

Research Article

## Relationship of Retinol Binding Protein Four Serum Level on Endometrial Hyperplasia and Endometrial Carcinoma

### *Hubungan Kadar Serum Retinol Binding Protein Empat pada Hiperplasia Endometrium dan Karsinoma Endometrium*

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#### Abstract

**Objective:** To determine the relationship between elevated serum retinol-binding protein 4 with abnormal uterine bleeding.

**Methods:** This study was an observational quantitative with cross-sectional methods, with all women who had abnormal uterine bleeding caused either by endometrial carcinoma or endometrial hyperplasia at RSUP Prof. Dr. R.D. Kandou, and affiliation hospitals from November 2016 until April 2017. Data were analysed with SPSS version 2.0 to see the significance level.

**Results:** Of 26 research subjects, 23 subjects with endometrial hyperplasia and three subjects with endometrial carcinoma. From the total of 26 malignancy and hyperplasia diagnoses, 21 had IMT > 25, and 23 were diagnosed with Endometrial Hyperplasia and 3 Carcinoma Endometrium. 18 subjects had elevated serum RBP4 levels, with 15 people with endometrial hyperplasia and 3 with endometrial carcinoma. With the Fischer Exact test statistic, serum retinol binding protein 4 levels were found in both endometrial hyperplasia and endometrial carcinoma  $p = 1.00$ , meaning no significant difference for the occurrence of abnormal uterine bleeding.

**Conclusion:** There was no significant association between serum retinol binding protein 4 between endometrium carcinoma and endometrial hyperplasia.

[Indones J Obstet Gynecol 2018; 6-4: 243-247]

**Keywords:** abnormal uterine bleeding, endometrial carcinoma, endometrial hyperplasia, serum retinol binding protein 4

#### Abstrak

**Tujuan:** Mengetahui adanya hubungan peningkatan kadar serum retinol binding protein 4 pada hiperplasia endometrium dengan karsinoma endometrium.

**Metode:** Penelitian ini adalah jenis kuantitatif observasional secara potong lintang, dengan semua perempuan yang mengalami perdarahan uterus abnormal yang disebabkan oleh hiperplasia endometrium atau karsinoma endometrium di Obstetri dan Ginekologi Rumah Sakit Umum Pusat (RSUP) Prof. Dr. R.D. Kandou, dan RS jejaring mulai November 2016 sampai April 2017. Data dianalisa dengan SPSS versi 2.0 untuk melihat tingkat kemaknaannya.

**Hasil:** Dari 26 subjek penelitian, 23 subjek dengan hiperplasia endometrium dan 3 subjek dengan karsinoma endometrium. Didapatkan data penelitian dari total keganasan diagnosa dan hiperplasia sejumlah 26 orang, sebanyak 21 orang memiliki IM T>25 dan sebanyak 23 orang didiagnosa dengan Hiperplasia Endometrium dan 3 orang karsinoma endometrium. Didapatkan sebanyak 18 subjek penelitian mengalami peningkatan kadar serum RBP 4, dengan 15 orang yang mengalami hiperplasia endometrium dan 3 orang dengan karsinoma endometrium. Dengan uji statistik Fischer Exact test, didapatkan kadar serum retinol binding protein 4 baik pada hiperplasia endometrium dengan karsinoma endometrium  $p=1.00$ , mengartikan tidak mempunyai perbedaan bermakna untuk terjadinya perdarahan uterus abnormal.

**Kesimpulan:** Tidak terdapat hubungan bermakna kadar serum retinol binding protein 4 antara karsinoma endometrium dengan hiperplasia endometrium.

[Maj Obstet Ginekol Indones 2018; 6-4: 243-247]

**Kata kunci:** hiperplasia endometrium, kadar serum retinol binding protein 4, karsinoma endometrium, perdarahan uterus abnormal

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## INTRODUCTION

In healthy women, the menstrual cycle and reproductive health depend on the balance between the hormones formed by the hypothalamus and the pituitary. The hypothalamus will produce gonadotropin-releasing hormone (Gn-

RH), this hormone will control the release of hormones released by the pituitary luteinizing hormone (LH) and follicle stimulating hormone (FSH). FSH and LH play a role in follicular maturation, ovulation and corpus luteum formation and synthesis of sex steroids.<sup>1</sup>

In reproductive-aged women, the reproductive and endocrine organs are functioning perfectly, at the age of perimenarche the hypothalamic-pituitary and ovarian axis are immature while at perimenopause the organ function begins to decline. Based on nutritional status, women with a BMI > 25 have a higher risk of menstrual cycle disturbance than women with nutritional status <25.1

RBP 4 is identified as one molecule of adipose molecule derivatives that contributes to the cause of insulin resistance in obesity and type 2 diabetes mellitus. RBP will cloak directly on objects with obesity or glucose intolerance, type 2 diabetes or with risk factors for cardiovascular pathology disorders.<sup>2</sup>

RBP4 is a protein compound derived from the lipocalin group and is a carrier-specific protein for retinol (vitamin A alcohol) in the blood. This compound will deliver retinol from the storage of the liver and then distributed throughout the body tissues. In plasma, the RBP 4 complex will bind to the transthyretin complex and will be filtered by the renal glomerulus. Lack of this RBP will affect vitamin A levels and distribution to all cells.<sup>2</sup>

Based on the background of changes in estrogen levels in women with abnormal uterine bleeding (PUA), malignancy and endometrial hyperplasia in which estrogen levels will be affected by nutritional status which is a representation of adipose tissue it is necessary to do research on levels of

Retinol Binding Protein 4 (RBP4) in patients Abnormal Uterine Bleeding (PUA) malignancy and endometrial hyperplasia to aid in more accurate diagnosis and management.

## METHODS

This was a cross-sectional study, with all women who had abnormal uterine bleeding caused either by endometrial carcinoma or endometrial hyperplasia at RSUP Prof. Dr. R.D. Kandou, and affiliation hospitals from November 2016 until April 2017. Data were analysed With SPSS version 2.0 to see the significance level.

Data collection was performed by following steps: Patients who meet the inclusion criteria are given an explanation of the action to be performed and the patient signs the consent statement following the research provided. Subject of this research is anamnesis, physical examination and then recorded in research status. Venous blood taken for RBP4 was examined, then reversed slowly about 5-10 times. Immediately within 30 minutes, the sample was centrifuged at 1000 g at a speed of about 3000 rpm for 15 minutes then the serum obtained was stored in a refrigerator with a temperature of -200C and then sent to Jakarta by using Ice pack to maintain the storage temperature before the examination of RBP 4 in the Laboratory Prodia Jakarta. Examination of serum RBP 4 level was done by quantitative method, using ELISA method.

**Table 1.** Distribution of Research Subject Characteristic

Characteristic	BMI > 25		BMI < 25		total
	n	%	n	%	
<b>Age</b>					
> 40	18	81.81	4	18.18	22
< 40	3	75	1	25	4
<b>Education</b>					
Elementary	1	50	1	50	2
Junior high school	2	40	3	60	5
High school	15	93.75	1	6.25	16
Diploma	2	100	0	-	2
Bachelor	1	100	0	-	1
<b>Occupation</b>					
Civil	1	100	0	-	1
Private	20	92.64	5	7.35	25

**Table 2.** Difference of serum RBP4 between Endometrial Hyperplasia and Endometrial Carcinoma

	BMI > 25		BMI < 25		
	RBP 4 <26.800 ng/ml	RBP 4 >26.800 ng/ml	RBP 4 <26.800 ng/ml	RBP 4 >26.800 ng/ml	
Hyperplasia	3	15	3	2	23 p=1.00
Carcinoma	0	3	0	0	3
	3	18	3	2	26

## RESULTS

From above data, it is found that the most age group obtained is at the age of 40 years (22 people) with 18 people have BMI >25. While the highest education status is high school with 15 subjects. A total of 20 subjects were private employees, and only one subject works as a civil servant.

Of the total 26 malignancy and hyperplasia diagnoses, 21 had a BMI of > 25 and 23 were diagnosed with Endometrial Hyperplasia and 3 Endometrial Carcinomas. 18 subjects had elevated serum RBP4 levels, with 15 people with endometrial hyperplasia and 3 with endometrial carcinoma.

## DISCUSSION

RBP 4 is identified as one molecule of adipose molecule derivatives that contributes to causing insulin resistance in obesity and type 2 diabetes mellitus. RBP will correlate directly to objects with obesity or glucose intolerance, type 2 diabetes or with risk factors for cardiovascular pathology disorders.<sup>2</sup>

As a system for retinol transport, a specific protein retinol binding will be produced in the liver and will mobilise retinol from the liver as the primary storage area. Once excreted from the liver, the retinol binding protein complex will circulate in the blood further binding to the plasma transthyretin. When retinol has reached the target tissue, the bone-specific retinol-free protein will be rapidly catabolized and excreted in the kidneys. The turning point of biological retinol binding protein has a half-life of 11.5 hours in adult humans.<sup>2</sup>

In Table 1. Characteristics of the subjects are seen by age, occupation, education, BMI and basic aetiology of AUB-M. All samples were patients who had been diagnosed with AUB-M. In Table 1 it was

found that the most age group obtained was at the age of 40 years (22 people) with 18 of them had IMT > 25. While the highest education status was high school with 15 samples. A total of 25 samples have private employment, and only one person works as a civil servant. According to Kovacs P. 2012. Endometrial bleeding will be more common at age ≥ 40 years than in women <40 years. Age 40 years is the beginning of perimenopausal age. Where at age 40 a woman will be more frequent to occur anovulation and based on it at age 40 menstrual cycle will be longer. In research in Australia, when the menstrual cycle has exceeded 42 days, menopause can be predicted to occur within 1 to 2 years. The duration of the follicular phase is the determinant of the length of the cycle. As discussed in the literature review, there are 2 estrogen receptors that simultaneously affect the body's estrogen levels. One of the estrogen receptors derived from the ovaries (E2 receptors) so that researchers need to choose the conditions in which E1 receptors (skin, fat, muscle, endometrium) can be assessed as a major factor in this study.<sup>2-6</sup>

In Table 2, the largest distribution of research samples obtained aetiology of endometrial hyperplasia and endometrial carcinoma is 26 people and of that 21 people have IMT > 25 and as many as 18 people have serum RBP 4 > 26.000. Adipose tissue is found on almost all body surfaces. Corrected directly with the surface area of the body which in this study is defined as the body mass index. Increasing IMT means that the broader the adipose network is owned by the individual. In the event of AUB-M with the aetiology of malignancy and hyperplasia has been explained and in the aetiology endometrial has been suspected one of the risk factors is the nutritional status, where will be directly related to endometrial status. Pathological nutrition status will affect the occurrence of metabolic syndrome. And this will affect the adipose network which will then affect

the level of an individual RBP. In endometrial hyperplasia with BMI < 25 serum RBP4 levels were found in 3 people with serum RBP 4 < 26.800. Then at the RBP 4 level of endometrial hyperplasia patients with BMI > 25, there were three subjects with serum RBP 4 < 26.800. This may be due to many things, where elevated serum RBP 4 levels are affected by various factors such as comorbidities, insulin sensitivity, dietary patterns, and other lab variables not examined in this study. The results of this study are consistent with those reported by Kovacs, P 2012, obesity, a risk factor often encountered in endometrial malignancies.<sup>6-10</sup>

Endometrial hyperplasia is a pathological condition in the endometrium in the form of increased proliferation of the endometrial glands resulting in changes in the ratio of glands and stroma, shape and size of the gland, the glandular structure increases to 2-3 layers and has the potential to be an atypical form of cells when there is no balance of inhibitors and the initiator of the proliferation of glandular cells.<sup>9-11</sup>

With the imbalance between estrogen and progesterone where there is a state of Unopposed Estrogen that will cause endometrial thickening. Estrogen hormones known to be produced in the ovaries are also produced on adipose tissue. Adipose tissue has been known as the organ that produces adipokine where one of the adipokines is RBP4. With increased adipose tissue will be associated with increased BMI.<sup>1</sup> In Table 3 the results of the Fischer exact test show  $p = 1.00$ . This means there is no significant difference between serum retinol binding levels of Protein 4 in endometrial hyperplasia and endometrial carcinoma. This may occur because in this study increased serum RBP 4 levels obtained as many as 17 people with both endometrial hyperplasia and endometrial carcinoma. Increased RBP 4 was associated with BMI levels > 25 in this study there were 21 people with BMI > 25. So that serum RBP 4 levels were > 50% increased by more than 26,800. So with the statistical test obtained  $p$ -value = 1.00. So it can be said there is no significant difference in serum RBP 4 levels between endometrial hyperplasia and endometrial carcinoma. While in BMI obtained in this study, showed that with total samples of hyperplasia and endometrial carcinoma with BMI > 25 were 21 people. Susanne et al. 2007, reported BMI associated with serum RBP 4 levels, the higher

the BMI then serum RBP 4 levels will also increase. In several other studies have also been done to see the relationship between serum RBP4 and BMI levels. This is in line with the results obtained in this study where elevated serum RBP 4 levels in BMI > 25 as many as 18 people. In addition, there have been several studies linking RBP with insulin resistance wherein insulin resistance will be associated with Diabetes Mellitus type 2.<sup>12-16</sup>

This research has advantages and disadvantages, the limitations that will be able to influence the research results, such as the lack of research samples, RBP in normal BMI patients and not experiencing AUB-M, cannot control confounding variables such as, GDS, dietary patterns, unexamined FSH-LH, estrogen, HOMA-IR levels. Moreover, some other mediators whom other studies have suspected will affect the results of our research. While the advantages of this study were all those examined were patients with AUB with endometrial hyperplasia aetiology and endometrial carcinoma, also this study used data obtained by Anatomical Pathology and ultrasound. Another advantage is that all samples were taken from September 2016 to April 2017 so that it can be used as an overview of PUA events with the aetiology of malignancy and endometrial hyperplasia.<sup>16-18</sup>

## CONCLUSION

There was a no correlation between serum RBP 4 between endometrial hyperplasia and endometrial carcinoma.

## SUGGESTION

Further studies with larger sample size and better study design are required.

## REFERENCES

1. Fritz MD, Marc A, Speroff L MD. Clinical Gynecologic Endocrinology and Infertility Eight Edition. Lippincott Williams & Wilkins. 2011: 590-1446.
2. Craig R. Retinol-binding protein 4 as a candidate gene for type 2 diabetes and prediabetic intermediate traits. *Mol Genetics Metabol*. 2006; 90: 338-44.
3. Legro R, Kunselman A, Dodson W, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. *J Clin Endocrinol Metabol* 1999; 84: 165-9.

4. Ehrmann D, Barnes R, Rosenfield R, Cavaghan M, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. *Diabet Care* 1999; 22: 141-6.
5. Aronne L & Segal K. Adiposity and fat distribution outcome measures: assessment and clinical implications. *Obesity Research* 2002;10 (Suppl 1): 14S-21S.
6. Kovacs P. The Link between Obesity and Endometrial Cancer. *Medscape OB/GYN*. 2012
7. Munkhtulga L, Nakayama K, Utsumi N, Yanagisawa Y, Gotoh T, Omi T, et al. Identification of a regulatory SNP in the retinol binding protein 4 gene associated with type 2 diabetes in Mongolia. *Hum Genetics* 2007; 120: 879-88.
8. Haider D, Schindler K, Prager G, Bodjahlia A, Luger A, Woltz M, Ludvik B. Serum retinol-binding protein-4 is reduced after weight loss in morbidly obese subjects. *J Clin Endocrinol Metabol*. 2006; 92: 1168-71.
9. Yang Q, Graham T, Mody N, Preitner F, Peroni O, Zabolotny J, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* 2005; 436: 356-62.
10. Graham T, Yang Q, Blugher M, Hammerstedt A, Claraldi T, Henry R, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *New England J Med*. 2006; 354: 2552-63.
11. Uzan J, Laas E, Alsamad I, Skalii D, Mansouri D, Haddad B, Touboul C. Supervised Clustering of Adipokines and Hormonal Receptors Predict Prognosis in a Population of Obese Women with Type 1 Endometrial Cancer. *Int J Mol Sci*. 2017; 18(5). pii: E1055. doi: 10.3390/ijms18051055.
12. Lee J, Nierstenhoefer M, Nolbot T, Yoo S, Smith L, Usheva A. Retinol Binding Protein Is A Major Carrier of Progesterone In Vitro And In Human Follicular Fluid. *American Society for Reproductive Medicine September 2008, Fertil Steril*. 2008; 90: s121.
13. Maxel T, Svendsen P, Smidt K, Lauridsen J, Brock B, Pedersen S, Rungby J, Larsen A. Expression Patterns and Correlations with Metabolic Markers of Zinc Transporters ZIP14 and ZNT1 in Obesity and Polycystic Ovary Syndrome. *Front Endocrinol (Lausanne)*. 2017; 2; 8: 38. doi: 10.3389/fendo.2017.00038. eCollection. 2017.
14. Hahn S, Backhaus M, Broecker M, Tan S, Dietz T, Kimmig R, Schmidt M, Mann K, Janssen O. Retinol-binding protein 4 levels are elevated in polycystic ovary syndrome women with obesity and impaired glucose metabolism. *Eur J Endocrinol*. 2007; 157: 201-7
15. Chen Y, Ping L, Mengkai D, Liang Z, Zhou M, Chen D. Increased Retinol Free RBP4 Contributes to Insulin Resistance in Gestational Diabetes Mellitus. *Arch Gynecol Obstet*. 296(1): 53-61. doi: 10.1007/s00404-017-4378-9. 2017.
16. Michelle W, Jorddan V, Lagas, Showell M, Wong N, Lensen S, Farquhar C. Obesity and Endometrial Hyperplasia and Cancer in Premenopausal Women: A Systematic Review. *Am J Obstet Gynecol*. 2016; 214(6); 689: E1-68.
17. Lethaby A, Suckling J, Barlow D, Farquhar C, Jepson R, Roberts H. Hormone replacement therapy in postmenopausal women: endometrial hyperplasia and irregular bleeding. *Cochrane Database Syst Rev* 2003; (4): CD000402.
18. Aviva B, Gerken A, Blaner W, Root J, McMahon D, Obersfield S. Metabolic Manifestations of Polycystic Ovary Syndrome in Nonobese Adolescent: Retinol Binding Protein 4 and Ectopic Fat Deposition. *American Society for Reproductive Medicine. Fertil Steril*. 2012; 97(4): 1009-15.