Reports of Biochemistry & Molecular Biology
Vol.11, No.2, July 2022

Original article

www.RBMB.net

Plasma Protein Profile of Lactating Women from Two Primary Health Centers in Jakarta, Indonesia

Ninik Mudjihartini*[1], Septian Ika Prasetya[2], Mohamad Sadikin[1]

Abstract

Background: Plasma protein profile test is a potential laboratory method to assess nutritional status especially albumin and globulins levels which reflects protein adequacy. The purpose of this study is to evaluate plasma protein profile of lactating women from two primary health centers in Jakarta.

Methods: A cross-sectional study was conducted involving lactating women attending routine maternal examinations in two public primary health centers in Jakarta, Indonesia. The mother's plasma total protein, albumin, globulin, and immunoglobulin levels were measured.

Results: Sixty lactating women were recruited, mostly were 28 years old, slightly overweight, bearing two children, and their recent children were 2 months old. The mean total protein level was 8.13 g/dl, albumin 5.00 g/dl, globulin 3.18 g/dl, albumin: globulin ratio 1.558, mean total IgG level of 1255.98 and mean total IgM level of 135.819. All the measured plasma protein parameters were shown to be not correlated with maternal age, maternal BMI, or maternal parity.

Discussion: The plasma total protein, albumin, globulin, as well as total IgG and IgM level of lactating women in Jakarta were within normal range. These biochemical parameters were shown to be not correlated with anthropometrical data such as maternal age and BMI. The small and relatively homogenous samples were supposed to be the cause of such findings.

Conclusions: The plasma protein profile of lactating women in Jakarta was adequate. Further studies are required to evaluate the eligibility of plasma protein profile as biochemical parameter of nutritional status in lactating women.

Keywords: Blood protein, lactation, protein, albumin/globulin, immunoglobulin M/G.

Introduction

Children stunting remains a significant problem and become a major public health priority in Indonesia. More preventive strategies were deployed to reduce stunting prevalence which had reduced among children aged less than two years from 32.8% in 2013 to 29.9% in 2018. Lactating women are among the crucial population to overcome stunting problem in which their adequate nutritional status will greatly determine the health of children in their first 1000 days of life. While specific and sensitive nutritional intervention remain the priority strategy in national strategy for acceleration of stunting prevention, assessment of lactating women's adequate nutritional status must also be conducted properly (1). The stunting eradication program must consider the adequacy status of protein profile of lactating women as the sole provider of nutrients in the infants' early life. Lactating women provide human milk to their infants which are the best nutritional source for the baby, enhancing the children's immune system as well as influencing their gut microbiota

1: Department of Biochemistry, Faculty of Medicine, Universitas Indonesia.
2: Hermina General Hospital Purwokerto, Indonesia.
***Corresponding author: Ninik Mudjihartini; Tel: +6285704470007; E-mail: ninikbiokim@gmail.com**
Received: 24 Dec, 2021; Accepted: 29 Dec, 2021

balance (2).

Although nutritional status can be assessed with simple method such as body mass index and body composition measurement, a laboratory test can provide more information about specific parameter in lactating women's blood, such as plasma protein profile. Plasma protein profile has been widely used as parameter of nutritional adequacy as well as indicator of several metabolic diseases. This study evaluated the plasma protein level comprising total protein level, albumin, globulin, and immunoglobulin levels of lactating women in two public primary health centers as a mean to obtain the nutritional adequacy status.

Materials and Methods

Study population

The studied population was lactating women with age range of 20 – 35 years old. The subjects were lactating women attending routine neonatal check in Petamburan PHC (West Jakarta) and Cilincing PHC (North Jakarta). The exclusion criteria were mothers undergoing or recently on specific diet program in the last 1 year, having any kidney disease, grade II obesity, history of preeclampsia/ eclampsia or other autoimmune/ inherited metabolic diseases based on medical history and simple physical examination by the PHC doctor.

Biochemical assay

Plasma total protein level was measured by means of Christian Warburg method (3). Meanwhile, plasma albumin level was evaluated by calorimetric assay kit/ bromocresol green method (4). Plasma immunoglobulin G and M were measured through radial immunodiffusion method. Plasma protein fraction was evaluated through electrophoresis method with cellulose acetate (5).

Measurement of plasma total protein

Plasma total protein level was measured by means of Christian – Warburg method (3). A 1 mL plasma sample (diluted 200 times) and 1 mL BSA with six different concentrations were prepared (0.05; 0.1; 0.15; 0.2; 0.3; 0.4 and 0.5 mg/mL). The samples mixtures were then measured for its absorbance with spectrophotometer with a wavelength of 280 nm. The obtained absorbance values then became the substitute for x in the $y= ax + b$ equation to find y. The obtained y values obtained from that substitution were then multiplied by dilution factor of 200 to find the total protein level in g/dL unit.

Measurement of plasma albumin

Plasma albumin level was measured with Bromocresol Green (BCG) kit. In a disposable cuvette, samples were prepared comprised of 5 µL plasma from each subject, distilled water as blank sample, and standard albumin solution. BCG reagent of 1250 µL was then poured into each cuvette and then was incubated for 10 minutes. The subjects' plasma samples, blank sample, and standard albumin sample were then measured for its absorbance by a spectrophotometer with a wavelength of 628 nm. The value of plasma albumin concentration (in mg/mL) was obtained from the ratio of subjects to standard albumin solution ratio multiplied by standard albumin concentration (4).

Measurement of plasma IgM and IgG level

The plasma total IgM and IgG level were measured using radial immunodiffusion (RID) kit (Kent). The RID gel plate was discarded from the storage (with a temperature of 4-8 °C) and then was thawed in a room temperature (20-24 °C) in a closed box. A 5 µL plasma sample from each subject which has been diluted four times with NaCl 0.9% and standard IgM solution were inserted into wells on RID gel plate. The plate containing samples was then incubated for 300 hours. The white circle formed on around the wells were then measured for its diameter and then were searched for appropriate IgM concentration on kit brochure for any given diameter obtained from the sample. The measurement of plasma IgG level was like that of plasma IgM with the same RID kit but the plasma samples from each subject was diluted 50 times and then were incubated for 48 hours (5).

Statistical analysis

The data was initially collected in Microsoft Excel software (Microsoft Corp. CA, USA) to construct the database. The data was then cleaned and coded to be analyzed using SPSS for Windows (IBM Inc, MA, USA) version 25. Since all the data was continuous in nature, the central tendency values for each variable were notated as mean±standard deviation for variables with normal distribution and was notated as median (minimum – maximum) for non-normally distributed data. Test for normality was using Shapiro-Wilk and the data was normally distributed if the p-value is> 0,05. The correlation test was performed using Pearson correlation test if the two variables being analyzed were normally distributed and using Spearman test for non-normally distributed variables. Statistical significance was set at< 0.05.

Ethical consideration

The overall study process was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization Guidelines for Good Clinical Practice. The study proposal had been reviewed approved by the Faculty of Medicine, University of Indonesia's Health Research Ethical Board with the approval letter number of KET-1008/UN2.F1/ETIK/PPM.000.02/2020. All the recruited subjects were given oral and written information about the study and those who were willing to be the participants had signed the written informed consent for study participation.

Results

Sixty lactating women were recruited. The subjects' baseline characteristics were provided in the table 1. The average age was 28 years old, slightly overweight, having two children, and their recent child were about 2 years old in average.

Table 1. The subjects' baseline characteristics.

Parameters	Data (n= 60)
Mothers' age (years)	28.0 (20–35)
Mother's body mass index (kg/m^2)	23.42 (15.25–39.55)
Parity	2,0 (1– 4)
Infants' age (months)	3.0 (1–5)
Total protein level (g/dL)	8.13 (6.64–13.06)
Total protein level (mg/ml)	81.28 (66.36–130.57)
Albumin level (g/dL)	5.00 (2.67– 6.12)
Albumin level (mg/dl)	50.03 (26.71– 61.16)
Globulin level (g/dL)	3.18 (1.29–7.46)
Globulin level (mg/dl)	31.84 (12.91–74.55)
Albumin/globulin ratio	1.558 (0.37–4.14)
Total IgG level	1255.98±230.243
Total IgM level	135.819±53.704

The total protein level and albumin level of all subjects were within normal level. A non-parametric/ Spearman correlation analysis revealed that all the measured parameter of plasma protein level was not correlated with maternal age, maternal BMI nor parity. Total protein level was shown to be significantly correlated with the infants' age.

Table 2. The correlation between maternal demographic characteristics with plasma protein and plasma total IgM/IgG levels. [s]Spearman rho correlation test, [*]denotes statistical significance.

	Maternal age		Maternal BMI		Maternal parity		Recent infant's age	
Parameters	**Correlation coefficient**	**p-value**	**Correlation coefficient**	**p-value**	**Correlation coefficient**	**p-value**	**Correlation coefficient**	**p-value**
Total protein level (g/dL)	0.013	0.932	0.133	0.323	0.066	0.628	0.311	0.019*
Albumin level (g/dL)	-0.018	0.894	0.156	0.246	-0.097	0.474	0.223	0.098
Globulin level (g/dL)	0.006	0.966	0.055	0.683	0.138	0.305	0.199	0.138
Albumin/globulin ratio	-0.002	0.990	-0.014	0.916	-0.139	0.303	-0.137	0.311
Total plasma IgG level	-0.072	0.593	-0.216	0.107	-0.136	0.313	-0.026	0.846
Total plasma IgM level	-0.059	0.661	-0.064	0.638	0.002	0.986	0.010	0.943

The mothers of babies 1 – 3 months old were having significantly lower total protein serum level compared to that of mothers of babies aging 4 months or older.

Table 3. The comparison of mean total protein level of the mother among two groups based on infant's recent age.

Infant's recent age	Mean total protein level	Mean difference	p-value
1–3 months old	7.92 (6.85–10.19)	-7.7214	0.043[M]
4 months old or more	8.35 (6.64–13.06)		

The comparison of all plasma protein parameters across different age groups were not statistically significant.

Table 4 The comparison of total protein, albumin, globulin, albumin/globulin ratio, IgM and IgG among different maternal age group.

Variable	Age groups			F	p-value
	20 – 25 years	26 – 30 years	31 – 35 years		
Total protein	8.09 (7.32–11.72)	7.92 (7.19–13.06)	8.27±0.959	0.203	0.679[k]
Albumin	5.02±0.539	4.86±0.496	5.04 (2.67–5.82)	0.344	0.655[k]
Globulin	3.12 (2.00 – 6.45)	2.95 (1.92–7.46)	3.13 (1.29–7.17)	0.067	0.939[k]
Albumin/ globulin ratio	1.61±0.59	1.70 (0.75–2.88)	1.56 (0.37– 4.14)	0.303	0.835[k]
IgM	137.13±58.139	130.01±44.83	135.59±58.78	0.086	0.918[a]
IgG	1284.88±252.98	1192.94±180.84	1274.96±259.17	0.828	0.442[a]

[a] one-way ANOVA [k]Kruskal-Wallis test

Discussion

Plasma total protein, albumin, globulin as well as total IgM and IgG level among lactating women in Jakarta region were generally within normal range. There were no cases of subject with neither hypoproteinemia nor hypoalbuminemia. The values of total protein and albumin were 8.12 g/dL and 4.00 g/dL, respectively. Therefore, from the protein adequacy perspective, the lactating women in the area had adequate protein status. Those values were similar with the data from study by Gomez-Cantarino et al involving 215 pregnant women in Spain. The mean plasma total protein and albumin level of all subjects in that study were 7.08±0.39 g/dL and 4.4±0.29 g/dL, respectively in the first

trimester of pregnancy (6). Several studies demonstrated the correlation of plasma protein level with various demographic and clinical characteristics. Some of the factors were socioeconomic status, gestational age of pregnant women, and nutritional intake. Study by Gomez-Cantarino revealed that plasma total protein and albumin level were significantly influenced by gestational age, socioeconomic status, and settlement environment. In that study, plasma total protein as well as albumin level decreased overtime as the gestational age advances and both parameters were significantly lower in rural areas than that of urban/semi urban residents. A study by Wulandari in Padang, a city in Sumatera, Indonesia involving 34 pregnant women showed that those who had chronic energy deficiency had relatively low mean plasma albumin level (3.9±0.60 g/dL). That plasma albumin level was significantly correlated with mean protein intake of 56.4±14.6 g/day, in which 30 (88.2%) of subjects were classified as having inadequate daily protein intake (7). A study by Pervaiz et al among lactating women and their neonates in Pakistan concluded that socioeconomic status was a significant determinant of serum total protein, albumin, and globulin level levels while maternal ages were not. The serum total protein levels of low, middle, and high socioeconomic lactating women were 4.6; 4.99; and 5.83 g/dL respectively. The patterns also applied to the serum protein profile of the neonates (8).

Gestational age is a significant factor which correlated with serum protein profile. Study by Adejeji et al in Nigeria age revealed that serum total protein, albumin, and globulin were all getting lower over the advancing gestational age. The albumin: globulin ratio also accordingly decreases from 1.4 in the first 8 weeks to 1.0 in the 33-36 weeks of pregnancy. With the mean values of serum proteins 58.2; 29.2; and 29.1 mg/dl, respectively in the near-term pregnant women, those values were significantly lower than that of values found in this study (9). Another study assessing the protein profile in pregnant women in India showed similar trend of significant decrement in serum albumin and globulin level but not in the total protein level. The values of each serum protein parameters in that study were 3.39; 4.11; and 7.51, respectively and albumin/globulin ratio of 0.824 in the third trimester group (10).

A study by Ahmad et al which evaluated the correlation between total plasma IgM and IgG level with C3 and C4 complement protein as well as the spontaneous abortion occurrence showed that the plasma mean total IgM and IgG level in pregnant women were 237.79±24.53 mg/dl and 1235±70.09 mg/dl, respectively which were not significantly different with non-pregnant nor mothers having spontaneous abortion (11). The mean IgG data from that study was similar with the finding of this study (1255.9±230.24 mg/dl) although in this study, the mean IgM level was significantly lower (135.82±53.7 mg/dl) (9). The pregnant women were often exposed to various infection and the other noxious stimuli hence their immunoglobulin level was higher than non-pregnant/ lactating population.

This study demonstrated that age, BMI, and parity were not correlated with plasma protein level profile which in part can be explained that the included subjects in this study were relatively homogeneous in terms of those anthropometric parameters. It was also the case for the socioeconomic status in which most of the subjects were lower-to-middle class living the outskirts of the town. Although unidentified in this study, differences in plasma protein level could also be affected by the presence of metabolic diseases such as diabetes. As observed in the study by Susith et al, there were significant differences in the level of ischemia-modified albumin diabetes only, diabetes with hypertension, and healthy population (12). Moreover, history of undergoing invasive diagnostic or therapeutic procedures should also be taken into consideration as the level of ischemia-modified albumin was also affected by contrast-enhanced computed tomography (CECT) (13).

There are several limitations in this study. First, this study did not evaluate the nutritional status of the mother by the other methods such as food recall method that assessed daily nutritional intake adequacy, anthropometric measurements such as body composition values with bioelectrical impedance analysis, or other objective parameters of subjects' nutritional status. Secondly, the limited, non-randomized number of samples from relatively narrow area may hinder the generalizability of the data to represent the whole lactating women population in the province. Third, there were so many laboratory parameters of kidney and liver functions which were not tested in this study while those values may be beneficial to exclude the presence of disease on those organs which might interfere the main findings of this study.

The plasma protein profile of lactating women in Jakarta region was adequate with normal level of total protein, albumin, globulin, total IgM, and IgG levels. The plasma protein profile was not correlated with maternal age and BMI. Further studies with broader inclusion criteria as well as women with specific nutritional problem are required to assess the applicability of plasma protein profile measurement as a parameter of protein nutritional intake in lactating women.

Acknowledgements

The research was funded by PUTI grant (Hibah Publikasi Terindeks Internasional) Universitas Indonesia with the grant number of NKB-1896/UN2.RST/HKP.05.00/2020.

The authors declared that there were no conflicts of interests.

References

1. Ministry of Health, Republic of Indonesia. Basic Health Research Indonesia 2018. Available from: https://kesmas.kemkes.go.id
2. Elwakiel M, Boeren S, Hageman JA, Szeto IM, Schols HA, Hettinga KA. Variability of serum proteins in Chinese and Dutch human milk during lactation. Nutrients. 2019;11(3):499.
3. Warburg O, Christian W. Isolierung und Kristallisation des Garungs ferments Enolase. Biochem Z. 1941;310:384-421.
4. Harding JR, Keyser JW. Bromocresol Green as a Reagent for Serum Albumin. Proc Assoc din Biochem. 1968;5:51-53.
5. Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. 1965;2(3):234-254.
6. Gomez-Cantarino S, Agullo-Ortuno M, Dios-Aguado M, Ugarte-Gurrutxaga M, Bouzas-Mosquera C. Prevalence of hypoproteinemia and hypoalbuminemia in pregnant women from three different socioeconomic populations. Int J Environ Res Public Health. 2020;17(17):6275.
7. Wulandari S, Defrin, Reza M. Relationship between protein intake and serum albumin levels in first trimester chronic energy deficient pregnant women in Padang city. Science Midwifery. 2021;9(2):449-453.
8. Pervaiz Sh, Rukh G, Qayyum M. Serum protein status in women of different age and socioeconomic background and their neonates. Int J Agri Biol. 2001;3(4):394-396.
9. Adedeji AL, Adedosu OT, Afolabi OK, Badmus JA, ie LO E, Fatoki JO, et al. Serum protein profile in Nigerian women: an analysis by gestation age. Clastogenic and toxicological assessment of cashew (Anacardium occidentale) nut bark extracts in Wistar rats. 2012;4(11):38-42.
10. Das A, Teli AB, Borkotoki S. Study of liver function tests in normal pregnancy: a hospital based study. Int J Health Sci Res. 2016;6(8):156-160.
11. Ahmad HA, Muhammdsalih MM, Khidir KA. Complement protein and immunoglobulins serum levels in normal pregnant and spontaneous aborted women. Kurdistan Journal of applied research. 2018;129-133.
12. Sushith S, Krishnamurthy H N, Reshma S, Janice D, Madan G, Ashok K J, et al. Serum Ischemia-Modified Albumin, Fibrinogen, High Sensitivity C- Reactive Proteins in Type-2 Diabetes Mellitus without Hypertension and

Diabetes Mellitus with Hypertension: A Case-Control Study. Rep Biochem Mol Biol. 2020;9(2):241-249.
13. Jha C, Ullas Kamath S, Dash S, Prabhu Attur R, Ramachandra L, Shenoy Kallya R. Ischemia-Modified Albumin, Creatinine, and Paraoxonase-1 Levels in Serum of Patients Undergoing Intravenous Contrast-Enhanced Computed Tomography and Its Association with Contrast-Induced Nephropathy. Rep Biochem Mol Biol. 2019;8(1):72-78.