Editorial

Nutrition versus Great Obstetric Syndrome: A Bridge too Far?

Hermanto T. Joewono

The term 'the great obstetrical syndromes' (not discrete entities, with more than one cause)[1] refers to preterm labor, preterm premature rupture of membranes, preeclampsia, small for gestational age (SGA), large for gestational age (LGA), stillbirth etc. The "Great Obstetrical Syndromes" are associated with defective deep placentation,[2] The same opinions mentioned (biomarkers) and as well [3,4] (added gestational diabetes on the list and biomarkers). More specifically explained that trigger for abnormal placental development and the subsequent cascade of events remains unknown.[5] Various dietary and lifestyle factors have been associated with an increased risk of preeclampsia; however, causality has been difficult to prove. Maternal interventions in dietary advice and modifications have lacked significant success in preventing FGR.[6]

There are at least two papers on nutrition in pregnancy, on behalf of FIGO released paper think nutrition first.[7] Women's nutrition and health can play a role in the intergenerational transmission of human health capital, ensuring future health, happiness, longevity, and economic progress. Epigenetic modifications, a modifiable risk factor, undernutrition and overnutrition may be associated with GOS[8,9]. Different findings found no significant associations were observed between dietary and infant's outcomes.[10] And that several nutrients and dietary factors previously believed to be implicated in the risk of pre-eclampsia have now been shown to have no effect on risk such as vitamins C and E, magnesium, salt, ω-3 long-chain polyunsaturated fatty acids (fish oils) and zinc.[11] Findings in other specific nutrition related group; Undernutrition group[12] confirmed relationship with fetal growth restriction, low birth weight (LBW) and preterm birth. Obese group[13] increased risk for several GOS, increasing risk of developing preeclampsia with OR 6.04 in women whose BMI was ≥40 kg/m^2. Vegan group[8,9] : Only potential concerns on neonatal adverse outocmes . Fasting group; Ramadan fasting: no adverse effects on birth weight or preterm birth rate. In Surabaya, some animal studies showed no effect of maternal fasting to the neuronal number and apoptotic index of newborn Rattus norvegicus cerebrum and cerebellum[14,15].

Important on nutrition in pregnancy;[8,9] limitations of available evidence, many questions remain unanswered due to the many challenges of performing high-quality research in pregnancy. Thus, many recommendations for intake are based on observational studies and expert consensus, and lack randomized trials to support them. Other unanswered questions on nutrition are; absorption, distribution, metabolism, excretrion or pharmacokinetics – dynamics of nutrition the same between potato dominant eaters(Western) versus rice dominant eaters(Asia)? Is it wise to treat fe deficiency anemia without measuring the blood albumin level? Of note also, finding in mechanism of autophagy that is essential for the cellular response to starvation and other types of stress that can be an answer to what happen during fasting state.[16] The most of the GOS result from non nutritional causes.[17] Markers for this GOS without nutritional related items. In conclusion: correlation between nutritional modification and great obstetrics syndrome is positive but large multi centre multi ethnicity, multi trimester with various group of nutrition studies are needed to further elaborate what have been found to specifically conclude causal relationship.

GOS: Great Obstetrical Syndrome LGA: Large for Gestational Age SGA: Small for Gestational Age DOHaD: Developmental Origins of Health and Disease FIGO: International Federation of Gynecologist & Obstetricians NIH: National Institute of Health LBW: Low Birth Weight FGR: Fetal Growth Restriction GDM: Gestational Diabetes Mellitus MDI: Mental Development Index.

REFERENCES

1. Di Renzo, GC. The Great Obstetrical Syndromes. *J Matern-Fetal Neonatal Med.* 2009; 22(8): 633–5.
2. Brosens I, Pijnenborg R , Vercruysse L, and Romero R, The "Great Obstetrical Syndromes" are Associated with Disorders of Deep Placentation. *Am J Obstet Gynecol.* 2011 ; 204(3): 193–201.
3. NIH. Biomarkers for the 'Great Obstetrical syndromes' . 2022.
4. Gauster M and Desoye G. Great obstetrical syndromes: It's all in the placenta. *Obgyn Key.*2020: 10.
5. Karumanchi SA, Lim K, August P. Preeclampsia: Pathogenesis. *UpToDate* Sep 2022.
6. Melamed M. on behalf of FIGO initiative on fetal growth: Best practice advice for screening, diagnosis, and management of fetal growth restriction. *Int J Gynecol Obstet.* 2021;152(Suppl. 1):3–57.
7. Hanson MA. The FIGO recommendations on adolescent, preconception, and maternal nutrition:."Think Nutrition First". *Int J Gynecol Obstet.2015;* 131(S4) : S213–S53.
8. Garner CD. Nutrition in pregnancy: Assessment and counseling. *Up to Date* Sep 2022.
9. de Seymour DV, Beck KL, Conlon CA, et al,. An Investigation of the Relationshsip between Dietry Patterns in Early Pregnancy and Maternal/Infant Health Outcomes in a Chinese Cohort. Front Nutr.2022;9:775557. doi;10.3389.
10. Perry A, Stephanou A, Rayman MP. Dietary factors that affect the risk of pre-eclampsia.*BMJ Nutrition, Prevention & Health* 2022;0:e000399. doi:10.1136/ bmjnph-2021-000399
11. Hendrixson DT, Manary MJ, Trehan I, Wall LL. Undernutrition in pregnancy: Evaluation, management, and outcome in resource-limited areas. *Up to Date* Sept 2022.
12. Ramsey PS, Schenken RS. Obesity in pregnancy: Complications and maternal management. *Up to Date Sept* 2022.
13. Ucik N H, Hermanto TJ, Muhammad Miftahussurur, et al,. Effect of fasting during pregnancy to the number of cerebral and cerebellar neuron of newborn Rattus norvegicus. *Jur Ilmiah Ked Wijaya Kusuma.* 2020; 9(1): 1- 8.
14. Yunita KS, Hermanto TJ, Muhammad Miftahussurur. Effect of fasting during pregnancy to the number of cerebral and cerebellar neuron apoptotic index of newborn Rattus norvegicus *Care. Jur Ilmiah Ilmu Kes.* 2021;.9(2): 298-308.
15. Yoshinori Ohsumi. *Autophagia.* Noble prize winner 2016 in Physiology or Medicine
16. Cui L, Quinn MJ, Zhang HJ. Origins of the great obstetrics and gynecologic syndromes. *Am J Obstet Gynecol.* 2021;225(3):349. doi: 10.1016.
17. Romero R, Jung E, Chaiworapongsa T, et al. Toward a new taxonomy of obstetrical disease: improved performance of maternal blood biomarkers for the great obstetrical syndromes when classified according to placental pathology. *Am J Obstet Gynecol.* 2022;227:615.e1-25.

Research Article

Sociodemographic Factors of Elective and Emergency Cesarean Delivery in the Referal Hospital: A cross-sectional study

Faktor Sosiodemografi pada persalinan seksio sesarea elektif dan emergensi di Rumah Sakit Rujukan: sebuah penelitian potong lintang

Restuaji B. K. Umam[1], Nur I. Purnamasari[2], I Putu Sudayasa[3], Juminten Saimin[2]

[1]Medical Program Faculty of Medicine
[2]Department of Obstetrics and Gynecology
3Department of Public Health
Faculty of Medicine Universitas Halu Oleo
Kendari

Abstract

Objectives: To analyze the correlation between sociodemographic factors and cesarean section delivery at Referral Hospital in Kendari.

Method: This was a cross-sectional study conducted at dr. Ismoyo Hospital in Kendari. Data were obtained from medical records of cesarean section delivery from January to December 2019. Sampling used simple random sampling techniques. Data were analyzed using the Chi-square test.

Results: There were 50.5% emergency cesarean and 49.5% elective cesarean. The maternal age was 18-44 years. Most cases are aged 20-35 years (78.5%), have a higher education level (65.1%), employees (53.2%), and multipara (56.5%). There was a correlation between age and cesarean section (p = 0.027). There was no correlation between education level (p = 0.618), occupation (p = 0.563), and parity (p = 0.365) with cesarean section.

Conclusion: There is a correlation between maternal age and cesarean section. Education, counseling, and antenatal care should be done for early detection.

Keywords: age, cesarean section, education level, occupation, parity.

Abstrak

Tujuan: *Menilai hubungan antara faktor sosiodemografi dan waktu tindakan seksio sesarea.*

Metode: *Penelitian ini menggunakan desain potong lintang, dilakuan di RS dr. Ismoyo Kendari. Data diperoleh dari rekam medik kasus persalinan seksio sesarea pada bulan Januari sampai Desember 2019. Sampel dipilih menggunakan teknik simple random sampling. Analisis data menggunakan uji Chi-square, dengan nilai kemaknaan p ≤ 0,05.*

Hasil: *Terdapat 50,5% seksio sesarea emergensi dan 49,5% seksio sesarea elektif. Rentang usia ibu adalah 18-44 tahun. Kasus terbanyak berusia 20-35 tahun (78,5%), memiliki tingkat pendidikan tinggi (65,1%), pegawai (53,2%), dan multipara (56,5%). Terdapat hubungan yang bermakna antara usia ibu dan waktu tindakan seksio sesarea (p=0,027). Tingkat pendidikan (p=0,618), pekerjaan (p=0,563), dan paritas (p=0,365) menunjukkan tidak memiliki hubungan yang bermakna dengan tindakan seksio sesarea.*

Kesimpulan: *Faktor sosiodemografi yang berhubungan dengan tindakan seksio sesarea adalah usia ibu. Edukasi, konseling, dan pemeriksaan antenatal harus dilakukan untuk deteksi dini.*

Kata kunci: *paritas, pekerjaan, seksio sesarea, tingkat pendidikan, usia.*

Correspondence author. Juminten Saimin. Department of Obstetrics
Faculty of Medicine Universitas Halu Oleo. Kendari
Email; inten_azis@yahoo.com

Received: August, 2021 Accepted: September, 2022 Published: October,2022

INTRODUCTION

The maternal mortality rate is the indicator of maternal health services.[1] Cesarean section reduces complications of pregnancy and childbirth to decrease the maternal mortality rate. Many factors influence cesarean section deliveries. Indications of the mother and fetus affecting cesarean section will be performed in an emergency or planned.[2,3]

According to the World Health Organization (WHO), the cesarean section rate in a country is 10-15%.[4] The overall cesarean section rate in the world is around 18.6%, in some countries is above 27.2%.[5] The cesarean section rate in Asia also increases, 34.9% in China6 and 23.2% in Malaysia.[7] Indonesia has increased, 12% in 2012 and 17% in 2017.[8] The cesarean section rate in Southeast Sulawesi also increased, from 3.3% to 7.7% in 2018.[9]

The National Health Insurance System, organized by Social Security Management Agency for Health (SCMAH), has been implemented in Indonesia since January 1, 2014. The National Health Insurance requires the implementation of a tiered health service and referral system. Participants receive health services at first-level health facilities, including Puskesmas, doctor practices, dental practices, general clinics, and hospitals class D. Participants are not allowed directly to go to the hospitals or advanced health facilities except in emergency conditions.[10] The tiered referral system has an impact on service quality and public health.[11] The number of cesarean sections in Referral Hospitals was 37.7%. The distribution of cases based on sociodemographic factors and treatment varied.[12]

The cesarean section at the referral hospital is high. Therefore, this study aims to analyze the correlation between sociodemographic factors and the time of cesarean section at a referral hospital in Kendari.

METHODS

This study used a cross-sectional design. Data obtained from medical records of cesarean section delivery at dr. Ismoyo Hospital from January to December 2019. Sample selection used a simple random sampling technique. The number of samples was 186 cases determined by the Slovin formula.

The time of cesarean delivery was a dependent variable. Age, education level, occupation, and parity were independent variables. Data analysis used the Chi-square test, with a significance value of $p \leq 0.05$.

RESULTS

Based on the time of cesarean section, there were 94 cases of emergency cesarean (50.5%) and 92 cases of elective cesarean (49.5%). In this study, the maternal age range was 18-44 years.

Table 1. Characteristics of the Subject

Characteristics	n	%
Age (year old)		
20-35	146	78.5
<20 and >35	40	21.5
Education level		
Low	14	7.5
Middle	51	27.4
High	121	65.1
Occupation		
Non-employees	87	46.8
Employees	99	53.2
Parity		
Primipara	81	43.5
Multipara	105	56.5

Table 1 shows that most cases of cesarean delivery are aged 20-35 years (78.5%) and have a higher education level (65.1%). The employees (53.2%) are almost equal to non-employees (46.8%). Multipara (56.5%) are higher than primipara (43.5%).

Table 2. The Correlation between Sociodemographic Factors and the Time of Cesarean Section

Variable	Cesarean section						*P-value*
	Emergency		Elective		Total		
	n	%	n	%	n	%	
Age (years old)							0.027
20-35	80	43.0	66	35.5	146	78.5	
<20 and >35	14	7.5	26	14.0	40	21.5	
Education level							0.618
Primary	8	4.3	6	3.2	14	7.5	
Secondary	23	12.4	28	15.1	51	27.4	
Higher	63	33.9	58	31.2	121	65.1	
Occupation							0.563
Non-employees	42	22.6	45	24.2	87	46.8	
Employees	52	28.0	47	25.3	99	53.2	
Parity							0.365
Primipara	44	23.7	37	19.9	81	43.5	
Multipara	50	26.9	55	29.6	105	56.5	

Table 2 shows that emergency cesarean is more often at 20-35 years (43.0%). Elective cesarean is more often at <20 and >35 years (14.0%). There is a correlation between maternal age and time of cesarean section (p=0.027).

Based on education level, an emergency cesarean is more often at higher education level (33.9%). Elective cesarean is more often in middle education (15.1%). However, there was no correlation between education level and time of cesarean section (p=0.618).

Employees had a more frequent emergency cesarean (28.0%), while non-employees had a more often elective cesarean (24.2%). There was no correlation between occupation and time of cesarean delivery (p=0.563).

Primiparas had a more frequent emergency cesarean (23.7%), and multiparas were more frequent elective cesarean (29.6%). However, there was no correlation between parity and time of cesarean delivery (p=0.365).

DISCUSSION

The age range for the cesarean section in this study was 18-42 years. Most cesarean deliveries are carried out at 20-35 years, and there is a correlation between maternal age and the time of cesarean section. This result is in line with several previous studies in several regions in Indonesia.[12-15]

Reproductive age affects the readiness of pregnant women to undergo pregnancy and childbirth. 20-35 years is a healthy reproductive age. At this age, pregnant women can be in a healthy condition both physically and psychologically. Several factors affect readiness for pregnancy and childbirth.[2,16]

Higher education level was the largest group undergoing cesarean section in this study. These results are different from previous studies conducted on health insurance users and in private hospitals.[13,15] Advances in technology make it easier for people to obtain information and access health services. Pregnant women with higher education levels are expected to increase their knowledge and awareness in anticipating complications during pregnancy or childbirth.[14] Caesarean section is performed based on several considerations, both maternal and fetal complications.[2,17]

In this study, employees underwent cesarean section more often, but there was no relationship between cesarean section and occupation. These results are not in line with other studies conducted in big cities. The reason behind the trend of cesarean delivery in big cities is work. Status as a worker encourages women to choose cesarean section because they can plan time to work after giving birth.14 The types of occupation in small towns are not varied, and the working time is more flexible so that pregnant women are more flexible in planning the delivery process.

In this study, multipara was the largest with cesarean section, although it was not statistically significant. Most women who experience cesarean section are multipara.[14] Another hand, a different study showed a relationship between parity and cesarean section.[18]

Mothers with higher parity have experience with childbirth. It makes pregnant women more concerned about their pregnancy and influences decision-making in determining childbirth.[12] Cesarean section is not a personal preference, but several obstacles both mother and fetus.[2,17]

CONCLUSIONS

Based on the results study, we concluded that there is a correlation between maternal age and the time of cesarean section. There is no correlation between education level, occupation, and parity with the time of cesarean section. Further research about the indication of cesarean section is needed to determine the intervention. Education, counseling, and antenatal care should be done for early detection.

REFERENCES

1. Kemenkes RI. Profil Kesehatan Indonesia tahun 2019. Jakarta: Sekretariat Jendral Kementerian Kesehatan RI. 2020.
2. Saifuddin, A.B., Rachimhadhi, T., Wiknjosastro H. Ilmu Kebidanan. Ed 4. Cet Keempat. Jakarta: Bina Pustaka Sarwono Prawirohardjo. 2014: 1-20.
3. Cunningham, F.G., Leveno, K.J., Bloom, S.L, et al. Obstetric Williams. 24th ed. Washington DC: McGraw-Hill Companies.2014
4. World Health Organization. WHO statement on cesarean section rates. Department of Reproductive Health and Research. World Health Organization. 2015: 1-8.
5. Betrán, A.P., Ye, J., Moller, A.B., Zhang, J., et al. The increasing trend in cesarean section rates: global, regional and national estimates: 1990-2014. PLoS One.2016;11(2): e0148343.
6. Hong T.L., Shunseng L., Trasande L., et al. Geographic variation and temporal trends in cesarean delivery rates in China 2008-2014. JAMA. 2017; 317(1): 69-76.
7. Karalasingam, S.D., Jeganatham, R., Jegasothy, R., Reidpath, D.D. Cesarean section rates from Malaysian tertiary hospitals using Robson's 10-group classification. BMC Preg Childbirth.2020; 20:64. https://doi.org/10.1186/s12884-020-2760-2.
8. BKKBN. Survei Demografi dan Kesehatan Indonesia Jakarta: Badan Kependudukan dan Keluarga Berencana Nasional. 2018
9. Kemenkes RI. Profil Kesehatan Indonesia Tahun 2018. Jakarta: Kementerian Kesehatan Republik Indonesia. 2019.
10. Kemenkes RI. Buku pegangan sosialisasi Jaminan Kesehatan Nasional (JKN) dalam Sistem Jaminan Sosial Nasional. Jakarta: Kementrian Kesehatan Republik Indonesia. 2014.
11. Yusuf S, Achmar N, Haniarti, et al. Revenue and financing of patients with national health insurance by the social security organizing agency to improve health services. Enferm Clin. 2020; Suppl 6:276–9
12. Saimin, J., Lianawati., Yohanis, M., Ridwan, S. Sosiodemografi Persalinan dengan Seksio Sesarea di RS dr. Ismoyo Kendari. Jur Medula. 2020; 7(2): 68-73.
13. Andayasari, L., Muljati, S., Sihombing, M., et al. Proporsi seksio sesarea dan faktor yang berhubungan dengan seksio sesarea di Jakarta. Bul Penelitian Kes. 2015;43(2): 105-16.
14. Sihombing N., Saptarini I., Putri D.S.K. Determinan Persalinan Sectio Caesarea di Indonesia (Analisis Lanjut Data Riskesdas 2013). Jur Kes Reprod. 2017; 8(1):63-75.
15. Widjayanti, T.B. Karakteristik Ibu Melahirkan Sectio Caesaria Peserta Jaminan Kesehatan Nasional di Rumah Sakit Profit X di Sekitar Jakarta. Jur Epidemiol Kes Indones. 2020;4(1): 23-8.
16. Saimin J. Pengantar Kesehatan Reproduksi. Surabaya: Airlangga University Press.2016.
17. Wiknjosastro, H. Saifuddin, A.B. Rachimhadi, T. Ilmu Bedah Kebidanan. Bina Pustaka Sarwono Prawirohardjo. Jakarta. 2010:133-40.
18. Pratiwi, R.A.B., Gunanegara, R.F., Ivone, J. Faktor-faktor yang Mempengaruhi Persalinan dengan Sectio Cesarea di RSUD Lembang pada Tahun 2017. J Med Health. 2019; 2(3): 838-46.

Research Article

The Effect of Serum Magnesium, Calcium, and Potassium Levels on the Event of Calf Muscle Cramps, According to the Perspective of Pregnant Women in the Third-Trimester of Pregnancy

Pengaruh Kadar Magnesium, Kalsium, dan Kalium Serum Terhadap Terjadinya Kram Otot Betis, Menurut Perspektif Ibu Hamil di Trimester Ketiga Kehamilan

Cut N. Maracilu, Cut M. Yeni, Hasanuddin, Hilwah Nora, Sarah I. Nainggolan

Department of Obstetrics and Gynecology
Faculty of Medicine, Universitas Syiah Kuala
Dr. Zainoel Abidin Regional General Hospital
Banda Aceh

Abstract

Objective: To determine the effect of magnesium, calcium, and potassium levels on calf muscle cramps in third-trimester pregnant women at Dr. Zainoel Abidin Banda Aceh.

Methods: This research is an observational analytic study with a case-control method by taking samples using a total sampling technique. The research sample was third-trimester pregnant women (28-40 weeks of gestation) who were treated in the maternity ward for the period September to December 2020.

Results: A total of 263 samples were involved in this study where 105 patients (40%) were obese, 229 patients (87%) were housewives, 161 patients (61%) did not experience muscle cramps and samples had magnesium levels below normal as much as 82 people, calcium levels below normal as many as 127 people and potassium levels below normal as many as 2 people. The mean levels of magnesium, calcium, and potassium were 1.8 mg/dL, 8.69 mg/dL, and 4.25 mmol/L, respectively. The results of this study are magnesium levels affect muscle cramps in pregnant women (p-value=0.000), while calcium levels (p-value=0.373) and potassium (p-value=0.062) do not affect muscle cramps in pregnant women.

Conclusion: There is a significant effect between magnesium levels on the incidence of calf muscle cramps in third-trimester pregnant women, while calcium and potassium levels do not affect them.

Keywords: calcium, magnesium, muscle cramps, potassium.

Abstrak

Tujuan: *Untuk mengetahui pengaruh kadar magnesium, kalsium dan kalium terhadap kram otot betis pada ibu hamil trimester III di RSUD Dr. Zainoel Abidin Banda Aceh.*

Metode: *Penelitian ini merupakan penelitian analitik observasional dengan metode case control dengan cara pengambilan sampel menggunakan teknik total sampling. Sampel penelitian ialah ibu hamil trimester III (usia kandungan 28-40 minggu) yang di rawat di kamar Bersalin periode September hingga Desember 2020.*

Hasil: *Sebanyak 263 sampel terlibat dalam penelitian ini di mana 105 pasien (40%) mengalami obesitas, 229 pasien (87%) adalah seorang ibu rumah tangga, 161 pasien (61%) tidak mengalami kram otot dan sampel yang memiliki kadar magnesium di bawah normal sebanyak 82 orang, kadar kalsium di bawah normal sebanyak 127 orang dan kadar kalium di bawah normal sebanyak 2 orang. Kadar rerata magnesium, kalsium, dan kalium masing-masing adalah 1,8 mg/dL, 8,69 mg/dL dan 4,25 mmol/L. Hasil dari penelitian ini adalah kadar magnesium berpengaruh terhadap kram otot pada ibu hamil (p-value=0,000), sedangkan kadar kalsium (p-value=0,373) dan kalium (p-value=0,062) tidak berpengaruh terhadap kram otot pada ibu hamil.*

Kesimpulan: *Terdapat pengaruh signifikan antara kadar magnesium terhadap kejadian kram otot betis pada ibu hamil trimester III, sedangkan kadar kalsium dan kalium tidak memiliki pengaruh.*

Kata kunci: *kalium, kalsium, kram otot, magnesium.*

Correspondence author. Cut M. Maracilu. Department of Obstetrics and Gynecology.
Faculty of Medicine Universitas Syiah Kuala/Dr. Zainoel Abidin General Hospital. Banda Aceh
Email; nondacut@yahoo.com

Received: June,2022 Accepted: September,2022 Published: October,2022

INTRODUCTION

Muscle cramps are sudden muscle contractions due to an increase in the frequency of motor action potentials that are painful, continuous, unconscious, and localized to certain muscle fibers. In general, cramps can last from a few seconds to several minutes[1,2]. The prevalence of cramps in pregnant women in China was 32.9% during the first trimester, the percentage was 11.6%, the second trimester, 28.2%, and the last trimester, 50.2%[3]. Pregnant women in India experience third-trimester muscle cramps with a percentage of 64.6%[4].

Electrolyte imbalances such as potassium, magnesium, and calcium deficiency can worsen the condition of muscle cramps[4,5]. Pregnancy is closely related to hypomagnesemia. Based on several previous studies, it has been known that serum magnesium levels decrease during the pregnancy period [6]. The need for calcium to strengthen the skeletal structure of the fetus during pregnancy also increases, especially during the third-trimester, which is around 25-30 g[7–9]. potassium functions to regulate electrolyte balance, maintain blood pressure regulation, reduce rest-leg muscle contraction syndrome, and even transmit nerve impulses[10]. There are no related studies that examine the effect of magnesium, calcium, and potassium levels on the incidence of calf muscle cramps in third-trimester pregnant women at the Regional General Hospital (RSUD) Dr. Zainoel Abidin Banda Aceh so that researchers are interested in researching this.

METHODS

This research is an observational analytic study with a case-control method. The sampling technique used in this study is non-probability sampling using a total sampling technique. The purpose of this study was to determine the effect of magnesium, calcium, and potassium levels on calf muscle cramps in third-trimester pregnant women at RSUD Dr. Zainoel Abidin Banda Aceh. This research was conducted in the Obstetrics and Gynecology Maternity Room at Dr. Zainoel Abidin Banda Aceh from September to December 2020.

The inclusion criteria in this study were mothers in the third trimester of pregnancy (28-40 weeks). As for pregnant women who take magnesium, calcium, and potassium supplements, mothers who smoke and consume alcohol, mothers who use a diuretic or nephrotoxic treatment therapy, mothers who experience medical diseases such as diabetes mellitus, acute pancreatitis, kidney disease, hypothyroidism, and cystic fibrosis (as stated in the investigations, and mothers with gastrointestinal disorders such as chronic diarrhea, vomiting, and gastrointestinal fistulas were excluded from this study.

Data collection was carried out directly by researchers through questionnaires to obtain information about the incidence of calf muscle cramps in pregnant women through respondents' answers. Data on serum magnesium, calcium, and potassium levels in pregnant women were obtained through laboratory tests. Blood sampling was conducted by health workers at RSUD Dr. Zainoel Abidin Banda Aceh on the subject and blood samples were taken from the median cubital vein by antiseptic treatment with 70% alcohol and allowed to dry, then 3 ccs of blood were taken, put into a yellow-top vacutainer blood tube for examination of blood chemistry (magnesium, potassium, and calcium).

RESULTS

During the period September to December 2020, a total of 263 research samples met the inclusion criteria. The general characteristics of respondents in this study are presented in table 1 below.

Table 1. General Characteristics of Respondents

General characteristics	Amount (n=84)	(%)
Body Mass Index (BMI)		
Status	8	3
Underweight	54	20
Normal	55	21
Overweight	105	40
Obesitas I	41	16
Obesitas II		
Occupations	229	87
Housewife	9	Non-Housewife
Civil servant	8	(13)
Teacher	4	
Private	3	
Honorary	3	
Contract employees	7	
Others		
Cramp Status Percentage	102	39
Yes	161	61
No		
Levels of Magnesium, Calcium, and Potassium (Below Normal Values)		
Magnesium	82	31
Kalsium	127	49
Kalium	2	0.7

Based on table 1, the study sample was dominated by pregnant women with BMI obesity I status of 105 people (40%) and housewives as many as 229 people (87%). A total of 161 people (61%) did not experience muscle cramps in this study. The values of magnesium, calcium, and potassium levels below normal were 82 people (31%), 127 people (49%), and 2 people (0.7%).

Binary Logistic Regression Analysis to See the Effect of Magnesium, Calcium, and Potassium Levels on the Perspective of the Incidence of Muscle Cramps in Pregnant Women

The initial stage in binary logistic regression analysis is looking for parameter estimates. Based on the results of data processing with software R version 3.6.3, the following information was obtained:

Table 2. The Estimated Value of the Parameter

Variables	The estimated value of the parameter
(Intercept)	15,902
Magnesium levels	-19,775
Calcium levels	1,002
Potassium levels	2,108

Based on the values presented in table 2, the logit conjecture model is obtained as follows:

$$g(x) = In\left[\frac{\pi}{1 - \pi}\right]$$

=15,902 - 19,775 Magnesium + 1,002 Kalsium + 2,108 Kalium

The logistic equations are

$$\pi(x_i) = \frac{e\ (15{,}902 - 19{,}775\ \text{Magnesium} + 1{,}002\ \text{Kalsium} + 2{,}108\ \text{Kalium})}{1 + e\ (15{,}902 - 19{,}775\ \text{Magnesium} + 1{,}002\ \text{Kalsium} + 2{,}108\ \text{Kalium})}$$

After estimating the parameters, the next step that must be done is to test the significance of these parameters. The parameter significance test was carried out in 2 ways, namely the simultaneous and partial parameter significance test. A simultaneous parameter significance test was conducted to see the effect of independent variables, namely levels of magnesium, calcium, and potassium on the occurrence of muscle cramps in pregnant women simultaneously. The test used to test the significance of the parameters simultaneously is the likelihood ratio chi-square test. The hypothesis for the simultaneous parameter significance test is as follows.

H_0: $\beta_1=\beta_2=\beta_i=0$ (Levels of magnesium, calcium, and potassium together do not affect the incidence of muscle cramps in pregnant women)

H_1: At least one of $\beta i \neq 0$ (at least one of the levels of magnesium, calcium, and potassium affects the incidence of muscle cramps in pregnant women) $\alpha=0.05$.

The test statistic used is the G^2 statistic which follows the chi-square distribution, so that to obtain a decision, it is compared with the chi-square value, a table with degrees of freedom = the number of independent variables. H_0 can be rejected if the test statistic value $G^2>\chi(\upsilon.\alpha)$.

Table 3. G Statistical Test

G^2 statistic	χ(3;0.05)
266.83	7.815

Based on the test results, the G^2 value is 266.83, while the value $\chi(3;0.05)$ is 7.815. The value of G^2 (266.83) > $\chi(3;0.05)$ (7.815) so H0 can be rejected. Thus, it can be concluded that at least one of the levels of magnesium, calcium, and potassium affects the incidence of muscle cramps in pregnant women.

The partial significance test aims to see the effect of each independent variable, namely levels of magnesium, calcium, and potassium on the incidence of muscle cramps in pregnant women. The partial test can be done with the Wald test with the following hypotheses:

H_0: $\beta_j = 0$ (Levels of magnesium, calcium, and potassium do not affect the incidence of muscle cramps in pregnant women)

H_1: $\beta_1 \neq 0$ (Magnesium levels affect the incidence of muscle cramps in pregnant women)

H_2: $\beta_2 \neq 0$ (Calcium levels affect the incidence of muscle cramps in pregnant women)

H_3: $\beta_3 \neq 0$ (Potassium levels affect the incidence of muscle cramps in pregnant women)

The Wald test uses the W^2 test statistic with criteria H_0 is rejected if $W^2>\chi(\upsilon.\alpha)$ or $|W| = {}^{Z\alpha}/_{2}$ with p-value$<\alpha=0.05$.

Table 4. Significance Value

Variables	*P-value*
Magnesium Levels	0.000
Calcium Levels	0.373
Potassium Levels	0.062

Based on the results of data processing, the p-value for the variable levels of magnesium, calcium, and potassium, respectively, is 0.000; 0.373, and 0.062. With a significance level of 0.05, H_0 is rejected if the p-value <0.05. So, the p-value for the variable magnesium content (0.000)<0.05. The p-value for the variable calcium content (0.373)>0.05, and the p-value for the variable potassium content (0.062)>0.05. Thus, H_0 can be rejected for the magnesium variable. It can be concluded that the variable magnesium levels affect muscle cramps in pregnant women, while calcium and potassium levels do not affect muscle cramps in pregnant women.

To test the feasibility of the obtained model, it can be done with a test called the deviance test. The deviance test compares the likelihood of the saturated model, which is a model that has a perfect match with the model obtained. The hypothesis of the model's feasibility test is as follows. H_0: The model is feasible (there is no difference between the observed results and the predicted results). H_1: The model is not feasible (There is a difference between the results of the observations and the predicted results).

The test statistic used is statistic D with rejection criteria H_0 is if $D>\chi(\alpha.n-p)$ which n is the number of observations and p is the number of parameters. Based on the results of data processing, the value of D = 84.42 and the value of $\chi(0,05;263-3)$ of 298,611 so that D (84,42) < $\chi(0,05;263-3)$. Thus, H_0 cannot be rejected. So, at the 0.05 level of significance, this model is feasible to use.

Testing the significance of the parameters partially gave the result that among serum magnesium, calcium, and potassium, only magnesium affected the incidence of muscle cramps in pregnant women. Thus, the previously obtained logistic regression model was revised. Serum calcium and potassium which did not have a significant effect on the incidence of muscle cramps in pregnant women were excluded from the model so that only magnesium remained.

The revised model obtained is

$$g(x) = \log\left\{\frac{\pi}{1-\pi}\right\} = 30{,}780 - 18{,}454\ Magnesium$$

With the following logistic equation

$$\pi(x_i) = \frac{e^{30{,}780 - 18{,}454\ Magnesium}}{1 + e^{30{,}780 - 18{,}454\ Magnesium}}$$

The value of $\pi(\chi_i)$ shows the probability value for muscle cramps in pregnant women. The closer the probability value to the number 1 means that the greater the chance for pregnant women to experience muscle cramps. The opportunity threshold value used in this study to be said to have cramp status is 0.98. It was found that the maximum magnesium level that can make a person experience muscle cramps is 1.457 mg/dL. This means that at least a pregnant woman must have a magnesium level of 1.457 mg/dL to avoid muscle cramps.

DISCUSSIONS

The general characteristics of respondents in this study were body mass index (BMI), cramp status, and patient occupation. In the characteristics of this study (table 1) that the study sample was dominated by pregnant women with BMI in the form of obesity I as much as 40%. This is not in line with the research conducted by Tinius et al. (2020) which states that pregnant women with an average BMI of 26.1 kg/m^2 or overweight will experience musculoskeletal complaints in pregnancy[11].

Furthermore, table 1 shows that 61% of the samples did not experience muscle cramps. Research shows that muscle cramps are the second most common complaint felt by pregnant women, especially in the second and third trimesters. These muscle cramps occur during sleep and are affected by magnesium and calcium deficiency[12]. In addition, stated that muscle cramps in the leg muscles are complaints that are often felt generally in women compared to men[13]. Another study stated that complaints such as low back pain occurred approximately 60% and muscle cramps 42% were the most common complaints experienced by pregnant women[14]. In another study showed that as many as 334 pregnant women who were the research sample experienced muscle cramps during pregnancy[15].

Another characteristic is the occupation of pregnant women, most of the pregnant women who were the sample of this study were dominated by housewives by 87%, while the other 13% worked as teachers, midwives, contract employees, civil servants, honorary, private, and others. Based on research, 50.9% of musculoskeletal disorders in pregnancy such as leg cramps occur in pregnant women whose daily work is other than being a housewife, so

in this study, it was stated that work status was related to musculoskeletal disorders in pregnant women[16]. It is different from the research which showed that 90% of pregnant women with musculoskeletal disorders that occur in pregnant women do not work[12]. The pathophysiology of calf muscle cramps is still not known with certainty. Based on previous research, one of the causes of muscle cramps due to electrolyte imbalance, in the form of magnesium, calcium, and potassium.

The results of this study indicate that based on a partial parameter significance test which aims to assess the effect of each of the independent variables, namely magnesium levels on the incidence of muscle cramps in pregnant women, the results of the test are that the p-value for magnesium levels is 0.000. The results of this statistical test can be interpreted that magnesium levels affect the incidence of muscle cramps in pregnant women. These results which states that if there is a magnesium deficiency in pregnancy it can have outcomes such as premature birth, intrauterine growth restriction (IUGR), uterine hyperexcitability, insulin resistance, and musculoskeletal disorders such as muscle cramps[17]. In another study, it was recommended that magnesium supplementation during pregnancy be the treatment of choice for the management of muscle cramps induced by pregnancy[18]. In addition, another study stated that pregnancy with a magnesium deficiency will cause hypertension in pregnancy, IUGR, and muscle cramps[19]. Oral magnesium supplementation during pregnancy did not reduce the incidence and frequency of episodes of leg cramps in pregnant women[20]. Another study stated that the administration of magnesium during pregnancy did not treat the incidence of muscle cramps in pregnant women, only reduced the incidence of muscle cramps[3].

The results of this study indicate that the p-value of calcium and potassium levels is 0.373 and 0.062, respectively. This means that calcium and potassium levels do not affect the incidence of muscle cramps in pregnant women. This can happen because when viewed from a descriptive analysis of the content of calcium levels in pregnant women are in the range of 8.0-9.5 mg/dL with the average calcium content in pregnant women is 8.689 or 8.7 mg/dL is still within the normal range. normal range (8.5-10.5 mg/dl)[21]. The potassium content in pregnant women is in the range of 3.2-5 mmol/L, the content of potassium levels in pregnant women when viewed on average is 4.25 mmol/L also still within normal limits (3.3-5.1 mmol/L)[21]. The results who stated that there was no significant effect of serum calcium and potassium levels in pregnant women with muscle cramps[22]. A study on the effects of calcium and potassium content on pregnant women showed that calcium and potassium levels cause hypertension in pregnancy such as preeclampsia-eclampsia but do not cause muscle cramps[23], also gave the results that giving calcium and potassium during pregnancy did not treat the incidence of muscle cramps in pregnant women, but only reduced pain when muscle cramps occur[3].

CONCLUSION

This study concludes that there is a significant effect of magnesium levels on the incidence of calf muscle cramps in third-trimester pregnant women. The minimum magnesium threshold value for a pregnant woman during the third trimester is 1.457 mg/dL, to avoid the occurrence of calf muscle cramps during pregnancy with a probability value of 0.98.

ACKNOWLEDGEMENT

The authors would like to thank the Department of Obstetrics and Gynecology, Faculty of Medicine, Syiah Kuala University/RSUDZA Banda Aceh, which has assisted and collaborated in completing this research. In this study, there is no conflict of interest.

REFERENCES

1. Bordoni B, Sugumar K, Varacallo M. Muscle Cramps. In: StatPearls Treasure Island, Florida. StatPearls Publishing. 2020.
2. Buttaravoli P. Minor Emergencies. Expert Consult. 2021: 465–518.
3. Luo L, Zhou K, Zhang J, Xu L, Yin W. Interventions for leg cramps in pregnancy. The Cochrane database of systematic reviews. 2020;12:CD010655.
4. Katzberg HD. Neurogenic muscle cramps. J Neurol. 2015;262(8):1814–21.
5. Group BP. BMJ Best Practice. BMJ Publishing Group Limited 2020. 2020. https://bestpractice.bmj.com/info/
6. Alalawi Y, M'Hiri I, Alrob HA, Khan A. Hypoparathyroidism in Pregnancy. Springer. 2020.
7. Ramachandra P, Maiya AG, Kumar P, Kamath A. Prevalence of musculoskeletal dysfunctions among Indian pregnant women. J Preg. 2015;2015: 437105. doi: 10.1155/2015/437105.

8. Katzberg HD. Case Studies in Management of Muscle Cramps. Neurol Clin. 2020;38(3):679–96. https://doi.org/10.1016/j.ncl.2020.03.011
9. Meena PK, Maheswari S. Serum Magnesium Level as a Predictive Tool for Preterm Labour. Ind J Obstet Gynecol Res. 2020;7(3):356–36.
10. Spatling L, Classen HG, Kisters K,. et al. Supplementation of Magnesium in Pregnancy. J Preg Child Health. 2017;04:01.
11. Tinius R, Nagpal TS, Edens K, Duchette C, Blankenship M. Exploring Beliefs about Exercise among Pregnant Women in Rural Communities. J Midwifery Women's Health. 2020;65(4):538–45.
12. Bakilan F, Zelveci DD. Musculoskeletal problems during pregnancy. J Clin Med Kazakhstan. 2020;6(60):53–5.
13. Asghar A, Ahmad A, Gillani SA. Leg cramps among older men and women. Rawal Med J. 2020;45(2):396–8.
14. Vyas H, Deviga T. Impact of Musculoskeletal Dysfunctions on Quality of Life of Pregnant Women. Int J Nurs Midwifery Res. 2020;7(2):27–31.
15. Hall H, Lauche R, Adams J, Steel A, Broom A, Sibbritt D. Healthcare utilization of pregnant women who experience sciatica, leg cramps and/or varicose veins: A cross-sectional survey of 1835 pregnant women. Women and Birth. 2016;29(1):35–40. http://dx.doi.org/10.1016/j.wombi.2015.07.184
16. Badri PRA, Novrikasari, Flora R. Analysis of Physical Activity Against Stress Levels in Pregnant Women at Plaju Health Center. Sriwijaya Int Conference of Public Health. 2020;25(Sicph 2019):51–63.
17. Fanni D, Gerosa C, Nurchi VM,. et al. The Role of Magnesium in Pregnancy and in Fetal Programming of Adult Diseases. Biol Trace Elem Res. 2020;199(10):3647-3657.doi: 10.1007/s12011-020-02513-0
18. Gaheen A, Sayed M, Shaban R. Knowledge, Attitude and Practices Regarding Calcium Intake among Pregnant Women in Tanta City. J Nurs Health Sci. 2020;9(1):55–69. http://www.academia.edu/download/62482125/E090108556920200325-86618-d5hffv.pdf
19. Zarean E, Tarjan A. Effect of Magnesium Supplement on Pregnancy Outcomes: A Randomized Control Trial. Advanced Biomed Res.2017;6(109). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5590399/
20. De Araujo CAL, De Lorena SB, De Sousa Cavalcanti GC, et al. Oral magnesium supplementation for leg cramps in pregnancy-An observational controlled trial. PLoS ONE. 2020;15(1):6–13.
21. Garg A, Parajuli P, Mamillapalli C. Pregnancy Complicated by Hypertension and Hypokalemia. Am J Kidney Dis. 2020;76(4):A21-2.
22. Majidi U, Amerian M, Khosravi F, et al,. The Effect of Gradual Reduction in Sodium Dialysate on Occurrence of Muscular Cramp, and the Serum Level of Calcium and Magnesium in Patients Undergoing Hemodialysis: A Cross-Over Clinical Trial. Int J Health Stud. 2020;6(1):1–5.
23. Brown LL, Cohen BE, Edwards E, Gustin CE, Noreen Z. Physiological Need for Calcium, Iron, and Folic Acid for Women of Various Subpopulations during Pregnancy and Beyond. J Women's Health. 2020;30(2):207–12.

Research Article

Assessment Serum Zinc Level in Pregnancy with Covid-19 Compare with Normal Pregnancy

Penilaian Kadar Seng Serum pada Kehamilan dengan Covid-19 Dibandingkan dengan Kehamilan Normal

Freddy.W.Wagey, Vivi Mamangkey, Frank.M.M.Wagey

Department of Obstetrics and Gynecology
Faculty of Medicine Universitas Sam Ratulangi
Prof. Dr. R. D. Kandou General Hospital
Manado

Abstract

Objective: The objective of this study was to assess serum zinc levels in pregnancy with COVID-19 compared with normal pregnancy.

Methods: The sampling method used was consecutive sampling. All subjects enrolled must meet eligibility inclusion and exclusion criteria with signed informed consent. The study was conducted from March 2021 to June 2021 in Manado. Pregnant women with COVID-19 and normal pregnant women serum zinc levels were measured.

Results: Sixteen pregnant patients with COVID-19 and 16 normal pregnant patients' serum zinc levels were measured. All pregnant women with COVID-19 have low serum zinc levels (100%), whereas in the normal pregnancy group only 8 subjects (50%) have low serum zinc levels and 8 subjects (50%) have normal serum zinc levels. In the pregnancy with COVID-19 group, the mean±SD value was 40.75±10.440, whereas in the normal pregnancy group the mean±SD value was 60.25±17.407. The analysis using an independent T-test shows a significant difference in serum zinc levels between pregnancies with COVID-19 and normal pregnancies (p=0.001).

Conclusion: There is a significant difference in serum zinc levels in pregnancies with COVID-19 and normal pregnancies.

Keywords: COVID-19, pregnancy with COVID-19, zinc in pregnancy.

Abstrak

Tujuan: *Untuk mengetahui kadar seng pada kehamilan dengan COVID-19 dibandingkan pada kehamilan normal.*

Metode: *Pengambilan sampel metode consecutive sampling setiap subjek yang sesuai dengan kriteria inklusi dan eksklusi penelitian dan telah menandatangani surat persetujuan. Penelitian dilakukan bulan Maret 2021 sampai Juni 2021 di Manado. Dilakukan pemeriksaan kadar seng dalam serum pasien hamil dengan COVID-19 dan pasien hamil normal.*

Hasil: *Enam belas pasien hamil dengan COVID-19 dan 16 pasien hamil normal dilakukan pemeriksaan kadar seng. Kelompok kehamilan dengan COVID-19, semuanya memiliki kadar seng rendah (100%). Sedangkan pada kehamilan normal kelompok kadar zinc rendah sebanyak 8 orang (50%) dan kadar seng normal sebanyak 8 orang (50%). Pada kelompok kehamilan dengan COVID-19 didapatkan nilai mean±SD sebesar 40,75±10,440, sedangkan pada kelompok kehamilan normal nilai mean±SD sebesar 60,25±17,407. Pada analisis uji beda independen menunjukkan bahwa terdapat perbedaan bermakna kadar zinc pada kehamilan dengan COVID-19 dan kehamilan normal (p=0.001).*

Kesimpulan: *Terdapat perbedaan bermakna kadar seng pada kehamilan dengan COVID-19 dan kehamilan normal.*

Kata kunci: *COVID-19, kehamilan dengan COVID-19, seng pada kehamilan.*

Correspondence author. Vivi Mamangkey. Department of Obstetrics and Gynecology Faculty of Medicine Universitas Sam Ratulangi. Prof.Dr. R.D. Kandou General Hospital Manado. Email;vivimamangkey@gmail.com

Received: September,2021 Accepted: September,2022 Published: October,2022

INTRODUCTION

Zinc is an essential micronutrient that plays a role in cell proliferation, differentiation, DNA and RNA synthesis, and cell membrane and structure stabilization.[1,2] Zinc plays an important role in modulating pro-inflammatory response by regulating inflammatory cytokines and controlling oxidative stress.[3] According to the study by Sonja et al. in pregnancy and fetal development, zinc supplementation may reduce the incidence of premature birth by 14%.

Novel coronavirus 2019 (SARS-CoV) is a new type of virus emerging from the coronavirus family, which causes the COVID-19 pandemic.[4] COVID-19 enters the host cell and triggers an immune response, including pro-inflammatory cytokines production, CD4 and CD8+ T-cell activation.[5] The general signs and symptoms of COVID-19 infection include acute respiratory distress such as fever, cough, and dyspnea. Acute respiratory distress syndrome is the severe symptom of this disease. Excessive cytokine production, termed cytokine storm, plays an important role in causing acute respiratory distress syndrome.[6]

Many studies had been done to control the pandemic, including the topic of vaccine and treatment. However, many things are still unknown such as the potential protective effect against the virus, and the effect of micronutrients such as vitamin D, vitamin B12, and zinc in 44 pregnant women with COVID-19.[7] The study found that reduced micronutrient levels affected the immune response of pregnant women with COVID-19. Studies regarding the role of zinc in pregnancies in Indonesia are mainly done in preeclampsia condition, or in the birthweight, mortality, and morbidity.[8,9] However, studies regarding the role of zinc in pregnancies with COVID-19 have never been reported. Therefore, the author wanted to investigate the zinc levels in pregnant women with COVID-19 compared with a normal pregnancy to provide crucial information regarding the role of zinc in pregnancy during the COVID-19 pandemic.

METHODS

This study was a cross-sectional study. The study population was pregnant women who presented to the RSUP Prof. dr. R. D. Kandou Manado and RS Pancaran Kasih Manado from March 2021 until June 2021, with a total subject of 32 patients. The subjects consisted of 16 pregnant women with COVID-19 and 16 normal pregnant women. All subjects met the inclusion and exclusion criteria and signed informed consent. The inclusion criteria were all pregnancies with a confirmed COVID-19 and normal pregnancy who presented to the emergency ward and Obstetrics and Gynecology outpatient polyclinic of RSUP Prof. dr. R. D. Kandou Manado and RS Pancaran Kasih Manado; and agreed to participate in the study with signed informed consent. The exclusion criteria were women suffering from autoimmune disease and HIV; women currently in immunosuppressive therapy; currently using zinc supplementation; refused to participate in the study. Data analysis was done by using the statistical data processing software program SPSS version 23.0. The study was conducted after obtaining the approval and recommendation from the Health Research Ethics Committee, Faculty of Medicine Universitas Sam Ratulangi RSUP Prof RD Kandou Manado.

RESULTS

The study was conducted in RSUP Prof. dr. R. D. Kandou Manado and RS Pancaran Kasih Manado from March 2021 until June 2021 with a total subject of 32 patients. The subjects consisted of 16 pregnant women with COVID-19 and 16 normal pregnant women who met the inclusion and exclusion criteria and had signed informed consent. The subjects' characteristics are shown in Table.

Table 1. Subjects' Characteristics

Characteristics	Pregnancy with COVID-19		Normal Pregnancy	
	N	%	N	%
Maternal age (y o)				
≤20	2	12.5	0	0
21-34	13	81.25	13	81.25
≥35	1	6.25	3	18.75
Parity status				
Primigravida	4	25	4	25
Multigravida	12	75	12	75
Trimester				
I	1	6.25	2	12.5
II	0	0	1	6.25
III	15	93.75	13	81.25

The data above showed that most pregnancies with COVID-19 occurred in the age range of 21-34 years, namely 13 subjects (81.25%). The age group of ≤ 20 consisted of 2 subjects (12.5%), and age group of ≥ 35 consisted of 1 subject

(6.25%). In the normal pregnancies, most subjects were in the age range of 21-34 years old, namely 13 subjects (81.25%), with the age ≥ 35 years consisting of 3 subjects (18.75%).

Table 2. Zinc level in Pregnancy with COVID-19 and Normal Pregnancy

Characteristics	Pregnancy with COVID-19		Normal Pregnancy	
	N	%	N	%
Zinc Levels (ug/dL)				
Low (<60)	16	100	8	50
Normal (60-130)	0	0	8	50
High (>130)	0	0	0	0

Based on the zinc level, all subjects of the pregnancies with COVID-19 group had low zinc levels (100%). In the normal pregnancies group, 8 subjects (50%) had low zinc levels, and 8 (50%) had normal levels.

Table 3. Zinc Levels Distribution in Pregnancy with COVID-19 and Normal Pregnancy

Variable	Pregnancy with COVID-19(n=16)		Normal Pregnancy (n=16)	
Zinc Levels	Mean	40.75	Mean	60.25
	Median	41.50	Median	56.00
	Std deviation	10.440	Std deviation	17.407
	Minimum	19	Minimum	42
	Maximum	57	Maximum	100

Table 3 showed a distribution of zinc levels in the pregnancies with COVID-19 and in normal pregnancies. In the pregnancies with COVID-19 group, the mean±SD value was 40.75±10.440. In the normal pregnancies group, the mean±SD value was 60.25±17.407. Mean zinc levels were lower in the pregnancy with COVID-19 group than that in normal pregnancy.

Table 4. Independent T-test of Zinc Levels in Pregnancy with COVID-19 and In Normal Pregnancy

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference Lower	Upper
Zinc	Equal variances assumed	3.449	.073	3.843	30	.001	19.500	5.074	9.137	29.863
	Equal variances not assumed			3.843	24.556	.001	19.500	5.074	9.039	29.961

Based on the Shapiro-Wilk normality test, the zinc levels in pregnancy with COVID-19 and in normal pregnancy data were normally distributed (p = 0.074 and 0.0955). Therefore, we conducted the independent samples T-test (T-test) (Table 4). Table 4 showed a significant difference in zinc levels in pregnancy with COVID-19 and in normal pregnancy (p=0.001).

DISCUSSION

SARS-CoV-2 infection is still uncontrollable; thus, it is still a global issue. As of July 12th, 2021, COVID-19 cases had reached 186.762.453 cases and 4.030.918 death cases globally. Approximately 2.527.203 cases and 66.464 death cases had been reported in Indonesia.8 In our study, we found that the age range of 21-34 years had the most subjects of pregnancy with COVID-19, namely 13 subjects (81.25%). The ≤20 years age group consisted of 2 subjects (12.5%), and the ≥35 years age group consisted of 1 subject (6.25%). In the normal pregnancy group, most subjects were in the 21-34 years age range group, namely 13 subjects (81.25%), whereas the ≥35 years age group consisted of 3 subjects (18.75%). This result is in line with the data from the predominant age group of infected patients in Indonesia, which is the 25-34 years age group.[8]

Based on the parity status in the pregnancy with COVID-19 group, the multigravida group had the most subjects, namely 12 subjects (75%), whereas the primigravida had 4 subjects (25%). While in the normal pregnancy group, the multigravida group also had the most subjects, namely 12 subjects (75%), whereas primigravida also had 4 subjects (25%). Based on the gestational age in the pregnancy with the COVID-19 group, the trimester III group had the most subjects, namely 15 subjects (93.75%), whereas trimester

I had 1 subject (6.25%). In the normal pregnancy group, trimester III also had the most, namely 13 subjects (81.25%), whereas trimester I had 2 subjects (12.5%), and trimester II had 1 subject (6.25%).

Previous studies of COVID-19 infection showed that patients with low zinc levels are strongly associated with the rate of severe complications, longer hospital stays, corticosteroid usage, and increased mortality rate.[9]

Zinc deficiency is strongly associated with the rate of severe respiratory viral infection.[10] Zinc has also been used as nutritional supplementation, both as single or combined supplementation, for the prophylaxis or therapy of COVID-19 infection.[11] Zinc has also been documented to play a role in preventing cellular damage. It has an antiviral effect that underlies the potential of zinc in the management of COVID-19.[12] Multiorgan damage and dysfunction that occurs in COVID-19 infections can also occur based on zinc deficiency, which affects the nerve, cardiovascular, immune, and endocrine systems.[13]

In our study, we found that all subjects in the pregnancy with COVID-19 group had low zinc levels (100%). In the normal pregnancy group, 8 subjects had low zinc levels (50%), and 8 subjects had normal zinc levels (50%). The mean±SD value in the pregnancy with COVID-19 group was 40.75±10.440, while the normal pregnancy group had 60.25±17.407. The independent t-test analysis in our study revealed a significant difference in zinc levels in pregnancy with COVID-19 and the normal pregnancy group (p=0.001). The mean zinc levels were lower in the pregnancy with COVID-19 group compared to the normal pregnancy group. Studied 100 pregnant women with COVID-19 and 100 normal pregnant women.[14] The study showed that zinc levels in the pregnancy with COVID-19 group were lower compared to the normal pregnancy in the first trimester (67.19±13.87 vs 55.97±16.57, p=0.004), in the second trimester (52.84±12.57 vs 46.38±12.66, p=0.005), and in the third trimester (54.37±13.57 vs 46.82±12.51, p=0.02). The study also concluded an association between zinc and magnesium on the acute phase reactant, especially in the first trimester.

A similar result was also found in the group of 44 pregnant women with COVID-19, which showed lower zinc levels in pregnancy with COVID-19 group compared to the normal pregnancy group (62.58±2.63, p<0.001).7 They concluded a positive correlation of zinc levels with better immune response in pregnant women by reducing inflammation, increasing mucociliary clearance, preventing lung injury in ventilator usage, and modulating antiviral and antibacterial effects.

Forty-seven pregnant women with COVID-19 also showed lower zinc levels compared to the levels of 45 normal pregnant women, with a median of 74,5 µg/dL vs. 105,8 µg/dL (p<0.001).9 Of 47 pregnancies with COVID-19, 27 subjects (57.4%) had zinc deficiency. There was a higher risk of complication in this group (p=0.02), including acute respiratory distress syndrome (18.5% vs 0%, p=0.06), increased corticosteroid usage (p=0.02), longer hospital stays (p=0.05) and increased mortality rate (18.5% vs 0%, p=0.06). The limitation of our study is that it is still unclear whether zinc supplementation can lower the severity of the infection. The Spike protein of the SARS-CoV-2 along with its receptors (ACE2) in the lung alveoli is the entry of the virus into the cell. Low zinc plasma levels would cause increased interactions between the viral protein and its receptor, and vice versa; increased zinc levels would cause reduced interactions.[15] Zinc also has an antiviral effect by obstructing RNA synthesis, viral replication, DNA polymerase, reverse transcriptase, and viral protease.[16] Zinc can provide an anti-inflammatory effect by inhibiting the NF-KB signal and modulating the regulation of T-cell function; therefore, reducing the aggravation of cytokine storm. Increased zinc levels can also lower the risk of secondary bacterial co-infections by increasing mucociliary clearance and barrier function of the respiratory epithelial, also with a direct antibacterial effect against S. pneumonia. Zinc status is known to be closely related to the risk factors of severe COVID-19 infections, such as older age, immune deficiencies, obesity, diabetes mellitus, and atherosclerosis.[17]

Based on the previous studies, the author did not find a study that connects zinc supplementation with the incidence of COVID-19 infection risk factors, the effect of zinc levels and zinc supplementation on clinical symptoms severity of COVID-19, and complications related to COVID-19 infection. COVID-19 infection is still a new infection; however, the impact of the pandemic would have a large effect globally. Therefore, further and profound studies regarding the role of zinc in COVID-19 infection are necessary.

CONCLUSIONS

Zinc levels in the pregnancy with COVID-19 group are lower with a mean ± SD value of 40.75±10.440 compared to the normal pregnancy group with a mean ± SD value of 60.25±17.407. Independent t-test showed a significant difference in zinc levels in pregnancy with COVID-19 and normal pregnancy (p=0.001).

SUGGESTIONS

Various factors strongly influence zinc levels in pregnancy with COVID-19. Therefore, more inclusion and exclusion criteria are necessary to be more selective on the study subjects. Moreover, further studies should include a larger sample size. Finally, studies on the effect of zinc levels and supplementation on the clinical severity, risk factors, and complications related to COVID-19 in pregnancy should be conducted.

REFERENCES

1. Rink L, Gabriel P: Zinc and the immune system. Proc Nutr Soc. 2020;59:541-52.
2. Maret W, Li Y: Coordination dynamics of zinc in proteins. Chem Rev. 2019;109:4682-4707.
3. Maret W: Zinc and human disease . Interrelations between Essential Metal Ions and Human Diseases. Sigel A, Sigel H, Sigel RK (ed): Springer, Dordrecht,. The Netherlands. 2013;13:389-414.
4. Li H, Liu SM, Yu XH, Tang SL, Tang CK: Coronavirus disease 2019 (COVID- 19): current status and future perspectives. Int J Antimicrob Agents. 2020;55:105951.
5. Tufan A, Avanoğlu Güler A, Matucci-Cerinic M: COVID-19, immune system response, hyperinflammation and repurposing antirheumatic drugs. Turk J Med Sci. 2020;50:620-32.
6. Ye Q, Wang B, Mao J. The Pathogenesis and Treatment of the Cytokine Storm in COVID-19 . J Infect. 2020;80:607-13.
7. Bahat YP, Talmac MA, Bestel A, Selcuki NF, Aydin Z, Polat I. Micronutrients in COVID-19 Positive Pregnancies. Cureus 12(9):e10609. DOI 10.7759/cureus.10609.
8. Gugus Tugas Percepatan Penanganan COVID-19. Peta sebaran. 2020. www.covid19.go.id/peta-sebar.
9. Jothimani D, Kailasam E, Danielraj S, et al,. COVID-19: poor outcomes in patients with Zinc deficiency. Int J Infect Dis. 2020;100:343–9.
10. Elshafeey F, Magdi R, Hindi N, Elshebiny M. A systematic scoping review of COVID-19 during pregnancy and childbirth. Int J Gynecol Obstet. 2020;150(1):47-52.
11. Roth DE, Richard SA, Black RE. Zinc supplementation for the prevention of acute lower respiratory infection in children in developing countries: meta-analysis and meta-regression of randomized trials. Int J Epidemiol. 2010;39:795–808.
12. Wessels I, Rolles B, Rink L. The potential impact of zinc supplementation on COVID-19 pathogenesis. Front Immunol. 2020;11:1712.
13. Prasad AS. Zinc: an antioxidant and anti-inflammatory agent: role of zinc in degenerative disorders of aging. J. Trace Elem. Med. Biol. 2014;28:364–71.
14. Anuk AT, Polat N, Akdas SS, Erol SA. The Relation Between Trace Element Status (Zinc, Copper, Magnesium) and Clinical Outcomes in COVID-19 Infection During Pregnancy. Biol Trace Elem Res. 2020 ;2:1-10.
15. Devaux CA, Roalin JM, Raoult D. ACE2 receptor polymorphism: Susceptibility to SARS-CoV-2, hypertension, multi-organ failure and COVID-19 disease outcome. J. Microbiol. 2020;53:425-35.
16. Read SA, Obeid S, Ashlenstiel C. The role of zinc in antiviral immunity. Adv Nutr. 2019;10(4):696-710.
17. Skalny AV, Rink L, Ajsuvakova OP, Aschner M, Gritsenko VA, Alekseenko SI, et al. Zinc and respiratory tract infections: Perspectives for COVID-19 (Review). Int. J. Mol. Med. 2020;46:17-26.

Research Article

Assosiation between Serum Cortisol Levels and Anxiety levels In Elective and Emergency Cesarean Section

Hubungan antara Kortisol Serum dan Tingkat Kecemasan pada Operasi Seksio Sesarea Elektif dan Darurat

Nadia O. S. Nurdin, John J. E. Wantania, R. A. A. Mewengkang

Departement of Obstetrics and Gynecology
Faculty of Medicine Universitas Sam Ratulangi
Prof. DR. R. D. Kandou General Hospital
Manado

Abstract

Objective: To detect anxiety before elective and emergency cesarean section.

Methods: This study was a cross-sectional study. The number of research samples was 42 samples consisting of 21 pregnant patients with an elective cesarean section plan and 21 pregnant patients with an emergency cesarean section at Prof. RSUP. Dr. R. D. Kandou Hospital Manado and Network Hospital in Manado from March 2021 to June 2021. Anxiety was assessed by examining serum cortisol and anxiety levels according to the Hamilton questionnaire (HAM-A). The data were then analyzed using SPSS version 23.0 software.

Results: Cortisol levels were higher in emergency cesarean section (mean 21.590±11.6392) compared to elective cesarean section (mean 10.586±4.9501). Anxiety levels according to HAM-A scores were higher in emergency cesarean section (mean 15.33±7.722) compared to elective cesarean section (mean 7.19±3.614).

Conclusion: There was a significant positive correlation between cortisol levels and anxiety levels based on HAM-A scores.

Keywords: cesarean section, cortisol, HAM-A score anxiety.

Abstrak

Tujuan: *Untuk mendeteksi kecemasan sebelum tindakan seksio sesarea elektif dan emergensi.*

Metode: *Penelitian ini merupakan suatu penelitian cross-sectional. Jumlah sampel penelitian 42 sampel terdiri dari 21 pasien hamil dengan rencana seksio sesarea elektif dan 21 pasien hamil dengan rencana seksio sesarea emergensi di RSUP Prof. Dr. R. D. Kandou Manado dan RS Jejaring di Kota Manado dari bulan Maret 2021 sampai bulan Juni 2021. Kecemasan dinilai melalui pemeriksaan kortisol serum dan tingkat kecemasan menurut kusioner Hamilton (HAM-A). Data kemudian dianalisis dengan menggunakan software SPSS versi 23.0.*

Hasil: *Kadar kortisol secara rata-rata lebih tinggi pada seksio sesarea emergensi (mean 21.590±11.6392) dibandingkan dengan seksio sesarea elektif (mean 10.586±4.9501). Tingkat kecemasan menurut skor HAM-A secara rata-rata lebih tinggi pada seksio sesarea emergensi (mean 15.33±7.722) dibandingkan dengan seksio sesarea elektif (mean 7.19±3.614).*

Kesimpulan: *Terdapat korelasi positif bermakna antara kadar kortisol dan tingkat kecemasan berdasarkan skor HAM-A pada semua subjek penelitian.*

Kata kunci: *kortisol, seksio sesarea, skor HAM-A. kecemasan.*

Correspondence author. Nadia O.S. Nurdin. Department of Obstetrics and Gynecology.
Faculty of Medicine Universitas Sam Ratulangi. Prof.Dr. R.D. Kandou General Hospital Manado
Email; aichikarai@gmail.com

Received: September, 2021 Accepted: September, 2022 Published: October, 2022

INTRODUCTION

Pregnancy and childbirth, both vaginally and cesarean section are considered physiological situations that cause anxiety and stress. The prevalence risk of depression or anxiety in dealing with vaginal delivery in Indonesia in 2015 was 10-25%. The incidence of depression or anxiety complicates the delivery process around 10-15%, but until now there is still little attention paid to anxiety disorders in pregnancy. ACOG recommends that obstetric care providers screen patients at least once during the perinatal period for the evaluation of anxiety and depression symptoms.[1,2]

Cesarean section can be performed as an elective or emergency procedure. Emergency cesarean section is performed in an emergency, with indications including fetal distress, cephalopelvic disproportion, failed induction, uterine inertia, and history of caesarean section. Elective cesarean section is a planned procedure usually performed at term pregnancy, where the incidence of neonatal tachypnea is less.[3]

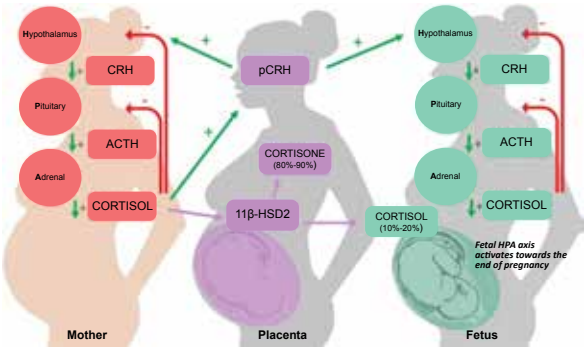

Figure 1. HPA axis dan cortisol level during pregnancy.[4]

Stress plays an important role in the onset and persistence of anxiety. Cortisol is released in response to stress and is a key physiological marker of the stress response. Increased cortisol during pregnancy is associated with worsening labor outcomes and affects fetal development and even lasts to adulthood. Cesarean section has various risks but is often considered a routine procedure, so the potential for side effects, especially psychological, are often overlooked. Cortisol in emergency and elective surgery increased, but in emergency surgery, there was a more significant increment. Another study using a questionnaire for the assessment of anxiety, also found that mothers who underwent emergency cesarean section experienced higher anxiety than electives. However, there has not been much research on anxiety before caesarean section by combining subjective (eg questionnaires) and objective (eg anxiety-related hormones) parameters.[4-6]

Based on the reasons above, to anticipate the occurrence of anxiety and its adverse effects on the mother and fetus, this study was conducted to examine the relationship between serum cortisol levels in pregnant women and anxiety during elective cesarean sections and emergency cesarean sections. We hoped that the results of this study can be applied to detect anxiety earlier so we can prevent adverse effects on mothers and babies undergoing cesarean section.

METHODS

This study was a cross-sectional study conducted at the Department of Obstetrics and Gynecology Prof. DR. R. D. Kandou Central General Hospital, Pancaran Kasih Hospital, Bhayangkara Hospital and Pramita Clinical Laboratory in Manado. This research was started from March to June 2021, sampling was carried out using a consecutive sampling technique, which included all respondents who met the inclusion criteria until the number of samples was met. The population in this study were pregnant women who came to RSUP Prof. Dr. R. D. Kandou Manado, network hospital (Pancaran Kasih Hospital, Bhayangkara Hospital) in Manado and who were planned for cesarean section during the period March to June 2021.

The inclusion criteria of this study were all pregnant women with gestational age more than 28 weeks who came to the ER or Polyclinic at the Obstetrics and Gynecology Section of Prof. Dr. R.D. Kandou Manado and network hospitals in Manado, that were decided for an emergency cesarean section, or those who came without signs of labor and were planned for an elective cesarean section. Patients willing to participate in this study were asked to sign an informed consent. The exclusion criteria in this study were patients who had a pregnancy with suspected/ probable/confirmed COVID-19, pregnancy with chronic disease, such as chronic hypertension, diabetes mellitus, kidney disease, heart disease, obesity, received steroid therapy in the last 72 hours, twin pregnancy, fetal death, congenital abnormalities of the fetus, non-obstetric problems such as family problems, domestic violence, financial problems or mothers who are not willing to participate in the study. The

number of subjects was 42 patients consisting of 21 pregnant patients with an elective cesarean section plan and 21 pregnant patients with an emergency cesarean section at Prof. RSUP. Dr. R. D. Kandou Manado and Network Hospital in Manado City.

The dependent variable in this study was the category of cesarean section which was carried out as an elective or emergency. While the independent variables in this study were serum cortisol levels and anxiety levels. The independent variable was assessed by examining serum cortisol which was carried out 2 days before an elective cesarean section or 2 hours after the decision for an emergency cesarean section. The collected serum was then determined for cortisol levels using the immulite cortisol method. Anxiety level was measured by the Hamilton questionnaire (HAM-A) consisting of 14 assessments. Interviews for filling out the questionnaires were conducted immediately after the blood sampling.

Data obtained from serum cortisol levels and anxiety levels through questionnaires were collected, processed and then analyzed using SPSS software 23rd version. To assess the relationship between cortisol levels during elective and emergency cesarean sections, a chi-square and t independent test was performed if the data were normally distributed, or alternatively, Fischer exact or Mann-Whitney test if they were not. Meanwhile, to assess the correlation of anxiety to elective and emergency cesarean sections, logistic regression statistical tests were carried out.

RESULTS

This study was conducted on a population of pregnant women with a total of 42 subjects. The research subjects consisted of 21 pregnant women with an emergency cesarean section and 21 pregnant women with an elective cesarean section who all met the inclusion and exclusion criteria and signed informed consent to participate in this study. The characteristics of the complete research subjects are shown in Table 1.

Table 1. Characteristics of Respondents

Characteristic	Elective Cesarean Section		Emergency Caesarean Section	
	N	**%**	**N**	**%**
Age (y o)				
20-34	14	67	12	57
≥ 35	7	33	9	43
Education				
Junior HS	1	5	2	10
Senior HS	15	71	12	57
University	5	24	7	33
Occupation				
Unemployed	18	86	10	48
Working	3	14	11	52
Gravid				
Primigravid	5	24	4	19
Multigravid	15	71	15	71
Grandmulti	1	5	2	10

The data above showed that in the elective and emergency cesarean section, the highest percentage was the age group of 20-34 years old. Based on education level, in the elective and emergency cesarean section, the highest percentage was Senior High School. Based on occupation, in the elective cesarean section group, the highest percentage was in the unemployed group, while in the emergency cesarean section group, the highest percentage was in the working group. Based on gravid status, in the elective and emergency cesarean section group, most groups were multigravid.

Table 2. Cortisol serum level in Elective and Emergency Cesarean Section

Cortisol Serum Level ≥ 19.4 µg	Elective Cesarean Section		Emergency Caesarean Section		*P-value*
	N	**%**	**N**	**%**	
Normal	21	100	15	71.43	0.021
Increase	0	0	6	28.57	
Total	21	100	21	100	

The results of Fischer's exact test, p value = 0.021 (p < 0.05), indicated a significant relationship between cortisol levels and the incidence of emergency cesarean section. In table 2, it can be seen that cortisol levels in the elective cesarean section group, all samples had normal cortisol levels, namely 21 people (100%). In the emergency cesarean section group, there were 6 respondents (28.57%) who experienced an increment in cortisol levels.

Table 3 showed the distribution of serum cortisol levels in elective and emergency cesarean sections. In the elective cesarean section, the mean was 10.586 ± 4.9501, while in the emergency cesarean section the mean was 21,590 ± 11,6392. Cortisol levels were higher on average in the emergency cesarean section compared to elective cesarean section.

Table 3. Mean Distribution of Cortisol Serum Level in Elective and Emergency Cesarean Section

Variable	Elective Cesarean Section (n=21)		Emergency Cesarean Section (n=21)	
Cortisol level	Mean	10.586	Mean	21.590
	Median	10.500	Median	18.200
	Std Deviation	4.9501	Std Deviation	11.6392
	Minimum	2.5	Minimum	9.6
	Maximum	17.1	Maximum	49.0

Furthermore, Table 4 showed the level of anxiety according to the Hamilton scale (HAM-A). In the elective cesarean section, none experienced anxiety (0%). In the emergency cesarean section group, 8 people (38%) did not feel anxiety, 8 people (38%) experienced mild anxiety, 3 people (14%) experienced moderate anxiety as much as 3 people, and 22 people (10%) experienced severe anxiety. The Mann-Whitney statistical test showed that there was a significant difference in HAM-A scores between the elective cesarean section and the emergency cesarean section (p = 0.001).

Table 4. Anxiety score (HAM-A) in Elective and Emergency Caesarean Section

Anxiety score	Elective Cesarean Section		Emergency Cesarean Section		*P-value*
	N	%	N	%	
No anxiety	21	100	8	38	0.001
Mild anxiety	0	0	8	38	
Moderate anxiety	0	0	3	14	
Severe anxiety	0	0	2	10	
Total	21	100	21	100	

In table 5, HAM-A scores were on average higher for emergency cesarean sections than for elective cesarean sections. The Mann-Whitney statistical test showed that there was a significant difference in HAM-A scores between the elective cesarean section and the emergency cesarean section (p = 0.001).

Table 5. Mean Distribution of Anxiety Score (HAM-A) in Elective and Emergency Cesarean Section

Variable	Elective Caesarean Section (n=21)		Emergency Cesarean Section (n=21)	
HAM-A score	Mean	7.19	Mean	15.33
	Median	7.00	Median	17.00
	Std Deviation	3.614	Std Deviation	7.722
	Minimum	0	Minimum	4
	Maximum	13	Maximum	29

The Spearman correlation test showed that there was a significant correlation between cortisol levels and HAM-A in all study subjects (r = 0.528 and p = 0.000). This can also be seen in the scatter plot image of the correlation between the two.

DISCUSSION

Childbirth is a phase that has a major psychological impact, especially on primiparous mothers. Surgery, especially cesarean section, generally causes anxiety due to fear of the procedure, as well as anesthetic drugs, operating

room, operating equipment, pain, infection, and postoperative inflammatory reactions. This will lead to stimulation of the HPA axis and further increase cortisol secretion. Emergency cesarean section is usually a situation that is more unpredictable, difficult to control, and therefore more prone to causing traumatic stress. Several aspects can affect this, such as age, culture, individual characteristics in dealing with stress, understanding of surgery, surgery history, waiting time for surgery, and anxiety about postoperative problems such as pain, changes in body shape, dependence on others, and possible lifestyle changes after surgery.[7-9]

The subjective assessment of anxiety was done through a cortisol examination. Cortisol levels were on average higher in emergency cesarean section compared to elective cesarean section. Cortisol in elective and emergency surgery and found that cortisol increased in both types of surgery. However, in emergency surgery, there was a more significant increment. Obtained similar results, where cortisol values were normal in the elective cesarean section on admission, increased significantly immediately before surgery, then decreased to baseline levels at 2 hours after surgery. Cortisol levels vary widely in each individual; therefore, the sampling time was carried out according to the provisions consistently to minimize bias due to diurnal variations in cortisol. If sampling is repeated according to diurnal variations or stress exposure, changes in the diurnal pattern may be more pronounced so that cortisol elevations can be better observed.[10,11]

In this study, subjective anxiety assessment was carried out with the HAM-A questionnaire, where the level of anxiety according to the HAM-A score was on average higher in emergency cesarean sections compared to elective cesarean sections. In this study, it could be concluded that there is a positive relationship between cortisol levels and anxiety levels according to the HAM-A score with either emergency or elective cesarean section. This is consistent with the research who found that mothers who underwent emergency cesarean section experienced higher anxiety than electives. In this study, there was no anxiety in the elective cesarean section group. There was anxiety in the elective cesarean section before and during hospital admission. Mothers did not experience anxiety when they were admitted to the hospital for elective cesarean section. However, the anxiety increased significantly during skin suturing and then decreased significantly at 2 hours postoperatively.[5,6]

Anxiety before surgery can lead to difficult venous access and an increased need for anesthetic drugs. Intraoperatively, anxiety can also cause hypertension, an increased heart rate that can increase the risk of bleeding. During the postoperative period, this anxiety can increase pain, nausea, vomiting, sleep disturbances, fatigue, and fear about postoperative recovery. If it continues, it can interfere with wound healing, increase the risk of infection, change sleep patterns, prolonging the length of treatment and increasing hospital costs.[8,9,12]

Preoperative education can reduce maternal anxiety. Interventions before and during surgery can calm the mother and even reduce the potential for pain, including music before and during surgery, acupuncture, and hypnosis.[13,11,14-16]

So far, there have been no studies that evaluate the subjective and objective anxiety simultaneously in mothers who will undergo elective and emergency cesarean sections. This study emphasized that pregnant women awaiting emergency cesarean section experience anxiety before surgery. Therefore, greater attention should be paid especially before emergency cesarean section to identify patients who are at risk of developing anxiety and requiring intervention. In this study, the characteristics of the subjects who vary in age, education level, and gravida can also affect the level of anxiety of each patient. Further research can be conducted to analyze the risk factors for anxiety, to determine the effect of anxiety on the condition of the mother, baby, postoperative care and recovery, and its interventions.

CONCLUSION

Different delivery methods have different stressor effects and neurotransmitter reactions. Surgery, especially cesarean section, generally causes anxiety due to fear of the procedure, as well as anesthetic drugs, operating room, operating equipment, pain, infection, and postoperative inflammatory reactions. Emergency cesarean section is usually a situation that is more unpredictable, difficult to control, and therefore more prone to causing traumatic stress.

Anxiety can be assessed objectively and subjectively. Objectively using cortisol levels and subjectively through a questionnaire. This study shows that there is a relationship between cortisol

levels and HAM-A scores with both emergency and elective cesarean sections. Cortisol levels and subjective anxiety were on average higher in emergency cesarean section compared to elective cesarean section.

REFERENCES

1. González-Ochoa R, Sánchez-Rodríguez EN, Chavarría A, et al,. Evaluating Stress during Pregnancy: Do We Have the Right Conceptions and the Correct Tools to Assess It? J Pregnancy. 2018;2018.
2. Casey BM, Sheffield JS, Cunningham FG, et al. Psychiatric disorders. In: Cunningham FG, Leveno KJ, Bloom SL, Spong CY, Dashe JS, Hoffman BL, et al., editors. Williams Obstetric. 24th ed. New York: McGraw-Hill Education.2018: 1204–12.
3. Yang XJ, Sun SS. Comparison of maternal and fetal complications in elective and emergency cesarean section: a systematic review and meta-analysis. Arch Gynecol Obstet. 2017;296(3):503–12.
4. Selimuzzaman S., Begum N, Islam N, Begum S. Comparative Study between Elective and Emergency Surgery. J Bangladesh Soc Physiol. 2007;2(2):28–33.
5. Bansal T, Joon A. A comparative study to assess preoperative anxiety in obstetric patients undergoing elective or emergency cesarean section. Anaesthesia, Pain Intensive Care. 2017;21(1):25–30.
6. Solgajová A, Sollár T, Zrubcová D, Vörösová G. Predictors of preoperational anxiety in surgical patients. Kontakt. 2015;17(4):e201–5.
7. Sun Y, Huang K, Hu Y, Yan S, Xu Y, Zhu P, et al. Pregnancy-specific anxiety and elective cesarean section in primiparas: A cohort study in China. PLoS One. 2019;14(5):1–13.
8. Maheshwari D, Ismail S. Preoperative anxiety in patients selecting either general or regional anesthesia for elective cesarean section. J Anesthesiol Clin Pharmacol. 2015;31(2):196–200.
9. Zeinab H. Relationship between Perceived Social Support and Anxiety before and after Cesarean in the Pregnant Women. Biomed J Sci Tech Res. 2021;34(2).
10. Selimuzzaman S., Begum N, Islam N, Begum S. Comparative Study between Elective and Emergency Surgery. J Bangladesh Soc Physiol. 2007;2(2):28–33.
11. Hepp P, Hagenbeck C, Burghardt B, Jaeger B, Wolf OT, Fehm T, et al. Measuring the course of anxiety in women giving birth by caesarean section: A prospective study. BMC Pregnancy Childbirth. 2016;16(1):1–7. http://dx.doi.org/10.1186/s12884-016-0906-z
12. Kalliyath A, Korula S, Mathew A, Abraham S, Isac M. Effect of preoperative education about spinal anesthesia on anxiety and postoperative pain in parturients undergoing elective cesarean section: A randomized controlled trial. J Obstet Anaesth Crit Care. 2019;9(1):14.
13. Kasirajan G, Shanmuga M, Sivagangai G, College M. Effect of preoperative advice on anxiety and intraoperative co-operation in primi mothers undergoing emergency caesarean. IJSR. 2018;(10):14–6.
14. Kocamer Şimşek B, Şahin Ş, Akyılmaz AA, Özkaplan ŞE, Özdurak İH. Effect of calming conversation on anxiety levels in Cesarean section. Cukurova Med J. 2019;44(Suppl 1):1–1.
15. Mubarokah RI, Prasetya H, Respati SH. The Effectiveness of Hypnotherapy to Reduce Anxiety in Pre-Caesarean Section Women. J Matern Child Heal. 2020;5(1):12–8.
16. Jiménez-Jiménez M, García-Escalona A, Martín-López A, De Vera-Vera R, De Haro J. Intraoperative stress and anxiety reduction with music therapy: A controlled randomized clinical trial of efficacy and safety. J Vasc Nurs. 2013;31(3):101–6.

Research Article

Level of Maternal Zinc Serum as Risk Factor of Preeclampsia

Kadar Seng Serum Maternal sebagai Faktor Risiko Preeklamsia

Tian Kaprianti[1], Putri Mirani[2], Abarham Martadiansyah[2], Peby M. Lestari[2], Theodorus[3]

[1]Department of Obstetrics and Gynecology
[2]Division of Maternal-Fetal-Medicine
[3]Department of Pharmacology
Faculty of Medicine Universitas Sriwijaya
Dr. Mohammad Hoesin General Hospital
Palembang

Abstract

Objective: To investigate the relationship between serum zinc levels and preeclampsia (PE).

Methods: This observational study used case control design. The population of this study was all pregnant women with singleton pregnancy and 28–42 weeks of gestation who were treated at Department of Obstetrics and Gynecology dr. Mohammad Hoesin General Hospital Palembang from August 2020 to November 2020. The study was divided into 2 groups, a case group of 30 pregnant women with PE and a control group of 60 pregnant women without PE. Serum zinc level examination was performed on all samples which met the study criteria. The data was processed by using SPSS software program version 20.0 Windows.

Results: Both case and control groups had no meaningful differences in the general characteristics of the patient. There were significant differences in the maternal serum of average zinc level in both study groups (p = 0.013; 43.90 ± 15.79 µg/dL). The level of serum zinc which had the best sensitivity and specificity was 45.5 µg/dL.

Conclusion: There was a meaningful relationship between serum zinc levels and PE. Pregnant women with serum zinc levels ≤ 45.5 µg /dL were significantly 3.2 times more risky towards PE than pregnant women with serum zinc levels > 45.5 µg/dL. In this case, it was necessary to give zinc earlier in pregnancy to reduce the risk of preeclampsia.

Keywords: case-control study, preeclampsia, zinc.

Abstrak

Tujuan: *Mengetahui hubungan antara kadar seng serum dan kejadian preeklamsia (PE).*

Metode: *Penelitian observasional ini menggunakan desain kasus kontrol. Populasi penelitian ini adalah seluruh ibu hamil dengan kehamilan tunggal dan usia kehamilan 28–42 minggu. Pasien dirawat di KSM/Bagian Obstetri dan Ginekologi RSUP dr. Mohammad Hoesin Palembang dari Agustus 2020 hingga November 2020. Penelitian dibagi menjadi 2 kelompok, kelompok kasus terdiri atas 30 ibu hamil dengan PE dan kelompok kontrol 60 ibu hamil tanpa PE. Pemeriksaan kadar seng serum dilakukan pada semua sampel yang memenuhi kriteria penelitian. Data diolah dengan menggunakan program software SPSS Windows versi 20.0.*

Hasil: *Tidak terdapat perbedaan yang bermakna mengenai karakteristik umum pasien antara kelompok kasus dan kontrol. Terdapat perbedaan yang signifikan dalam rata-rata kadar seng serum ibu pada kedua kelompok penelitian (p = 0,013; 43,90 ± 15,79 g/dL). Kadar seng serum yang memiliki sensitivitas dan spesifisitas terbaik adalah 45,5 g/dL*

Kesimpulan: *TTerdapat hubungan yang bermakna antara kadar seng serum dan PE. Ibu hamil dengan kadar seng serum ≤ 45,5 µg/dL secara signifikan berisiko 3,2 kali lebih besar mengalami PE daripada ibu hamil dengan kadar seng serum > 45,5 µg/dL. Dalam hal ini, pemberian seng perlu diberikan lebih awal pada kehamilan untuk mengurangi risiko preeklamsia.*

Kata kunci: *preeklamsia, seng, studi kasus-kontrol.*

Correspondence author. Tian Kaprianti. Department of Obstetrics and Gynecology.
Faculty of Medicine Universitas Sriwijaya. dr. Mohammad Hoesin General Hospital. Palembang.
Email;tiankaprianti91@gmail.com

Received: August,2021 Accepted: September,2022 Published: October,2022

INTRODUCTION

Preeclampsia (PE) is defined as gestational hypertension with the presence of proteinuria ≥ 300 mg/24 hours or protein ratio: creatinine ≥ 0.3 or with dipstick (+), or the presence of thrombocytopenia with platelets < 100,000/ µL, renal insufficiency i.e. creatinine > 1.1 mg/dL, involvement of liver function, serum transaminases increased 2-fold, symptoms of increased intracranial pressure such as dizziness, blurred vision, and seizures.[1] Incidence of preeclampsia varies globally. Based on a secondary analysis of the World Health Association (WHO) Global Survey on Maternal and Perinatal Health in 24 countries in the world, the prevalence of preeclampsia was 10,754 out of 276,388 mothers (4%).

Preeclampsia and eclampsia are significant risk factors for maternal death, perinatal death, premature birth, and low birth weight. Nearly 1 in 10 maternal deaths in Asia and Africa are associated with hypertension in pregnancy. Preeclampsia and eclampsia have the most significant impact on maternal morbidit, mortality, and newborns. Based on the Health Profile of South Sumatra Province in 2019, hypertension in pregnancy was the second most common cause of maternal death at South Sumatera in 2019 with a total of 29 out of 120 maternal deaths.[2-4] Oxidative stress is one of PE etiopatogenesis.[1] Zinc is an essential mineral for many biological functions including protein synthesis, cellular cleavage, nucleic acid metabolism, and one of the trace elements directly involved in oxidative/antioxidant balance. One of the pathogenesis processes in PE that relies heavily on dietary habits and supplements. Some studies have found significantly lower serum zinc levels in PE patients compared with non-PE pregnancies. Serum zinc levels were 43% lower in women with preeclampsia when compared with normal pregnant women in Turkey. Research on Indians found that serum zinc levels in mild and severe preeclampsia women were lower at 12.72 µmol/L in mild preeclampsia and 12.04 µmol/L in severe preeclampsia compared with 15.64 µmol/L in normal pregnancy.[5-8] Research related with the benefits of zinc in preeclampsia had not been conducted in Indonesia, especially in dr. Mohammad Hoesin General Hospital Palembang. Because of that, authors are interested in proving the relationship between zinc and preeclampsia in the pregnant women population at dr. Mohammad Hoesin General Hospital Palembang.

METHODS

This study used case-control design to determine the relationship between zinc level in the third trimester pregnant women with preeclampsia and normal pregnancy. This study was conducted at Department of Obstetrics and Gynecology, dr. Mohammad Hoesin General Hospital (RSMH) Palembang from August 2020 to November 2020. The study population was all pregnant women with singleton pregnancy, 28–42 weeks of gestation who were treated at Department of Obstetrics and Gynecology dr. Mohammad Hoesin General Hospital Palembang. Inclusion criteria for the case group are preeclampsia, singleton live fetus, 28–42 weeks of gestation, willing to follow study, and signing informed consent sheet. For the control group, inclusion criteria include 28–42 weeks of gestation, singleton live fetus, getting the same treatment as other normal pregnancies, willing to follow study, and signing informed consent sheet. Exclusion criteria for both groups are pregnancy <20 weeks of gestation, multiple pregnancies, pregnancy with complications, such as antepartum hemorrhage, liver disease, kidney disease, heart disease, diabetes mellitus, metabolic disorder, intrauterine fetal death, intrauterine growth retardation, and patients who refused to participate in the study. Samples that met the inclusion and exclusion criteria are included in this study. The sample was selected by using consecutive sampling according to the day patient is diagnosed with preeclampsia (time of diagnosis). In the sample, anamnesis, physical examination (vital signs, head to toe), gynecological examination (external and internal examination or according to indication), and additional examination (ultrasound and laboratory examination) in accordance with hospital protocol. Samples that met the study criteria were given informed consent to follow the study. Samples who agreed to participate in the study were randomly grouped into control (non-preeclampsia women) and case (preeclampsia women). All samples were then examined for zinc serum level at Prodia Laboratory, Palembang. Tools and materials needed during the study, both consumable and fix, consisted of tourniquet, stetoscope, weight scale, meter, and Aglient 7700. Consumable kits include 3 cc syringes, trace element blood sample tubes, alcohol swabs, and Randox kits.

All data were captured and tabulated in the

data table and also matched based on age, body mass index (BMI), education level, marital status, obstetric status, socioeconomic level, address, occupation, and maternal disease. We matched demographic variables data (bias variables). Data were obtained in the form of a main table. Tabulation, coding, and calculation of statistical data were performed using SPSS software program version 20.0 Windows. Data analysis would be conducted according to the type of data and data dissemination (Kolgomorov Smirnov test). Chi Square/Fisher's tests were conducted for nominal and categorical data. T-test or Mann Whitney U-test was performed for interval data. The results were presented in the form of tables and graphs with a 95% confidence interval (CI). The successful parameter of this study was the relationship of serum zinc and preeclampsia level with odds ratio of > 2 ($p < 0.05$).

RESULTS

This study was conducted from August 2020 to November 2020 at Department of Obstetrics and Gynecology RSMH Palembang and Prodia Laboratory Palembang. As many as 90 pregnant women met the inclusion criteria. There were 60 samples of PE in the case group and 30 samples in the control group. In this study, the average age of PE patients was 31.23 ± 5.83 years (20 to 41 years of age) and most of them was in the > 30 years (53.3%) group. The average age of patients without PE was slightly younger (29.25 ± 6.77 years (17–44 years of age)) with the most age category was > 30 years (48.3%). Although the average age of patients without PE was slightly younger but the difference was not statistically significant ($p = 0.138$). There was no age category difference between patients with and without PE ($p = 0.266$).

Most of pregnant women with and without PE had high school (90%, 83.3%) education level and were housewives (80%, 85%). Moreover, there were no differences in education ($p = 0.342$) and occupation ($p = 0.319$) between patients with and without PE. Most of patients with PE were overweight (73.3%). There was no BMI difference between patients with and without PE ($p = 0.246$). In addition, the majority of patients with and without PE were 37–42 weeks of gestation (75.9%, 63.3%) and multiparity (43.3%, 43.3%). There was no difference in gestational age ($p = 0.347$) and parity ($p = 0.940$) between patients with and without PE (Table 1).

Table 1. Characteristics of Subjects in the Case and Control Group (n = 90)

Variables	Case group (n= 30)	Control Group (n= 60)	*P-value*
Age (years)			
Mean ± SD	31.23 ± 5.83	29.25 ± 6.77	0.138[a]
Median (min–max)	32 (20–41)	30 (17–44)	
Age categories (years old) (%)			
< 20	0 (0.0)	5 (8.3)	0.266[b]
20–30	14 (46.7)	26 (43.3)	
> 30	16 (53.3)	29 (48.3)	
Education level			
Primary School	1 (3.3)	4 (6.7)	0.342[b]
Junior High School	2 (6.7)	1 (1.7)	
Senior High School	27 (90.0)	50 (83.3)	
Diploma	0 (0.0)	1 (1.7)	
Bachelor Degree	0 (0.0)	4 (6.7)	
Occupation			
Housewife	24 (80.0)	51 (85.0)	0.319[b]
Enterpreneur	3 (10.0)	5 (8.3)	
Private employee	2 (6.7)	0 (0.0)	
Civil employee	0 (0.0)	2 (3.3)	
Farmer	1 (3.3)	1 (1.7)	
Student	0 (0.0)	1 (1.7)	
BMI (kg/m²)			
Normoweight	8 (26.7)		
Overweight	22(73.3)	25(41.7)	0.246[c]
Gestational age, (weeks)		35(58.3)	
< 37	7 (24.1)	22 (36.7)	0.347[d]
37–42	22 (75.9)	38 (63.3)	
Parity			
Nulliparous	9 (30.0)	15 (25.0)	0.940[b]
Primiparous	7 (23.3)	16 (26.7)	
Multiparous	13 (43.3)	26 (43.3)	
Grand multiparity	1 (3.3)	3 (5.0)	

[a]*Mann Whitney Test, p-value = 0.05*
[b]*Pearson Chi Square Test, p-value = 0.05*
[c]*Continuity correction, p-value = 0.05*
[d]*Chi Square Test, p-value = 0.05*

The mean serum zinc levels in the case and control group were 43.90 ± 15.79 µg/dL (ranged from 15 to 86 µg/dL) and 48.77 ± 10.54 µg/dL (ranged from 22 to 83 µg/dL), respectively. There was a significant difference in serum zinc level between cases and controls ($p = 0.013$). The serum zinc levels of pregnant women with PE were lower than without PE (Table 2).

Table 2. Serum Zinc Level (n = 90)

Variables	Cases (n= 30)	Controls (n= 60)	*P-value*
Serum zinc level			
Mean ± SD	43.90 ± 15.79	48.77 ± 10.54	0.013
Median (min–max)	41 (15–86)	49.5 (22–83)	

Mann Whitney Test, p = 0.05

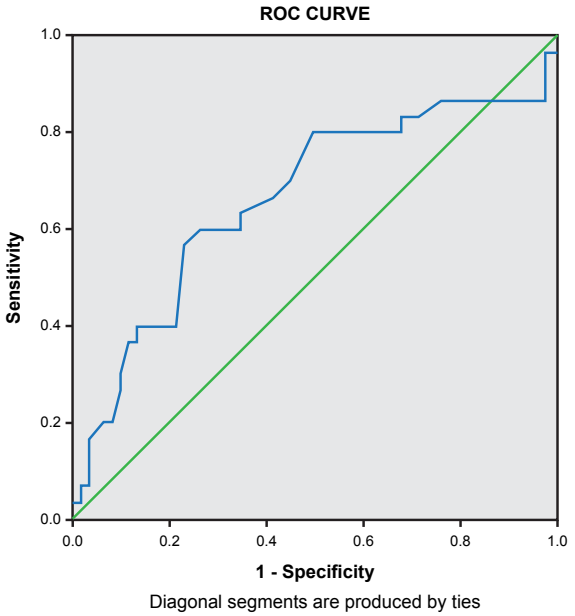

Figure 1. ROC of serum zinc levels

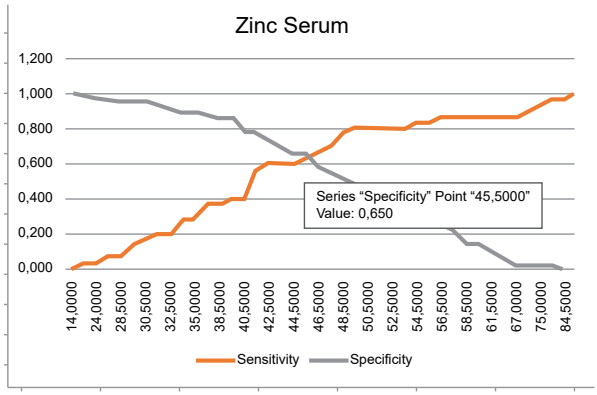

Figure 2. Cut-off curve of sensitivitya and specificity of serum zinc level

In the PE group, there were 19 out of 30 people (63.3%) who had serum zinc level ≤ 45.5 µg/dL. Meanwhile, in the non-PE group, there were 21 out of 60 people (35%) who had serum zinc level ≤ 45.5 µg/dL. There was a significant relationship between serum zinc level and PE. Pregnant women with serum zinc level ≤ 45.5 µg/dL was significantly 3.2 times more risky towards PE than pregnant women with serum zinc levels > 45.5 µg/dL (OR = 3.208 (CI95% 1.288–7.990); p = 0.020) (Table 3).

Table 3. The Association between Serum Zinc Level and PE

Characteristic	PE		Total	OR (CI95%)	*P-value*
	Yes	No			
Serum zinc level (µg/dL)					
≤ 45.5	19	21	40	3.208	0.020
> 45.5	11	39	50	(1.288–7.990)	
Total	30	60	90		

Chi Square test, p-value = 0.05

DISCUSSION

Hypertension in pregnancy is used to describe blood pressure disorders in patients who may only experience an increase in mild blood pressure or severe hypertension with some organ dysfunctions including chronic hypertension, gestational hypertension, superimposed preeclampsia, preeclampsia, eclampsia, and hemolytic syndrome in the form of an increase in liver enzymes and a decrease in the number of platelets (HELLP syndrome).[9] Severe preeclampsia criteria is when there are one or more symptoms of systolic blood pressure of more than 160 mmHg and diastolic equal to or more than 110 mmHg, proteinuria more than 2 grams/24 hours or +2.[10] Age has an important influence on the incidence of hypertensive disorder in pregnancy. A woman who is 35 years of age or older during pregnancy is defined as "advanced maternal age (AMA)" or "elderly mother".[11] Maternal and preeclampsia age relationship form U-shaped curve, the lowest frequency in women aged 25–29 years. Meanwhile, the high frequency was in women with less than 20 years of age and over 35 years.[12] In this study, the average PE patient was 31.23 ± 5.83 years of age (20–41 years) and the majority of patients with age > 30 years was 53.3%. From 95 pregnant women who suffered from PE, the average age was 31.3 ± 5.1 years.[13] Severe preeclampsia patients had an average age of 31 ± 5.0 years and from 131 preeclampsia patients, the average age was 30.9 ± 5.0 years.[14,15]

Preeclampsia in morbid obese women increased by 3.97 times. Preeclampsia incidence in obese women with BMI 27.5–30.0 kg/m^2 increased by 3.25 times and preeclampsia incidence in women with BMI 25.0–27.5 kg/m^2 increased by 1.60 times.[16] In this study, PE patients with overweight were 73.3%. The average BMI from 80 PE patients in both treatment groups was 28.0 ± 5.0 kg/m^2 and 27.0 ± 6.0 kg/m^2.[12] From meta-analysis of 92 studies involving 25,356,688 pregnant women, pregnant women with BMI before pregnant > 30 had 2.8 times risk of preeclampsia (RR = 2.8; CI95% 2.6–3.1).[17] Preeclampsia is often experienced by young and nulliparous women. The incidence of preeclampsia in multiparous women also varies but lower than nulliparous. Nulliparous women are 1.8 times more at risk of preeclampsia compared with primiparous (RR = 1.78; p = 0.000).16 Study reported preeclampsia occurred in 2.7% of nulliparous women and 1.9%

of multiparous women.[12] However, in this study, the majority of PE patients were multiparous (43.3%). Similarly, the majority of PE patients were multiparous (57.1%).[13]

In this study, there were no differences in age, age category, BMI, education, occupation, and parity between groups with and without PE, so both groups were eligible to be compared. Adequate maternal nutrition before and during pregnancy is essential for maternal and child health. Poor nutrition in pregnancy can interfere with maternal and neonatal health. Each year, 3.5 million deaths in women and children are associated with malnutrition.[18] Zinc acts as an intracellular signalling molecule which is capable of communicating between cells by converting extracellular stimuli into intracellular signal and controlling intracellular action. Changes in zinc homeostasis and dysfunction in signal function of zinc can lead to pathogenesis of some diseases. Some studies have shown that lower zinc plasma levels are associated with disruption towards pregnancy outcomes such as fetal malformation, intrauterine growth retardation, preterm birth, preeclampsia, and post partum hemorrhage.[19] Preeclampsia is caused by several factors and associated with an imbalance of increased lipid peroxide (LPO) and decreased antioxidant. Zinc also acts as an antioxidant, so zinc deficiency can cause lipid peroxidation to increase. Several studies have found significantly lower level of serum zinc in preeclampsia patients compared with pregnancies without preeclampsia.[6] In this study, the average zinc levels of PE patients were 43.90 ± 15.79 mcg/dL (15–86 mcg/dL), significantly lower than patients without preeclampsia which was 48.77 ± 10.54 mcg/dL (22–83 mcg/dL).

There were significant differences in zinc level between pregnant women with preeclampsia and normal pregnancies (902.50 ± 157.15 µgm/L vs 1153.33 ± 67.09 µgm/L; $p < 0.000$).[20] Moreover, there were significant differences in zinc level between pregnant women with preeclampsia and control (76.49 ± 17.62 µg/dL vs 100.61 ± 20.12 µg/dL; $p < 0.001$).[21] In 2010, a study reported significant differences in zinc level between pregnant women with preeclampsia and control (8.6 ± 1.4 µmol/L vs 9.4 ± 0.8 µmol/L; $p < 0.05$).[9] In addition, there were significant differences in zinc level between pregnant women with preeclampsia and control (0.77 ± 0.05 mg/dL versus 0.98 ± 0.03 mg/dL; $p = 0.000$).[22] In this study, there was lower zinc level compared with other studies. The difference could be caused by the study samples taken at 28–42 weeks of gestation. Meanwhile, the other study took samples from 20 weeks of gestation.

Chemically, zinc has its own uniqueness because it works in regulatory, catalytic, and structural cells that are important in various biological systems. Zinc plays a role in the metabolism of carbohydrate, lipid, and protein as well as the synthesis and degradation of nucleic acid through their role in carbonic anhydrous enzymes (metabolism of CO_2 and HCO_3), thimidin kinase/DNA and RNA polymerase (synthesis of nucleic acids and proteins). Zinc is important for a variety of functions including growth and development, reproductive function, sensory and immune functions, antioxidants, and membrane stabilization.[23,24] Another important function of zinc is its role in the structure and function of biomemurity. Some researchers have proven that reduced concentrations of zinc in biomemance underlie some of the mess associated with zinc deficiency. Zinc becomes an important component of several enzymes that regulate cell growth, protein and DNA synthesis, energy metabolism, gene transcription regulation, hormone level, and growth factor metabolism.[25,26] In the preconception period, zinc supplementation is used to promote fertility and healthy childbirth. Poor maternal zinc status is associated with fetal malformation, intrauterine growth retardation, preterm birth, preeclampsia, and postpartum hemorrhage.[27]

Oxidative stress of the placenta is considered an intermediary in the pathogenesis of pre-eclampsia. There is a lot of evidence to suggest the contribution of oxidative stress to endothelial dysfunction leading to preeclampsia. In human, there are three forms of SOD (Superoxide Dismutases), namely cytosic Cu/Zn-SOD, mitochondrial Mn SOD and extracellular SOD. Superoxide dismutases are metalloenzymes that catalyze superoxide dismutation into oxygen and hydrogen peroxide molecules and thus form an important part of the cellular antioxidant defense mechanism.[28] Increased concentration of oxidative stress markers and decreased antioxidant concentrations in maternal and placental circulation of women with preeclampsia. Increased biomarker lipid peroxidation (MDA) is accompanied by reduced SOD and GPx in cord blood pre-eclampsia and pregnancy eclampsia compared with normal pregnancy. Antioxidant enzyme SOD has been shown to be reduced in

patients with preeclampsia and eclampsia in the same study. Certain substrates and co-factors are necessary for adequate antioxidant enzyme function. Glutathione is a substrate for enzymes that catalyze the reduction of reactive and radical-free oxygen species.[28]

Zinc is one of the regulators of angiogenesis because it is related to cell proliferation, differentiation and apoptosis.[29] Zinc is also a major biomembracy component and is essential for membrane structure and function. Zinc modulates signal transduction in endothelial cells that affect angiogenesis.[30] A study proves that zinc has relationship with endothelial dysfunction. Zinc has a membrane stabilizing effect that helps control cell damage due to apoptosis. Some studies have found significantly lower serum zinc level in PE patients compared with patients without PE.6 By using the ROC, the cut-off point of zinc in preeclampsia is 45.5 mcg/ dL. In the PE group, there is 47.5% with zinc level ≤ 45.5 mcg/dL while in the non-PE group, there is 22% with zinc levels ≤ 45.5 mcg/dL. Pregnant women with zinc level ≤ 45.5 mcg/dL have 3.2 times risk of PE compared with pregnant women with zinc levels > 45.5 mcg/dL. Zinc level can be used as a good preeclampsia marker because it obtains an ideal cut-off point to help enforce the diagnosis of preeclampsia.

CONCLUSION

There were significant differences in level of serum zinc between PE and non-PE patients (p = 0.013). The level of zinc serum of pregnant women with PE was lower than without PE. Based on the cut-off point curve of serum zinc level, the value that had the best sensitivity and specificity is 45.5 mcg/dL. There was a significant relationship between serum zinc level and PE. Pregnant women with serum zinc level ≤ 45.5 mcg/dL were significantly 3.2 times more risky towards PE than pregnant women with serum zinc levels > 45.5 mcg/dL (OR = 3,208 (CI95% 1,288–7,990; p = 0.020). There were no significant differences in the average age, age category, and gestational age. Further study should be conducted to provide zinc supplementation early in pregnancy to reduce the risk of preeclampsia.

REFERENCES

1. Cunningham FG, Leveno KJ, Bloom SL, Spoong CY, Dashe JS, Hoffman BL, et al. Hypertensive disorders. Williams Obstetric. 24th ed. New York: McGraw-Hill. 2014:729–31.
2. V Dadelszen P, Vidler M, Tsigas E, Magee LA. Management of preeclampsia in low-and middle-income countries; Lessons to date, and questions arising, from the PRE-EMPT and relative initiatives. Maternal-Fetal Med. 2021;3(2):136–50.
3. World Health Organization. WHO recommendations for prevention and treatment of preeclampsia and eclampsia implications and actions. https://apps. who.int/iris/bitstream/handle/10665/119627/WHO_ RHR_14.17_eng.pdf?sequence=1 Dinas Kesehatan Provinsi Sumatera Selatan.
4. Kementerian Kesehatan. Profil kesehatan Provinsi Sumatera Selatan tahun 2019. In: https://www. kemkes.go.id/resources/download/profil/PROFIL_KES_ PROVINSI_2018/06_Sumsel_2018.pdf, accessed in July 20th, 2021.
5. Subandrate, Faisal MEPA, Anggraini NW, Sinulingga S. Malondialdehyde levels are higher and glutathione levels are lower in preeclampsia than in normal pregnancies. Universa Medicina. 2017;36:179–86.
6. Tesfa E, Nibret E, Munshea A. Maternal serum zinc level and pre-eclampsia risk in African women: A systematic review and meta-analysis. Biol Trace Elem Res. 2021;199:4564–71.
7. Elmugabil A, Hamdan HZ, Elsheikh AE, Rayis DA, Adam I, Gasim GI. Serum calcium, magnesium, zinc and copper levels in Sundanese women with preeclampsia. PLoS One. 2016;11(12):e0167495.
8. Darkwa EO, Boasiako CA, Djagbletey R, et al,. Serum magnesium and calcium in preeclampsia: A comparative study at the Korle-Bu teaching hospital, Ghana. Integr Blood Press Control. 2017; 10:9–15.
9. Chen Y, Ou QX, Chen Y,. et al. Association between trace elements and preeclampsia: A retrospective cohort study. J Trace Elem Med Biol. 2022;72:126971.
10. American College of Obstetricians and Gynecologists. Gestational hypertension and preeclampsia. ACOG Practice Bull No. 222. Obstet Gynecol. 2020;135(6):e237-e60.
11. Londero AP, Rossetti E, Pittini C, et al,. Maternal age and the risk of adverse pregnancy outcomes: A retrospective cohort study. BMC Preg Childbirth. 2019;19(261):1–10.
12. Hutcheon JA, Stephansson O, Cnattingius S, et al,. Pregnancy weight gain before diagnosis and risk of preeclampsia. Hypertens. 2018;72(2):433–41.
13. Siddiqui A, Deneux-Tharaux C, Luton D,. et al. Maternal obesity and severe pre-eclampsia among immigrant women: A mediation analysis. Sci Rep. 2020;10(1):1–9.
14. Nakimuli A, Nakubulwa S, Kakaire O,. et al. The burden of maternal morbidity and mortality attributable to hypertensive disorders in pregnancy: A prospective cohort study from Uganda. BMC Preg Childbirth. 2016;16(205):1–8.
15. Bokslag A, Teunissen PW, Franssen C,.et al. Effect of early-onset preeclampsia on cardiovascular risk in the fifth decade of life. Am J Obstet Gynecol. 2017;216(5):523.e1-e7.

16. Opitasari C, Andayasari L. Luaran maternal dan neonatal pada preeklampsia berat perawatan konservatif di RSUD Dr. Soetomo Surabaya. Obgynia. 2019;2(2).
17. Bartsch E, Medcalf KE, Park AL, Ray JG. Clinical risk factors for pre-eclampsia determined in early pregnancy: Systematic review and meta-analysis of large cohort studies. Bri Med J. 2016;353:i1753.
18. Wilson RL, Grieger JA, Bianco-Miotto T, Roberts CT. Association between maternal zinc status, dietary zinc intake and pregnancy complications: A systematic review. Nutrient. 2016;8(10):641.
19. Fukada T, Kambe T. Welcome to the world of zinc signaling. Int J Mol Sci. 2018;19(3):785.
20. Chababa L, Mukosha M, Sijumbila G. Relationship between Serum Zinc Levels and Preeclampsia at the University Teaching Hospital, Lusaka, Zambia. Med J Zambia. 2016;43(3):139–44.
21. Al-Jameil N, Tabassum H, Ali MN, et al,. Correlation between serum trace elements and risk of preeclampsia: A case controlled study in Riyadh. Saudi Arabia. Saudi J Bio Sci. 2017;24(6):1142–8.
22. Jin S, Hu C, Zheng Y. Maternal serum zinc level is associated with risk of preeclampsia: A systematic review and meta-analysis. Front Public Health. 2022;10:968045.
23. Hara T, Takeda T-a, Takagishi T, Fukue K, Kambe T, Fukada T. Physiological roles of zinc transporters: molecular and genetic importance in zinc homeostasis. J Physiol Sci. 2017;67:283–301.
24. Olechnowicz J, Tinkov A, Skalny A, Suliburska J. Zinc status is associated with inflammation, oxidative stress, lipid, and glucose metabolism. J Physiol Sci. 2018;68(1):19–31.
25. Kambe T, Tsuji T, Hashimoto A, Itsumura N. The physiological, biochemical, and molecular roles of zinc transporters in zinc homeostasis and metabolism. Physiol Rev. 2015;95:749–84.
26. Bafaro E, Liu Y, Xu Y, Dempski RE. The emerging role of zinc transporters in cellular hemostasis and cancer. Sig Transduct Target Ther. 2017; 2:17029.
27. Iqbal S, Ali I. Effect of maternal zinc supplementation or zinc status on pregnancy complications and perinatal outcomes: An umbrella review of meta-analyses. Heliyon. 2021;7(7):e07540.
28. Eze SC, Ododo NA, Ugwu EO, Enebe JT, Onyegbule OA, Eze IO, Ezem BU. Serum selenium levels of pre-eclamptic and normal pregnant women in Nigeria: A comparative study. PLoS ONE. 2020;15(8):e0238263.
29. National Center for Biotechnology Information. PubChem Compound Summary for CID 62640, Zinc sulfate heptahydrate. In: https://pubchem.ncbi.nlm.nih.gov/compound/Zinc-sulfate-heptahydrate, accessed in January 20th, 2021.
30. Michalczyk M, Celewicz A, Celewicz M, Woźniakowska-Gondek P, Rzepka R. The role of inflammation in the pathogenesis of preeclampsia. Mediators inflamm. 2020:1–9.

Research Article

Calsium Supplementation with Rasbora sp. to Prevent Loss of Bone Mineral Density during Gonadotropin Releasing Hormone Agonist Long-term Treatment

Suplementasi Kalsium dengan Ikan Seluang (Rasbora sp.) untuk Pencegahan Kehilangan Kepadatan Mineral Tulang selama Penggunaan Jangka Panjang Agonis Gonadotropin-Releasing Hormone

Hardyan Sauqi[1], Enrico Hervianto[2], Farida Heriyani[3], Juhairina[4]

1Division of Reproductive Endocrinology and Infertility
2Department of Obstetrics and Gynecology
3Department of Public Health
4Department of Nutrition
Faculty of Medicine Universitas Lambung Mangkurat
Banjarmasin

Abstract

Objective: To observe the benefit of calcium supplementation overcoming the hypoestrogenic effect of *Gonadotropin-releasing hormone* (GnRH) agonist, using a standardized regimen and *Rasbora sp.* (local: Seluang fish) as a natural source of calcium.

Methods: This study included 24 reproductive-aged (15-49 years old) females on subcutaneous agonist GnRH leuprolide acetate 11,25 mcg regimen. Bone mineral density was measured twice within three months, before and after the first agonist GnRH treatment. Subjects were classified into three groups of daily supplementations: placebo containing Saccharum lactis, ground powder of 500 mg of calcium, or ground powder of dried Rasbora sp containing 500 mg of calcium. Measurement pre- and post-supplementation were performed using the bone *quality index* with Osteosys of Sonost 3000.

Results: Following three months of observation, subjects receiving placebo had a decreased bone mineral density of -22,7201 compared to those who received 500 mg calcium supplementation and fish powder with a calcium content of 500 mg (-4,4570 and -3,3634, respectively). The homogeneity test showed a significance level of 0.031, and ANOVA resulted in a significant difference between the three classes. *Post Hoc* showed a significant difference between calcium lactate supplementation 18,26 + 3,20 (p = 0.001) and Seluang fish powder 19,36 + 3,20 (p = 0.000). Both forms of calcium lactate and fish powder supplementation had no significant differences.

Conclusion: Both calcium supplementations of calcium lactate powder and natural resources help maintain bone mineral density during GnRH agonist treatment. Seluang fish (Rasbora sp.), which is abundant in Indonesia and commonly consumed by the Indonesians, especially in South Borneo, has a similar potency to pharmaceutical 500 mg calcium lactate products. Fishery product contains other beneficial trace elements, such as 84 mg of calcium (Ca), 6,81 % of magnesium (Mg), 13,4 mg of iron (Fe), and 3.97 % of zinc (Zn).

Keywords: bone mineral density, calcium supplementation, GnRH agonist, *rasbora Sp.*

Abstrak

Tujuan: *Untuk mengetahui manfaat suplementasi kalsium dalam mengatasi efek hipoestrogenik oleh GnRH agonis menggunakan sediaan kalsium terstandar dan Rasbora sp. (lokal: Ikan Seluang) sebagai sumber kalsium alami.*

Metode: *Penelitian ini melibatkan 24 perempuan berusia reproduktif 15-49 tahun yang menjalani terapi GnRH agonis dengan regimen Leuprolide 11,25 mcg subkutan. Pengukuran terhadap densitas massa tulang dilakukan dua kali berselang 3 bulan sebelum dan sesudah pemberian regimen. Dalam waktu pengamatan, subjek dibagi secara acak dalam tiga kelas masing-masing berisi 8 orang yang mendapatkan suplementasi harian berisi salah satu dari plasebo (saccharum lactis), gerusan tablet 500 mg kalsium, dan tepung ikan seluang (Rasbora sp) dengan kandungan setara 500 mg kalsium. Hasil pengukuran sebelum dan sesudah pemberian regimen dihitung menggunakan indeks kualitas tulang dengan alat Sonost 3000 dari Osteosys.*

Hasil: *Perempuan pengguna GnRH agonis yang menerima plasebo mengalami penurunan kepadatan densitas tulang sebesar -22,7201 dibandingkan dengan mereka yang mendapatkan suplementasi 500 mg kalsium tablet dan tepung ikan dengan kalsium setara 500 mg, masing – masing -4,4570 dan -3,3634 setelah 3 bulan. Uji homogenitas menemukan signifikansi sebesar 0.031 dan ANOVA menunjukkan perbedaan bermakna antara ke tiga kelas tersebut yakni p=0.000. Uji Post Hoc menemukan perbedaan terjadi pada suplementasi kalsium tablet dibandingkan dengan placebo sebesar 18,26313 (p = 0.001) dan pengguna suplementasi tepung ikan seluang (Rasbora Sp.) sebesar 19,35675 (p = 0.000). Sedangkan suplementasi kalsium tablet dengan tepung ikan yang setara 500 mg, tidak terdapat perbedaan bermakna 1,09362 (p = 0.948).*

Kesimpulan: *Suplementasi kalsium baik dalam bentuk sediaan tablet maupun bahan alami membantu menjaga kepadatan massa tulang selama pemberian GnRH. Seluang (Rasbora sp.) yang melimpah dan dikonsumsi luas oleh masyarakat Indonesia khususnya di Kalimantan Selatan, memiliki potensi sama baiknya dengan produk farmasi berupa kalsium laktat setara 500 mg. Produk ikan, mengandung trace element lain yang baik untuk tubuh, selain 8.4 mg kalsium, terdapat juga 6.81 mg Magnesium (Mg), 1,339 mg Besi (Fe), dan 3.97 mg Zinc (Zn).*

Kata kunci: *GnRH Agonis, kepadatan massa tulang, Rasbora Sp., suplementasi kalsium.*

Correspondence author. Enrico Hervianto. Department of Obstetrics and Gynecology.
Faculty of Medicine Universitas Lambung Mangkurat. Banjarmasin.
Email; hervianto.enrico@gmail.com

Received: July.2021 Accepted: September,2022 Published: October,2022

INTRODUCTION

Long-term GnRH agonist therapy is used in several medical conditions related to both sex and growth hormone abnormalities. The pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are stimulated by GnRH agonist, especially as leuprolide acetate, thus increasing steroidogenesis of ovaries and testes and resulting in increased female estrogen production.[1] The hypoestrogenic condition could be a double-blade sword; while helping reduce the size of the benign gynecologic mass, including endometriotic cyst, due to its ability to decrease growth factor,[2] it could decrease the density of bone as it reduces proliferation and causes more prominent osteoclast activity than osteoblast.[3]

Bone mineral density starts to decrease when GnRH is suppressed and causes osteoclast to be more active, and residual loss of media percentage is highest after 12 months. Add-back therapy using calcium supplementation seems to reduce the rate of BMD reduction significantly.[4]

Quantitative ultrasound (QUS) has gained popularity as a technique to assess bone health since its introduction in 1984. It is highly accessible due to its portability, low cost, and simplicity of handling. In addition, no ionizing radiation is produced by QUS compared to dual-X-ray absorptiometry (DXA).[5] For precision of measurement, there isn't any difference in assessing bone mineral density and predicting osteoporosis.[6]

Supplementation of calcium during GnRH agonist therapy was observed and advised to prevent loss of bone mineralization.[7] Rasbora sp. is mainly found in the deep river around Nusantara (Indonesia's historical area),[8] whereas it is endemic, especially in the riverside plain on several main islands of Nusantara.[9] The species is considered to be the main source of protein or trace elements for indigenous people, who consume it as a daily diet.[10] Rasbora sp. is considered a genus of tiny fish of Cyprinidae that inhabit freshwater in South and Southeast Asia. They are small, with lengths ranging from 10 to 17 cm with many dark horizontal stripes.[11] The Indigenous name for this fish is Seluang, which has been believed to be a good source of calcium and protein for a long time.[12]

Figure 1. Silver rasbora or *Rasbora argryotaenia* (Local: Seluang fish).

METHODS

Subjects included females of reproductive age (15-49 years old) who presented to the gynecologic outpatient clinic of Ulin District Hospital in Banjarmasin, South Borneo, and were treated with GnRH agonist under any medical reason. The participants were selected using inclusion and exclusion criteria. Counseling and informed consent were obtained after detailed explanations to the patient and guardians.

Data included evaluation of age, blood pressure, and body mass index. During the study, patients were not restricted to any food but were randomly checked for a full-week food diary twice during the study. Later, participants were distributed into one of three groups. All study procedures were carried out according to the Human Research Ethics Committee from Ulin District Hospital.

A randomized controlled trial was applied to this single-centre, randomized, double-blinded, single-dose study. Data of bone quantity index (stiffness index) was examined in subjects before and after treatment using supplementation of either calcium or placebo.

Either subject and regiment were numbered and randomly distributed one sample for one subject. The supplementation regimen was classified into three classes consisting of the same number of samples: Saccharum lactis or fructose as placebo, ground powder of 500 mg lactate calcium, and ground powder of Seluang fish (Rasbora sp.) containing 500 mg calcium in equivalent, extracted and quantified by Certified Biochemistry Laboratory of Lambung Mangkurat University in Banjarbaru, South Borneo.

Every sample was packed into a capsule with the same size, colour, and weight. Each subject takes a daily capsule, which could consist of the following: placebo, ground powder of 500 mg lactate calcium, or ground powder of Seluang fish containing 500 mg calcium in equivalent for three months. The fish is processed into powder within three steps; the fish is minced together without separating flesh and bones, and the mixture is then steamed within 15 minutes and pressed to extract the water. Later on, it is dried under sun rays for 1 day, and it is further blended and ground into powder.[13]

Then, extracts were verified in a biochemistry laboratory for the calcium portion in the sample. Based on the sample in 065/UN.8.1.17.2.2/ PP/2019 fishery product, this sample contains trace elements, which are beneficial (consisting of 84 mg of calcium (Ca), 6.81 %mass of magnesium (Mg), 13,4 mg of iron (Fe), and 3.97 %mass of zinc (Zn) for every 100 mg powder of extract).

The bone quality index (BQI) was evaluated using Osteosys of Sonost 3000 before any treatment was given. Evaluation of the second attempt to retrieve bone mineral density data was done after three months of supplementation following the first subcutaneous injection of GnRH agonist depot consisting of 11,25 mcg Leuprolide acetate that lasted for three months. Patients' complaints were solved based on symptoms and documented.

The bone mineral density (BMD) is expressed in the stiffness index or called the bone quantity index. We used the Student's t-test or one-way ANOVA (analysis of variance) to determine the difference between BMD before and after GnRH agonist and supplementations. We subsequently performed posthoc Duncan's multiple range test ($p<0.05$ is considered significant). We used Leven's test to determine the homogeneity of variance for ANOVA and t-test. Statistical Product and Service Solutions (SPSS) 25th version released by IBM was used for all statistical analyses. The odd ratio is analyzed for each group to find the quantitative difference between classes.

RESULTS

This study included twenty-four subjects who completed the randomized, single-dose, closed-label, single-centre, oral supplementation study within 3 months. All characteristics data were presented in table 1. The result showed that age, parity, and BMI did not differ between groups. Hence, it shows that participants were fairly distributed.

Subjects receiving placebo had a decreased bone mineral density of -22,7201 compared to those who received 500 mg calcium supplementation and fish powder with a calcium content of 500 mg (-4,4570 and -3,3634, respectively).

Table 1. Basic Characteristics Data of Participants.

Characteristic (Mean ± SE)	Group 1 Placebo	Group 2 Oral pill (500 mg calcium)	Group 3 Ground seluang (500 mg calcium)	Significance *(P-value)*
Age (mean, years old)	38.00 ± 2.260	42.75 ± 1.461	38.13 ± 1.977	0.164
Parity (mean)	0.75 ± 0.313	0.75 ± 0.366	0.88 ± 0.398	0.961
0 (*n*)	4	4	4	
1 (*n*)	2	3	2	
> 2 (*n*)	2	1	2	
BMI (mean, kg/m^2)	26.83 ± 1.447	25.71 ± 1.652	27.66 ± 1.922	0.717
Random calcium intake (mg/day)	56.33 ± 3.402	53.21 ± 2.843	55.16 ± 3.221	0.653
BQI before therapy	76.75 ± 5.681	79.22 ± 4.210	82.19 ± 6.378	0.785
Change in BQI	- 22.72 ± 1.775	- 4.46 ± 2.967	- 3.36 ± 1.855	0.000

The stiffness index or bone quantity index showed a significant difference; therefore, the result is tested using ANOVA, homogeneity using Levene's test, and post hoc comparison between groups using the Bonferonni test.[14] Correlation in classes from paired T-Test was significant for each class showing that data were taken from the same sample, while changes were significant only in the placebo class, showing that paired data has significant changes in that group only.

Table 2. Differences in changes in BQI between groups

Group (Mean ± SD)	before therapy and supplementation (*p* of Normality)	after therapy and supplementation (*p* of Normality)	Changes (*p* of Homogeneity)	Paired T Test *p1* of Correlation test *p2* of Changes test
Placebo	76.75 ± 16.07 (0.918)[a]	54.03 ± 20.33 (0.670)[a]	22.72 ± 5.02 (0.667)[b]	*p1* = 0.000[c] *p2* = 0.000[d]
Oral pill (500 mg calcium)	79.22 ± 11.91 (0.068)[a]	74.76 ± 7.04 (0.998)[a]	4.46 ± 13.95 (0.199)[b]	*p1* = 0.043[c] *p2* = 0.117[d]
Ground seluang (500 mg calcium)	82.19 ± 18.04 (0.231)[a]	78.83 ± 19.01 (0.465)[a]	3.36 ± 5.24 (0.913)[b]	*p1* = 0.000[c] *p2* = 0.113[d]

Note: [a] using Saphiro Wilk, [b] using Levene, [c] and [d] using correlation and changes in paired T-test

The homogeneity test showed a p-value of 0.031. The ANOVA showed a significant difference between the three classes. Mean and SE differences in stiffness index or bone quantity index were presented in figure 2.

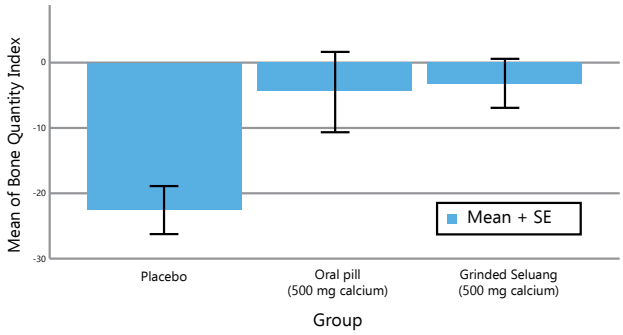

Figure 2. Mean and Standard Error of difference in bone quantity index (BQI) from each group of calcium supplementation.

Data were taken before and after receiving GnRH agonist for 3 month. Calcium supplementation lowered loss in BQI during GnRH agonist therapy. Compared to placebo, *post hoc* test using Bonferonni formula showed a calcium lactate supplementation of 18.26 + 3.20 (p = 0.001) and ground Seluang powder of 19.36 + 3.20 (p = 0.000). Calcium lactate supplementation and ground Seluang powder did not have a significant difference.

DISCUSSION

Long studied by Treber et al. of bone calcium loss during GnRH agonist therapy,[15] current guidelines recommend supplementation of calcium to prevent the further possibility of osteoporosis due to loss of bone calcium.[16] Calcium supplementation could potentially provide hormonal add-back therapy to prevent blood loss. But several conditions that need hypoestrogenic conditions are not suited to this add-back therapy.4 GnRH agonist therapy in dysregulated puberty, either late or precocious, and follicle stimulation could have an advantage over non-calcium supplementation.[17]

Seluang was recommended as calcium supplementation since it contains abundant organic calcium.[18] Result from other studies[19,20] showed that 100 grams of Seluang contain 80 mg calcium, 224 mg phosphorus, and 4.7 mg iron. Organic calcium comes with other trace elements that could enhance or benefit participants.

CONCLUSION

Calcium supplementation helps to maintain bone mineral density while patients are in GnRH agonist treatment. A daily dose of 500 mg whether in calcium lactate or organic calcium extracted from Seluang fish (Rasbora sp.), the primary fish group particularly found in South Borneo, has a similar potency to 500 mg pharmaceutical calcium lactate. Local people can benefit from daily intake of seluang fish as an economic resource of calcium while taking medication. For recommendation, further studies of multi-center calcium supplementation using a prepared regimen or local resources are necessary to assess ethnic-related calcium metabolism—while using calcium supplementation when undergoing GnRH agonist medication.

REFERENCES

1. Seo JW, Lee DY, Kim SE, Yoon BK, Choi DS. Comparison of long-term use of combined oral contraceptive after gonadotropin-releasing hormone agonist plus add-back therapy versus dienogest to prevent recurrence of ovarian endometrioma after surgery. Eur J Obstet Gynecol and Reprod Biol. 2019;236:53-7.
2. Rafique S, Decherney AH. Medical Management of Endometriosis. Clin Obstet Gynecol. 2017;60(3):485-96.
3. Panday K, Gona A, Humphrey MB. Medication-induced osteoporosis: screening and treatment strategies. Ther Adv Musculoskelet Dis. 2014;6(5):185-202.
4. Osuga Y, Torng PL, Jeng CJ. Clinical studies investigating the use of leuprorelin in Asian women with endometriosis: a review. J Obstet Gynaecol. 2019;1:1-7

5. Chin KY, Ima-Nirwana S. Calcaneal quantitative ultrasound as a determinant of bone health status: what properties of bone does it reflect?. Int J Med Sci. 2013;10(12):1778-83.
6. Newcomer, Beth R. Comparison of Two Bone Mineral Density Pre-Screening Tools: QUS and RA, to the DXA. Electronic Theses and Dissertations. 2010:106.
7. Sandra BA, Francisco AP, Rosângela AV, et al. Bone mineral density and body composition in girls with idiopathic central precocious puberty before and after treatment with a gonadotropin-releasing hormone agonist. Clin. 2012;67(6):591-6.
8. Alpiyah N, Purnengsih I. Karakter Gajah Mada: Simbol Kejayaan Majapahit. Visual Heritage. Jur Kreasi Seni dan Budaya. 2019;1(2):1-3
9. Dina R, Samir O, Lukman, Haryani GS, Nasution SH. Fish and fisheries of Bada (Rasbora spp.) in Lake Maninjau, West Sumatra. IOP Conf. Ser. Earth Environ. Sci. 2018:306
10. Partan RU, Salim E, Hidayat R. The Potential Role of Seluang Fish Oil (Rasbora Spp) in Improving Calcium, Zinc and Vitamin D3 Deficiencies. Int J Oceans Oceanography. 2017;11(2): 285-91.
11. Muchlisin ZA. Morphometric Variations of Rasbora Group (Pisces: Cyprinidae) in Lake Laut Tawar, Aceh Province, Indonesia, Based on Truss Character Analysis. HAYATI. J Biol Sci. 2013;20(3):138-43.
12. Triawanti, Yunanto A, Sanyoto D. The potential of seluang fish (Rasbora spp.) to prevent stunting: The effect on the bone growth of Rattus norvegicus. Proceedings of the 4th BIBMC (Bandung International Biomolecular Medicine Conference) 2016 and the 2nd ACMM (Asean Congress on Medical Biotechnology and Molecular Biosciences). 2017
13. Napitu N. Pembuatan Ekstrak Dan Tepung Ikan Gabus Sebagai Substitusi Pengolahan Biskuit Untuk Menaikkan Kadar Albumin Pada Balita Gizi Buruk. Jurnal Pengabdian Kepada Masyarakat. 2016;16(60):42-6.
14. Davison ML, Sharma AR. ANOVA and ANCOVA of pre- and post-test, ordinal data. Psychometrika. 1994;59:593–600.
15. Waibel-Treber S, Minne HW, Scharla SH, Bremen T, Ziegler R, Leyendecker G. Reversible bone loss in women treated with GnRH-agonists for endometriosis and uterine leiomyoma. Hum Reprod. 1989;4(4):384-8.
16. Carrie Armstrong. ACOG Updates Guideline on Diagnosis and Treatment of Endometriosis. Am Fam Physician. 2011;83(1):84-5.
17. Kaya A, Cayir A, Turan MI, Ozkan B. An Examination of the Effects of Leuprolide Acetate Used in the Treatment of Central Precocious Puberty on Bone Mineral Density and 25-Hydroxy Vitamin D. West Ind Med J. 2015;64(2):104-7.
18. Putri AS, Kusfriyadi MK. Pengaruh Substitusi Tepung Selpis (Seluang Dan Pisang) Terhadap Kadar Protein, Kalsium, Daya Terima Dan Mutu Organoleptik. Jur Ris. 2020;1(1):1-3.
19. Triawanti, Yunanto A, Sanyoto DD, Nur'amin HW. Nutritional Status Improvement in Malnourished Rat (Rattus norvegicus) after Seluang Fish (Rasbora spp.) Treatment. Curr Res Nutr Food Sci. 2018;6(1):127-34.
20. Triawanti, Noor MS, Nur'amin HW, Sanyoto DD. The seluang fish (Rasbora spp.) diet to improve neurotoxicity of endosulfan-induced intrauterine pup's brain through of oxidative mechanism. ClinNutr Experimental. 2019;28:74-82.

Research Article

miRNA519a-3p and NKG2DL in Endometriosis

Ekspresi miRNA519a-3p dan NKG2DL pada Endometriosis

Cepi T. Pramayadi, Muharam Natadisastra, Kanadi Sumapradja, Aria Kekalih

Division of Reproductive Health
Department of Obstetrics and Gynecology
Faculty of Medicine Universitas Indonesia
Dr. Cipto Mangunkusumo General Hospital
Jakarta

Abstract

Objective: To investigate the correlation between miRNA-519a-3p expression with NKG2D ligands (MICA, MICB, ULBP 1-6) on endometriosis and non-endometriosis patients.

Methods: This was a cross-sectional study held in five centers: dr. Cipto Mangunkusumo General Hospital, Pelni Hospital, Bunda Hospital, YPK Mandiri Hospital, and Primaya Evasari Hospital from October 2020 to July 2021. miRNA and NKG2DL analysis were done in *Human Reproduction, Infertility and Family Planning* (HRIFP) cluster at IMERI FKUI.

Results: We obtained 19 patients in each study groups. NKG2D ligands and miRNA519a-3p relative expressions were not significantly different ($p > 0.05$). Increased miRNA519a-3p expression negatively affected NKG2D ligands expression. A decrease in ULBP1 and an increase in ULBP2 increased the probability for endometriosis. NKG2D ligands expression may be influenced by infection, pro-inflammatory cytokine production, dan polymorphism. NKG2D ligands expression level can be different depending on the origin of the sample. Lower expression of miRNA519a-3p indirectly inhibits tumor apoptosis by lowering NKG2D ligands, caspase, or mRNA.

Conclusion: We did not manage to establish a correlation between NKG2D ligands with miRNA519a-3p in endometriosis and non-endometriosis patients.

Keywords: apoptosis, endometriosis, immunologic cytotoxicity, miRNA519a-3p, NK cells, NKG2D ligands.

Abstrak

Tujuan: *Mengetahui korelasi antara ekspresi miRNA-519a-3p dengan ligan NKG2D (MICA, MICB, ULBP 1-6) pada pasien endometriosis dan non-endometriosis.*

Metode: *Studi ini merupakan studi potong lintang yang diadakan di lima pusat Kesehatan: Rumah Sakit (RS) dr. Ciptomangunkusumo, RS Pelni, RS Bunda, RS YPK mandiri, dan RS Primaya Evasari dari Oktober 2020 hingga Juli 2021. Analisis dattat mirNA dan NKG2DL dilakukan di cluster Human Reproduction, Infertility, and Family Planning (HRIFP) IMERI FKUI.*

Hasil: *Studi ini diikuti oleh 19 pasien di setiap kelompok. Ekspresi relatif ligan NKG2D dan miRNA519a-3p tidak bermakna secara signifikan ($p > 0.05$). Peningkatan ekspresi miRNA519a-3p menurunkan ekspresi ligan NKG2D. Penurunan ULBP1 dan peningkatan ULBP2 berhubungan dengan meningkatnya kemungkinan endometriosis. Ekspresi ligan NKG2D dapat dipengaruhi oleh infeksi, produksi sitokin pro-inflamasi, dan polimorfisme. Tingkat ekspresi ligan NKG2D dapat berbeda-beda berdasarkan asal sampel jaringan. Ekspresi miRNA519a-3p yang rendah secara tidak langsung meenghambat apoptosis tumor melalui penurunan ligan NKG2D, caspase, atau mRNA.*

Kesimpulan: *Tidak terdapat korelasi antara ekspresi miRNA519a-3p dengan ligan NKG2D pada pasien endometriosis dan non-endometriosis.*

Kata kunci: *apoptosis, sitotoksisitas imun, endometriosis, ligan NKG2D, miRNA519a-3p, sel NK.*

Correspondence author. Cepi T. Maryadi.Department of Obstetrics and Gynecology.
Faculty of Medicine Universitas Indonesia. Dr. Cipto Mangunkusumo General Hospital. Jakarta
Email:cteguh@yahoo.com

Received: September,2021 Accepted: September,2022 Published: October,2022

INTRODUCTION

Endometriosis accounts for 15-25% cause of female infertility in Indonesia and is commonly found in reproductive age.[1,2] Undetected endometriosis are represented with persistent and progressive symptoms which negatively affect patients' wellbeing and comfort.[3] Literatures have shown that 70% of endometriosis patients may present with chronic pelvic pain and 15-20% are in risk of developing endometrioma. Infections are quite common in endometriosis patients, which can be found in 30-40% patients. Depression and anxiety disorders are not uncommon in endometriosis patients.[1,4]

miRNAs are specialized in controlling human innate and adaptive immune systems, mainly in autoimmune diseases and cancers.[5,6] Studies have shown several types of miRNAs effect on regulating natural killer (NK) cells activity. For instance, miRNA-150 and miRNA-180 may inhibit Nemo like kinase (NLK) to prevent NK cell maturation. Another type of miRNA, such as miRNA-203, miRNA494-3p- and miRNA-155 may affect PTEN-AKT-mTOR pathway to affect apoptosis.[7–9] miRNA-519 has been found to correlate with ovarian carcinoma and breast cancer. High expression of particular member of miRNA-519, miRNA-519a-3p, has been found to lower TNF-related apoptosis inducing ligand (TRAIL)-R2, caspase-7, and caspase-8 in breast cancer to avoid TRAIL or Fas ligand-mediated apoptosis. miRNA-519a-3p can bind to some ligands of NK cell receptor group 2 member D (NKG2D), namely ULBP2 and MICA to intercept tumour recognition, NK cell activation, and provides the tumour cell with resistance to granzyme B.[5,10]

Binding of NKG2D receptor in NK cells with its ligands maintains cell structure integrity as a response to DNA damage and promotes NK-cell mediated apoptosis.[11,12] However, overexpression of NKG2D ligands in ectopic endometriosis cells may signify a cytotoxic avoidance mechanism of NK cells.[13] Previous studies have shown various different results on the effect of NKG2D ligands and miRNA expression in neoplasms.[14,15]

METHODS

This was a cross-sectional study conducted from October 2020 through July 2021 in five different centers: Dr. Cipto Mangunkusuomo General Hospital, Pelni Hospital, Bunda Hospital, YPK Mandiri Hospital, and Primaya Evasari Hospital. This study has been approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia – Dr. Cipto Mangunkusumo Hospital (KET-441/UN2.F1/ETIK/PPM.00.02/2021) in June 2020. Subjects included in this study were female with or without endometriosis aged 20-45 years old, not in pregnancy, confirmed not having other malignancies such as cervical cancer, severe cervical stenosis, nor other genital tract malignancies, and has agreed to informed consent. We targeted a minimum sample size of 19 samples using correlative analytic formula. Sampling was done through consecutive sampling method. Both endometriosis and endometrium samples were obtained through operative hysteroscopy and laparoscopy. miRNA and NKG2D ligands analysis were done through standard quantitative real-time PCR method. Data analysis were done through non-parametric analysis using IBM Statistical Package for the Social Sciences (SPSS) version 26.0.

RESULTS

We obtained a total of 29 patients with endometriosis and 26 patients without endometriosis. Average age of patients in endometriosis and control group were 33 years old and 36 years old, respectively. Patients in control group had higher body mass index (BMI) (25.63 + 4.27 kg/m^2) than those in endometriosis group (24.92 + 4.89 kg/m^2). Our endometriosis patients had menarche at slightly later age (12.52 + 0.99 years old) rather than those in control group (12.48 + 1.24 years old). Most samples were taken during the proliferative phase of endometrium. Subjects' characteristics can be seen on Table 1.

Table 1. Subjects Characteristics

Variables	Endometriosis (n = 29)	Control (n = 26)
Age (years old)	33.89 + 5.33	36.27 + 6.79
Weight (kg)	61.78 + 12.22	63.90 + 10.18
Height (m)	1.57 + 0.05	1.58 + 0.06
BMI	24.92 + 4.89	25.63 + 4.27
Menarche age (years old)	12.52 + 0.99	12.48 + 1.24
Endometrium phase		
Proliferative	13	9
Secretory	10	7
Unknown	6	10

Relative expression and fold changes for each NKG2D ligands and miRNA-519a-3p was assessed through Livak's formula. We excluded

10 subjects from endometriosis group and 7 from control group due to extreme cycle threshold (CT) value which may produce inaccurate results. We divided the samples into two groups: tumour compared to control (TC) and endometriosis endometrium compared to control (EC). MICA expression in endometriosis tissue was found to be 2.4 times lower (0.42 + 0.79) than in control group. We found that MICB expression in endometriosis tissue was 100 times lower (0.01 + 0.02) than control group. ULBP1, ULBP2, ULBP3, and ULBP6 were found to be 3.7 times (0.27 + 0.46), 33.3 times (0.03 + 0.06), 5 times (0.20 + 0.52), and 12,5 times (0.08 + 0.11) lower than control group. Higher expression of MICA (1.09 times; 1.09 + 3.12), ULBP1 (5.64 times; 5.64 + 13.51), ULBP4 (7.49 times; 7.49 + 12.91), and ULBP5 (5.75 times; 5.75 + 8.90) were found in endometrium tissue of endometriosis patients. Relative expression of miRNA-519a-3p were 11.1 times lower in tumour tissue (0.09 + 0.24) and 5.55 times lower in endometriosis patients' endometrium (0.18 + 0.40).

Table 2. Relative Expression of NKG2D Ligands and miRNA519a-3p Compared to Control

Variables	TC	EC
MICA	0.42 + 0.79	1.09 + 3.12
MICB	0.01 + 0.02	0.02 + 0.05
ULBP1	0.27 + 0.46	5.64 + 13.51
ULBP2	0.03 + 0.06	0.04 + 0.05
ULBP3	0.20 + 0.52	0.25 + 0.42
ULBP4	1.65 + 2.40	7.49 + 12.91
ULBP5	2.99 + 5.05	5.75 + 8.90
ULBP6	0.08 + 0.11	0.44 + 0.80
miRNA-519a-3p	0.09 + 0.24	0.18 + 0.40

There was no significant difference of NKG2D ligands and miRNA-519a-3p expression between TC and EC (p value > 0.05). Lower median value of ULBP1, ULBP3, ULBP4, ULBP5, and miRNA-519a-3p were observed in tumour tissue compared to control group.

Table 3. Comparative Analysis of NKG2D Ligands and miRNA519a-3p

NKG2D ligands	TC	EC	*P- value*
MICA	0.17 (0.01-3.53)	0.17 (0.02-13.74)	0.804
MICB	0.02 (0.0003-0.11)	0.01 (0.00-0.19)	0.589
ULBP1	0.11 (0.01-1.89)	0.19 (0.002-50.21)	0.397
ULBP2	0.01 (0.0008-0.22)	0.01 (0.0004-0.17)	0.579
ULBP3	0.05 (0.005-2.31)	0.07 (0.001-1.84)	0.226
ULBP4	0.65 (0.00-8.82)	0.82 (0.02-50.21)	0.274
ULBP5	1.36 (0.03-21.56)	1.43 (0.01-31.34)	0.358
ULBP6	0.04 (0.0007-0.42)	0.03 (0.001-3.12)	0.422
miRNA-519a-3p	0.01 (0.001-1.01)	0.03 (0.0002-1.72)	0.405

Correlative analysis in this study was carried using Spearman's analysis. This research found that miRNA-519a-3p correlates negatively with MICA (r = -0.305), MICB (r = -0.009), ULBP1 (r = -0.107), ULBP2 (r = -0.128), ULBP3 (r = -0.128), and ULBP5 (r = - 0.126) expression in endometrium tissue of endometriosis patients. Higher miRNA519a-3p expression may depress most of NKG2D ligands expression save for MICB (r = 0.088) and ULBP 1 (r = 0.126) in endometriosis tumour tissue.

Table 4. Correlation of miRNA-519a-3p and NKG2D Ligands Expression

Variables	TC		TC	
	r	*p-value*	r	*p-value*
MICA	-0.125	0.611	-0.305	0.204
MICB	0.088	0.721	-0.009	0.972
ULBP1	0.126	0.606	-0.107	0.663
ULBP2	-0.082	0.737	-0.128	0.601
ULBP3	-0.244	0.314	-0.128	0.601
ULBP4	-0.047	0.847	0.074	0.764
ULBP5	-0.040	0.870	-0.126	0.606
ULBP6	-0.144	0.557	0.100	0.684

DISCUSSION

This study reported the correlation and differences in expression between miRNA519a-3p and the NKG2D ligands in endometriosis and non-endometriosis patients. Ectopic endometrial tumour tissue had higher expression of NKG2D and miRNA519a-3p ligands than endometrial eutopic tissue in similar patients. ULBP4 and ULBP5 are NKG2D ligands were elevated in tumour and eutopic endometrial tissue of endometriosis patients compared to control group. Enhanced expression of ULBP4 and ULBP5 did not increase the likelihood of endometriosis, except for ULBP1 and ULBP2. Difference in relative expression of NKG2D ligands and miRNA519a-3p between ectopic endometriosis and endometrial tissue of endometriosis patients was not statistically significant. Relative expression of miRNA519a-3p tends to negatively correlated with most of NKG2D ligands, although this correlation is statistically insignificant. The expression of NKG2D ligands and miRNA519a-3p plays a major role in determining the diagnosis of endometriosis.

We found an increase in relative expression of MICA in endometrium tissue (1.09 + 3.12) and tumour tissue of endometriosis patients (0.42 + 0.79) compared to control. Further analysis showed that the difference in MICA expression was not statistically significant (p = 0.804). This finding is reciprocal with a study in 2012 [16] which found lower expression of MICA and ULBP2 in breast cancer cells due to the influence of miRNA-519a-3p could attenuate the activation and cytotoxicity of NK cells. Our findings are in contrary with study by Xu and colleagues[15] which found no change in the expression of MICA, MICB, and ULBP-1 in endometriosis patients. The discrepancy between the results of this research and other studies may be due to histological factors and differences in the ELISA method used in the previous study.[17]

MICB expression was found to be lower in the endometrium in the endometriosis group (0.02 + 0.05) and in the endometrial tumour tissue (0.01 + 0.02) compared to the control and not statistically significant (p = 0.589). Similar findings were found in a study [15] which found that MICA/MICB protein expression did not show any difference/ increase, compared to normal endometrial tissue. This finding is in contrast [18] who stated that soluble MICB levels were found to be elevated in serum in many patients with gastric, colon, and rectal carcinomas. Any difference in soluble MICA and MICB levels might be due to differential degradation in human serum.[19]

MICA, ULBP 1, 2, and 3 can be increased in infection or tumour transformation, Increased MICA, ULBP1, 2, and 3 may promote activation of NK cells.[20] Our results found that endometrial tissue of endometriosis patients had higher ULBP1 expression (5.64 + 13.51) than tumour tissue of the same patient (0.27 + 0.46) and control patients. The difference between these ULBP1 relative expression was not statistically significant (p = 0.397). DNA damage may induce ULBP transcription.[21]

Decreased ULBP2 expression was found in endometrial tissue (0.04 + 0.05) and ectopic tumour tissue of endometriosis tumour (0.03 + 0.06) compared to control with no statistical significance in difference (p = 0.579). This finding is in line who found that the expression of ULBP2 in endometriosis patients was much lower than in patients without endometriosis.[15] Decreased expression of ULBP2 and ULBP3 may help patients' eutopic/ectopic endometrial cells escape immune surveillance, and may also play a role in the reduction of NKG2D-mediated NK cell cytotoxicity. Contradicting evidence was found in a study by Gonzalez-Foruria et al., (2015)[17] who found that ULBP2 levels were found to be significantly higher in 27 endometriosis patients compared to controls (p = 0.012). Disparity between RNA and protein expression occurs in the process of translation. This implies that protein molecules are separated from the cell membrane, and therefore, cannot be detected in the endometrium.[15]

Endometrial ectopic tissue was found to have lower ULBP3 expression than endometrial tissue in endometriosis and non-endometriosis patients.[15] Our study produced a similar result, in which we observed a decreased ULBP3 expression in endometrial tissue (0.25 + 0.42) and in endometriosis tumour tissue of patients with endometriosis (0.20 + 0.52) compared to control. The disparity of ULBP3 expression held no statistical significance (p = 0.226). Contrary to our finding, found high ULBP3 expression in ovarian carcinoma tissue. Elevated ULBP3 expression was positively and significantly associated with ovarian carcinoma recurrence and lower survival rates compared to patients with low ULBP3.[22] Differences in ULBP3 expression in this study with previous studies could be influenced by histological factors. NK cells mediated immune system may act differently in certain epithelial

tissues.

We managed to found higher average ULBP4 expression in endometrial tissue of patients with endometriosis (7.49 + 12.91) and in subsequent tumour tissue endometriosis (1.65 + 2.40) compared to control. This difference was not statistically significant (p = 0.274). ULBP4 expression is generally only found in human monocyte cells and cannot be found in large numbers in NK cells. ULBP4 expression was increased in cervical cancer tissue and squamous carcinoma of the larynx, renal pelvis, colon, thyroid, and ovaries[23]. ULBP4 expression in malignant cells is affected by levels of TGFβ. Decreased TGFβ expression enhances ULBP4 and other NKG2D ligands such as MICA and ULBP2. TGFβ produced by malignant cells suppresses NKG2D receptors expression on NK cells through paracrine pathways and decrease NKG2D ligands on cancer cells surface through autocrine pathways. These two pathways provide invisibility from NK cells.[24]

The average of ULBP5 expression was found higher in endometrial tissue of patients with endometriosis (5.75 + 8.90) and in tumour tissue of patients with endometriosis (2.99 + 5.05) than in control, but was not statistically significant (p = 0.358). The expression of ULBP5 in patients without malignancy is generally low in healthy tissues.[25] The increase in ULBP5 can be caused by increased succinate, inhibition of fumarate hydratase activity, and decreased glutathione activity. Fumarate accumulation causes oxidative stress that triggers the expression of NKG2D ligands such as ULBP2 and ULBP5.[26]

Suppressed ULBP6 expression was found in endometrial tissue of patients with endometriosis (0.44 + 0.80) and in tumour tissue of patients with endometriosis (0.08 + 0.11). We found no statistical significance in difference between these two samples (p = 0.422). Our result contradicted another study which showed a 5-fold increase in ULBP6 on patients with haematological malignancy compared to ULBP6 in those without.[27] Variations in ULBP6 expression are regulated by ULBP6 gene polymorphisms. Single nucleotide mutation on V52F gene was found to reduce the production and cytotoxic activity of ULBP6.[28] Increased NKG2D ligand expression is widely known to reinforce NK cell activity in suppressing tumour growth. However, overly alleviated expression of ULBP6 may cripple NK cell action.

MiRNA519a-3p expression decreased the production of mRNA, caspase, and NKG2D ligand in breast cancer cells. This activity inhibited cancer cell apoptosis and NK cell cytotoxicity.[5] Our study found diminished miRNA519a-3p expression in endometrial tissue (0.18 + 0.40) and tumour tissue endometriosis patients compared to the control group. Healthy patients were found with higher miRNA519a-3p expression than tumour tissue (0.09 + 0.24). Similar results were expressed who found a decrease in miRNA-519a-3p expression in breast cancer patients.[29] Different result was presented on liver cancer cells. Increased miRNA519a was associated with a larger cancer size, metastases, higher grading, and worse outcome.[30]

Polymorphisms or other gene mutations related to NKG2D ligand expression may be the discrepancy source of this study's results with those of previous studies. We did not conduct an investigation at gene level, which might be one of several flaws of this study. We were only able to obtain samples from patients with stage 2 to stage 4 endometriosis, making this study not able to profile the expression of NKG2D ligand and miRNA519a-3p in patients with stage 1 endometriosis. Another shortcoming of this study was due to coronavirus disease 2019 (COVID-19) pandemic, which hampered the sampling process due to the limited use of general anaesthesia in order to prevent the spread of the virus. Results from this study require further study with larger sample to provide more valid results.

CONCLUSION

Increased miRNA-519a-3p may be followed by low NKG2D ligands expression. We did not find any significant difference between relative expressions of NKG2D ligands and miRNA-519a-3p in endometrium and tumour tissue on endometriosis patients. miRNA-519a-3p and NKG2D ligands assay may be a plausible future non-invasive diagnostic method for endometriosis.

ACKNOWLEDGEMENTS

The authors declared no conflicts of interest/ competing interest.

REFERENCES

1. Mukti P. Faktor Risiko Kejadian Endometriosis. *Unnes J Public Heal.* 2014;3(3):1–10.
2. Fuldeore MJ, Soliman AM. Prevalence and Symptomatic Burden of Diagnosed Endometriosis in the United States: National Estimates from a Cross-Sectional Survey of 59,411 Women. *Gynecol Obstet Invest.* 2016;82(5):453–61.
3. Agarwal SK, Chapron C, Giudice LC, et al. Clinical diagnosis of endometriosis: a call to action. *Am J Obstet Gynecol.* 2019;220(4):1–12.
4. Sourial S, Tempest N, Hapangama DK. Theories on the Pathogenesis of Endometriosis. *Endometr Sci Pract.* 2014;2014:453–61.
5. Breunig C, Pahl J, Küblbeck M, et al. MicroRNA-519a-3p mediates apoptosis resistance in breast cancer cells and their escape from recognition by natural killer cells. *Cell Death Dis.* 2017;8(8):1–12.
6. Momen-Heravi F, Bala S. miRNA regulation of innate immunity. *J Leukoc Biol.* 2018;103(6):1205–17.
7. Pesce S, Greppi M, Ferretti E, et al. miRNAs in NK Cell-Based Immune Responses and Cancer Immunotherapy. *Front Cell Dev Biol.* 2020;8:1–13.
8. Pesce S, Squillario M, Greppi M, et al. New miRNA signature heralds human NK cell subsets at different maturation steps: Involvement of miR-146a-5p in the regulation of KIR expression. *Front Immunol.* 2018;9:1–18.
9. Beaulieu AM, Bezman NA, Lee JE, et al. MicroRNA function in NK cell biology. *Immunol Rev.* 2013;253(1):40–52.
10. Bassani B, Baci D, Gallazzi M, et al. Natural killer cells as key players of tumor progression and angiogenesis: Old and novel tools to divert their pro-tumor activities into potent anti-tumor effects. *Cancers (Basel).* 2019;11(4):1–29.
11. Zingoni A, Molfetta R, Fionda C, et al. NKG2D and its ligands: "One for all, all for one." *Front Immunol.* 2018;9:1–12.
12. Duan S, Guo W, Xu Z, et al. Natural killer group 2D receptor and its ligands in cancer immune escape. *Mol Cancer.* 2019;18(1):1–14.
13. Yang S, Wang H, Li D, et al. Role of endometrial autophagy in physiological and pathophysiological processes. *J Cancer.* 2019;10(15):3459–71.
14. Kim TH, Kim YK, Kwon Y, et al. Deregulation of miR-519a, 153, and 485-5p and its clinicopathological relevance in ovarian epithelial tumours. *Histopathol.* 2010;57(5):734–43.
15. Xu H. Expressions of natural cytotoxicity receptor, NKG2D and NKG2D ligands in endometriosis. *J Reprod Immunol.* 2019;136(February):1–7.
16. de Kruijf EM, Sajet A, van Nes JGH, et al. NKG2D ligand tumor expression and association with clinical outcome in early breast cancer patients: An observational study. *BMC Cancer.* 2012;12(24):1–20.
17. González-Foruria I, Santulli P, Chouzenoux S, et al. Soluble ligands for the NKG2D receptor are released during endometriosis and correlate with disease severity. *PLoS One.* 2015;10(3):1–15.
18. Salih HR, Rammensee H-G, Steinle A. Cutting Edge: Down-Regulation of MICA on Human Tumors by Proteolytic Shedding. *J Immunol.* 2002;169(8):4098–102.
19. Salih HR, Goehlsdorf D, Steinle A. Release of MICB Molecules by Tumor Cells: Mechanism and Soluble MICB in Sera of Cancer Patients. *Hum Immunol.* 2006;67(3):188–95.
20. Eleme K, Taner SB, Önfelt B, et al. Cell Surface Organization of Stress-inducible Proteins ULBP and MICA That Stimulate Human NK Cells and T Cells via NKG2D. *J Exp Med.* 2004;199(7):1005–10.
21. Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science (80-).* 2008;321(5897):1801–6.
22. Lee GH, An HJ, Kim TH, et al. Clinical impact of natural killer group 2d receptor expression and that of its ligand in ovarian carcinomas: A retrospective study. *Yonsei Med J.* 2021;62(4):288–97.
23. Lazarova M, Steinle A. Impairment of NKG2D-Mediated Tumor Immunity by TGF-β. *Front Immunol.* 2019;10:10–3.
24. Fujita H, Hatanaka Y, Sutoh Y, et al. Immunohistochemical Validation and Expression Profiling of NKG2D Ligands in a Wide Spectrum of Human Epithelial Neoplasms. *J Histochem Cytochem.* 2015;63(3):217–27.
25. Ruan GT, Wang S, Zhu LC, et al. Investigation and verification of the clinical significance and perspective of natural killer group 2 member D ligands in colon adenocarcinoma. *Aging (Albany NY).* 2021;13(9):12565–86.
26. Høgh RI, Droujinine A, Møller SH, et al. Fumarate Upregulates Surface Expression of ULBP2/ULBP5 by Scavenging Glutathione Antioxidant Capacity. *J Immunol.* 2020;204(7):1746–59.
27. Zuo J, Willcox CR, Mohammed F, et al. A disease-linked ULBP6 polymorphism inhibits NKG2D-mediated target cell killing by enhancing the stability of NKG2D ligand binding. *Sci Signal.* 2017;10(481):1–15.
28. Soremekun OS, Soliman MES. From genomic variation to protein aberration: Mutational analysis of single nucleotide polymorphism present in ULBP6 gene and implication in immune response. *Comput Biol Med.* 2019;11:103354. https://doi.org/10.1016/j.compbiomed.2019.103354
29. Ren L, Li Y, Zhao Q, et al. MiR-519 regulates the proliferation of breast cancer cells via targeting human antigen R. *Oncol Lett.* 2020;19(2):1567–76.
30. Tu K, Liu Z, Yao B, et al. MicroRNA-519a promotes tumor growth by targeting PTEN/PI3K/AKT signaling in hepatocellular carcinoma. *Int J Oncol.* 2016;48(3):965–74.

Research Article

Effectiveness of Participation in Pregnancy Classes to Reduce the Incidence of Obstetric Labor Complications and Cesarean Section

Efektivitas Keikutsertaan Kelas Ibu Hamil untuk Mengurangi Kejadian Komplikasi Persalinan dan Operasi Sesar

Apriska M. Sutowo Putri[1], Muhammad P. Mahindra[2], Muhammad P. Mapindra[2], Mahendra T. A. Sampurna[2], Rozi A. Aryananda[3], Fitria K. Khotima[3]

[1]Faculty of Medicine, Universitas Sebelas Maret Surakarta
[2]Department of Pediatrics
[3]Department of Obstetrics and Gynecology
Faculty of Medicine Universitas Airlangga
Surabaya

Abstract

Objective: To assess the effectiveness of participation in pregnancy classes to inform such a prenatal program for physicians and midwives, focusing to reduce the incidence of obstetric labor complications and cesarean section.

Methods: This study was done using an analytic observational approach, using a cross-sectional study and consecutive sampling. One hundred and ninety-four participants of this study were women aged 20–35 years who gave birth between January and November 2019 in balarejo sub-district, East Java, Indonesia. The subjects were divided into two groups: participation (four times) and non-participation (less than four times) in the pregnancy classes. The analyzed outcomes are labor complications (prolonged labor, postpartum hemorrhage, postpartum fever, premature rupture of membrane), and cesarean section birth.

Results: The results of this study indicate that participants of pregnancy classes have a significantly lower probability of suffering prolonged labor (OR=0.05, 95% CI=0.01-0.23, p<0.05), postpartum hemorrhage (OR=0.20, 95% CI=0.04-0.95, p<0.05), and postpartum fever (OR=0.13, 95% CI=0.16-1.08, p<0.05) compared with non-participants. Also, participating women have a lower chance of undergoing cesarean section (OR=0.08, 95% CI=0.03-0.21, p<0.05).

Conclusion: The incidence of obstetric labor complications and cesarean section can be effectively reduced by the participation of pregnant woman class.

Keywords: cesarean section, obstetric labor complications, prenatal education.

Abstrak

Tujuan: *Mengetahui efektivitas keikutsertaan kelas ibu hamil, sebuah program prenatal oleh dokter atau bidan, yang berfokus untuk mengurangi kejadian komplikasi persalinan dan operasi sesar.*

Metode: *Penelitian ini menggunakan pendekatan observasional analitik, studi potong lintang dengan konsekutif sampling. 194 subjek penelitian adalah perempuan berusia 20-35 tahun yang melahirkan antara Januari-November 2019 di wilayah kerja Puskesmas Balerejo, Jawa Timur, Indonesia. Subjek dibagi menjadi dua kelompok partisipasi (empat kali kunjungan), dan non-partisipasi (kurang dari 4 kali kunjungan) dalam kelas ibu hamil. Hasil yang dianalisis adalah komplikasi persalinan (partus memanjang, perdarahan pascasalin, demam pascasalin, ketuban pecah dini), dan persalinan dengan metode operasi sesar.*

Hasil: *Hasil penelitian ini menunjukkan bahwa partisipasi kelas ibu hamil telah menurunkan secara bermakna probabilitas kejadian partus memanjang (OR=0.05, 95% CI=0.01-0.23, p<0.05), perdarahan paska salin (OR=0.20, 95% CI=0.04-0.95, p<0.05), dan demam pascasalin (OR=0.13, 95% CI=0.16-1.08, p<0.05) dibandingkan dengan non-partisipan. Selain itu, wanita yang mengikuti kelas ibu hamil juga menurunkan kejadian persalinan melalui operasi sesar (OR=0.08, 95% CI=0.03-0.21, p<0.05).*

Kesimpulan: *Kejadian komplikasi persalinan dan persalinan dengan operasi sesar secara efektif dapat dikurangi dengan keikutsertaan program kelas ibu hamil.*

Kata kunci: *kelas ibu hamil, komplikasi persalinan, operasi sesar.*

Correspondence author. Mahendra T.A. Sampurna. Department of Pediatrics
Faculty of Medicine Universitas Airlangga. Surabaya. Email; mahendra.tri@fk.unair.ac.id

Received: March,2021 Accepted: September,2022 Published: October,2022

INTRODUCTION

The maternal mortality rate (MMR) in Indonesia remains high, occurs around 305 per 100,000 live births. This figure is much higher than the MDG's target. The MDG's target in 2015 was to reduce the MMR number to 102 per 100,000 live births.[1] East Java Province in 2017 showed that the incidence of MMR had reached 91.92, an increase on the previous year that saw 91 per 100,000 live births. Maternal deaths occur mostly in developing countries. Dominated by low-income countries, South-Eastern Asia indicated that there were 16.000 mothers death, which comprised a point estimate of 137 in MMR.[2]. The most common causes are bleeding, eclampsia, sepsis, abortion, and labor obstruction.[3] As a part of East Java, the Madiun Regency had been reported with an MMR at around 65.80.[4] in East Java, the two most common causes of MMR are pre-eclampsia/ eclampsia (28.9%) and postpartum hemorrhage (28.28%).

The maternal mortality rate has not been significantly reduced, it has actually increased every year. One of the government programs to reduce the MMR that has been running since 2010 is the class for pregnant women. These pregnancy classes are about women learning about pregnancy health together, in the form of face-to-face groups. This program aims to increase knowledge5 and skills regarding pregnancy, prenatal care, childbirth, postnatal care, newborn care, myths, infectious diseases, and compliance in routine antenatal care visits, and the maximum number of participants per class is ten pregnant women of gestational ages between 20 and 32 weeks.[5,6] The classes are facilitated by midwives or health workers who have trained as class facilitators for pregnant women. The program comprises four meetings with a different material in each at the beginning of each meeting there is a pretest, and at the end of each meeting there is a posttest, according to the material provided and pregnancy exercises.

Prenatal education provides training on adaptations in pregnancy, preparation for childbirth, preparation for potentially dangerous situations, postpartum contraception, and breastfeeding.[7] In Sweden, participants of pregnancy classes feel safer and more ready for the delivery process.[8] Prenatal education is expected to identify disease history and various risk factors for pregnancy and labor complications.[9] Labor complications are circumstances that deviate from normal conditions, which can cause morbidity and death of the mother and baby as a direct result of childbirth. According to the Indonesian Demographic Health Survey 2007, the types of labor complications are prolonged labor (37%), bleeding (9%), fever (7%), seizures (2%), and other complications (4%).[10] To address this problem, a study in Indonesia stated that there was a significant relationship between pregnancy classes and labor complications.[11]

Pregnancy classes aim to increase the readiness of pregnant women for the delivery process. Previous studies have reported that women who participate in prenatal education can reduce and control their fear, anxiety, and stress during childbirth.[7,12] The fear felt by a pregnant woman, if not controlled properly, can prolong the labor process and increase the incidence of cesarean section. A mother who participated in prenatal education reported having a higher readiness for labor, so she had a lower risk of cesarean section delivery.[7] Research conducted to evaluate childbirth education classes showed that there were benefits to pregnancy classes, to reduce the incidence of delivery via cesarean section by 10% and therefore have a significant effect on vaginal delivery (non-section).[13]

The problem of maternal mortality, including complications in childbirth and the increasing incidence of deliveries via cesarean section, has prompted the authors of this study to conduct studies that assess the effectiveness of pregnancy class participation in reducing labor complications and cesarean sections.

METHODS

An analytic observational cross-sectional study with 194 respondents was carried out, using the consecutive sampling technique. The inclusion criteria were women aged 20–35 years who gave birth between January and November 2019 in Balarejo-sub district, East Java, Indonesia. The exclusion criteria were women who had a history of cesarean delivery and those who had cephalopelvic disproportion. The subjects were divided into two groups: participation (four times) and non-participation (less than four times) in pregnancy classes. The dependent variables included complications in labor and delivery through cesarean section. The operational definition of pregnancy class participation (prenatal education) was participation in a pregnancy class at least four

times, whereas the operational definition of labor complications was a situation that deviates from normal conditions, which can cause morbidity and mortality of mothers and babies as a direct result of childbirth. In this study, the four main complications of labor were prolonged labor, postpartum hemorrhage, postpartum fever, and premature rupture of membranes. The chi-square test was used to compare both groups. Odds ratios were calculated for each delivery outcome with a 95% confidence interval (CI).

This study has been approved by Balerejo Primary Health Care (No. 445/ 322/ 402.102.05/ 2020) that covers the waiver of consent and the statements of minimum risk of study to the subjects. The data of all respondents' who agreed to participate in this study was concealed and thus only used for study purposes.

RESULTS

Table 1. Characteristics of Participating Mothers

	Amount	%
Age (in years)		
20–24	51	26.3
25–29	95	48.9
30–35	48	24.8
Education Qualification		
No degree	59	30.4
Diploma	80	41.2
Graduate	55	28.4
Financial Status		
Low–middle income	116	59.8
Middle–high income	78	40.2
Parity		
Nulliparous	104	53.6
Primiparous	63	32.5
Multiparous	27	14.0
Delivery Method		
Cesarean section	47	24.2
Vaginal birth	147	75.8
Prenatal education		
Participating	98	50.5
Not participating	96	49.5

Table 1. Distribution of Delivery Complications in the Case Group and the Control Group

Delivery Outcomes	Pregnancy Class			
	Participant (n=98) 50.5 (%)	**Non Participant (n=96) (49.5) %**	**OR (95% CI)**	***P-value***
Obstetric Labor Complication				
Prolonged labor	2 (6.9)	27 (93.1)	0.05 (0.01-0.23)	0.032
Postpartum hemorrhage	2 (18.2)	9 (81.8)	0.20 (0.04-0.95)	0.034
Postpartum fever	1 (12.5)	7 (87.5)	0.13 (016-1.08)	0.116
Premature rupture of membrane	1 (16.7)	5 (83.3)	0.18 (0.02-1.63)	
Delivery Method				0.000
Cesarean section	6 (12.8)	41 (87.2)	0.08 (0.03-0.21)	0.000
Vaginal birth	92 (62.6)	55 (37.4)	11.43 (4.55-28.66)	

This study selected a total of 194 participants who were eligible for analyses. Table 1 shows the characteristics of mothers. The participants were mostly composed of women aged 25–29 years old, which made up 48.9% of the total, while 53.6% had no prior history of giving birth (nulliparous). Regarding socioeconomic status, the participants were predominantly women who had a diploma qualification and were in the middle- or lower-income range, comprising 41.2% and 59.8%, respectively. Just over half of the women (50.5%) attended the educational pregnancy course. Furthermore, roughly three quarters (75.8%) of the total proportion gave birth spontaneously, the remainder giving birth via the mode of cesarean section and comprising almost one quarter. In this study, prolonged labor accounted for most of the complicated labor cases leading to cesarean section, accounting for more than half of the total number of women who gave birth via this method. The other common labor complications were postpartum hemorrhage, postpartum fever, and premature rupture of the membrane, comprising no more than one-third of the total proportion. Furthermore, statistical analysis was used to determine the odds ratio (OR) for included women with labor complications.

The evidence of statistical results showing the delivery outcome probability in women who participated in pregnancy classes compared to non-participants can be seen in Table 2, which includes the OR and 95% CI. In this study, women who attended the pregnancy classes program had a significantly lower chance of experiencing prolonged labor [(OR=0.05, 95% CI=0.01-0.23) $p<0.05$]. Postpartum hemorrhage probability was also demonstrated to be significantly lower in the participant group [(OR=0.20 95% CI=0.04-

0.95) p<0.05]. Also, mothers who signed up for the program had a significantly lower chance of developing a fever after delivery [(OR=0.13, 95%CI=0.16-1.08) p< 0.05]. Conversely, premature rupture of the membrane was reported to be not significant, although the odds were lower for women who participated in the classes [(OR=0.18, 95%CI=0.02-1.63) p>0.05]. Following labor complications, participants of the pregnancy classes were not only reported to have a lower probability of delivering their babies abdominally compared to non-participants [(OR=0.08, 95%CI=0.03-021) p<0.05], they also had a higher chance of being given the option of a vaginal delivery [(OR=11.43, 95%CI=4.55-28.66) p<0.05]. Overall, the study results show that there was a significant difference between participants and non-participants in terms of labor complications and cesarean section.

DISCUSSION

This study found that participant women had significantly lower odds of experiencing prolonged labor, postpartum hemorrhage, and postpartum fever than those in the non-participant group. With regards to delivery method, women in the participant group had significantly lower odds of delivering their babies abdominally. A comparative study on pregnant women in Yogyakarta and the rural area of Semarang described how prenatal education can expand the knowledge of pregnant women about the obstetric labor complications and delivery method, according to the evaluation of pretests and posttests completed after classes.[14,15] Lack of support and knowledge are considered essential factors that can decrease the eagerness of pregnant women to participate in such classes.[9] Other factors, including the provision of visual media in prenatal education knowledge transfer, might attenuate the process of information translation.[16]

Our study implied the antenatal education plays a significant role to reduce the odds of various labor complications, namely prolonged labor, postpartum hemorrhage, and postpartum fever. A study regarding pregnancy classes in Indonesia reported the same significance by demonstrating case-control studies that resulted in the reduction of labor complications via prenatal education.[11] Another similar study conducted in Central Java also indicated this result, describing the multiparity status and lack of education of pregnant women as causative factors that impact labor complications.[17] The effectiveness of labor preparation class, a training program consisting of how to do various techniques stretching exercise, relaxation, breathing patterns, and massage during labor, found that the class can enhance the positive attitudes and vitality toward normal delivery in the participant group.[18] A study on women's experience revealed that certain activities such as efficient education absorbed by pregnant women to understand more detailed information on vaginal delivery and prenatal yoga exercise can benefit them in their physique readiness and breathing control during the labor process.[19] These learned abilities are pretty rewarding to spare the energy needed for the delivery process and therefore prevent the arrests of labor.

A similar program to pregnancy classes is defined differently in many countries worldwide. Regarding the delivery method preferred for women, our study resulted in the decreased odds for cesarean section and otherwise increased the odds to deliver babies via vaginal delivery. Our study is in line with a few previous studies in which results demonstrated the significant impacts of prenatal education on the probability of vaginal birth and assisted birth, but did not cause the rates of cesarean birth to increase.[20-22] However, a study in British Columbia reported that women who attended childbirth education classes had a lower rate for cesarean surgery, whether it was arranged by the obstetrician or due to patients' requests.[23] Nonetheless, there was another study involving Italian women given prenatal education. This study showed a higher incidence of cesarean delivery in non-participants compared to participants, although proving that prenatal education can reduce the risk of cesarean section. Nevertheless, the study was considered weak because the definition of prenatal education was not correctly standardized.[12] Evaluated the psychologic effect, prenatal education is proven to be effective in reducing the fear of delivery in primiparous women.[24] Meanwhile, there was a study that stated such pregnancy classes to be not beneficial in reducing cesarean section incidence. In contrast with our findings, a retrospective study involving one term of childbirth education classes to manage the risk of labor difficulty reported that the classes were significantly effective in lowering the risk of vacuum extraction, but did not have the same effect on reducing the cesarean rate.21 A study evaluating prenatal education stated

that the program could not be carried out to maximize the benefits because there would be many considerations, such as the cultural and community background of the women and the healthcare providers' method of encouraging pregnant women to join.[25]

Therefore, pregnancy classes conducted according to the guidelines is aiming for the detection, prevention, and elimination of abnormalities in pregnancy, which can be complicating factors during the delivery process. The implementation of the program has its weaknesses the role of the Health Service has not been optimal in encouraging the implementation of this program, including a lack of operational support facilities and intensive facilitator training, which can be burdensome for primary healthcare (PHC) services.[5,26]

CONCLUSIONS

Pregnancy class participants can lower the probability of cesarean section delivery and fewer labor complications, such as prolonged labor, postpartum hemorrhage, and postpartum fever. Hence, pregnancy classes are really advised to be obtained by pregnant women to prepare vaginal birth. However, due to the lack of similar studies reporting on prenatal education programs, this study can be used as an evaluation of the effectiveness of such programs and as one of the beneficial programs related to women's health in primary healthcare settings. However, confounding factors such as economic class and degree of education are thought to affect the ongoing program. Thus, a further study evaluating these confounding factors should be carried out to clarify the efficacy of pregnancy classes in managing pregnant women.

REFERENCES

1. United Nations. Resolution adopted by the General Assembly, 55/2. United Nations Millennium Declaration. http://www.un.org/millennium/declaration/ares552e.pdf.
2. World Health Organization. Trends in Maternal Mortality 2000 to 2017. Estimates by EHO, UNICEF, UNFPA, World Bank Group, and the United Nations Population Division 2019. https://apps.who.int/iris/bitstream/handle/10665/327596/WHO-RHR-19.23-eng.pdf?sequence=13&isAllowed=y
3. Dinas Kesehatan Provinsi Jawa Timur. Profil Kesehatan Provinsi Jawa Timur 2018. Kementerian Kesehatan Republik Indonesia. 2018.
4. Das S, Munshi A. Research advances in Apert syndrome. J Oral Biol Craniofacial Res . 2018;8(3):194–9.http://dx.doi.org/10.1016/j.jobcr.2017.05.006
5. Kementerian Kesehatan Republik Indonesia. Pedoman Pelaksanaan Kelas Ibu Hamil.pdf. In: Pedoman Pelaksanaan Kelas Ibu Hamil. Jakarta. 2014: 1–16.
6. Runjati, Susanto H, Sawitri DR, Thaufik S. The Effect of Antenatal Class Plus Coping Skill Training on the Level of Stress and Childbirth Self-Efficacy. Adv Sci Lett. 2017;23(4):3329–33.
7. Seyhan Ç, Bülent Ş. Effects of Antenatal Education on Fear of Birth, Depression, Anxiety, Childbirth Self-Efficacy, and Mode of Delivery in Primiparous Pregnant Women: A Prospective Randomized Controlled Study. Clin Nurs Res. 2020
8. Ahldén I. Parents ' Expectations About Participating in Antenatal Parenthood Education Classes.2017. :11–7.
9. Sutopo P, Ayun S. Determinants of Mothers' Participation in Antenatal Classes. J Adm Kes Indones. 2019;7(2).
10. National Population and Family Planning Board (BKKBN), Statistics Indonesia (BPS), Ministry of Health (Kemenkes) and II. Indonesia 2017 DHS. In: Indonesia Demographic Health Survey 2017. 2017th ed. Jakarta. 2017: 588.
11. Faridah H, Ni Nyoman M, Endah W. Hubungan Usia, Paritas, dan Kelas Ibu Hamil dengan Komplikasi Persalinan di RSKB Sayang Ibu Balikpapan. Mahakam Midwifery J. 2019;2(5):361–74.
12. Spinelli A, Baglio G, Donati S, et al,. Do antenatal classes benefit the mother and her baby ? J Matern Neonatal Med. 2018;13:94–101.
13. Cantone D, Lombardi A, Assunto DA, et al. A standardized antenatal class reduces the rate of cesarean section in southern Italy. 2018:1–5.
14. Arulita Ika F, Muhammad A. Model kelas ibu hamil untuk pemetaan risiko kehamilan dan pencegahan komplikasi persalinan. Abdimas. 2016;20(1):11–8.
15. Setyaningsih D, Zakiyah Z. The Implementation of Antenatal Class to Improve Mother's Knowledge of Pregnancy. 2019;(3):759–64.
16. Widiar RP, Deliana SM, Uniska AK. The Effectiveness of Pregnancy Training Class towards the Childbirth Preparation in Kendal Regency. 2018;3(99).
17. Maryani S, Respati SH, Astirin OP. Association between Pregnant Woman Class and Pregnancy Complication in Tegal District . Central Java. 2016;1:214–9.
18. Parvin B, Fariba A, Hamidreza O, Mahboubeh V. The effect of participating in the labor preparation classes on maternal vitality and positive affect during the pregnancy and after the labor. Iran J Nurs Midwifery Res. 2010;15(Suppl 1):331–6. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3208940/
19. Harahap D, Afiyanti Y. Women' s experiences of the prenatal group class in Indonesia . 2019:29.
20. Afshar Y, Wang ET, Mei J, et al. Childbirth Education Class and Birth Plans Are Associated with a Vaginal Delivery. 2016:1–6.
21. Gluck O, Zvia TP, Hanny H, Bar J, Kovo M. The impact of childbirth education classes on delivery outcome. 2020:1–5.
22. Soriano-Vidal F, Vila-Candel R, Soriano-Martín P,et al,. The effect of prenatal education classes on the birth expectations of Spanish women. Midwifery. 2018; https://doi.org/10.1016/j.midw.2018.02.002

23. Stoll KH, Hall W. Childbirth Education and Obstetric Interventions Among Low-Risk Canadian Women. Is There a Connection 2019 :229–37.>>> thn?
24. Kacperczyk-bartnik J, Symonides A, Sroka-ostrowska N, et al,. Association between antenatal classes attendance and perceived fear and pain during labour. Taiwanese J Obstet Gynecol 2019;58:492–6.
25. Nurdiyan A, Machmud R. Artikel Penelitian Analisis Sistem Pelaksanaan Kelas Ibu Hamil di Puskesmas Malalak dan Biaro Kabupaten Agam2018. 4(1):1–9
26. Fuada N, Setyawati B. Pelaksanaan kelas ibu hamil di indonesia. 2015::67–75.

Research Article

Sexual Function in Female of Reproductive Age Posttotal Hysterectomy

Fungsi Seksual pada Perempuan Usia Reproduksi Pascahisterektomi Totalis

Yonathan Onibala, Bismarck J. Laihad, Rudy A. Lengkong

Department of Obstetric and Gynecology
Faculty Of Medicine Universitas Sam Ratulangi
Prof. Dr. R D Kandou General Hospital
Manado

Abstract

Objective: To determine the effect of sexual function on women of reproductive age after a total hysterectomy.

Method: This study is a descriptive retrospective study with a cross-sectional study design.

Results: Based on the data of 23 samples that met the inclusion criteria. With 11 samples increasing FSFI score, 11 samples with a decreasing of FSFI score and 1 sample had an unchanged sexual function before and after surgery. Then from those 23 samples, 16 samples with Female Sexual Dysfunction (FSD) have FSFI score of ≤ 26.55 before surgery, and 17 samples with FSD after surgery. Based on the T-Test, there were significant differences between the Female Sexual Function Index (FSFI) before and after the surgery. Most of the respondents experienced decreasing sexual satisfaction (14 samples), while only five samples experienced increased sexual satisfaction and the other four samples had unchanged sexual satisfaction. In our study, most of the respondents (17 samples) claimed that there were better or decreased pain after the hysterectomy procedure. While there were only three samples that claimed an increase of pain, and only three samples claimed that there was unchanged pain scale.

Conclusion: There were changes in sexual function before and after hysterectomy as measured by Female Sexual Function Index (FSFI).

Keywords: after hysterectomy, before hysterectomy, fsfi, sexual function.

Abstak

Tujuan: *Untuk mengetahui pengaruh fungsi seksual wanita usia reproduksi pasca histerektomi totalis.*

Metode: *Penelitian ini merupakan penelitian deskriptif retrospektif dengan menggunakan rancangan potong lintang.*

Hasil: *Berdasarkan data yang telah diambil sebanyak 23 sampel yang memenuhi kriteria inklusi. Dengan total 11 orang mengalami peningkatan skor FSFI, 11 orang mengalami penurunan skor FSFI dan 1 orang tidak terdapat perubahan. Kemudian dari total 23 sampel, terdapat 16 sampel yang memiliki Female Sexual Dysfunction (FSD) yaitu mendapatkan skor FSFI ≤ 26,55 sebelum operasi, dan 17 sampel yang memiliki FSD sesudah operasi. Berdasarkan Uji T, menunjukkan perbedaan yang bermakna antara skor Female Sexual Function Index (FSFI) pada saat sebelum operasi dan sesudah operasi. Sebagian besar responden merasakan penurunan kepuasan seksual (14 sampel), sedangkan yang merasakan peningkatan kepuasan seksual hanya 5 responden dan sisanya tidak merasakan adanya perubahan yaitu 4 responden. Dalam penelitian ini sebagian besar responden mengaku terjadi perbaikan atau penurunan rasa nyeri setelah operasi histerektomi yaitu sebanyak 17 responden. Sedangkan hanya 3 responden yang mengaku terjadi peningkatan rasa nyeri dan hanya 3 responden yang mengaku tidak terdapat perubahan.*

Kesimpulan: *Terdapat perubahan fungsi seksual sebelum dan sesudah histerektomi yang diukur berdasarkan Female Sexual Function Index (FSFI).*

Kata kunci: *FSFI, fungsi seksual, sebelum histerektomi, sesudah histerektomi.*

Correspondence author. Yonathan Onibala. Department of Obstetrics and Gynecology
Faculty of Medicine Universitas Sam Ratulangi. Prof.Dr. R.D. Kandou General Hospital
Manado

Received: February,2021 Accepted: September,2022 Published: October,2022

INTRODUCTION

Sexuality is an essential part of most women life. Although it is essential, most of them find it challenging to share their sexual problems with their doctors, and many doctors also inconvenient to discuss sexual problems with their patients.[1] Hysterectomy is one of the most commonly performed surgical procedure. In the United States, hysterectomy is the second most common major surgical procedure after section Caesarea.1 About 600,000 women underwent hysterectomy annually in the United States, and this number remains constant.[2] Incidence of hysterectomy performed mostly in women aged 40 to 49-year-old with the mean age of 46,1 years old.[1]

The impact of hysterectomy towards sexual function is still uncertain. Many women reported an improvement of sexual function after hysterectomy, which may be because of the relieving symptoms, while others complained about sexual dysfunction after hysterectomy. Female Sexual Function Index is a multidimensional instrument that consists of a validated self-report questionnaire and reliable in calculating the sexual function in women.[3-6]

Sexuality is an essential factor in strengthening a marriage, so our study aims to determine the effect of sexual function on women of reproductive age who have undergone a total hysterectomy in Prof. dr. R. D. Kandou Manado Provincial General Hospital.

METHODS

This study is a descriptive retrospective study with a cross-sectional study design. This study's target population are women who have undergone total hysterectomy with/or without unilateral salpingo-oophorectomy, whether by transabdominal or transvaginal approach without vaginal reconstructive surgery.

Inclusion criteria, women with active sexual life, women in their 30s to 50s, and were married for minimal two years before the surgery. Live at Manado or its surrounding area, with full address and reachable through phone call or home visit. Underwent total hysterectomy for ≥ three months before the study started. No sexual diversion, lived with their husbands, and could do sexual intercourse at least six months before the study. Willing to participate in this study by signing the informed consent. Exclusion criteria: Was in the medical treatment of any other disease. Total hysterectomy by transabdominal approach with bilateral salpingo-oophorectomy. Total hysterectomy by transvaginal approach with vaginal reconstruction. Radical hysterectomy. Have other comorbidities such as diabetes mellitus or other degenerative diseases.

Women who underwent total hysterectomy with/or without unilateral salpingo-oophorectomy, transabdominal or transvaginal approach with vaginal reconstructive surgery, and have met the inclusion and exclusion criteria. The samples were then picked by using the consecutive sampling methods with minimal 21 samples.

RESULTS

Table 1. Changes of FSFI after a Total Hysterectomy Procedure

Changes of FSFI after the Surgery	Total	%
Increased	11	47.83
Decreased	11	47.83
No change	1	4.34

Table 2. The T-Test (dependent variable/paired T-test)

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Group-FSFI	-22.46739	5.18198	76404	-24.00625	-20.92853	-29.406	45	.000

Thus, it can be concluded that total hysterectomy is closely related to female sexual function .

Based on the data of 23 samples that met the inclusion criteria. With 11 samples increasing FSFI score, 11 samples with a decreasing of FSFI score and 1 sample had an unchanged sexual function before and after surgery. Then from those 23 samples, 16 samples with Female Sexual Dysfunction (FSD) have FSFI score of ≤ 26.55 before surgery, and 17 samples with FSD after surgery. Based on the T-Test, there were significant differences between the Female Sexual Function Index (FSFI) before and after the surgery. Most of the respondents experienced decreasing sexual satisfaction (14 samples), while only five samples experienced increased sexual satisfaction and the other four samples had unchanged sexual satisfaction. In our study, most of the respondents (17 samples) claimed that there were better or decreased pain after the hysterectomy procedure. While there were only three samples that claimed an increase of pain, and only three samples claimed that there was unchanged pain scale.

DISCUSSION

We have collected 23 samples that have to meet the inclusion criteria of our study. With a total of 11 people experiencing an increase of their FSFI score, 11 people experiencing a decrease in their FSFI score and one person with no change in FSFI score as can be seen in table.1.

There were 11 samples (47.83%) with elevated FSFI after the hysterectomy procedure in our study. These findings were similar to a study by Schiff L et al. and other studies. Besides, there were 11 samples (47.83%) with decreased FSFI after the hysterectomy procedure. These findings show that sexual function could be affected by various physiological factors and psychological factors of each woman. Whereas only one sample had an unchanged sexual function before and after surgery.[3,7] Based on the T-Test, there were significant differences between the Female Sexual Function Index (FSFI) before and after the surgery.

CONCLUSION

There was the transformation of sexual function before and after hysterectomy calculated by the Female Sexual Function Index (FSFI). The transformation could be increased or decreased of the sexual function and could be affected by various factors.

T-Test's data analysis showed significant differences between FSFI scores before and after the surgery.

Various factors influenced female sexual functions, so it is essential to do counselling with patients and their husband before the hysterectomy procedure. Further research with different methods and more samples were needed for comparison to our study.

REFERENCES

1. Berek J. et al. Sexuality and Sexual Dysfunction. Berek & Novak's Gynecology. 15th edition. Wolter Kluwer Health: Lippincott Williams & Wilkins. 2012. p. 409-430.
2. Cohern S, Hollingsworth A, Rubin M, Graff B, Thomas J, Wehry L. Psychosocial adaption during recovery from hysterectomy. Webmed Central obstetrics and Gynecology. 2011;2(3):1-8.
3. Briedite I, Ancana G, Rogovska I, Lietuviete N. Quality of Female Sexual Function after Conventional Abdominal Hysterectomy – Three Months Observation. 2014. 26-31.
4. Wulandari, Rachman, Nisam. Seksualitas Pada Wanita Setelah Total Abdominal Histerektomi. Journal of Nursing Care and Biomolekular. Vol 1.38. 2016.
5. Illiano, Ester, et al. Hysterectomy and Sexuality. Tersedia http://www.avidscience.com/wp-content/uploads/2017/01/HYST-16-02.pdf
6. Wiegel M, Meston C, Rosen R. The Female Sexual Function Index (FSFI): Cross-Validation and Development of Clinical Cuttoff Scores. Journal of Sex & Marital Therapy 2015; p. 1-20.
7. Tatuncu B, Yildiz H. The Influence on Women's Sexual Function of Education According to the PLISSIT Model after Hysterectomy. Procedia-Social and Behavioral Sciences. 2012; 47(2012): 2002-2004

Case Report

Successful Pregnancy after hCG/hMG Treatment in an Azoospermic Male with Idiopathic Hypogonadotropic Hypogonadism

Keberhasilan Kehamilan setelah Pengobatan hCG/hMG pada Pria Azoospermia dengan Hipogonadisme Hipogonadotropik Idiopatik

Cheyon Park, Gyun-Ho Jeon

Department of Obstetrics and Gynecology
University of Inje College of Medicine
Haeundae Paik Hospital
Busan, South Korea

Abstract

Objective: To report a rare case of idiopathic hypogonadotropic hypogonadism (IHH), one of the correctable causes of male infertility, with previous literature reviews.

Methods: A case report with previous literature reviews.

Case: A 29-year-old male was referred due to primary infertility with azoospermia. Laboratory investigations revealed low serum gonadotrophin levels and testosterone with normal appearance of pituitary gland magnetic resonance imaging. 9 months of human chorionic gonadotropin (hCG) treatment was followed by human menopausal gonadotropin (hMG) treatment under the suspected diagnosis of IHH. Spermatozoa were firstly detected after 10 months from the initial treatment and sperm concentration rose in 14 x106/ml after 18 months treatment. After succeeding in twin pregnancy with IVF-ET procedure and fullterm live birth, his hCG/hMG treatment was changed into testosterone replacement therapy because they did not want an additional pregnancy with maintaining an approlelakite male hormone level.

Conclusion: This case report shows that a thorough and careful examination of whether it is a reversible cause is necessary and important in the approach to male infertility. In addition, it further proves that, in the case of IHH, a continuous long-term gonadotrophic stimulation therapy contributes to successful pregnancy and may need a testosterone replacement therapy after childbirth.

Keywords: azoospermia, gonadotropin, human chorionic gonadotropin, hypogonadotrophic hypogonadism, infertility.

Abstrak

Tujuan: *Untuk melaporkan kasus langka hipogonadisme hipogonadotropik idiopatik (IHH), salah satu penyebab infertilitas laki-laki yang dapat diperbaiki, dengan tinjauan literatur sebelumnya.*

Metode: *Sebuah laporan kasus dengan tinjauan literatur sebelumnya.*

Kasus *Seorang laki-laki berusia 29 tahun dirujuk karena infertilitas primer dengan azoospermia. Pemeriksaan laboratorium menunjukkan kadar gonadotropin serum yang rendah dan testosteron dengan gambaran normal dari pencitraan resonansi magnetik kelenjar pituitari. 9 bulan pengobatan human chorionic gonadotropin (hCG) diikuti dengan pengobatan human chorionic gonadotropin (hMG) di bawah dugaan diagnosis IHH. Spermatozoa pertama kali terdeteksi setelah 10 bulan dari pengobatan awal dan konsentrasi sperma meningkat pada 14 x106/ ml setelah 18 bulan pengobatan. Setelah berhasil hamil kembar dengan prosedur IVF-ET dan kelahiran hidup cukup bulan, pengobatan hCG/hMG-nya diubah menjadi terapi penggantian testosteron karena mereka tidak menginginkan kehamilan tambahan dengan mempertahankan kadar hormon laki-laki yang sesuai.*

Kesimpulan: *Laporan kasus ini menunjukkan bahwa pemeriksaan menyeluruh dan hati-hati apakah penyebab reversibel diperlukan dan penting dalam pendekatan infertilitas laki-laki. Selain itu, lebih lanjut membuktikan bahwa, dalam kasus IHH, terapi stimulasi gonadotropik jangka panjang yang berkelanjutan berkontribusi pada keberhasilan kehamilan dan mungkin memerlukan terapi penggantian testosteron setelah melahirkan.*

Kata kunci: *azoospermia, gonadotropin, human chorionic gonadotropin, hipogonadotropik hipogonadisme, infertilitas.*

Correspondence author. Gyun H. Jeon. Department of Obstetrics and Gynecology
University of Inje College of Medicine. Haeundae Paik Hospital. Busan, South Korea
Emai; jeon285@@hotmail.com

Received: July,2021 Accepted: September,2022 Published: October,2022

INTRODUCTION

Idiopathic hypogonadotropic hypogonadism (IHH) is rare and its incidence is known as 1-10 cases per 100,000 births.[1,2] IHH is caused by deficient production, secretion or action of gonadotropin-releasing hormone (GnRH) and can be diagnosed through the presence of low testosterone and low gonadotropin levels without any structural or functional abnormalities in the hypothalamic-pituitary-gonadal axis.[3] Gonadotropin replacement is known as the treatment of choice to induce spermatogenesis in a male with hypogonadotropic hypogonadism.[2]

Most IHH patients go through the state of azoospermia if not treated with gonadotropin stimulation and testosterone therapy. Exogenous gonadotropin therapy can be a practical tool for desired clinical pregnancy of many infertile couples as it was demonstrated that the presence of case where progressively motile and normally formed sperm produced was observed in patients who received the therapy. Exogenous gonadotropin therapy includes follicle stimulating hormone (FSH)/ luteinizing hormone (LH) preparations that can be used for the treatment of idiopathic spermatogenic failure. Since human chorionic gonadotropin (hCG) has the biologic activity of LH but a longer half-life in the circulation; it stimulates the Leydig cells of the testes to synthesize and secrete testosterone, hCG is used to replace LH in men who have secondary hypogonadism and desire to become fertile. Testosterone plays a role in helping the Sertoli cells, which line the seminiferous tubule and contain androgen receptors, to produce spermatozoa.[4]

Hypogonadotropic hypogonadism (HH) leads to not only infertility but also to a significantly decreased quality of life and problems such as reduced muscle mass, energy, libido, facial and pubic hair, small genitalia, failure of voice to deepen, etc. Furthermore, the patients might also develop low self-esteem, depression and osteoporosis later in life because of low testosterone level in their bodies. Testosterone replacement can be applied to improve these problems in case who do not wants the pregnancy.[5]

To the best of our knowledge, there are no established guidelines of therapeutic method for IHH yet, and no information on reversal rate of IHH after hormone replacement therapy although there were a few reports on hormonal restoration of IHH.[3,6] Here, we present a rare case of IHH who had a successful experience of pregnancy after hCG/hMG treatment with previous literature reviews.

CASE

A 29-year-old male was referred due to primary infertility, with no known comorbidity. He was married two years ago and had been started to try conception without contraception for 2 years. He had no previous medical and surgical history and did not have any familial genetic abnormality history. Also, he did not notice anything abnormal with his sexual function except that watery semen was found. He had no history of headache, deficiency in smell, visual problems, and trauma.

On physical examination, his blood pressure was normotensive and his height and weight were 174 cm and 82.2 kg. His Tanner scale of external genitalia and pubic hair were all 2. The testicular volume was small, about 5ml in size. He had shown no sign of gynecomastia and other abnormalities were not detected on physical examination.

The initial laboratory finding showed low levels of LH, FSH and testosterone as 0.6 mIU/mL, 0.6 mIU/mL and 1.17 pg/mL, respectively. The levels of prolactin, thyroid stimulating hormone (TSH) and free thyroxine 4 (fT4) were normal (Table1). The semen analysis test could not be performed due to the patient's failure in ejaculation. Chromosome analysis revealed a normal male karyotype (46, XY). The enhanced pituitary gland magnetic resonance imaging (MRI) scan revealed no abnormal growths or abnormalities in pituitary, hypothalamus and suprasellar areas.

Table 1. Initial Laboratory Investigation of the Patient

	P-value	**normal range**
LH(mIU/mL)	0.6	1.2-7.8
FSH(mIU/mL)	0.6	1.5-15.4
Free Testosterone(pg/mL)	1.17	8.90-42.50
Testosterone(ng/mL)	0.058	2.49-8.36
DHEAS(ug/dL)	117.6	160-449
Prolactin(ng/dL)	7.54	2.0-18
TSH(uIU/mL)	1.08	0.4-4.2
free T4(ng/dL)	1.11	0.89-1.76
ACTH(pg/mL)	73.0	7.2-63.3
Cortisol(ug/dL)	9.5	4.82-11.9

A gonadotrophin treatment was started with hCG (5,000 IU, IM) alone, three times a week for 9 months. Along with hCG treatment, serum testosterone level was measured monthly. For semen analysis, a computer-assisted semen analysis (CASA) was also executed monthly since the beginning of treatment. The patient's serum testosterone level increased to normal range along with his subjective sexual function and also his sexual contentment increased during the hCG treatment. Semen could be collected after one month of hCG treatment, however, azoospermia was sustained. Therefore, human menopausal gonadotropin (hMG) therapy, (75IU, SC, three times a week) was added after 9 months of initial hCG treatment, with reference to ASRM guidelines.[7]

Two months after the initiation of hCG hormonal therapy, patient's serum testosterone level increased to the normal range of 6.03 pg/mL (Figure. 1). Thus, we continued the hCG therapy with same dose of 5,000 IU. Although serum testosterone level was maintained in normal range for over 6 months, azoospermia was still sustained (Figure. 1~3a) and then hMG injection was added to the hCG therapy. From 2 months after additional hMG therapy, sperm was recognizable for the first time in the patient's semen (Figure. 3b), and 4 months later, more sperms with moderate motility were spotted (Figure. 3c). Semen analysis was performed serially at an interval of one or two months. As a result, we could observe an increasing in sperm count of 14.0 million/ml and its motility of 30.8% at 9 months after additional hMG therapy. (Figure. 2).

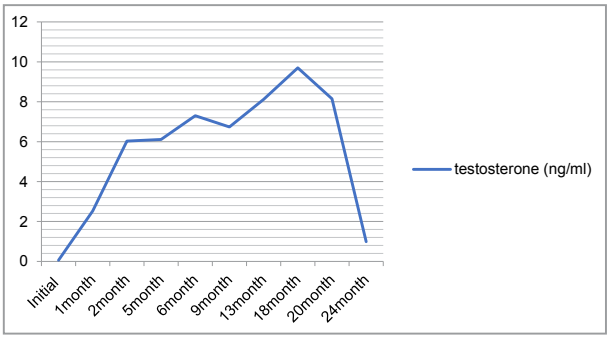

Figure 1. Change of Serum Testosterone Levels during hCG/hMG Treatment

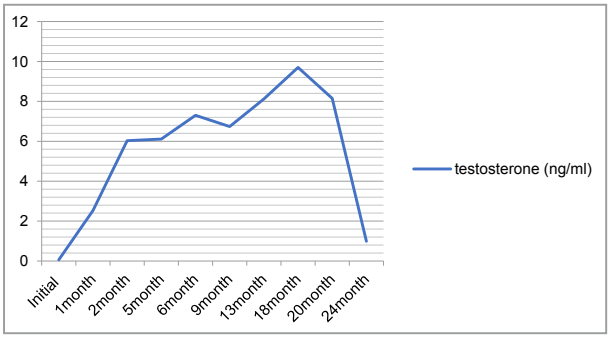

Figure 2. Serial Results of Computer Assisted Semen Analysis

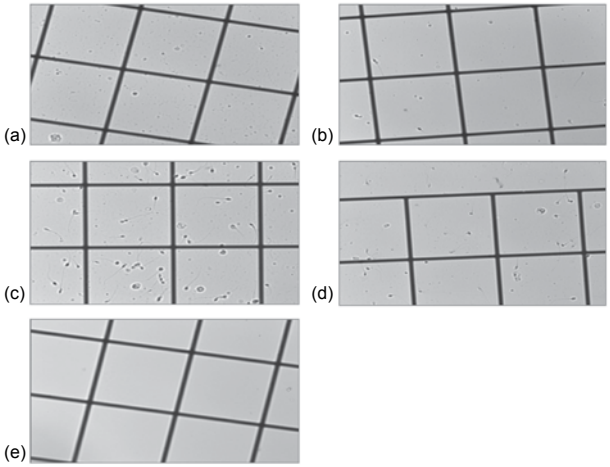

Figure 3. Gross findings of computer assisted semen analysis. (a) Initial analysis, (b) 11 months from initial treatment (; 2 months after hCG/hMG treatment), (c)15 months from initial treatment (; 6 months after hCG/hMG treatment), (d) 1 month after discontinuation of hCG/hMG treatment, (e) 3 month after discontinuation of hCG/hMG treatment

Because his wife had bilateral tubal obstruction at the hysterosalpingography, they underwent an IVF-ET procedure after 9 months of the hCG/hMG therapy. And then they succeeded in twin pregnancy and full term delivery. Immediately after successful pregnancy, the husband wanted to discontinue gonadotrophin treatment for burden of self-injection and no further pregnancy plan. In follow-up tests three weeks after the discontinuation of hCG/hMG therapy, the serum free testosterone level decreased dramatically as 1.40 pg/mL. 3 months later from discontinuation of hCG/hMG therapy, semen analysis revealed decrease in sperm counts and azoospermia again (Figure. 3 d,e). Patient started testosterone replacement therapy with testosterone undecanoate 80mg, daily after 6 months from discontinuation of hMG/hCG therapy. From initiation of testosterone replacement, we had checked patient's testosterone level of serum at 6 months interval, which has been sustained in range of 1.24- 2.48 pg/mL

DISCUSSION

In this case, we noted his deficiencies of secondary sexual development by the Tanner stages and low testosterone and low gonadotropin levels from the initial laboratory findings without any structural or functional abnormalities. There was no secondary factor – endocrine pathology, central neural pathology, etc - of hypogonadotropic hypogonadism found through multidimensional approaches in history taking and examination; hence, the diagnosis of IHH in this patient was confirmed.

The most common presentation of IHH is the complete absence of pubertal development with minimal testicular growth. The testes in such cases may be maldescended.[8] Testicular volume is usually < 4ml and hormone replacement treatment rarely results in complete normalization of sperm production. However, patients with hypogonadism of adult or post-pubertal onset are rare.[9] Although the case of our patient had also minimal pubertal development and testicular growth, his delayed referral at the age of 29 years can be regarded as unusual.

Multiple pathological factors have been known to cause HH. It includes genetic abnormality and any disease that affects the hypothalamic-pituitary axis. The relation between the failure of pulsatile gonadotropin releasing hormone (GnRH) secretion and gene mutation has been revealed in a few studies. However, genetic testing in IHH is challenging, given the genetic and allelic heterogeneity, as well as complex oligogenic inheritance patterns.[10] It is because we only tested karyotyping on the patient for genetic analysis and it was unclear to identify if the patient with IHH had a genetic impact.

Hormonal therapy for spermatogenesis can be applied with either gonadotrophins or pulsatile GnRH.[11] Exogenous pulsatile GnRH stimulates the FSH and LH secretion from the pituitary gland.[12,13] However, because of complex and time-consuming pulsatile therapies, today, only a few patients with hypogonadotropic hypogonadism are treated with pulsatile GnRH. In addition, pulsatile GnRH therapy seems to have no proven advantage over FSH plus hCG therapy in patients with hypothalamic HH. The lack of sufficient well-designed and randomized prospective studies did not allow firm conclusions on the best therapy for infertility in these patients.[14]

The exogenous substitution of testosterone is to maintain all androgen-dependent functions. This therapy has been well established over decades, relatively convenient for male patients and comparably inexpensive.[15] In the case where patients desire offspring, the testosterone substitution therapy is no longer sufficient and has to be interrupted. The patients should then be treated with FSH preparations, in addition, with pharmacological preparation such as LH to stimulate intratesticular testosterone production by the Leydig cells. As the LH preparation hasn't been yet approved for male HH, patients are usually treated with hCG preparations with not identical but similar bioactivity. LH, by the use of its substitute hormone hCG, is always replaced before FSH. Because stimulation of hCG alone may be sufficient for spermatogenesis and hCG is considerably less expensive than exogeneous FSH preparations.[16,17] In our case, 9 months initial hCG therapy was not enough to spermatogenesis thus we added FSH preparation using hMG which is less expensive than recombinant FSH and had also proved as an effective alternative treatment.[18] In our case, we also got successful result of spermatogenesis with hCG/hMG therapy.

In some cases, reported that 15 patients had sustained reversal of HH even after the discontinuation of the therapy.[3] Five of them received testosterone treatment alone, three were treated with only pulsatile GnRH therapy and the rest received a mixed regimen, including testosterone, gonadotropins or GnRH. Although the mechanism of reversal is unclear, it is inferred that the GnRH therapy somehow modified the hypothalamic neurons responsible for producing GnRH. This might lead to reversal of HH and initiation of spermatogenesis. A case of HPG axis recovery after testosterone therapy.[6] Although our patient had succeeded in spermatogenesis and pregnancy, his HPG axis was not recovered after gonadotrophin or testosterone treatment. Gonadotrophin therapy was followed by testosterone substitution therapy to maintain the androgen-dependent functions including sexual ability of erection and ejaculation.

CONCLUSION

This study is a good example that shows the importance of differentiating reversible cause of infertility from irreversible cause by a thorough and careful examination. In addition, it further proves that, in the case of IHH, a continuous long-term gonadotrophic stimulation therapy (hMG/hCG) contributes to successful pregnancy and

childbirth. Further reports and related research will be needed for the cases with IHH reversal and established guidelines for therapeutic method of IHH.

Conflicts of Interest Statement and Funding/ Support Statement/Ethical clearance

The author(s) have no conflicts of interest and no funding/support relevant to this article. The Institutional Review Board of Inje University Haeundae Paik Hospital approved this study (No. 2021-03-007).

REFERENCES

1. Boehm, U., Bouloux, P., Dattani, M. et al. European Consensus Statement on congenital hypogonadotropic hypogonadism-pathogenesis, diagnosis and treatment. Nat Rev Endocrinol. 2015; 11:547-64
2. Bianco, S., Kaiser, U. The genetic and molecular basis of idiopathic hypogonadotropic hypogonadism. Nat Rev Endocrinol. 2009;5:569-76.
3. Raivio T, Falardeau J, Dwyer A, et al. Reversal of idiopathic hypogonadotropic hypogonadism. N Engl J Med. 2007;357:863-73.
4. Finkel DM, Phillips JL, Snyder PJ. Stimulation of spermatogenesis by gonadotropins in men with hypogonadotropic hypogonadism. N Engl J Med. 1985;313(11):651.
5. Bassil N, Alkaade S, Morley J. The benefits and risks of testosterone replacement therapy: a review. Ther Clin Risk Manag. 2009;5:427-48
6. Rashid O, Ram N, Farooq S, et al. Idiopathic hypogonadotropic hypogonadism reversal after testosterone replacement in a 34-year-old male. BMJ Case Rep. 2017: bcr2016218729.
7. Practice committee of the American society for Reproductive Medicine. Evidence-based treatments for couples with unexplained infertility: a guideline. Fertil Steril. 2020;13(2):305-22.
8. Finkel DM, Phillips JL, Snyder PJ.Stimulation of spermatogenesis by gonadotropins in men with hypogonadotropic hypogonadism. N Engl J Med. 1985;313(11):651.
9. Matsumoto, A.M. Hormonal therapy of male hypogonadism. Endocrinol. Metabol Clin. N. Am. 1994;23:857-75.
10. Whitcomb, R.W. and Croweley, W.F. Diagnosis and treatment of isolated gonadotropin-releasing hormone deficiency in men. J. Clin. Endocrinol. Metabol. 1990;70:3-7.
11. Sumko D, Weise SE, Wong L. Congenital idiopathic hypogonadotropic hypogonadism: a case report. J Clin Case Reports. 2014;4:371.
12. Schophl, J., Mehltretter, G., von Zumbusch, R. et al. Comparison of gonadotropin-releasing hormone and gonadotropin therapy in male patients with idiopathic hypothalamic hypogonadism. Fertil. Steril. 1991;56:1143-6.
13. Behre HM, Nieschlag E, Partsch C-J, Wieacker P, Simoni M. Diseases of the hypothalamus and the pituitary gland. Andrology. Male Reproductive Health and Dysfunction. 3rd ed. Berlin: Springer 2010:169-92.
14. Young J, Xu C, Papadakis GE, , et al. Clinical management of congenital hypogonadotropic hypogonadism. Endocrinol Rev. 2019;40:669-710.
15. Mao JF, Liu Zx, Nie M, et al,. Pulsatile gonadotropin-releasing hormone therapy is associated with earlier spermatogenesis compared to combined gonadotropin therapy in patients with congenital hypogonadotropic hypogonadism. Asian J Androl. 2017;19:680-5.
16. Behre HM, Clinical Use of FSH in Male Infertility, Front. Endocrinol. 2019;10:322.
17. Casarini L, Lispi M, Longobardi S, et al,. LH and hCG action on the same receptor results in quantitatively and qualitiatively different intracellular signalling. PLoS ONE. 2012;7:e466682.
18. Bucher D, Behre HM, Kliesh S, Nieschlag E. Pulsatile GnRH or human chorionic gonadotropin/human menopausal gonadotropin as effective treatment for men with hypogonadotropic hypogonadism: a review of 42 cases. Eur J Endocrinol. 1998;139:298-303.

Case Report

Management of Isolated Tubal Torsion (ALADIN) during Emergency Laparotomy in Infertile Women

Tata Laksana dari Torsio Tuba Terisolasi (ALADIN) selama Laparotomi Emergensi pada Perempuan Infertil

Bella Aprilia[1], Raymond Surya[1], Bram Pradipta[2]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine Universitas Indonesia
[2]Department of Obstetrics and Gynecology Urban Hospital Koja Jakarta

Abstract

Objective: To report ITT and primary infertility patient which came with acute abdominal pain.

Methods: Case report.

Case: A 36 yo female came to Koja Hospital with acute abdominal pain without sign of infections. Ultrasound findings show cystic mass on bilateral adnexal sized 76x28x39 mm and 31x51x43 mm with minimal ascites. During laparotomy, proximal right fallopian tube was torsioned four times clockwise. There was hydrosalping founded on contralateral tube, while right and left ovaries and uterus were normal. We performed salpingectomy.

Discussion: The clinical presentation of ITT is non-specific and it has become a challenge to physician to develop preoperative diagnosis. The spectrum of imaging findings may be wide range depending on adnexal pathology, degree of severity, and the duration of adnexal torsion. The recommendation of primary approach to tubal torsion is conservative management considering **ALADIN** (m**AL**ign**A**ncy-**D**eath tissue, **IN**fertility).

Conclusion: The diagnosis is rarely be made before operation, due to non-specific clinical symptoms and imaging findings. The considerations to perform conservative management are malignancy, death tissue/necrotic, and infertility.

Keywords: hydrosalping, isolated tubal torsion malignancy-death tissue infertility.

Abstrak

Tujuan: *Untuk membahas tentang pasien torsio tuba terisolasi dan infertilitas primer yang datang dengan keluhan nyeri abdomen akut.*

Metode: *Laporan kasus.*

Kasus: *Seorang perempuan 36 tahun datang ke RS Koja dengan nyeri abdomen akut tanpa tanda infeksi. Pemeriksaan USG menunjukkan massa kistik pada adneksa bilateral dengan ukuran 76x28x39 mm dan 31x51x43 mm dengan asites minimal. Selama laparotomi, tuba falopi proksimal kanan mengalami torsio empat kali searah jarum jam. Selain itu, ditemukan hidrosalping pada tuba kontralateral dan sementara ovarium kanan, kiri, dan uterus dalam batas normal. Kami melakukan salpingektomi.*
Diskusi: Presentasi klinis dari torsio tuba terisolasi tidak spesifik dan menjadi tantangan dalam diagnosis preoperatif. Gambaran penunjang dapat bervariasi bergantung pada patologi adneksa, derajat keparahan, dan lama torsio adneksa. Rekomendasi pendekatan torsio tuba ialah konservatif melalui ALADIN (mALignAncy-Death tissue, INfertility).

Kesimpulan: *Diagnosis dapat dilakukan sebelum operasi karena pemeriksaan klinis dan gambaran penunjang yang tidak spesifik. Pertimbangan melakukan tatalaksana konservatif ialah keganasan, jaringan yang nekrotik, dan infertilitas.*

Kata kunci: *hidrosalping, keganasan – infertilitas, torsio tuba terisolasi.*

Correspondence author. Bella Aprilia. Department of Obstetrics and Gynecology.
Faculty of Medicine Universitas Indonesia. Dr. Cipto Mangunkusumo General Hospital. Jakarta
Email; bellaraphael.md@gmail.com

Received: May,2021 Accepted: September,2022 Published: October,2022

INTRODUCTION

Isolated tubal torsion (ITT) is rare cases with incidence 1 in 1.5 million women. The challenge of diagnosing ITT preoperatively is its non-specific symptoms such as lower abdominal pain, while in acute condition it is accompanied with nausea, vomiting[1] Moreover, imaging findings for ITT are non-specific commonly diagnosed as ovarian cysts. One factor that may cause ITT is hydrosalping and it is often correlated with infertility condition. Therefore, management regarding ITT should be carefully decided based on each patient. However due to delayed diagnosis, surgical salpingectomy is commonly performed as decision during intraoperative findings. Nevertheless, there are very few cases managed with detorsion of fallopian tube.[2] Here we reported case of ITT patients and primary infertility came to hospital with acute abdominal pain in order to discuss its diagnostic features and surgical management.

CASE

A 36 yo female came to Koja Hospital with acute abdominal pain without sign of infections. Ultrasound findings show cystic mass on bilateral adnexal sized 76x28x39 mm and 31x51x43 mm with minimal ascites (Figure 1), both ovaries were difficult to identified due to the abdominal pain. Laboratory results show normal hemoglobin and leucocytosis. During laparotomy, there was minimal ascites found on the pelvic floor with proximal right fallopian tube was torsioned four times clockwise. There was hydrosalping founded on contralateral tube, while right and left ovaries and uterus were normal. (Figure 2A-C). The right fallopian tube was gangrenous seen with hemorrhagic fluids enlarged to sized 7x4x3cm. Salpingectomy was performed due to the necrotic condition. Histopathology result shows hydrosalping and paratubal cyst (Figure 3A-B).

Figure 1. Ultrasound findings of bilateral adnexal masses with minimal ascites

Figure 2-A. Right fallopian tube was torsioned four times clockwise *(arrow);* 2-B. Further exploration: right and left ovaries and uterus were normal, with left hydrosalping; 2-C). Right salpingectomy was performed

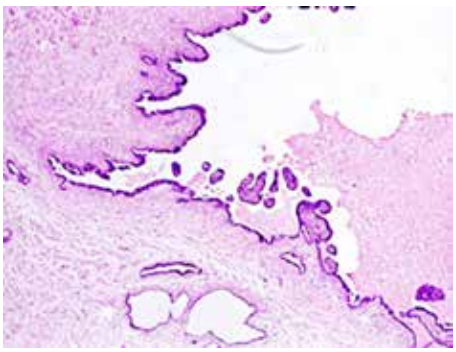

Figure 3-A. Histopathology finding of the fallopian tube showed distortic plicae lined by ciliated simple columnar epithelium. Acute and chronic inflammatory cells infiltrated the stroma. (Magnification 40x

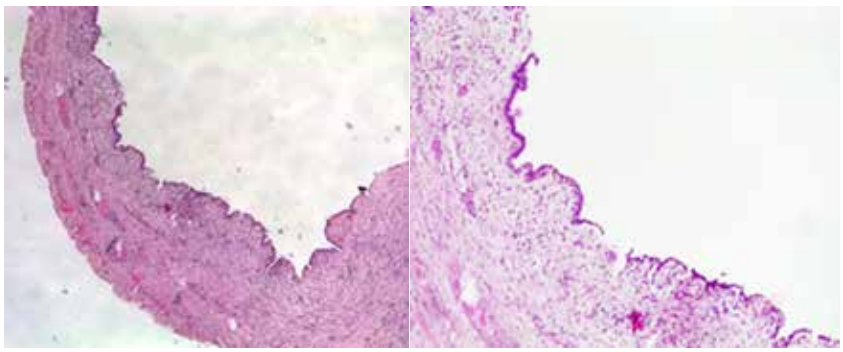

Figure 3-B. Histopathologic findings of the fallopian tube showed a cystic space lined by simple columnar epithelium. Chornic inflammatory cells infiltrated the stroma (Magnification *Left:* 40x, Right : 100x)

DISCUSSION

Isolated tuba torsion (ITT) is a very rare entity and comprises of minor group among etiologies of abdominal pain in adolescents. It is torsion of fallopian tube without ovarian torsion involvement. This condition can be precipitated by intrinsic and extrinsic factors. Intrinsic factors are pelvic inflammatory disease (PID), hydrosalping, prior tubal surgery, primary fallopian tube malignancy, and abnormal length of mesosalping and spiral course of the salping. The extrinsic pathologies are scarring from endometriosis, prior pelvic surgery, gravid uterus, malignancy or tumor of the adjacent structures, and paraovarian or paratubal cysts.[3,4] ITT may be considered as one causes of unexplained infertility. Two hundred and thirteen of four hundred and nine (52.1%) unexplained infertility patients were found to have hydatid of Morgagni cysts[5] Through impeding ovum pick-up, hydatid of Morgagni may be considering as new factors in infertility. This cyst can trigger the isolated torsion of morgagni hydatids with or without fimbria.[6] One theory that may include in this pathology findings is stimulation of reproductive axis in high follicle stimulating hormone (FSH) levels long before the onset of menses in a case with asymptomatic distal occlusion of the tube and asymptomatic pelvic inflammation in adnexal area.[7] Hydrosalping might be a secondary finding in ITT cases and might not be responsible as the main causes of ITT.[3, 4] But in this case, hydrosalping might be consider as one underlying cause due to the left hydrosalping findings.

The clinical presentation of ITT is non-specific and it has become a challenge to physician to develop preoperative diagnosis. Acute severe lower abdominal pain is commonly present, with or without accompanied by nausea, vomiting, and fever. Physical and laboratory exams are also not specific to diagnosed ITT. Some findings show leukocytosis caused by necrosis condition.[8, 9] ITT is predominantly appears on the right side due to mild immobilization of left tube by the proximity to sigmoid mesentery. Thus, patients with right lower abdominal pain will be undergoing operation faster than left side because suspicion of appendicitis.[10] However, 6 cases in which ITT occurred on the left side.[11]

The spectrum of imaging findings may be wide range depending on adnexal pathology, degree of severity, and the duration of adnexal torsion. Normal fallopian tube is rarely visible on ultrasonography because of its narrow diameter and lack of clear echogenic features. Thus, dilated tube might show thickened, echogenic walls, with internal debris or echogenic mass. It is recommended to use color Doppler ultrasound, to differ normal ovary with other pelvic cyst. The whirlpool sign represents tissue mass twisted around central axis, by moving transvaginal ultrasound probe back and forth along axis of suspected torsion.[1,9] CT-scan might not be superior compare to transvaginal ultrasound, therefore ultrasound might considered as first line of diagnostic imaging.[12] The progression of torsion causes hematosalping, tubal rupture, and peritubal hematomas, which become more complicated and harder to diagnose. This heterogenous mass may confused with ruptured ectopic pregnancy. [1]Magnetic Resonance Imaging (MRI) is valuable in recognizing adnexal torsion with most common findings thickening

tube, ascites and uterine deviation to twisted side. Whirlpool sign might be observed in T2-weighted imaging.13

The recommendation of primary approach to tubal torsion is conservative management considering **ALADIN** (m**AL**ign**A**ncy-**D**eath tissue, **IN**fertility). M**AL**ign**A**ncy, if imaging/ intraoperative findings shows possibility of malignancy then it should be performed radical surgery (referred to oncologist if possible).[9] **D**eath tissue/necrotic, Due to delayed diagnosis, most cases were presented with necrotic tube. If detorsion was performed then reperfusion (color appearance), vascularization, and viability. This salvation has risk of thromboembolic events. Thus, salpingectomy was preferred in most necrotic cases.[2] Infertility, fertility status is important to consider the conservative management in this patient, Fertility-sparing surgery may be performed in infertility patients, using laparoscopic detorsion of fallopian tube to preserve fertility.[9] After detorsion, it should be watched for reperfusion and associating pathologies. The salvage of the tube should be evaluate its viable because of future fertility.[2]

Early laparoscopy is reference standard in diagnosis and treatment. Recovery after laparoscopy is faster and causes fewer post-operative adhesions. Timing of diagnosis is very important in ITT which delay may cause irreversible consequences; therefore best treatment can be performed for the patients.[9] After laparoscopic detorsion, follow up using ultrasound must be performed to document possibility of recurrent torsion or hydrosalping.[3]

CONCLUSION

Gynecologist should be aware of isolated tubal torsion as differential diagnosis in lower abdominal pain. Possible complications possess a risk for future fertility. The diagnosis is rarely be made before operation, due to non-specific clinical symptoms and imaging findings. The considerations to perform conservative management are malignancy, death tissue/ necrotic, and infertility (**ALADIN**). Thus, laparoscopic may be consider as diagnostic tools and laparoscopic detorsion may be consider as primary conservative management to preserve fertility.

REFERENCES

1. Fadıloğlu E, Dur R, Demirdağ E, Öztürk Caa, Fadıloğlu Se, Kaplan M, et al. Isolated tubal torsion: Successful preoperative diagnosis of five cases using ultrasound and management with laparoscopy. Turk J Obstet Gynecol. 2017;14:187-90.
2. Gunal YD, Bahadir GB, Boybeyı O, Cıl AP, Aslan MK. A rare cause of acute abdominal pain in children: Isolated Tubal Torsion; a case series. Turk J Emerg Med. 2017;17:73-6.
3. Ardıçlı B, Ekinci S, Oğuz B, Haliloğlu M, Tanyel FC, Karnak I. Laparoscopic detorsion of isolated idiopathic Fallopian tube torsion: conservative treatment in a 13-year-old girl. Turk J Pediatr. 2013;55:451-4.
4. Orazi C, Inserra A, Lucchetti M, Schingo P. Isolated tubal torsion: a rare cause of pelvic pain at menarche. Pediatr Radiol. 2016;36:1316-18.
5. Rasheed SM, Abdelmonem AM. Hydatid of Morgagni: a possible underestimated cause of unexplained infertility. Eur J Obstet Gynecol Reprod Biol. 2021;158:62-6.
6. Cebesoy FB, Kutlar I, Dikensoy E, Yazicioglu C, Kalayci H. Morgagni hydatids: a new factor in infertility? Arch Gynecol Obstet. 2010;281:1015-17.
7. Boukaidi A, Delotte J, Steyaert H, al e. Thirteen cases of isolated tubal torsions associated with hydrosalping in children and adolescents, proposal for conservative management: retrospective review and literature survey. J Pediatr Surg. 2011;46:1425-31.
8. Zielinska D, Rzepka-Gorska I. Isolated fallopian tube torsion in a teenager-a case report. Ginekol Pol. 2011;82(12):933-5.
9. Jokic R, Lovrenski J, Lovrenski A, Trajkovic V. Isolated Fallopian Tube Torsion – A Challenge for the Timely Diagnosis and Treatment. Srp Arch Celok Lek. 2015;143(7-8):471-5.
10. Harmon J, Binkovitz L, Binkovitz L. Isolated fallopian tube torsion in an adolescent: CT features. Pediatr Radiol. 2018;38(2):175-9.
11. Wong S, Suen S, Lao T, Chung K. Isolated fallopian tube torsion: a series of six cases. Acta Obstet Gynecol Scand. 2020;89(10):1354-6.
12. Kardakis S, Barranca A, Vitelli A, Amore I, Trento F, Caccia G. Isolated fallopian tube torsion. Case Rep Obstet Gynecol. 2013;2013:479698.
13. Aydin R, Bildircin D, Polat A. Isolated torsion of the fallopian tube with hydrosalping mimicking a multiloculated ovarian cyst: whirlpool sign on preoperative sonography and MRI. J Clin Ultrasound. 2014;42(1):45-8.