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Association of Serum Cancer Antigen 125 and Carcinoembryonic Antigen with Menstrual Irregularities in Young Non-Hospitalized Females

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Abstract

Menstrual irregularities affect a substantial proportion of young females in South Asia and are often accompanied by mesothelial endometriosis, pelvic inflammatory disorders, and polycystic ovary syndrome (PCOS). This study aimed to document serum carcinoembryonic antigen (CEA) and serum cancer antigen 125 (CA 125) levels in young, non-hospitalized female students with menstrual irregularities across higher education institutions in Islamabad and Rawalpindi. A cross-sectional study was conducted on 200 female students (aged 18 to 25) with pre-screened menstrual irregularities. A structured form documented demographics and clinical parameters. Venous blood samples were collected, and the serum concentrations of both markers (CA 125 and CEA) were quantified using enzyme-linked immunosorbent assay (ELISA), with rigorous statistical validation employing Chi-square categorical association ($p < 0.05$). In the population, 2.5% (n = 5) had elevated CEA levels (>5.0 ng/mL), and 7.0% (n = 14) had abnormal CA 125 antigen levels (>35.0 U/mL). Abnormally high CA 125 levels were strongly associated with severe dysmenorrhea ($\chi2 = 6.31$, $p = 0.012$) and increased body mass index (25.0 kg/m^2). Elevated CA 125 in young females was associated with dysmenorrhea severity and increased body mass index, a pattern consistent with benign pelvic pathology such as endometriosis or pelvic inflammatory disease rather than malignancy. However, this cross-sectional sample lacked clinical and imaging correlation, and these findings should be confirmed in longitudinal studies.

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Introduction

Menstrual irregularities are among the most neglected health concerns among young females worldwide, affecting a substantial number (20-30%) of females during the reproductive years. In South Asian countries, especially Pakistan, polymenorrhea, oligomenorrhea, and severe dysmenorrhea are the most widely reported menstrual abnormalities among university-enrolled females across higher education institutions. These abnormalities are indirectly related to structural or functional pelvic disorders, including, but not limited to, early-stage endometriosis, benign ovarian cysts, polycystic ovarian syndrome (PCOS), or pelvic inflammatory disorders (PID). Despite this high burden, young females in resource-restricted settings in Pakistan rarely undergo preliminary biomarker screening or any diagnostic evaluations until symptoms compromise their fertility.

Cancer antigen (CA 125) is a dense, high-molecular-weight glycoprotein used as a preliminary biomarker for evaluating epithelial cells related to ovarian cancer. However, this diagnostic marker is not limited to ovarian-related anomalies; its expression is upregulated during mechanical stretching, mesothelial regulation of the peritoneum, cytokine dysregulation, and interleukin-induced inflammation. Consequently, elevated serum CA 125 levels (>35 U/mL) are documented in various non-malignant conditions, including uterine fibroid formation, active endometriosis, phases of the menstrual cycle, and pelvic infections. This non-specific mesothelial release of CA 125 is a better predictor of inflammation than an exact indicator of ovarian-related abnormalities. Apart from CA 125, carcinoembryonic antigen (CEA) is another glycoprotein analyzed during the management of colorectal, mucinous, and gastrointestinal disorders, with mild increases in baseline CEA (>5.0 ng/mL) observed during systemic inflammation, exposure to passive smoking, and metabolic dysfunctions [7-9].

While the diagnostic utility of CEA and CA 125 in late-stage oncology is well established in tertiary care facilities across various districts of Pakistan, screening among young and non-hospitalized groups remains underreported [10]. Evaluating these antigenic markers among university students with menstrual irregularities provides a valuable baseline for assessing epidemiological data. It also helps identify benign pelvic pathologies, warranting early, non-invasive clinical follow-up across diagnostic laboratories [11]. This study aims to quantify serum CEA and CA 125 among female university students with menstrual irregularities in Islamabad and Rawalpindi and assess their relationship with body mass index (BMI) and symptom severity.

Materials and Methods

Study design and setting

We conducted a cross-sectional observational analysis at four major higher education institutions in Pakistan's twin cities: IQRA University, Pir Mehr Ali Shah Arid Agriculture University (PMAS-AAUR), National University of Sciences and Technology (NUST), and Fatimah Jinnah Women University (FJWU). The laboratory procedures followed standard medical laboratory technology protocols issued by the National Institute of Health (NIH) and the National Bioethics Committee (NBC).

Population and eligibility criteria

The observed population comprises female university students aged 18 to 25 years who have been diagnosed with persistent menstrual irregularities. All 200 analyzed participants had menstrual irregularities, and no regular-cycle comparison group was enrolled, so all analyses are within-cohort. For the evaluation of serum CEA and CA 125 levels, venous blood (3 ml) was collected, along with a well-structured questionnaire to assess lifestyle factors and gynecological history.

Inclusion and exclusion criteria

Participants were unmarried females aged 18 to 25 years who reported menstrual irregularities persisting for at least three consecutive months, categorized as either Polymenorrhea (cycle duration <21 days) or oligomenorrhea (cycle duration >35 days). All participants voluntarily provided written informed consent before enrollment and sample collection. Females with a prior diagnosis of systemic, cancer, or pelvic disorders were excluded. We also excluded students with active or recent pregnancy. We also excluded students with autoimmune, inflammatory, or chronic hepato-renal conditions.

Sample size

The sample size for this cross-sectional study was calculated using the World Health Organization (WHO) [12]. The confidence interval, prevalence of menstrual irregularities, and the error margin were set to 95%, 15%, and 5%, respectively. The required sample was 196; to account for potential hemolysis, 200 students were enrolled.

Clinical scoring: data collection

Before blood collection, students completed a well-structured, pretested questionnaire to collect data on age, weight, height, dysmenorrhea severity (mild, moderate, or severe based on analgesic use and restrictions in daily activity), menstrual irregularity, and first-degree family history of anomalies. Body mass index was calculated as kg/m^2 and further stratified as per the criteria of WHO [13, 14]: underweight: (<18.5 kg/m^2); normal: (18.5–24.9 kg/m^2) and overweight: (≥25.0 kg/m^2)

Laboratory procedure

Blood samples (3mL) were extracted from each participant using sterile equipment (aseptic conditions) and transferred into gel-barrier serum separator tubes (SST). Samples were allowed to clot at room temperature (27^0C) for 30 minutes and centrifuged at 3000 rpm for 10 minutes to separate serum from plasma. The serum aliquots were immediately stored at 20^0C till further analysis. Serum CA-125 (Cat No. ELH-CA125-1, RayBiotech, Inc., Peachtree Corners, GA, USA) and CEA (Cat No. KE00281, Proteintech Group, Inc., Rosemont, IL, USA) levels were quantified via sandwich ELISA following standard manufacturer incubation, washing, and TMB substrate protocols. Absorbance was recorded at 450 nm using a microplate reader (Model xMark, Bio-Rad Laboratories, Inc., Hercules, CA, USA), with concentrations calculated in duplicate against standard curves in accordance with standard biosafety and clinical protocols [15-17].

Ethical considerations

The departmental institutional review board (IRB) at IQRA University Chak Shehzad approved the proposed protocol and questionnaire (approval no IRB/IUCS/AHS/2026/011, dated: May 04, 2026), and strict measures were taken to obtain written informed consent from participants. Confidentiality of data was maintained through de-identification; unique numbers were assigned to each specimen, and access was given only to authorized investigators [18].

Statistical analysis

Data were computed using SPSS Version 26.0 (IBM Corporation. Categorical variables (types of irregularities, BMI groups, biomarker elevations) were presented as percentages, means, and frequencies. Pearson Chi-Square (x^2) test was used to determine categorical associations between elevated serum levels and observed clinical parameters. Expected cell counts were verified before applying the Pearson chi-square test; where any expected count was below 5 (family history of cancer and the BMI comparison), Fisher's exact test was used, and exact p-values are reported. The *p*-value was set to <0.05 for the data to be statistically significant. No correction for multiple comparisons was applied because the analyses were exploratory and hypothesis-generating; p-values should therefore be interpreted with caution.

Results

Population baseline profile and clinical parameters

The mean age of all subjects was 22.4 ± 1.9 years, and most subjects (n = 116, 58.0%) were between 19 and 22 years. Regarding BMI, 114 participants (57.0%) were within the optimal range, while 58 participants (29% of the population) were in the overweight category. About the menstrual patterns, most of the subjects were dealing with oligomenorrhea (n = 92, 46.0%), followed by painful cycles of dysmenorrhea (n = 72, 36.0%) and mild Polymenorrhea (n = 36, 18.0%) (Fig. 1). Moreover, a positive family history of subsequent anomalies was also documented in n = 22 subjects (11.0%) of the cohort under observation (Table 1).

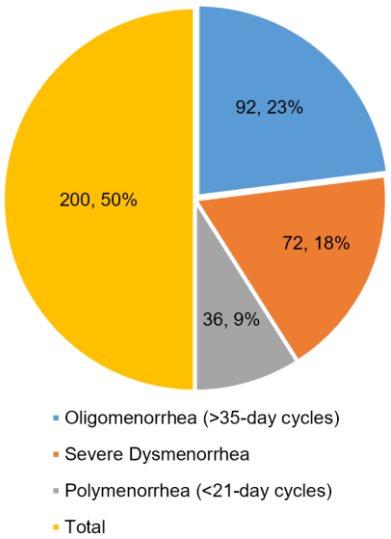


Fig. 1 Distribution of menstrual irregularity types in the study cohort (n = 200). Oligomenorrhea was the most common irregularity (46.0%, n = 92), followed by severe dysmenorrhea (36.0%, n = 72) and polymenorrhea (18.0%, n = 36). Percentages represent the proportion of the total cohort (n = 200). Numbers in parentheses indicate the number of participants in each category.

Table 1 Anthropometric measures, baseline characteristics and irregularity profile of population under observation.

Variable	Category	Frequency (n)	Percentage (%)
Age Group	18–21 years	116	58.0
	22–25 years	84	42.0
BMI Stratification	Underweight (<18.5 kg/m²)	28	14.0
	Normal (18.5–24.9 kg/m²)	114	57.0
	Overweight (≥ 25.0 kg/m²)	58	29.0
Dominant Menstrual Irregularity	Oligomenorrhea (>35-day cycles)	92	46.0
	Polymenorrhea (<21-day cycles)	36	18.0
	Severe Dysmenorrhea	72	36.0
Family History of Cancer	Present (First-degree relative)	22	11.0
	Absent	178	89.0

Table 2 Dysregulation of serum CA-125 and CEA levels among participants (n = 200).

Biomarker	Mean ± SD	Reference Cutoff	Normal (≤ Cutoff) n (%)	Elevated (> Cutoff) n (%)
Serum CA-125	18.4 ± 11.2 U/mL	35.0 U/mL	186 (93.0)	14 (7.0)
Serum CEA	1.8 ± 1.1 ng/mL	5.0 ng/mL	195 (97.5)	5 (2.5)

Prevalence and tumor marker quantification

The mean serum CEA concentration was 1.8 ± 1.1 ng/mL, and the mean serum CA-125 concentration was 18.4 ± 11.2 U/mL. Both markers were right-skewed; the median (IQR) was 15.6 (10.2–24.8) U/mL for CA-125 and 1.6 (1.0–2.4) ng/mL for CEA, with observed ranges of 4.1–58.7 U/mL and 0.3–7.9 ng/mL, respectively. Serum CA-125 was elevated (>35.0 U/mL) in 14 participants (7.0%), and serum CEA was elevated (>5.0 ng/mL) in 5 participants (2.5%) (Table 2).

Bivariate analysis: categorical association

Cross-tabulation analysis revealed a statistically significant association between dysmenorrhea severity and abnormal CA 125 levels ($\chi^2 = 6.31$, $p = 0.012$). Furthermore, elevated CA 125 levels were present in 9 of 72 participants with severe dysmenorrhea (12.5%) compared with 5 of 128 participants with mild/moderate dysmenorrhea (3.9%) ($\chi^2 = 6.31$, $p = 0.012$). Moreover, serum CA 125 levels were elevated in participants with higher BMI (≥ 25.0 kg/m²), with 7 (12.1%) overweight participants showing elevated titers compared with 7 (4.9%) normal ones ($\chi^2 = 4.31$, $p = 0.038$). No statistically significant association was observed between family history of malignancies and CA 125 ($p = 0.345$) (Table 3).

Discussion

The current study documented the serum CEA and CA 125 profiles in a cohort of non-hospitalized female university students aged 18 to 25 years, presenting with menstrual irregularities in public and private higher education sectors in Islamabad and Rawalpindi. The main goal was to establish screening profiles to determine elevations of subclinical biomarkers with menstrual parameters and sociodemographic factors. Because this was a cross-sectional study with no imaging or surgical confirmation, the associations reported here are unadjusted and cannot establish causality or a specific underlying diagnosis.

Our findings highlighted elevations of serum CEA (n= 5) and CA 125 (n = 14) among the cohort. In asymptomatic participants, higher levels of CA 125 are primarily driven by non-malignant pelvic disorders rather than cancer-related disorders [19, 20]. The elevated levels of CA 125 are secreted by the mesothelial linings of the fallopian tubes and peritoneum walls in response to abnormal levels of cytokines, pelvic remodeling, and microhemorrhage (tiny, localized bleeding) [21]. A critical finding of this investigation is the significant link between severity of dysmenorrhea and higher levels of CA 125 ($p = 0.012$). Females with extreme, disabling pain during the late stage of the menstrual cycle showed a 12.5% rate of serum CA 125 elevation [22-24]. This result aligns with reported pathophysiological profiles, highlighting CA 125 as a secondary biomarker for initial screening of adenomyosis, pelvic inflammatory disorders (PID), and mesothelial-related endometriosis [25, 26]. Furthermore, during early stages of endometriosis, endometrial implants are documented to produce a localized immune response (upregulated cytokines and interleukins that support inflammation) during late-stage menstruation, leading to retroperitoneal shredding with subsequent vascular permeability, allowing CA 125 to move into systemic circulation

Table 3 Bivariate cross-tabulation analysis of factors associated with serum CA-125 elevation (n = 200).

Clinical Parameter	CA-125 Normal (≤ 35 U/mL) n (%)	CA-125 Elevated (> 35 U/mL) n (%)	Total n (%)	χ^2	p-value
Dysmenorrhea Severity				6.31	0.012
Mild/Moderate (n = 128)	123 (96.1)	5 (3.9)	128 (100)		
Severe (n = 72)	63 (87.5)	9 (12.5)	72 (100)		
BMI Category				4.31	0.038
Underweight/Normal (n = 142)	135 (95.1)	7 (4.9)	142 (100)		
Overweight (n = 58)	51 (87.9)	7 (12.1)	58 (100)		
Family History of Cancer				0.89	0.345
Present (n = 22)	20 (90.9)	2 (9.1)	22 (100)		
Absent (n = 178)	166 (93.3)	12 (6.7)	178 (100)		

Pearson chi-square was used for dysmenorrhea severity; Fisher's exact test was used for BMI category and family history of cancer because of expected cell counts below 5

allowing CA 125 to move into systemic circulation [27]. In unadjusted analysis, elevated CA 125 levels were associated with BMI ($p = 0.038$); excess adipose tissue is metabolically active and secretes adipokines and TNF-α, producing chronic low-grade inflammation [28, 29], which may explain this association. Because no multivariable adjustment was performed, this relationship may be confounded by age or dysmenorrhea severity [28, 29]. This metabolic condition is closely related to polycystic ovarian syndrome (PCOS), where low-grade inflammation in the pelvis, along with follicular arrest, is associated with low-to-high biomarker fluctuations [30]. In contrast, CEA levels did not surpass the minimum threshold (97.5% reference limit) in the studied population. This comparatively low prevalence (2.5%) of elevated serum CEA levels reflects the specific nature of this antigenic marker for mucinous carcinomas and mucosal lesions in the gastrointestinal tract, which are very rare in young females [10, 31]. Minor variation in CEA levels may reflect limited environmental exposure or non-specific dietary factors among the enrolled participants.

From the perspective of a laboratory technologist, these findings reveal the diagnostic value of integrating enzyme-linked immunosorbent assay (ELISA) screening in combination with clinical symptom scoring for better gynecological outcomes. Detecting CA 125 elevations on subclinical grounds in young non-hospitalized females with dysmenorrhea provides a non-invasive screening protocol for better identification of candidates requiring either gynecological evaluation or pelvic ultrasonography.

Limitations

The study has many limitations, including failure to establish a causal relationship over varying time frames (cross-sectional study design) and the inability to perform ultrasonographic procedures to better underscore expected conditions like polycystic ovarian syndrome or endometriosis. Prospective studies should focus on multicenter longitudinal designs that integrate imaging modalities with biomarker-based diagnostics.

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Conflicts of Interests

The authors share no competing interests in the preparation of this manuscript.

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