Variation of Hematological profile in Postmenopausal Women - A preliminary study

Mohd Adil[1*], Nirmala Devi MK[1], Nikhat Shaikh[1], Shah Alam[1], Ziaur Rahman[1], Piyush yadav

[1]*Research Officer, Regional Research Institute of Unani Medicine (RRIUM), Mumbai*

ABSTRACT
Menopause is the permanent cessation of menses for 1 year and is physiologically correlated with the decline in Oestrogen secretion resulting from the loss of follicular function. Females attained menopause naturally after the age of 40 years or more, as it is due to hormonal changes occurring in the body that makes them vulnerable to deal with many consequences physiological and sometime pathological too. Classical Unani literature mentioned Ehtibase Tams i.e. cessation of menstruation which can be correlated with Sinne Yaas (age of natural termination of menstruation). Ibn Sina stated that it is the decline of Quwate Dafiya i.e. faculty of excretion of the body which alter and ultimately stops the cycle. Average age of menopause is 47.5 years in Indian women with an average life expectancy of 71 years. Several studies reveal that Ovarian functions in menopausal women gets declines that leads to the alterations in metabolism, vascular endothelium function, fibrinolytic and coagulative changes in the blood. As a result this age brings women at risk of Ischemic Heart Diseases thus increasing the morbidity and mortality. In view of the associated risk, present study was undertaken to screen the menopausal women to evaluate the relationship between menopausal age and their hematological profile. Patients were randomly selected from the General and Geriatric OPDs and were analyzed.
Keywords: Menopause; Ehtibase Tams; Sinn-i-Yaas; Hematological profile; Unani Medicine.
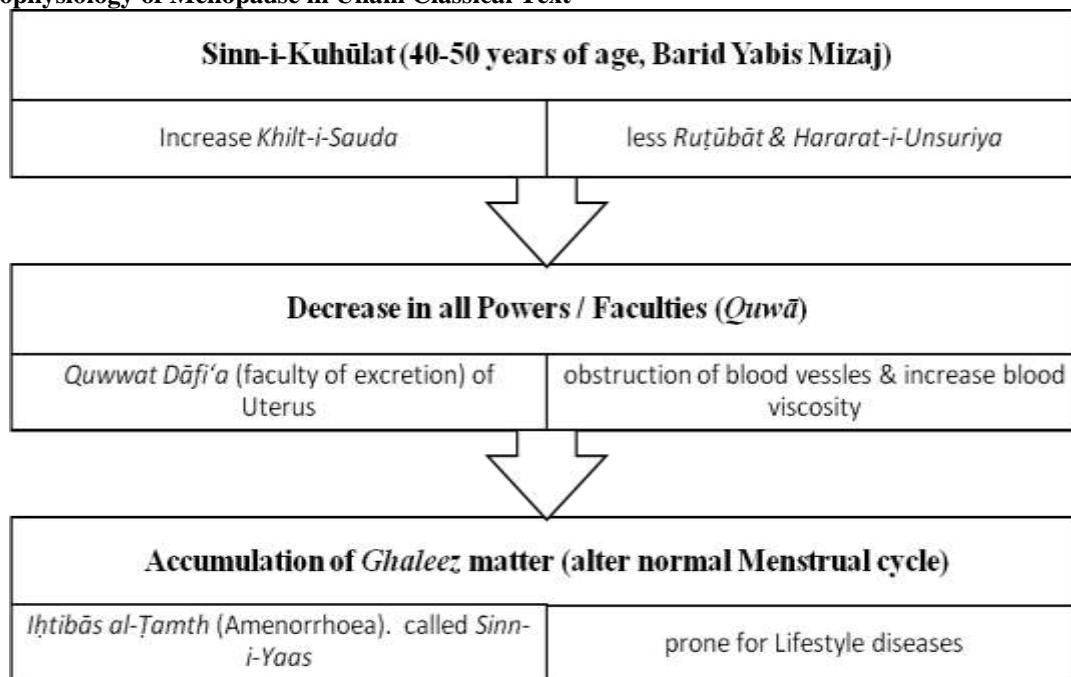
I. INTRODUCTION
Word Menopause derives from Greek word "Menos" (i.e. menstruation) and "pause" stands for cessation; thus menopause is defined as the 'time of stoppage of menstruation'. Most commonly females attained menopause after the age of 40 years or more, as it is due to hormonal changes occurring in the body that makes them vulnerable to deal with many consequences physiological and sometime pathological too. (Shahid et. al; 2016)

Indian menopausal society reported that there are 43 million menopausal women in India. Projected figures in 2026 have estimated that population in India will be 1.4 billion, with people over 60 years of age 173 million, and menopausal population 103 million. Average age of menopause is 47.5 years in Indian women with an average life expectancy of 71 years (Unni J. 2010).

A majority would spend one third of their life in the postmenopausal stage (Monika et. al 2014). Hot flashes, irregular periods, sleep disturbances, low energy levels, weight gain, or emotional disturbances are major issues in menopausal females. In majority of cases physicians are frequently using hormone-based therapies to treat the menopausal syndrome. However long-term usage of the therapy causes venous thromboembolism, coronary heart disease, stroke, and breast cancer. Due to a decrease in bone density, menopausal women are more prone to fractures. Additionally, sarcopenia (loss of skeletal muscle mass) and a reduction in muscle strength may result. During this time, changes in body mass increase the prevalence of other metabolic and lifestyle diseases.

Pathophysiology of Menopause in Unani Classical Text

Sinn-i-Kuhūlat (40-50 years of age, Barid Yabis Mizaj)	
Increase *Khilt-i-Sauda*	less *Ruṭūbāt & Hararat-i-Unsuriya*

Decrease in all Powers / Faculties (*Quwā*)	
Quwwat Dāfi'a (faculty of excretion) of Uterus	obstruction of blood vessels & increase blood viscosity

Accumulation of *Ghaleez* matter (alter normal Menstrual cycle)	
Iḥtibās al-Ṭamth (Amenorrhoea). called *Sinn-i-Yaas*	prone for Lifestyle diseases

According to Unani Medicine Sinn-i-Yaas has been described as the age of attaining long standing Iḥtibās al -Ṭamth (Amenorrhoea). Menopause usually occurs at about the age of 50 years at which a female attains Sinn-i-Kuhūlat having Barid Yabis Mizāj. At this stage Khilt-i-Sauda excessively produced in the body that decreases the production of Ruṭūbāt -i-Unsuriya and Hararat-i-Unsuriya resulting in the weakness of all Quwā (Powers) of the body (Bhat et. al.2015).

Due to weakness of All Quwā of the body Quwwat Dāfi'a (Expulsive Power) of the Uterus becomes weak resulting in the retention of morbid matter inside the body. These morbid matters admix with blood, resembles like Sauda (Black bile) and produces symptoms like Iztirab-i-Nafsani (emotional disturbance), Kasrat-i-Arq (hot flashes/sweating), Sahar (Insomnia), Ṣudā' (Headache), Waja' al -Mafāṣil Saw dāwī (Joint pain), Ḥikka al -Raḥim (Itching and dryness of uterus) and Yaboosat-i-Mehbal (Dryness of vagina) (Jurjani, 2010)

Razi described the causes of Sinn-i-Yaas and stated that Uterine vessels get obstructed due to predominance of Baroodat and increased viscosity resulting in the permanent cessation of Menses (Razi, 2001) while Ibn-e-Sina mentioned the cause of Sinn-i-Yaas as the weakness of Quwwat Dāfi'a resulting in the alteration of normal menstrual cycle (Kantoori, 2010).

Objectives
1. Primarily objective is to compare the variation of hematological values (RBC indices which include red blood cell count, packed cell volume, haemoglobin concentration and mean corpuscular volume) within postmenopausal population.

II. MATERIAL AND METHODS

The data was collected from the patients attended Geriatric OPD at Regional Research Institute of Unani Medicine (RRIUM), Mumbai. Ethical clearance was obtained from IEC. Informed consent was obtained from each participant. Total 100 females were screened and those having the history of stoppage of menstruation for a minimum of 12 months or more were selected for the study, thus constituting a sample size of 35 only. Blood samples were collected through venipuncture and analyzed using Sysmex pocH-100i hematology auto-analyzer. The principle for counting blood cells is based on electric resistance detection method and hemoglobin concentration is determined by a photometric measuring method. The Hb%, RBC count, ESR, PCV, Platelet count, and Reticulocyte count were study variables. While Complete Blood Count, Urinalysis for rapid detection of Protein/Glucose in urine, Stool for Ova/Cyst, Renal Function Test, Liver Function Test, Random Blood sugar, Peripheral smear for

malarial parasite was conducted to exclude the patients.

Inclusion Criteria
- Age group of 50-65 year of post-menopausal females
- History of natural menopause without any hormonal or surgical intervention

Exclusion Criteria

- Fertile, pregnant and lactating females were excluded
- Perimenopausal & Premature menopausal women (Completing menopause before age 40)
- History of any major disorders
- History of tobacco chewing and smoking
- Known cases of Diabetes Mellitus and Hypertension, surgically induced menopause such as Oophorectomy, history of Cerebrovascular disease, history of Coagulopathies, Thyroid diseases, and medications known to affect the hematological values were excluded from the study

- Women with a history of recent blood transfusion, those are taking Vitamin, Iron supplements or who were on medication which can alter RBC indices like Immunosuppressive Anti-epileptic, Anti-retroviral or Chemotherapeutic agents were excluded from the study.

III. STATISTICAL ANALYSIS

Results were presented as mean ± SD while data was analyzed using unpaired Student t-test and one way analysis of variance (ANOVA). Results were considered statistically significant with $p<0.05$. The mean red blood cell count, Packed cell volume and Haemoglobin concentration of the postmenopausal women was lower than that of the control and was statistically significant. The Mean corpuscular volume of the menopausal women was significantly higher in women who attained menopause >10years back ($p<0.05$). (Give data of age at which menopause was attained in study group)

Observations and Results

Table no: 1- Age wise Hemoglobin level in Menopausal women (normal: 12.0-15.2 gm% in Female)

Age in years	Haemoglobin gm %				
	<7-8.5gm%	8.6-10.5gm%	10.6-12gm%	>12gm%	Total (n=35)
< 50 yrs	05	03	01	04	13(37.14%)
50-55 yrs	02	02	02	00	06(17.14%)
55-60 yrs	03	03	00	01	07(20.00%)
60-65 yrs	03	01	05	00	09(25.71%)
Total	13(37.14%)	09(25.71%)	08(22.85%)	05(14.28%)	35
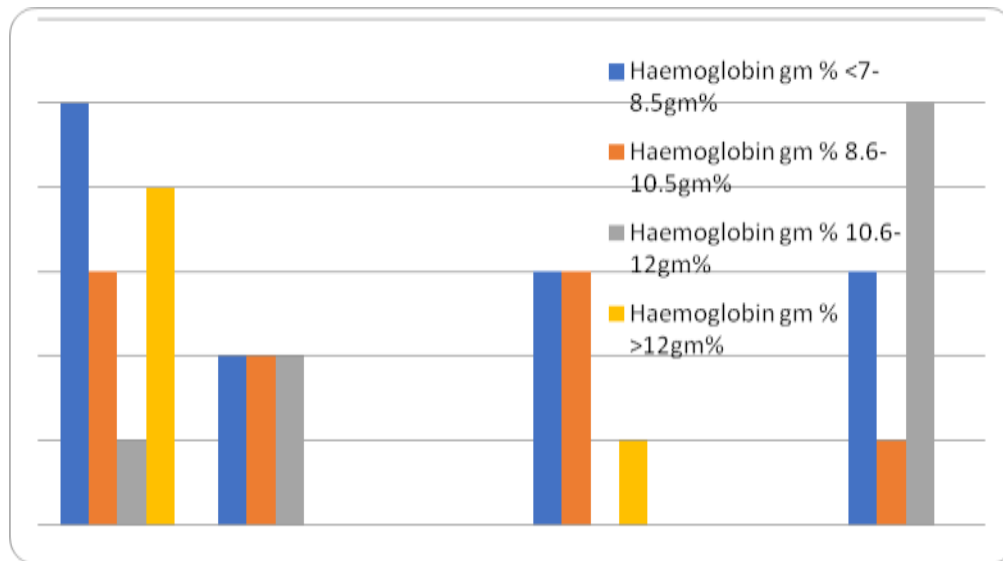
Figure no: 1 shows that 37.14% of the study population showed haemoglobin within the range of 7-8gm% whose duration of menopause was 4-5 years. <u>Give details</u>

Table no: 2- Age wise Haematocrit (Hct) in menopausal women (normal: 37-46% in Female)

Age in years	Haematocrit %				
	<30%	>30-36%	>37-46%	>46%	total(n=35)
< 50	02	05	01	05	13(37.14%)
50-55 yrs	01	03	01	01	06(17.14%)
55-60 yrs	02	01	01	03	07(20.00%)
60-65	01	03	05	00	09(25.71%)
Total	06(17.14%)	12(34.28%)	08(22.85%)	09(25.71%)	35
	(51.42%)				
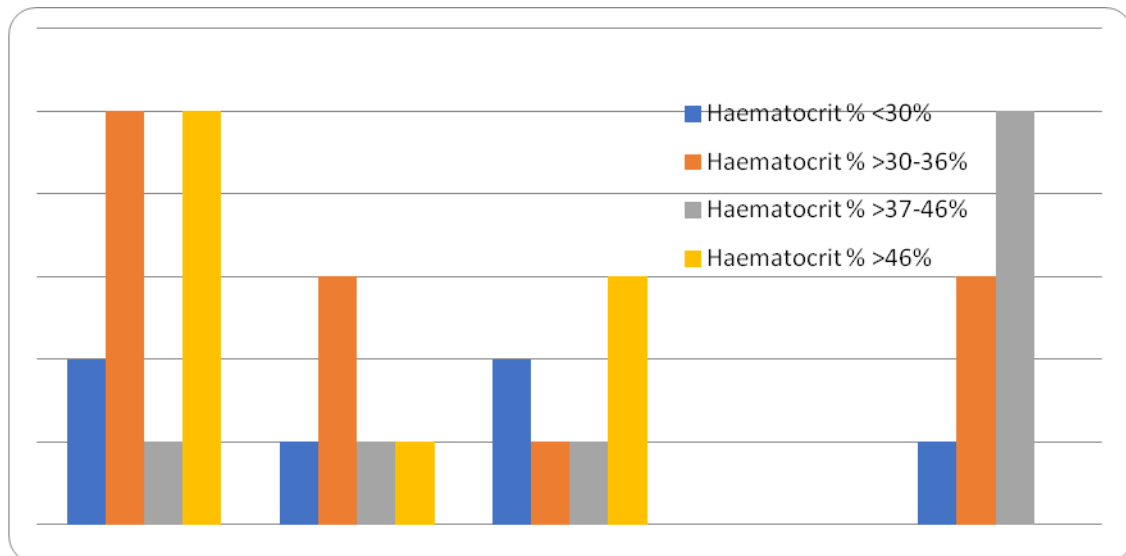
Figure no.02: shows that 51.42% of study population had Haematocrit lesser than the normal range of 37-46%.

Table no:3 shows that 48.57% of study population showed RBC count <3.80x10^6/ µl. These values are contradictory to those of other studies where the values (specifically hemoglobin) were higher in menopausal women (Milman et al., 1992; Castro et al., 1985).

Age in years	R.B.C. Count				
	<3.80x10^6/ µL	>3.80-4.50x10^6/ µL	>4.50-5.50x10^6/ µL	>5.50x10^6/ µL	Total (n=35)
< 50 yrs	08	02	01	02	13(37.14%)
50-55 yrs	02	02	01	01	06(17.14%)
55-60 yrs	03	02	00	02	07(20.00%)
60-65 yrs	04	01	03	01	09(25.71%)
Total	17(48.57%)	07(20.00%)	05(14.28%)	06(17.14%)	35

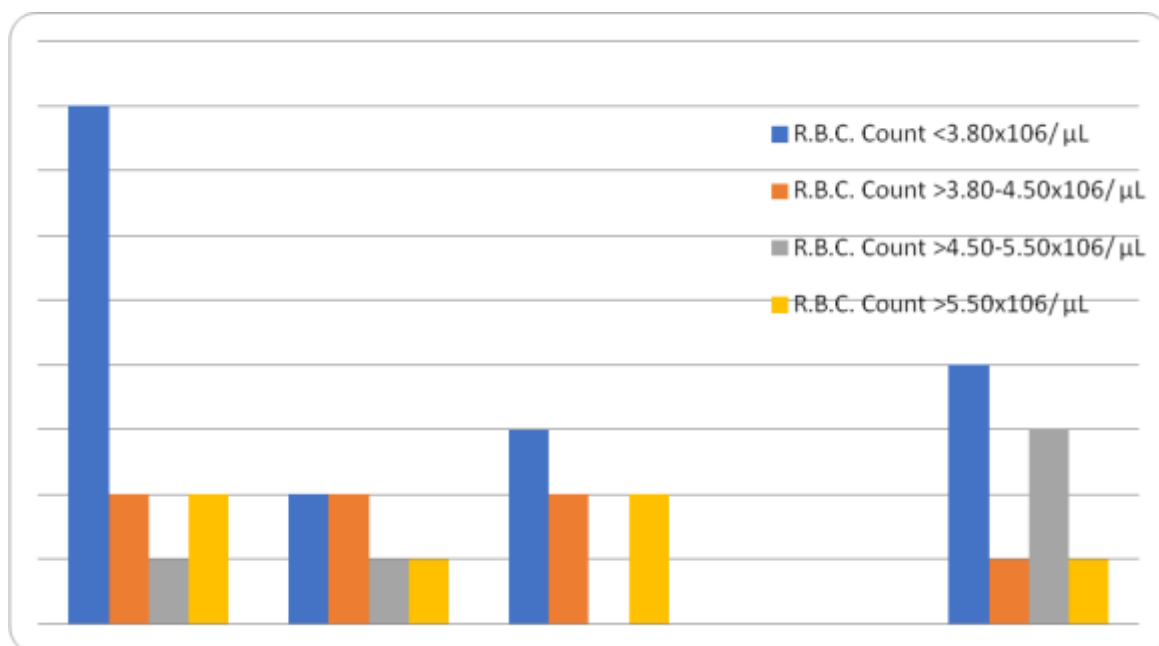**Figure no.03:** RBC count

Table no: 4 Age wise MCV menopausal women (normal: 78-101 fL (Female)

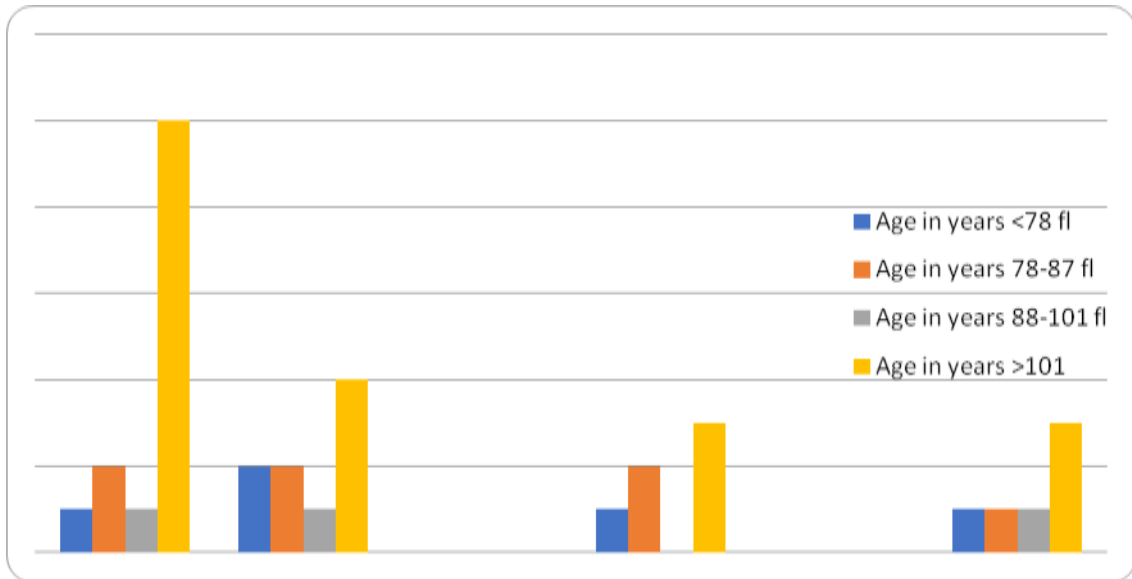| Mean corpuscular volume					
Age in years	<78 fl	78-87 fl	88-101 fl	>101	Total(n=35)
< 50	01	02	01	10	13(37.14%)
50-55 yrs	02	02	01	04	06(17.14%)
55-60 yrs	01	02	00	03	07(20.00%)
60-65	01	01	01	03	09(25.71%)
Total	05(14.28%)	07(20.00%)	03(08.57%)	20(57.14%)	35

Figure no.04: Mean Corpuscular Volume

Table no: 4 shows that 57.14% of study population shows increase in the mean corpuscular volume. This is in agreement with the findings of (Chalmers et al., 1979) who observed an increase in MCV following the menopause. The MCV is used to classify anemia according to the size of the red cell. Increased MCV is found in pernicious and megaloblastic anemia caused by deficiency of vitamin B12 and folic acid. They are essential for the synthesis of DNA. The increase in MCV in this study was statistically significant (p = 0.042) in the >10 years postmenopausal women. Hence a need for age specific reference ranges in this population for efficient detection of abnormal results (Shibata et al., 2005).

Table no: 5a-Descriptive statistics of age of premenopausal and menopausal women

Parameters	Control n=35	Menopausal women n=35
Age in years	25.51±5	53.59±6.11
Age at menopause(years)	-	46.16±3.45
Age at menopause(median)	-	46.00
Parameters	Control n=35	Menopausal women n=35
Age in years (Mean ±SEM)	25.51±5	53.59±6.11
Age at menopause(years)	-	46.16±3.45
Age at menopause(median)	-	46.00

Insufficient information regarding the control group, Whole script lack control group data

Table no: 5b- Haematological parameters observed in premenopausal and menopausal women

RED BLOOD CELLS INDICES	Control n=35	Menopausal women n=35	P value < 0.05 (at 5%level of significance)
RBC count(x10^6/µL)	4.9±0.52	4.5±0.8	Significant
Hb (g/dl)	12.68±1.23	10.5±1.45	Highly significant
PCV%	39.5±3.6	38.1±4.7	Low significance
MCV(fl)	83.8±3.1	86.6±3.6	Significant

Table 5a: Mean age in years of control (n = 35) and menopausal women (n = 35) was 25.51±5.1 and 53.59±6.11 respectively. Age at menopause median 46.00 years (46.16±3.45). This value is lower than that for Caucasians (51.4 years) (Gold et al., 2001). An earlier age at menopause as observed in the study group is known to be associated with increased morbidity (Milman et al., 1992; Castro et al., 1985).

Table 5b: Red blood cell indices for the control (n = 35) and menopausal women (n = 35) showed that the RBC (Red blood cell) count, Hemoglobin, PCV (Packed cell volume) were lower in the menopausal women as compared to the control group and it was statistically significant (p<0.05).The ethnic variations to the menopause experience is said to be due to some variables (like Parity, Menstrual cycle) which differ in the different ethnic groups (Ozumba, et al.,2004).There is an increase in MCV (86.6±3.6) as compared with the control (83.8±3.1) and it is statistically significant (p<0.05)

IV.   DISCUSSION

In this study menopausal women were found to have lower red cell indices with a predisposition for developing anemia. The differences indicate the need to develop reference values with concurrent developing of strategies to tackle the morbidity in the postmenopausal population. Studies to assess serum levels of iron, folic acid and vitamin B12 of menopausal women along with RBC indices incorporating larger study group as multicentric studies in other states of the country should be carried out to develop reference range for postmenopausal women. It is suggested that estrogen has a possible suppressive effect on erythropoietin induction (Horiguchi et al., 2005). Our findings indicated there was decrease in red cell indices specifically RBC count, HB and PCV in the menopausal women as compared to control.

Only after the onset of menstruation does a specific difference in the RBC indices emerge (Rushton and Barth, 2010. Not until 10 years after the menopause does this situation revert in women, when the hemoglobin concentration becomes similar to that of aged matched men (Rushton et al., 2001). In a study of the variations of the hemoglobin concentrations with age, sex and in blood donors, it was observed that from the fifth decade to the age of 65 there was a progressive and significant decrease of hemoglobin concentration in women. Reasons for the difference were related to the effects of the hormonal environment of menopause (Cruikshank, 2008). However, studies also found that there is significant difference in the RBC indices between premenopausal and postmenopausal women (Vazquez et al., 2009). Studies indicate that the difference in red cell indices with menopause is usually noticed in women who were at least 10 years postmenopausal (Bain, 2006).

Menstruation and menopause have both been shown to significantly affect the concentration of a number of biologically important plasma constituents, including blood counts.

In this study, we observed that menopausal women had significantly lower red blood cell count (RBC) as compared control (P value < 0.05) table 5b.PCV and hemoglobin concentration were significantly lower in menopausal group (p = <0.05 , respectively, Table 5b). Previous report had suggested that haemoglobin concentration may decrease in menopausal women, mainly due to high prevalence rates of nutritional anemia in the elderly.

This was contradicted by the report of Milman et al., which indicated that haemoglobin concentration may actually be higher in menopausal women, most probably due to the cessation of menstruation.

In this study, we observed that menopausal women had significantly higher mean corpuscular volume as compared to control (P value < 0.05) table 5b. Even though the increase in MCV did not extend into the macrocytic range, it is important to observe that these increases were observed to occur. It may thus appear that our study population might have had pre-existing subclinical nutritional anemia which then became corrected with the onset of menopause, leading to significant increases in MCV. This line of thought appears to be favored by the documented high prevalence of nutritional deficiency (especially B12 deficiency) in the environment of this research. Previous studies among Nigerian and Caucasian women populations supported our observation. Interestingly, the Nigerian study observed increases in MCV even up to the microcytic range in menopausal women and concluded that this may be due to concurrent nutritional (B12/folate) insufficiency. It will be interesting to evaluate for the true prevalence of nutritional anemia in our pre- and postmenopausal women population in a subsequent follow-up study.

V. CONCLUSION

The study established that menopause is associated with a decrease in red cell indices which was significant. The study shows an increase in Mean Corpuscular Volume (MCV) following the menopause. The MCV is used to classify anemia according to the size of the red cell. Increased MCV is found in pernicious and megaloblastic anemia caused by deficiency of vitamin B12 and folic acid which are essential for the synthesis of DNA.

The findings are supported by a study of elderly Nigerians which revealed that women were more vulnerable to under nutrition than their male counterparts (Olayiwola et al., 2006).

The menopausal women were also prone to constitute people of a low socioeconomic group in this study. Some of them were retirees (being older), others were widows and also unemployed. That might have affected their nutrition and could have contributed to the finding of lower RBC count, Hb and PCV parameters in the menopausal women.

The differences indicate the need to develop reference values with concurrent developing of strategies to tackle the morbidity in the postmenopausal population. Studies to assess serum levels of iron, folic acid and vitamin B12 of menopausal women along with RBC indices incorporating larger study group as multi-centre studies in other states of the country should be carried out to develop reference range for postmenopausal women.

The higher MCV observed in the study population during menopause is believed to be related to B12 deficiency additionally, this study established that menopause is associated with a significant decrease in the red cell indices. While we believe that this finding may be related to possible pre-existing nutritional anemia in our population of menopausal women, it is important that this observation is further validated in follow up studies, so as to enable possible institution of epidemiological interventional programs that could address the issue

REFERENCES)

[1]. Bain, B.J., 2006. Blood Cells: A practical Guide. 4th Edn., Blackwell, Oxford: 177-187.

[2]. Bhat S.A., Raza A., Wani P., Shahabuddin M. 2015. Understanding Basics of Menopause in Greeco-Arab Medicine: A Review; Public Health and Preventive Medicine; Published by American Institute of Science; Vol 1 (2) : 58-61

[3]. Castro, O.L., T.B. Haddy, S.R. Rana, K.D. Worrell and R.B. Scott, 1985. Electronically determined red blood cell values in a large number of healthy black adults. Subpopulations with low hemoglobin and red blood cell indices. Am. J. Epidemiol., 121(6): 930-936.

[4]. Chalmers, D.M., A.J. Levi, I. Chanarin, W.R.S. North, and T.W. Meade, 1979. Mean cell volume in a working population: The effects of age, smoking,

alcohol and oral contraception. Br. J. Haematol., 43(4): 631-636.

[5]. Cruikshank, J.M., 2008. Some variations in the normal haemoglobin concentration. Br. J. Haematol., 18(5):523-530.

[6]. Gold, E.B., J. Bromberger, S. Crawford, S. Samuels, G.A. Greendale, S.D. Harlow and J. Skurnick, 2001.Factors associated with age at natural menopause in a multiethnic sample of midlife women. Am. J. Epidemiol., 153(9): 865-874.

[7]. Horiguchi, H., E. Oguma and F. Kayama, 2005. The effects of iron deficiency on estradiol- induced suppression of erythropoietin induction in rats: Implications of pregnancy-related anemia. Blood, 106(1): 67-74.

[8]. Howkins and Bourne Shaw's Textbook of gynaecology15th edition, 60-67. (incomplete)

[9]. Jurjani I, Zakheera Khawarzam Shahi (Urdu translation by Khan, HH), Vol-8, Idara Kitab-us-Shifa, New Delhi, 2010, pp 599-600

[10]. K. Monika, Meena K. L, V Prabhaker. Review of menopausal syndrome and scope of Rasayan Therapy in its management Punarna V. An International Peer Reviewed Ayurvedic Journal, 2014, 1-15.

[11]. Kantoori Allama Ghulam Hussain. Al Qanoon Fit tib Urdu translation (Original author Ibn-e-Sina). New Delhi: Idara Kitab-us-Shifa (2010)1095-1097.

[12]. Lewis, S.M., B.J. Bain and I. Bate, 2008. Dacie and Lewis Practical Haematology. 10th Edn., Reed Elsevier India Private Limited, New Delhi, India, pp:43-193, 595-607.

[13]. Milman, N., M. Kirchhoff and T. Jorgensen, 1992. Iron status markers, serum ferritin and hemoglobin in 1359 Danish women in relation to menstruation,hormonal contraception, parity, and postmenopausal hormone treatment. Annal. Haematol., 65(2): 96-102. Asian J. Med. Sci., 3(8): 154-157, 2011.

[14]. Olayiwola, I.O. and A.O. Ketiku, 2006. Sociodemographic and nutritional assessment of the elderly Yorubas in Nigeria. Asia. Pac. J. Clin. Nutr., 15(1): 95-101.

[15]. Ozumba, B.C., S.N. Obi, E. Obikili and P. Waboso, 2004.Age, symptoms and perception of menopause among Nigerian women. Obstet. Gynecol. Ind., 54(6): 575-578.

[16]. Razi AMB Zakariya, Kitab Al-Hawi Fit Tib (Urdu translation) Vol.9 CCRUM 2001; 153-154

[17]. Research on the menopause, 1981. Report of a WHO scientific group. World Health Organization Technical Report Series, Geneva, 670: 1-120.

[18]. Rushton, D.H. and J.H. Barth, 2010. What is the evidence for gender differences in ferritin and haemoglobin? Cr. Rev. Oncol. Haematol., 73(1): 1-9.

[19]. Rushton, D.H., R. Dover, A.W. Sainsbury, M.J. Norris,J.J.H. Gilkes and I.D. Ramsay, 2001. Why should women have lower reference limits for haemoglobin and ferritin concentrations than men? Br. Med. J., 322(7298): 1355-1357.

[20]. Shibata,T., Y. Ogushi, T. Ogawa and T. Kanno, 2005. Estimation of sex-age specific clinical reference ranges by nonlinear optimization method. Connecting medical informatics and Bio-Informatics:Proceedings of MIE2005 - The XIXth International congress of the European Feder. Med. Inf., 116: 229-234.

[21]. Thirup, P., 2003. Haematocrit: Within subject and seasonal variation. Sports Med., 33(3): 231 -243.

[22]. Valberg, L.S., J. Sorbie, J. Ludwig and O. Pelletier, 1976.Serum feritin and the iron status of Canadians.Canad. Med. Associat., 114(5): 417-421.

[23]. World Health Organization1996.Resaerch on the menopause in 1996.technical report scr 866, Geneva, Switzerland.

[24]. Shahid M., Naaz I., Fatima S., Ahmad T. 2016. Menopause and its Management in Unani System of Medicine- An Update ; Int. J. of Institutional Pharmacy & Life Sciences 6(4): 1-9

[25]. Unni J. 2010. Third consensus meeting of Indian Menopause Society (2008): A summary. J Midlife Health. 1(1): 43–47. doi: 10.4103/0976-7800.66987. PMC3139265.