ISSN (Print) : 0974-6846
Indian Journal of Science and Technology, *Vol 8(15), 55224, July 2015* ISSN (Online) : 0974-5645

Association between Primary Amenorrhea and Early Maternal Age: A Population Study

Poulami Majumder[1*], Sujoy Ghosh[2] and Subrata Kumar Dey[1]

[1]Department of Biotechnology, Centre for Genetic Studies, School of Biotechnology and Biological Sciences, West Bengal University of Technology, BF-142, Sector I, Salt Lake City, Kolkata, West Bengal 70064, India; plm.majumder@gmail.com

[2]Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Kolkata, West Bengal 700019, India; g_sujoy@yahoo.com, skd.humgenet@gmail.com

Abstract

Primary Amenorrhea (PA) is one of the most common reproductive disorders found in women can be characterized by the absence of menstruation cycle during puberty as well as throughout life that lead to infertility. The major cause of PA includes hypothalamic disorder and ovarian disorder. In this study a promising association between early maternal age and PA is found. This present study was carried out to perceive the effect of early maternal age (age of conception) in the etiology of PA. A population based study of approximately 993 PA cases and 1027 controls have been investigated in our laboratory (from 2004 and 2013). PA cases born to mothers of age less than 20 years are 71.70% and the mean age of mother is 16.15 years. A highly significant association of primary amenorrhea patient with the younger mother (≤ 20 years age) (OR = 5.51; 95% CI = 4.47- 6.79; $p < 0.0001$) has been found. On the other hand, association with older women is not significant (≥ 30 years) (OR = 1.17; 95% CI = 0.86-1.61; $p < 0.315$). Unpaired t-test analysis has shown statistical significant impact of younger maternal age on PA occurrence. The partial correlation of coefficient between maternal age and PA occurrence while keeping paternal age constant is 0.593. Statistical assessment shows a significant association between younger mother and daughter facing primary amenorrhea at puberty. There are different causes of PA which have been revealedbut the allianceof early maternal age with PA may be highlighted as the etiology of PA.

Keywords: Etiology, Hypothalamic, Infertility, Maternal Age, Menstruation Cycle, Primary Amenorrhea, Puberty, T-Test

1. Introduction

Menstruation is the result of progression of reproductive events which marks the onset of sexual maturity and ovulation among women. Maturation of hypothalamus initiates a cascade of events resulting in occurrence of normal menstrual cycle[1]. The sequential molecular mechanism of maturation maintains a functional axis involving hypothalamus, anterior pituitary, ovary and reproductive tract. Amenorrhea refers the absence of menstruation due to break up in one or more places in this axis or malfunction in any participating organ. Females suffer from amenorrhea exhibit some under developed secondary sexual characters which include ill developed breast, absent or sparse pubic hair, blind vagina[2]. Depending upon the degree of clinical manifestation, amenorrhea is of two types Primary Amenorrhea (PA) and Secondary Amenorrhea (SA). PA can be diagnosed if a patient has ordinary normal secondary sexual characteristics but no menarche by 16 years of age or if patient has no secondary sexual characteristics and no menarche; PA can be diagnosed as early as 14 years of age[2]. PA is one of the most common disorders seen gynecological problems in adolescent girls[3]. PA refers to the total absence of menstruation during puberty and beyond. The clinical findings of PA include the developmental impairments like mullerian agenesis or MRKH syndrome (Mayer-Rokitansky-Kuster-Hauser Syndrome), gonadal dysgenesis, streak gonad, vaginal atresia, hypoplastic uterus, imperforate hymen[4]. The release of the Gonadotropin Releasing Hormone (GnRH) regulates the activity of set of hormones collectively

* *Author for correspondence*

control the menstruation cycle. The impaired structure of reproductive tract causes hormonal imbalance like Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Gonadotropin Releasing Hormone (GnRH), prolactin, estrogen, progesterone and Thyroid Stimulating Hormone (TSH) secretions[5]. Hypothalamic amenorrhea is associated with primary amenorrhea as it is characterized by a condition in which menstruation stops for several months due to a problem involving the hypothalamus. The hypothalamus in the center of the brain controls the process of reproduction. It produces Gonadotropin Releasing Hormone (GnRH) that signals the production of other necessary hormones for the eggs to mature and successful ovulation such as Follicle Stimulating Hormone (FSH) and after ovulation, progesterone and Luteinizing Hormone (LH)[6]. Sometimes the hypothalamus stops producing GnRH, which in turn reduces the amount of other hormones produced (FSH, LH and estrogen). Ovulation and menstruation get stop resulting in infertility. Hence it's clearly assumed that hypothalamus plays a crucial role in PA. Corrine et al. found that the hypothalamic disruption may occur due to energy deficits that means the levels of the adipocyte-secreted hormone leptin[7]. Numerical and structural chromosomal aberrations have been recognized to be associated with PA. This includes aneuploidy such as Turner syndrome (2n =45, XO) and structural abnormalities like presence of a small size of Y chromosome (2n = 46, XY) results PA. Merin et al. suggests the mutations in some genes like FSHβ gene, FSHR gene, and LHR gene are associated with menstrual abnormality[8] though frequency of such incidence is low. Prevalence of spontaneous abortion in female with PA has also been reported in some literatures stating the premature ovarian failure as causative factor[9]. In 1942, Fuller Albright first described the Primary Ovarian Insufficiency (POI) in amenorrhea which is relevant to PA to some extent[10]. Stein et al. reveals the polycystic ovaries as a cause of PA[11]. The present study has been performed to determine as the etiologic factor responsible for PA on the basis of epidemiological data collected at the time of clinical and laboratory investigations. Owing to the fact that there is no significant report on maternal hereditary factor related or responsible for PA, this investigation has been carried out to understand the implication of maternal age on the origin of PA. Though there is no such significant work on hereditary status especially the maternal factor related or responsible for PA is available till date, we have tried out to understand the role of maternal age on the origin of PA. We have made a preliminary work to find out the association of PA occurrence to maternal age.

2. Material and Methods

2.1 Study Population

From the January 2004 to December 2013, approximately 1135 PA patients have been registered to our laboratory. On the basis of careful examinations and laboratory investigations the clinical status of PA among the patients was confirmed and included in our study. We exclude 101 cases due to lack of complete information regarding parental age data and other 41 cases due to insufficient information regarding hormonal status and clinical diagnosis. Rest 993 cases have been included in this study and the age range of those patients is 14-25 years. The chromosomal profile of PA cases is ascertained through karyotyping, the chromosomal analysis. During the data collection process, we found a large number of PA patients whose mothers' age during conception are mostly ≤ 20 years. To detect the effect of maternal age on PA, we have taken 1027 controls which we have identified and selected randomly from healthy woman population without any history of menarche or menstrual hazards. We have stratified three different maternal age groups: younger group ≤ 20years; middle age ≥ 21 to ≤ 29 years and the rest ≥ 30 years. Same public hospitals have been preferred for case and control selection to ensure maximum possible similarity in demographic distribution of both populations. Sincere care has been taken while selecting of control to maintain maximum similarity in girl's age, ethnicity, language, religions, maternal age and socio-economic status between cases and controls. Controls have been chosen to be approximately age-matched with cases. The epidemiological data have been recorded for all the participant families after obtaining consents in privacy manner. The questionnaire covers all the details about parental age at conception especially maternal age, pregnancy loss, life style, birth control preference, addictions and all other aspects related to reproductive health of patient's mother. The confidentiality of all information is maintained very carefully at our laboratory. The participating cohort sample consisting of Bengali-speaking families from West Bengal and most of them are from communities like Hindu and Muslim.

2.2 Clinical Examinations

All PA patients have been reported from hospitals

of Kolkata and its surrounding localities and their gynecological features got assessed by the concerned doctors. Their common clinical findings include MRKH syndrome, absence of axilliary and pubic hair, blind imperforated vagina and non-initiation of menstrual cycle yet. Their USG (Ultra-Sono Graphy) reports show different developmental obstructions like rudimentary uterus, hypoplastic ovaries, mullerian dysgenesis and streak gonad. Their breast development is not proper. Most of the cases the tanner stages are not clearly mentioned which caused us to exclude the data for breast development from our study. According to these clinical finding we separate them into three classes; Class A includes PA patients with MRKH syndrome, Class B refers Turner syndrome (both pure and mosaic Turner syndrome) and gonadal dysgenesis and Class C. Another group of PA patients neither have MRKH syndrome nor turner syndrome. The frequency of occurrence of those clinical conditions associated with PA is measured with those stratified maternal age groups to ascertain any acquaintance present in between these variables.

2.3 Karyotype Analysis

Karyotyping for all the patients has been carried out from lymphocyte culture in our lab by following conventional air drying method. We consider all chromosomal status found in PA patients revealed normal diploid count 2n = 46, XX; monosomy 2n = 45, XO and mosaicism (2n = 45, XO/ 46, XX). Only one (1) patient is reported with menstrual absence whose karyotyping analysis confirms the chromosomal profile of X chromosome with a small part of Y chromosome as well as whose mother's age of conception is 17 years. A molecular diagnosis is carried out to confirm the above state fact which concludes the frequent involvement of SRY gene in this case. As only one (1) PA patient shows the SRY involvement so we do not include that particular data in further calculation.

2.4 Hormonal Status

Plasma hormonal titre is important key indicator of PA. We use these hormonal data in our study to distinguish between effects of ovarian and hypothalamic-pituitary causes. We take titer data of three hormones; ovarian hormone, Gonadotropin Releasing Hormone (GnRH), Thyrotropin Releasing Hormone (TRH) against PA patients. Cytogenetically different PA patients show distinct range of hormonal level and we considered the mean range of those levels. It has been reported that cytogenetically different PA individuals show unusually different hormone levels we analyzed them for our study (Table 5). The hormonal range of all patients is taken according to the standard fixed by American Association of Clinical Endocrinologist and the data provided by NIH.

2.5 Statistical Analysis

We take on maternal age factor and the participating mothers of PA for epidemiological analysis into three groups: younger age group (≤ 20 years), normal age group (21-29 years) and older age group (≥ 30 years). We also stratify all possible cytogenetic status of PA patients against their maternal age groups. Maternal age of conception is considered as proxy for oocyte age, as direct estimation of the same has been beyond the scope of the present study. We design a case-control model to find out the association of maternal age with occurrence of PA of their daughters. We perform unpaired t-test to verify significant effect of early maternal age on PA occurrence statistically. Additionally we calculate partial correlation coefficient to find out the interaction of maternal age with PA cases. During this analysis we have considered paternal age and other external factors as constant.

3. Result

Marked variation has been noticed amongst the age of participating mother (age of conception of PA child). Maternal age is considered as the factor may affect the menstruation of their daughters. On the basis of ten years crude data, it is found that 71.7% PA patients to have their mothers with conception age of ≤ 20 years (Figure 1). We stratify the cases in seven different age groups to detect the maternal age distribution in respect to PA. Within 15 to 17 years of maternal age show the maximum girl child birth face PA during their puberty and the mean age of mother within this age group was 16.15 years in the PA cases (Figure 2). In case control analysis we find that ≤20 years age group is more likely to give birth of daughter having PA. The estimated odds ratio is significant for ≤ 20 years of maternal age (OR = 5.51, CI = 4.47-6.79; p>0.0001) compared to ≥ 30 years maternal age group (OR = 1.17, 0.86-1.61; p > 0.315). The partial correlation coefficient between young maternal age and PA cases is r=.593 which indicates the increased risk of PA occurrence to early aged mother. 16.15 years of mean age has been calculated in younger maternal age group which causes about 71.7%

of PA cases (Table 1). The unpaired t-test has revealed a significant association of PA occurrence with younger maternal age ($p<0.0001$) whether the other maternal age groups do not show any significance (Table 2). According to available clinical data, lower maternal age group shows the prevalence of those stratified classes are 67.13%, 53.44%, 70.51% respectively which is quite frequent than the other age group (Table 3). When a cytogenetic analysis of PA is carried put it is obtained that 77.26% of PA patients with normal diploid count (2n = 46, XX) belong to ≤ 20 years maternal age group in contrast of 13.47% PA cases towards ≥ 21 to ≤ 29 maternal age group and 9.27% cases in ≥ 30 years maternal age (Table 4). On the other hand 68.63% PA patients with Turner syndrome (2n =45, XO) and 63.15% with mosaicism (2n = 45, XO/ 46, XX) also belong to young maternal age group where 17.28% cases of monosomy and 15.79% cases of mosaicisms undergo the ≥ 21 to ≤ 29 maternal age group. In ≥ 30 years group 9.27% PA case with normal cytogenetics, 14.09% monoploidy and 21.05% mosaicisms cases has been found. So, all the cytogenetically varied PA cases have possibly a significant and strong correlation with young maternal age i.e. ≤ 20 years. Hormonal range has also been estimated and tabulated. A huge difference is seen in comparison to normal range of hormones (Table 5).

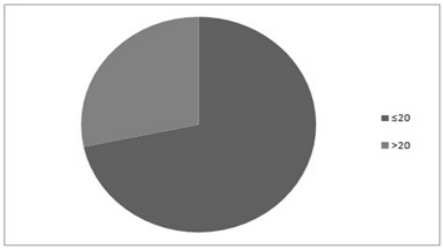

Figure 1. Percentage frequency of PA cases with maternal age ≤20 and ≥20 years.

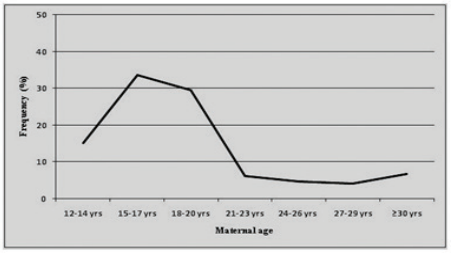

Figure 2. Occurrence of PA cases in five different groups of maternal age where the second age group (15-17 years) shows the highest PA occurrence frequency.

Table 1. Maternal age distribution and mean maternal age in PA case frequency

Maternal age (in years)	PA cases			Control		
	Frequency (%)	Mean Maternal age (years)	SD*	Frequency (%)	Mean Maternal age (years)	SD*
≤20	71.70	16.15	2.17	36.9	18.92	1.02
21-29	13.12	26.73	1.94	45.84	26.37	2.15
≥30	15.18	34.56	1.76	17.26	34.41	1.84

SD*= Standard Deviation

Table 2. t-test analysis showing the significance effect of younger maternal age group whether the rest groups are not quite statistically significant for PA occurrence

Maternal age	t-test value	95% CI# value	p≠ value
≤ 20	23.50	-3.0016 to - 2.5384	<0.0001
21-29	1.725	-0.0500 to 0.7700	0.0851
≥30	0.751	-0.2431 to 0.5431	<0.50

CI# = Confidence interval; p≠ value = Probability value

Table 3. Maternal age distribution in three stratified classes of PA (Class A=MRKH syndrome; Class B=Turner syndrome; Class C= Other group)

	PA cases frequency (%)	Maternal age		
		≤20	21-29	≥30
Class A	42.21	67.13%	18.94%	13.93%
Class B	29.4	53.44%	21.64%	24.92%
Class C	28.39	70.51%	9.83%	18.66%

Table 4. Maternal age distributions in cytogenetically analyzed PA cases

	PA cases frequency (%)	Maternal age		
		≤20	21-29	≥30
Normal Karyotype (2n=46, XX)	67.27	77.26%	13.47%	9.27%
Turner syndrome (2n=45, XO)	19.47	68.63%	17.28%	14.09%
Mosaic turner syndrome (2n=45, XO/46, XX)	13.27	63.15%	15.79%	21.05%

Table 5. Mean range of hormonal level of cytogenetically analyzed PA cases

	Normal Karyotype (2n=46, XX) (Mean range)	Turner syndrome (2n=45, XO) (Mean range)	Mosaic turner syndrome (2n=45, XO/46, XX) (Mean range)	Normal range of hormone[£]
Ovarian hormone				
Estradiol	65.29pg/ml	7.12pg/ml	5.67pg/ml	30 - 400pg/ml
GnRH				
LH	21.96lU/L	35.15IU/L	40.29IU/L	5 to 25IU/L
FSH	44.28mlU/ml	31.63mIU/ml	22.50mIU/ml	0.3 - 10.0 mIU/ml
TRH				
TSH	2.90 mlU/L	8.22 mlU/L	3.65 mlU/L	0.4 - 4.0 mIU/L
Prolactin	6.01 ng/ml	42.26 ng/ml	31.23 ng/ml	2-29 ng/ml

[£]Normal ranges of hormones have taken according to given data in http://www.nlm.nih.gov and AACE.

4. Discussion

A number of works have been carried out on etiology of primary amenorrhea mostly based on hormonal imbalance and reproductive developmental impairment[12]. Some studies show that the ovarian failure has varied manifestations of amenorrhea, primary amenorrhea or dysfunctional uterine bleeding[10,13]. Meduri et al. shows the novel mutation in human follicle stimulating hormone receptor results PA[14]. The granulose cell ovarian tumor is also found to be one of the root causes of PA prevalence[15]. However, there is no available significant reference highlights on the maternal age factor in PA. In this study we have observed that early maternal age (≤ 20 years) as an etiological factor of primary amenorrhea (PA) through epidemiological studies. Early maternal age arise different kinds of problems associated with pregnancy and show the detrimental effect on health of both mother and her child. In this study we consider maternal age with PA prevalence as we found a remarkable number of PA patients having their mothers getting married at early age (mostly < 18 years). This is due to the unscientific social practices done especially in rural areas of India where girls are compelled to get married at early age and consecutively giving birth of babies at the age of 16 to 19 years (< 20 years). Additionally, it is to be notified that the most of the families belong to low income group which causes the girls to refrain from getting necessary supplement of nutrition. Prakash et al. manifests the pernicious effect of early maternal age on reproductive health which affects the pregnancy as well as the embryonic health status[16]. So the insufficient nutrition and immature reproductive health of mother may also effect on embryonic development including the immature germ cell development. It is assumed that early maternal age cannot give a sufficient environment for fetus to get a proper development in uterus. At the same time it is believed that oogonia is formed in large numbers by mitosis happened early in fetal development from primordial germ cells[17]. In case of humans, they start to develop between weeks 4th to 8th and are present in the fetus between weeks[18] 5th and 30th. During the 6th and 8th week of female embryonic development, the primordial germ cells grow and begin to differentiate into oogonia. Oogonia proliferate via mitosis during the 9th to 22nd week of embryonic development and generally 7 million oogonia are formed during third trimester[19]. After the seventh month of embryonic development a number of oogonia drop simultaneously and most oogonia die, while the remaining oogonia enter the first meiotic division[19]. Of the millions of primary oocytes present at birth, only about 400 mature during a woman's lifetime. These latter cells, called the primary oocytes, progress through the first meiotic prophase until the diplotene stage, where they are maintained till puberty. With the onset of adolescence, groups of oocytes periodically resume meiosis. Thus, in human female the first part of meiosis begins in the embryo, and the signal to resume meiosis is not given until roughly 12 years later. Early aged mothers go prone with various problems related to reproductive health. Their weak reproductive health might effect on development of oogonia in female fetus body, a study in Netherlands has shown that extremely low diet during the war did not have a great impact on daughters' fertility, though[20].

This study reveals that more than 70% of PA patients are born to young mothers of ≤ 20 years and less than 30% born to mothers age > 21 years (Figure 1). The result

of this study provides primary support to the anticipation that maternal age of conception is a correlate of occurrence of PA among daughters. More investigations on larger population sample size are required to confirm the notion of early age of conception as a risk factor for PA in daughters. Such data would also be helpful in better assessment of the hypothesis involving hormonal imbalance. A multidisciplinary approach to know the PA occurrence and influence of maternal age is also required. Besides maternal age, other factors as maternal hormonal imbalance, addictions, spontaneous abortion, types of pregnancy terminations might highlight the findings of the etiological factors of PA. As we consider the paternal age and other external factors to be constant, the second query has no chance of creating ambiguity in analysis.

5. Conclusion

This study provides a novel approach to find out the etiology of PA related to maternal age. We carry out a basic preliminary epidemiological work. Statistical assessments show significant association between younger mother and their daughter facing PA at their puberty. Further research works at molecular level are required to understand the relation between maternal inheritance and PA cases in better way. We wish that proper research attempts will decipher the exact etiological picture and identify the risk factors responsible for occurring PA which could help to prevent the disorder and smoothen the livelihood of women.

6. Acknowledgement

We are thankful to Department of Biotechnology (DBT), India for financial support.

7. Conflict of Interests

Authors declare no conflict of interest.

8. References

1. Doody KM, Carr BR. Amenorrhea. Obstst Gynecol Clinics of North America. 1990; 17: 361–87.
2. Hunter TM, Heiman DL. Amenorrhea: evaluation and treatment. Am Fam Physician. 2006; 73(8):1374–82.
3. Mittal P, Saxena P, Jain AL. Adolescent gynecological issues. In: Salhan S, editor. Textbook of Gynaecology. New Delhi: Jaypee Publishers; 2011. p. 69–70.
4. Jabbar S. Frequency and causes of primary amenorrhoea at Civil Hospital, Karachi Pak J Surg. 2004; 20(1):35–7.
5. Forhad QE, Tansim S, Begum A. Primary amenorrhoea: analysis of 44 cases. Bangladesh J Obstet Gynaecol. 2008; 23(2):46–50.
6. Cann CE, Martin MC, Genant HK, Jaffe RB. Decreased spinal mineral content in amenorrheic women. JAMA. 1984; 251(5):626–9. doi:10.1001/jama.1984.03340290040017
7. Welt CK, Chan JL, Bullen J, Murphy R, et.al. Recombinant human leptin in women with hypothalamic amenorrhea. N Engl J Med. 2004; 351:987–97. doi: 10.1056/NEJMoa040388
8. Merin T, Rema D, Preetha T, Amudha S, Jayalakshamma J, Mary M. Amenorrhea: cytogenetic studies and beyond. Am J Mol Cel Biol. 2012; 1:25–32.
9. Rebar RW, Erickson GF, Yen SS. Ideaopathic premature ovarian failure: clinical and endocrine characteristics. Fertil Steril. 1982; 37(1):35–41.
10. Albright F, Smith PH, Fraser R. A syndrome characterized by primary ovarian insufficiency and decreases stature: report of 11 cases with a digression on hormonal control of axillary and pubic hair. Am J Med Sci. 1942; 204(5):625–48.
11. Stein IF, Leventhal ML. Amenorrhea associated with bilateral polycystic ovary. Am J Obstet Gynecol. 1935; 29:181–91.
12. Howlett TA, Wass JAH, Grossman A, Plowman PN, Charlesworth M, Touzel R. Prolactinomas presenting as primary amenorrhea and delayed or arrested puberty: response to medical treatment. Clin Endicrinol. 1989; 30:131–40.
13. Wright CS, Jacobs HS. Spontaneous pregnancy in a patient with hyper gonadotrophic ovarian failure. Br J Obstet Gynaecol. 1979; 86(5):389–92.
14. Meduri G, Touraine P, Beau I, Lahuna O, Desroches A, Vacher-Lavenu MC et al. Delayed puberty and primary amenorrhea associated with a novel mutation of the human follicle-stimulating hormone receptor: clinical, histological and molecular studies. J Clin Endocrinol Metabol. 2003; 88(8).
15. Shaikh NB, Sirichand P. Granulosa cell ovarian tumor as cause of primary amenorrhea. JLUMHS. 2005; 77–9.
16. Prakash R, Singh A, Pathak PK, Parasuraman S. Early marriage, poor reproductive health status of mother and child well-being in India. J Fam Plann Reprod Health Care. 2011; 1–10.
17. Pinkerton JHM, McKay DG, Adams EC, Hertig AT. Development of the human ovary: a study using histochemical techniques. Obstet. Gynecol. 1961; 18:152–81.
18. Jones RE. Human Reproductive Biology, 2nd ed. San Diego: Academic Press, Elsevier; 1997. p. 26–40, 90-7, 117–25.
19. Chassot AA, Gregory EP, Lavery R, Taketo MM, de Rooij DG, et al. RSPO1/β-catenin signaling pathway regulates oogonia differentiation and entry into meiosis in the mouse fetal ovary. PLoS ONE. 2012; 6(10).
20. Roseboom TJ , van der Meulen Jan HP, Ravelli ACJ, Osmond C, Barker DJP, Bleker OP. Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. Molecular and Cellular Endocrinology. 2001; 185:93–8.