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SCOPE OF LAPAROSCOPIC OVARIAN BIOPSY VERSUS MULTIPLE
SERUM GONADOTROPIN ESTIMATIONS IN THE DIAGNOSIS OF
SECONDARY AMENORRHOEA

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Introduction

Secondary amenorrhea could be the end result of divergent etiopathological factors. At one end of the spectrum it can be the symptom of a serious underlying disease like pituitary tumour, or genital tuberculosis, while at the other end it could just be a reflection of a psychosomatic disorder. In order to localise the lesion, several investigative approaches have been suggested for the

diagnosis of secondary amenorrhoea (Kletzy *et al* 1975; Lunefed and Insler 1974; Speroff *et al* 1973). The object of the present study was to specially evaluate the scope and limitations of (1) laparoscopy and laparoscopy-directed ovarian biopsy and (2) multiple serum gonadotropin estimations in the investigative approach to secondary amenorrhoea.

Material and Method

Thirty-seven women with secondary amenorrhoea attending the endocrine clinic of the Institute from January 75 to December 75 constituted the clinical material for the present study. After recording history according to a well planned proforma, physical and pelvic exami-

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nation a protocol was followed for investigations which included complete haemogram, radiological and ophthalmological examination and karyotyping. Duration of amenorrhoea ranged from 3 months to 9 years. Their ages at the time of investigation ranged between 19 and 35 years. Two patients complained of episodes of hot flushes, one patient was amenorrhoeic after having ingested oral contraceptives for 2 years. In 3 patients menstrual bleeding had not returned after the last delivery 1½ years, 6 years and 7 years ago. Five patients had an associated problem of galactorrhoea and in one patient amenorrhoea followed dieting to reduce weight.

Endometrial biopsies were evaluated for their development as well as to rule out tuberculous endometritis. Besides the conventional parameters, the following investigations were carried out for their diagnostic significance in cases of secondary amenorrhoea.

Laparoscopy and Laparoscopic Ovarian Biopsy

Laparoscopy was performed in 30 patients. Twenty-four of them underwent ovarian biopsy. Uterus, tubes and ovaries were visualized. Chromopertubation with 1% methylene blue for testing tubal patency was carried out at the time of laparoscopy. Ovarian biopsy was taken by means of a biopsy forceps from both ovaries. The sections were assessed for the capsular thickness, stromal cellularity, presence of follicles and their development and for corpora albicans.

Serum Gonadotropin Levels

Blood was drawn on 2 consecutive days each week for 2 weeks at a fixed time of the day for the estimation of serum gonadotropins and prolactin. A precaution was taken to collect blood only after 6

weeks of discontinuation of hormonal therapy, if received by the patient prior to her attending the clinic. Sera were separated and stored at -20°C until assayed. To reduce the influence of interassay variation all samples from each patient were assayed at the same time. Serum FSH and LH were measured by the double antibody technique of Midgley (Midgley, 1966).

Results

The diagnostic classification of 37 cases of secondary amenorrhoea as reached by a comprehensive evaluation is shown in Table I. In the present series there were

TABLE I
Diagnostic Classification

Diagnosis	No. of patients
I. Genital tuberculosis	5
II. Ovarian (a) Premature Ovarian Failure	12
(b) Polycystic ovary syndrome	3
III. Hypothalamo-Pituitary Lesion	
(a) Pituitary tumour	3
(b) Hypothalamo - pituitary failure	2
(c) Hypothalamo - pituitary dysfunction	12
	37

5 cases of genital tuberculosis, 12 of Premature Ovarian Failure (P.O.F.), 3 of Polycystic Ovary Syndrome (P.C.O.), 3 of Pituitary tumour, 2 of Hypothalamo-pituitary failure and 12 of hypothalamo-pituitary dysfunction.

Laparoscopy and Ovarian Histopathology

Laparoscopy was performed in 30 cases. Of the remaining 7 cases, 3 got spontaneous periods during investigations, 2 were cases of pituitary tumour and the remaining 2 did not wish to undergo laparos-

copy. Pelvic findings were normal in 25 of them. Genital tuberculosis was diagnosed in 5 patients who revealed a shaggy uterus, blocked tubes, tubal adhesions and/or blue uterus (Motashaw *et al* 1974). An extra-genital tuberculous focus was detected in only 2 of them.

Table II gives the gross and microscopic appearance of the ovaries in 24 patients who underwent ovarian biopsy at laparoscopy. Eleven out of 12 cases of P.O.F. underwent laparoscopy. In 9 patients the ovaries looked small and corrugated and in 2 they were normal. Laparoscopic ovarian biopsies were obtained in 10 of them. Four ovarian biopsies where the ovaries looked small and corrugated were afollicular, while the remaining 4 of this group contained some atretic follicles. Ovarian biopsies in the remaining 2 individuals who had normal ovaries displayed occasional primary follicles. Two patients, one with pituitary tumour and the other with genital tuberculosis, who had normal ovaries displayed no follicles microscopically. Eugonadotropic status and x-ray skull helped to differentiate those cases from those of P.O.F. Two out of 3 cases of P.C.O. syndrome revealed microcystic ovaries. The remaining patients with hypothalamo-pituitary dysfunction and hypothalamo-pituitary failure had normal looking ovaries, which contained follicles at different stages of development in some cases and few follicles in others.

Serum Gonadotropin Levels

Mean values of FSH and LH in individual patients of different etiological groups are shown in Figure I. Values of FSH and LH overlap in between groups and there is no sharp demarcation except in group I—that of P.O.F. Twelve patients had a hypergonadotropic status. Isolated determinations of FSH and LH in

TABLE II
Gross and Microscopic Appearance of Ovaries in the Different Diagnostic Groups

Diagnostic Group	No. of Pts.	Gross Appearance of Ovary			Microscopic Appearance of Ovaries									
		Normal	Enlarged	Small & corrugated	Follicles		Stroma		Corpus albicans		Capsule			
					Afol.	Few folli.	No. of follic.	Normal	Fibrous	+		-	Normal	Thick
Genital tuberculosis	3	3	—	—	1	—	2	3	—	—	—	—	—	—
P.O.F.	10	2	—	8	4	6	—	3	7	4	6	10	—	—
P.C.O. syndrome	3	1	2	—	—	—	3	3	—	—	3	2	1	—
Pituitary tumor	1	1	—	—	1	—	—	1	—	—	—	1	1	—
Hypothalamo-pituitary failure	1	1	—	—	—	—	1	1	—	—	—	1	1	—
Hypothalamo-pituitary dysfunction	6	5	—	1	—	—	6	6	—	—	—	6	6	—

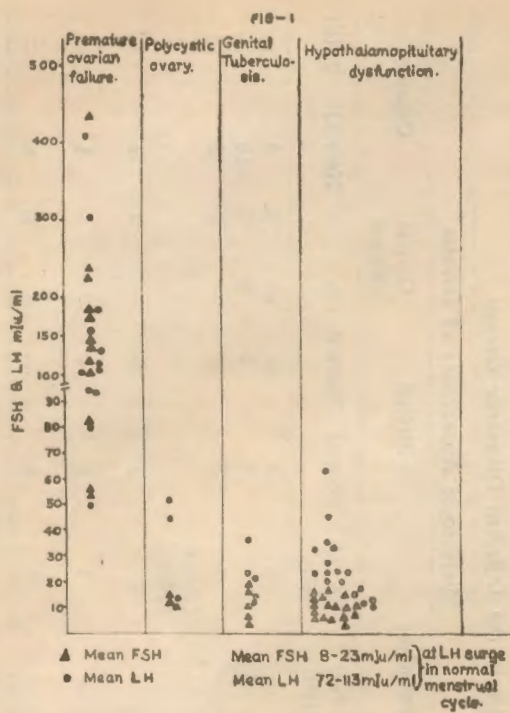


Fig. 1
Serum Gonadotropin levels in the different diagnostic groups of 37 cases of secondary amenorrhoea.

few of these cases were within the normal physiological range. The diagnosis of P.O.F. was reached only when two or more values of FSH were found in the menopausal range. Moderately elevated levels of LH and normal FSH constituted the picture in 2 of totally 3 cases of P.C.O. syndrome who also had the problem of hirsutism. All 17 patients with the lesion at the hypothalamo-pituitary level were eugonadotropic. Occasional values of LH and FSH in some patients were very low but not consistently low so as to classify them in the hypogonadotropic status. Three of these patients were cases of pituitary tumour with radiological and ophthalmological evidences. Pituitary gonadotropin reserve test using 25 μ gm of synthetic LHRH intravenously was carri-

ed out in the remaining 14 patients. The response was poor in 2 patients and normal in 12 of them. Cases of H.P. failure were differentiated from those of H.P. Dysfunction because of their poor response to synthetic LHRH (Haji *et al* 1975).

There were totally 5 patients of secondary amenorrhoea with galactorrhoea. Serum prolactin levels in 3 of them were high (120 to 333 ng/ml). Two of these were confirmed to be cases of pituitary tumour on radiological and ophthalmological evidences. The 3rd case of pituitary tumour in the present series had an associated acromegaly.

Discussion

Secondary amenorrhoea is a common symptom with a variety different causes. A series of investigations are necessary to pin-point the diagnosis. Since the popularization of laparoscopy, it has been widely used in recent years as a diagnostic tool in cases of secondary amenorrhoea. The value of laparoscopy in the diagnosis of genital tuberculosis is undoubted (Motashaw *et al* 1974; Rozin 1960). When combined with ovarian biopsy a better assessment of the ovarian function can be made. Ovarian biopsy has made a major contribution to the diagnosis, prognosis, and management of a patient with secondary amenorrhoea (Black and Govan 1972; Steele *et al* 1970; Sykes and Ginsburg 1972). Other authors have shown the limitations of laparoscopic ovarian biopsy. Sutton (1974) noted that ovarian sampling by this type of procedure was not necessarily representative of the histologic appearance of the whole ovary. Laparoscopy and laparoscopy-directed ovarian biopsy were evaluated for their diagnostic significance in the present study. Five cases

of genital tuberculosis were diagnosed on laparoscopy. Genital tuberculosis often silent can be diagnosed by an important sign, "the blue uterus" at laparoscopy (Motashaw *et al* 1974). The endometrium is destroyed up to the basal layer due to tuberculous endometritis. When chromopertubation is carried out using 1% methylene blue it permeates into the venous, lymphatic and interstitial spaces of the myometrium, giving the uterus a blue colour. In the present study a blue uterus was seen in 2 patients.

Laparoscopic ovarian biopsies were carried out in 24 patients. In 20 out of 24 cases the ovarian biopsy picture tallied with the other parameters to reach the final diagnosis. Where the ovaries are small and corrugated on gross appearance and afollicular microscopically the diagnosis of P.O.F. can be made without any doubt, as in 4 of our patients of P.O.F. When similar looking ovaries reveal occasional follicles the diagnosis of P.O.F. can be confirmed in the presence of a hypergonadotropic status (Emperaire, *et al*, 1970; Kettle Bradbury, 1964; Starup 1973). This was seen in the other 4 patients of P.O.F. When ovaries are normal looking and afollicular or with a poor follicular stock as in some of our patients the serum gonadotropin levels again are essential to reach the final diagnosis. In 2 of our patients of P.O.F. ovaries looked normal and contained few follicles. This was the type of microscopic picture seen in few of our patients of H.P. dysfunction. In such situations multiple serum gonadotropin estimations are crucial; for clinching the diagnosis of P.O.F. Ovarian biopsy has been used for its prognostic value (Sykes and Ginsburg, 1972), in cases of H.P. Dysfunction. A good number of follicles indicate a better prognosis. Sykes and Ginsburg, 1972 have reported return

of menstruation or pregnancy on therapy in patients who had a good follicular stock. One of the major criticisms of ovarian biopsy is the danger of complications namely haemorrhage and injury to the bowel or ureter. In the present series there were no complications in any of the patients. One of us (N.D.M.) has an extensive experience with laparoscopy and laparoscopic directed ovarian biopsy. In the hands of an experienced gynaecologist it is safe and relatively easy and should be performed whenever possible as tissue diagnosis is invaluable to substantiate a clinical impression.

Gonadotropin secretion in healthy individuals with intact hypothalamo-pituitary-gonadal axis show short term variations besides cyclic fluctuation with monthly periodicity (Friedman, 1972). Because of this inherent variation in serum gonadotropins the diagnosis in cases of secondary amenorrhoea based on isolated determinations of gonadotropin levels from a single blood sample is of limited value (Freidman, 1972; Vaidya, 1975). Friedman (1973) measured FSH and LH levels in multiple daily blood samples from cases of secondary amenorrhoea. Sequential FSH and LH determinations in these cases did not show any characteristic pattern in various groups. Distinct hormonal patterns were the basis of classifying 21 patients of secondary amenorrhoea in a series reported by Kletzky *et al*, (1975). This was achieved only by obtaining serial blood samples at 15 minutes intervals for a period of 4 hours. However, neither daily samples for sequential determination nor serial determination every 15 minutes for a period of 4 hours is a practical proposition in a busy endocrine clinic and an overburdened endocrine laboratory. Much information can be obtained from

multiple serum gonadotropin levels especially to rule out or confirm the diagnosis of P.O.F. Isolated determinations of FSH in few of the cases in the present series were within the normal physiological range. Clinical usefulness of serum gonadotropin estimation is more important than ovarian biopsy for the diagnosis of P.O.F. Two out of 3 cases of P.C.O. syndrome showed moderately elevated LH and normal FSH. Yen *et al* (1970) have shown high levels of LH associated with low FSH levels in cases of polycystic ovarian syndrome. Friedman (1973) found elevated LH levels with low to normal FSH in cases of P.C.O. syndrome. It has been postulated that hypothalamic disturbance may play a role in the pathogenesis of P.C.O. Multiple serum gonadotropin estimations have clear limitations in the other diagnostic groups. The remaining patients with lesion at the hypothalamo-pituitary level were eugonadotropic. Occasional values of LH and FSH were low but not consistently low. It may be that if one was to do serial estimations in these cases every 15 minutes for 4 hours, one may discover that the total amount of gonadotropin secreted over 4 hours is low indicating hypothalamo-pituitary failure rather than dysfunction.

It is obvious that multiple serum gonadotropin estimations would not help to distinguish between H.P. dysfunction and Hypothalamo-pituitary failure. The differential diagnosis between these two groups could be achieved by either serial estimation of gonadotropins as achieved by Kletzky (1975) or by pituitary gonadotropin reserve test using synthetic LHRH (Bohnet *et al*, 1976; Haji *et al*, 1975).

Summary and Conclusion

(1) Thirty-seven cases of secondary

amenorrhoea were comprehensively evaluated and the scope and limitations of laparoscopic ovarian biopsy and multiple serum gonadotropin estimations are evaluated.

(2) The diagnostic classification included 5 cases of genital tuberculosis, 12 of P.O.F., 3 of P.C.O. syndrome, 3 of pituitary tumour, 2 of Hypothalamo-pituitary failure and 12 of Hypothalamo-pituitary dysfunction.

(3) For the diagnosis of P.O.F. both serum gonadotropin estimations and ovarian histopathology are important. Where the ovaries are small and corrugated on gross appearance and a follicular microscopically the diagnosis of P.O.F. can be made without doubt. When ovaries are normal looking and a follicular or with a poor follicular stock, estimations of serum gonadotropins are essential for the final diagnosis.

(4) In hypothalamic secondary amenorrhoea, ovarian biopsy has a prognostic value. The presence of enough number of follicles would indicate a better prognosis than when the follicles are absent or reduced in number.

(5) Isolated determinations of FSH and/or LH in few of our cases of P.O.F. were within the normal physiological range. This suggests the need for estimations of minimum of two blood samples for serum gonadotropins.

(6) Cases of Hypothalamo-pituitary dysfunction cannot be distinguished from those of Hypothalamo-pituitary failure by multiple serum gonadotropin estimations. This can be achieved by using pituitary gonadotropin reserve test using synthetic LHRH or by serial estimation of serum gonadotropins.

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