PROGNOSTIC USE OF HORMONAL PROFILE AND BONE MARKERS IN THE ASSESSMENT OF MENOPAUSAL AND PERIMENOPAUSAL SUBJECTS AT LOKOJA INTERNATIONAL MARKET, LOKOJA, KOGI STATE, NIGERIA

***[1]Emeje, P. I.,[2] Mary, O.A., Shedrack, E. A., [1] S. A. Abubakar and [3]Zakari, U.**
[1]Department of Medical Laboratory Science, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria
[2]Department of Community Medicine, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria
[3]The Commissioner for Health, Kogi State Ministry of Health, Lokoja, Kogi State, Nigeria
*Corresponding Author: Emeje Paul Isaac; Email: emeje.pi@ksu.edu.ng, 07063539000.
Received: 10th Dec., 2023 **Accepted**: 13th Jan., 2024 **Published:** 1st June, 2024

ABSTRACT
Background: Menopause is defined as the time in a woman's life when menstrual cycle ends due to the natural depletion of ovarian oocytes from aging and the deficiency of estrogen. It marks the end of fertility and it usually begins between ages 51 and 52, but can develop before or after this age. Menopause is diagnosed after 12 months of absence of menstruation.
Aim: This study aimed at evaluating the Prognostic use of Hormonal profile and Bone Markers in the assessment of Menopausal and Perimenopausal Subjects at Lokoja International Market, Lokoja, Kogi State, Nigeria.
Methodology: Participants aged between 16 and 65 years without any known infertility challenges in their life were recruited for this study while women aged between 16 and 64years with previously known fertility challenges were excluded from this study. All the hormones were measured using enzyme linked immunosorbent assay. Alkaline phosphatase, serum Calcium, and serum phosphorus were measured spectrophotometrically.
Results: There were significant correlation between the hormonal profile and the bone markers amongst the study participants. The hormonal levels in menopausal and perimenopausal participants (groups A and B) were significantly different from hormonal levels of participants of women of reproductive age (group C) ($P \leq 0.05$).
Conclusion: From the findings, the study thus conclude that hormonal profile and bone markers could be significant markers for the assessment and monitoring of perimenopausal and menopausal symptom in patients.
Keywords: Menopause, Perimenopause, Hormonal profile, Bone markers, Osteoporosis.

INTRODUCTION
Menopause is defined as the time in a woman's life when menstrual cycle ends due to the natural depletion of ovarian oocytes from aging and the deficiency of estrogen. It marks the end of fertility and it usually begins between ages 51 and 52, but can develop before or after this age (Flores *et al.*, 2022). Menopause is diagnosed after 12 months of absence of menstruation. Hormonal changes and clinical symptoms occur over a period of time and it is immediately followed by menopause, this period is frequently termed perimenopause or menopausal transition (Kapoor *et al.*, 2022). Menopause occurs due to the following factors; age, premature ovarian failure, hysterectomy, chemotherapy and radiation therapy, oophorectomy.

The stages of menopause include; perimenopausal stage (between 40 to 47years), early menopausal stage (between 50 to 55 years), menopausal phase (55 to 60 years) and post-menopausal stage (60 years and above) (Manson *et al.,* 2022). In advanced setting, the tests done to diagnose menopause are; hormonal assay, transvaginal ultrasound, hysteroscopy, computed tomography or magnetic resonance imaging (Su *et al.,* 2022).During menopausal transition, physiological changes occurs and it causes a range of symptoms which includes; insomnia, vaginal dryness, mood changes, weight gain and bloating, depression, increased urination, painful or stiff joints, osteoporosis, hot flashes and reduced bone mass (Janaka*et al.,* 2019).

Differentiating amenorrhea from menopause; amenorrhea describes complete absence of menses; no menstrual bleeds for greater than 6 months in the absence of pregnancy. Primary amenorrhea is defined as absence of onset of menses by 16 years of age, and secondary amenorrhea as absence of periods for at least 6 months if the patient has previously had regular periods, and 12 months if she has previously had oligomenorrhoea. In contrast, oligomenorrhoea describes infrequent periods, with bleeds less than every 6 weeks but at least one bleed in 6 months (Edmonds *et al*., 2001).

Therefore, it is important to employ a diagnostic evaluation that differentiate amenorrhea from menopause which segregates causes of amenorrhea into the following levels: disorders of the outflow tract, disorder of the ovary, disorders of the anterior pituitary and disorders of the hypothalamus or central nervous system [Edmond *et al.*, 2001]. The causes of amenorrhea are best remembered by reference to the levels within the hypothalamus- pituitary-ovarian axis, at which problems can develop.

In woman of advanced age, cessation of menses is commonly used to diagnose menopause in resource-limited settings. This has been found to have its setbacks or limitation. Therefore, identifying and evaluating biochemical markers that will aid in the proper diagnosis of menopause will help in the management of menopausal symptoms. These biochemical parameters will help to rule-out pseudomenopause in which women of reproductive age stops ovulating or menstruating due to hormonal interruption (Koch *et al.,* 2022). Also, little is known about the health effects of this natural biological occurrence in our resource – limited settings (Wilson *et al.,* 2022).

Menopausal symptoms vary in therein severity from person due to the effects of confounding factors such as lifestyles, social status, body composition and psychological status (Kapoor *etal.,* 2022). Menopausal symptoms, especially the vasomotor and sexual symptoms, are associated with impaired quality of life in women (Jennifer *et al.*, 2022). Quality of Life is "an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns (Erin *et al.*, 2022).

Menopause is estimated to be about 50 million cases worldwide. Menopausal women are projected to increase to 1.2 billion worldwide by the year 2030 (Grandi *et al.,* 2022). In Nigeria, menopausal women are estimated to be about 20 million occurring between the ages of 45 to 52 years of age. In rare cases in Nigeria, menopause can occur betweenthe ages of 36 to 40years and this can be referred to as early menopausal stage. In Nigeria, about 100,000 women enter menopause annually (UNAIDS, 2014). However, there is paucity of data related to menopause in Kogi State perhaps due to lack of interest in this field of research.

The complications of menopause are: cardiovascular diseases, osteoporosis, urinary incontinence, sexual dysfunction, weight gain. These complications usually occur if the diagnosis are not made early for proper management.

Management of menopause involves hormonal replacement therapy, vaginal estrogen, low-dose antidepressants, gabapentin, and medications to prevent osteoporosis, exercise, practice relaxation technique, and dietary measures (Cagnacci*et al.*, 2022).

MATERIALS AND METHODS

Study Area

The study was carried out at International Market, Lokoja, Kogi State. The Lokoja International market is located at opposite Ibro Park, Felele express road, Lokoja. International market was established in the year 18th March, 2010 by Governor Ibrahim Idris Administration. It is a market placed at the bypass section of the Lokoja township. It is a standard market with both buyers and sellers from far and nearby State like Niger, Edo, Benue, Enugu, Ondo, Ekiti, Kano, Gombe and Abuja. Food items are also relatively cheap compare to other market within Kogi State. Lokoja is situated at 7.8^{o}North Latitude, 6.74^{o}C East longitude and 55meters elevation above the sea level. Lokoja is a town in Nigeria, the capital of Kogi State, having about 60,579 inhabitants.

Study Population

The study population consist of 552 women aged between 16 and 64 years that were randomly recruited for the study. They were grouped as follows:

Group A: 138 women of menopausal age group that served as test participants.

Group B: 138 women of perimenopausal stage that served as test participants.

Group C: 276 women of reproductive age that served as control.

Ethical Approval

Before the commencement of the prospective cross-sectional study, ethical approvals were obtained from Kogi State Ministry of Health Ethical Review Committee.

Informed Consent

Informed consent was obtained from all participants before the commencement of the study. Participants aged between 16 and 65 years without any known infertility challenges in their life were recruited for this study while women aged between 16 and 64years with previously known fertility challenges were excluded from this study.

Specimen Collection and Analysis

Eight milliliters of venous blood was collected from each participant at the point of joining research. Each sample taken was dispensed into a 10ml plain bottle. The sample was allowed to clot, dislodged and was centrifuged immediately at 3000 rpm for 5 minutes, serum obtained was aliquoted into two cryovial bottles and were stored at -20^{o}C until analysis. All the samples for hormones and bone markers were analyzed at Federal Medical Centre, Lokoja, Kogi State, Nigeria.

Methods

All the hormones were measured using enzyme linked immunosorbent assay (Stowell*et al.*, 1991).

Alkaline phosphatase was measured according to Moss *et al.*, 2016.

Serum Calcium was measured according to Schwarzenbach*et al.*, 1955

Serum phosphorus was measured according to *Tietz*, 1983

Serum Calcitonin was measured according to Felsenfield, 2015

Statistical Analysis

Values obtained were expressed as mean plus or minus standard deviation (SD) using SPSS Version 20.0. All numerical results were analyzed with one way ANOVA with post hoc multiple comparisons tests, while spearmen correlation analysis between parameters was done amongst menopausal participants. P value below 0.05 was considered statistically significant.

RESULTS

Table 1 showed the characteristics of the study population (mean age and residential status).Table 2 showed the hormonal levels

amongst the participants (groups A, B and C). The hormonal levels in menopausal and perimenopausal participants (groups A and B) were significantly different from hormonal levels of participants of women of reproductive age (group C) ($P \leq 0.05$). Table 3 showed the bone markers amongst the participants (groups A, B and C). The levels of bone markers in menopausal and perimenopausal participants were significantly different from the levels of bone markers in women of reproductive age ($P \leq 0.05$).Table 4 showed correlation analysis between hormonal profile and bone markers in group A participants (menopausal women). There were significant associations between hormonal profile and bone markers, all were found significant ($P \leq 0.05$).

Table 1: **Characteristics of the study population**

Variables	All subjects N=552	Group A: n=138	Group B: n=138	Group C: n=276	P values
Mean age in years (± SD)	39.91 (± 13.30)	58.41 (± 8.11)	49.11 (± 7.30)	33.4 (± 7.40)	0.03
Residential status					
i. Semi Urban	282 (51.09%)	48 (34.78%)	71 (51.45%)	150 (54.35%)	0.05
ii. Rural	270 (48.91%)	90 (65.22%)	67 (48.55%)	126 (45.65%)	0.05

SD: Standard Deviation

N: Total population, n: Group population; A: Group A participant (menopausal women); B: Group B participant (Perimenopausal women); C: Group C participants (Reproductive women)

Table 2: Mean (± SD) values of hormonal levels amongst the participants

Group	N	FSH (mIu/ml)	LH (mI/u/ml)	PRL (ng/ml)	TSH (µIu/ml)	Inh (pg/ml)	AMH (ng/ml)	E_2 (pg/dl)	PROG (ng/ml)	Calcitonin (pg/ml)
A	138	95.00	51.00	2.00	11.00	3.00	0.70	20.00	0.06	4.00
B	138	61.00	30.00	3.50	9.00	8.00	1.20	25.00	0.07	6.00
C	276	5.00	4.80	17.0	3.6	34.00	4.10	175.00	1.70	10.00
F-Value		21.03	20.00	31.05	40.05	41.03	41.06	21.03	45.31	38.51
P-Value		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.02
A V. B		0.005	0.025	0.112	0.05	0.02	0.25	0.06	0.190	0.025
A V. C		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.05
B V. C		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.05

FSH: Follicle stimulating hormone
LH: Leutinizing hormone
PRL: Prolactine
TSH: Thyroid stimulating hormone
N: Group population
A: Group A participant (menopausal women)
B: Group B participant (Perimenopausal women)
C: Group C participants (Reproductive women)
Inh: Inhibin
AMH: Anti mullerian hormone
E_2: Estradiol
Prog: Progesterone

Table 3: Mean (± SD) values of Bone markers amongst the participants

Group	N	Ca (mmol/l)	PO^3_4 (mmol/l)	ALP (Iu/ml)
A	138	0.70	1.60	191.00
B	138	0.90	1.50	151.00
C	276	1.3	1.11	70.00
F – Value		22.80	21.52	40.30
P – Value		0.002	0.002	0.030
Post Hoc:				
A Vs B		0.05	0.007	0.035
A Vs C		0.001	0.001	0.001
B Vs C		0.001	0.001	0.001

A: Group A participant (menopausal women)
B: Group B participant (Perimenopausal women)
C: Group C participants (Reproductive women)
N: Group population

Ca: Calcium
PO_4^{3-} : Phosphate
ALP: Alkaline phosphatase

Table 4: **Correlation analysis between Hormonal profile and Bone markers amongst menopausal participants (N=138)**

Parameters	**N**	**R**	**P**	**S**	**Significant**
FSH Vs Ca	138	-.765	.001	S	P<0.05
FSH Vs $P0^{3-}_4$	138	.805	.001	S	P<0.05
FSH Vs Calc.	138	-.655	.001	S	P<0.05
LH Vs Ca	138	-.701	.001	S	P<0.05
LH Vs $P0^{3-}_4$	138	.673	.001	S	P<0.05
LH Vs Calc.	138	-.803	.001	S	P<0.05
Prl Vs Ca	138	+.745	.001	S	P<0.05
Prl Vs $P0^{3-}_4$	138	-.700	.001	S	P<0.05
Prl Vs Calc.	138	.613	.003	S	P<0.05

Table 4 continue

TSH Vs Ca	138	-.551	.003	S	P<0.05
TSH Vs $P0^{3-}_4$	138	.615	.003	S	P<0.05
TSH Vs Calc.	138	-.621	0.002	S	P<0.05
Inh Vs Ca	138	0.771	0.001	S	P<0.05
Inh Vs $P0^{3-}_4$	138	-0.573	0.004	S	P<0.05
Inh Vs Calc.	138	0.591	.003	S	P<0.05
Amh Vs Ca	138	0.685	.003	S	P<0.05
Amh Vs $P0^{3-}_4$	138	-.716	.001	S	P<0.05
Amh Vs Calc.	138	.681	.001	S	P<0.05
E2 Vs Ca	138	.895	.001	S	P<0.05
E2 Vs $P04^{3-}_4$	138	-.816	.001	S	P<0.05
E2 Vs Calc.	138	-.943	.001	S	P<0.05
Prog Vs Ca	138	0.905	.001	S	P<0.05
Prog Vs $P0^{3-}_4$	138	-.911	.001	S	P<0.05
Prog Vs Calc.	138	.895	.001	S	P<0.05

R: Correlation; N: Number of menopausal women; S: Significant

DISCUSSION

The present study focused on some indices that could be used to diagnose menopause and monitoring the management of menopause related symptoms. The indices used were hormonal and bone markers. The hormonal profile are follicle stimulating hormone, luteinizing hormone, prolactin, thyroid stimulating hormone, inhibin, antimullerian hormone, estradiol, progesterone and calcitonin while the bone markers are calcium, phosphorus, alkaline phosphatase.

In this study, gonadotropin hormones (follicle stimulating hormone, FSH and luteinizing hormone, LH) Concentration in menopausal and perimenopausal subjects were dramatically high when compared to that of women of reproductive age. The follicle stimulating hormone (FSH) levels was higher than luteinizing hormone (LH) levels and both FSH and LH rise to even higher values than those seen in the surge during normal menstrual cycles. The observed elevated FSH and LH levels could be due to decline or declining ovarian function as a result of exhaustion of the pool of primary ovarian follicles and decreased steroidogenesis.

This affects the pituitary – hypothalamic feedback mechanisms causing an increase in pituitary gonadotropins. This finding is supported by the work of Sherman *et al.*, (1976) that loss of oocytes and follicles ultimately results in a series of endocrine changes in the hypothalamic – pituitary – gonadal axis and gradual diminution of estrogen and inhibin occurs. (Sherman *et al.*, 1976) research findings further revealed that decreased levels of inhibin result in raised levels of follicle-stimulating-hormone which is the first laboratory indication of the perimenopause and that the increased FSH induces rapid follicular development with consequent shortening of cycles. This change in menstrual cycle is due to a shortening of the follicular, but not luteal phase and may be the first clinical sign of the perimenopause. This showed that FSH and LH could be used as potential markers of menopause.

In this study, the serum prolactin levels in menopausal and perimenopausal subjects were significantly lower when compared with prolactin level of women within reproductive age. This suggest that prolactin concentration drops at menopausal stage of life and could be used as a potential marker for the diagnosis of menopause. This finding concur with the study done by Eleni*et al.*, (2022) that dopamine restrains the production of prolactin while estrogen increases it; that estradiol send message to the pituitary gland primarily indicating whether to begin the production of prolactin. In addition, Estradiol do not only promote prolactin synthesis but also decreases the production of luteinizing hormone and follicle stimulating hormone from the pituitary gland. This finding is also supported from another study that in pregnancy and just after parturition, estrogen and progesterone stimulate prolactin production (Nwankwo *et al.*, 2001) but at menopause, there is a significant decrease in estradiol and progesterone and so there is no stimulation of prolactin secretion. Similarly, this study concurs with the study done by Balint *et al.*, (2009) that prolactin levels decrease significantly during menopause.

The study observed an increase in thyroid stimulating hormone amongst perimenopausal and menopausal participants when compared to thyroid stimulating hormone (TSH) of women within the reproductive age. This suggests that TSH concentration increases at menopause and could be used to indicate laboratory symptoms of perimenopause or menopause. It is important to say from this study that elevated TSH level was due to menopause. The findings of the present study could suggest that TSH levels could be used as an independent predictive marker of osteoporosis in menopausal and perimenopausal participants.

This study also observed the significant decrease in the levels of progesterone, estradiol, inhibin and antimullerian hormone in perimenopausal and menopausal subjects when compared to levels of progesterone, estradiol, inhibin and antimullerian hormone of women of reproductive age. This finding could indicate decline or complete cessation of ovarian function at menopause due to exhaustion of the pool of primary ovarian follicles. Inhibin, unlike gonadotropin surge inhibiting factor, selectively suppresses pituitary release of FSH. Inhibin is a non-steroidal inhibitor present in follicular fluid and its peptides moiety is synthesized by the granulosa cells and secreted into the follicular fluid. The decrease in inhibin secretion by the ovarian follicles begins early at around 35 years of age but accelerates after the age of 40 (Buckler *et al.,* 1991). Similarly, low levels of estrogen and progesterone is the primary basis for the progressive decrease and complete cessation of the cyclic function of the female reproductive organs, this deficiencies in estrogen and progesterone appears to lie in the ovary itself (Jin*et al.*, 2016). There is continuing loss of the primordial follicles during intrauterine life and throughout the reproductive years until menopause. After about 35 years, the human ovary begins to decrease in weight and size, and contains much fewer oocytes and follicular structures and more atretic and degenerating follicles (Seok*et al.*, 2016).

It is important to realize that the feedback mechanisms may cause readjustments between the pituitary and ovary as long as there are follicles remaining in the ovary to respond. Also over a period of 1 or 2 years, reversal of laboratory findings as well as clinical signs and symptoms may occur (Seung*et al.*, 2016). In the menopausal ovary, although ovarian estradiol, antimullerian hormone and progesterone secretions are sharply reduced, the ovary is nevertheless capable of substantial steroidogensis. The ovarian stroma cells as well as the adrenal cells have a steroidogenic capacity for producing androstenedione which is converted by the skin and appendages to oestrone (Buckler *et al.,* 1991).

The primary steroidogenic element of the menopausal and postmenopausal ovary is the stroma, which frequently contains islands of thecal cells and may have the appearance of a generalized bilateral thecal hyperplasia. The steroids secreted by the menopausal and postmenopausal ovary in response to the stimulus from high concentrations of LH are primarily androgens (androstenedione, testosterone), but some estradiol may also be produced in insignificant concentrations. Hence, the ovarian stroma continues to be stimulated by LH to produce androstenedione and testosterone, with oestrone accounting most of the circulatory estrogen in the postmenopausal women (Eleni*et al.,* 2022). From this study, it is therefore important to say that menopausal women have an estrogen milieu that is lower than necessary for reproductive function. It is also important to say that Antimullerian hormone (Amh) levels is one of the important markers of ovarian reserve and it is highly associated with ovarian follicular development.

Also in this study, the levels of calcitonin and calcium were significantly lower while levels of phosphorus and alkaline phosphatase were significantly higher amongst perimenopausal and menopausal participants when compared to calcitonin, calcium, phosphorus and alkaline phosphatase of women of reproductive age. A marked decrease in calcium and calcitonin with a marked increase in phosphorus and alkaline phosphatase amongst the menopausal women could be due to significant decrease in estradiol level. The decreased estradiol leads to release of a cytokine (receptor activator nuclear factor kappa B ligand; RANK L) which stimulate osteoclastic cascade leading to osteoporosis. Perhaps, the osteoporosis could be the cause of elevated alkaline phosphatase (ALP) and phosphorous with corresponding decrease in calcium and calcitonin.

Since estradiol is an inhibitor of RANK L that prevent binding of RANK L to RANK. Hence the binding of Estradiol to RANK stimulate the secretion of calcitonin and calcitonin in turn stimulate osteoblast for born formation. This study is supported by the work done by Sherri *et al.,* 2018, that long period of estrogen lack such as in menopause and postmenopause may be associated with a more pronounced calcitonin deficiency. This exaggerated deficiency of estradiol could be an important factor in the pathogenesis of postmenopausal bone loss due to bone resorption. This work was supported by another similar study by Carolyn *et al.,* 2018, on the effects of estrogen treatment on circulating levels of calcitonin, parathyroid hormone and vitamin –D metabolites in postmenopausal women. The most striking change was a sharp rise in plasma calcitonin. Estrogen prevents postmenopausal bone loss, and it is suggested that this effect could be mediated, at least in part, through control of calcitonin secretion. Calcitonin may prove effective in the prevention of postmenopausal bone loss. (Arun *et al.,* 2018).

CONCLUSION

The present study thus concludes that elevated levels of FSH, LH, and TSH with decreased levels of E2, Prl, Inh, Amh, progesterone and calcitonin were associated with perimenopause and menopause. This suggests that hormonal profiles are good markers for the diagnosis and management of symptoms of menopause and perimenopause. The study observed increased activities of ALP with elevated level of phosphorus while decreased calcium level was observed. From these findings, hormonal profile and bone markers could be significant markers for the assessment and monitoring of perimenopausal and menopausal patients. Also, screening and assessing risk for menopausal and perimenopausal women would be helpful before the developments of certain clinical symptoms such as osteoporosis, spondylosis and many others symptoms of menopause. The decline in E2, AMH, Prog, Inh and calcitonin can also be used to identify women at risk of early menopause. Further studies should be carried out to establish alternative, appropriate, easy and simpler indices and methods suitable for assessing or diagnosing menopause in resource-limited settings.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

This work was carried out in collaboration between all authors. Author EPI, MOA, and ASA designed the study and performed the statistical analysis. Authors EPI, MOA, SEA, ASA, and ZU conducted and managed the Laboratory analysis. All authors read and approved the final manuscript.

Acknowledgement

The authors wish to acknowledge all the participants who voluntarily gave their written informed consent for this work and also the authors are immensely grateful to Kogi State Ministry of Health for all the supports throughout the research period.

REFERENCES

Allain, C., Poon, S., Chan, G., Fu, P.(1974). Enzymatic determination of serum cholesterol. *Clinical Chemistry*, **20**: 470 -475.

Arun, S. K., Sherri-Ann, M. B., Carolyn, J.C.(2018). Bone health during the menopause transition and beyond. Journal of the Obstretic and Gynecology clinics of North America, 45(4): 695 – 708.

Baber, R. , Abdulla, H. ,Studd, J. (1991). The prematremenopause.in stud, J. *Progress in Obstetrics and Gynaecology, Edinburgh: Churchill Living stone*, **9**:209-226.

Bayanjargal, O., Namsrai, Z.,Sievert, L.L (2022). The menopausal transition in mongolia. *The Journal of the American Menopause Society*, **29**(1): 96-100.

Boardman, H.M. ,Hartley,L. , Eisinga, A. ,Main, c . , Roque, I. , Figuls, M. , Bonfill C. X., Gabriel, S.R., Lnight , B.[2015] . Hormone therapy for preventing cardiovascular disease in post- menopausal women. *Journal of National Library of Medicine*, **10**(3):1-2.

Buckler, H.M., Evans, A., Mamlora, H. (1991). Gonadotropin, steroid, and inhibin levels in women with incipient ovarian failure during anovulatory and ovulatory rebound cycles. Journal of Clinical Endocrinology and Metabolism, **72**: 116 – 124.

Bucolo,G., David, H. (1973). Quantitative determination of serum triglycerides by use of enzymes. *Journal of Clinical Chemistry,* 19: 476 – 482.

Bukulmez, O., Yareli, H., Gurgan, T. (1999). Total corporal synechiqe due to tuberculosis cary a very poor prognosis following hysteroscopicsynechialysis. *Human Reproduction*, **14**: 1960-1996.

Cagnacci, A.,Xholli, A.,Fontanesi, F.,Neri, I., Facchinetti, F. ,palma, F. (2022). Treatment of menopausal symptoms: concominant modification of cortisol. *The Journal of North American Menopause Society*,**29**(1):23-27.

Edmonds, D. K., (2001). Vaginal and uterine anomalies in the paediatic and adolescent patient. *Journal of current opinion of obstetrics and Gyneologists*, **13**: 463-467.

EL Khoudary, S. R., Qi, M., Chen,X., Matthews, K., Allshouse, A. A. , Crawford, S. L., Derby, C . A., Thurston, R. C., Kazlauskaite, R., Barinas- Mitchell, E., Santoro, N. (2022). Patterns of menstrual cycle length over the menopause transition are associated with subclinical atherosclerosis after menopause. *The Journal of North AmericanMenopause Society,* **29**(1):8-5.

Eleni, D., Deena, A., Nidhi, A., Anne, K., Janet, S. (2022). Hyperprolactinemia; diagnosis, treatment and prevention. Journal of Endocrine Society, **22**: 234 – 245.

Elsabagh, E.E.M, and AbdAllah, E.S. [2019]. Menopausal symptoms and the quality of life among pre and post-menopausal women from rural area in Zagazigcity. *Life science journal*, **9**(2) :283-291.

Fenton, A., Panay, N. [2012]. Does routiene gynecological surgery contribute to an early menopause? *Journal of climacteric*, **15**: 1-2.

Fores, V.A., Manson, J. E.(2022). Patterns of menstrual cycle length over the menopause transition – a novel marker for cardiovascular risk? *The Journal of North Americanwomenmenopause society*, **29**(1):1-2.

Frank, R. T. (1938). Formation of artificial vagina without operation. *American Journal of Obstetrics and Gynecologists*, **35**:1053.

Garcia-Alfaro, P., Garcia, S., Rodriguez, I. Perez-Lopez, F. (2022). Handgrip strength, dynapenia, and related factors in postmenopausal women. *The Journal of North American Menopause Society*.**2**(1):16-22.

Gloria, B., Richard, J.S (2020). Clinical manifestations and diagnosis of genitourinary syndrome of menopause (Vulvovaginal atrophy). *Journal of Revista media Sinergra*, **5**(09): 1-17.

Grandi, G. , Fiocchi, F. , Cortesi, L. , Toss, A. , Boselli, F. ,Sammarini, M. , Sigholfi, G. , Facchinetti, F. (2022). The challenging screen detection of ovarian cancer in BRCA mutation carries adhering to a 6-months follow –up program: results from a 6-years surveillance. *The Journal of the American Menopause Society,* **29**(1):63-72.

Hilser, J.R, Hartiala,J.A., Sriprasert, I., Kono, N., Cai, z., Karim, R., De Young, J., Mack, W.J., Hodis, H.N, Allayee, H.[2022]. Effect of menopausal hormone therapy on methylation levels in early and later post menopausal women. *Journal of clinical epigenetics*, **14**(1):90-95.

Janaka, L., Gayani, A, Sarath, L. [2019]. Prevalence and severity of menopausal symptoms and the Quality of life in middle aged women: A study from Sri Lanka. *Journal of Nursing research and practice*, **10**(1155): 20 – 3.

Jeong , H.G. ,Park , H. [2022]. Metabolic Disorders in menopause.*Journal of National LibraryMedicine*, **12 (**10):954-955.

Jones, G. S. ,DeMoraes, R. M.(1979). A new syndrome of amenorrhoea in association with hypergonadotripism and apparently normal ovarian follicular apparatus. *American Journalof Obstetrics and Gynecologists*, **104**: 597.

Kapoor, E. (2022). Menopause symptoms and the cortisol response. *The Journal of NorthAmerican Menopause society*, **29**(1): 6-7.

Karvonen-Gutierrez,C.A., Park, S.K., Kim, C. [2016]. Diabetes and menopause. *Journal of Current Diabetes Reports*, **16**(4):20-22.

Lambrinoudaki, I., Paschou, S.A., Armeni, E., Goulis, D.G [2022]. The interplay between diabetes mellitus and menopause: clinical implications. *Journal of Nature Reviews of Endocrinology*, **18** (10):608-622.

Li ,S. ,Rosenberg ,L. , Wise ,L.A. , Boggs, D.A. , LaValley , M. ,Palmer, J.R. [2013]. Age at natural menopause in relation to all-cause and cause-specific mortality in a follow up study of united states black women. *Journal of National Library of Medicine*,**75(**3): 246-252.

Lobo, R.A. ,Davis, S.R . , De Villers, T.J . , Gompel, A., Henderson, V.W. , Hodis, H. N. ,Lumsden, M.A. , Mack, W.J. ,Shapiro, S. ,Baber, R. J. [2014]. Prevention of disease after menopause. *Journal of National Library of Medicine* ,**17**(5) : 540-556.

Makarch – studzinska, M. T .,Krys-Noszczyk, K. M. ,Jakiel ,G .[2014].Epidemiology of the symptoms of menopause – an intercontinental review. *National center for BiotechnSology Information Journal*, **13** (3):203-211.

Marjoribanks , J. ,Farquhar, C. , Roberts, H. , Lethaby, A. [2012]. Long term hormone therapy for perimenopausal and postmenopausal women. *Journal of National Library of Machine*, **11**(7): 1-2.

Monferrosa- Castro, A., Blumel,J.E., Portela-Buelvas, K. (2013).Type II diabetes mellitus and menopause: a multinational syudy.*Journal of Climacteric*, **16** : 663-672.

Moretti, G., Troiani, E., Sarlo, F., Baroni, S., Urbani, A. (2022). Analytical Performance Evaluation of a New Calcitonin Assay. *Journal of Applied Laboratory Medicine,***7**(2): 568 – 574.

Nwankwo, E. L., (1996). Applied Anatomy and Physiology (2nd edition). Ibadan: Sibon Books.

Olesen, C., Koch T., Uldbjerg, C. S. ,Gnegersen, L. S. , Christensen, J. , Dehlendorff, C. , Priskorn, L. , Wilson, L. F., Lim, Y. H. , Jorgensen, J. T. , Anversen, Z. T. , Juul, A. , Abildgaard, J. , Hickey, M. , Brauner, E.V (2022). Cardiovascular mortality after bilateral oophorectomy: a prospective cohort study. *The Journal of the North American MenopauseSociety,***29**(1):28-34.

Panay, N. (2015). Genitourinary syndrome of menopause-dawn of a new era? *Open accessJournals*,**18**(1) :13-17.

Panay, N., Ylikorkala, D.F., Archer, R.G, Lang, E. (2007). Ultra-low-close estradiol and norethisterone acetate: effective menopausal symptoms relieve. *Open access Journals*, **10**(2): 120-131.

Pelosi , E , Simonsick, E. , Forabosco, A. , Garcia-ortiz , J. E , Schlessinger, D [2015]. Dynamics of the ovarian reserve and impact of genetic and epidermiological factors on age of menopause. *Journal of biology of reproduction, 92* :130.

Roberts, C. P, Haber, M. J., Rock, J. A. (2001). Vaginal creation for mullerian agencies. *American Journal of Obstetric and Gynecologist,* **185**:1345-1352.

Sanchez-Borrego, R.(2022). A strong handshake! Do not forget to measure grip strength in menopause: a simple way to predict general frailty or impairment. *The Journal of North American menopause society,* **29**(1):3-5.

Saxena,R . , Bjones , A.C., Georgopoulos, N.A. , Koika, V. , Pandis D. , Welt, C. K.[2015]. Gene varialsassociated with age at menopause are also associated with polyrystic ovary syndrome , gonadotrophins and ovarian volume. *Journal of Human reproductivesciences*, **30** : 1697-1703.

Sethi ,N.J. , Safi, S. K. , Hrobigartsson, A. , S koog, M. , Gluud, C. , Jacobsen , J. C. [2021] . Antibiotics for secondary prevention of coronary heart disease. *Journal of National Library* of Medicine, **2**(2): 1-2.

Seung, J. C., Jin, Y. H., Seok, K. S.(2016). Serum thyroid stimulating hormone levels are associated with the presence of coronary atherosclerosis in healthy postmenopausal women. Journal of menopausal Medicine, **22**(3): 146 – 153.

Sherman, B.M., West, J.H., Koreman, S.G. (1976). The menopausal transition: analysis of LH, FSH, Estradiol, and progesterone concentrations during menstrual cycles of older women. Journal of Clinical Endocrinology and Metabolism, **42**: 629 – 636.

Speroff, L., Glass, R., Kase, N. (1994). Amenorrhoea in clinical Gynaecologic Endocrinology and Infertility, 5th Edition. *Battimole: Williams and Willians*, 401-456.

Stowell, L.H., Sharman, L.E., Hamel, K,(1991). An Enzyme-Linked Immunosorbent Assay (ELISA) for hormonal assay. *Forensic Science International Journal*,**50**: 125 -138.

Su, J., Jogamoto, A., Yoshimura, Yang, L.J.(2022). Menopausal symptoms among Chinese and Japanese Women: differences and similarities. *The Journal of the American MenopauseSociety*,**29**(1):73-81.

The Joint United Nations programme on menopause [2014]. Women aged 50 years and older. The Gap Report 2014, Geneva, Switzerland,2014.

Trounson, A., Leeton, N.,Besanko, M.,C., Conti, A. (1983). Pregnancy established in an infertile patient after transfer of a donated fertilized in vitro. *British Medical Journal*, **286**:835-838.

Vankasteren, Y. M,Schoemaker, J. (1999). Premature ovarian failure: a systematic review on therapeutic interventions to restore ovarian function and achieve pregnancy. *Journal ofHuman Reproduction*,**5** :483-492.

Wang R., Su, Q. , Yan, Z.(2021). Excising the neovagina due to introitalartesia and closed neovaginal loop after sigmoidvaginoplasty :A case report. *Medicine (Balttimore)*,**100**(9) :5

World Health Organization [1996]. Research on the menopause in the 1990s.*Report of a World Health Organization Scientific Group,*1996.

Ye, X. P., Yang, Y. Z., Sun, x.x. (2015). A retrospective analysis of the effect of salpingectomy on serum anti-mullerian hormone level and ovarian reserve. *American Journal of obstetricand Genealogists*, **212** (53): 1-10.

Yixve, N., Jennifer, S. W., Erin, K,W., Alison, K.S. , Maureen, J. M, Baraa, K.A. [2022]. Roles of Hormonal Replacement Therapy and Menopause on Osteoarthritis and Cardiovascular Disease Outcomes:A narrative review. *Journal of National Libraryof Meicine*, **28**(3): 10-15.

Zakeriya, L., Rand, W. M. , Wurtman, R.J.(1976). A prospective study of sexual development and growth in American girls: the statistics of menarche. *Obstetrics and Gynecology Survey*, **31**:325