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**CORRELATION BETWEEN MALONDIALDEHYDE AND NERVE GROWTH FACTOR SERUM LEVEL IN
PATIENT WITH DIABETIC PERIPHERAL NEUROPATHY**

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ABSTRACT

Aim

To identify the correlation between malondialdehyde (MDA) level with nerve growth factor (NGF) in the serum among patients with diabetic peripheral neuropathy.

Methods

A cross-sectional study was conducted to observe the inpatients and outpatients diabetic patients in Dr. M. Djamil Hospital, Padang, West Sumatra. Sample size was calculated using a formula to find a single correlation. The serum NGF level was analysed using ELISA method for human nerve growth factor- β . The concentration of MDA was measured using Beuge method with thiobarbituric acid. Peripheral neuropathy was defined when the Michigan Neuropathy Screening Instrument (MNSI) score ≥ 7 and the foot examination score > 2 .

Results

Mean score of the thirty subjects for the neuropathy score was 3.53 (± 0.91). The mean of MDA level was 2.16 (± 2.89) nmol/ml, while for NGF level was 10.56 (± 2.89) pg/dl. There were significant correlations between the MDA and the NGF serum level ($r = -0.037$, $p = 0.044$), the MDA and the neuropathy score ($r = 0.364$, $p = 0.048$) and the NGF level with the neuropathic score ($r = -0.59$, $p = 0.001$).

Conclusion

Malondialdehyde and nerve growth factor have a role in the neuropathy development among diabetes patients, nerve growth factor has a stronger role in the neuropathy development.

Keyword: Malondialdehyde, Nerve Growth Factor, Diabetic Peripheral Neuropathy

ABSTRAK

Latar belakang

Untuk mengetahui hubungan kadar malondialdehid (MDA) serum dengan kadar *nerve growth factor* (NGF) serum pada pasien neuropati perifer diabetes.

Metode

Penelitian ini adalah penelitian observasional dengan metode potong lintang yang dilakukan di Instalasi Rawat Inap dan Rawat Jalan Penyakit Dalam RSUP Dr. M. Djamil Padang. Besar sampel ditetapkan dengan menggunakan rumus besar sampel untuk korelasi tunggal. Pemeriksaan NGF serum dengan metode ELISA untuk *human nerve growth factor-β*. Kadar malondialdehid diukur menggunakan metode Beuge dengan menggunakan asam tiobarbiturat. Neuropati perifer ditentukan dengan skor MNSI ≥ 7 dan skor pemeriksaan fisik kaki > 2 .

Hasil

Rerata skor neuropati dari tiga puluh pasien adalah 3,53 ($\pm 0,91$), sedangkan rerata kadar MDA adalah 2,16 ($\pm 0,28$) nmol/ml, serta NGF 10,56 ($\pm 2,89$) pg/dl. Terdapat korelasi bermakna antara kadar MDA serum dengan kadar NGF serum ($r = -0.37$, $p = 0,044$), kadar MDA dengan skor neuropati ($r = 0.364$, $p = 0,048$) dan kadar NGF dengan skor neuropati ($r = -0.59$, $p = 0,001$).

Kesimpulan

Malondialdehid dan *nerve growth factor* memiliki peran terhadap terjadinya neuropati perifer diabetik, dalam hal ini *nerve growth factor* memiliki peran yang lebih kuat pada neuropati perifer diabetik.

Kata kunci: Malondialdehid, Nerve Growth Factor, Neuropati Perifer Diabetes

INTRODUCTION

Diabetes Mellitus is a chronic disease in which the prevalence is the highest in the world. This may cause a long term health problem and multiple quality of life depriving complications, i.e. diabetic neuropathy.¹ Diabetic peripheral neuropathy has a serious impact to the quality of life and may increase the cost of the health care. Patients with painful neuropathy compared with the painless ones, show a decreasing quality of life and increasing cost of living in long time.

Chronic hyperglycemia inflicts oxidative stress, damaging endothelium, and causing microvascular and hemorheological disturbances. By the end it will diminish the nutrient and oxygen supply for the nerve cells, resulting in interfering the life of the nerve and the Schwann cells and increasing the cell degeneration.^{7,3} Nerve Growth Factor (NGF), produced by the neurons and the Schwann cells, is an important protein to maintain and for the survival of the neurons. It works by increasing cell regeneration and decreasing the degeneration. Insufficiency of the NGF induces peripheral nerve lesions in the form of axonal atrophy, demyelination, and reduced number of nerve fibers, causing uncontrollable peripheral neuropathy.^{Error! Reference source not found.,5} Pittinger and Vinik (2003) found that dwindling of NGF in diabetes correlates with the clinical symptoms of small nerve fiber dysfunction.⁶ Yasuda et al (2003) discovered a positive correlation between decreasing NGF level and the neuron regeneration.⁷

Increasing number of free radical oxygen escalates the level of oxidative stress marker, such as malondialdehyde (MDA) and lipid hydroperoxides. The MDA, product of the lipid peroxidation, has been accepted as one of the reliable biological marker for oxidative stress, based on BOS (Biomarker Oxidative Stress) Study in 2002.^{Error! Reference source not found.,3,10} The oxidative stress may impact peripheral nerve especially among those who are at risk, in this

case: diabetes. Screening for peripheral neuropathy is crucial as an early detection of the nerve damage, but also useful to evaluate the treatment result. This will be beneficial to identify risk of ulcer and therefore, preventing amputation. It can be done using instrument like Michigan Neuropathy Screening Instrument (MNSI).^{13,12} The objective of this study is to identify the correlation between the MDA and the NGF serum level among patients with diabetic peripheral neuropathy.

METHODS

Study design

This was a cross-sectional study, conducted in internal medicine ward and outpatient unit in Dr. M. Djamil Hospital, Padang, West Sumatra.

Subjects

The subject was diabetes mellitus type II (T2DM) patients with peripheral neuropathy, aged 18-59 years old. All subjects agreed to participate and signed the informed consent form. Patients with chronic renal disease, cirrhosis, stroke, alzheimer's disease, allergy, asthma, rheumatoid arthritis, psoriatic arthritis, systemic lupus erythematosus, systemic sclerosis and other autoimmune disorder, leprosy, anemia, thrombocytosis, patients currently using antituberculosis drugs, cytostatic drug, steroid, alcohol, and patients with foot ulcer that is difficult to examine were excluded in the study.

The sample size was calculated using a formula to estimate a single correlation, and resulted in thirty subjects.¹³

Data collection

The peripheral neuropathy was defined by MNSI score '7' or higher and the foot examination score was more than two. The MNSI is a self-fulfilled questionnaire, but could be interviewed by the examiners. The score was calculated thereafter, and whenever the score reached seven or higher, the patients proceeded to the foot examination.¹¹

The foot examination was inspection, vibratory sensation using 128 Hz tuning fork, ankle jerk reflex, and 10g monofilament testing. The foot inspection was scored 1 when the skin was dry, callused, fissured, ulcerated, or deformed. The vibratory sensation was examined by placing the fork on the projection of distal interphalangeal joint of the hallux. With closed eyes, subjects would tell the examiner whether they felt the vibration when it disappeared. When subjects did not report any vibration, the score was zero. However, if the subjects felt the vibration less than 10 seconds, they would get score 0.5. Score 1 was given for the achilles reflex when there was no reflex. A reflex appeared only after assisted by Jendrassic maneuver was scored 0.5. The monofilament testing was done on ten points in each feet. The monofilament was perpendicular and pressed for two seconds only, while the subjects closed their eyes. All points would be tested three times. Score zero would be given for eight right answers or more, and half point for one two seven right answers.¹²

About 3 mL blood samples were taken from the cubital vein for MDA and NGF using a anticoagulant-free container. When serum was formed, NGF level was analysed using ELISA method for human nerve growth factor- β . The concentration of MDA was measured using Beuge method with thiobarbituric acid.

Data analysis

The categorical variables of the demographic data of the subjects was presented in proportion

of the subjects, while the numerical data would be presented as mean and standard deviation. The normality test was performed only for the numerical data using Kolmogorov-Smirnov test.

Correlation between MDA and NGF, also with neuropathy score, were examined using Spearman correlation test, as the number of the subjects was considered small. Coefficient correlation was calculated and the level of significance for the correlation was counted.

RESULT

The characteristics of the subjects was presented in table 1. The study involved subjects with age ranged from 41 to 59 years old. There were 8 out of 30 subjects (27%) with foot ulcer although no patients with neuropathic score of more than 7 in this study. The range of the serum MDA in this study was between 1.6 nmol/mL to 3.01 nmol/ml while while the NGF concentration ranged from 6.8 pg/dl up to 15.7pg/dl.

Figure 1 shows a weak negative correlation was significantly found between MDA serum level and NGF serum level ($r = -0.37$, $p = 0.044$). A positive correlation was proved between the MDA serum level, as the increasing of the oxidative stress increased the neuropathy score (figure 2), although the correlation was weak ($r = 0.364$, $p=0.048$).

The correlation between NGF serum and neuropathy was the strongest among all other correlation (figure 3). Moreover this model might explained 50% of the findings among the subjects, compared to the other linear correlation of the other variables ($r = -0.59$, $p=0.001$).

DISCUSSION

This study found potential used of MDA among the T2DM patients to predict peripheral neuropathy, as positive correlation was found significant among MDA level and the

neuropathy scores. As expected the MDA level had a negative correlation with NGF serum level. This showed the pathogenesis of neuropathy might be affected by oxidative stress beside the progress of diabetes itself. Tosaki et al (2008) reported that the Schwann cell planted on high-glucose culture media, had low concentration of NGF.¹⁴ Yilmaz et al (2013) obtained the result of lower NGF production by the Schwann cell of diabetic subjects.¹⁵ The mean MDA serum level of this study was 2.16 (0.28) nmol/ml (normal value 0.9-1.59 nml/ml). Maitreyess (2011) and Gallan (2003) has also found that MDA serum level on DM type 2 with complication was higher than the no complication group.^{16,17} The mean score for the serum level of NGF was 10.56 pg/dL in this study. Lang et al (2003) reported the mean of NGF serum 71.37 (335) pg/ml, significantly higher as compared with healthy population, (18.6 (6.8) pg/dl).¹⁸ Mahmoud et al in 2009 found a significant lower NGF serum level among patients with peripheral neuropathy.¹⁹ Obrosova et al (2001) had shown that NGF level in diabetic patient was lower than control.²⁰

Though there was a positive findings in this study, potential confounder was not well adjusted in the model. That might be the reason of weak correlation of the findings. Age of the subjects would have an influence with the neuropathy , as people grow older. The average age of the subject was 53.9 years old, closed to the Noha and Gamal (2012), 50.1 (1.8) years old.²¹ Lu et al (2010) reported that about 61.8% of the subject was above thirty years old²², while Gregg et al (2004) reported that all the cases were in the age of more than forty, the same as our subjects.²²

Moreover, older subjects tended to had longer diabetes and this might influence the peripheral neuropathy. Papanus and Zigler (2012) presented that the peripheral neuropathy is linked with the long duration of the diabetes itself.^{24,25} It is confirmed with the subjects of

this study. Mean duration of diabetes was 7.6 (4.2) years.

The second potential confounder was whether the blood glucose level was controlled. It had been found that the mean of the HbA1c in this study was 8.8%. Hussain et al (2013) showed in their study that the uncontrolled hyperglycemia (HbA1c 7.5 %) was proportional to the diabetic neuropathy.^{26,27} Of all subjects in this study, 23 persons had concentration of HbA1C more than 7.5%.

The next potential confounder was body mass index. The mean score for the BMI in this study was 24.6 kg/m². A study showed that BMI is a risk factor for polyneuropathy, with obesity is a independent factor for peripheral neuropathy in diabetes.²⁵ Similarly with the above findings, there were 22 subjects had BMI higher than 23.9 Kg/m², the cut-off level to be considered normal BMI. There were even subjects with BMI more than 30 Kg/m².

CONCLUSION

Malondialdehyde and nerve growth factor have a role in the neuropathy development among diabetes patients, nerve growth factor has a stronger role in the neuropathy development.

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Table 1. Characteristics of the subjects

| Variable (unit) | n (%) | mean (SD) |
|--------------------------------------|---------|--------------|
| Age (years) | | 53.9 (5.7) |
| Sex | | |
| Male | 13 (43) | |
| Female | 17 (57) | |
| Body Mass Index (Kg/m ²) | | 24.6 (3.7) |
| Overweight or obesity | 19 (63) | |
| Foot ulcer | 8 (27) | |
| Neuropathic score | | 3.53 (0.91) |
| HbA1c (%) | | 8.8 (2.0) |
| MDA (nmol/mL) | | 2.16 (0.28) |
| NGF (pg/dL) | | 10.56 (2.89) |

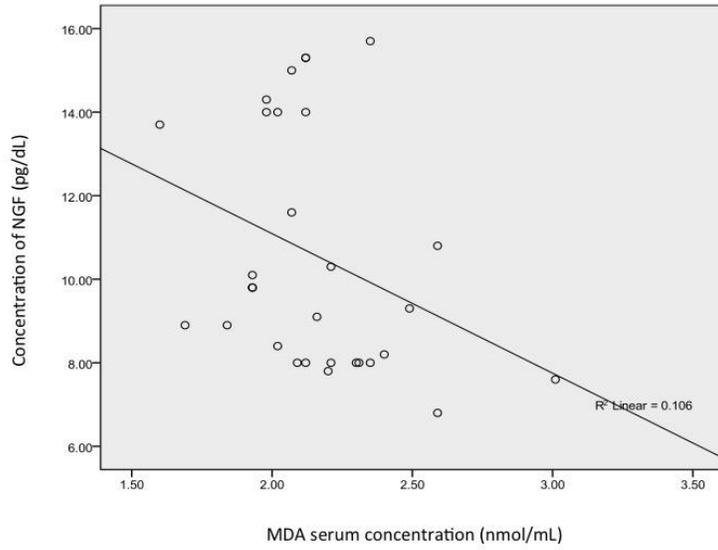


Figure 1. Correlation between MDA serum level and NGF serum level. Spearman correlation test $r=-0.37$ and $p = 0.044$

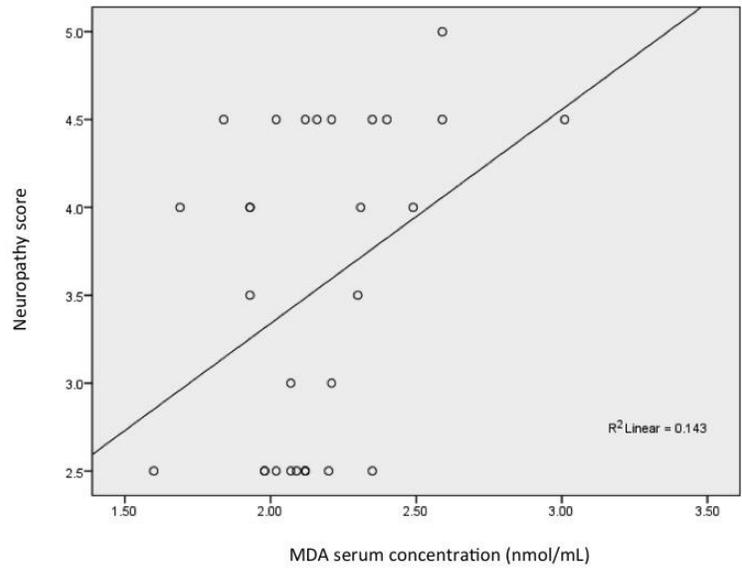


Figure 2. Correlation between the MDA serum level and the neuropathy score ($r=0.364$ and $p = 0.048$)

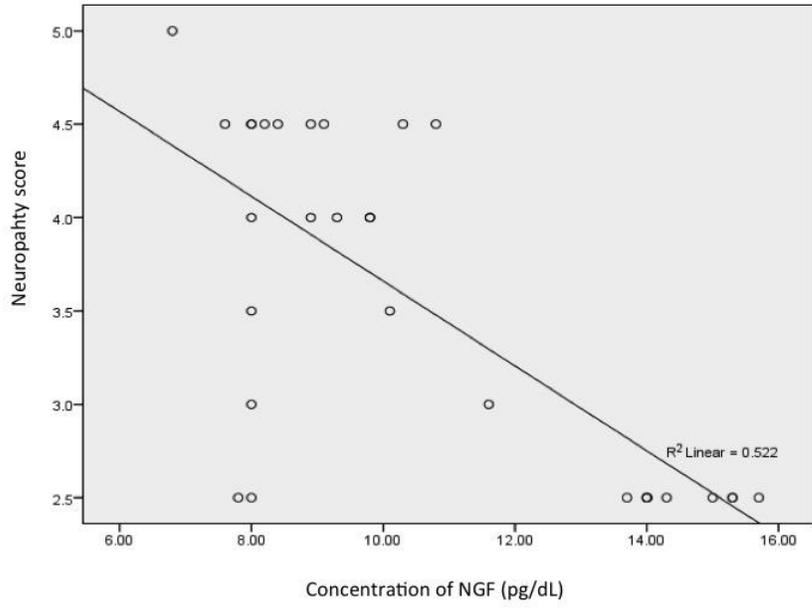


Figure 3. Correlation between NGF Serum and neuropathy score ($r=-0.59$ and $p=0.001$)