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The relation between vitamin D and the adolescents' mid-luteal estradiol and progesterone

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Abstract. – OBJECTIVE: The aim of this study was to detect the effect of vitamin D (Vit. D) intake on the mid-luteal estradiol (E_2), and progesterone (P), and the relation between vit. D, and the adolescents' mid-luteal E_2, and P.

PATIENTS AND METHODS: Eighty-five (85) adolescents were recruited for this cohort study after obtaining informed consent. After a detailed history and clinical examination, the body mass index (BMI) of the studied participants was calculated, followed by pelvic sonography to exclude any pelvic pathology. Participants' blood samples were collected on days 21-22 of the menstrual cycle (mid-luteal) to measure the thyrotropin (TSH) (i.e., to exclude hypothyroidism), prolactin (i.e., to exclude hyperprolactinemia), glycosylated hemoglobin (HbA1C), (i.e., to exclude diabetes), E_2, P, and 25(OH)D. Participants received 50,000 IU of vit. D weekly for two months, and on the 3rd month, the mid-luteal E_2, P, and 25(OH)D were measured. The mid-luteal E_2, P, and 25(OH)D were compared before and after the vit. D intake to detect the effect of vit. D intake (50,000 IU weekly for 2 months) on the mid-luteal E_2 and P (primary outcome). Additionally, the relations between vit. D and the adolescents' mid-luteal E_2 and P were detected as secondary outcomes using the correlation analysis (Pearson's correlation).

RESULTS: The mid-luteal E_2 and P statistically decreased from 109.3±15.7 pg/mL and 9.8±1.01 ng/mL, respectively to 40.7±10.52 pg/mL, and 5.2±0.73 ng/mL, respectively, after vit. D intake (p=0.00015; 95% CI: 64.5, 68.6, 72.7, and p=0.0016; 95% CI: 4.3, 4.6, 4.87, respectively). Significant negative correlations between the 25(OH)D, and both the mid-luteal E_2 (r -0.661; p<0.00001), and P (r -0.521; p<0.00001) were detected in this study.

CONCLUSIONS: The mid-luteal E_2 and P statistically decreased after vit. D intake (50,000 IU of vit. D weekly for 2 months). Significant negative correlations between the 25(OH)D, and both the mid-luteal E_2 and P were detected in this study. The relation between vit. D and ovarian steroids, and the effect of vit. D intake on ovarian steroids need further larger studies.

Key Words:
Vitamin D, Vit. D, Adolescents, Mid-Luteal, Estradiol, Progesterone.

Introduction

The Relation Between Vitamin D and the Adolescents' Mid-Luteal Estradiol and Progesterone

The adequate vitamin D (Vit. D) concentrations have been associated with a range of reproductive outcomes[1,2].

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Previous studies[3,4] suggest a possible relation between vit. D and polycystic ovary syndrome (PCOS).

Granulosa cells contain vit. D receptors (VDR), and *in-vitro* stimulation of human ovarian cells with $1,25(OH)_2D$ (1,25-dihydroxy vit. D) affects the ovarian steroidogenesis[5].

Experimental studies[5,6] reported an influence of vit. D metabolites on the reproductive hormones production.

Mouse models[7] showed an association between VDR defect and decreased aromatase enzyme (which converts the androgen to estrogen) activity and expression.

Moreover, the $1,25(OH)_2D$ deficiency was associated with reduced corpus luteum function and progesterone (P) production[6].

In-vitro studies[8] on the porcine granulosa cells showed no effect of $1,25(OH)_2D_3$ on basal P production. At the same time, other studies[9] showed a reduced progesterone synthesis after $1,25(OH)_2D_3$ treatment.

The experimental effect of vit. D on estrogen is clearer[10]. Studies on human[11], porcine[12], and goat[13] granulosa cells revealed a stimulatory effect of $1,25(OH)_2D_3$ on both the estradiol (E_2) and aromatase enzyme.

Moreover, the calcium metabolism hormones, including vit. D levels are changed with endogenous E_2 changes (i.e., pregnancy and menopause), and with the use of oral contraceptive estrogen-containing tablets[6].

The relation between the 25-hydroxy vit. D [25(OH)D] and steroid hormones in normal[14], and PCOS women[15] was inconsistently reported.

The role of vit. D on ovarian steroids is not intensively studied. Therefore, the current study is designed to detect the effect of vit. D intake on the mid-luteal E_2, and P, and the relation between vit. D, and the adolescents' mid-luteal E_2, and P.

Patients and Methods

Eighty-five (85) adolescents were recruited for this prospective cohort study, which was conducted in Aktobe-West Kazakhstan over two years (2021-2022), to detect the effect of vit. D intake on the mid-luteal E_2, and P, and the relation between vit. D, and the adolescents' mid-luteal E_2, and P.

Participants were recruited for the current prospective cohort study, after the approval from the ethics committee of West Kazakhstan Medical University (No. 10, dated 04.10.2020) and after obtaining the informed consent from the adolescents themselves, and their guardians. The study followed ethics guidelines of the Helsinki Declaration.

After a detailed history, and clinical examination, the body mass index (BMI) of the studied participants was calculated, followed by a pelvic sonography to exclude any pelvic pathology (i.e., especially PCOS).

Inclusion criteria include adolescents (12-18 years old), with regular menstrual cycles, normal BMI 18.5-24.9 Kg/m^2, and vit. D deficiency.

Exclusion criteria include adolescents <12 years old or >18 years old, underweight (18.5 Kg/m^2 BMI), overweight (25-29.9 Kg/m^2) or obese (BMI >30 Kg/m^2)[16,17], with irregular menstrual cycles, known medical disorders (i.e., diabetes, or hypertension), known endocrine disorders (i.e., PCOS, thyroid, or hyperprolactinemia), received exogenous hormones or vit. D supplement within the 6 months, and/or refused to participate.

Regular menstrual cycles were defined as menstrual flow on a regular basis every 21-35 days. The serum 25(OH)D is a reliable indicator for the vit. D status[18]. It reflects the cutaneous production of vit. D and the vit. D intake and it has a long half-life[18].

The normal serum vit. D was defined as 25(OH)D >30 ng/mL, while <20 ng/mL 25 (OH) D was defined as vit. D deficiency[18].

The serum 25(OH)D was measured using the spectrophotometric method since it forms a pink chloroform that can be read at 500 nm wavelength when it binds with the antimony trichloride.

Diabetes was defined according to the American diabetic association (ADA) as a group of metabolic disorders characterized by hyperglycemia resulting from either deficient insulin secretion and/or insulin action[19].

Diabetes is diagnosed when the HbA1c ≥6.5% or fasting plasma sugar ≥126 mg/dL (7 mmol/L), or 2-hrs plasma glucose ≥200 mg/dL (11.1 mmol/L)[19].

Hypertension is ≥140 mmHg systolic blood pressure, and/or ≥90 mmHg diastolic blood pressure (on two different days)[20].

The diagnosis of PCOS was based on two criteria of the following ESHRE/ASRM criteria[16,17], 1) polycystic ovaries by ultrasound, 2) oligo-/anovulation, and 3) clinical evidence of hyperandrogenism (hirsutism and acne).

Participants' blood samples were collected on days 21-22 of the menstrual cycle (mid-luteal)

to measure the thyrotropin (TSH) (i.e., to exclude hypothyroidism), prolactin (i.e., to exclude hyperprolactinemia), glycosylated hemoglobin (HbA1C), (i.e., to exclude diabetes), E_2, progesterone (P), and 25(OH)D.

The normal serum TSH ranges from 0.4-4.0 mIU/mL[21], normal serum prolactin <29 ng/mL[22], and normal HbA1C <6.5%[23].

The normal mid-luteal E_2 ranges from 60-200 pg/mL, and the normal mid-luteal P in adolescents with regular menstrual and ovulatory cycles is >7 ng/mL[24].

The participants received 50,000 IU of vit. D (Lamyra Pharmacare, Harrow, Middlesex, England) weekly for two months according to the hospital's protocol, and on the 3rd month, the mid-luteal E_2, P, and 25(OH)D were measured.

The mid-luteal E_2, P, and 25(OH)D were compared before and after the vit. D intake to detect the effect of vit. D intake (50,000 IU weekly for 2 months) on the mid-luteal E_2 and P (primary outcome). The secondary outcome measured the relation between vit. D and the adolescents' mid-luteal E_2 and P.

Statistical Analysis

The G Power 3.1.9.7 (Heinrich Heine Universität, Düsseldorf; Germany)[25], with 0.05 probability, 0.95% power, 0.5 sample size, and Student's *t*-test for statistical analysis was used for sample size calculation[26,27]. Student's *t*-test, and correlation analysis (Pearson's correlation) were used for statistical analysis. $p<0.05$ was considered significant.

Results

Eighty-five (85) adolescents were recruited for this cohort study to detect the effect of vit. D intake (50,000 IU weekly for 2 months) on the mid-luteal E_2 and P (primary outcome), and the relation between vit. D and the adolescents' mid-luteal E_2 and P (secondary outcome).

Table I. Participants` characteristics, 25(OH)D, mid-luteal estradiol, and progesterone.

Variables	Studied participants (n = 85 adolescents)
Age (Years)	15.4 ± 1.3
Weight (Kg)	59.92 ± 4.3
Height (Cm)	159.0 ± 2.75
BMI (Kg/m^2)	23.3 ± 1.4
25(OH)D (ng/mL)	13.4 ± 3.01
Estradiol (pg/mL)	109.3 ± 15.7
Progesterone (ng/mL)	9.8 ± 1.01

25(OH)D: 25-hydroxy vit. D; BMI: Body mass index; vit. D: Vitamin D. Data presented as mean ± SD (standard deviation).

The normal serum vit. D was defined as 25(OH)D >30 ng/mL, while <20 ng/mL 25 (OH) D was defined as vit. D deficiency[18].

The mean age, weight, height, and BMI of the studied participants are presented in Table I.

The mean 25(OH)D of the studied participants before vit. D intake was 13.4±3.01 ng/mL. The mid-luteal E_2 and P of the studied participants before vit. D intake were 109.3±15.7 pg/mL and 9.8±1.01 ng/mL, respectively (Table I).

The 25(OH)D of the studied participants statistically increased from 13.4±3.01 ng/mL to 58.5±2.07 ng/mL after vit. D intake (p=0.00036; 95% CI: -45.9, -45.1, -44.32).

The mid-luteal E_2 and P statistically decreased from 109.3±15.7 pg/mL and 9.8±1.01 ng/mL, respectively to 40.7±10.52 pg/mL, and 5.2±0.73 ng/mL, respectively, after vit. D intake (p=0.00015; 95% CI: 64.5, 68.6, 72.7, and p=0.0016; 95% CI: 4.3, 4.6, 4.87, respectively), (Table II).

Significant negative correlations between the 25(OH)D, and both the mid-luteal E_2 (r -0.661; $p<0.00001$), (Figure 1), and P (r -0.521; $p<0.00001$), (Figure 2) were detected in this study.

Table II. Participants' 25(OH)D, mid-luteal estradiol, and progesterone before and after vit. D intake.

Variables	Before vit. D intake (n = 85 adolescents)	After vit. D intake (n = 85 adolescents)	*p*-value (95% CI)
25(OH)D (ng/mL)	13.4 ± 3.01	58.5 ± 2.07	0.00036* (-45.9, -45.1, -44.32)
Estradiol (pg/mL)	109.3 ± 15.7	40.7 ± 10.52	0.00015* (64.5, 68.6, 72.7)
Progesterone (ng/mL)	9.8 ± 1.01	5.2 ± 0.73	0.0016* (4.3, 4.6, 4.87)

*: Significant difference. 25(OH)D: 25-hydroxy vit. D; CI: Confidence Interval; vit. D: Vitamin D.Data presented as mean ± SD (standard deviation). Student's t-test was used for statistical analysis.

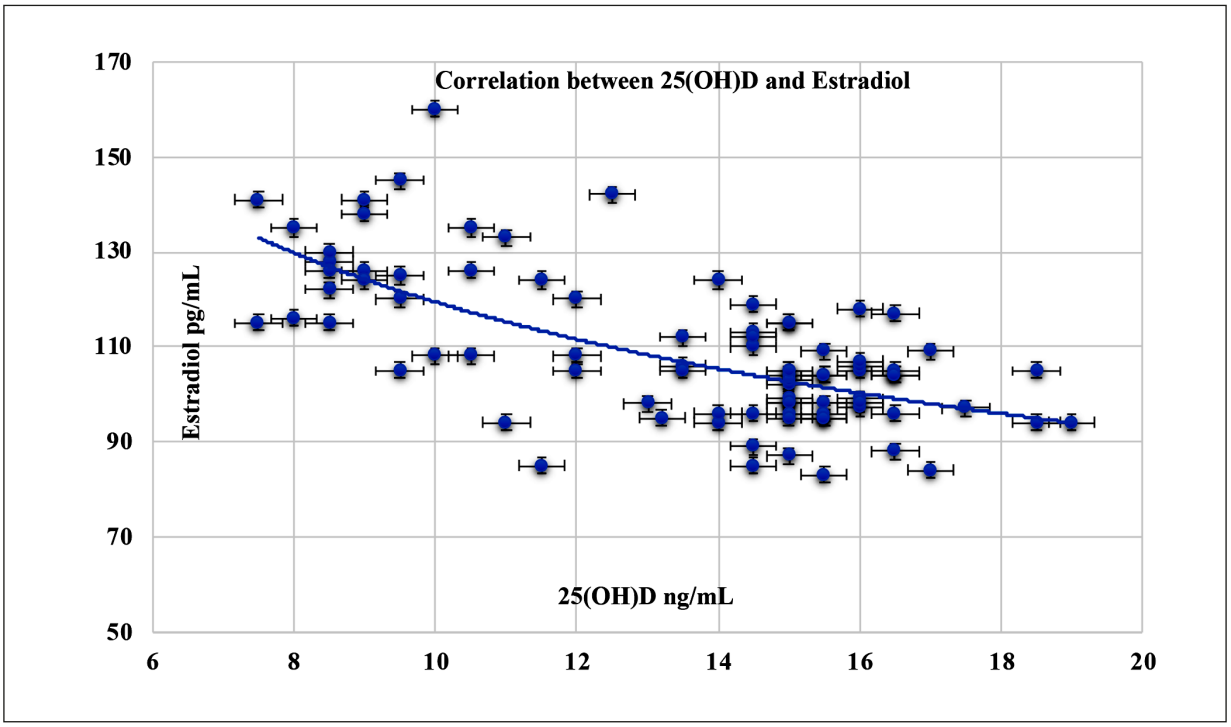

Figure 1. Correlation between 25(OH)D and estradiol.

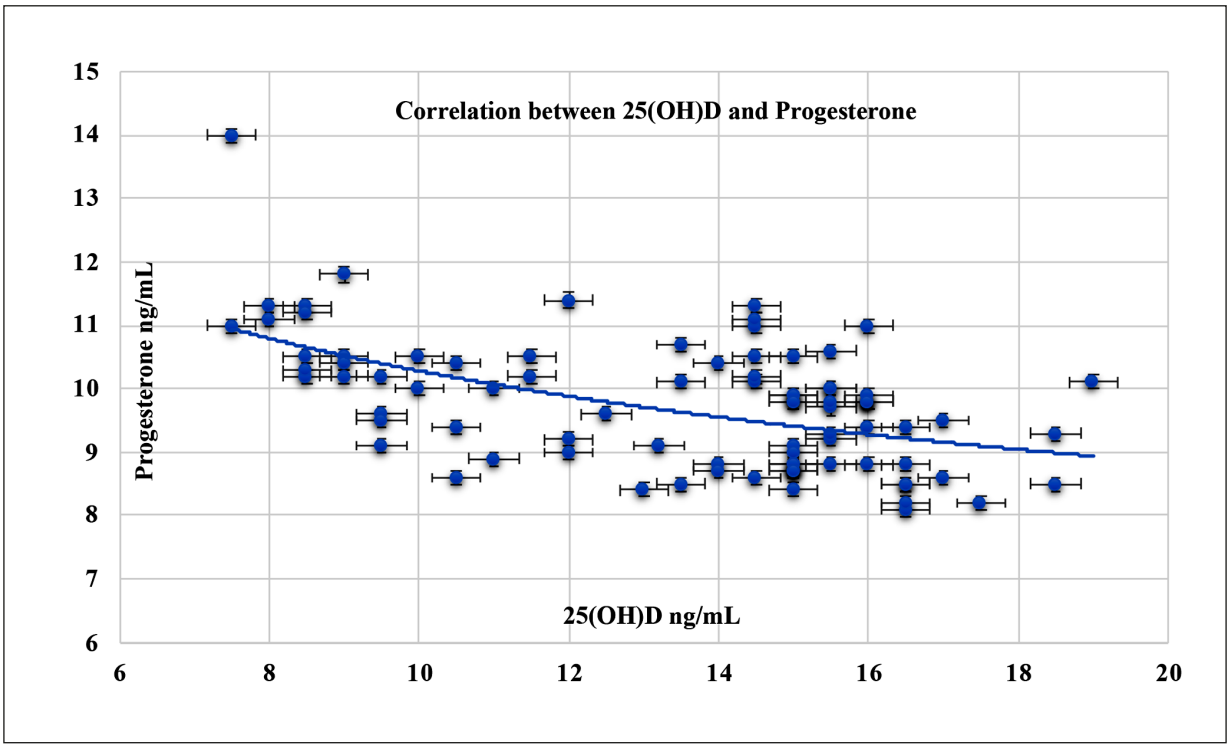

Figure 2. Correlation between 25(OH)D and progesterone.

Discussion

The animals' or the humans' studies regarding the effect of vit. D and/or its metabolites on ovarian steroids are limited.

The relation between 25(OH)D and steroid hormones in normal women was inconsistently reported[14], and the role of vit. D on ovarian steroids is not intensively studied.

Therefore, eighty-five (85) adolescents were recruited for this cohort study. Participants received 50,000 IU of vit. D weekly for two months, and on the 3rd month, the mid-luteal E_2, P, and 25(OH)D were measured.

The mid-luteal E_2, P, and 25(OH)D were compared before and after the vit. D intake to detect the effect of vit. D intake (50,000 IU weekly for 2 months) on the mid-luteal E_2 and P.

The relations between vit. D and the adolescents' mid-luteal E_2 and P were detected as secondary outcomes using the correlation analysis.

Although Hong et al[12] found the vit. D_3 increased E_2 production from the porcine cultured granulosa cells, Parikh et al[11] found that 1,25-$(OH)_2D_3$ stimulates the P production by 13%, and E_2 production by 9%, in human ovarian cells.

This study found that the mid-luteal E_2 and P statistically decreased from 109.3±15.7 pg/mL and 9.8±1.01 ng/mL, respectively, to 40.7±10.52 pg/mL, and 5.2±0.73 ng/mL, respectively, after vit. D intake (p=0.00015 and 0.0016, respectively).

Significant negative correlations between the 25(OH)D, and both the mid-luteal E_2 (p<0.00001, and <0.00001, respectively) were detected in this study.

Knight et al[28] reported significantly lower luteal P and reduced E_2 at higher 25(OH)D levels, with an inverse relationship between the 25(OH)D and both the E_2 and P.

The vit. D effect on the ovarian steroids is mediated through the *CYP19* expression, which results in low aromatase enzyme with subsequent decreased E_2 production[28].

The identification of the vit. D receptors in the ovaries, uterus, and pituitary gland, supports the role of vit. D in ovarian steroidogenesis[28].

A review article reported an inverse relation between the 25(OH)D, and both the E_2 and P with decreased both E_2 and P after vit. D intake[29].

The same review[28] found the vit. D was linked to obesity and the metabolic syndrome of PCOS. Additionally, Hong et al[9], found that vit. D_3 decreased the production of P from the porcine-cultured granulosa cells.

Moreover, Horii et al[30], found that high doses of 1,25$(OH)_2$D, reduce the corpus luteum function, and P production in female rats.

Vit. D has a beneficial role in insulin resistance (IR), and endometrial receptivity, but high levels and incorrect vit. D administration time, seem to have a detrimental effect on oocyte maturation. Therefore, Menichini et al[31], encourage a low dose of vit. D (400-800 IU/day), particularly in vit. D deficient PCOS women, with metabolic syndrome. Additionally, they suggested vit. D supplementation in selected women only during the luteal phase of the ovarian cycle (as luteal phase support).

Recently, Kiani et al[32] found that vit. D decreases the IR in PCOS. Similarly, inositol (carbocyclic sugar isomer) lowers the IR, improves oocyte maturation, and restores normal ovulation in PCOS[32].

Benelli et al[33], found that myo-inositol (MI), and D-chiro-inositol (DCI) significantly increased the E_2 (from 47.06±18.2 to 107.4±92.86 pg/mL), (p<0.01) in obese PCOS women. They concluded that the MI plus DCI combination was effective with improved endocrine parameters in obese PCOS women.

The combination of MI and DCI improves the PCOS features[34]. The alpha-lipoic acid (ALA) also has anti-inflammatory, antioxidant, and insulin-sensitizing activities[34]. Few studies[34-36] have suggested strengthening the MI effect in PCOS women by combining the ALA with MI. Laganà et al[37], concluded that in the absence of strong evidence, the ALA should not be recommended in the routine management of PCOS, even if combined with MI.

A placebo-controlled trial compared the effect of MI plus folic acid (treated group) to the folic acid (placebo) and found that the ovulation frequency was significantly higher (p<0.01), and the time of first ovulation was significantly shorter (p<0.05) in the treated group[38]. The same study found that the effect of MI on follicular maturation was rapid, with increased circulating E_2 from the first week of MI treatment[38].

Gullo et al[39] found that cultured embryos in embryo culture media rich in MI showed more physiological cleavage rate, and MI depletion may be responsible for the poor oocyte quality, and increased risk of ovarian hyperstimulation syndrome for PCOS women.

Moreover, Dell'Edera et al[40] found that the combination of MI and DCI could prevent ma-

ternal gestational diabetes mellitus (GDM) and macrosomia in women with >92 mg/dl fasting blood sugar during the first trimester of pregnancy.

Beck-Fruchter et al[41], reported a significant association between the live birth and progesterone (14.65 *vs.* 11.62 ng/ml), (p=0.001) as well as E_2 levels (355.12 *vs.* 287.67 pg/ml), (p=0.001).

Recently, the innovation of Artificial intelligence (AI) in the field of assisted reproductive technology (ART) could improve infertility treatment for better outcomes and higher success rates[42]. The introduction of AI in ART treatment requires certain precautions, to maintain the ethical frameworks, prioritizing human dignity, privacy, and data protection[42]. The AI should be under human control since it impacts the well-being of unborn children[42].

Abdelazim et al[16], reported increased risks of hypothyroidism, and hyperprolactinemia in PCOS women. They concluded that hypothyroidism and hyperprolactinemia are common endocrine disorders observed in PCOS women, and should be treated before starting the ART treatment for PCOS women.

The relation between hypothyroidism and hyperprolactinemia in PCOS women was explained by the high Thyrotropin-releasing hormone in hypothyroidism (TRH act as a dopamine antagonist)[43]. The hyperprolactinemia predisposes to anovulation, luteinized unruptured follicle syndrome (LUFS), luteal phase defect, and defective steroidogenesis with subsequent ovarian hyperandrogenism, increased PCOS severity, and infertility[43].

The relation between vit. D and ovarian steroids, and the effect of vit. D intake on ovarian steroids need further larger studies.

The current cohort study was the first conducted in Aktobe-West Kazakhstan to detect the relation between vit. D and the adolescents' mid-luteal E_2 and P, and the effect of vit. D intake (50,000 IU weekly for 2 months) on the mid-luteal E_2 and P.

This study found that the mid-luteal E_2, and P statistically decreased after vit. D intake (p=0.00015 and 0.0016, respectively), with significant negative correlations between the 25(OH) D, and both the mid-luteal E_2 (p< 0.00001, and <0.00001, respectively).

The short-term follow-up (because of the cohort nature of the study), and adolescents refused to participate were the limitations of this study.

Conclusions

The mid-luteal E_2 and P statistically decreased after vit. D intake (50,000 IU of vit. D weekly for 2 months). Significant negative correlations between the 25(OH)D, and both the mid-luteal E_2 and P were detected in this study. The relation between vit. D and ovarian steroids, and the effect of vit. D intake on ovarian steroids needs further larger studies.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Informed Consent

Informed consent was obtained by the participants themselves or their guardians.

Ethics Approval

The study was approved by the West Kazakhstan Medical University Ethics Committee (Approval No. 10, dated 04.10.2020).

Availability of Data and Materials

The data analyzed during this cohort study are submitted with the manuscript.

Authors' Contribution

AD, AA, and AZ are responsible for the study concept, and design, literature review, data collection, final revision before publication. IAA, AK and ES are responsible for the literature review, Microsoft editing, and final revision before publication. RN, GG, SS, DA, and ZK are responsible for literature review, Microsoft editing, update of the references, and final revision before publication. IIS is the corresponding author responsible for literature review, Microsoft editing, and drafting, final revision before publication and submission for publication. All the authors have read and agreed to the published version of the manuscript.

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