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**The potential role of MMP-9 and VEGF-C as predictors of lymph node involvement in papillary thyroid carcinoma**

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**Correlation between platelet distribution width and mean platelet volume with the stage of hepatocellular carcinoma based on Barcelona Clinical Liver Cancer criteria**

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**Factors related to mortality in geriatric patients after elective surgery: a retrospective cohort study**

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**Relationship between Beighton score, genders, body mass index, and quadriceps angle in East Java Training Athletes**

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**The impact of transcutaneous auricular vagus nerve stimulation on C-reactive protein in patients with chronic low back pain**

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**The percentage of positive biopsy cores in predicting biochemical recurrence and adverse pathology in prostate cancer patients after radical prostatectomy: a systematic review and meta-analysis**

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**Helicobacter pylori detection in gastric biopsy: immunohistochemistry and toluidine blue comparison**

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**Brain-derived neurotrophic factor (BDNF) and the capute scales in offspring of mothers with normal and deficient vitamin D levels**

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**The effect of low-intensity intradialytic aerobic exercise on heart rate variability in maintenance hemodialysis patients**

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**Effect of Spiramycin and Moringa leaf feeding in improving placenta, heart, histopathological neurons and glia in fetal models with toxoplasmosis**

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**Balinese purnajiwa (Kopsia arborea Blume.) extract stimulates male rats' sexual behavior and plasma testosterone level**

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**Understanding of home care and interest level of nursing students working as home care nurses**

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**Relations between the incidence of endometriosis and type 1 epithelial ovarian cancer at M Djamil Central Hospital of Padang Indonesia**

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**Tumour infiltrating lymphocytes (TILs) as an excellent predictive factor for neoadjuvant chemotherapy response on locally advanced rectal cancer patient**

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**Prevalence and susceptibility profile of carbapenem-resistant pseudomonas aeruginosa (CRPA) at Dr. Soetomo Public Hospital, Surabaya, from January to December 2021**

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**Validity test of pre-screening developmental questionnaire (PDQ) and mother and child health (MCH) handbook for determining children's readiness to enter elementary school**

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**Postoperative complications of Mathieu incised plate versus standard tubularized incised plate urethroplasty for distal hypospadias: a systematic review and meta-analysis**

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**Correlation of 5-minutes heart rate variability with health performance of resident physicians in covid 19 pandemic**

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**Adjusted enhanced recovery after surgery (ERAS) protocol in colorectal surgery at dr. Cipto Mangunkusumo General Hospital, Jakarta**

Andre Setiawan Suryadi, Vania Myralda Giamour Marbun, Arnetta Naomi Louise Lalisang, Wifanto Saditya Jeo, Yarman Mazni, Toar Jean Maurice Lalisang

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**Effectiveness of topical turmeric extract compared to topical zinc on fibroblast count and collagen density in clean-contaminated wound: a study on new zealand white rabbit**

Made Walmiky Budi, Adria Hariastawa, Fendy Matulatan

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**Post-chemotherapy response of paclitaxel and carboplatin regimens in advanced stage cervical cancer types of histopathology squamous cell carcinoma and adenocarcinoma**

Yohanes Adhitya Prakasa Sukoco, Meitria Syahadatina Noor, Ferry Armanza, Hariadi Yuseran, Renny Aditya

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**Effects of Lactobacillus plantarum IS-10506 on gastric protective mucosal factors: an ibuprofen-induced gastric mucosal injury**

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**The relationship between metabolic acidosis as a predictive factor of mortality in Ludwig's Angina patients at Dr. Soetomo General Hospital, Surabaya, Indonesia**

Alif Rakhman Prima Adi, Marjono Dwi Wibowo, Dwi Hari Susilo

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**Association between serum homocysteine levels and the frequency of epilepsy-related cognitive dysfunction: a cross sectional study in patients with epilepsy in Mataram, Indonesia**

Herpan Syafii Harahap, Mohammad Rizki, Yanna Indrayana, Sri Budhi Rianawati

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**Sarcopenia as an indicator of nutritional status and outcome predictor for colorectal cancer in Javanese ethnic**

Damianus Hipolitus, Adeodatus Yuda Handaya, Agus Barmawi

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**Comparison of chlorhexidine 0.7% and modified Petroff's method on sputum decontamination for culture method to detect Mycobacterium tuberculosis**

Rizky Hurriah Lumbessy, Ni Made Mertaniasih, Lindawati Alimsardjono, Soedarsono Soedarsono

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**Comparison of agility between the flat foot and normal foot in East Java Puskatda athletes**

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**Exercise improving balance function in the older adult with locomotive syndrome stage 1: a randomized clinical trial**

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**Development of social skill training design for preschool in social distancing pandemic COVID-19**

Machmudah, Sa'dun Akbar, Nur Eva, Ika Andriani Farida, Fifi Khoirul Fitriyah, Siti Nurjanah, Nafi'ah

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**The impact of COVID-19 vaccination for healthcare workers on the SARS-CoV-2 antibody titers in a tertiary hospital in Jakarta, Indonesia**

Rika Bur, Febby Elvanesa Sandra Dewi, Virmandiani, Yosanti Elsa Kartikawati, Meutia Gebrina, Aditya Rifqi Fauzi, Duddy Mulyawan, Ni Sayu Dewi Budhiyani

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**Factors that determine inpatient satisfaction on nursing care: a cross-sectional study**

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**Development of nursing learning program for COVID-19 patients with e-learning method for nursing department students Poltekkes Ministry of Health Jayapura Papua**

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**Performance comparison between Conventional Polymerase Chain Reaction and AbTes Real-time polymerase chain reaction with the gold standard of microscopy imaging detection method**

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**Profiles of patients with maxillofacial fractures in emergency department of head and neck surgery division of Dr. Soetomo General Hospital Surabaya in 2021 during COVID-19 pandemic**

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**Research gap and trend in neurological therapy related to nanotechnology based on bibliometric analysis**

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**Efficacy of transcutaneous electrical nerve stimulation on slow transit constipation in children with cerebral palsy**

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**The Efficacy of Blood Flow Restriction Training for Grip Strength and Disability in Lateral Epicondylitis**

Gede Parta Kinandana, Anak Ayu Nyoman Trisna Narta Dewi, I Made Niko Winaya, Ni Komang Ayu Juni Antari, Ari Wibawa, I Putu Gde Surya Adhitya

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**The effect of added Transcutaneous Vagus Nerve Stimulation (tVNS) on quality of life in patients with chronic low back pain: a randomized controlled trial study**

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**The association between biofilm formation ability and antibiotic resistance phenotype in clinical isolates of gram-negative bacteria: a cross-sectional study**

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**Placental apoptotic index on COVID-19 and non-COVID-19 cases: a cross sectional study**

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**The impact of bullying on adolescent self-concept**

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**Association between IL-6 rs1800795 and IL-1 $\beta$  rs 16944 gene polymorphisms with clinical severity of COVID-19 patients in Surakarta, Central Java, Indonesia**

Hendrastutik Apriningsih, Betty Surtawati, Reviono, Tonang Dwi Ardyanto -, Dewi Pratiwi, Hafi Nurinasari

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**The clinicopathological characteristics of borderline ovarian tumors at Dr. Soetomo Hospital, Surabaya: a single center study**

Setyo Teguh Waluyo, Brahmata Askandar Tjokropawiro

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**Predictors of neonatal mortality with congenital heart disease**

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**The frequency and determinants of executive dysfunction among patients with subacute phase of ischemic stroke in West Nusa Tenggara, Indonesia**

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**Integrated antenatal care services with breastfeeding counseling practices to improve pregnant women's knowledge, skills and readiness for exclusive breastfeeding**



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**Correlation between age, the clinical status of patients, operator characteristics, and surgical landmarks with the incidence of tracheotomy complications**

Evan Anindito, Muhtarum Yusuf, Titeik H Ahadiah

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**Comparison of conventional and electronic cigarette on formaldehyde in blood and alveolus histopathology in vivo**

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**High ratio of neutrophils to lymphocytes and high triglyceride levels in serum as risk factors for pre-eclampsia**

Anak Agung Ngurah Jaya Kusuma, Endang Sri Widiyanti, Anak Agung Gde Marvy Khrisna Pranamartha, Adytia Theopani Bineredo Damanik

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**The validity and reliability of various footprint analysis in flatfoot diagnosis of competitive athletes**

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**The effect of Curcuma longa extract on fibroblast count and collagen density in intestinal anastomosis: an experimental study on New Zealand rabbits**

Sarwendah Pratiwi Budiman, I Gusti Bagus Adria Hariastawa, Fendy Matulatan

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**Bacteriocin potency test of lactic acid bacteria (LAB) isolated from rumen of Bali cattle against low pH and bile salt**

Hamong Suharsono, I Wayan Suardana, Komang Januartha Putra Pinatih

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**Infant temperament with anxiety postpartum mothers in primary health care Sleman, Indonesia**

Riski Oktafia, Ema Anindita Berliyanti, Nur Azizah Indriastuti, Rini Rahmayanti

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**Relationship between knowledge and medication adherence among patients with tuberculosis: a cross-sectional survey**

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**Declining in estimated glomerular filtration rate of chronic kidney disease patients after Ramadan fasting is independent of interleukin-6 serum level**

Abdullah, Kurnia Fitri Jamil, Dharma Lindarto, Desi Salwani, Muhsin, Andri Baftahul Khairi, Maimun Syukri

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**Overview of prolactin levels in patients with schizophrenia during antipsychotic therapy at HB. Saanin Mental Hospital Padang, Indonesia**

Dita Hasni, Bayu Eka Surya, Mutiara Anissa, Debie Anggraini

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**Postoperative supplementation effect of zinc and vitamin C in primary colonic anastomosis on peritonitis condition: A study on New Zealand rabbit**

Luh Putu Neolita Pradnya Wineni, Adria Hariastawa, Ariandi Setiawan

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**Healthy food for pregnant mother**

Gde Ngurah Indraguna Pinatih

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**Mesona palustris BL: the potential antioxidant**

Rahmah, Harsono Salimo, Brian Wasita, Eti Poncorini Pamungkasari, Risyia Cilmiaty, Soetrisno

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**Revealing the artemisinin and piperazine treatment failure: a systematic review**

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**Curcumin, a potential oral herbal male contraceptive: a review article**

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**Effectiveness of Small Group Discussions (SGD) to Improve Learning Outcome and Critical Thinking in Medical Student: a Literature Review**

I Gusti Ayu Sri Darmayani

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**Exclusive breast milk by working mothers as a new habit: a literature review**

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**Knowledge, attitude, and skills of partograph utilization among health workers: a systematic review**

Alfun Dhiya An, Ova Emilia

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**Low dose mannitol versus high dose mannitol to achieve brain relaxation in brain tumor surgery: a meta-analysis**

Gunna Hutomo Putra, Irwan Barlian Immadoel Haq, Rahadian Indarto Susilo, Joni Wahyuhadi

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**Massage stimulation's effect on melatonin levels in preterm infants via vagal activity**

Diah Budiarti, Ahmad Suryawan

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**Acute Lymphoblastic Leukemia (ALL)-L2 in an adult woman: a case report**

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# Relationship between angiotensin-like-protein-2 levels and anti-mullerian hormone levels in polycystic ovary syndrome of reproductive age

Dyan Asih Rachmawati<sup>1\*</sup>, Ashon Sa'adi<sup>2</sup>, Budi Utomo<sup>2</sup>, Arif Tunjungseto<sup>2</sup>

## ABSTRACT

**Introduction:** Polycystic Ovary Syndrome (PCOS) is the most prevalent metabolic disease affecting reproductive-age women and is often present with insulin resistance. Angiotensin-like-protein-2 (ANGPTL2) is an angiogenic factor influencing insulin resistance in PCOS, manifesting in the anti-mullerian hormone (AMH). There are no previous studies regarding the relationship between ANGPTL2 levels and AMH in PCOS. The study aims to analyse the relationship between ANGPTL2 and AMH levels in PCOS and to compare ANGPTL2 and AMH levels between PCOS phenotypes.

**Methods:** This cross-sectional study on 43 women aged 18-40 diagnosed with PCOS according to the Rotterdam criteria. Subjects were recruited consecutively and categorized into four PCOS phenotypes. Serum levels of ANGPTL2 and AMH were measured and analysed by correlation and comparison test using SPSS 26.

**Results:** A total of 43 PCOS samples were included in the study within 4 months. Based on the severity, phenotype A (anovulation, hyperandrogenic and polycystic ovaries) showed the highest levels of ANGPTL2 and AMH. There was no significant difference in ANGPTL2 levels between the PCOS phenotypes. There was a significant difference in AMH levels between phenotypes, where the highest value was between phenotypes A and B. There was a positive correlation between ANGPTL2 and AMH levels.

**Conclusion:** There is a positive relationship between ANGPTL2 and AMH serum levels. The ANGPTL2 serum levels were not significantly different. In contrast, the AMH serum levels significantly differed across the four PCOS phenotypes, with the highest level found in phenotype A.

**Keywords:** angiotensin-like protein 2, Anti-Mullerian hormone (AMH), phenotype, polycystic ovary syndrome.

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## INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common metabolic disease in women of reproductive age. The prevalence of PCOS is known to be 5-10% and around 15-20%, according to the European Society for Human Reproduction and Embryology/American Society for Reproductive.<sup>1-4</sup> Insulin resistance is the most common condition in PCOS patients, which can be measured by the Homeostatic Model Assessment of Insulin Resistance (HOMA IR). Angiotensin-like-protein-2 (ANGPTL2) is an angiogenic factor that plays a role in the pathogenesis of insulin resistance in the PCOS condition. Anti-mullerian hormone (AMH) is a hormone

that is expressed in small pre-antral and antral follicles. The increased number of PCOS is closely related to impaired folliculogenesis.<sup>5,6</sup>

The diagnosis of PCOS was made according to the Rotterdam criteria, where at least 2 of 3 criteria were found: anovulation, hyperandrogenism and morphology of polycystic ovaries. Grouped into phenotype A, if all three symptoms are present, phenotype B (anovulation and hyperandrogenic), phenotype C (hyper androgen and polycystic ovarian morphology) and phenotype D (anovulation and polycystic ovarian morphology). Angiotensin-like-protein-2 is an angiogenic factor that mediates the occurrence of insulin

resistance through obesity (chronic low-grade inflammation) and without obesity (serine phosphorylation) pathways. In both conditions, there can be an increase in ANGPTL2 serum levels and insulin resistance occurs. The anti-mullerian hormone is a good biochemical marker of ovarian function, produced by preantral and small antral follicle's granulosa cells. In PCOS, AMH levels will increase and positively correlate with ovarian morphology.<sup>5,6</sup> The study aims to analyse the relationship between ANGPTL2 serum levels and AMH serum levels in PCOS and the differences between ANGPTL2 serum levels and AMH serum levels in PCOS based on their phenotype.



**Table 1. Patients Demographics.**

Demographic	Phenotype A (%); (min-maks)	Phenotype B (%); (min-maks)	Phenotype C (%); (min-maks)	Phenotype D (%); (min-maks)	Total (%); (min-maks)
<b>Age (year old)</b>	27.82±4.38*	29.71±4.46	29.29±5.28	29.92±2.71	28.95±4.12
Median	28 (19-34)**	31 (24-36)	30 (22-39)	29 (26-34)	29 (19-39)
18-25	3 (7)	2 (4.7)	1 (2.3)	0	6 (14)
26-30	9 (20.9)	1 (2.3)	4 (9.3)	7 (16.3)	21 (48.8)
31-35	5 (11.6)	3 (7)	1 (2.3)	5 (11.6)	14 (32.6)
35-40	0	1 (2.3)	1 (2.3)	0	2 (4.7)
Total	17 (39.5)	7 (16.3)	7 (16.3)	12 (27.9)	43 (100)
<b>Residence</b>					
Surabaya	11 (25.6)	6 (14)	2 (4.7)	10 (23.3)	29 (67.4)
Out of Surabaya	6 (14)	1 (2.3)	5 (11.6)	2 (4.7)	14 (32.6)
Total	17 (39.5)	7 (16.3)	7 (16.3)	12 (27.9)	43 (100)
<b>BMI</b>					
Mean	31.08±4.9*	30.49±6.34	23.27±1.70	25.29±5.48	28.1±5.82
Median	30.3 (21.5-37.8)**	28.65 (21.23-40.18)	22.66 (21.08-26.04)	24.33 (18.67-37.88)	27.73 (18.67-40.18)
18.5-24.9	2 (4.7)	1 (2.3)	6 (14)	7 (16.3)	16 (38.1)
25-29.9	6 (14)	3 (7)	1 (2.3)	3 (7)	13 (31)
30-34.9	4 (9.3)	1 (2.3)	0	1 (2.3)	6 (14.3)
35-39.9	5 (11.6)	1 (2.3)	0	1 (2.3)	7 (16.3)
> 39.9	0	1 (2.3)	0	0	1 (2.4)
Total	17 (39.5)	7 (16.3)	7 (16.3)	12 (27.9)	43 (100)
<b>Infertility</b>					
Not married yet	3 (7)	0	1 (2.3)	0	4 (9.3)
No infertility	0	1 (2.3)	0	1 (2.3)	2 (4.7)
Primary infertility	10 (23.3)	6 (14)	5 (11.6)	8 (18.6)	29 (67.4)
Secondary infertility	4 (9.3)	0	1 (2.3)	3 (7)	8 (18.6)
Total	17 (39.5)	7 (16.3)	7 (16.3)	12 (27.9)	43 (100)
<b>ANGPTL2</b>					
Mean	1.92±0.69*	1.30±0.28	1.48±0.43	1.64±0.41	1.67±0.56
Median	1.82 (0.65-3.1)**	1.35 (0.84-1.62)	1.53 (0.72-2.13)	1.55 (0.83-2.16)	1.59 (0.65-3.10)
<b>AMH</b>					
Mean	10.31±4.18*	4.18±0.46	7.02±1.35	7.51±3.03	7.99±3.77
Median	9.61 (5.46-21.52)**	4.15 (3.74-5.06)	6.73 (5.21-8.84)	6.10 (4.12-11.92)	6.73 (3.74-21.52)

**Table 2. Normality Test (Shapiro-Wilk).**

Variable	n	p
ANGPTL2	43	0.179
AMH	43	<0.001

## METHODS

### Study Design

This analytic observational study with a cross-sectional design was conducted at the obstetrician-gynaecologists private practice clinic and Dr. Soetomo Hospital in Surabaya.

### Participants and Settings

The patients diagnosed with polycystic ovary syndrome according to the Rotterdam criteria between August until November 2022 who met the inclusion and exclusion criteria. Subjects were recruited by consecutive sampling and

categorised into four PCOS phenotypes, then serum levels of ANGPTL2 and AMH were measured. Eligibility criteria for the participants in this study are patients with polycystic ovary syndrome according to the Rotterdam criteria aged 18-40 years. The exclusion criteria are the patient's history and clinical complaints about vascular abnormalities, kidneys, livers, tumours and who had been threatened before.

**Table 3. Spearman Correlation Test.**

		AMH
ANGPTL 2	Correlation Coefficient	0.355
	<i>p</i> (2-tailed)	0.019
	N	43

**Table 4. Anova Test.**

	n	F	<i>p</i>
Between Groups	3	2.651	0.062
Within Groups	39		
Total	42		

**Table 5. Kruskal Wallis Test.**

Phenotype	AMH Average	<i>p</i>
A	10.308	<0.001
B	4.177	
C	7.017	
D	7.509	

**Table 6. Mann Whitney Test.**

Group	Different Average	<i>p</i>	
A and B	6.131	<0.001	Significantly different
A and C	3.291	0.033	Significantly different
A and D	2.799	0.057	Not Significantly different
B and A	-6.131	<0.001	Significantly different
B and C	-2.840	0.002	Significantly different
B and D	-3.332	0.002	Significantly different
C and A	-3.291	0.033	Significantly different
C and B	2.840	0.002	Significantly different
C and D	-0.492	0.735	Not Significantly different

### Sample Size

The sample size was calculated using a cross sectional study formula based on a proportion of 50% and an error of 0.15. The sample size was 43 patients, collected using a consecutive sampling technique.

### Test Methods

The PCOS patient list from 2021 until 2022, after participants agreed to informed consent, demographic data were collected from anamnesis, the physical examination to measure BMI, Ferriman Galleway Score and ovarian morphology USG. ANGPTL2 and AMH serum have been collected from intravenous serum as much as 5 ml.

### Analysis

All subjects were given the same treatment, performed anamnesis, physical examination and ultrasound to assess the morphological picture of the ovaries. Then a 5 ml venous blood sample was taken

and the ANGPTL2 and AMH levels were measured. The data were analysed using SPSS software version 26.

## RESULTS

### Demographics

In this study, there were 43 patients with demographic characteristics shown in [Table 1](#) majority of the patients were 26-30 years old. The average body mass index (BMI) was  $31.08 \pm 4.9$  kg/m<sup>2</sup>. The most common BMI categories were obesity and overweight (27 people (62.8%)). Based on the result, most research subjects have phenotype A (17 respondents (40.5%)), followed by phenotypes D, B and C of 12 respondents (27.9%), 7 respondents (16.3%) and 7 respondents (16.3%). The available data shows the most phenotype, namely phenotypes A (39.5%). The highest BMI in phenotype A is  $31.08 \pm 4.9$ . The median AMH level in the PCOS group

was 9.61 (5.46-21.52) ng/ml. The highest mean ANGPTL2 level and median AMH level results for phenotype A were  $1.92 \pm 0.69$  and 9.61 (5.46-21.52).

### Correlation Study

Based on the results in [Table 2](#), the *p*-values of ANGPTL2 and AMH level data were 0.179 and <0.001, respectively. The probability value of AMH data is smaller than the 0.05 significance level and the probability value of ANGPTL2 data is greater than the 0.05 significance level. This means that the assumption of normality is not fulfilled.

[Table 3](#) shows a significant relationship between ANGPTL2 and AMH (*p* = 0.019), which refers to the basis for decision-making, with the result that H<sub>0</sub> was rejected. The correlation coefficient is 0.355 and has a positive value indicating an adequate and unidirectional relationship.

### Comparative Study

Based on [Table 4](#), the probability value is 0.062. This probability value is greater than the significance level of 0.05. Referring to the basis of decision-making, H<sub>0</sub> is accepted or there is no significant difference in the average ANGPTL2 between phenotypes, so the Post-hoc test is unnecessary.

Based on the Kruskal Wallis test results in [Table 5](#), the probability value is <0.001. The probability value is smaller than the 0.05 significance level. Referring to the basis of decision-making, H<sub>0</sub> is rejected or there is a significant difference in the average AMH for each phenotype. The Mann-Whitney test was performed to compare each phenotype's mean difference in AMH level. The basis for decision-making is the same as the Kruskal-Wallis test. [Table 6](#) shows the most significant differences in phenotype A and B groups, but there were no significant differences between phenotypes A and D; C and D phenotypes.

## DISCUSSION

Based on the result, most research subjects have phenotype A. Previous studies, such as in Wiweko et al., found phenotype A in 31.2% of subjects, Gursu et al. in 44.8% of subjects, and Gupta et al. in 38% of subjects. Ozay et al. found phenotype A

as 33.4%, and Carmina and Lobo as much as 54% of their total PCOS respondents. This result is consistent with the existing theory of hyperandrogenic conditions. Oligo or anovulation and polycystic ovary morphology are corresponding sequences. Hyper-androgens can be caused by central factors (LH hypersecretion) or peripheral factors. Insulin resistance is the most dominant peripheral factor causing hyperandrogenism. LH and insulin hypersecretion will cause premature luteinization and stop the maturation process in the ovarian follicles. The cessation of the ovarian follicle maturation process in its development will increase the number of follicles and produce a polycystic ovarian morphology.<sup>7-11</sup> This study found the highest average BMI in the phenotype A group compared to other phenotypes, and the most common BMI categories were obesity and overweight. This result is in line with the research by Wiweko et al. that most PCOS were found with obesity (50.4% of respondents). Obesity plays an important role in the pathophysiology of PCOS through insulin resistance.<sup>7</sup>

### Relationship between Angiotensin-like-protein-2 levels and Anti-Mullerian Hormone in Polycystic Ovary Syndrome

There are no studies regarding the relationship between ANGPTL2 and AMH directly. From the existing studies, Rahmani et al. examined 26 PCOS samples and 26 controls and found a significant positive correlation between ANGPTL2, insulin and HOMA IR. Wiweko et al. studied 125 PCOS and found a positive correlation between AMH serum and HOMA levels IR. The study by Dai et al. using 102 PCOS samples and 100 controls, found an increased regulation of ANGPTL2 in the serum of obese and non-obese PCOS patients. Based on this explanation, there is a relationship between serum levels of ANGPTL2 and AMH in PCOS.<sup>7,12,13</sup>

Angiotensin-like-protein-2 is an angiogenic factor produced predominantly by adipose tissue associated with obesity-related-insulin-resistance, insulin-resistance-without-obesity, oocyte development and maturation. Insulin

resistance plays an important role in the occurrence of PCOS through two different pathways. The PI3-kinase pathway mediates metabolic effects (including glycogen formation), and the MAP kinase pathway mediates cell growth and steroidogenic effects. On the other hand, insulin resistance and hyperandrogenemia conditions can occur.<sup>14</sup> Other insulin resistance pathways are related to obesity, which can cause adipocyte hypertrophy, compression of stromal blood vessels, and increased free fatty acids in adipose tissue, which can result in hypoxia. Further, vascular remodelling will stimulate macrophage and inflammation mediators, including ANGPTL2 release into tissues. An increase in ANGPTL2 will trigger chronic low-grade inflammation, exacerbating insulin resistance and hyperandrogenemia. This condition will affect impaired folliculogenesis, which will increase circulating AMH levels.<sup>14</sup>

### Differences in Angiotensin-like-protein-2 Levels between Phenotypes in Polycystic Ovary Syndrome

This research found the highest average ANGPTL 2 level found in phenotype A group. This study conducted a comparative test of ANGPTL2 serum levels, and there is no significant difference in ANGPTL2 levels between polycystic ovary syndrome phenotypes. In line with a study by Tabata et al., circulating levels of ANGPTL2 are closely related to adiposity, systemic insulin resistance, and inflammation.<sup>15</sup> A study by Dai et al. found increased levels of ANGPTL2 in PCOS in the obesity and without obesity group.<sup>13</sup> Until now, there is no data regarding ANGPTL2 levels in each PCOS phenotype.

This study found the highest levels of ANGPTL2 and the highest BMI average in phenotype A. Obesity can exacerbate PCOS conditions through the expansion of adipose tissue mass, vascular vasoconstriction and increased release of free fatty acids into the circulation so that the supply of oxygen to adipocytes decreases. Micro-hypoxia induces a chronic inflammatory response, increases the ANGPTL2 serum level, and causes pathological adipose tissue remodelling, resulting in insulin resistance.<sup>15</sup>

### Differences in Anti-Mullerian Hormone Levels between Phenotypes in Polycystic Ovary Syndrome

The phenotype A group had the highest median AMH level compared to other phenotype groups. Research by Wiweko et al., Gupta et al., Gursu et al., Ozay and Lobo. and Carmina et al. showed that the highest median was in phenotype group A., namely 11.7 ng/ml. 11.68 mg/mL. 9.72 ± 5.6 (2.32-27) ng/mL. 9.17 ± 4.56 ng/mL and 10.2 ± 5 ng/mL.<sup>7-11</sup> This study found significant differences in AMH levels between phenotypes A-B, phenotypes A-C, phenotypes B-C and phenotypes B-D. This study found the most significant difference in AMH levels between phenotypes A and B with a probability value of <0.001 (p <0.05). While between A and D phenotypes and between C and D phenotypes, there were no significant differences. This is in line with research by Gupta et al. that the median difference in AMH levels was significant between phenotype A and phenotype B (p = 0.018).<sup>9</sup>

The increase in AMH serum levels varies in PCOS subgroups, which can be used to differentiate between clinical subgroups because AMH is closely related to PCOM but not to hyperandrogenism.<sup>16</sup> The most significant difference was found between the A and B phenotype groups because phenotype A was the most severe form of phenotype. The phenotype B was a form of PCOS phenotype in which polycystic ovary morphology was not found. The AMH levels reflect the activity of granulosa follicular cells, whose levels increase with a small number of preantral and antral follicles. In PCOS, there is an increase in the number of small preantral and antral follicles due to the influence of insulin resistance and hyperandrogenism. There is no difference between AMH levels in phenotypes A and D and C and D, in which phenotype D is a phenotype without hyperandrogenism. This can be explained by the fact that the hyperandrogenism that occurs is central hyperandrogenism, which is not influenced by the number of follicles in the ovary but by an increase in LH.

### CONCLUSION

There is a positive relationship between ANGPTL2 and AMH serum levels.

The ANGPTL2 serum levels did not significantly different, while AMH serum levels were significantly different across the four PCOS phenotypes. The highest values were found in phenotype A.

## DISCLOSURE

### Author Contribution

All authors have contributed to this research process, including conception and design, analysis and interpretation of the data, article drafting, critical revision of the article for important intellectual content, and final approval.

### Funding

This study uses independent funds from researchers.

### Conflict of Interest

There are no conflicts of interest to declare.

### Ethical Consideration

This research was approved by the Ethics Commission of the Dr. Soetomo General Hospital, Surabaya, Indonesia No. 0486/KEPK/IX/2022.

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