ACTA FAC MED NAISS

UDC 618.173

Original article

ACTA FAC MED NAISS 2008; 25 (4): 183-188

Zoran Protrka[1], Olivera Protrka[2], Mirjana Varjacic[1], Momcilo Djordjevic[1]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, University of Kragujevac, Kragujevac, Serbia

[2]Health Center Kragujevac, Serbia

PRIMARY OVARIAN INSUFFICIENCY

SUMMARY

Primary ovarian insufficiency or premature menopause is one of the stressful problems in women younger than 40, which may cause numerous early or late psychological and physical complications. Failure of germ cell development is associated with complete ovarian insufficiency, while their decreased number is more likely associated with partial ovarian insufficiency, which leads to secondary amenorrhea.

The aim of this study was to assess 11 young women in whom premature menopause occurred between 14 and 25 years of age.

Menopausal symptoms, total urinary gonadotropins, therapy, ultrasonography and laparoscopy of the gonads were investigated and the patients were referred to a genetic counselor for cytogenetic tests and counseling.

Laparascopically, in four rudimentary types of ovarian streaks, probably a representative form of gonadal dysgenesis was found. In four patients, the ovaries were typical of the corrugated wrinkled ovaries of postmenopausal women. The chromosomal nuclear sex was positive in 10 and the karyotype was normal in 9 of 11 patients in whom these tests were performed.

This finding has substantial implications for understanding human primary ovarian insufficiency and for developing effective alternative therapies.

Key words: **premature ovarian failure, secondary amenorrhea, karyotype**

INTRODUCTION

Primary ovarian insufficiency (POI) is a condition causing secondary amenorrhea, hypoestrogenism, and elevated gonadotrophins in women younger than 40 years. POI will occur in 1% of women before the age of 40 and in 0.1% or 1 per 1000 women before the age of 30 years (1). POI can be familial, genetically inherited, or sporadic where there has been no family history of the disorder. Even though there have been many advances into the cause of POI in recent years, especially in the field of molecular genetics, the cause of POI in most cases remains a mystery. Most women presented with idiopathic POI have normal menstrual history, age of menarche, and fertility prior to the onset of the condition. It was once thought that POI was irreversible in all cases as in menopause, however, intermittent ovarian insufficiency has been reported, and pregnancy can occur in approximately 10% of patients subsequent to diagnosis (2).

The most immediate concern for women

Corresponding author • Zoran Protrka • Tel: + 381 34 342482; mobtel. 064 1129859 • E-mail: protrka@eunet.yu

with POI is the menopausal symptoms they experience due to decreased circulating oestradiol coupled with the psychological implications of these symptoms. The menopausal symptoms include hot flushes, night sweats, insomnia, palpitations, headaches, incontinence, and dyspareunia as a result of vaginal dryness. The psychological implication of POI does not only include those associated with menopause such as forgetfulness, poor concentration, irritability and mood swings. A second consequence of POI is the loss of fertility. Even though some women will spontaneously ovulate and achieve a natural pregnancy, most women with POI will not. Infertility treatment is difficult, as in many cases the ovary does not have any follicles left. In the cases where follicles can be detected by biopsy, the ovary has become unresponsive to FSH. Therefore, most women with POI can either choose to adopt children or undergo donor egg IVF. However, obtaining donor eggs can be difficult and the procedure can be very expensive.

A wide spectrum of pathogenic mechanisms may lead to the development of POI including chromosomal, genetic, autoimmune, metabolic (galactosaemia), infectious (mumps) and iatrogenic (anticancer treatments) causes. In a large proportion of cases no cause is found and they are classified as idiopathic or karyotypically normal, spontaneous ovarian insufficiency (3), whereas up to 30% of cases may have an autoimmune cause (4).

In the embryo, germ cells migrate from the urogenital ridge to the primitive ovary where they proliferate to form $3.5x10^6$ oocytes in each ovary by about 20 weeks of intrauterine life. Most of these germ cells are destroyed through apoptosis (5). The ovary is endowed with a fixed number of primordial follicles at the time of birth, about $1x10^6$ in each ovary. This number steadily dwindles throughout life as a result of atresia and recruitment towards ovulation (6). Fewer than 500 of the original $7x10^6$ (0.007%) oocytes are released in the entire reproductive life span of a woman.

In idiopathic POI, there may be involvement of as yet unknown mechanisms affecting the rate of oocyte apoptosis. This may lead to a reduced complement of oocytes in the ovaries at birth or accelerated atresia. Using ultrasound, follicles have been reported in up to 40% of POI patients (7). However, ultrasonography or ovarian biopsies are not helpful in the prognosis of future ovulation and fertility.

In a recent thought-provoking article, Johnson et al. have challenged the concept that each woman is endowed with an irreplenishable number of gametes in the ovary. Through three different sets of experiments they came to a conclusion that ovarian germ cells are a dynamic population and undergo constant renewal. Such a novel concept that challenges the central dogma in reproductive sciences is likely to stir a flurry of debate and to be followed by further studies exploring the issue (8).

MATERIAL AND METHODS

The prospective study was carried out during 2005 and 2007, at the Department of Obstetrics and Gynecology, Faculty of Medicine in Kragujevac and the experimental part was performed at the Laboratory for Experimental and Clinical Immunology of the Faculty of Medicine, Kragujevac. The research included 11 young women in whom premature menopause occurred between 14 and 25 years of age. We examined menopausal symptoms, total urinary gonadotropins, therapy, ultrasonography and laparoscopy of the gonads and the patients were referred to a genetic counselor for undergoing cytogenetic tests and counseling.

Blood samples for chromosomal analysis were taken after obtaining informed consent of patients in accordance with the Declaration of Helsinki and recommendations of the World Health Organization (WHO) for experiments on human material and after obtaining the approval of the Ethics Committee.

RESULTS AND DISCUSSION

Several features are common to these 11 patients, as well as to those recently reported in the literature as having undergone primary ovarian insufficiency. Secondary amenorrhea, with or without menopausal symptoms, is the chief complaint. After a normal menarche, the patients experienced more or less regular periods for various lengths of time before the cessation, often abruptly, of menses *(Figure 1)*.

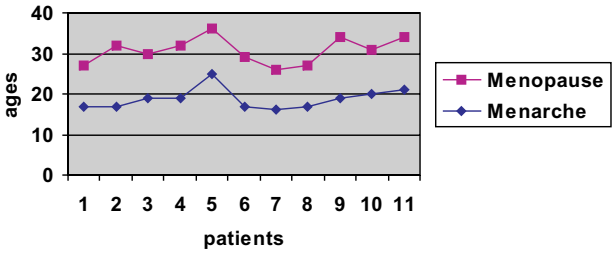

Figure 1. Periods of menarche and menopause

In 6 of our 11 cases, menopausal symptoms were present *(Figure 2)*.

Since hot flushes, as a rule, occur only after several years of hypothalamic conditioning by estrogens, the lack of this symptom in two cases is understandable.

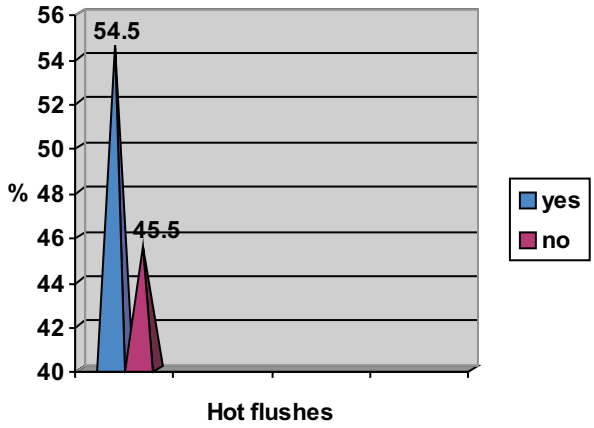

Figure 2. Presence of menopausal symptoms

The individuals composing this series were, in general, normally developed women, without notable bodily aberrations. In absence of previous therapy, their estrogenic function was rather poor, as judged by vaginal cytology *(Figure 3)*.

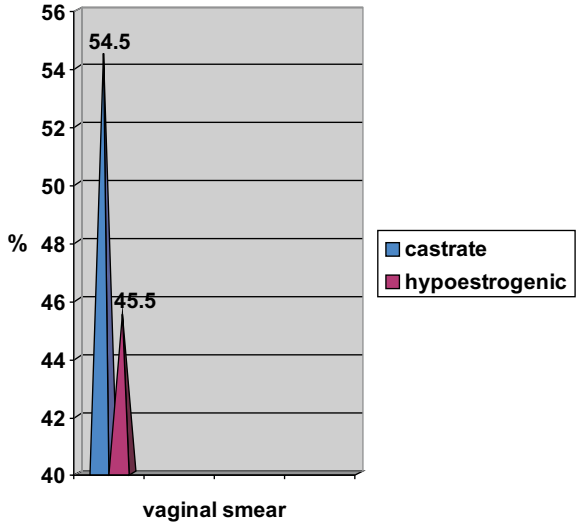

Figure 3. Vaginal cytology

In primary ovarian insufficiency, urinary gonadotropin titers are usually elevated. Several recent studies confirm their very high levels both by bioassay and immuno- or radioimmunoassays, after the physiologic menopause (9). Increased levels in primary ovarian insufficiency were also reported in recent studies and were present in five of the cases *(Table 1)*.

Two of our patients did not exhibit high values because the assays were performed while they were taking estrogen or soon after discontinuation of estrogenic therapy. However, this very useful criterion does not always prove confirmatory, as shown in two cases. The bioassay method, because of errors in the technique or sensitivity of the animals, may not reflect the true gonadotropin levels.

Several authors have attempted to confirm the diagnosis of POI by the lack of responsiveness of the ovary to exogenous gonadotropin stimulation. But this non-responsiveness to a certain dosage is not a criterion of POI (10). In secondary hypoovarianism, the reaction of the resting ovary to exogenous gonadotropins is variable, a phenomenon also observed following ACTH stimulation of the adrenal gland. On the other hand, in one case, several courses of Clomid resulted in three ovulatory cycles *(Table 2)*.

Table 2.

Therapy	/	Estrogens	Estrogens + Clomid
No (%)	6 (54.5%)	3 (27.3%)	2 (18.2%)

Laparoscopically, the ovary may present several appearances: it may resemble the rudimentary streak of dysgenetic gonads (four cases), it may be atrophic, with a smooth surface, but normal in shape (three cases) or it may present the typical appearance of the menopausal ovary-small and corrugated (four cases). These three aspects are not likely, however, to represent different entities *(Figure 4)*.

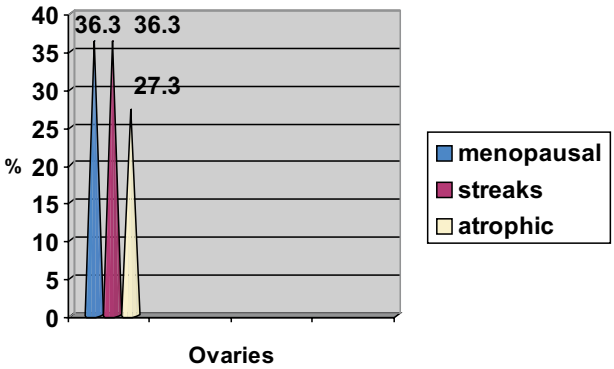

Figure 4. Laparoscopy

Table 1. Levels of urinary gonadotropin

Total urinary gonadotropins (mouse unit/24h)	<6.6	6.6-52	52-104	104-208	>208
No (%)	1 (9.1%)	4 (36.3%)	1 (9.1%)	4 (36.3%)	1 (9.1%)

Ultrasonography of the gonads shows two important features: few rare primordial follicles and no primordial follicles *(Figure 5)*.

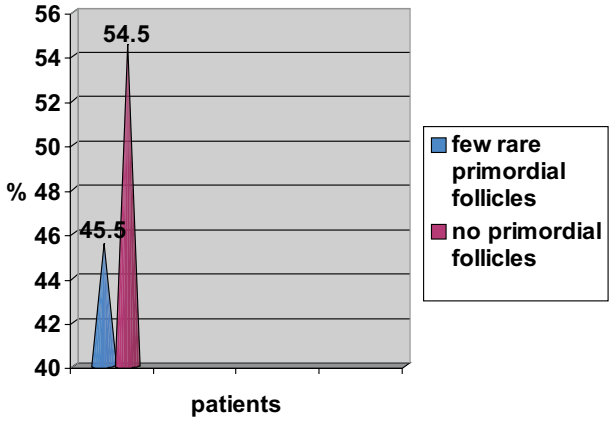

Figure 5. Ultrasonographic examination

The histoligic examination of the gonad is actually the only proof for the diagnosis of premature menopause. When the histologic appearance of the ovaries of these women is in harmony with this description, it is possible to speak about actual premature menopause (11). In addition, Arlt W. reports the rarity of theca interna around the occasional follicles and thus feels that the ovarian stroma might be genetically abnormal (12).

In summary, secondary amenorrhea in a well-developed woman under 30 years of age with elevated urinary gonadotropins should suggest a diagnosis of POI. Substantiation can only be obtained by study of histological sections of ovarian biopsies.

Physiopathologic considerations: the actual causes of premature menopause remain conjectural. There is no single cause to account for all the cases on record. However, two predisposing factors have been recognized.

-*Familial occurrence*: several earlier reports described families in whom the menopause occurred before 40 years of age in most of the female members. Although such reports should be received with a degree of caution, this fact has long been known. One of our patients may be considered as belonging to this category, as three other members in the family history revealed abnormally early menopause.

-*Genetic anomalies*: all the most recent reports have been concerned with cytogenetic studies. Indeed, it has been found that patients with gonadal dysgenesis might have menstrual periods. Armitage M. et al. reported the case of a woman who menstruated regularly for 17 years and then stopped. Her karyotype was 45/XO (13). Three sorts of anomalies of the karyotype are described in occasional cases of POI: a) 45/XO. Increasing numbers are appearing in literature. b) Triple-X syndrome (14). POI, however, is not a common feature in these individuals. Tonacchera M et al. reviewing 38 cases of 47/XXX women, did not find a single occurrence of this entity among them (15). c) Mosaicisms: XX/XO, XX/XX, and even XO/XX/XXX/XXXX (16). The best known anomaly is the 45/XO variety, linking these cases to gonadal dysgenesis. The question arises as to the mechanism by which this chromosomal anomaly could lead to both entities. Until recently, it was assumed that poor endowment to the primitive gonad could result from abnormalities in migration of the germ cells. In fact, it is well-documented that the ovary of the 45/XO embryo is indistinguishable, until at least the third month, from the ovary of the normal embryo of the same age (17). The physiologic drop in the germ cell population at about the fifth month of intrauterine life might eventuate in a massive degeneration in the 45/XO embryo. Various degrees in the intensity of this process could lead to various extremes in ovarian function from primary amenorrhea to the later development of premature insufficiency. There were abnormal cytogenetic findings in our group. However, a karyotype, or at least a buccal smear, should be done on such patients. Indeed, these secondary amenorrheic 45/XO subjects do not, as a rule, present clinical features to call attention to their genetic anomaly. The chromosomal nuclear sex was positive in 10 cases and the karyotype was normal in 9 of the 11cases in which these tests were performed *(Table 3)*.

However these secondary amenorrheic 45/XO subjects do not, as a rule, present clinical features to call attention to their genetic anomaly.

-*No evident cause*: however, no specific cause may be found in patients undergoing premature menopause. Here, only hypotheses are possible.

Table 3. Nuclear sex chromatin and karyotype

Patient	1	2	3	4	5	6	7	8	9	10	11
Nuclear sex chromatin	55%	32%	42%	-	55%	35%	32%	40%	50%	45%	50%
Karyotype	46/xx	46/xx	46/xx	45/xo	46/xx	46/xx	46/xx	46/xx	45/xo	46/xx	46/xx

Some authors believe in a congenital defect of the stromal cells of the ovary (18). Furthermore, karyotype from blood culture does not always rule out a mosaicism. Ovarian tissue cultures might be more revealing.

Germ cells may be destroyed by several agents in pre- or postpuberal life. Radiations, certain viruses, and some drugs are notorious for their deleterious effect. There are probably other unknown agents and the concept of autoimmune processes has been proposed.

The mechanisms concerned with ova maturation, release or degeneration are still poorly understood. It has been assumed that a hypothalamo-pituitary dysfunction, through an abnormal pattern of gonadotropin secretion, could bring about a premature exhaustion of the germ cells of the ovary. Maturation of an excessive number of follicles during each cycle could result in massive follicular atresia.

In five cases of ovarian failure in women with Addison's disease, ranging in age from adolescence to 26 years, Smith S. et al. (19) suggested that immunologic aspects were at play. This suspicion was confirmed by the finding of antibodies reactive to the theca interna of the ovary in these patients. Since it is now generally accepted that many cases of hypothyroidism and Addison's disease result from autoimmune processes, consideration now must be given to the possibility that certain cases of primary ovarian insufficiency are olso due to autoimmune disease.

CONSLUSION

It is possible that primary ovarian insufficiency is the consequence of poor endowment of primordial follicles or other factors presently poorly understood.

This finding has substantial implications for understanding human POI and for developing effective alternative therapies to treat the condition.

REFERENCES

1. Bodega B, Porta C, Crosignani PG, Ginelli E, Marozzi A. Mutations in the coding region of the FOXL2 gene are not a major cause of idiopathic premature ovarian failure. Mol Hum Reprod 2004; 10: 555–7.

2. Abdalla HI, Baber RJ, Kirkland A, Leonard T, Studd JW. Pregnancy in women with premature ovarian failure using tubal and intrauterine transfer of cryopreserved zygotes. Br J Obstet Gynaecol 1989; 96: 1071–5.

3. Anasti JN, Kimzey LM, Defensor RA, White B, Nelson LM. A controlled study of danazol for the treatment of karyotypically normal spontaneous premature ovarian failure. Fertil Steril 1994; 62: 726–30.

4. Alper MM, Garner PR. Premature ovarian failure: its relationship to autoimmune disease. Obstet Gynecol 1985; 66: 27–30.

5. Aaltonen J, Laitinen MP, Vuojolainen K, Jaatinen R, Horelli-Kuitunen N, Seppa L, et al. Human growth differentiation factor 9 (GDF-9) and its novel homolog GDF-9B are expressed in oocytes during early folliculogenesis. J Clin Endocrinol Metab 1999; 84: 2744–50.

6. Abir R, Fisch B, Nitke S, Okon E, Raz A, Rafael B. Morphological study of fully and partially isolated early human follicles. Fertil Steril 2001; 75: 141–46.

7. Asch R, Balmaceda J, Ord T, Borrero C, Cefalu E, Gastaldi C, et al. Oocyte donation and gamete intrafallopian transfer as treatment for premature ovarian failure. Lancet 1987; 1: 687.

8. Johnson J, Canning J, Kaneko T, Pru JK, Tilly JL. Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature 2004; 428: 145–50.

9. Morgante AM, Costa SS. Premature ovarian failure is associated with maternally and paternally inherited premutation in Brazilian families with fragile X. Am J Hum Genet 2000; 67: 254–5.

10. Betterle C, Rossi A, Dalla PS, Artifoni A, Pedini B, Gavasso S, et al. Premature ovarian failure: autoimmunity and natural history. Clin Endocrinol 1993; 39: 35–43.

11. Pal L, Santoro N. Premature ovarian failure (POF): discordance between somatic and reproductive aging. Ageing Res Rev 2002; 1: 413–23.

12. Arlt W. Management of the androgen-deficient woman. Growth Horm IGF Res 2003; 13: 85–9.

13. Armitage M, Nooney J, Evans S. Recent concerns surrounding HRT. Clin Endocrinol 2003; 59: 145–55.

14. Van Kasteren Y. Treatment concepts for premature ovarian failure. J Soc Gynecol Investig 2001; 8: 58–9.

15. Tonacchera M, Ferrarini E, Dimida A, Agretti P, De Marco G, De Servi M. et al. Gonadotrophin receptor blocking antibodies measured by the use of cell lines stably expressing human gonadotrophin receptors are not detectable in women with 46,XX premature ovarian failure. Clin Endocrinol 2004; 61: 376–81.

16. Takakura K, Takebayashi K, Wang HQ, Kimura F, Kasahara K, Noda Y. Follicle-stimulating hormone receptor gene mutations are rare in Japanese women with premature ovarian failure and polycystic ovary syndrome. Fertil Steril 2001; 75: 207–9.

17. Shibanuma K, Tong ZB, Vanderhoof VH, Vanevski K, Nelson LM. Investigation of KIT gene mutations in women with 46, XX spontaneous premature ovarian failure. BMC Womens Health 2002; 2: 8.

18. Rebar RW, Connolly D. Clinical features of young women with hypergonadotropic amenorrhea. Fertil Steril 1990; 53: 804–10.

19. Smith S, Hosid S. Premature ovarian failure associated with autoantibodies to the zona pellucida. Int J Fertil Menopausal Stud 1994; 39: 316–19.

PRIMARNA OVARIJALNA INSUFICIJENCIJA

Zoran Protrka[1], Olivera Protrka[2], Mirjana Varjačić[1] i Momčilo Đorđević[1]

[1]Odeljenje za ginekologiju i akušerstvo, Medicinski fakultet
Univerzitet u Kragujevcu, Kragujevac, Srbija
[2]Zdravstveni centar Kragujevac, Srbija

SAŽETAK

Primarna ovarijalna insuficijencija ili prevremena menopauza predstavlja jedan od stresnijih problema za žene mlađe od 40 godina koji može prouzrokovati brojne rane ili kasne psihološke i fizičke komplikacije. Insuficijencija u razvoju germinativnih ćelija je udružena sa kompletnom ovarijalnom insuficijencijom, dok je njihov smanjeni broj najverovatnije udružen sa parcijalnom ovarijalnom insuficijencijom, koja dovodi do sekundarne amenoreje.

Cilj ove studije bio je da se ispita 11 mladih žena kod kojih se prevremena menopauza dogodila između 14. i 25. godine života.

Ispitivani su menopauzalni simptomi, totalni urinarni gonadotropin, terapija, ultrasonografski i laparoskopski status gonada a bolesnice su upućivane u genetsko savetovalište radi citogenetskih analiza.

Laparoskopski je kod 4 rudimentirana tipa ovarijalnih traka pronađena verovatno reprezentativna forma gonadalne disgenezije. Kod 4 bolesnice su ovarijumi bili izbrazdanog izgleda, tipičnog za žene u menopauzi. Hromozomski pol bio je pozitivan kod 10, a normalan kariotip pronađen je kod 9 od 11 ispitivanih žena.

Naša istraživanja mogu biti od bitnog značaja za razumevanje humane primarne ovarijalne insuficijencije i za razvoj efektivne alternativne terapije.

***Ključne reči*: prevremena ovarijalna insuficijencija, sekundarna amenoreja, kariotip**