and reproductive fitness. These findings are in a good agreement with other authors findings which showed a significant increase in vaginal dryness from early to late perimenopause, i.e, from 13% to 16% respectively (Nancy *et al*., 2005).

Chinese women concur with studies in Caucasian women reporting a significant increase in vasomotor symptoms during late perimenopausal period, which is conversely with Sudanese women, and the elevation of such symptoms for some years after menopause (Gold *et al*., 2000; Dennerstein *et al*., 2000; Holte and McKinlay, 1991; McKinlay *et al*., 1992) agree to some extend with the present study. These

observations have suggested that vasomotor symptoms are related to hormonal changes during this period of life (Suzanne *et al*., 2003). Hot flashes was (12.3%) in Japan (31%) in Canada.

In the current study, few Sudanese women reported urogenital symptoms which include sexual behavior symptoms (loss of sexual desire which is almost 25% in peri- and 30% in the post). These findings may not necessarily indicate that the women did not experience these symptoms, but this may be attributed to cultural believes in Sudan that loss of sexual desire is related to aging and consider the sex some thing don't talked about. Muscle-joint-bone pain also shows a general tendency to an increase in incidence from peri- (37.5%) to post-menopausal life (57%).This is compatible with study in Turkey in which, muscle-joint-bone pain was the symptom most often associated with menopause (Seckin *et al*., 1998), whereas in Japan, shoulder stiffness, ringing in the ears, and a heavy feeling in the head were commonly associated with the menopausal transition (Kaufert and Lock 1998; Lock and Kaufert, 2001). The most currently reported symptoms for perimenopausal Indian women are psychosomatic symptoms such as tiredness and headaches (Sharma and Saxena, 1981).

Generally awareness about menopausal symptoms among Sudanese women should be increased, because women deal with these symptoms as a disease rather than its relevance to specific transition.

4.6. Relationship between hormonal changes and aging:

Extracting conclusions about the relationship between hormonal changes and aging is difficult to do with data obtained from cross sectional studies and age groups. This is simply because each age group is not a homogeneous group but a heterogeneous group with variable hormonal and clinical manifestations. For example the age group of 35-40 years may contain some women with elevated hormonal levels that will change the mean level during calculation. On the other hand the age group of 45-50 years may contain some women with normal hormonal levels that will tend to lower the mean in face of increasing age. Therefore, the relationship of hormonal levels with aging in women is not a simple and straight forward relationship and it cannot be done unless a longitudinal study is performed.

4.7. Conclusions:

- FSH has a significant potential to be used as a marker of peri-menopausal status. Elevated FSH level in presence of normal LH indicates peri-menopause.
- Elevation of LH levels in presence of elevated FSH "signals" the beginning of the post-menopausal life.
- FSH alone cannot tell whether the lady is post menopausal or not, and only the combined use of both FSH and LH can be used reliably.
- The study of the relationship between hormonal variation and aging in women is not a simple and straight forward and can not be done unless menopausal status is identified or a longitudinal study is performed.
- Any study requiring registration of a woman's age is difficult to conduct in Sudan and any conclusions derived from it, will remain erratic.
- Studying factors affecting the age at menopause (age at menarche, age at marriage, parity and weight) should be taken with due care as women do not tend to tell their exact ages.
- The average menopausal age of Sudanese women in this study was obtained from the mean ages of the subclasses surrounding menopause, namely mean ages of the late peri- and the early post- menopausal women, and it was found to be 43.0 ± 4.2 years.
- E2/E1 ratio, as derived from this cross-sectional study, cannot be used reliably as a clear "cut-off point" for the prediction of the menopausal onset time or the menopausal status of women.

4.8. Recommendations:

(1) In any future longitudinal study, LH level combined with FSH can be used as a reliable marker for the exact determination of menopausal onset time.

(2) More research need to be conducted concerning the other menopause related factors like age at menarche, age at marriage, parity, education, work, number of

pregnancies, and number of miscarriages, but a mechanism of extracting the exact age is needed as women do not tend to tell their true ages.

(3) It is highly recommended that any study related to age should be conducted under strict conditions to verify that age is correctly reported even if that requires asking for the certificate of birth.

(4) Awareness about menopausal symptoms should be increased among Sudanese women as most of their regular complains, like osteoporosis and recurrent urinary tract infections are related to menopause.

CHAPTER FIVE

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Appendix

Appendix 1: Reference range of reproductive hormones for females (follicular phase):

Hormone	Reference range
PRL	80 – 650 µIU/ml
FSH	2 – 18 mIU/ml
LH	1.5 – 12 mIU/ml

Appendix 2 :Reference range of sex hormones for females (follicular phase):

Hormone	Reference range
E1 Pmol/L	925 – 12950 pmol /L
E2 Pmol/L	55 – 220.2 pmol /L
Testosterone nmol/L	0.35- 3.5 nmol / L

Appendix 3: Table 3.8: Comparison of hormones between menopausal stages

HORMONE / STAGE	MEAN	SD
PRL pre	245.9	245.8
early peri	176.1	172.4
late peri	299	432.7
early post	174.6	101.9
late post	160.9	140.1
FSH pre	8.7	4.4
early peri	9.4	4.2
late peri	29.7	10.3
early post	52.5	14.9
late post	57.4	14.2
LH pre	5.8	5.7
early peri	8.0	6.7
late peri	7.5	3.0
early post	30.8	13.9
late post	30.5	15.3
TESTO pre	2.09	0.98
early peri	2.06	1.3
late peri	1.4	0.7
early post	3.0	1.8
late post	2.4	1.8
E2 pre	37.0	43.3
early peri	47.2	71.4
late peri	13.4	7.3
early post	16.0	19.6
late post	11.6	14.9
E1 pre	142.3	89.2
early peri	151.4	112.1
late peri	90.2	33.3
early post	122	90.7
late post	88.5	104

Appendix 4: Questionnaire

Hormonal identification of menopausal status of Sudanese women

Name:

Residence: **Block No:** **House No:**

Tel No:

Age:

Marital Statues:

Weight……Height…….

Menstrual History:

Age at menarche:

Cycle Length:

Duration of bleeding:

Amount of bleeding:

Slight…….

Normal…….

Heavy……

Have experienced decrease in menstrual cyclicity……. Yes/No

Have you had a menstrual period in the last three month?.....Yes/No

Have you had an operation which caused your periods to stop? ……..Yes/No

Hormonal treatment……

personal history

Age at marriage…… years

Age when first child was born……..years

Number of pregnancies……

Number of miscarriage……..

Breast feeding:

less than one year…….

More than one year……..

Duration of lactational amenorrhoea if any……

Change in cycle length with age……

Amenorrhoea…….Onset…….

Galactorrhoea…….Onset……..

Surgical intervention

Uterus…… ovaries……. Breast…….

History of endocrine disease

Diabetes mellitus……

Hyper tension…….

Thyroid disease……..

<u>Family history</u>

Diabetes mellites……Father……..Mother……

Hypertension……. Father……..Mother……..

Thyroid disease……. Father……..Mother……..

Symptom:

Hot flashes (Wave of heat)…….

Shortening in cycle length……

Irregular menses……

Night sweats…….

Nausea…….

Headache…….

Palpitations……..

Insomnia (Sleeplessness)……..

Fatigue……

Depression……..

Irritability……

Joint and muscle pain…….

Difficulty concentrating……

Dizzy spells……

Nervous tension……

Changes in sex drive…….

Vaginal dryness…….

Bladder control problems……..

Numbness in arms or hands……..

Signs:

Facial hair…….

Weight gain……..

Hair loss…….

Skin and hair dryness and wrinkles…….