IV. DISCUSSION

Menstruation is an integral, prime biological signal noticed as a biomarker event to confirm gestation in a woman’s fertile age. It is the opening ceremony of the reproductive age and an eye opener discerning the reproductive health of women. This cyclical event decides the continuation of generations in menstrual animals. The integration of hormonal, neural and endometrial factors designs the fate and type of menstrual cycles.

Menses

The female human menstrual cycle represents a complex interplay of hormones (Fritz MC., and Speroff L. 1983). Menstruation is an integral part of the female reproductive age which starts functioning when a girl reaches sexual maturity during puberty. The endocrine system controlled menstrual cycle consists of three phases (viz) the follicular phase, ovulatory phase and luteal phase (Greenberg Jerrold S., et. al., 2007).

The median healthy menstrual cycle length is 28±3 days and the average duration of menstrual flow is 5±2 days with a blood loss averaging 130ml. A complex interaction is involved between the hypothalamus, pituitary and ovary for the successful completion of this rhythmic event.

This cyclical process which requires clear communication between the participating glands is regulated in part by complex changes in the concentration of five hormones: gonadotropin releasing hormone (GnRH), follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E) and progesterone (P). The interplay of the hormones is highly complicated with the steroid hormones (E and P), exerting both negative and positive feed back effects on gonadotropin secretion (LH and FSH).
The release of LH and FSH from the pituitary is dependent on the secretion of GnRH from the hypothalamus which is modulated by the feedback effects of E and P. LH and FSH in turn, are important in stimulating secretion of E and P. Virtually all hormones are released in short bursts or pulses at intervals of 1-3 hrs, so constant levels are not observed in the circulation. The frequency and amplitude of the pulses are modulated by steroid hormones and vary throughout the cycle (www.acudoc.com/Healthy%20Cycle.PDF).

**Phases of the menstrual cycle**

Each month the reproductive system repeats a regular pattern of events controlled by hormones. The menstrual cycle is defined as the time from the first day of a woman’s period to the first day of her next period. Menstrual cycle comes as a routine event but it necessarily takes place once a month. The average cycle time for women is 28 days, but it may last from 21-35 days and still be normal.

**The menstrual phase (menstruation)**

The menstrual phase is a woman’s monthly bleeding, commonly referred to as a period.

**The follicular phase**

During this phase the combined action of follicle stimulating hormone (FSH), estrogen and LH decides the process ‘ovulation’ (i.e.) the release of an egg either on the right or left side of the ovary. In woman with regular 28 day menstrual cycles, ovulation usually occurs on day 14.

**The luteal phase**

After ovulation, the follicle becomes a hormone-producing structure called the corpus luteum synthesizing estrogen and large amounts of progesterone, which is essential for the implantation of a
fertilized egg. Absence of pregnancy results in degeneration of corpus luteum about two weeks after ovulation followed by a drop in the level of progesterone promoting the starting of another cycle.

During this phase of the menstrual cycle, if there is pregnancy, the egg moves into the uterus and attaches to the lining and absence of pregnancy favours the lining of the uterus shed through the vaginal opening. Then a new menstrual cycle begins (www.always.com/en-us/lifestyle/every-woman/pad-menstrual-cycle/phases-of-menstrual-cycle.aspx).

**Reproductive hormones and menses**

A normal, natural and monthly menstrual cycle is only possible by the successive positive and negative co-ordination existing between the reproductive hormones. During the first 14 days of the (follicular phase) menstrual cycle, estrogen is high and progesterone is low (www.netnutritionist.com/18.htm), and a decrease of estradiol and progesterone is noticed in the late luteal phase (Welt CK., *et. al.*, 2003).

In most women, the LH pulse amplitude begins to increase after ovulation takes place (Reame N., *et. al.*, 1984) and reaches its peak during ovulation (Pauerstein CJ., *et. al.*, 1978).

One study shows that LH is low during the early follicular phase and begins to rise by the mid-follicular phase due to the +ve feedback from the rising estrogen levels (Young JR and Jaffe RB. 1976).

It is evident from a study that an increase of FSH is noticed during the last few days of the menstrual cycle (Groome NP., *et. al.*, 1996). Reports say FSH levels begin to decline after menses due to the negative feedback of estrogen (Groome NP., *et. al.*, 1996; Tsafiri A. 1994; Sawetawan C., *et. al.*, 1996; Welt CK., *et. al.*, 1997). Our study also corroborates with these results. The Menstrual phase specific increase or
decrease of reproductive hormones such as estrogen, progesterone, LH and FSH is noticed in our study subjects.

Studies show that the length and regularity of menstrual cycles reflect changes in ovarian steroid production (Kato I., et. al., 1999; Harlow SD and Ephross SA. 1995). Our study too supports this view.

One report says there is a significant correlation between testosterone and menstrual irregularities (Van Anders SM and Watson NV. 2006). Our study too supports this view. It is well known from a study that highly elevated levels of prolactin decrease the levels of estrogen in women (Mann WA. 2011). Studies reveal that there is a higher level of prolactin during the luteal phase than the early follicular phase (Vekesmans M., et. al., 1997; Cole EN., et. al., 1977; Sheth NA., et. al., 1975). Similar results are also seen in our study.

**Normal menstrual cycle**

The length of a menstrual cycle is the number of days between the first day of menstrual bleeding of one cycle to the onset of menses of the next cycle. The median duration of a normal menstrual cycle is 28 days with most cycle lengths between 25-30 days (Treloar AE., et. al., 1967; Presser HB. 1974). A highly significant (P<.001) menarche age Vs duration of menstrual cycles are noticed in our study subjects.

Patients who experience menstrual cycles that occur at intervals less than 21 days are called polymenorrheic and prolonged cycles with more than 35 days are called oligomenorrheic. The typical volume of blood lost during menstruation is approximately 30ml (Hallberg A. et. al., 1966). Any amount >80ml is considered as abnormal (Hallberg A. et. al., 1966). The menstrual cycle is typically most irregular around the extremes of reproductive life (menarche and menopause) due to anovulation and inadequate follicular development (Lenton A., et. al., 1984; Fraser IS., et.
al., 1973; Apter D., et. al., 1987). The percentage prevalence of irregular cycles (polymenorrheic and oligomenorrheic) in our pre-teen menarche subjects are 7.32 while it is 11.99 in teenage menarche girls.

One study reveals that the variability of menstrual cycle length is highest for women under 25yrs of age and is lowest (i.e.) most regular for ages 35-39 yrs, subsequently the variability increases slightly for women aged 40-44 yrs (Chiazze Jr.L., et. al., 1968). Another study indicates that the length variation between 8 and 20 days in women is considered as moderately irregular menstrual cycles and 21 days or more is considered as very irregular cycles (John K. and Kippley S. 1996). Our study subjects (5000 cases) fall under the age 17-22 yrs and the mean age of the population is 18.89±1.57 yrs with 6.46% cases having irregular and 93.54% show regular cycles.

Studies suggest that breast cancer risk is directly related to the cumulative number of regular ovulatory cycles. Regular vigorous physical activity is one method of reducing the frequency of ovulatory cycles and such exercise could markedly reduce a woman’s lifetime risk of developing breast cancer (Henderson BE., et. al., 1985; Berstein L., et. al., 1987).

A positive association between breast cancer risk and the cumulative number of ovulatory menstrual cycles a woman has experienced is reported by many researchers (Mac Mahon B., et. al., 1982; Vihko R. and Apter D. 1984; Henderson BE., et. al., 1985; La Vecchia C., et. al., 1985). Report says women who report lifelong patterns of very short or very long cycles may have decreased risk of breast cancer (La Vecchia C., et. al., 1985; Henderson BE., et. al., 1985).

**Factors influencing menarche age at menarche**

In western societies the onset of puberty and the age at menarche had been declining from the 19th to 21st centuries with a rate of

In developing countries, the age of both onset of puberty and menarche continues to decline (Malina RM., *et. al.*, 2004; Rao S., *et. al.*, 1998; Huen KF., *et. al.*, 1997) and India is not an exemption to this change. One report says the mean menarcheal ages of the mothers are higher than their daughters (Ersoy B., *et. al.*, 2005). Our study too supports this view.

Earlier studies reveal that there is a slight difference in the mean menarche age in girls (Khanna A., *et. al.*, 2005; Dasgupta A. and Sarkar M. 2008; Amita Singh., *et. al.*, 2008; Khadilkar VV., *et. al.*, 2006; Chumlea WC., *et. al.*, 2003; Demir SC., *et. al.*, 2000; Chavarro J., *et. al.*, 2004). In the present study the mean menarche age of the study subjects is 13.14±1.8 yrs, while it is 14.48±1.4 yrs in their mothers (Anushiya Devi K. and Murugan A. 2012).

The mean menarcheal age of our study subjects’ mothers is higher than the mean menarcheal age of the girls (P<0.001). Two way ANOVA test further confirms that there is a significant difference among the mothers’ and the subjects’ menarche age (P<0.001). Linear regression model against subjects’ and their mothers’ menarche age has clearly proved that subjects’ menarche age is greater than their mothers’ menarche age.

Menarche is one of the most important biological signals in the life of a woman. Menarche is a biological variable which is of multifactorial in
nature. The differences in the menarcheal ages in the subjects and their mothers persisted independent of the socio-economic status, nutritional state and physical activity of the individuals.

The study further confirmed that the study subjects’ and their mothers’, study subjects’ and their first sisters’ and study subjects’ and their second sisters’ menstrual cycles are totally independent (P>0.05), hence of null hypothesis is fully accepted in these cases.

Age at menarche serves both as an indicator of ovarian function onset and a predictor of ovulatory frequency. A relatively early or late age at menarche can be used in part, to predict future disease risk. For (e.g) each 1 yr delay in the age at menarche is associated with a 5% decreased risk of breast cancer (Kelsey JL., et. al., 1993).

**Heredity and menarche**

Comparison of the menarcheal age of mothers and their daughters are complicated, since the data on the two generations are not really comparable. Popenoe P., (1928) found a correlation of (r) 0.4±0.03 between mothers’ and daughters’ menarcheal ages. Bolk L. (1923) reported that, daughters’ menarcheal age is comparably lower than their mothers. In our study the mean menarcheal age of the study subjects is 13.14 ± 1.18 yrs, while it is 14.48 ± 1.4 yrs in their mothers.

**Socio-economic factors and menarche**

Socio-economic level is compounded of many factors including nutrition, public and individual health, family size, urban or rural living.

1) **Diet**: Age at menarche is an important event which is related to the reproductive maturity of a woman and modified by determinants like diet (www.womenshealth.gov//publications/our-publications/factsheet/menstruation.cmf#f). Nutrition has always been considered as a
major influential factor in pubertal growth period. Several studies have been attempted to trace the relationship of diet and onset of menarche.

Several early studies suggested that meat or protein intake had a menarche promoting effect. Previous studies emphasize the relationship between non-vegetarian diet and the early onset of menarche in girls (Kralj-Cercek L. 1956; Padmavati V., et. al., 1984; Shastree US., et. al., 1974).

Vegetarianism (Sanchez A., et. al., 1981) and higher consumption of meat analogues or nuts and beans (Kissing er D., and Sanchez A., 1987) to be associated with delayed menarche (Kus Y., et. al., 2006). In the present study a positive correlation \( (r=1) \) is noticed between our pre-teen and teen menarche subjects of vegans and mixed diet users. It is frequently suggested that vegetarian diet is associated with menstrual disturbances (Bakan R., et. al., 1993; Brooks SM., et. al., 1984; Pirke KM., et. al., 1986; Pederson AB., et. al., 1991; Slavin J., et. al., 1984). It is true in our study also.

2) Economic status and habitation in menarche

In the modern world a positive impact of socio-economic status on early menarche in girls is reported by many researchers (Wronga I., et. al., 2005; Ersoy B., et. al., 2005; Chavarro J., et. al., 2004; Attallah NL. 1998). Reports say girls from families with high socio-economic status experience menarche at an earlier age than girls from families with lower economic status (Wronga I., and Pawlinska-Chamara R. 2005; Bielicki T., et. al., 1986; Adadevoh SW., et. al., 1989; Hughes RE., and Jones E. 1985; ICMR. 1972; Bai KL., and Vijayalakshmi B. 1973; Eiben O. 1972; Rao S., et. al., 1998). A positive correlation \( (r = 1.0) \) is noticed between our pre-teen and teen menarche subjects in different socio-economic categories.
Better nutrition, improved hygiene, increased social stimulation or sexual stimulation and other factors influence the urban residents to predispose early maturation. The impact of urbanisation on early menarche is reported in some studies (Wronga I., and Pawlinska-Chamara R. 2005; Adadevoh SW., et. al., 1989; Wilson DC. and Sutherland I. 1955; Madhavan S. 1965; Anushiy Devi K., and Murugan A. 2012). In the present study our urban subjects are with lowest mean menarcheal age (12.9 yrs) than the semi-urban (13.02 yrs) and rural girls (13.5 yrs). A strong positive association is seen between our pre-teen and teen menarche subjects with urban, semiurban and rural backgrounds (r = 0.95).

**Anthropometric measures and the onset of menarche**

Anthropometric measures such as bodymass index (BMI), and waist-hip ratio (WHR) show a profound effect on the early onset of menarche in girls. Persons with a value of BMI ≥ 30 or WHR ≥ 0.86 is considered as obese. Obesity is thought to be the mother of all sorts of health related issues in man.

Studies reveal that obesity has been found to be associated with precocious menarche in girls (Shuttleworth FK. 1937; Mc Neil D. and Livson N. 1963). Reports say the greater body weight is associated with a greater likelihood of early menstruation (Soriguer FJ., et. al., 1995; Petridou E., et. al., 1996; Meyer F. et. al., 1990). One report says there is an inverse association between age at menarche and obesity or over weight (Al-Awadhi N., et al., 2013).

Huen KF., et.al. 1997 reported that BMI is an important factor in triggering menarche. Reports say BMI is found to be one of the most important predictors of early onset of menarche in girls (Ersoy B., et. al., 2004; Petridou E., et. al., 1996; Meyer F., et. al., 1990). In the present study correlation for pre-teen BMI Vs teen BMI menarcheal subjects is
0.96. A positive association between pre-teen WHR Vs teen WHR menarcheal subjects \((r = 0.87)\) is found in our study.

**Blood groups and age of menarche**

Reports on blood groups and age of menarche are very rare to elucidate its role. However, one earlier report shows a positive association between blood groups and menarche age (Jean Grant. 1956). A strong association \((r = 0.94)\) is found between our pre-teen and teen menarche subjects and ABO blood groups. The study further emphasize that, irrespective of the Rh groups (i.e) either +ve (or) -ve group about two third of the subjects have attained menarche in their teenage period.

**Menstrual disorders**

Menstrual patterns are influenced by a number of host and environmental characteristics. Even slight increases in BMI have observable impacts on menstrual patterns. Stress or physical activity both of which are important risk factors for menstrual cycle irregularities (Harlow SD. 2000; Harlow SD., and Ephros SA. 1995; Harlow SD and Matanowski GM. 1991; Fenster L., et. al., 1999).

The menstrual cycle is a hormonally controlled process, although several factors may influence its length and regularity (Harlow SD., and Ephross SA. 1995). BMI and physical activity were constantly shown to be associated with menstrual function (Sterfeld B., et. al., 2002; Harlow SD., and Ephross SA. 1995). One study shows that cycle length has been negatively associated with age because of shortening of follicular phase (Harlow SD., and Ephross SA. 1995). Another study demonstrated that over weight is associated with the possibility of long cycles in college women (Harlow SD., and Matonoski GM. 1991).

Menstrual disorders and abnormal uterine bleeding or menorrhagia are among the most frequent gynecologic complaints of the
adolescent (Caufrirz A., 1991; Deligeoroglou E., 2006). Menorrhagia is defined as blood loss over 80ml during a period and it is a very common gynecological problem in adolescence (Anne CF., 1981; Albert A., 1977). In the present study around 1.42% subjects experience menorrhagia.

Dysmenorrhea refers to the syndrome of painful menstruation that interferes with daily activities of reproductively active women. The prevalence in adolescent females is estimated to be 67.2% in one study (Sharma P., et. al., 2008) and up to 90% in adolescents and 25% in women is reported by another study (Durain D. 2004).

Lee LK., et. al., (2006) explained that menstrual disorders are common presentation by late adolescence and 75% of girls experience some problems associated with menstruation. French L (2005) reported that primary dysmenorrhea is a painful menses in women with normal pelvic anatomy usually beginning during their adolescence. The prevalence of dysmenorrhea is found as 27.24% in our subjects. Hypermenorrhagia, oligomenorrhea, polymenorrhea, amenorrhea and spotting are the other types of menstrual disorders, are seen in minor levels in our study subjects.

Eumenorrhea, the normal regular menstruation usually lasts for 3-5 days (www.4woman.gov/faq/menstru.htm). A great majority of our subjects (i.e) 68.5% are with normal cycles. Menorrhagia or hypermenorrhea denotes prolonged menstrual period at regular intervals lasting longer than 7 days (www.en.wikipedia.org/wiki/Hypermenorrhea). Hypomenorrhea is a regular normal short cycle lasting for less than 3 days (www.en.wikipedia.org/wiki/Hypomenorrhea). 28.62% and 2.88% subjects in the present study show long and short cycles respectively.

**Premenstrual syndrome (PMS) or perimenstrual syndrome**

PMS, a common cyclic disorder of young and middle aged women, is characterized by emotional and physical symptoms that consistently
occur during the luteal phase of menstrual cycle (Lori MD., et. al., 2003). PMS is a physical, cognitive and behavioural symptoms that occur cyclically during the luteal phase of the menstrual cycle and resolve quickly at or within a few days of the onset of menstruation (Braverman PK. 2007). It is experienced by upto 90% of women of child bearing age (Mishell DR. Jr., 2005).

The prevalence of PMS or premenstrual tension (PMT) is 37.2% (Delara M., et. al., 2012), 61.4% (Derman O., et. al., 2004), 76% (Lee AM., et. al., 2005), 35% (Serfaty D., and Magneron AC. 1997), 19-30% (Dean BB., et. al., 2006), 85% (Steiner M., and Born L. 2000), 69.6% (El-Defrawi MH., et. al., 1990) and 96.6% (Rasheed P., and Al-Sowilem LS. 2003). A moderate to very high level of incidence of PMS is reported by the above mentioned authors. In the present study it is around 13.3%.

Studies reveal that PMS symptoms are more intense in 16-18 yrs (Cleckner-Smith CS., et. al., 1998) and 18-20 yrs (Bhakshani N., et. al., 2009) age groups. It is true in our study also. Reports say abdominal bloating is found as the most frequently reported symptom (Braverman PK. 2007, Khella AK. 1992) and stress and nervousness are observed as the most common symptoms (Derman O., et. al., 2004). Physical symptoms, mood disturbances and behavioural problems are the frequently noticed PMS in our subjects.

Reports say severe degree of PMS leads to physical impairment such as impairment of daily activities (Dean BB., et. al., 2006, Antai B., et. al., 2004), academic absence and low achievement (Montero P., et. al., 1999, Tenkir A., et. al., 2003) and severe mental distress associated with greater burden on mental and physical health (Yang M., et. al., 2008).

**Premenstrual dysphoric disorder (PMDD)**

PMDD consists of symptoms of similar to but more severe than PMS and primarily mood related may include physical symptoms such as
bloating. It is classified as a repeating transitory cyclic disorder with similarities to unipolar depression and several antidepressants are approved as therapy (www.dsm5.org/Proposed Revision/Pages/proposed revision.aspx).

PMDD is cycle related problem which is common in almost all women in their fertile age (Moline ML and Zendell SM. 2000; Vigod SN. 2009). It is due to lack of serotonin, a neurotransmitter and mediated by the fluctuations of the levels of sex hormones (progesterone, estrogen and testosterone) in the luteal phase of menstrual cycle (Eriksson O., et. al., 2006).

Reports say the incidence of PMDD is 37.2% (Delara M., et. al., 2012), 50.2% (MC Hichialami KH., et. al., 2002) and 13-18% (Halbreich U., et. al., 2003) in women. The percentage prevalence of PMDD in our subjects is about 23.24% (Anushiya Devi K., and Murugan A. 2012).

PMDD is a medical condition in which a woman has severe depression symptoms, irritability and tension before menstruation (Vigod SN. 2009). Depression, anxiety and physical symptoms are the major form of PMDD noticed in our cases. PMS and PMDD are related to poor quality of life (Delara M., et. al., 2002). Both PMS and PMDD evoke severe degree of physical impairments in menstruating women.

**Biomarkers and menstrual cycle**

A biomarker or biological marker generally refers to a measured characteristic which may be used as an indicator of biological state or condition. Biomarkers are used in many scientific fields, they are often measured and evaluated to examine normal biological process (www.en.wikipedia.org/wiki/Biomarker).

The cardiovascular biomarker blood pressure is more during menstruation than at most other phases of the cycle (Dunne FP., et. al.,
Lakshman R., et. al., (2009) and Hartge P. (2009) reported that early age at menarche is associated with increased risks of adverse health outcomes such as breast, ovarian and endometrial cancer, hypertension, Type-2 diabetes, and cardiovascular disease. Our study partially agrees with these findings, and only 43% early menarche subjects are having hypertension.

Profound physiological changes occur in females during the monthly menstrual cycle (Speroh L., and Vande Wiele RL. 1971). These include changes in blood pressure (Korubo Owiye T. and Uzor GC. 1999), white cell count (Alizadeh SA., et. al., 2012, Mathur J., et. al., 1979; Rutter SM., and Peterson CM. 1994), no differences in RBC count (Makinoda S., et. al., 1996) and erythrocyte sedimentation rate (Dapper DV., and Didia BC. 2002; Alizadeh SA., et. al., 2012).

Inflammatory markers such as leucocyte, ESR are measured and compared using student – t test on different phases of menstrual cycle. Our findings show that there are significant differences in the inflammatory markers in healthy college girls during the different phases of menstrual cycle. Raised ESR could be associated with anemia.

A prothrombin time test measures how long it takes for a clot to form in a blood sample. One study says around 20 – 30% adolescent girls are having major bleeding diathies (Classens EA., and Cowell CA., 1981). The present study shows a significant difference in PT is noticed in menstrual phase when compared to other phases of the menstrual cycle.

Besides heavy menstrual blood loss, nutritional deficiencies or imbalances in Fe (iron), Zn (zinc) and Vit-B12 contribute greatly to anaemia in females. Subclinical iron-deficiency anemia occurs when iron stores are depleted while Hb levels remain within normal range (www.irondisorders.org/women). One study explains menorrhagia are more to iron deficiency than the average person (Harvey LJ., et. al., 2005).
Ferritin has been known as an index for body iron stores and also as an inflammatory marker. It serves to store iron in a non toxic form to deposit it in a safe form, and to transport it to areas where it is required (Seckback J. 1982). The present study reveals elevated levels of Hb and ferritin in ovulatory and luteal phases compared to menstrual phase of the subjects. Monitoring serum Hb, and ferritin values and diet to assure that the right balance of iron is maintained, thereby anemia can be prevented.

Immunoglobulin G antibodies are found in all body fluids. It is an antibody created by the immune system to help fight infection and disease. Approximately 75% of Igs in normal person’s immune system are molecules of IgG. One study shows that menstrual cycle had no effect on immunoglobulin concentrations in serum (Lu FX., et. al., 1999). Franklin RD., and Kutteh WH. 1999 reported that an increasing level of immunoglobulins are associated with the increasing levels of estradiol. Our results contradict with Franklin RD., et. al., 1999 and support the findings of Lu FX. et.al. 1999.

Apseloff G., et.al. (2000) reported that a significant level of increase of total protein is observed in luteal phase compared to follicular phase. A marked level of increase of serum albumin in proliferative and luteal phase is reported by Kim I., et.al. (1993). Malipatil BS., and Shilpapatil (2013) report the same findings and explained that changes in serum total protein and albumin may be due to the influence of progesterone as a protein anabolic effect on synthetic mechanism in liver. Our result contradicts these findings and there is no noticeable level of increase of serum total protein, albumin and globulin.

Microalbuminuria, an urinary marker is closely associated with hyperglycemia, obesity and hypertension (Yang X., et. al., 2008). Our results did not find any significant differences in the levels of
microalbuminuria in different phases of menstrual cycle when compared to the menstrual phase.

Studies on serum electrolytes level in the various phases of menstrual cycle are very rare. Reports say there is a definite relationship between ovarian hormone activity and the blood constituents especially calcium, magnesium and inorganic phosphorus during various phases of menstruation (Christiansen C., and Riis BJ. 1990; Czaja JA. 1978).

A low level of serum calcium is noticed in menstrual phase compared to pre and ovulatory phase (Tsai PS., and Yucha CB. 1991) and a noticeable level of low sodium in menstrual phase compared to the other phases (Pandya AK., et. al., 1995; Mira M., et. al., 1984). Our study too supports this view.

One report says the elevated level of serum magnesium in proliferative phase compared to other phases may be due to high estrogen (Pandya AK., et. al., 1995). It is true in our study too.

Magnesium deficiency leads to a few of the unpleasant symptoms of PMS like head-ache, bloating, irritability, anxiety, fatigue, insomnia, palpitation, constipation, migraine, asthma, diabetes, obesity, osteoporosis, high BP, menstrual cramps etc., which affects millions of women every month (Tong GM., and Rude RK. 2005; Johnson S. 2001). Magnesium supplements such as calcium, zinc, Vit – B6 will solve PMS at a greater extent (www.mg12.info/magnesium-in-iochemistry/magnesium-and-women-html).

Estrogen production is essential for the production of serotonin which decides the mood disorders. A brain chemical serotonin (neurotransmitter) influences women’s moods, playing a big role in anxiety, depression and obsessional states. Estrogen is responsible for maintaining the orderly firings in the brain of a number of
neurotransmitters – among them are dopamine, acetylcholine, nor-
epinephrine and serotonin.

The impact of both estrogen and progesterone creates changes in
the brain that can lead to disturbed mood. Estrogen – serotonin are the
two critical vital components to sustain mental health in women. Estrogen
can be thought of as the body’s own antidepressant and mood stabilizer.
When estrogen levels rise, serotonin levels rise too and mood improves.
When estrogen level drops (and along with it serotonin) the reverse
happens, mood becomes affected negatively (www.womens-wellbeing-and-
mental-health.com/anxiety_therapy_new_york_city.html).

When estrogen level is elevated, serotonin is elevated and life is
smooth. The final 14 days make up the luteal phase when progesterone is
elevated. When progesterone is high serotonin decreases, endorphin and
dopamine levels drop. When these brain chemicals are in low supply
mood and appetite control are affected (www.netnutritionist.com/18.htm).
Serotonin is probably thought to be a contributor to feelings of well being
and happiness (Young SN. 2007). In the present study a significant level
of increase of serotonin is noticed in preovulatory, ovulatory and luteal
phases compared to the menstrual phase.

Lead produces various health effects including reproductive
problems. Reports say environmental exposure to lead may delay growth
and pubertal development in girls (Selevan SG., et. al., 2003; Wu T., et.
al., 2003). Studies have shown that blood lead levels may increase as
estrogen levels decrease and more amount of lead is eliminated during
menses (Yang YH., et. al., 2007; Yang Y. 2007). Our study fully supports
this view. There is a remarkable level of increase of blood lead
concentrations in various phases compared to the menstrual phase of our
respondents.
One recent study shows a positive association between endogenous estrogen with total cholesterol and high density lipoprotein cholesterol and an inverse association with low density lipoprotein cholesterol (Mumford SL., et. al., 2010). It is true in our study also.

Lipoprotein cholesterol levels varied across the menstrual cycle in response to changing reproductive hormone levels. TC and LDL-C tend to be highest during the follicular phase and decline during the luteal phase. HDL-C is most often highest during the late follicular and periovulatory phases (Mumford SL., et. al., 2011). Our study also corroborates these findings and a significant level of variations is found between menstrual and other phases and it is verified by student – t test. There is a highly significant level of variation in Tg in preovulatory phase, a marginal level of increase in ovulatory and a decline in luteal phase compared to menstrual phase.

Thyroid dysfunction is associated with a range of menstrual abnormalities including oligomenorrhea, amenorrhea and menorrhagia. The connection between thyroid hormone levels and the menstrual cycle is mainly mediated by thyrotropin releasing hormone (TRH) which has a direct effect on the ovary. Increased levels of TRH may raise prolactin levels, contributing to the amenorrhea associated with hypothyroidism (Speroff L., and Fritz MA. 2005).

Weeke J., and Hansen AP (1975) reported that TSH and fT3 were found to be unchanged through out the normal menstrual cycles. Our results not support this view. There is a noticeable level of increase and a remarkable level of decrease of TSH in both of our preteen and teen menarche subjects’ ovulatory and luteal phase compared to the menstrual phase.

A narrow level of decrease of fT3 is seen in ovulatory and luteal phase of our subjects with respect to their menstrual phase. There is a
continuous decrease of fT4 in ovulatory and luteal phase of our subjects with respect to the menstrual phase.

CA-125 (cancer antigen-125) (or) Carbohydrate antigen – 125 also known as mucin – 16 (or) MUC – 16 is a protein that in humans is encoded by the MUC – 16 gene (Yin BW., et. al., 2002). It is the most frequently used biomarker for ovarian cancer detection (Suh KS., et. al., 2010). Studies reveal that the serum CA-125 levels are significantly elevated during menstruation when compared to other phases (Lehtovitra P., et. al., 1990; Oshaughnessy A., et. al., 1993; Grover S. et. al., 1992). Our results show there is a slight increase of CA-125 in preovulatory and simultaneous decrease in ovulatory and luteal phases, but the values are within the reference range.

Lactate dehydrogenase (LDH) is a ubiquitous cytosolic enzyme present in all tissues (Maekawa M. 1988). Routine serum measurement of LDH is of clinical use in the diagnosis and monitoring of certain diseases including cancer (Iglesias J., et. al., 1988). There is no significant difference in LDH level in different phases of the cycle when compared to the menstrual phase and the LDH concentrations are within the normal range.

Pseudocholinesterase (or) plasma cholinesterase (or) butrylcholinesterase is found primarily in liver. One report says there is an elevation of plasma cholinesterase in 90.5% of cases with acute myocardial infarction (Chatterjea MN., and Rana Shinde. 2005). Giannini AJ., et. al. (1985) reported that, it is a possible marker trait for anxiety and found elevated in severe PMS cases than in mild PMS subjects. There is no noticeable level of differences in preovulatory, ovulatory and luteal phase of our subjects when compared to the menstrual phase.
Interpretation of anthropometric measures by Computer Simulation Models

The output of the linear model is calculated in ‘R’ using response variable subjects’ menarche age the explanatory variables mothers’ menarche age, body mass index and waist hip ratio indicates that there is a positive relationship between the subjects’ menarche age, mothers’ menarche age and waist hip ratio. We also found that there is a negative relationship between subjects’ menarche age and body mass index. The output from the linear model calculation in R can be represented using Ordinary Least Square Regression (OLS) method as follows.

Subject’s Menarche Age = 10.08 -0.007*Body Mass Index + 2.714*Waist Hip Ratio + 0.04*Mothers Menarche Age

In statistics, OLS is mainly used to demonstrate the relationship between response and explanatory variables with the error values. The formula for OLS is \( Y = a + \beta_1X_1 +, \beta_2X_2 +, \beta_3X_3... \beta_nX_n \). In our case the value \( a \) is 10.08, which indicates the menarche age of the subject when all of our explanatory variables are zero. Prediction of menarche age with all the response variables equal to zero is having limited usefulness. The values \( \beta_1, \beta_2, \beta_3 \) indicates the average change in menarche age of the study subjects associate with the each unit increase in explanatory variable \( X_1 \) (Body Mass Index), \( X_2 \) (Waist Hip Ratio), \( X_3 \) (Mothers Menarche Age) respectively.

The graphical representation of the linear model, shows that the data set is normally distributed. The line of best fit on the Q-Q plot is varying slightly from 45 degree. The Kernel density estimate represents a good estimate of population density and how much it varies from the average.

Confidence interval for the linear model is around 97.5 %. For a given statistic calculated for a sample of observations (e.g. the mean), the confidence interval is a range of values around that statistic that are
believed to contain, with a certain probability (e.g. 95%), the true value of that statistic (i.e. the population value).

The Durbin Watson Test value for the linear model is 1.88756, which indicates that the residuals are non-auto correlated to each other. A value of 0 indicates there is a strong positive correlation and a value of 4 indicates there is a strong negative correlation. The results also highlight that the alternative hypothesis is rejected.

Both Confidence Interval and Durbin Watson Test indicates the selected linear model might fit to the sample data and can be used to calculate the menarche age of any study subject. On the other hand the output from R’s gvlma which is used to validate the assumptions of the linear model indicates the selected model might not be an accurate fit. But the assumption validations indicates a linearity of relationship between the variables selected are acceptable.

One of the constraints of the linear model is, it always assumes all the variables used in the model are having fixed effects. Ignoring the random effects in any model might lead to incorrect assumptions and it is also not accurate to ignore random effects. In the present study three random variables are selected (CASTE, RELIGION, and COMMUNITY) and introduced one by one to analyze the statistical model using Generalized Linear Mixed Model. The output of glmer function from R using CASTE as a random effect is widely used for Generalized Linear Mixed Model calculations. It shows that the variance and standard deviation are zero (ie) CASTE alone does not have any impact on the relationship of the response and explanatory variables. The results are same after including COMMUNITY. But we experience a slight impact after including RELIGION as a random variable.

The present population based study is to try to identify and analyse the related causes of age at onset of menarche and menstrual cycle
characteristics in college girls by proper ‘statistical tools’, and ‘computer simulation models’. Computer simulation on menarche data shows that not all the anthropometric measures are having the same impact on this biological event.

Since the age at onset of menarche and menstrual cycle disorders are multifactorial in nature, large scale studies are unavailable to solve the unsolved facts about the biomarker events in the reproductive age of women.