Ultrasonographic Evaluation and Clinico-biochemical Association in Patients with Polycystic Ovarian Disease in a Tertiary Care Hospital: A Cross-sectional Study

SINDHU REDDY DWARAMPUDI[1], GITANJALI SATAPATHY[2], KAMAL KUMAR SEN[3], SANGRAM PANDA[4]

ABSTRACT

Introduction: Polycystic Ovarian Syndrome (PCOS) is an endocrinological disorder characterised by a combination of polycystic ovarian morphology, diverse clinical features, and abnormal biochemical indices that affects women of reproductive age.

Aim: To assess the sonographic appearance of ovaries and endometrium in clinically suspected cases of PCOS and to determine the association between the sonographic appearance of ovaries and endometrium with the clinical and hormonal profile of the patient.

Materials and Methods: This cross-sectional study included 53 female patients in the reproductive age group (18-35 years) who were referred to the Department of Radiology, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India for pelvic ultrasonography in clinical suspicion of PCOS from September 2020 to September 2022. All patients underwent transabdominal sonography, only married individuals with abdominal obesity underwent transvaginal ultrasonography. Complete biochemical hormonal analysis was performed. Association between volume, size of the ovary, number of follicles, and stromal thickness with hormones like Follicle Stimulating Hormone (FSH), Luteinising Hormone (LH), testosterone, prolactin, Thyroid Stimulating Hormone (TSH) were analysed. All continuous variables were expressed as mean±standard deviation and categorical variables as percentages. Chi-square test was used for statistical analysis.

Results: The mean age was 25.60±4.09 years and the majority 26 (49.1%) were between 18 to 25 years and presented with menstrual abnormalities. Volume of ovary was ≥10 cc in 41 (93.18%) patients with LH >6.7 IU/mL which was statistically significant (p=0.03) and LH/FSH ratio was >2:1 in 30 patients of which 28 (93.3%) had ovarian volume ≥10 cc which was statistically significant (p=0.03). A number of follicles >12 showed a significant association with LH (p=0.04), LH/FSH (p=0.01), testosterone (p=0.002). Stromal thickness also showed significant association with LH (p=0.018), LH/FSH (p=0.001), testosterone (p=0.04).

Conclusion: Predominant patients with PCOS had enlarged ovaries with multiple (>12), small-sized (<9 mm) peripherally arranged follicles and low FSH and elevated LH, LH/FSH ratio, testosterone, TSH, and prolactin levels.

Keywords: Abdominal obesity, Luteinising hormone, Prolactin, Testosterone, Thyroid stimulating hormone

INTRODUCTION

The PCOS is a complex endocrinologic disorder characterised by a combination of polycystic ovarian morphology, diverse clinical features, and abnormal biochemical indices that affects women of reproductive age [1]. According to the World Health Organisation (WHO), PCOS affects 116 million women (3.4%) worldwide [2]. Prevalence varies greatly around the world, ranging from 2.2-26%. PCOS affects approximately 10% of the women in India [3]. PCOS affects 50% of women who visit infertility clinics [4]. Women with PCOS present with three main clinical complaints-menstrual irregularities (mean incidence 70%), infertility (mean incidence 40%), and hyperandrogenism (mean incidence of Hirsutism 70%, acne 15-30%) [5]. PCOS is distinguished by low levels of FSH and elevated levels of LH. Low FSH levels cause anovulation, elevated LH levels cause hyperandrogenism, and insulin resistance symptoms range from simple cystic acne, cephalic hair loss, or mild facial hirsutism to oligomenorrhoea or amenorrhoea, sterility, and severe generalised hirsutism [6,7]. In the 1970s, the development of pelvic ultrasonography increased the diagnostic specificity of PCOS recognition. Following the Rotterdam Consensus meeting in 2003, ultrasound diagnostic criteria for PCOS diagnosis were developed [8].

Thus, from a clinical, biochemical, and imaging standpoint, the PCOS has progressed through many stages. Transvaginal sonography has improved our understanding of the internal structure of the ovary and endometrial morphology. In light of this, the current study was designed to identify and evaluate the sonological features in patients with clinical and biochemical features of PCOS. This study minimises the burden of expensive hormonal analyses in PCOS patients by assessing the association between hormonal status and ultrasonographic features.

MATERIALS AND METHODS

This cross-sectional study was done from September 2020 to September 2022 on 53 female patients in the reproductive age group (18-35 years), who were referred to the Department of Radiology, Kalinga Institute of Medical Sciences Bhubaneswar, Odisha, India for pelvic ultrasonography in clinical suspicion of PCOS (infertility, menstrual abnormalities, hirsutism, obesity). This study was approved by Ethics Committee (KIIT/KIMS/IEC/440/2022), and each woman gave informed consent. Consecutive sampling technique was adopted to recruit the study population.

Inclusion criteria: Patients with symptoms of menstrual irregularities, obesity, hirsutism, acne and infertility and have undergone ultrasound and hormonal investigations were included in study.

Exclusion criteria: Patients with ovarian hyperstimulation syndrome, pregnancy, patients with underlying endocrine disorders and on treatment were excluded from the study.

Study Procedure

All patients underwent transabdominal ultrasound and only married patients with abdominal obesity underwent transvaginal ultrasound. Ultrasound scans were performed between days 3 and 7 of the menstrual cycle by using GE-VOLUSON S10 ultrasound scanner using curvilinear probes of frequency ranging from 1 to 5 MHZ and transvaginal sectorial probe with frequency ranging from 5 to 9 MHZ. Volume and stromal thickness of the ovary was measured [Table/Fig-1a,b]. The total number of follicles were counted manually when scanning from one ovarian margin to the other, and it contain those with a diameter of 2 to 9 mm [Table/Fig-1c]. Whether follicles were mostly dispersed in a peripheral pattern or unevenly across the stroma is determined by the follicle distribution pattern. The diagnosis was made by Rotterdam criteria [9,10]. Fasting blood samples were collected and chemoluminescent assay was used to measure FSH and LH. TSH and serum prolactin levels were determined using Enzyme Linked Immunosorbent Assay (ELISA). By using high-performance liquid chromatography mass spectrometry testosterone was detected.
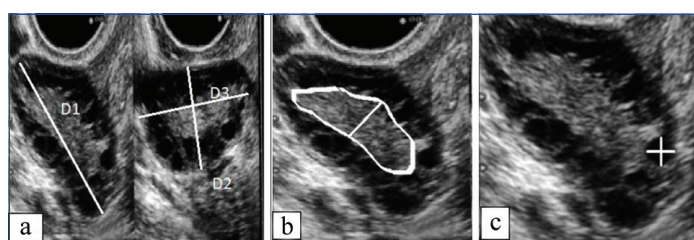
[Table/Fig-1]: a) Ovarian volume was calculated for each ovary using the formula for a prolate ellipsoid: P/6 (D1 D2 D3), where D represented the maximum diameter in transverse, antero-posterior and long section; b) The stromal thickness was obtained by outlining the peripheral profile of the stroma with a calliper; c) Follicle size was calculated by two perpendicular diameters, one of which represented the largest dimension of each follicle.

Outcome measured was to assess the association between volume of the ovary, size and number of follicles, and stromal thickness with hormones like FSH, LH, testosterone, prolactin, TSH.

STATISTICAL ANALYSIS

Categorical data was represented in the form of frequencies and proportions. Chi-square test was used to assess association between volume, size of the ovary, number of follicles, and stromal thickness with hormones like FSH, LH, testosterone, prolactin, TSH. Continuous data was represented as mean and standard deviation. The p-value of <0.05 was considered as statistically significant after assuming all the rules of statistical tests. All the analyses were done using Statistical Package for the Social Sciences (SPSS) software version 22.0.

RESULTS

The study included 53 participants with the mean age being 25.60±4.09 years and majority 26 (49.1%) were in between 18 to 25 years, 19 (35.8%) were in the age group of 26 to 30 years, and the rest were in age group 31 to 35 years. The common clinical presentation was oligomenorrhoea, seen in 39 (73.6%) of study population. On ultrasonography of abdomen, 48 (90.5%) study had ovarian volume of >10 cc, with follicles arranged peripherally in all patients. More than 12 follicles were observed in 32 (60.4%) of patients with majority 51 (96.1%) had follicle size of <9 mm. Ovarian stromal thickness of >10 mm was observed in 46 (86.79%) patients and endometrium was thickened and heterogenous in 1 (1.9%) patients while rest had homogenous endometrium [Table/Fig-2].

In this study, out of 44 patients with LH >6.7 IU/mL, volume of ovary was >10 in 93.18% patients (p-value=0.03). LH/FSH ratio was >2:1 in 30 patients of which 93.3% had ovarian volume >10 cc which was statistically significant (p-value=0.03) [Table/Fig-3].

Parameter	N=53
Age (years) M±SD	25.60±4.09
Female, n (%)	53 (100%)
Clinical features, n (%)	
Oligomenorrhoea	39 (73.6)
Obesity	24 (45.3)
Acne	14 (26.4)
Infertility	10 (18.9)
Hirsutism	8 (15.1)
Amenorrhoea	7 (13.2)
Ovarian characteristics	
Ovarian volume (cc)	13.64±3.32
Number of follicles	14.79±4.65
Follicular size (mm)	4.47±1.87
Stromal thickness (mm)	13.32±2.6
Hormonal analysis	
FSH (IU/L)	5.74±1.9
LH (IU/L)	11.03±3.17
LH:FSH	2.15±0.75
Testosterone (ng/mL)	9.93±2.54
TSH (IU/mL)	2.15±0.75
Prolactin (pg/mL)	14.15±3.22

[Table/Fig-2]: Baseline characteristics of patients included in this study.
FSH: Follicle stimulating hormone; LH: Luteinising hormone; TSH: Thyroid stimulating hormone

Hormonal analysis		Total number (N=53)	Volume of ovary (in cc)		p-value
			<10 (n=5)	≥10 (n=48)	
FSH (IU/L)	<10.5	51 (96.2%)	5 (9.8%)	46 (90.2%)	0.642
	>10.5	2 (3.8%)	0	2 (100%)	
LH (IU/L)	<6.7	9 (17%)	2 (22.2%)	7 (77.8%)	0.03*
	>6.7	44 (83.01%)	3 (6.8%)	41 (93.2%)	
LH/FSH	<2:1	23 (43.4%)	3 (5.7%)	20 (37.7%)	0.03*
	>2:1	30 (56.6%)	2 (6.7%)	28 (93.3%)	
Testosterone (ng/mL)	<10	19 (35.8%)	3 (15.7%)	16 (84.3%)	0.381
	>10	34 (64.2%)	2 (5.9%)	32 (94.11%)	
TSH (IU/mL)	<6.5	21 (39.6%)	4 (19%)	17 (81%)	0.51
	>6.5	32 (60.4%)	1 (3.1%)	31 (96.9%)	
Prolactin (pg/mL)	<20	45 (84.9%)	3 (6.7%)	42 (93.3%)	0.190
	>20	8 (15.1%)	2 (25%)	6 (75%)	

[Table/Fig-3]: Association between volume of ovary on ultrasound with biochemical hormonal analysis in PCOS patients.
*A p-value <0.05 is considered to be statistically significant

Out of 53 patients, majority (96.2%) had ovarian follicular size of <9 mm of which 86.2% had LH >6.7 IU/mL (p-value=0.001) [Table/Fig-4]. Number of follicles >12 was observed in 32 patients of which 26 patients had LH >6.7 IU/mL (p-value=0.04), LH/FSH >2:1 was seen in 21 patients (p=0.01), testosterone >10 ng/mL was seen in 27 patients (p-value=0.002) were statistically significant [Table/Fig-5]. Stromal thickness >10 mm was observed in 46 patients of which 43 patients had LH >6.7 IU/mL (p=0.018), LH/FSH >2:1 was seen in 29 patients (p<0.001), testosterone >10 ng/mL was seen in 32 patients (p-value=0.04) were statistically significant [Table/Fig-6].

DISCUSSION

In this study, almost half of the patients in 18-25 years of age group presented with clinical features like-oligomenorrhoea, obesity, acne, infertility, hirsutism, and amenorrhoea in descending order. Upon detailed evaluation, volume of ovary had statistically significant association with LH, LH/FSH ratio while ovarian follicular size had only with LH. Follicular number and stromal thickness of ovary had

Hormonal analysis		Total number (N=53)	Follicular size (in mm)			p-value
			<2 (n=5)	2.0-9.0 (n=46)	>9 (n=2)	
FSH (IU/L)	<10.5	51 (96.2%)	5 (9.8%)	45 (88.2%)	1 (2%)	0.680
	>10.5	2 (3.8%)	0	1 (50%)	1 (50%)	
LH (IU/L)	<6.7	9 (17%)	2 (22.2%)	5 (55.6%)	2 (22.2%)	0.001*
	>6.7	44 (83.01%)	3 (6.8%)	41 (93.2%)	0	
LH/FSH	<2:1	23 (43.4%)	3 (13%)	18 (78.3%)	2 (8.7%)	0.65
	>2:1	30 (56.6%)	2 (6.7%)	28 (93.3%)	0	
Testosterone (ng/mL)	<10	19 (35.8%)	4 (21.1%)	13 (68.42%)	2 (10.5%)	0.71
	>10	34 (64.2%)	1 (2.9%)	33 (97.1%)	0	
TSH (IU/mL)	<6.5	21 (39.6%)	3 (14.3%)	16 (76.2%)	2 (9.5%)	0.65
	>6.5	32 (60.4%)	2 (6..2%)	30 (93.8%)	0	
Prolactin (pg/mL)	<20	45 (84.9%)	2 (4.44%)	43 (95.66%)	0	0.397
	>20	8 (15.9%)	3 (37.5%)	3 (37.5%)	2 (25%)	

[Table/Fig-4]: Association between size of follicle (maximum) of ovary on ultrasound with biochemical hormonal analysis in PCOS patients.
*A p-value <0.05 is considered to be statistically significant

Hormonal analysis		Total number (n=53)	Number of follicles		p-value
			<12 (n=21)	≥12 (n=32)	
FSH (IU/L)	<10.5	51 (96.2%)	20 (39.2%)	31 (60.8%)	0.76
	>10.5	2 (3.8%)	1 (50%)	1 (50%)	
LH (IU/L)	<6.7	9 (17%)	3 (33.3%)	6 (18.7%)	0.04*
	>6.7	44 (83.01%)	18 (40.9%)	26 (59.1%)	
LH/FSH	<2:1	23 (43.4%)	12 (52.2%)	11 (47.8%)	0.01*
	>2:1	30 (56.6%)	9 (30%)	21 (70%)	
Testosterone (ng/mL)	<10	19 (35.8%)	14 (73.7%)	5 (26.3%)	0.002*
	>10	34 (64.2%)	7 (20.6%)	27 (79.4%)	
TSH (IU/mL)	<6.5	21 (39.6%)	14 (66.7%)	7 (33.3%)	0.53
	>6.5	32 (60.4%)	7 (21.9%)	25 (78.1%)	
Prolactin (pg/mL)	<20	45 (84.9%)	18 (40%)	27 (60%)	0.618
	>20	8 (15.1%)	3 (37.5%)	5 (62.5%)	

[Table/Fig-5]: Association between number of follicles in an ovary on ultrasound with biochemical hormonal analysis in PCOS patients.
*A p-value <0.05 is considered to be statistically significant

Hormonal analysis		Total number (n=53)	Stromal thickness (in mm)		p-value
			<10 (n=7)	≥10 (n=46)	
FSH (IU/L)	<10.5	51 (96.2%)	6 (11.8%)	45 (88.2%)	0.117
	>10.5	2 (3.8%)	1 (50%)	1 (50%)	
LH (IU/L)	<6.7	9 (17%)	6 (66.7%)	3 (33.33%)	0.018*
	>6.7	44 (83.01%)	1 (2.3%)	43 (97.7%)	
LH/FSH	<2:1	23 (43.4%)	6 (26.1%)	17 (73.9%)	<0.001*
	>2:1	30 (56.6%)	1 (3.3%)	29 (96.7%)	
Testosterone (ng/mL)	<10	19 (35.8%)	5 (26.3%)	14 (73.7%)	0.04*
	>10	34 (64.2%)	2 (5.9%)	32 (94.1%)	
TSH (IU/mL)	<6.5	21 (39.6%)	4 (19.1%)	17 (80.9%)	0.12
	>6.5	32 (60.4%)	3 (9.4%)	29 (90.6%)	
Prolactin (pg/mL)	<20	45 (84.9%)	1 (2.2%)	44 (97.8%)	0.621
	>20	8 (15.1%)	6 (75%)	2 (25%)	

[Table/Fig-6]: Association between stromal thickness of ovary on ultrasound with biochemical hormonal analysis in PCOS patients.
*A p-value <0.05 is considered to be statistically significant

significant association with serum LH, testosterone levels and LH/FSH ratio.

In a prospective study with 214 patients by Jonard S et al., concluded that, median age was 27 years with majority in 21-34 years age group [11]. The most common presentation was oligomenorrhoea, seen in 39 (73.6%) patients while amenorrhoea was seen only in 7 (13.2%). In a study by Sangabathula H and Varaganti N in a similar setting observed that 87% had oligomenorrhoea and 13%

had amenorrhoea which was consistent with the present study [12]. While another study by Peri N and Levine D with 245 patients with polycystic ovarian disease stated that 146 (59.5%) of these patients had irregular menstrual cycles, 38 (15.5%) had hirsutism and 15 (6.1%) had infertility. This difference was due to geographical variations and inclusion of older women in the study ranging from 16-49 years [4].

Of 50 patients, the mean volume of the ovary was 13.64±3.32 cc with ≥10 cc in 48 (90.5%) patients while the rest had <10 cc. Adams J et al., provided support for this finding that the majority 70% of PCOS patients displayed 10 cc [13]. In a study by Hann LE et al., with 28 patients concluded what 71% of them had enlarged ovarian volume with mean volume at 14 cc and 71% had peripherally arranged follicles which was consistent with the present study [14].

On detailed examination of follicle, the mean size of follicle was 4.47±1.87 mm with ≤9 mm in majority of patients with only 2 (2.4%) had >9 mm. In a study by Adams J et al., they concluded that 2-9 mm follicles were seen in 60% of patients which was consistent with current study [13]. Evaluation of stroma revealed thickened and echogenic in 46 (86.79%) of patients. In a study by Ardaens Y et al., with majority (64.9%) had abnormal stroma which was consistent with the present study [15]. Majority had normal and homogenous endometrium while only around 2% had thickened and heterogenous which was consistent with findings of a study by Peri N and Levine D with 245 patients concluded that 93% patients had homogenous endometrium [4].

Ultrasonographic features when correlated with biochemical hormonal analysis revealed that larger ovarian volume had significant association with LH level and LH/FSH ratio. In a study by Nardo LG with 23 patients, concluded that ovarian volume had significant association with LH, while stromal volume had no significant association [16]. In a study by Sidhmalswamy AG et al., with 74 PCOS patients, concluded that ovarian volume had a significant association with hyperandrogenism [17].

Ovarian follicular size had a statistically significant association with LH, which was consistent with a study by Pache TD et al., [18]. Follicular number and stromal thickness had significant association with LH, testosterone and LH/FSH ratio. Takahashi K et al., noted a positive correlation between number of small follicles (2-8 mm) and serum testosterone level [5].

In a study by Dolz M et al., concluded that follicular number had significant association with LH, LH/FSH ratio [19]. In a study by Loverro G et al., with 24 PCOS patients stated that increased ovarian stomal thickness had significant association with elevated serum LH, and testosterone levels [20].

Limitation(s)

Antimullerian hormone level an important parameter, was not evaluated in the study and the patients underwent biochemical hormonal analysis on multiple days of menstrual cycle.

CONCLUSION(S)

Patients of PCOS had enlarged ovaries (>10 cc) with multiple (>12) small sized (<9 mm) peripherally arranged follicles. Upon hormonal analysis patients had low FSH and elevated LH, LH/FSH ratio, testosterone, TSH, and prolactin levels. Sonographic features had significant association with LH, LH/FSH, testosterone levels. So, it is concluded that clinicians should advise screening ultrasonography in cases of suspected PCOS for early diagnosis and prompt management.

REFERENCES

[1] Belinda MS, Richard PD. Polycystic ovarian syndrome and the metabolic syndrome. Am J Med Sci. 2005;330(6):336-42.

[2] Kabel AM. Polycystic ovarian syndrome: Insights into pathogenesis, diagnosis, prognosis, pharmacological and non-pharmacological treatment. Pharm Bioprocess. 2016;4(1):07-12.

[3] Inan C, Karadag C. Correlation between ovarian morphology and biochemical and hormonal parameters in polycystic ovary syndrome. Pak J Med Sci. 2016;32(3):742-45.

[4] Peri N, Levine D. Sonographic evaluation of the endometrium in patients with a history or an appearance of polycystic ovarian syndrome. J Ultrasound Med. 2007;26(1):55-58; quiz 59-60. Doi: 10.7863/jum.2007.26.1.55. PMID: 17182709.

[5] Takahashi K, Eda Y, Abu Musa A, Okada S, Yoshino K, Kitao M. Transvaginal ultrasound imaging, histopathology and endocrinopathy in patients with polycystic ovarian syndrome. Human Reproduction. 1994;9(3):1231-36.

[6] Kambale T, Sawaimul KD, Prakash S. A study of hormonal and anthropometric parameters in polycystic ovarian syndrome. Annals of African Medicine. 2023;22(1):112.

[7] Lancuba S, De Marco MJ, Thomson MS, Tesone M. Endocrinological causes of female infertility. In Management of Infertility. 2023 (pp. 65-70). Academic Press.

[8] Adam HB, Joop SE, Tan SL, Dewailly D. Ultrasound assessment of the polycystic ovary: International consensus definitions. Human Reproduction Update. 2003;9(6):505-14.

[9] Smet ME, McLennan A. Rotterdam criteria, the end. Australas J Ultrasound Med. 2018;21(2):59-60. Doi: 10.1002/ajum.12096. PMID: 34760503; PMCID: PMC8409808.

[10] Arpitha KJ, Ravi N. Transvaginal ultrasound diagnosis of polycystic ovarian syndrome and its correlation with biochemical parameters. Int J Contemp Med Sur Radiol. 2020;5(2):B21-B26.

[11] Jonard S, Robert Y, Cortet-Rudelli C, Pigny P, Decanter C, Dewailly D. Ultrasound examination of polycystic ovaries: Is it worth counting the follicles? Human Reprod. 2003;18(6):598-603.

[12] Sangabathula H, Varaganti N. Clinical profile polycystic ovarian syndrome-100 cases. J Comtemp Med Res. 2017;4(6):1249-53.

[13] Adams J, Polson D, Abdulwahid N, Morris D, Franks S, Mason H, et al. Multifollicular ovaries: Clinical and endocrine features and response to pulsatile gonadotropin releasing hormone. The Lancet. 1985;326(8469-8470):1375-79.

[14] Hann LE, Hall DA, Mc Ardle CR, Seibel M. Polycystic ovarian disease: Sonographic spectrum. Radiology. 1984;150(2):531-34.

[15] Ardaens Y, Robert Y, Lemaitre L, Fossati P, Dewailly D. Polycystic ovarian disease: Contribution of vaginal endosonography and reassessment of ultrasonic diagnosis. Fertility and Sterility. 1991;55(6):1062-68.

[16] Nardo LG. Three-dimensional assessment of ultrasound features in women with clomiphene citrate-resistant polycystic ovarian syndrome (PCOS): Ovarian stromal volume does not correlate with biochemical indices. Human Reprod. 2002;17(4):1052-55.

[17] Sidhmalswamy AG, Ghongdemath JS, Venkatesh S. Clinical, ultrasonographical and hormonal correlation in women with polycystic ovarian syndrome. Int J Reprod Contracept Obstet Gynecol. 2018;7(12):5134.

[18] Pache TD, Wladimiroff JW, Hop WC, Fauser BC. How to discriminate between normal and polycystic ovaries: Trans-vaginal ultrasound study. Radiology. 1992;183(2):421-23.

[19] Dolz M, Osborne NG, Blanes J, Raga F, Abad-Velasco L, Villalobos A, et al. Polycystic ovarian syndrome: Assessment with color doppler angiography and three dimensional ultrasonography. J Ultrasound Med. 1999;18(1):303-13.

[20] Loverro G, De Pergola G, Di Naro E, Tartagni M, Lavopa C, Caringella AM. Predictive value of ovarian stroma measurement for cardiovascular risk in polycyctic ovary syndrome: A case control study. J Ovarian Res. 2010;3:25.

PARTICULARS OF CONTRIBUTORS:
1. Junior Resident, Department of Radiodiagnosis, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India.
2. Professor, Department of Radiodiagnosis, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India.
3. Professor, Department of Radiodiagnosis, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India.
4. Assistant Professor, Department of Radiodiagnosis, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Sangram Panda,
Assistant Professor, Department of Radiodiagnosis, Kalinga Institute of Medical Sciences, Patia, Bhubaneswar-751024, Odisha, India.
E-mail: sangram.aju@gmail.com

AUTHOR DECLARATION:
• Financial or Other Competing Interests: None
• Was Ethics Committee Approval obtained for this study?   Yes
• Was informed consent obtained from the subjects involved in the study?   Yes
• For any images presented appropriate consent has been obtained from the subjects.   Yes