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Ethanolic extract of the *Centella asiatica* (L.) Urb. leaf decreases cerebellar brain-derived neurotrophic factor (BDNF) levels in rats after chronic stress

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ABSTRACT

Chronic stress produces glucocorticoid-induced neurotoxicity that may lead to alterations of the brain-derived neurotrophic factor (BDNF) concentration in the brain. Cerebellum is known to be severely affected by glucocorticoids-associated oxidative damage. *Centella asiatica* (L.) Urb. may protect neurons from oxidative damage. This study aimed to investigate the effect of ethanolic extract of *C. asiatica* (L.) Urb. leaf on the rat cerebellar BDNF levels following stress. Twenty young-adult male Sprague Dawley rats were randomly assigned into four experimental groups. The stress control group received aquadest, and the other groups were treated with different doses of the *C. asiatica* (L.) Urb. extract i.e 150 (CeA150), 300 (CeA300) and 600 (CeA600) mg/kg body weight/day orally, respectively and followed by chronic footshock stress for 28 days. Upon completion of the experimental period, all animals were sacrificed and the cerebellar was isolated. The BDNF levels from the cerebellar tissue lysate was measured using ELISA. The mean BDNF levels of the cerebellar tissue in the stress control, CeA150, CeA300 and CeA600 groups were 1217.10 ± 301.40; 771.46 ± 241.45; 757.05 ± 268.29; and 627.00 ± 246.02 pg/mL, respectively. Post-hoc analysis showed a significant difference between the control and treatment groups ($p < 0.05$). In conclusion, the ethanolic extracts of the *C. asiatica* (L.) Urb. leaf decrease the cerebellar BDNF levels in rats after chronic stress.

ABSTRAK

Stress kronis menyebabkan terjadinya sekresi glukokortikoid sehingga menginduksi terjadinya neurotoksisitas dan memicu terjadinya perubahan konsentrasi *brain-derived neurotrophic factor* (BDNF) pada otak. Cerebellum sangat dipengaruhi oleh glukokortikoid yang berhubungan dengan stres oksidatif. *Centella asiatica* (L.) Urb. dikenal dapat melindungi neuron dari kerusakan akibat stres oksidatif. Tujuan penelitian ini adalah mengkaji pengaruh *C. asiatica* (L.) Urb. terhadap konsentrasi BDNF di cerebellum pada kondisi stres. Dua puluh tikus jantan Sprague Dawley dibagi menjadi 4 kelompok perlakuan, yaitu kontrol, ekstrak *C. asiatica* (L.) Urb. 150 (CeA150), 300 (CeA300), 600 (CeA600) mg/kg berat badan/hari dan diikuti oleh pemberian stress kronik (*footshock stress*) selama 28 hari berturut-turut. Diakhir perlakuan, semua hewan coba diterminasi

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dan dilakukan isolasi protein dari cerebellum. Konsentrasi BDNF dari cerebellum diukur dengan menggunakan metode ELISA. Rerata konsentrasi BDNF untuk kelompok kontrol, CeA150, CeA300, CeA600, berturut-turut adalah 1217,10 ± 301,40; 771,46 ± 241,45; 757,05 ± 268,29 dan 627,00 ± 246,02 pg/mL. Hasil uji analisis post-hoc menunjukkan terdapat perbedaan yang signifikan antara kelompok kontrol dan perlakuan ($p < 0,05$). Dapat disimpuakan, ekstrak etanol *C. asiatica* (L.) Urb. menurunkan konsentrasi BDNF pada cerebellum tikus setelah diinduksi stres kronis.

Keywords: BDNF - *Centella asiatica* (L.) Urb. - cerebellum - chronic stress - prevention

INTRODUCTION

Centella asiatica (L.). Urb. or 'Gotu Kola' is a tropical medicinal herb plant that flourishes in swampy areas of Asian countries. The primary active constituents of *C. asiatica* (L.) Urb that have important medicinal applications are triterpenoids and flavonoids.[1] These triterpenes which include asiatic acid and asiaticoside, exert a significant neuroprotective effect on the rat brain against oxidative damage[2] due to their high antioxidant activity and the reduction of the permeability of the blood-brain barrier.[3] Previous studies showed that the administration of ethanol extracts of *C. asiatica* (L.) Urb. in rats following chronic stress affects the concentration of serum and hippocampal brain-derived neurotrophic factor (BDNF), which is an endogenous neuroprotective agent in the brain.[4-6] The brain is the central organ for stress adaptation and is also a target of stress.[6] Chronic stress may result in abnormal changes in brain plasticity, including dendritic retraction, neuronal toxicity and suppression of neurogenesis, as well as axospinous synaptic plasticity.[7] Repetitive stress exposure will gradually change the electrical characteristic, morphology, and proliferative capacity of neurons.[8] Several studies on animals have examined the involvement of the oxidative status in the adaptive response of the animals to stress. Physical or psychological stressors cause oxidative damage by inducing an imbalance between the pro-oxidant and antioxidant status.[9]

Of the brain regions, the cerebellum is known to be severely affected by oxidative damage associated with glucocorticoid levels due to high levels of glucocorticoid receptors localized in the external granular layer.[10] Several reasons might contribute to its high vulnerability: the large quantity of oxidizable lipids; high iron content; and relatively low level of antioxidant defense molecules, mainly glutathione and vitamin E.[11] A robust increase in basal cerebellar oxidative stress causes abnormal changes, such as poor dendritic arborization of Purkinje cells, alterations of the cellular organelles and neuronal death.[12] Chronic stress has been shown to down-regulate the glucocorticoid receptors of mRNA in the granular and Purkinje cell layers.[13]

BDNF, a member of the neurotrophin family, is known to be a strong survival promoting factor and plays a critical role in cell proliferation and differentiation, neuronal protection, and regulation of synaptic functioning in the central nervous system.[14] BDNF is highly expressed in the cerebellum, mainly in granular cells. Both granular and Purkinje cells express the BDNF receptor tropomyosin-receptor kinase B (TrkB).[15] Gene deletion of mouse BDNF increases granular cell death, stunts the growth of Purkinje cell dendrites, and impairs the foliation patterns. Previous studies have shown that BDNF can

also protect neurons from injuries caused by hypoglycemia, ischemia, hypoxia and neurotoxicity.[16]"

Acute and chronic stress alter the expression of BDNF and TrkB in the brain.[17] Some studies have shown that chronic stress decreased BDNF mRNA and protein expression in the hippocampus, but acute stress has the opposite effect.[18] In contrast, chronic multiple stress has been shown to induce a significant increase in BDNF and TrkB protein expression in the hippocampus.[19] The up/down-regulation of BDNF is affected by the type of stressors, the intensity, the duration, and the number of exposures.[20]

Although the impact of stress on the BDNF levels displayed different results, BDNF is believed to protect neurons from injuries caused by stress. However, previous clinical trials have reported that BDNF had poor penetration through the blood brain barrier, making it very difficult to use as a drug.[21] Therefore, we investigated the neuroprotective effect of ethanolic extract of *C. asiatica* (L.) Urb. leaf on the cerebellar tissue BDNF levels in chronically stressed rats.

MATERIALS AND METHODS

Animals

Twenty young-adult (8 weeks) male Sprague Dawley rats weighing 100-150 g were obtained from the Animal Model Care Unit, Universitas Gadjah Mada, Yogyakarta, Indonesia and were housed under standard laboratory conditions (at 25-30°C; 50%-60% humidity; with 12 h light and dark cycles). All animals were given access to the diet and tap water *ad libitum*. The rats were placed in glass cages, with two animals per cage, and were allowed to acclimatize one week prior to treatment.

The animals were randomly assigned into four experimental groups, with five rats per group: the stress control, CeA150, CeA300 and CeA600 groups. The control group received aquadest alone, and the other groups were treated with different doses (mg/kg BW/day, p.o.) of *C. asiatica* (L.) Urb. leaf ethanolic extracts: 150 (CeA150), 300 (CeA300) and 600 (CeA600), followed by chronic footshock stress for 28 days. All experimental procedures were approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Gadjah Mada University (Ref. KE/FK/657/EC).

Administration of ethanolic extracts of *C. asiatica* (L.) Urb.

The fresh leaves of *C. asiatica* (L.). Urb. were procured from the commercial herb manufacturer (Merapi Farma Herbal, Sleman, Yogyakarta), and samples of the plant were identified and authenticated for their correct botanical identity at the Faculty of Biology, Universitas Gadjah Mada. Ethanolic extracts of *C. asiatica* (L.) Urb. were obtained using maceration methods from the Integrated Testing and Research Labortory, Universitas Gadjah Mada. The leaves were cleaned, air-dried, and made into powder. The powder was soaked in a 70% ethanol solution in a shaking incubator for 2 days. The extracted solution was filtered through filter paper and concentrated. The concentrate was evaporated through a vacuum rotary evaporator at 70°C followed by a water bath. The filtrate from this process was weighed and dissolved in a sterile aquadest to create the various dose-dependent preparations (150, 300, and 600 mg/kg BW). The ethanolic extracts of the *C. asiatica* (L.) Urb. leaf were administered orally using an oral gastric tube for 28 consecutive days with weekly weight-adjusted doses.

Stress induced procedure

Thirty min after the oral administration of *C. asiatica*, (L.) Urb. each rat was subjected to footshock stress using a plexiglass rodent shock box. The plexiglass rodent shock box consisted of a box containing an animal space which is positioned on a metallic grid floor connected to a shock generator. Each rat was placed in the box and received 10 min/day inescapable footshock stress (a footshock of 0.8 mA/50 V in intensity and 10 s in duration with a 15-s interval). Footshock stress was given chronically for 28 consecutive days.

Fecal pellets procedure

The fecal pellets were quantified after the rats got electrical stress. The fecal pellets were directly collected after the rats underwent electrical stress in the plexiglass rodent shock box. Each fecal pellets was collected with a small forcep and counted it manually.

Cerebellar tissue collection

Upon completion of the experimental period, the animals were decapitated according to the Institutional Animal Care and Use Committee (IACUC) instructions. Brain tissue was dissected immediately over an ice pack, and the cerebellar tissue was separated manually from the other brain region. Cerebellar tissue was then stored in safe lock tubes at -80°C until they were used for immunoblotting and ELISA. For immunohistochemistry (IHC) study, the animals were perfused with normal saline. After that the brains were quickly removed and stored in 4% paraformaldehyde fixative solution.

Immunohistochemistry procedure

Before proceeding to the staining protocol, the tissue sections were deparaffinized and rehydrated with xylene and alcohol. Endogenous peroxidase was blocked by preincubating the tissue sections in 0.3% H_2O_2 with methanol. Antigen retrieval was done by heating the slides in a sodium citrate buffer. Finally, immunohistochemistry staining was processed with an HRP universal detection kit (Starr Trek from Biocare Medical®; Cat. No. STUHRP700 H, L10). Inappropriate protein was blocked by a background sniper (protein blocker), and we applied the rat BDNF-antibody (Abcam®; Cat. No. ab80436) overnight. The tissue sections were washed with PBS buffer and then incubated with secondary antibody. The HRP conjugate was applied, and subsequently, the tissue sections were incubated in betazoid DAB chromogen. The counter stain solution (hematoxyllin Meyer) was applied, and the dehydrated tissue with alcohol and xylene was mounted/coverslipped.

The extraction of cerebellar tissue protein

Protein from the cerebellar tissue was extracted using the Pro-Prep™ (Intron Biotechnology; Cat. No. 17081) protein extraction solution according to the manufacturer's instructions. Twenty mg of cerebellar tissue were mashed and homogenized with approximately 600 µL of Pro-Prep™ solution. The homogenates were incubated for 30 min at -20°C and were centrifuged at 12,000 rpm at 4°C for 5 min. The supernatants were stored in safe lock tubes at -80°C until they were assayed.

The measurement of cerebellar tissue BDNF concentrations

The cerebellar tissue BDNF levels were measured using a Rat BDNF enzyme-linked immunosorbent assay (ELISA) kit (Boster Immunoleader; Cat. No. EK0308) according

to the manufacturer's instructions. A standard curve ranging from 31.2 to 2000 pg/mL of BDNF was obtained by the absorbance at 450 nm. This curve was used to create a formula to determine the BDNF levels from the optical density.

Statistical analysis

The data are presented as the mean ± standard deviation (SD). Differences among groups were calculated by the parametric one-way ANOVA test using SPSS v 16.0 software. Post hoc analysis was conducted to determine differences between each group. A p value of less than 0.05 were considered statistically significant.

RESULTS

Body weight rats

Comparisons of the mean body weights before and after the stress induction for all of the groups are presented in TABLE 1. A significant difference in the mean body weight before and after the stress induction was observed ($p < 0.05$) indicating that the chronic footshock stress significantly increased body weight for all of the groups.

TABLE 1. The body weights (mean ± SD g) before and after the stress procedures

Group	n	Before stress induction	After stress induction	p
Control	5	158±25.88	192±17.88	0.042
CeA150	5	140±7.07	192±8.36	0.034
CeA300	5	184±15.16	220±24.49	0.042
CeA600	5	176±8.94	206±13.41	0.039

Fecal pellet

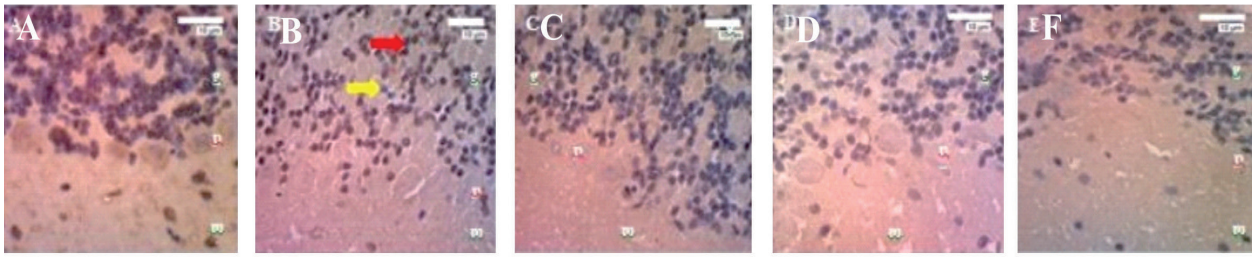
The means of the fecal pellets during the stress induction are presented in TABLE 2. The data show that chronic footshock stress induced defecation reflexes in all groups. The group with the most fecal pellets was the CeA300 group (5.8±1.08 units), and the group with the least fecal pellets was the Control group (4.32±0.77 units).

TABLE 2. The fecal pellet production during the stress induction

Group	n	Fecal pellet (mean ± SD units)
Control	5	4.32 ± 0.77
CeA150	5	4.87 ± 0.36
CeA300	5	5.80 ± 1.08
CeA600	5	5.15 ± 0.66

Cerebellar tissue BDNF immunohistochemistry examination

BDNF protein was expressed in the granular, Purkinje, and molecular cell layers of the cerebellar cortex hemisphere (FIGURE 1). Chronic footshock stress increased the cerebellar tissue protein expression in rats demonstrated the higher BDNF protein expression in the control group (B) compared to that the negative control/non-stressed rat (A). The administration of ethanolic extract of *C. asiatica* (L.) Urb. leaf in CeA150 (C), CeA300 (D) and CeA600 (E) followed by chronic footshock stress decreased cerebellar tissue BDNF expression.



light microscope with a 10x magnification level. (A) negative control group (non-stressed rat); (B) Control stress group (aquadest); (C) CeA150; (D) CeA300; (D) CeA600; (g) granular cell layer; (p) Purkinje cell layer; (m) molecular cell layer; Red mark displayed BDNF expression; Yellow mark did not display BDNF expression.

Cerebellar tissue BDNF levels

The mean cerebellar tissue BDNF levels for all groups are presented in FIGURE 2. A significant difference of mean cerebellar tissue BDNF levels between groups was observed ($p<0.05$). Furthermore, the cerebellar tissue BDNF levels were significantly lower after ethanolic extract of *C. asiatica* (L.) Urb. treatment (groups CeA150, CeA300, and CeA600) compared to that the control group ($p < 0.05$). However, the cerebellar tissue BDNF levels were not significantly different between the dose of ethanoic extract of *C. asiatica* (L.) Urb. treatment groups ($p>0.05$).

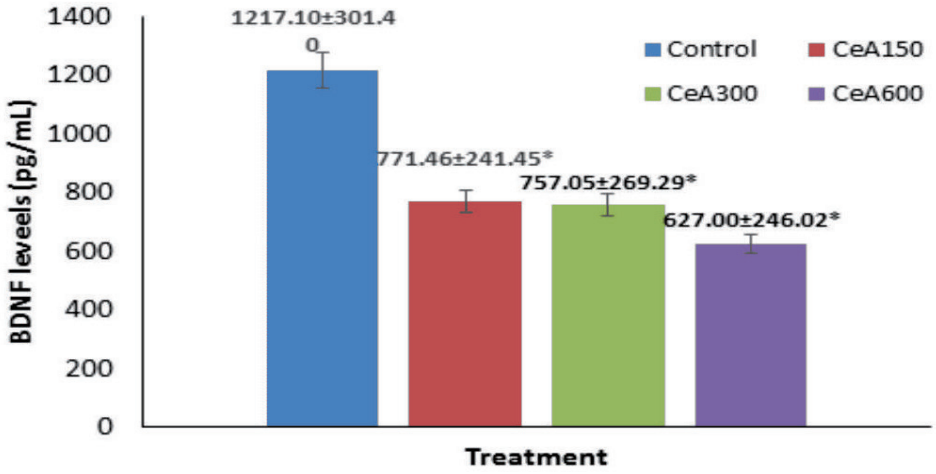


FIGURE 2. Rats cerebellar tissue BDNF levels after the administration of ethanolic extract of *C. asiatica* leaf followed by chronic footshock stress. *significantly different compared to control ($p<0.05$).

DISCUSSION

In this study, we assumed that the chronic footshock stress procedure caused functional changes in rats, such as increased body weight and induced defecation. We also assumed that the brain, especially the cerebellum, responded to this chronic stress procedure through structural and chemical changes. Prolonged or

extreme stressors induce abnormal changes in brain plasticity that, paradoxically, impair the ability of the brain to regulate and respond to subsequent stressors.[8]

Both acute and chronic stress can influence eating patterns. The severity of the stressor seems to influence food intake in the rat model. Chronic stress may increase food intake and body weight when the foods offered are highly palatable (high-fat diet).[22] This phenomenon is believed to be due to a greater preference for energy and nutrient dense food during and after stressors. Increased ingestion of palatable food may reflect a pleasing activity that reduces the discomfort of stress.[23] Chronic stress induces the activation of the HPA axis and may stimulate orexigenic neuropeptides, including neuropeptide-Y (NPY) and agouti related peptide (AgRP).[24] On the other hand, glucocorticoids may inhibit the release of an anorexigenic hormone, such as the corticotropin-releasing hormone (CRH), and cause hyperphagia.[23,24]

The gastrointestinal tract is particularly sensitive to stress. Both acute and chronic stress induce colon mucosal hyper responsiveness.[25] Previous studies showed that central CRH signaling mediates gastrointestinal responses to stress.[26] Chronic stress exposure has been proven to increase colon motility and induce the defecation reflex.[27] It is also believed to be due to the release of peripheral 5-HT mediated by CRH. A previous study showed that the administration of a 5-HT3 receptor antagonist inhibited CRH-induced defecation in a dose-dependent manner.[28] This finding has clinical relevance for symptoms of irritable bowel syndrome (IBS) caused by the activation of CRH pathways.[26]

In this study, chronic footshock stress might increase the cerebellar tissue BDNF leves in rats, which has been demonstrated through the immunohistochemistry staining of the cerebellar cortex layers with a rat BDNF-antibody (FIGURE 1). The rat BDNF protein expression in the stress control group was higher than that in the negative control group (non-stressed rats). A previous study reported that early postnatal repeated maternal deprivation caused a transient increase in rat BDNF mRNA and protein levels.[29] In contrast, some studies have shown that chronic stress decreased rat BDNF mRNA and protein expression in some brain areas.[30,31] These results prove that stress has an important role in BDNF regulation.

There are some possible explanations for the different results in this study. First, the increase of cerebellar BDNF levels in response to chronic stress may be due to the sex, strain, and age of the rat at the time of stress exposure. Previous studies reported that the cerebellum is more sensitive to stress four to nine days after birth.[32]

Second, it is possible that the discrepancy in the effect of chronic stress is due to differences in the stress protocols applied in the different studies. The effect of stress on cerebellar BDNF expression in the cerebellum appears to be dependent on several factors, such as the type of stressor, the intensity, the duration, the frequency and the number of exposures.[20] Increases in the cerebellar BDNF levels are believed to be due to alterations of the glucocorticoid receptor activity induced by stress. Both mineralocorticoid (MR) and glucocorticoid receptors (GR) mediate the bidirectional action of neuronal cells in response to stress.[33]

Third, although speculative, it is possible that exposure to chronic stress activates mechanisms in the cerebellum that reflect an adaptive protective response.[20] The up-regulation of the cerebellar BDNF levels induced by stress may be a compensatory adaptation to repeated stress. BDNF has

been reported to protect neurons from oxidative damage caused by stress.[14] The anti-apoptosis activity of BDNF is considered to be the underlying mechanism behind the neuroprotective effect of this protein. BDNF may reduce neuron apoptosis by increasing the expression of the Bcl-2 anti-apoptosis protein and inhibiting intracellular calcium overload.[34]

BDNF increases glucose transport by inducing the expression of GLUT3 and also increases Na^+- dependent amino acid transport and protein synthesis.[35] BDNF up-regulates antioxidant enzymes and enhances the repair of damaged DNA in neurons. This study also suggests that the up-regulation of cerebellar BDNF levels could be induced by the stress-induced specific activation of some BDNF gene promoters in cerebellar macrophages or in neurons. The dopaminergic neurons that sprout as a result of stress appear to activate macrophage BDNF secretion.[36]

In this study, the administration of ethanolic extract of *C. asiatica* (L.) Urb. leaf ethanol extracts followed by chronic footshock stress significantly decreased the cerebellar tissue BDNF levels of rats in all groups. The decrease of the cerebellar tissue BDNF level is believed to be due to the down-regulation process of neurons and glia in response to the neuroprotective effect of ethanolic extract of *C. asiatica* (L.) Urb. It is still possible that the neuroprotective effect of ethanolic extract of *C. asiatica* leaf (L.) Urb. is mediated by other neurotrophic factors, such as neural growth factor (NGF), basic fibroblast growth factor (bFGF), glial-derived neurotrophic factor (GDNF), neurotrophin-3 (NT-3), and NT-4/5. It was reported that the major chemical compounds in *C. asiatica* (L.) Urb., including asiatic acid, madecassic acid, asiaticoside, madecassoside and madasiatic acid, play an important role in neuroprotective activity.[37] It is believed to be due to its high anti-oxidant and anti-apoptosis activity, which is mediated by decreasing the blood-brain barrier permeability.[3]

Administrations of ethanolic etract of *C. asiatica* (L.) Urb. prevent lipid peroxidation and limit the protein carbonyl content in the rat brain region.[38] The extract is also able to protect neuron cells from oxidative stress by inhibiting the activation of the caspase-9 pathway. The anxiolytic, antidepressant and anti-stress effects of *C. asiatica* (L.) Urb. may alter glucocorticoid activities and the adaptation response to stress.[38-41] Although a speculation, the reciprocal relationship between the cerebellum and HPA-axis was recently established by the discovery of dense glucocorticoids binding sites on the vermis.[42] In this study, we added biochemical evidence that *C. asiatica* (L.) Urb. has a role as a neuroprotective agent in the brain, mainly in the cerebellum.

CONCLUSION

In conclusion, the administration of ethanolic extract of *C. asiatica* (L.) Urb. leaf decreases the cerebellar tissue BDNF protein levels in rats after chronic stress. The BDNF protein is expressed in the Purkinje, granular, and molecular cell layers of the cerebellar cortex. It would be interesting to examine possible contributions from other neurotrophic factors, such as NGF, bFGF, GDNF, NT3 or NT-4/5.

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The effects of ethanolic extract of *Phaleria macrocarpa* (Scheff.) Boerl leaf on macrophage phagocytic activity in diabetic rat model

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ABSTRACT

Diabetic patients suffer from inflammation and immune deficiency due to the decrease macrophage activity that lead to vulnerable to infection. *Phaleria macrocarpa* (Scheff.) Boerl leaf extract has been proven increase macrophage activity and to have antiinflammatory effect. This study was conducted to investigate effect of ethanolic extract of *P. macrocarpa* (Sheff.) Boerl leaf (EEPML) on macrophage phagocytic activity in diabetic rat model. In addition, the M1 and M2 macrophage ratio was also evaluated. This was quasi experimental study with post test only control group design. Forty five male Sprague Dawley rats aged eight weeks were grouped into non diabetic control group, diabetic control group and diabetic treatment group which given with 7; 14; 28 mg/200 g body weight (BW) of EEPML respectively, orally once a day for 3, 14 or 25 days. Diabetic rats were induced by intraperitoneal injection of streptozotocin (STZ) at a dose of 65 mg/kg BW and nicotimamide at 100 mg/kg BW. Peritoneal fluid was isolated on day 3, 14 and 25 and cultured for the assay of macrophage phagocytic activity with latex beads. M1 and M2 macrophage percentages were analyzed by flowcytometry with anti CD40 and CD206 antibody. The results showed that the mean of active macrophages and macrophages phagocytic index of EEPML treatment groups on day 3, 14 and 25 were significantly higher than those in the diabetic control group ($p<0.05$). Moreover, the mean of M1 macrophage percentage of EEPML treatment groups were significantly higher on day 14 but significantly lower on day 25 than that in the diabetic control group ($p<0.05$). In addition, the mean of M2 macrophage percentage was not significantly difference among the groups ($p>0.05$). In conclusion, the ethanolic extract of *P. macrocarpa* (Sheff.) Boerl leaf administration can increase macrophage phagocytic activity in diabetic rats. In addition, it also can increase M1 macrophage percentage on .day 14

ABSTRAK

Penderita diabetes mengalami inflamasi dan penurunan sistem imun akibat menurunnya aktivitas sehingga rentan terhadap infeksi. Ekstrak daun mahkota dewa (*Phleria macrocarpa* (Scheff.) Boerl) telah dibuktikan dapat meningkatkan aktivitas makrofag dan mempunyai efek antiinflamasi. Penelitian ini bertujuan untuk mengkaji efek ekstrak etanol daun mahkota dewa (EEMD) terhadap aktivitas fagositosis makrofag pada tikus model diabetes. Selain itu rasio makrofag M1 dan M2 juga dihitung. Penelitian ini adalah

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penelitian eksperimental semu dengan rancangan uji akhir dan kelompok control. Empat puluh lima tikus jantan Sprague Daley berumus delapan minggu dikelompokkan menjadi kelompok kontrol non diabetes, kelompok kontrol diabetes dan kelompok perlakuan EEMD dosis 7, 14 dan 28 mg/kg berat badan secara oral sekali sehari selama 3, 14 atau 25 hari. Tikus dibuat diabetes dengan diinduksi streptozotosin dosis 65 mg/kg berat badan dan nikotinamid 100 mg/kg berat badan secara intraperitonial. Cairan peritonial tikus diisolasi pada hari ke 3, 14 dan 25 dan dikultur untuk pengujian aktivitas fagositosis makrofag terhadap partikel lateks. Persentase makrofag M1 dan M2 dianalisis dengan flositometri menggunakan anti CD40 and antibody CD206. Hasil penelitian menunjukkan rerata makrofag aktif dan indeks fagositosis makrofag kelompok perlakuan EEMD pada hari ke 3, 14 dan 25 lebih tinggi secara bermakna dibandingkan dengan kontrol diabetes ($p<0.05$). Selanjutnya, rerata persentase makrofag M1 kelomppok perlakuan EEDM pada hari ke 14 lebih tinggi secara bermakna dibandingkan dengan kontrol diabetes ($p<0.05$), akan tetapi lebih rendah secara bermakna pada hari ke 25 ($p<0.05$). Sedangkan persentase makrofag M2 tidak berbeda nyata diantara kelompok uji ($p>0.05$). Dapat disimpulkan, pemberian ekstrak etanol daun mahkota dewa dapat meningkatkan aktivitas makrofag pada tikus diabetes. Selain itu, pemberian ini juga dapat meningkatkan persentase makrofag M1 pada hari ke 14.

Keywords: diabetes mellitus - peritoneal macrophage – phagocytosis - M1 macrophage - M2 macrophage

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of endocrine characterized by hyperglycemia as the result of insulin deficiency, decrease in tissue insulin sensitivity or both.[1] The prevalence of DM worldwide increased dramatically in the last 20 years. The World Health Organization (WHO) estimated that the people with DM will increased to be 300 million in 2025 in the world.[2] Indonesia is the fourth country with the highest people with DM in the world after India, China and America. In Indonesia, the people with DM reached 12.2 million in 2013, it will be 21.3 million in 2030.[3]The pathogenesis of DM is associated with the inflammatory process which oxidative stress and chronic inflammation can induce insulin resistance. Diabetes mellitus is also the manifestation of inflammatory response such as migration and macrophage infiltration into the islets of Langerhans of pancreas, the increase in C-reactive protein, fibrinogen, pro-inflammatory cytokines (IL-6 and TNFα), and the decrease in IL-10. Oxidative stress in diabetes also increases the death of cells and apoptosis.[4]Macrophage is phagocytic cells in innate immunity system that plays an important role in inflammatory process and body immunity sytem. It consists of two sub-populations, M1 and M2 macrophages. M1 macrophages play their role in the inflammatory stage to produce pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-12, IL-18 and IL-23. They are transformed into M2 macrophages in the resolution phase of inflammation. M2 macrophages produce anti-inflammatory cytokines such as IL-10, TGFβ, IL-1Ra and IL-18BP.[5]

Macrophages play their role in inflammation and infection in diabetic case. Activation of M1 macrophages induces inflammation and insulin resistance. M2 macrophage activation can forestall pancreatic β cell auto antigen in type I diabetes mellitus and inhibit the development of type II diabetes.[6,7] Dysfunction of innate immunity system and the decrease in phagocytic

activity of macrophages can increase the risk for infection in DM.[8] *Phaleria macrocarpa* (Scheff.) Boerl, locally well known as *mahkota dewa*, is an endogenous medicinal plant from Papua, Indonesia that widely used by Indonesian and Malaysian people. This plant is traditionally used to treat DM, allergy, liver diseases, vascular diseases, cancer, kidney failure, stroke, and hypertension. The parts of the plant used as medicine are stems, leaves, and fruit.[9] The toxicity and mutagenicity of the *P. macrocarpa* (Scheff.) Boerl. fruit have been evaluated and showed that this fruit is safe for use as medicine.[10,11]

Phaleria macrocarpa (Scheff.) Boerl. leaf contains the active compounds including flavonoids, polyphenols, saponins, tannin, and steroids which have anti-microbial effects.[12,13] Phalerin is a specifically active compound found in its leaf as an anti-inflammatory agent.[14,15] The extract of its leaf has been reported to have antihyperglycemic effects through the inhibition of α-glucosidase activity which is a carbohydrate-digesting enzymes.[16] This plant also has antioxidant activity, tyrosinase inhibition, and analgesic effect.[17,18] Ethanolic extract of *P. macrocarpa* (Scheff.) Boerl. leaf can increase splenic NK1.1 cells activity and macrophage phagocytic activity in mice.[19] In this study we reported the effect of ethanolic extract of *P. macrocarpa* (Sheff.) Boerl leaf (EEPML) on macrophage phagocytic activity in diabetic rat model. Moreover, the M1 and M2 macrophage ratio was also determined.

MATERIALS AND METHODS

Extract preparation

Phaleria macrocarpa (Scheff.) Boerl leaf was collected from Bantul District, Yogyakarta and identified in Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, then dried and powdered. Powdered leaves were macerated with 70% ethanol at room temperature for 24 h and then filtered to separate filtrate from residue. The residue was remacerated three time and filtrates obtained were collected and dried using vacuum rotary evaporator to obtain dried extract. Extract preparation for testing was prepared in 5% polyethylene glycol (PEG) solution.

Diabetes animal model

Forty five male Sprague Dawley rats aged eight weeks with the body weight (BW) of 200 to 230 g obtained from the Integrated Research and Testing Laboratory, Universitas Gadjah Mada, Yogyakarta were used in this study. The rats were housed at room temperature under 12 hours cycles of dark and light and fed a standard food as well as provided an access to aquadest *ad libitum*. After an acclimatization period of one week, the rats were then grouped into non diabetic or normal control group, diabetic control group and diabetic treatment group which given with 7; 14; 28 mg/200 g BW of EEPML respectively, orally once a day for 3, 14 or 25 days according to their terminating groups. Diabetic rats were induced by single intraperitoneal injection of streptozotocin (STZ) at a dose of 65 mg/kg BW and nicotimamide at 100 mg/kg BW. Fasting blood glucose level was examined on day 8 after STZ induction. Diabetic rats were diagnosed if blood glucose level was >170 mg/dL. Protocol of the study was approved by the Ethical Commission for Preclinical Research of the Integrated Research and Testing Laboratory, Universitas Gadjah Mada, Yogyakarta.

Isolation and culture of peritoneal macrophages

The rats were sacrificed on day 3, 14 or 25 according to their terminating groups. The

rats were anaesthetized by xylazin ketamine injection intramuscularly on their left thighs and cervical dislocation was performed on supine position. The abdomen skin was opened and the peritoneum sheath was cleaned up with 70% of ethanol. Approximately 10 mL of cold RPMI medium were injected into the peritoneal cavity during 3 min while they were shaked slowly. Peritoneal fluid was excreted from peritoneal cavity by pressing the organ with two fingers. Aspirated fluid using injecting tube was conducted on non-fat part and which was far from intestine. Aspirated centrifugation was at 1,200 rpm at the temperature of 4°C in 10 min. The established supernatant was removed and 3 mL of complete RPMI medium on the pellet was added. The number of cells was counted with hemocytometer and their viability was determined by using trypan blue solution. The solution was re-suspended with complete RPMI medium so that cell suspension with the density of $2.5x10^6$ cells/ mL was obtained. The calculated cell suspensions were inoculated in a 24-well plate, equipped with cover-slip with each well contained 200 mL suspension ($5x10^5$ cells). The cells were incubated in a 5% CO_2 incubator at the temperature of 37°C in 30 min, and 1 mL of complete RPMI medium was added in each well, followed by incubation within 2 h. The cells were washed with RPMI-1640 twice and 1 mL of complete RPMI medium was added in each well, followed by incubation within 24 h.

Macrophage phagocytic activity test

Latex beads were resuspended in PBS with $2.5x10^7$/mL concentration. Peritoneal macrophages that had been cultured in the previous day were washed twice with RPMI-1640. Suspension of latex beads (200 mL/ well) and the sample (200 mL/well) were added to each well and then were incubated in 5% CO2 incubator at the temperature of 37°C in 60 min. Following after the incubation, cells were washed with PBS three times to remove the excess of latex beads, dried at a room temperature, and fixed with absolute methanol in 30 sec. The cells attached to cover slips were tinged with 20% of Giemsa in 20 min then washed with distilled water. The percentage of 100 examined macrophage cells that phagocyte latex beads and the number of latex beads phagocyted by macrophages were counted by using a light microscope with 400x magnification.

Flowcytometry analysis

Peritoneal macrophage cell suspension which contained approximately $5x10^5$ cells was added with anti-CD40 antibody FITC (Ebioscience 11-0402-82) and anti-CD206 antibody/MRC1 (BIOSs bs-4727R-Cy5.5) and incubated in 30 min at the temperature of 4°C. The cells were then washed twice, resuspended into pharmigen stain buffer, examined by using flowcytometer FACS Calibur, and analyzed by using Cell Quest software. The data obtained from the flowcytometry analysis constituted the percentage of M1 macrophages and M2 macrophages.

Statistical analysis

Data of the percentage of active macrophages (AM), phagocytosis index (PI), and the percentage of M1 and M2 macrophages were presented as mean ± standard deviation (SD). Normality of data distribution was tested by using the Shapiro-Wilk. One way analysis of variance (Anova) and Tukey post hoct tests were used for normal distributed data. Non-parametric Kruskal Wallis and Mann Whitney post hoc tests were used for abnormal distributed data.

RESULTS

The morphology of macrophages in normal control group and in the EEPML treatment groups were bigger with protruded cytoplasm and phagocyted many latex beads, while macrophages in diabetes control group were smaller and rounded and phagocyted fewer latex beads (FIGURE 1).

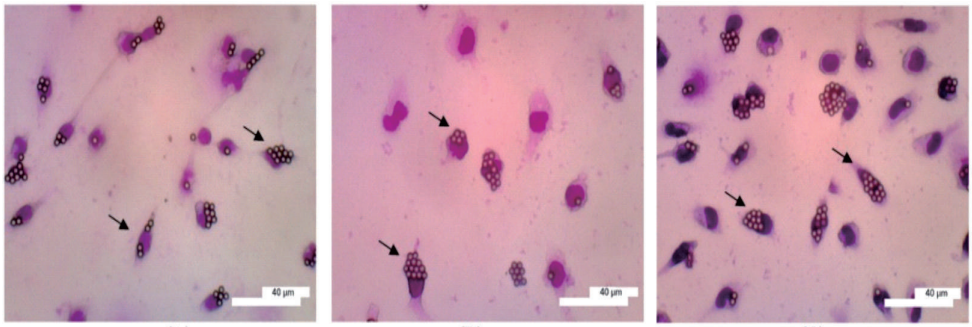

FIGURE 1. Macorphage phagocytic assay of normal control group (A) and diabetic treatment group (C) showed more active phagocyte latex beads (arrow) than diabetic control group (B). Giemsa staining, 400x magnification, scale 1:400µm.

The mean of active macrophages (AM), phagocytic index (PI), M1 and M2 macrophages of all groups on day 3, 14 and 25 are presented in TABLE 1.

TABLE 1. The mean of active macrophages (AM), phagocytic index (PI), M1 and M2 macrophages of all groups on day 3, 14 and 25

Groups		Parameter			
Termination	Treatment	Active Macrophage (%)	Phagocyitic Index	M1 Macrophage (%)	M2 Macrophage (%)
	N	88.3±0.6	526.7±25.7	45.7±7.9	64.5±8.6
	DM	77.3±1.2	323.3±30.9	28.8±7.8	57.4±17.6
Day 3	EEPML1	81.3±1.2	350.0±20.4	23.5±15.4	52.0±23.0
	EEPML2	78.7±2.1	369.3±33.8	38.9±4.6	70.2±13.1
	EEPML3	82.7±2.3	407.7±62.6	38.6±10.6	62.0±11.3
	N	87.3±2.3	473.3±59.8	50.7±20.5	60.1±26.2
	DM	65.0±2.7	275.3±59.3	48.1±24.3	59.1±28.0
Day 14	EEPML1	72.0±3.6	325.0±44.0	60.7±10.0	77.1±0.7
	EEPML2	87.3±5.0	383.0 ±56.7	69.4±7.1	76.1±2.3
	EEPML3	83.0±5.6	321.3±30.0	51.9±6.2	72.4±2.0
	N	92.0±3.5	525.7±98.3	51.2±2.2	74.8±3.4
	DM	76.3±6.0	259.7±30.1	45.1±6.1	75.0±4.4
Day 25	EEPML1	90.0±1.0	384.7±19.6	46.5±4.9	74.7±9.1
	EEPML2	90.7±3.1	341.0± 68.6	24.7±6.1	74.3±0.6
	EEPML3	84.7±1.5	335.0±87.7	46.4±16.0	74.1±1.6
p		0.000**	0.000*	0.002*	0.165**

Normality test with Saphiro-Wilk, performed by the mean value of ± SD. N: Normal control group; DM: diabetic control group; EEPML: diabetic treatment group at doses 7, 14 and 28 mg/200 g BW.*One way Anova, $p<0.05$ significant, **Non parametric Kruskal Wallis, $p<0.05$ significant.

The mean of AM of the normal control group was significantly higher than that of the diabetic control group on all day of examination and than that of the diabetic treatment groups at all doses on day 3 ($p<0.05$). However, it was not significantly different compared to that of the diabetic treatment groups at doses of 14 and 28 mg/200g BW on day 7 and at all doses on day 14 ($p>0.05$). The means of AM of the diabetic treatment groups at all doses on day 3 and at dose of 7 mg/200g BW on day 7 were not significantly different compared to those of the diabetic control group ($p>0.05$). However, at doses of 14 and 28 mg/200g BW on day 7 and at all doses on day 14, they were significantly higher than those of diabetic control group ($p<0.05$). The highest of the mean of AM in diabetic treatment groups was observed at dose of 14 mg/200g BW on day 25 (FIGURE 2.A).

The mean of PI of the normal control group was significantly higher than that of the diabetic control group on all day of examination and than that of the diabetic treatment groups at all doses on all day of examination ($p<0.05$). In addition, the means of PI of the diabetic treatment groups at all doses on all day of examination were significantly higher than that of the diabetic control group ($p<0.05$) (FIGURE 2.B).

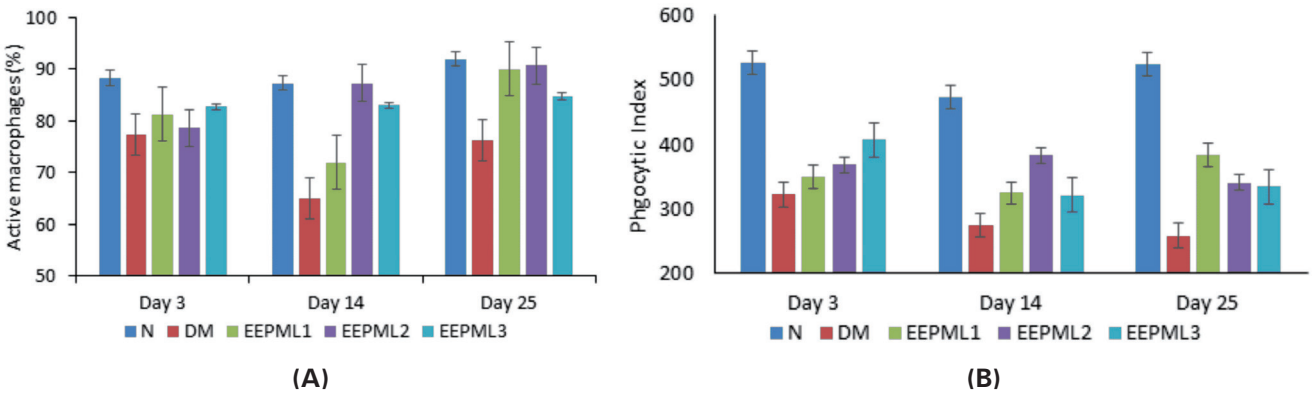

FIGURE 2. A) The mean of active macrophage (AM) and B) phagocytic index (PI) on day 3, 14 and 25; N: Normal control group; DM: diabetic control group; EEPML: diabetic treatment groups at doses of 7, 14 and 28 mg/200 g BW.

The mean of M1 macrophage percentage of the normal control group on day 3 was significantly higher than that of the diabetic control group on day 3 ($p<0.05$). However, it was not significantly different compared to that the diabetic control group on day 7 and 14 ($p>0.05$). The means of M1 macrophage percentage of the diabetic treatment groups at doses of 14 and 28 mg/200g BW on day 3 and at doses of 7 and 14 mg/200g BE on day 14 were significantly higher than that the diabetic control group on day 3 and 7, respectively ($p<0.05$). However, the means of M1 macrophage percentage of all groups on day 25 were not significantly different ($p>0.05$), except the mean of M1 macrophage percentage at dose of 14 mg/200g BW which was significantly lower than others groups ($p<0.05$) (FIGURE 3.A). Furthermore, the means of M2 macrophage percentage of all groups on all day of examination were not significantly different ($p>0.05$) (FIGURE 3.B).

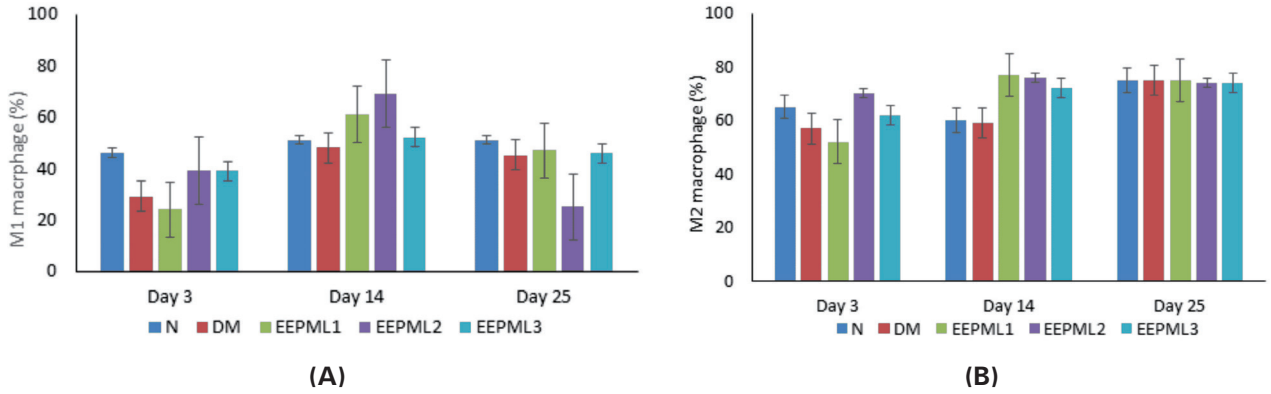

FIGURE 3. A) The mean value of M1 macrophage and B) M2 macrophage percentage on day 3, 14, and 25. *post hoc Tukey (p<0,05). N: Normal control group; DM: diabetic control group; EEPML: diabetic treatment groups at doses of 7, 14 and 28 mg/200 g BW.

DISCUSSION

Diabetes mellitus is associated with the innate immune system disturbance that caused patients with DM exhibit increased susceptibility to infection, impaired wound healing and other inflammatory or degenerative manifestations. Macrophages play important roles in controlling innate immune system.[8,20] In this study, the decrease in macrophages activity was observed in diabetic rats as indicated by lower of the mean of AM and PI compared to normal rats on day 3 and continued on day 14 and 25 (TABLE 1 and FIGURE 2 and 3).

The decrease in the macrophages activity may be caused by hyperglycemia condition in DM which leads to oxidative stress and glycation induced the cells death. Hyperglycemia also increases advanced glycation end product (AGE) formation that will be bound to the AGE receptor (RAGE) on the membrane of various cells, including macrophages. The bond of AGE-RAGE with macrophage cells can inhibit the macrophages phagocytic activity.[21] In addition, inflammatory condition in DM can also induce the formation of inflammatory mediator of high mobility group box 1 (HGMB1) which can bind to phosphatidylserine on the membrane of apoptotic cells. This condition can inhibit the macrophage capacity to identify apoptotic cells and to perform efferocytosis on the apoptotic cells lead to inhibition of tissue repair.[22]

The EEPML is potential to increase the peritoneal macrophage phagocytic activity and improve the function of immunity system in diabetic condition. The three doses of EEPML have similar effects to increase macrophages activity. However, the most effective dose of EEPML was 14 mg/200g BW. This dose EEPML could increase the macrophages activity similar to that normal control group on day 3, 14 and 25 (TABLE 1 and FIGURE 2 and 3). The immunomodulatory effect of *P. macrocarpa* (Scheff.) Boerl. leaf extract has been reported in previous studies. Ghufron *et al.*[19] reported that ethanolic extract of *P. macrocarpa* (Scheff.) Boerl. leaf can increase splenic NK1.1 cells activity and macrophage phagocytic activity in mice. Abood *et al.*[20] reported an immunomodulatory effect of *P. macrocarpa* (Scheff.) Boerl. and their isolated fraction by increasing macrophage cell proliferation and significant induce of cytokines INF-γ, IL-6 and IL-8 intracellular expression.

Macrophages play an important role in the inflammatory process in diabetic condition. This study showed the percentage of peritoneal M1 macrophages in diabetic control group significantly decreased on day 3 compared to normal control group, while the percentage of peritoneal M2 macrophages was unchanged (FIGURE 3.A. and B). The decrease of the peritoneal M1 macrophage percentage was caused by the decrease in the macrophages phagocytic activity due to the immunity system dysfunction in diabetic condition. M1 macrophages are pro-inflammatory macrophages that play an active role in performing phagocytosis while M2 macrophages are anti-inflammatory macrophages that actively perform effecytosis to clean up apoptotic cells for tissue repair.[6,21] M2 macrophages are commonly accumulated in normal energy metabolism process involving mitochondria. Meanwhile, M1 macrophages are generally accumulated at edema, hypoxia, anaerobic glycolysis, inflammation, and insulin resistance condition.[22]The EEPML administration influenced the percentage of peritoneal M1 and M2 macrophages in diabetic condition. It could increase the mean of the percentage of peritoneal M1 macrophages especially on day 14 (FIGURE 3.A). The increase of the percentage of peritoneal M1 macrophages can maximize inflammatory state of diabetes, together with the increase in the macrophage phagocytic activity induced by the EEPML administration. Moreover, it could also increase the mean of the percentage of peritoneal M2 macrophages on day 14, however it was not significantly different (FIGURE 3.B). The increase of the percentage of peritoneal M2 macrophages is caused by the increase of percentage of peritoneal M1 macrophages and its activity inducing the M1 macrophages polarization into M2 macrophages to relieve inflammatory conditions in diabetes.

The result of this study is different from the previous study that reported the topically EEPML ointment administration on diabetic wound on rats can increase the M1 and M2 macrophages on day 3, however it decrease on day 14.[23] The different may be caused by the macrophages used in these study. The inflammatory process in diabetic wound involves the tissue-resident macrophages distributed in the epidermis. In this study, we used peritoneal macrophages containing the combination of the tissue-resident macrophages and monocytes derived macrophages from the systemic circulation.[24,25] The two types of macrophages might have the different time of activation.

CONCLUSION

In conclusion, the ethanolic extract of *P. macrocarpa* (Sheff.) Boerl leaf administration can increase macrophage phagocytic activity in diabetic rats. In addition, it also can increase M1 macrophage percentage on day 14. This immunomodulatory effect of the plant extract could be used to improve the immunity system of diabetic conditions.

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Chronic wound mitomycin-c-induced animal models

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ABSTRACT

A chronic wound is a problem often encountered in medical care, especially in areas that do not have adequate health facilities. Several factors causing the injury are mechanical, chemical, electrical, or heat. Chronic inflammation and bacterial infections are two significant factors that affect the process of wound chronicity. Mitomycin-C (MMC) is widely used as intravenous, oral, or topical anti-cancer drug. Mitomycin-C that is topically administration to an injury can cause cross-linking and decrease or stop the DNA transcription process lead the injury will not reach the healing phase. Mitomycin-C works as an inhibitor of fibroblast proliferation that can inhibit wound healing process. This study aimed to investigate the effect of topical MMC administration in chronic wound animals model. Eight female Wistar rats aged 70 to 90 days and weighing between 300 to 350g were used in this study. Wound model was made in the back area with a diameter of approximately 2 cm. Rats were then divided into 2 groups. The first group as treatment group which the wound rats were compressed using sterile gauze moistened with 0.5 mg/mL of MMC for five min and rinsed with 10 mL of saline solution. The second group as control which the wound rats were compressed by using sterile gauze moistened with saline for 5 minutes. On day 3, 5, and 15, the wound profiles were observed consisting of wound diameter, necrosis, and consistency. The rat wound after MMC administration showed a slower decrease in diameter, fewer scar tissue formation, and development of necrotic tissue. In addition, the rat wound appeared as brownish-black, dry, thick chronic wounds and took longer to heal compared to those saline control. In conclusion, MMC administration inhibits the wound healing process on rat surgical wound model.

ABSTRAK

Luka kronis merupakan suatu masalah yang sering dihadapi terutama di daerah dengan fasilitas kesehatan yang tidak memadai. Faktor penyebab terjadinya luka adalah mekanik, kimia, listrik, atau panas. Inflamasi kronik dan infeksi bakteri merupakan dua faktor utama yang mempengaruhi proses kronisitas luka. Mitomisin-C (MMC) merupakan sediaan intravena, oral, dan topikal yang banyak digunakan pada keganasan. Pemberian MMC topikal pada luka dapat menyebabkan pertautan silang dan menurunkan atau menghentikan proses transkripsi DNA sehingga dapat menghambat penyembuhan luka. Mitomisin bertindak sebagai penghambat proliferasi fibroblast yang dapat menghambat penyembuhan luka. Penelitian ini bertujuan mengkaji efek pemberian MMC topical pada model hewan luka kronis. Delapan ekor tikus Wistar betina umur 70 hingga 90 hari, dengan berat antara 300 sampai 350g digunakan dalam penelitian ini. Luka dibuat pada

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daerah punggung yang bebas rambut dengan ukuran diameter sekitar 2 cm. Tikus dibagi menjadi dua kelompok secara acak. Kelompok pertama sebagai kelompok perlakuan dikompres dengan kasa steril yang dibasahi MMC 0,5 mg/mL selama 5 menit kemudian dibilas dengan larutan salin 10 mL. Kelompok kedua sebagai kelompok kontrol dikompres dengan kasa steril yang dibasahi dengan salin saja selama 5 menit. Pada hari ke-3, 5, dan 15 diamati profil luka tikus yang meliputi diameter luka, nekrosis, dan konsistensinya. Luka tikus setelah pemberian MMC menunjukkan penurunan diameter luka lebih lambat, pembentukan jaringan parut lebih sedikit dan terjadinya pembentukan jaringan nekrosis. Selain itu luka tampak berwarna lebih hitam kecoklatan, kering dan tebal dibandingkan kelompok kontrol. Dapat disimpulkan, pemberian MMC menghambat proses penyembuhan luka pada tikus.

Keywords: chronic wound – profile – mitomycin-c – wound healing - topical

INTRODUCTION

Chronic wounds are common medical problems, especially in areas where there are not adequate health facilities. Some factors that cause injury are mechanical, chemical, electrical, or heat. The wound healing process in the skin is a complicated cellular process, involving keratinocytes, fibroblasts, blood vessels, endothelial cells, and immune cells. Chronic injuries begin with acute onset injuries. Chronic wounds do not typically go beyond the initial stage of inflammation. An acute injury may become a chronic injury if there is no development in the wound healing process through hemostasis, inflammation, proliferation, and tissue maturation. The acute injury becomes chronic injury if it fails to improve within four weeks and does not show wound healing within eight weeks. Chronic inflammation and bacterial infection are two major causes that affect the chronicity of a wound.[1-3] The ability to recognize chronic wounds by health care workers is essential, so that wound treatment is more appropriate, making wound healing faster and reducing wound care costs.

Mitomycin-C (MMC) is an anti-proliferative agent that inhibits DNA synthesis. It is widely used as an intravenous, oral, or topical anti-cancer drug. Mitomycin-C is given topically on the wound to form crosslinks and stop the DNA transcription process so that the wound will not undergo the healing process. Topical application of MMC to scar tissue will act as aninhibitor of proliferative fibroblast, thus inhibits wound healing.[4-6]

Mitomycin-C has a broad-spectrum effect in inhibiting the proliferation of many cell types, and when used topically, the biological effect is affected by the concentration and duration of exposure. Larger concentration and longer duration of exposure result in greater biological effects. Its clinical use in inhibiting scar tissue formation has been extensively studied. The rats are often used in animal model of wound healing studies, but there are limitations involving the anatomical differences in human skin and rats, the wound healing process and the immune system. Regarding the anatomy, rats have denser hair follicles and thinner dermis layers than the human skin. It is also showed that the rat skin heals through contractions, causing the edges of the wound to be closer to each other, forming bag zippers, and re-epithelization. In contrast, human skin heals the wound by re-epithelization only, where keratinocytes accumulate on the granulation tissue to cover

the wound. With these differences, the study using rats are ideal for wound healing model.[6-7] This preliminary study aimed to investigate the effect of topical MMC administration in chronic wound animals model.

MATERIALS AND METHODS

Animal model

Eight female Wistar rats aged 70 to 90 days, weighing between 300 and 350 g were used in this study. The animals were kept in separate cages in the Integrated Research and Testing Laboratory (LPPT), Universitas Gadjah Mada, Yogyakarta. Rats were given anesthesia using an intramuscular dose of ketamine 30 mg/kg of body weight. Following after anesthesia, the hairs on the back were shaved after they were cleaned by using 10% povidone-iodine. The wound was created in the back area that was free of hair with a diameter of approximately two centimeters in full thickness (FIGURE 1).

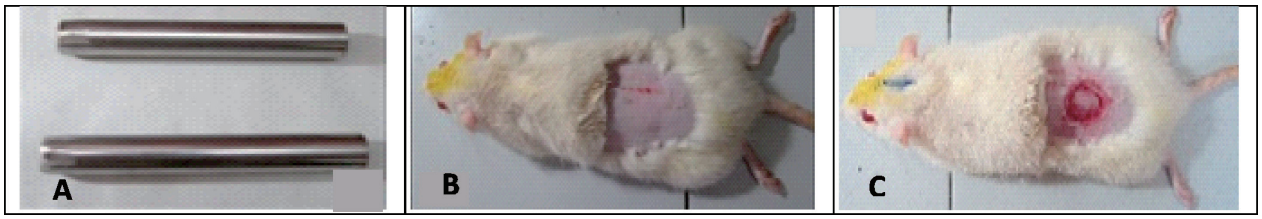

FIGURE 1. A: skin puncher; B: prepared and draped female rat; C: initial wound

Animal treatment

Eight rats were given a number from one to eight and randomly divided into two groups using the lottery method. In the first group as treatment group, the wound was compressed using sterile gauze moistened with 0.5 mg/mL of MMC for five minutes and rinsed with 10 mL of saline solution. The topical concentration value was adopted from the current literature.[6] In the second group as control, the wound was compressed using sterile gauze moistened with saline solution without MMC for 5 minutes.

On the third day, the wound diameter was measured in both groups. We performed a necropsy on one of rats from each group; then we repeated the pretreatment for the rest of the rats in each group. On the 7th and 15th day, the wound skin diameters were measured in both groups. During the study, there were no sick or dead rats. The sample tissues were observed to notice any differences in the diameter of the wound, necrosis, scar formation and tissue color. This study was approved by the Ethical Committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital under permit (KE/FK/594/EC/2015).

Statistical analysis

The wound skin diameters were analyzed by means of descriptive statistics following by visual and macroscopic analysis.

RESULTS

The results show that the wound skin diameters decreased more slowly in rats given with MMC, suggesting that the healing process took a longer time compared to the rats that given with saline solution (FIGURE 2 and TABLE 1).

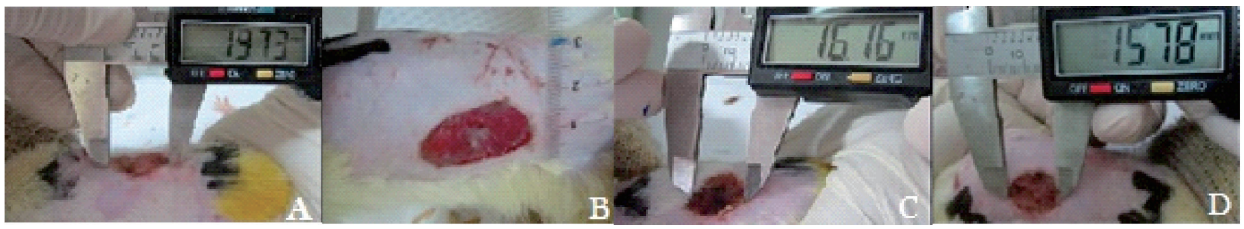

FIGURE 2. Groups of rats that received mitomycin-C (A: Day 0; B: day 3; C: day 7; D: day 15)

The formation of scar tissue in rats after MMC administration was smaller indicated by decreased scar tissue formation in chronic wounds. The rats after topical MMC administration showed a brownish color on the wound, and by day of 15 some of the lesions appeared to be black, dry, and hard, which were characteristics of the necrotic wounds. The rats after saline solution administration did not reveal any necrotic tissue. The wound consistency in rats after topical MMC administration appeared brownish and after 15 days, coated by dark brown, dry, and hard brown tissue with surrounding tissue under local edema. The wound skin characteristics are shown in TABLE 1.

TABLE 1. The wound analysis based on wound diameter, scar formation, necrosis and wound consistency

Days	Rats	Wound Diameter (mm)	Scar formation	Necrosis	Wound consistency
Day-0	1	19.73	-	-	Red, moist
	2	19.09	-	-	Red, moist
Day-3	3	19.00	-	+	Brownish, dry
	4	19.00	+	-	Pink, moist
Day-7	5	16.16	-	++	Blackish brown, dry, thick
	6	17.13	++	-	Pink, soft
Day-15	7	15.78	+	+++	Blackish, hard
	8	10.78	+++	-	Slightly pale, soft

DISCUSSION

Our study showed that for the macroscopic parameters analyzed from the rats after topical MMCs administration was decreased in diameter of the wound, the fewer scar tissue formation and formed necrotic tissue, with hard consistency and blackish brown wounds. This results were in accordance with the previous studies which showed MMC administration topically in rats decreased wound strength and reduced scarring process indicating a chronic wound healing occurred.[6,8] However, the mechanism of MMC in inhibiting fibroblast proliferation remains unclear.

Wound healing process consists of three phases that overlap each other, namely inflammation, proliferation and remodeling.[9-11] After a skin injury, platelets are activated in the ruptured blood vessels and initiate clot formation to stop the bleeding. Platelets release a factor that stimulates the arrival of immune cells into the wound area of the blood circulation. Polymorphonuclear neutrophils

(PMN) come first, followed by monocytes that rapidly differentiate into macrophages. Neutrophils produce many reactive oxygen species (ROS), proteases and proinflammatory cytokines to clean wounds. When the process is over, neutrophils will undergo apoptosis and be phagocytosed by macrophages. Macrophages will also phagocyte bacteria and debris to clean wounds. When the wound is clean, the proliferation process will begin, which is the process of tissue growth. Cells in the wound area will proliferate and migrate to replace lost tissue. Some of the cells involved are the extracellular matrix (ECM) or also called granulation tissue, which contains keratinocytes. During the remodeling phase, the final phase of the wound healing process, the granulation tissue becomes mature, and its mechanical strength increases. The complete process of wound healing occurs when myofibroblast and vascular cells start to apoptosis, leaving scar tissue rich in collagen. In chronic wounds, the proliferative and remodeling phases are not yet fully initiated nor established so that the wound remains in the inflammatory phase. The absence of tissue regeneration causes the wound to be healed incompletely.[7] Observations in this study were conducted on days 0, 3, 7, and 15 because the process of wound healing in rats is actually faster than humans. The rats only take about 10-14 days to heal their wounds.[12] Based on our observations, the macroscopic wound healing becomes slower as we predicted earlier.

Some examples of chronic wounds are diabetic ulcers, venous ulcers, and pressure ulcers. Microscopically, chronic wounds have many inflammatory cells infiltrated, mainly neutrophils. It is thought that the cause of the chronic wound healing process is that fibroblasts lose their migration capacity and become unresponsive to growth factor signals. This change is indicated by the decrease of TGF-β receptor levels and subsequent downward cascades in the wound healing process are disrupted. In previous studies, it was found that in chronic wounds, pro-inflammatory macrophages (M1) persist and did not transition into anti-inflammatory macrophages (M2), thus appeared to contribute to tissue repair disorders. The increased matrix of metalloproteinase (MMP) in chronic wounds is also thought to be the cause of decreased signaling and growth factor responsiveness. The process of re-epithelization also becomes impeded by the decrease in the capacity of immune cells in chronic wounds, resulting in the buildup of debris cells.[7,13]

Formed scar tissues depend on the degree of fibrosis of the wound. The formation of fibrin during the hemostasis phase will be replaced by granulation tissue consisting of fibroblasts, macrophages and endothelial cells. Fibroblasts have an essential role in the wound healing process. Fibroblasts can synthesize collagen for an extracellular matrix useful as the foundation for epithelial cells and tissue epithelization. The study by Gray *et al.*[14] reported that mitomycin-c has the ability to inhibit DNA synthesis to prevent the proliferation of fibroblasts in the long term. Experimental study on animals conducted by Lampus *et al*.[15] reported that topical mitomycin-c was able to reduce total fibroblast in anoplasty wound healing trials in Wistar rats. Another study by Su *et al*.[16] also shows the same result, finding topical MMC over 0.3 mg/mL can delay the wound healing via anti-cell proliferation effect and also that MMC inhibits angiogenesis of the wound via inhibition of vascular endothelial growth factor (VEGF).

The novelty of this study is that no macroscopic observation has been done with

necrotic variables and wound consistency in chronic wound-induced animal models using MMC in any previous study. Typically, in the past, the assessed variables were degree of fibrosis, vascular proliferation and wound strength.[6]

Although this study is preliminary, it has shown that the application of topical MMC to surgical wounds in the rats resulted in disrupted healing process. Our long-term aims that motivate this study are to make an ideal chronic wound animal model that could be used to study diseases that are linked with chronic wound healing such as diabetic, venous, and pressure ulcers. We also intend to evaluate the possible adverse effects that might be caused by topical MMC on chronic wound healing models using different concentrations and more prolonged exposure over extended periods of time. Further studies are needed to examine these important salutogenesis issues. However, our small sample size could not provide sufficient power for our results and consequently larger samples are needed to provide clarification and confirmation of better results. We recommend that further research with microscopic tissue examination be performed with clinical trials involving human subjects.

CONCLUSION

In conclusion, the MMC inhibits wound healing process on rat surgical wound model as indicated by the formation of less scarring and longer wound healing duration that seen from slower diameter reduction. In addition, the wound tissue appears dry, hard, thick, and the surface appears colored blackish.

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Neonatal outcomes in *in vitro* fertilization (IVF) pregnancies

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ABSTRACT

In vitro fertilization (IVF) has been associated with poor neonatal outcomes. Preterm birth, small-for-gestational age (SGA), and low birth weight (LBW) rates are approximately twice as high in IVF pregnancies than in natural pregnancies. The IVF procedures have become more routine in recent years in Indonesia, but there have been few assessments of neonatal outcomes. The study aimed to evaluate the risk of preterm birth, SGA, and LBW in IVF infants. This was a retrospective cohort study performed in Dr. Sardjito General Hospital, Yogyakarta from January 2012 to December 2016. Pre-coded questionnaires were used to collect data from medical records. The relative risk of preterm birth, SGA, and LBW among IVF infants were calculated and compared to naturally conceived infants. A total sampling method was used for the IVF infants and a simple random sampling method was used for naturally conceived infants, who were born on the same day as an infant in the IVF group.

A total of 108 infants were recruited, consisting of 54 IVF infants and 54 naturally conceived infants. The IVF infants had increased risk of preterm birth (RR = 2.0; 95%CI 0.52 - 7.58) and LBW (RR = 1.25; 95%CI 0.53 - 2.92). However, the IVF infants did not have an increased risk of SGA (RR = 1.0; 95%CI 0.21 - 4.73). In conclusion, the risk of preterm birth and LBW in IVF infants are higher than in naturally conceived infants, but not statistically significant. However, there is no increased risk of SGA in IVF infants.

ABSTRAK

Fertilisasi *in vitro* (FIV) dihubungkan dengan luaran neonatus yang rendah. Tingkat kelahiran preterm, bayi kecil untuk usia kehamilan, berat badan lahir rendah sekitar dua kali lebih tinggi pada kehamilan FIV dibandingkan kehamilan normal. Teknik FIV telah rutin dilakukn di Indonesia beberapa tahun belakangan ini, tetapi sedikit dilakukan penilaian terhadap luaran neonatusnya. Penelitian ini dilakukan bertujuan untuk mengkaji risiko kelahiran preterm, bayi kecil untuk usia kehamilan, berat badan lahir rendah pada anak dengan FIV. Penelitian ini merupakan penelitian kohort retrospektif yang dilakukan di RSUP Dr. Sardjito, Yogyakarta dari Januari 2012 sampai Desember 2016. Kuesioner berkode digunakan untuk mengumpulkan data dari rekam medik. Risiko relatif kelahirn preterm, bayi kecil untuk usia kehamilan, berat badan lahir rendah dihitung dan dibandingkan dengan bayi lahir normal. Metode sampling total digunakan untuk bayi dengan FIV dan metode sampling acak sederhana digunakan untuk bayi normal yang lahir pada hari yang sama. Total sebanyak 108 bayi direkrut yang terdiri dari 54 bayi dengan FIV dan 54 bayi normal. Fertilisasi *in vitro* meningkatkan risiko kelahiran preterm (RR = 2,0; 95%CI 0,52 – 7,58) dan berat badan lahir rendah (RR = 1,25; 95%CI 0.53 – 2,92). Namun demikian,

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FIV tidak mempunyai risiko bayi kecil untuk usia kehamilan (RR = 1,0; 95%CI 0,21-4,73). Dapat disimpulkan, risiko kelahiran preterm dan berat badan lahir rendah pada FIV lebih tinggi daripada bayi normal, tetapi tidak berbeda nyata. Namun demikian, tidak ada kenaikan risiko terjadinya bayi kecil untuk usia kehamilan.

Keywords: *in vitro* fertilization – preterm - small for gestational age - low birth weight – relative risk

INTRODUCTION

In vitro fertilization (IVF) is one of several assisted reproductive technologies (ART). The number of babies conceived through this procedure is increasing, with an estimated 3-5 million IVF babies worldwide.[1–3] As the number of newborns increase, several studies have been conducted to evaluate neonatal outcomes of low birth weight (LBW), preterm birth, and small-for-gestational age (SGA). These outcomes occur almost twice as often compared to natural pregnancies, even in singleton IVF pregnancies.[4–7]to provide guidelines to optimize obstetrical management and counselling of Canadian women using ART, and to identify areas specific to birth outcomes and ART requiring further research.\\n\\nOPTIONS:Perinatal outcomes of ART pregnancies in subfertile women are compared with those of spontaneously conceived pregnancies. Perinatal outcomes are compared between different types of ART.\\n\\nOUTCOMES:This guideline discusses the adverse outcomes that have been recorded in association with ART, including obstetrical complications, adverse perinatal outcomes, multiple gestations, structural congenital abnormalities, chromosomal abnormalities, imprinting disorders, and childhood cancer.\\n\\nEVIDENCE:The Cochrane Library and MEDLINE were searched for English-language articles from 1990 to February 2005, relating to assisted reproduction and perinatal outcomes. Search terms included assisted reproduction, assisted reproductive technology, ovulation induction, intracytoplasmic sperm injection (ICSI Studies in Taiwan and India reported that the prevalence of preterm birth was increased in IVF pregnancies compared to natural pregnancies.[8,9]

A study in eight infertility centers in Indonesia showed a pregnancy success rate of 29.46%.[10] In 2014, the IVF program at Dr. Sardjito General Hospital, Yogyakarta, had a 25.4% pregnancy success rate, while the percentage of live births was 19.8%. These percentages increased in 2015 to 30.9% and 25.1%, respectively.[11,12] The IVF program in Indonesia has existed for approximately 29 years with an increasing percentage of live births, but the data on neonatal outcomes remains unclear. Hence, this study aimed to evaluate the risk of poor neonatal outcomes in IVF infants.

MATERIALS AND METHODS

Subjects

This retrospective cohort study was done from January 2012 to December 2016. Infants who were born at Dr. Sardjito General Hospital, Yogyakarta, through IVF pregnancies (IVF group) and natural pregnancies (natural group) were recruited as subjects. The infants whose mothers underwent IVF procedures outside this hospital and those whose medical records were not found or incomplete were excluded. The data were collected from medical records using questionnaires.

Protocol of study

A total sampling method for IVF infants and a simple random sampling method for the natural group were used. Naturally conceived infants born on the same day as an IVF infant underwent simple random sampling for inclusion. If no naturally conceived infant was found on the same dayas an IVF infant, retrieval was extended to up to three days later.

A minimum of 64 subjects was required for each group to obtain 90% power with 5% significance level. In this study, dependent variables were neonatal outcomes, i.e., birth weight, gestational age, and birth weight according to gestational age, while independent variables were the process of fertilization (IVF/ natural). Confounding variables were maternal age, parity, and placental abnormalities.

The definition of LBW was weight at birth of less than 2,500 g, regardless of the gestational age; the definition of SGA was birth weight according to gestational age of less than 10th percentile on the Lubchenco curve. Gestational age was defined as the length of pregnancy until the time of delivery. By the Dubowitz score, preterm was defined as <37 weeks gestational age.[13] Maternal age was defined as the age of the mother at the time of delivery. Parity was defined as the number of previous pregnancies that reached viable gestational age. Placental abnormalities included placental abruptio or placenta previa. Faculty of Medicine, Universitas , Yogyakarta

Statistical analysis

Statistical analysis was done using SPSS Statistics 20. Univariate analysis was performed on numerical data and was shown as mean or median, while categorical data was displayed in proportion. Chi-square or Fisher's exact tests were used to compare independent and dependent variables, with relative risk as a measure of the strength of the relationship. Statistical significance was defined to be $p<0.05$. Multivariate analysis was performed using logistic regression.

RESULTS

Fifty-eight IVF infants were initially screened for this study, but three infants were excluded due to their IVF not having been performed in Dr. Sardjito General Hospital and one due to loss of the medical records. Hence, 54 subjects were included in the IVF group. No infants born from natural pregnancies were excluded, for a total of 54 naturally conceived subjects (FIGURE 1).

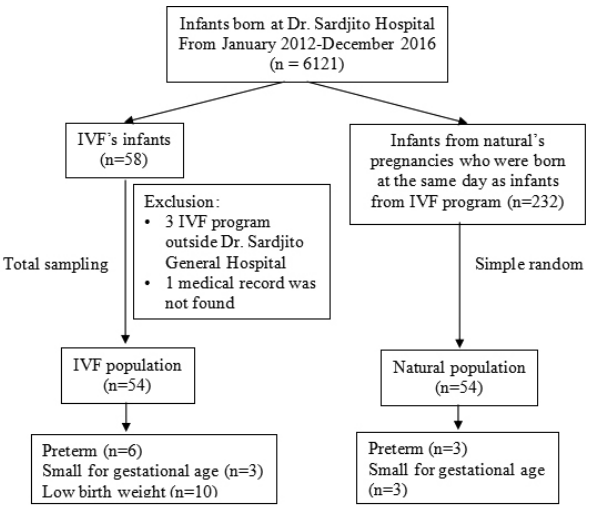

FIGURE 1. Flowchart of subjects recruitment

The characteristics of subjects are shown in TABLE 1. The IVF group (88.9%) had a greater proportion of parity 0 (nulliparous) than the natural group (38.9%). In addition, the IVF group (22.2%) had a greater proportion of multiple pregnancies than the natural group (7.40%), though the proportion of singletons (77.8%) was still greater than the proportion of multiples (22.2%) in the IVF group. Moreover, the IVF group (92.6%) had a greater proportion of caesarean section deliveries than the natural group (48.1%).

TABLE 1. Characteristics of IVF and naturally conceived subjects

Characteristics	IVF (n = 54)	Natural (n = 54)
Maternal age [n (%)]		
• 20-34 years	34 (63.0)	36 (66.7)
• ≥35 years	20 (37.0)	18 (33.3)
Maternal age (mean ± SD years)	33.2 ± 3.74	31.4 ± 5.53
Parity prior to this pregnancy [n (%)]		
• 0	48 (88.9)	21 (38.9)
• ≥1	6 (11.1)	33 (61.1)
Gestational age, n (%)		
• Full term	48 (88.9)	51 (94.4)
• Preterm	6 (11.1)	3 (5.60)
Number of fetuses [n (%)]		
• Single	42 (77.8)	50 (92.6)
• Multiple	12 (22.2)	4 (7.40)
Sex [n (%)]		
• Male	27 (50.0)	34 (63.0)
• Female	27 (50.0)	20 (37.0)
Birth weight, n (%)		
• Normal	44 (81.5)	46 (85.2)
• Low	10 (18.5)	8 (14.8)
Birth weight [median (min-max)/mean (SD)]	2,875.0 (600-3850)	2,928.4 (400)
Birth weight for gestational age, n (%)		
• AGA	51 (94.4)	51 (94.4)
• SGA	3 (5.60)	3 (5.60)
Mode of delivery [n (%)]		
• Vaginal	4 (7.4)	28 (51.9)
• Caesarean section	50 (92.6)	26 (48.1)
Placental abnormalities, n (%)	4 (7.4)	0 (0)

AGA=appropriate for gestational age; SGA=small for gestational age

TABLE 2 shows the bivariate analysis between the independent variables and preterm. The IVF infants had two times the risk of preterm birth than natural infants. However, it was not significantly different (95%CI 0.52-7.58; p=0.48). Other independent variables such as maternal age, parity, and placental abnormalities had no significantly association with the occurrence of preterm birth (p>0.05).

TABLE 2. Bivariate analysis of independent variables and preterm

Variables	Preterm (n=9)	Full term (n=99)	Bivariate RR	Bivariate 95% CI	p
Fertilization process [n (%)]					
• IVF	6 (11.1)	48 (88.9)	2.0	0.52-7.58	0.48
• Natural	3 (5.6)	51 (94.4)			
Maternal age [n (%)]					
• 20-34 years	5 (7.1)	65 (92.9)	0.67	0.19-2.37	0.71
• ≥35 years	4 (10.5)	34 (89.5)			
Parity prior to pregnancy [n (%)]					
• Nulliparous	6 (8.7)	63 (91.3)	1.13	0.29-4.27	1.00
• Multiparous	3 (7.7)	36 (92.3)			
Placental abnormalities [n (%)]					
• Yes	1 (25)	3 (75)	3.25	0.52-20.1	0.29
• No	8 (7.7)	96 (92.3)			

Bivariate analysis of the independent variables and SGA revealed no increased risk of SGA in IVF infants (RR=1.0; 95%CI 0.21- 4.73; p=1.0). Other independent variables such as maternal age, parity, and placental abnormalities also had no significant association with the incidence of SGA (TABLE 3).

TABLE 3. Bivariate analysis of independent variables and SGA

Variable	SGA (n=6)	AGA (n=102)	Bivariate RR	Bivariate 95%CI	p
Fertilization process [n (%)]					
• IVF	3 (5.6)	51 (94.4)	1.0	0.21- 4.73	1.0
• Natural	3 (5.6)	51 (94.4)			
Maternal age [n (%)]					
• 20-34 years	3 (4.3)	67 (95.7)	0.54	0.11- 2.56	0.66
• ≥35 years	3 (7.9)	35 (92.1)			
Parity prior to this pregnancy [n (%)]					
• Nulliparous	3 (4.3)	66 (95.7)	0.56	0.12- 2.67	0.66
• Multiparous	3 (7.7)	36 (92.3)			
Placenta abnormalities [n (%)]					
• Yes	0 (0)	4 (100)			1.00
• No	6 (5.80)	98 (94.2)			

SGA=small for gestational age; AGA=appropriate for gestational age

Bivariate analysis of the independent variables and LBW revealed that IVF infants had 1.2 times higher risk of LBW compared to naturally conceived infants. However, it was was not significantly different (95%CI 0.53 - 2.92; p= 0.6). Other independent variables such as maternal age, parity, and placental abnormalities were not also significantly associated with LBW incidence (TABLE 4).

TABLE 4. Bivariate analysis of independent variables and LBW

Variable	LBW (n=18)	NBW (n=90)	Bivariate RR	Bivariate 95%CI	p
Fertilization process [n (%)]					
• IVF	10 (18.5)	44 (81.5)	1.25	0.53- 2.92	0.60
• Natural	8 (14.8)	46 (85.2)			
Maternal age [n (%)]					
• 20-34 years	10 (14.3)	60 (85.7)	0.67	0.29 -1.57	0.37
• ≥35 years	8 (21.1)	30 (78.9)			
Parity prior to pregnancy [n (%)]					
• Nulliparous	11 (15.9)	58 (84.1)	0.88	0.37 -2.10	0.78
• Multiparous	7 (17.9)	32 (82.1)			
Placenta abnormalities [n (%)]					
• Yes	1 (25)	3 (75)	1.53	0.26- 8.82	0.52
• No	17 (16.3)	87 (83.7)			

LBW: low birth weight; NBW: normal birth weight

Multivariate analysis could not be performed in this study due to no variables had p values <0.25 after bivariate analysis performed.

DISCUSSION

The proportions of preterm infants were 11.1% and 5.6% in the IVF and natural groups, respectively. Similarly, a previous study reported that the prevalence of preterm ranged from 7.8 to 16.1% in IVF population and 4.5% to 8.0% in natural population.[14] Nevertheless, no significant association between IVF and preterm (p=0.48) was observed, whereas several previous studies showed significant results.[14,15] Koivurova *et al*.[16] found no significant association between singleton IVF and preterm (OR 1.5; 95%CI 0.7 - 3.2), in which the control was only singleton pregnancies taken from the general population. However, this result became significant when both singleton and multiple pregnancies were taken as control subjects (OR 5.6; 95%CI 3.7 - 8.6). As such, sample diversity is an important factor in the incidence of preterm.[16]

All infants from ART procedures may be predisposed to preterm birth. Previous studies divided ART into subgroups, i.e., fresh with frozen embryos, oocyte donors with own oocytes, standard IVF with intracytoplasmic sperm injection (ICSI), and third day with fifth day embryo showed greater risk of preterm, LBW and VLBW in each subgroup.[17,18]maternal morbidity and mortality among Swedish women giving birth after in vitro fertilisation (IVF However, Romundstad *et al*.[19] also compared natural conception with ART in the same mothers and found no significant difference. They concluded that ART did not harm the perinatal outcome, but genetics was more likely to be an underlying factor of preterm incidence. Another study mentioned that ART pregnancies were generally more closely monitored, such that birth was more frequently subject to induction and caesarean section. These ART interventions also have been associated with SGA incidence, increased perinatal mortality, and VLBW.[14]

The proportion of SGA in our study was similar in both the IVF and natural groups (5.6%). Previous studies reported SGA in

12.7% of IVF pregnancies and 13% of natural pregnancies,[20] and 2.89% in both IVF and natural newborns.[21] In this study, there was no increased risk of SGA in IVF infants. However, this finding may not be conclusive, as a previous meta-analysis stated that the risk of SGA in IVF pregnancies was 1.3 times higher compared to natural pregnancies (95%CI 1.27 to 1.53).[22] In contrast, other studies stated that there was no increased risk of SGA in IVF infants.[20,21,23] Wen *et al*.[23] noted that general and more diverse populations tended to have significant influences on SGA incidence.

The proportions of LBW in our study were 18.5% and 14.8% in the IVF and natural groups, respectively. Similarly, a previous study reported LBW of 11.2% in IVF and 11.6% in natural newborns.[20] Our bivariate analysis revealed no significant association between IVF and LBW incidence (p=0.60), similar to previous studies that compared LBW and preterm in ART and non-ART groups.[20,24] In contrast, a recent meta-analysis suggested that LBW tended to occur in the IVF population compared to the natural population, even if the baby is full term.[25] Differences in the size of the research scale may explain our lack of association between IVF and LBW, as small-scale research tended to get no significant results.[20]

Placental abnormalities (all placenta previa) were only found in our IVF group (7.4%), but there were not significantly different from the natural population. IVF procedures such as cervical catheterization, or mechanically- inducing uterine contractions may play a major role in implantation in the lower uterine segment, thus leading to placenta previa.[19] Another study explained that high concentrations of estradiol hormone in the IVF cycle increased complications associated with placental aberration by affecting endometrial growth and decidualization.[26]

Several previous studies have noted that the underlying factors of preterm, SGA, and LBW remain unclear.[7,27,28] To date, the underlying factors are maternal age, infertility/ subfertility, genetics, as well as the IVF technique itself, but we were unable to assess for these variables in our study. Furthermore, women who undergo ART may differ from the general population, as these women usually have high socioeconomic status, good nutritional status, better antenatal care, and sufficient rest during pregnancy. These factors are believed to positively affect pregnancy and its outcome.[24] Another study reported that most pregnancies from the IVF program had no complications and resulted in the birth of healthy babies.[29]

Several limitations of our study should be noted. The small and potentially inadequate sample size as well as the retrospective study design may have led to information bias. The selection of no intervention populations (natural populations) appropriate to the IVF population WAS also a weakness. Women who underwent IVF treatment in this study generally had middle to upper economic status (because the IVF program in Indonesia is not guaranteed by insurance), more routine control, consultation, and treatment during pregnancy, especially by the obstetrician. Hence, the IVF group may have received better attention and care than the natural group. Another weakness in our study was that some important variables such as socioeconomic status and maternal education were not recorded completely in the medical records, so they were not included in the analysis.

CONCLUSION

In conclusion, the risk of preterm birth and LBW in IVF infants tend to be higher than in naturally conceived infants. However, they

are not statistically significant. In addition, there is no increased risk of SGA in IVF infants. Further research using a larger sample size is needed for more representative data of the actual conditions in the population.

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Ephaptic crosstalk in painful diabetic neuropathy: an electrodiagnostic study

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ABSTRACT

Painful Diabetic Neuropathy (PDN) is a common complication of diabetes mellitus (DM) which significantly causes pain and distress in patients. Release of factors from degenerating fibers activating adjacent fibers to produce ephaptic crosstalk have been proposed as one of the pain mechanism in PDN. Here we aim to detect ephaptic crosstalk between small fibers and large fibers in PDN subjects by comparing the electrodiagnostic result of patients with PDN and patients without PDN.

This study used cohort prospective design. Patients with type 2 DM or impaired glucose tolerance (IGT) without PDN from several health facilities in Yogyakarta were followed for 12 months for the occurrence of PDN. Demographic, clinical, laboratory and electrodiagnostic data from all patients were collected and analyzed.

One hundred and forty-one subjects (58 men, 83 women) with an average age of 51 years (range, 40–61 years), were enrolled in this study. After 48 weeks of observation, 12 subjects were found to have PDN. The differences of distal latency between PDN and non-PDN group were significant when measured in median sensory nerve (4.47 ms $\pm$2.43 versus 3.39 ms $\pm$1.79, p = 0.002), tibial motor nerve (6.96 ms $\pm$3.07 versus 5.90 ms $\pm$2.17, p = 0.041), and sural sensory nerve (6.02 ms $\pm$3.56 versus 3.55 ms $\pm$2.90, p <0.001). Among all parameters measured in this study, the H-reflex had higher abnormality persentage compared to other electrodiagnostic variable (H latency = 30%, H amplitude = 71%, H/M Ratio = 88%, and H-M IPL = 15%).

Our result shows that small fiber neuropathy in PDN can be detected by electrodiagnostic study which measures large fibers function. This indicates that ephaptic crosstalk between small fiber and large fiber happens in PDN.

Keyword **:** Painful diabetic neuropathy – NCS – ephaptic– Diabetes Mellitus

INTRODUCTION

Painful diabetic neuropathy (PDN) and post herpetic neuralgia (PHN) are the most frequent painful neuropathies.[1] Painful diabetic neuropathy significantly causes pain and distress in patients with diabetes mellitus (DM). Approximately 30% of patients with DM develop PDN.[2] The pain is typically worse at night and characterized by typical neuropathic pain descriptors (burning, tingling, aching, dysesthesia). Sometimes sensory loss can also be found. Over 50% of patients report significant problem in performing activities of daily living, including mobility, work, sleep, recreation and social activities.[3] This condition can lead to serious

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psychological problem. Sleep deprivation following prolonged periods of intense pain can lead to apathy towards life in general, and/or self-imposed social isolation. Some may also experience reduced memory retention, mood swings and suicidal tendencies.[2]

Diabetic neuropathy is characterized by progressive distal neurodegeneration. This condition adds an additional layer of complexity in identifying pathogenic mechanisms for neuropathic pain in diabetic patients. This nerve degeneration has been proposed as a mechanism responsible for pain generation in neuropathy.[4] Assessment of the most distal regions of sensory axons have shown the possibility that mechanisms by which nerve degeneration may cause pain include ectopic activity by destabilized degenerating fibers and the release of factors from degenerating fibers activating adjacent fibers to produce ephaptic crosstalk.[4]

Ephaptic (electrical) crosstalk has been long proposed as a mechanism of neuropathic pain. It is a form of fiber-to fiber interaction found in neuromas and demyelination plaques in nerves and spinal roots.[5]the normal anatomy and physiology of pain; second, the pathophysiology of damaged sensory neurons; and third, the diagnosis and treatment of patients with neuropathic pain. The book begins with a discussion of neural mechanisms relevant to pain perception along with a brief review of neuropathic pain. This is followed by separate chapters on hyperalgesia following cutaneous injury; the importance of peripheral processes in the etiology of neuropathic and radiculopathic pain; and mechanisms by which sympathetic efferent fibers contribute to the occurrence of pain. Subsequent chapters cover the diagnosis and treatment of reflex sympathetic dystrophy; pain in generalized neuropathies; surgical treatment of pain; clinical features and management of postherpetic neuralgia; diagnosis of cancer pain syndromes; and drugs in the management of chronic pain. Nerve conduction study is commonly used to evaluate large myelinated sensory and motor nerve fibers, but is ineffective in diagnosing small fiber neuropathies.[6] In case of PDN however, ephaptic crosstalk will result in activation of large fibers near the demyelinated C fibers. This phenomenon should be able to be detected by electrodiagnostic (EDx) study. The aim of this study was to detect ephaptic crosstalk between small fibers and large fibers in PDN subjects by comparing the electrodiagnostic result of patients with PDN and patients without PDN.

MATERIALS AND METHODS

Subjects

This was an observational study using cohort prospective design. The inception cohort was diabetes patients free from PDN (ID pain score less than 2), followed by a 12-months disease follow-up. The subjects of this study were diabetic patients admitted to Diabetes Outpatient Clinic in Dr. Sardjito General Hospital, Yogyakarta, members of Indonesian Diabetic Association at PKU Hospital Yogyakarta, staffs of Health Science Academy of Aisiyah, Yogyakarta and prediabetic patients involving in the research conducted in Department of Clinical Pathology, Dr. Sardjito General Hospital, Yogyakarta. The protocol of study was approved by the Medical and Health Research Ethics Committees of Faculty of Medicine, Gadjah Mada University.

Protocol of study

On the appointed day, subjects were gathered to be selected. Explanations

concerning the background, objective, benefit of the study was provided. Subject who meet the inclusion and exclusion criteria and were willing to involve in the study were provided an informed consent to be signed. The inclusion criteria were men and women aged 20 to 60 years old, with type 2 DM or impaired glucose tolerance (IGT), as defined by American Diabetes Association (ADA) criteria. The following were reasons for exclusion: 1) anatomical deformities on extremities that would interfere with electrodiagnostic study protocol; 2) pregnancy or lactation; 3) a documented history of lumbosacral surgery that would interfere with electrodiagnostic study protocol; 4) other diseases known to be associated with pain, especially chronic pain in the feet that the investigator believed would interfere with the assessment of pain associated with diabetic neuropathy, like cancer pain, lumbosacral abnormality or other entrapment neuropathy; 5) any acute or underlying serious illness that are likely to interfere with completion of the trial.

Clinical and electrodiagnostic examinations were performed in all patients. Baseline assessments, consisted of ID pain, neuropathy symptom score (NSS), diabetic neuropathy symptom (DNS), review of nerve conduction study of upper and lower extremity, and soleus H-reflex study, were done at week 1. Nerve conduction study and H reflex were conducted using an MEB-2300K ENMG machine (Nihon Kohden, Tokyo, Japan). The evaluation and recording of PDN occurrence were carried out every week up to 12 months by self-assessment which was monitored by the doctors.

Statistical analysis

Data were presented as mean ± standard deviation (SD) or percentage or range. Student t test was used to compare between PDN and non PDN groups. A p value < 0.05 was considered as significant.

RESULTS

One hundred and forty-one subjects (58 men, 83 women) with an average age of 51 years (range: 40–61 years), were enrolled in this study. The hyperglycemia state were diabetic in 65% and IGT in 35% of the subjects. The mean glucose level was 116 mg/dL (range: 78 - 200 mg/dL) for fasting, 170 mg/dl for 2 h post prandial (range: 90 – 250 mg/dL). The mean value of HbA1c was 6.9 % (range: 4 - 7 %). Screening by NSS and DNS scores at admission found that 57 subjects (40.4%) were diagnosed as neuropathy (DN) according to NSS, while 68 (48.2%) were diagnosed as neuropathies according to DNS. There were no subjects diagnosed as PDN. However, upon completion of 48 weeks of observation, 12 subjects were found to have PDN.

The electrodiagnostic study showed that there were prolonged distal latencies in PDN group, while there was no abnormality found in non-PDN group (TABLE 1). The differences of distal latency between PDN and non-PDN group were significant when measured in median sensory nerve (4.47 ± 2.43 ms versus 3.39 ± 1.79 ms, $p = 0.002$), tibial motor nerve (6.96 ± 3.07 ms versus 5.90 ± 2.17 ms, $p = 0.041$), and sural sensory nerve (6.02 ± 3.56 ms versus 3.55 ±2.90 ms, $p < 0.001$). There was no significant difference in median motor nerve (4.90 ± 0.84 ms versus 4.44 ±1.46 ms, $p = 0.119$).

In the measurement of H-reflex, the H-latency prolonged in both groups, but significantly longer in PDN group (38.90 ± 5.94 ms versus 34.82 ± 6.32 ms, $p = 0.004$). The M-latency was also significantly longer

(p = 0.003) in PDN group (9.37 ± 3.01 ms) compared to non-PDN group (7.51 ± 2.74 ms). Nerve conduction velocity in PDN group decreased significantly compared to the non-PDN group (p<0.05). The median nerve conduction velocity in PDN group was 44.69 ±10.78 m/s, and 51.51 ±9.08 m/s in non-PDN group (p = 0.001). The tibial nerve conduction velocity was 33.52 ± 14.86 m/s in PDN group, and 41.45 ± 9.64 m/s in non-PDN group (p = 0.001).

There were significantly smaller distal amplitudes in PDN group compared to non-PDN group in median motor nerve (3.45 ± 1.82 mV versus 8.37 ± 6.27 mV, p <0.001), median sensory nerve (6.02 ± 10.32 µV versus 14.95 ±15.95 µV, p = 0.008), tibial motor nerve (4.47 ± 3.45 mVversus 7.22 ± 4.47 mV, p = 0.004), and sural nerve (4.37 ± 4.16 µV versus 13.89 ± 14.09 µV, p = 0.001). There was also significant difference (p = 0.001) between the H-reflex amplitude in PDN group and non-PDN group. The H-reflex amplitude was abnormal in PDN group (0.29 ± 0.42 mV) while normal in non-PDN group (1.32 ±1.52 mV).

The PDN group also had significantly longer H-M Inter Peak Latency (32.21 ± 6.56 ms) compared to non-PDN group (27.53 ± 5.87 ms) (p = 0.0020. The H/M Ratio decreased for both groups, but significantly lower (p = 0.016) in PDN (11.23% ±17.896) compared to non-PDN (25.53% ±27.97). Among all parameters measured in this study, the H-reflex had higher abnormality persentage compared to other electrodiagnostic variable (H latency = 30%, H amplitude = 71%, H/M Ratio = 88%, and H-M IPL = 15%) (Table 2).

TABLE 1. The analysis of electrodiagnostic parameters value towards PDN status.

Parameter	Variable	Non-PDN (±SD)	PDN (±SD)	p	CI 95%
Distal Latency	Median motor (ms)	4.44 (1.46)	4.90(0.84)	0.119	-0.12 – 1.08
	Median sensory (ms)	3.39 (1.79)	4.74 (2.43)	0.002	0.52 – 2.19
	Tibial motor (ms)	5.90 (2.17)	6.96 (3.07)	0.041	0.04 – 2.09
	Sural sensory (ms)	3.55 (2.90)	6.02 (3.56)	<0.001	1.15 – 3.79
Nerve Conduction	Median (m/s)	51.51 (9.08)	44.69 (10.78)	0.001	-10.91– -2.72
Velocity	Tibial (m/s)	41.45 (9.64)	33.52 (14.86)	0.001	-12.68–3.17
Distal	Median motor (mV)	8.37 (6.27)	3.45 (1.82)	<0.001	-7.40– -2.40
Amplitude	Median sensory (µV)	14.95 (15.95)	6.02 (10.32)	0.008	-15.50– -2.30
	Tibial motor (mV)	7.22 (4.47)	4.47 (3.45)	0.004	-4.60– -0.87
	Sural (µV)	13.89 (14.09)	4.37 (4.16)	0.001	-15.15– 0. 89
Amplitude	H-reflex (mV)	1.32 (1.52)	0.29 (0.42)	0.001	-1.65– -0.42
Latency	H-reflex (ms)	34. 82 (6.32)	38.90 (5.94)	0.004	01.35–6.81
M-latency	H-reflex (ms)	7.51 (2.74)	9.37 (3.01)	0.003	0.64–3.08
H-M IPL	H-reflex (ms)	27.53 (5.87)	32.21 (6.56)	0.002	1.60–6.84
H/M Ratio	H-reflex (%)	25.53 (27.97)	11.23(17.896)	0.016	-25.84- -2.73

This table shows that all electrodiagnostic parameters differ significantly between the group with and without PDN (p<0.05), except for distal latency of median nerve. PDN= painful diabethic neuropathy.

TABLE 2. Abnormality percentage of electrodiagnostic variable

Variable	Parameter	Result		p	Abnormality %
		Non-PDN	PDN		
Median (motor)	Amplitude	normal	decrease	sig.	35
	D. Latency	normal	prolonged	ns.	-
	NCV	normal	slowed	sig.	14
Tibial (motor)	Amplitude	normal	normal	sig.	0.6
	D. Latency	normal	prolonged	sig.	16
	NCV	normal	slowed	sig.	20
Median (sensory)	Amplitude	normal	decrease	sig.	50
	Latency	normal	prolonged	sig.	35
Sural (sensory)	Amplitude	normal	normal	sig.	0
	Latency	normal		sig.	50
H-reflex	H Latency	prolonged	prolonged	sig.	30
	H Amplitude	normal	decrease	sig.	71
	H/M ratio	decrease	decrease	sig.	88
	H-M IPL	normal	prolonged	sig.	15

This table reveals that, generally, electrodiagnostic parameters in non PDN group are still normal, while almost all parameters in PDN group are abnormal. The highest percentage of abnormality was found in the decrease of H reflex amplitude (88%). *sig*= significant ; *ns*= not significant.

DISCUSSION

The study population in this research was patients with abnormality in blood glucose level (DM or IGT). The clinical neurological complication focused in this study was state of being neuropathy with and without PDN. After observation for almost 12 months, we found around 40% of the subjects with neuropathy and 12% with PDN. Electrodiagnostic study was performed in all subjects in the initial recruitment of this study. Motoric and sensoric nerve conduction study and the H reflex study were conducted. According to Skljarevski & Malik,[7] attributes of nerve conduction are reliable, reproducible, and objective primary outcome measures in trials evaluating pharmaceutical treatment of diabetic peripheral neuropathy. The principal factors affecting nerve conduction velocity (NCV) are: the integrity and degree of myelination of the largest diameter fibers; the mean axonal cross-sectional diameter; the representative internodal distance, and the distribution of nodal ion channels.[7] Our study found that almost all EDx variables in non PDN subjects were normal. This result indicated that the large myelin fibers investigated were intact.

In healthy peripheral nerves, individual myelinated axons form discrete and highly isolated conduction channels.[5],a stable electrical (ephaptic[8] Each sensory neuron functions as an independent conduction channel until it reaches the synapse. In injured nerve, however, disruption of glial ensheathment allows adjacent denuded axons to make contact, permitting both electrical (ephaptic) and chemical (via a diffusible substance) cross-excitation.[9] This phenomenon can be seen in PDN subjects. Prolonged latency and decreased amplitudo were found in almost all parameters of EDx in PDN subjects, except in the latency of median motor nerve. Orstavik[10] has previously reported about altered properties of afferent C-fibres in a chronic painful condition. This phenomenon

can happen in chronic hyperglycemic subjects. Previous researches have shown that ephaptic crosstalk can be the source of the pathologic pain.[11] Acute transection of a neuron has been shown to be able to short-circuit neighboring axons in a nerve so that the current from the cut end of one fiber can excite the others.[5]a stable electrical (ephaptic[8] The same mechanism happen in PDN. Electrical impulse running through demyelinated axons in PDN can trigger demyelinated axons near it to also fire. Ephaptic crosstalk between fibers mediating light touch (Aβ) and those involved in the generation of pain (C and Aδ) may account for the generation of pain by light tactile stimulation.[12,13]

Collateral sprouting from primary afferent fibers, which induces ephaptic or physical crosstalk between different types of fibers, have been proposed to be involved in reorganization mechanisms of spinal neuronal circuits for pain transmission.[13] The damage to the nerves can cause regeneration of nerve sprouts, called neuromas, at the stump. The sprouting of the new nerves in all directions causes damage to healthy nerves nearby and expands the sensitized area. Hyper excitability in the neuroma generates ectopic impulses that affect neighboring intact afferents and the cell bodies of the dorsal root ganglion.[14] Lysophosphatidic acid (LPA), which is released at the dorsal root, demyelinates the Aδ- and Aβ-fibers on the dorsal root through the LPA_1 receptor, followed by physical (or ephaptic) crosstalk between the C-fiber and Aδ-fiber, and between the Aδ-fiber and Aβ-fiber.[13]

Decreased conduction velocity and increased activity dependent slowing are possible pathological features of a small fiber neuropathy like PDN.[10,15] Our research shows that the processes can also be detected by EDx study which assess large fibers. This fact supports the idea that ephaptic crosstalk between small fibers with large fibers really happens in PDN. After ephaptic transmission, the action potentials of the two cells were conducted at almost the same velocity along the axons. The conduction velocity of the action potential was reduced from 0.30 ± 0.11 cm/s to 0.15±0.05 cm/s by ephaptic transmission of the action potential. “container-title”:”Plant and Cell Physiology”,”page”:”575-579”,”volume”:”31”,”issue”:”5”,”source”:”pcp.oxfordjournals.org”,”abstract”:”When two separated Chara internodal cells were kept in contact over a length of 14 mm or more in moist air, an action potential of one cell could be transmitted to the other cell in about 40% of cases (ephaptic transmission

CONCLUSION

In conclusion, our result shows that small fiber neuropathy in PDN can be detected by EDx study which measures large fibers function. This indicates that ephaptic crosstalk between small fiber and large fiber happens in PDN. To our knowledge this is the first report of altered properties of afferent C fibers in a chronic painful condition captured by electrodiagnostic study which actually measures the large fibers function.

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The impact of malaria in pregnancy on infant susceptibility to malaria infection

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ABSTRACT

Malaria infection during pregnancy is a significant global health problem with substantial risks for pregnant women, her foetus, and the newborn child. Infant malaria is a major public health concern in Timika, Papua. The aim of the study was to investigate the impact of malaria during pregnancy on infant's susceptibility to malaria infections, the timing of its occurrence, the number of malaria infections during pregnancy. This was a cohort prospective study conducted in Timika, Papua from October 2013 to September 2016. Malaria investigation was done by microscopic and PCR methods. Demographic data and malaria status of mother-infant pairs were collected and analyzed by SPSS 22.0 version. One hundred seventy-eight infants consisting of 95 (53.37%) infants born to mothers with malaria and 83 (46.63%) without malaria 91 (51.12%) boys and 87 (48.88%) girls were involved in the study. The mean of mothers' ages were 25.35 ± 6.30 vs. 26.0 ± 5.69 years. At the ages of 6 and 12 months, infants born to malaria positive mothers were more susceptible to malaria infections compared to infants born to malaria negative mothers with RR = 3.49; 95%CI: 1.02-11.96; p = 0.03 and RR = 8.74; 95%CI: 1.14-66.81; p = 0.01, respectively. Independent risk factors of infant susceptibility to malaria infection during the first year of life were malaria in pregnancy (MiP) in 2nd trimester (RR = 4.50; 95%CI: 1.5-13.49; p = 0.07), pregnant women who only got malaria infection 1 time during pregnancy (RR = 2.95; 95%CI: 1.04-8.33; p = 0.04), and Papuan ethnicity (RR = 3.58; 95%CI: 1.22-10.59; p = 0.02). In conclusion, infant susceptibility to malaria is associated with maternal malaria status during pregnancy. MiP in second trimester, pregnant women who only had malaria once and Papuan ethnicity were independent risk factors for infant's increased susceptibility to malaria infection.

ABSTRAK

Infeksi malaria selama kehamilan merupakan masalah kesehatan global yang signifikan dengan risiko besar bagi ibu hamil, janin, dan bayi yang dilahirkan. Malaria pada bayi merupakan masalah kesehatan masyarakat utama di Timika, Papua. Penelitian ini bertujuan untuk mengkaji dampak malaria selama kehamilan terhadap kerentanan bayi pada infeksi malaria, waktu terjadinya infeksi malaria pada ibu, jumlah infeksi malaria selama kehamilan. Penelitian ini merupakan penelitian kohort prospektif yang dilakukan di Timika, Papua dari bulan Oktober 2013 sampai September 2016. Pemeriksaan malaria pada wanita hamil dan bayi dilakukan dengan metode mikroskopik dan PCR. Data demografi dan status

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malaria pasangan ibu-bayi dikumpulkan dan dianalisis dengan SPSS versi 22.0. Sebanyak 178 bayi yang terdiri dari 95 (53,37%) bayi yang lahir dari ibu dengan malaria dan 83 (46,63%) tanpa malaria dan 91 (51,12%) bayi laki-laki dan 87 (48,88%) bayi perempuan dilibatkan dalam penelitian. Rata-rata usia ibu adalah 25,35 ± 6,30 vs 26,0 ± 5,69 tahun. Pada usia 6 dan 12 bulan, bayi yang lahir dari ibu positif malaria lebih rentan terhadap infeksi malaria dibandingkan dengan bayi dari ibu negatif malaria (RR = 3,49; IK 95%: 1,02-11,96; p = 0,03 dan RR = 8,74; IK 95%: 1,14-66,81; p = 0,01). Faktor risiko independen pada kerentanan bayi terhadap infeksi malaria selama tahun pertama adalah malaria pada kehamilan saat trimester kedua (RR = 4,50; IK 95%: 1,5-13,49; p = 0,07), wanita hamil yang hanya terinfeksi malaria 1 kali selama kehamilan (RR = 2,95; IK 95%: 1,04-8,33; p = 0,04), dan etnis Papua (RR = 3,58; IK 95%: 1,22-10,59; p = 0,02). Dapat disimpulkan, kerentanan bayi terhadap malaria berhubungan dengan status malaria ibu selama kehamilan. Malaria pada kehamilan trimester kedua, wanita hamil yang hanya terinfeksi malaria satu kali selama kehamilan dan etnis Papua adalah faktor risiko independen terkait peningkatan kerentanan bayi terhadap infeksi malaria.

Keywords: Malaria - pregnancy - infant susceptibility - Timika Papua – risk factors -

INTRODUCTION

Malaria is a mosquito-borne infectious disease caused by the parasite Plasmodium. The predominant species are *Plasmodium falciparum* and *P. vivax* with an estimated 182.2 million clinical cases of *P. falciparum* malaria, 15.8 million clinical cases of *P. vivax* malaria and 584,000 deaths attributable to malaria every year.[1] The greatest burden of disease is reported in young children and pregnant women.[2] Annually, 88.2 (70%) of 125.2 million pregnancies in malaria endemic regions occur in the Asia-Pacific area.[3]

Malaria in pregnancy (MiP) or pregancy-associated malaria, is defined as peripheral or placental infection by Plasmodium. The MiP presents as a major public health concern due to significant adverse health effects on both the mother and the fetus.[4] The effects of MiP on infants include intra-uterine growth retardation (IUGR) and pre-term delivery, low birth weight (LBW), abortion and stillbirth, congenital malaria and foetal anaemia.[5] The risk of IUGR associated with malaria was greatest after three or more cumulative infections (RR 3.3; 95%CI: 1.3–8.2) and was two to eight-fold higher among women with evidence of undernutrition.[6] Submicroscopic malaria infections in pregnant women were associated with significantly increased risks of low birth weight in primigravidae and premature births in multigravidae.[7]

According to Poespoprojo[8] infant malaria is a major public health issue in Timika, Papua (Indonesia) and the risk starts at birth with the majority of malaria cases going undiagnosed, being mostly asymptomatic. Newborns and young infants (less than 6 months of age) are thought to be relatively protected from symptomatic malaria.[9] This protection has been considered to be primarily mediated by maternal antimalarial IgG antibodies transferred to the foetus in the last trimester of pregnancy.[10]

Antimalarial IgG antibodies may have three roles: first blocking sporozoite invasion of hepatocytes and merozoite invasion of erythrocytes; second, opsonize merozoites and infected erythrocytes expressing variant surface antigens (VSA) on their surface for phagocytosis; and third, fixing and activating complements on the merozoite surface with resultant parasite lysis.[11] Primarily in the third trimester, transplacental transfer of maternal IgG antibodies to the foetus occurs and is

mediated by the neonatal Fc receptor.[12] After birth, all isotypes of maternal antibodies, except for IgA, are transferred to infants in breast milk, despite the fact that these are not systemically absorbed and act primarily in the gut.[13] The waning of maternal antimalarial IgG antibodies by 6–9 months of age had been reported previously, which coincides with the period of time in which the risk for malaria infection and clinical disease in infants begins to increase.[14]

Beside transplacental transfer of protective antibodies from mother to foetus, several factors that allegedly contribute to the susceptibility of infants to malaria are innate mechanisms including haemoglobin foetus (HbF),[15] para amino benzoic acid (PABA)-deficiency and transforming growth factor (TGF) in breast milk, exclusive breast feeding,[16] neonatal responses to priming by transplacental transfer of parasites or products,[17] placental malaria,[18] infant nutritional status, high exposure to Plasmodium,[19] and proper implementation of insecticide-treated nets and intermittent preventive treatment.[20]

In placental intervillous spaces the parasite specific adhesion may contribute to placental insufficiency which may relate, either directly or indirectly, to foetal growth restriction and premature birth. Susceptibility of women to placental malaria is attributed to increased parasites sequestered in the placenta mediated by chondroitin sulfate A binding to the trophoblast[21] and pregnancy-associated suppression of inflammatory responses caused by hormonal changes.[22] Pregnant women experience immunological and hormonal changes, particularly oestrogen and progesterone which are partly driven by increased levels of pregnancy-associated hormones.[23] A recent study found the role of soluble human leukocyte antigen G (sHLA-G) in infant susceptibility to malaria during pregnancy.[24]

There were some evidences of the effect of placental malaria on infants, which also includes greater susceptibility to malaria and anaemia in those born to mothers with a parasitized placenta.[17, 25] All these studies have focused on placental infection at delivery, with no exploration of the mother's history of infection earlier during pregnancy. Our study aimed to investigate the impact of MiP on infant susceptibility to malaria infection, taking into account the timing of its occurrence, the number of MiP infections and demographic data during the first year of life.

MATERIALS AND METHODS

Subjects

The study was conducted from October 2013 to September 2016 in lowland Timika, Papua Indonesia, an area where *P. falciparum* and *P. vivax* malaria are similarly prevalent. This was a nested study from a cluster randomized trial of maternal malaria prevention and treatment. Pregnant women with gestational age < 24 weeks were enrolled at their first antenatal visit at *Posyandu* (community health centre) after giving written informed consent.

The subjects of this study were the infants born to these mothers who were enrolled consecutively until the number of sample size was sufficient. Inclusion criteria were healthy term newborns and consenting mothers living in the study areas for the duration of follow-up. The babies would be excluded if preterm (< 37 weeks gestation), sick newborns requiring hospitalization, withdrawal by the parent/mother, and loss to follow-up for more than 6 months.

Sample size of infants was based on the cohort prospective method[26] with RR = 2.13 assumed as significant, where the prevalence of malaria in infants in group without risk factor was (P2) = 0.123[27] with α= 0.05; power

80%; zα=1.96; and zβ= 0.842, then the project required a sample size of 162 infants (81 per arm). Ethics approval was obtained from the Medical and Health Research Ethics Committee at the Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Protocol

Pregnant women were followed-up 3 or 4 times (3 if enrolled after 24 weeks and 4 times if enrolled between 16-23 weeks) and again at delivery. At those times blood samples were taken from the finger prick for malaria investigation. Infants born to a mother with either MiP or no MiP would be followed for 1 year and we performed malaria laboratory diagnosis at 6 and 12 months.

The diagnosis of malaria both in mothers and infants were based on PCR examination. Demographic data from the mother included: age, gravidity, ethnicity and bed net utilization while from the baby data included: gender, birth weight, birth length and head circumference. Gestational age at birth were assessed from neuromuscular and physical maturity score of the newborn according to Ballard methods. The procedures were performed by a trained research nurse.

Statistical analysis

Statistical analysis using SPSS version 22.0 (UGM online license). Numerical data with normal distribution were analyzed using student t test or one way analysis of variance (Anova). If there was an abnormal distribution then it would be analyzed using Mann-Whitney U test. Categorical data were analyzed using chi square test with Yates correction or Fisher exact test. Statistical significance was considered if $p < 0.05$. To control confounding variables and look for independent risk factors from malaria in infants, bivariate and multivariate tests were be performed.

RESULTS

One hundred and ninety three mother–infant pairs were enrolled between October 2013 and September 2016. There were 4 parental refusals. Among 193 infants, 9 infants did not complete the 12 months follow up (4 infants from mother without MiP and 5 infants from mother with MiP), and 2 infants died from mothers with MiP due to sepsis and watery diarrhoea (FIGURE 1).

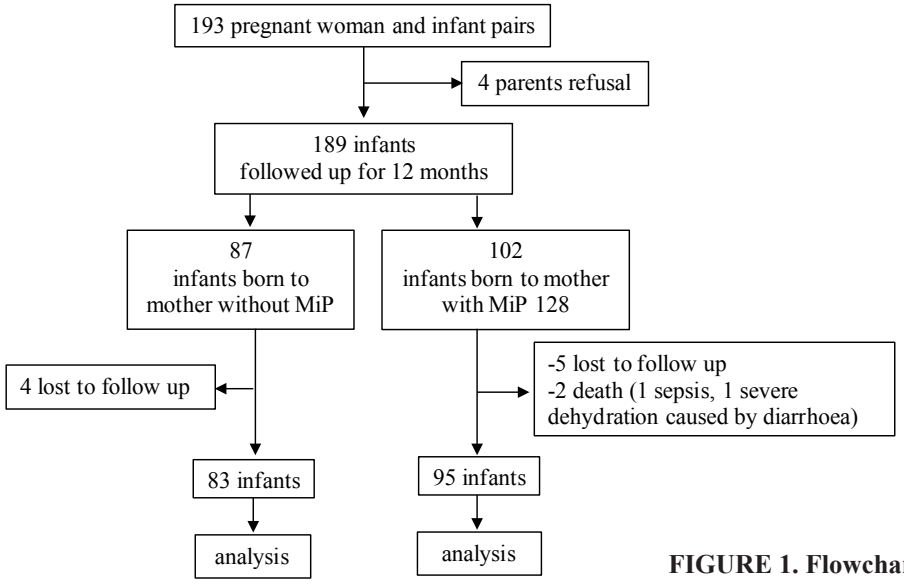

FIGURE 1. Flowchart of subject selection.

During the pregnancy, 46 women were infected during the 1st trimester, 54 during the 2nd trimester, and 14 during the 3rd trimester. There were 48 (27%) pregnant women that had at least one malaria infection, 29 (16.3%) had two infections, 14 (7.98%) had 3 or more infections and 87 (48.9%) had no infection. During delivery there were 44 mothers who had malaria infection, where 42 mothers had positive placental infection and 22 mothers had peripheral parasitemia. The mean of mother age was 25.35 ± 6.30 vs 26.0 ± 5.69 years, and the youngest was 12 years old and the oldest was 42 years old (TABLE 1). There were no significant differences in baseline characteristics between mothers with MiP and without MiP.

TABLE 1. Mother baseline characteristics

Characteristic	Malaria in pregnancy (+) n = 95	Malaria in pregnancy (-) n = 83	p*
Age (years ± SD)	25.35 ± 6.30	26.0 ± 5.69	0.14
Gravidity, n (%)			
• Primigravidae	31 (17.4)	17 (9.6)	
• Secundigravidae	25 (14.0)	34 (19.2)	0.69
• Multigravidae	39 (21.9)	32 (17.9)	
Ethnic Group, n (%)			
• Papuan	48 (27.0)	38 (21.3)	0.53
• Non Papuan	47 (26.4)	45 (25.3)	
Bed net utilization			
• Yes	28 (15.7)	24 (13.5)	0.94
• No	67 (37.6)	59 (33.2)	

*Fisher exact test, significant if p <0.05

TABLE 2 shows that there were 91 (51.12%) boys and 87 (48.88%) girls. Birth weight, head circumference and low birth weight were more likely to occur in infants from mothers with MiP positive compared with MiP negative mothers, but these results were not significant (p>0.05).

TABLE 2. Characteristics of infant

Characteristics	Malaria in Pregnancy (+) n = 95	Malaria in Pregnancy (-) n = 83	p*
Gender, n (%)			
• Boy	50 (28.1)	41 (23.0)	0.67
• Girl	45 (25.3)	42 (23.6)	
Birth weight (mean ± SD)	3028.95 ± 450.87	3051.21 ± 388.13	0.32
Birth length (mean ± SD)	49.13 ± 2.08	49.02 ± 2.06	0.68
Head circumference (mean±SD)	32.73 ± 1.56	33.02 ± 1.62	0.54
Gestational age (mean ± SD)	37.76 ± 1.07	37.53 ± 0.89	0.45
Low birth weight, n (%)			
• Yes	11 (6.2)	3 (1.7)	0.55
• No	84 (47.2)	80 (44.9)	

*Fisher exact test, significant if p < 0.05

TABLE 3 shows a bivariate analysis of the effects of MiP on infant malaria infections at age 6 and 12 months in which infants born to mother with MiP were more susceptible to malaria infection than infants born to mother without MiP (p = 0.03; RR = 3.49; 95% CI 1.02-11.96) and (p = 0.01; RR = 8.74; 95% CI 1.14-66.81) respectively.

TABLE 3. Malaria infant in 6 and 12 months old

Variable		Malaria Infant, n (%) (+)	Malaria Infant, n (%) (-)	p*	RR	95% CI
6 months old	MiP (+)	12 (12.63)	83 (87.37)	0.03	3.49	1.02-11.96
	MiP (-)	3 (3.61)	80 (96.39)			
12 months old	MiP (+)	10 (10.53)	85 (89.47)	0.01	8.74	1.14-66.81
	MiP (-)	1 (1.21)	82 (98.79)			

*Fisher exact test, significant if p < 0.05

We performed a multivariate analysis between all malaria infections over a period of 12 months with malaria-infected timings and maternal demographics (TABLE 4). In this study we found that MiP which occurred in second trimester, pregnant women who only had malaria infection 1 time before delivery and Papuan ethnicity were independent factors related to the infant susceptibility to malaria infection.

TABLE 4. Factors related to susceptibility infant to malaria infection at 6 and 12 months old

Characteristics	Infant Parasitemia (+)	Infant Parasitemia (-)	Crude RR	Crude 95%CI	Crude p	Logistic Regression RR	Logistic Regression 95%CI	Logistic Regression p
Malaria during pregnancy								
1st Trimester								
• Yes	2 (20.0)	8 (80.0)	1.77	0.48-6.55	0.41	2.76	0.41-18.43	0.29
• No	19 (11.3)	149 (88.7)						
2nd Trimester								
• Yes	12 (25.0)	36 (75.0)	**3.61**	**1.63-8.02**	**0.00**	**4.21**	**1.36-13.60**	**0.01**
• No	9 (6.9)	121 (93.1)						
3rd Trimester								
• Yes	5 (13.5)	32 (86.5)	1.19	0.47-3.04	0.72	0.99	0.27-3.74	0.99
• No	16 (11.3)	125 (88.7)						
Malaria infection 1 time								
• Yes	9 (18.8)	39 (81.2)	2.03	0.91-4.51	0.81	**3.39**	**1.09-10.57**	**0.03**
• No	12 (9.2)	118 (90.8)						
Parasitemia at Delivery								
Peripheral								
• Yes	4 (16.7)	20 (83.3)	1.51	0.56-4.11	0.43	2.43	0.18-33.64	0.51
• No	17 (11.0)	137 (89.0)						
Placental								
• Yes	6 (14.6)	35 (85.4)	1.34	0.55-3.22	0.51	2.99	0.41-22.12	0.28
• No	15 (10.9)	122 (89.1)						

Primigravidae								
• Yes	6 (12.5)	42 (87.5)	1.08	0.45-2.63	0.86	1.63	0.47-5.69	0.44
• No	15 (11.5)	115 (88.5)						
Mother Age								
• ≤18 years	2 (11.1)	16 (88.9)	0.94	0.24-3.19	0.48	0.53	0.09-3.09	0.48
• >18 years	19 (11.9)	141 (88.1)						
Ethnicity								
• Papuan	15 (17.4)	71 (82.6)	**2.67**	**1.09-6.58**	**0.03**	**3.35**	**1.09-10.28**	**0.03**
• Non Papuan	6 (6.5)	86 (93.5)						
Bednet Utilization								
• Yes	8 (14.4)	44 (84.6)	1.49	0.66-3.38	0.34	2.53	0.78-8.19	0.12
• No	13 (10.3)	113 (89.7)						

DISCUSSION

We found that at 6 and 12 months old, MiP was related to malaria infection in infants. This study is consistent with previous studies that found a significant relationship between MiP and infant susceptibility to malaria infection[27] and it also correlated with the increasing of malaria episodes during infancy.[28] Infants born to positive MiP mothers are more susceptible to malaria, presumably because placental malaria causes the transfer of maternal antibody decrease[29] leading to increase of infant susceptibility to malaria infection.

Our study found that after logistic regression analysis, MiP occurrence in the second trimester was independently associated with infant susceptibility to malaria infection in the first year of life (RR = 4.21; 95% CI 1.36-13.60; p=0.01) (TABLE 4). This finding is in contrast with the result of a previous study[18] that found MiP in the third trimester increased the risk of malaria infection during the first year of life. The association between MiP in the second trimester and infant susceptibility to malaria infection was thought to be related to the duration of malaria exposures in the mother.[22] Malhotra *et al.*[17] reported a tolerant immune process that occurred depending on the type of malaria antigen in contact with the foetus, the number of parasites and the duration of exposure and timing during pregnancy. This finding supports the premise that although the mother was exposed to malaria but the foetus has not been sensitized, so the infants are more susceptible to malaria infection.[17] Boudova *et al.*[18] reported that only placental infection during pregnancy is associated with increased risk of malaria in infancy, but in this study we found there was no significant correlation between placental malaria and infant susceptibility to malaria.

Another study reported that primigravidae are at greater risk of MiP compared to multigravidae[30] and their offspring are also more susceptible to malaria infection mainly as a result of reduced antibody transfer.[31] Yet in this study we did not find support for this conclusion. However, this study found that pregnant women who suffered from malaria as much as 1 time before delivery were associated with infant susceptibility to malaria infection. Among 26 cases of malaria in infants at 6 and 12 months of age, there were 4 infants who had malaria twice. The remaining of 21 cases consisted of 5 infants born to mothers without MiP, 7 infants born to mothers with MiP who had a different plasmodium species as their mothers and 9 infants born to mothers with MiP had the same plasmodium species as their mothers.

In all of the 9 infants who have the same species as their mothers, malaria infection of the mother occurred 1 time during the second trimester. This phenomenon corresponds to the theory that naturally acquired immunity develops over time after repeated infections[10] and the development of antimalarial antibodies as well as maternal antimalarial antibodies transferred to the foetus in the last trimester are thought to play a crucial role.[28] In malaria endemic areas like in Timika Papua, individuals develop naturally acquired immunity to both *P. falciparum* and *P. vivax* after repeated infections.[2] This immunity does not generally protect against infection per se, but protects against the development of high parasite densities and clinical symptoms.[32] HLA-G might play an important role in infant susceptibility to infection. A high level of soluble HLA-G (sHLA-G), a non-classical HLA class Ib antigen, with important immune-regulatory functions[33] was significantly associated with a higher incidence ratio of malaria in children.[34] It was consistent with the fact that the inhibition of immune responses by HLA-G expression could lead to a greater susceptibility to malaria.[24] In this study infant susceptibility to malaria infection besides being associated to MiP was also significantly correlated to Papuan ethnic (RR=3.35; 95%CI 1.09-10.28; p=0.03) (TABLE 4). This finding is consistent with previous research that found an association between Papuan ethnicity and the risk of parasitemia in pregnant women.[8]

Although a complete explanation of the pathophysiology of MiP has not yet been developed, exposure to malaria intrauterine probably correlates with placental sequestration of erythrocytes. The immune tolerance process then depends on the type of malaria antigen in contact with the foetus, the amount and the duration of the exposure, and the timing of exposure during pregnancy. The timing of malaria episodes during pregnancy results in different effects on both the mother and the foetus, where parasitaemia appears to be higher during the first and second trimesters, although follow-up on *P. falciparum* parasitaemia during the first trimester has seldom been complete.[35]

CONCLUSIONS

Infant susceptibility to malaria is associated with maternal malaria status during pregnancy. MiP in the second trimester, pregnant women who only had malaria once and Papuan ethnicity are independent risk factors for infant's increased susceptibility to malaria infection.

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The correlation between occurrence of dental caries and oral health-related quality of life on elderly population in Yogyakarta Special Region

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ABSTRACT

Dental caries is the most common oral disease affecting humans. Based on Indonesia Basic Health Research in 2013, dental caries prevalence increased up to 53.2% compared to 43.4% in 2007. One of the two most increasing prevalence occurred in population of more than 65 years. This disease might affect oral health-related quality of life (OHRQoL) since it causes pain, physical and psychological discomfort. The aim of study was to investigate the correlation between occurrence of dental caries and OHRQoL of elderly population in Yogyakarta Special Region. Occurrence of dental caries and OHRQoL were determined using Decay-Missing-Filling Teeth (DMFT) Index and Geriatric Oral Health Assessment Index (GOHAI), respectively for 118 elderly aged 60-84 years consisting 73 female and 45 male. The data then were classified into very low, low, moderate and high DMFT and low, moderate and high GOHAI. Spearman's rank correlation test was conducted to determine correlation between occurrence of dental caries and OHRQoL. Mean scores of DMFT Index and GOHAI were 16.61 ± 7.16 and 47.97 ± 9.03, respectively. Very low, low, moderate, and high DMFT Index were experienced by 4 (3.38%), 13 (11.02%), 25 (21.19%) and 76 (64.41%) of 118 elderly, respectively. Low, moderate and high GOHAI were experienced by 71 (60.17%), 25 (21.19%) and 22 (18.64%) of 118 elderly, respectively. The significantly correlation between dental caries and OHRQoL was observed in this study (r = -0,265; p = 0.004). In conclusion, there is a negative moderate correlation between the occurrence of dental caries and OHRQoL of elderly population in Yogyakarta Special Region.

ABSTRAK

Karies gigi merupakan penyakit mulut yang paling umum pada manusia. Berdasarkan data Riset Kesehatan Dasar Indonesia 2013, prevalensi karies gigi meningkat hingga 53,2% dibandingkan 43,4% pada 2007. Salah satu dari dua peningkatan prevalensi paling tinggi terjadi pada populasi usia di atas 65 tahun. Penyakit ini dapat mempengaruhi kualitas hidup terkait kesehatan mulut (*oral health-related quality of life* = OHRQoL) karena menyebabkan nyeri, ketidaknyamanan fisik dan psikologi. Tujuan penelitian ini adalah untuk mengkaji hubungan antara kejadian karies gigi dan OHRQoL pada populasi usia lanjut di Daerah Istimewa Yogyakarta (DIY). Kejadian karies gigi dan OHRQoL diukur berturut-turut dengan indeks karies gigi (*Decay-Missing-Filling Teeth* = DMFT) dan indeks penilaian kesehatan mulut usia lanjut (*Geriatric Oral Health Assessment Index* = GOHAI) terhadap 118 usia lanjut berumur antara 60-84 tahun yang terdiri dari 73 wanita dan 45 laki-laki. Data yang diperoleh dikelompokkan menjadi indeks DMFT sangat

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rendah, rendah, sedang dan tinggi dan GOHI rendah, sedang dan tinggi. Uji korelasi rank Spearman dilakukan untuk menentukan hubungan antara kejadian karies dan OHRQoL. Rerata skor indeks DMFT dan GOHAI berturut-turut adalah 16,61 ± 7,16 dan 47,97 ± 9,03. Indeks DMFT sangat rendah, rendah, sedang dan tinggi dialami oleh berturut-turut 4 (3,38%), 13 (11.02%), 25 (21,19%) dan 76 (64,41%) dari 118 usia lanjut. GOHAI rendah, sedang dan tinggi dialami berturut-turut oleh 71 (60,17%), 25 (21,19%) dan 22 (18,64%) dari 118 usia lanjut. Hubungan secara nyata antara karies gigi dan OHRQoL ditunjukkan dalam penelitian ini (r = -0,65; p = 0.004). Dapat disimpulkan terdapat hubungan negatif sedang antara karies gigi dengan kualitas hidup terkait kesehatan mulut pada populasi usia lanjut DIY.

Keywords: dental caries - DMFT index – oral health – elderly – quality life

INTRODUCTION

Despite advancements in oral disease science, dental caries continues to be a worldwide health concern, affecting humans of all ages. Approximately 2.3 billion people (32% of the population) have dental caries in their permanent teeth worldwide.[1] Dental caries is one of the most common oral diseases and it is linked to bacteria in the dental plaque overlying the dental hard tissue. Although acid generating bacteria are the etiologic agents, dental caries has been thought of as multifactorial since it is influenced by dietary and host factors as well. In addition, the role of saliva as a defense system against dental caries is well documented. These defense systems include clearance, buffering, antimicrobial agents, and calcium and phosphate delivery for remineralization.[2]

The first and most common symptom of dental caries is toothache. This is typically an infection or irritation of the tooth pulp usually causes the pain. Tooth pain or achy feeling, particularly after sweet, hot, or cold foods and drinks are first indicator. If dental caries is more severe, it can cause eating difficulty.[3] Dental caries can also cause bad breath and foul tastes.[4] In highly progressed cases, an infection can spread from the tooth to the surrounding soft tissues. Complications may include inflammation of the tissue around the tooth, tooth loss, and infection or abscess formation.[3] The earliest sign of a new carious lesion is the appearance of a chalky white spot on the surface of the tooth, indicating an area of demineralization of enamel. Visible pits or holes in the teeth are strong positive indicator of tooth decay.[2]

Recently, health is defined by a complete physical, mental and social well-being, not merely the absence of disease. Thereby the quality of life of a patient is taken into account. Oral health-related quality of life (OHRQoL) is defined as a multidimensional construct that reflects people's comfort when eating, sleeping, and engaging in social interaction; their self-esteem; and their satisfaction with respect to their oral health.[5] The OHRQoL is usually assessed by studying how factors such as function, pain, psychological, and social aspects affect the well-being of an individual.[6] Oral health-related quality of life is a more holistic approach in health care, in order to improve the oral related satisfaction and quality of life of patients, and not merely the eradication of disease.[7]

The elderly population have significantly increased in recent years. About 80% of the world elderly population is found in developing countries.[8] World Health Organization (WHO) predicted that the population of elderly in Indonesia will reach 11.34% or 28.8 million

people in 2010.[9] It is also predicted that the population of elderly in Indonesia will be up to 25% in 2050.[10] Amongst 33 provinces in Indonesia, Yogyakarta Special Region is a province with the highest number of elderly that reaches up to 14.02% in 2010. Moreover, Yogyakarta Special Regions is a province with the longest life expectancy as well i.e. up to 74.2 years in 2010 and in 2035 will be predicted up to 75.5 years.[11] The longer life expectancy of population in Yogyakarta Special Region is contributed by following factors : (i) comfortable environment; (ii) very good social support for elderly activities; (iii) very good community care; (iv) a relative cheap of life expenditure; (v) adequate health care facilities for elderly; (iv) accessible health care facilities.[12]

Health problem of elderly varies as consequences of physiologic or pathologic processes. Elderly people prone to chronic diseases and acute infections. This condition is deteriorated by decreasing immune system in elderly. Elderly at least have one chronic medical disturbance, so increasing elderly population might increase percentage of chronic diseases as well.[13] It is common that polymedication is experienced by elderly. Majority of elderly at least is taking one prescribed medication.[14] Polypathology and polymedication result from aging and disease processes. Medication for systemic diseases and systemic disease itself in elderly might cause hyposalivation either with or without xerostomia. It has been reported that 80% of prescribed medication cause xerostomia.[15]

On the other hand, oral health and function deteriorate as long as getting older.[16] Poor oral health in elders is caused by edentulism, dental caries, periodontal disease, xerostomia, dysfunction of salivary gland and oral mucosal lesion including oral precancer.[8] All these findings may give badly impact for daily life of elderly that results in decreasing of oral function, self confidence and social life that eventually affect OHRQoL. According to report of Indonesia Basic Health Research in 2013, prevalence of dental caries in Indonesia in 2013 increased up to 53.2% compared to 43.4% in 2007. One of the two most increasing prevalence occurred in population of more than 65 years.[17] The aim of this research was to evaluate the correlation between occurrence of dental caries and OHRQoL of elderly population in Yogyakarta Special Region.

MATERIALS AND METHODS

Subjects

This was an observational community-based cross-sectional study. A total of 118 elderly (60-84 years) consist of 45 males and 73 females from six representative urban and rural areas of Yogyakarta Special Region participated in this study. Three community health station for elderly (*Posyandu Lansia*) representing urban area i.e. Wirobrajan, Sewon and Minomartani and rural area i.e. Pundong, Moyudan and Berbah were randomly chosen for this study participants recruitment. Fifty nine subjects were recruited from eh area. Rural-urban characteristic was based on the criterions published by the Indonesian National Board of Statistics in 2010. A scoring technique which corresponds with the population density, proportion of agricultural-related profession, and the existence of public-leisure facilities was used to establish the criterion.[11] The protocol of the study was approved by the Medical and Health Research Ethics Committee (MHREC) of Faculty of Medicine, Universitas Gadjah Mada and Dr. Sardjito General Hospital, Yogyakarta (Approval number : KE/FK/441/EC/2016).

Protocol of study

Subjects were gathered and explained concerning the goal, the significance and the course of the study. Subjects who willing to participate in the study were given written informed consent to be signed. The subjects were then conducted clinical intraoral examination to determine DMFT index (the total number of decayed/D, missing/M and filled/F permanent teeth in an individual) using dental diagnostic instrument. Intraoral examination was carried out by four trained dentists under sufficient illumination with artificial light. Dentition status to measure the DMFT was examined using the procedures guided by the WHO Basic Oral Health Survey 2013 method. The examiners were calibrated before and during the survey, and inter-examiner reliability was assessed. According to replicated examinations of 10 patients, the Kappa value ranged from 0.75 to 0.9 which corresponds with substantial to almost perfect agreement according to the WHO Basic Oral Health Survey Method.18 The classification of DMFT index was very low (<5.0), low (5.0-8.9), moderate (9.0-13.9), and high (>13.9). The maximum score of DMFT index is 32 whereby a higher score indicates a more prevalence of dental caries (WHO, 2013).[18] Oral health-related quality of life was determined using GOHAI.[19] The 12-item questionnaire of GOHAI was developed to assess three dimensions of OHRQoL i.e. physical function, pain or discomfort and psychosocial function. It consists of a six point Likert scale from never, seldom, sometimes, often, very often and always with the score ranging from 0 to 5. The final score ranges from 0 to 60 whereby a higher score indicates a better OHRQoL. The classification of GOHAI score was high (57-60), moderate (51-56) and low (≤50).

Data analysis

Data were presented as mean ± standar deviation (SD) or percentage. Spearman's rank correlation test was conducted to determine the correlation between occurrence of dental caries and OHRQoL using software of SPSS of 16.0 version by computer. A p value < 0.05 was considered as signicant.

RESULTS

Clinical intraoral examination to determine DMFT index was conducted to all subjects using dental diagnostic instrument. The DMFT index value are presented in TABLE 1. Mean of DMFT index for all subjects was16.61 ± 7.16 with the range between 2 up to 32.

TABLE 1. Result of DMFT index (n=118)

DMFT index	Classification	Number of subjects	
		n	%
< 5.0	Very low	4	3.38
5.0 – 8.9	Low	13	11.02
9.0 – 13.9	Moderate	25	21.19
> 13.9	High	76	64.41

Oral health-related quality of life determined based on GOHAI score is presented TABLE 2. Mean of GOHAI score for all subjects was 47.97 ± 9.03 with the range between 5 up to 60.

TABLE 2. Result of OHRQoL based on GOHAI measurement (n=118)

GOHAI score	Classification	Number of subjects	
		n	%
≤50	Low	71	60.17
51 - 56	Moderate	25	21.19
57 - 60	High	22	18.64

Spearman's rank correlation test showed a negative moderate significant correlation between the occurrence of dental caries and OHROoL of elderly population in Yogyakarta Special Region (r = -0.265; p = 0.004).

DISCUSSION

Majority of subjects (64.41%) had high DMFT index (≥13.9) with the mean value of 16.61 ± 7.16. This DMFT index was higher than that in New Delhi's elderly (13.8) that obtained from total of 452 participants.20 Other DMFT index of New Delhi's elderly that obtained from 448 people aged ≥60 years was 14.4.[21] According to Indonesia Basic Health Research the prevalence of dental caries in Indonesia in 2013 increased up to 53.2% compared to 43.4% in 2007. The two most increasing prevalence occurred in population of more than 65 years (14.3%) and in children of 12 years (13.7%).

Oral and dental disease is the most disease suffered by people with the prevalence up to 61%. Dental caries and periodontal (tooth supporting tissue) disease were the two most oral and dental diseases experienced by Indonesian population.[17] These diseases are caused by dental plaque (biofilm) as a result of poor oral hygiene which leads to bacteria spreading across the tooth's surface. Biofilm accumulates in the oral cavity causes dental caries and periodontitis.[22]

Only a few countries have national data on oral hygiene habits among older people. Tooth brushing remains the most popular oral hygiene practice worldwide. However, according to the country reports this practice is less frequent in developing countries than in developed countries. Meanwhile, traditional oral self-care by use of chew sticks or powder is common in developing countries. Within regions, substantial variation is reported in the percentage of older people performing regular oral hygiene.[23]

Aging is a natural and progressive process capable of producing limitations and changes in the functioning of the body making the individual more vulnerable and susceptible to chronic diseases such as osteoarthritis, osteoporosis or Parkinson's disease.24 Severity of osteoarthritis in the hands is correlated with impaired functional ability resulting in unable to maintain proper oral hygiene that leads to plaque accumulation which increases the likehood of dental caries.[25,26] Parkinson's disease is characterized by dementia and loss of cognitive abilities cause patients having difficulties to memorize oral hygiene practice.[27] In addition, in the early stages patients may present the inability to perform functions and their motor skills that makes patients have difficulty to maintain the oral health care.[28]

Other commonly oral problem experienced by elderly is xerostomia. Xerostomia is subjective feeling of dry mouth either accompanied with hyposalivation (saliva secretion per minute <0.1 mL) or not.[29] It is estimated that about 30% of the population older than 65 suffer from xerostomia.30 Medications and systemic disease are aggravating factors that contribute to xerostomia in the elderly.[31] Xerostomia has a variety of possible causes. In recent years, the most common cause of xerostomia is medications. Xerostomia has been associated with more than 500 medications. Xerostomia can be caused by many factors such as diseases, medications, complications of radiation-therapy or chemotherapy, dehydration, psychological conditions such as anxiety and stress, complication of chronic graft-versus host disease (cGVHD), malnutrition and mouth breathing.[32,33] Xerostomia-associated diseases could be Sjogren syndrome, sarcoidosis, diabetes mellitus, primary biliary cirrhosis,

rheumatoid arthritis, stroke, Alzheimer's, depression, and chronic anxiety. Some medication that can cause xerogenic effects such as analgesics, antianxiety/ sedative/ hypnotics, anticonvulsants, antidepressants, antihypertensives, antihistamines, bronchodilators, diuretics, gastrointestinal drugs, antispasmodics, cytotoxic drugs, skeletal muscle relaxants.[34-35] Patient with hyposalivation or xerostomia also are susceptible to oral infection including candidiasis, dental caries, periodontal disease and tooth loss.[36] Without enough saliva, oral environment cannot be maintained in optimal pH, so the mouth is colonized rapidly with cariogenic bacteria and oral self-cleansing cannot be implemented that causes bad oral hygiene,[32] in turn, someone will be more susceptible having dental caries. So, the high prevalence of dental caries in this study might be contributed as well by medications consumed and diseases experienced by the subjects. In this study 19 subjects consumed antihypertensives, eight subjects consumed analgesics/anti-inflammatory medications. Six subjects consumed antihistamines and five subjects consumed gastrointestinal drugs. Besides that, it was detected that eight subjects suffered from diabetes mellitus. Osteoarthritis, rheumatoid arthritis and stroke, each was also experienced by one subject. Another cause of dental caries is poor oral hygiene since the biofilm will more accumulated in oral cavity. In this study, 51 of 118 subjects (43.22%) had poor oral hygiene that made them prone experiencing dental caries.

The majority of elderly (60.17%) had low OHRQoL that might be caused by poor oral health condition in this study (TABLE 2). This findings supported the statements that deterioration of oral health and function go along with the increasing age of people.16 From this result it seemed that the care towards oral health was still low in elderly in which this condition was probably influenced also by ageism concept that was believed by almost all elderly.[37] In this concept, elderly believes that deterioration of oral condition was natural process and occurs for all elderly, so it makes elderly having less effort to improve their oral condition.

To assess OHRQoL in this study was something so difficult since concept of quality of life is elusive and abstract. Quality of life can be intuitively understood however, it is very difficult to be defined. Perception of quality of life is influenced by many factors such as socio-economic condition, level of education, cultural, political, practical contexts in where the quality of life is implemented and measured. Talking about quality of life, someone should think with multidimensional and complex orientation since quality of life does not have a clear border and is very subjective. Quality of life assessment is full of life values.[38]

A negative moderate significant correlation between the occurrence of dental caries and OHRQoL of elderly population in Yogyakarta Special Region was observed in this sudy. It indicates that an increase score of DMFT index declines in the OHRQoL. The negative moderate significant correlation meant the more dental caries the more impact on GOHAI score by decreasing the OHRQoL. Or it can be concluded that the higher score of DMFT, the lower the OHRQoL of the elderly population in Yogyakarta Special Region.

Person with dental caries will have a symptom of pain. The pain is getting severe along with the more progressive caries process. When the enamel and dentin are destroyed, the cavity becomes more noticeable. Once the decay passes through enamel, the dentinal tubules, which have passages to the nerve of the tooth, become exposed, resulting in pain that can be transient, temporarily worsening

with exposure to heat, cold, or sweet foods and drinks. A tooth weakened by extensive internal decay can sometimes suddenly fracture under normal chewing forces. When the decay has progressed enough to allow the bacteria to overwhelm the pulp tissue in the center of the tooth, a toothache can result and the pain will become more constant. Death of the pulp tissue and infection are common consequences. The tooth will no longer be sensitive to hot or cold, but can be very tender to pressure. Dental caries can also cause bad breath and foul tastes. In highly progressed cases, an infection can spread from the tooth to the surrounding soft tissues.[3]

By understanding the chronological process of tooth decay, it was clear that dental caries will cause pain and the pain will influence the GOHAI assessment. There was three dimensions of OHRQoL i.e. physical function, pain or discomfort and psychosocial function that was assessed in GOHAI. If the dental caries was still untreated, the dental pulp will be non vital, and then the infection will spread to the periodontal tissue causing of tender to pressure. The latter will result in eating difficulty that was associated with the oral dysfunction. Dental caries becomes area of focal infection if still untreated. And it has to be extracted to prevent the spreading of the infection. Dental caries and periodontal disease is the two most oral disease that cause tooth loss. Tooth loss will impair mastication function of oral tissue. Unrehabilitated tooth loss may influence psychological condition of someone. Psychosocial aspect includes a lower self esteem, restrictions to daily life and worrisome towards oral problems. In the psychosocial aspect, speech and eating difficulties can impair social interactions which may cause some patients to avoid social engagements where it affects the OHRQoL.[30] Social interactions may also be affected due to a decreased self-esteem caused by difficulties in speech and mastication.[39] All of those impacts of dental caries in turn affect OHRQoL negatively.

This is the first study conducted in Yogyakarta Special Region to correlate the occurrence of dental caries and OHRQoL in elderly population. The results of this study might be considered by Indonesian government especially in Yogyakarta Special Region to plan the better oral health management for elderly, in turn, it can increase OHRQoL. To improve the oral health or to reduce the occurrence of dental caries in elderly requires inter-professional collaboration of health personnel since dental caries is a multi-factorial disease modulated by many aspects of health and behavior not only oral ecology.

Finally, the limitations of our study should be taken into consideration. The exact mechanism of this relationship was not clarified in this study and it needs to be further explored in longitudinal studies. Since this study was a cross sectional, which was conducted on modest sample size of 118 subjects, study with larger sample sizes needs to be carried out in the future to endorse the results observed in our study. Future work with larger, more diverse populations and more complete information would be essential to complete our findings. Furthermore, as the nature of the sample size used in this study, the result generalisability might not be completely dependable.

CONCLUSIONS

In conclusion, there is a negative moderate significant correlation between the occurrence of dental caries and OHRQoL of elderly population in Yogyakarta Special Region. The higher score of DMFT index, the lower the OHRQoL of the elderly population in Yogyakarta Special Region.

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Life style risk factors for femoral neck fracture in Dr. Sardjito General Hospital, Yogyakarta

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ABSTRACT

As life expentancy improved, the incidence of femoral neck fracture, as one of the most common traumatic injuries in the elderly, has also increased. Risk for fracture is not only determined by age and sex but also by the degree of osteoporosis and certain life-styles. The purpose of this study was to investigate life-style risk factors for femoral neck fracture in Dr. Sardjito General Hospital, Yogyakarta. In this case-control study, all patients with femoral neck fractures admitted to the Orthopedic Division and Traumatology, Department of Surgery in 2013 – 2014 was included as cases. Controls were subjects without fracture of similar age and sex. Data of corticosteroid use, habitual coffee consumption, visual acuity disorders, habitual use of slippers and engagement in routine sport activity were collected with questionnaires. We invited 63 patients (51 females and 12 males) and 63 controls. Corticosteroid use, habitual coffee consumption, visual acuity disorder and habitual use of slippers were risks factors for fractures, OR (95% CI) = 7.5 (2.9-21.6), $p < 0.001$; 7.5 (2.9-21.6), $p < 0.001$; 3.2 (1.6-6.8), $p < 0.001$ and 5.7 (2.7-12.6), $p < 0.001$, respectively. Engagement in routine sport activity was a protecting factor, OR (95% CI) = 0.10 (0.02-0.33), $p < 0.001$. In conclusion, corticosteroid use, habitual coffee consumption, visual acuity disorder and habitual use of slippers are risk for fractures, while engagement in routine sport is a protecting factor.

ABSTRAK

Seiring dengan meningkatnya usia harapan hidup, meningkat pula angka kejadian fraktur colum femur pada orang tua. Risiko terjadinya fraktur tidak hanya ditentukan oleh usia dan jenis kelamin tetapi juga derajat osteoporosis dan gaya hidup tertentu. Tujuan penelitian ini adalah untuk mengkaji hubungan antara berbagai faktor risiko gaya hidup terhadap kejadian fraktur colum femur di RSUP Dr. Sardjito Yogyakarta. Pada studi kasus-kontrol ini, semua penderita fraktur colum femur di Departemen Ortopedi tahun 2013-2014 dimasukkan sebagai kasus. Sebagai kontrol adalah subjek dengan jenis kelamin dan kelompok umur setara yang belum pernah mengalami fraktur. Data riwayat penggunaan steroid, kebiasaan minum kopi, gangguan visus, kebiasaan menggunakan sandal dan kebiasaan berolah raga dikumpulkan dengan pengisian kuesiener. Sebanyak 63 kasus (51 wanita and 12 laki-laki) serta 63 kontrol dilibatkan dalam penelitian. Riwayat penggunaan steroid, kebiasaan minum kopi, gangguan visus dan kebiasaan menggunakan sandal merupakan faktor risiko fraktur dengan rasio odds (IK 95%) berturut-turut: 7.5 (2.9-21.6), $p < 0.001$; 7.5 (2.9-21.6), $p < 0.001$; 3.2 (1.6-6.8), $p < 0.001$ dan 5.7 (2.7-12.6), $p < 0.001$. Sebaliknya, kebiasaan berolah raga merupakan faktor protektif dengan rasio odds

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(IK 95%): 0.10 (0.02-0.33), p < 0.001. Dapat disimpulkan, riwayat penggunaan steroid, kebiasaan minum kopi, gangguan visus dan kebiasaan menggunakan sandal merupakan faktor risiko fraktur, sedangkan kebiasaan berolah raga merupakan faktor protektif.

Key words: femoral neck fracture – corticosteroid – coffee - visual acuity - sport

INTRODUCTION

As life expectancy increased, the incidence of hip fractures, especially fracture of the femoral neck has also increased. In thirty years, this improvement of life expectancy will double the proportion of those ages more than 65 years. Without proper preventive measures, the increase in the proportion of elderly will naturally double the incidence of femoral neck fractures.[1] Old age was a well known risk factor for osteoporosis and the decrease in muscle volume and strength. The incidence of femoral neck fractures was highest between the age of 70-80 years. It was also much more common in women, with ratio of 3 to 1. The difference in the prevalence between gender, might be associated with longer life expectancy in women and higher prevalence of osteoporosis after menopause. Femoral neck fractures were also associated with any diseases that lead to the decreases in bone or muscle strength, such as osteomalacia, diabetes mellitus or stroke.[2]

Femoral neck fractures were less common in Africans compared to Asians or Caucasians. The reason for this lower incidence was not yet clear, however, it was known that Africans had higher bone mass and underwent slower bone loss after menopause. They also had sighly different bone structures.[1] Beside age, gender and race associated risks for femoral neck fractures, there were also life styles' associated risk factors, such as the use of coticosteroid, habitual coffee consumption, etc. These risk factors were important to be looked into since they might be preventable.[3]

This study was conducted to investigate life-style risk factors for femoral neck fracture in patients presenting to Dr. Sardjito General Hospital Yogyakarta. In this study we focused on corticosteroid use, habitual coffee consumption, visual acuity disorders, habitual use of slippers or footwears and engagement in routine sport.

MATERIALS AND METHODS

Subjects

This was a case control study. The cases were all patients admitted to the Division of Orthopedic and Traumatology, Department of Surgery, Dr. Sardjito General Hospital due to femoral neck fracture between January 2012 to December 2014. Controls were subjects from the general population who had never had any femoral fracture, with similar age, gender and area of living. We excluded subjects with dementia or history of stroke or any other cerebrovascular problems.

Protocol of study

We used questionnaires to obtain data on age, sex and area of living as well as history of life-style risk factors. History of corticosteroid use was regarded as positive if subjects used any corticosteroid at least once per month. The corticosteroid use included those prescribed by medical doctor for any indication, i.e. allergy or asthma, etc., or those obtained illegaly over the counter.

History of coffee consumption was regarded as positive when subjects drank at least one glass or one cup of coffee daily.

History of visual accuity disturbance was regarded as positive when subjects had to use eyeglasses or contact lenses, as well as medical diagnoses of cataract. Subjects were regarded as engaging in routine sport activities if he/she did it at least weekly. Furthermore, there was also question on the habitual use of slippers or other footwears at home. The study had obtained ethical approval from the Medical and Health Research Ethics Committee, Faculty of Medicine, Universitas Gadjah Mada/Dr. Sardjito General Hospital. Written informed concent were obtained from all participants.

Statistical analysis

Odd ratio or OR (95%CI) were calculated from every risk factors. Stastical significance was set at $p<0.05$. Analyses were performed with open epi at www.openepi.com.

RESULTS

In a period of Januari 2012 to December 2014, 63 patients with femoral neck fractures were obtained. Most of the patients (51 or 81%) were female. They were between 37 to 89 years old. The same number of control with similar age and gender distribution were invited from the general population. Most of the subjects (90%) came from the rural area. All patients had fractures associated with fall, either in the bathroom (27%), bedroom (24%), kitchen (21%) or in other places, such as in the living room, in the garden or on the street. More than 75% of the fall occured indoor.

Corticosteroid were commonly used. Overall, 44.4% of the cases and almost 10% of the controls used corticosteroid at least once a month. Similarly, habitual coffee consumption were also a common practice. Around half of the subjects drank coffee daily. Since most of the subjects were elderly, disorder in visual acuity were common. Most of the visual inacuity was due to the need to wear reading eyeglasses. Engagement in regular sport activity, on the other hand, were scarcely done. The odds for having femoral neck fracture for every life-style risk was presented in TABLE 1.

TABLE 1 shows that corticosteroid use, habitual coffee consumption, visual acuity disorder and habitual use of slippers were risk for fractures, while engagement in routine sport was a protecting factor.

TABLE 1. Association between life-style and femoral neck fractures

Variables		Case n=63 (%)	Control n=63 (%)	OR	95% CI	p
Corticosteroid use	Yes	28 (44.4)	6 (9.5)	7.48	2.91-21.6	<0.001
(at least once a month)	No	35 (55.6)	57 (90.5)			
Habitual coffee consumption	Yes	52 (82.5)	10 (15.9)	24.1	9.72-64.7	<0.001
(at least daily)	No	11 (16.5)	53 (84.1)			
Disorder of visual acuity	Yes	43 (68.3)	25 (39.7)	3.24	1.56-6.83	<0.001
(use of eyeglasses or contact lenses)	No	20 (31.7)	38 (60.3)			
Habitual use of slippers	Yes	43 (68.3)	17 (27.0)	5.73	2.68-12.6	<0.001
	No	20 (31.7)	46 (73.0)			
Regular sport activity	Yes	13 (20.6)	21 (33.3)	0.10	0.02-0.33	<0.001
(at least weekly)	No	50 (79.4)	42 (66.7)			

DISCUSSION

This study observed that corticosteroid use was risk factor for fracture. The finding was not suprising since corticosteroid was known to be strongly associated with osteoporosis. However, osteoporosis only explain around half of the incidence of proximal femoral fractures in the elderly.[4] What was surprising was the prevalence of corticosteroid use in the population studied, more than 40% of the cases and almost 10% of the controls use corticosteroid regularly. Population based study in the United states observed only around 1.2% of the population studied were using corticosetroid at the time of the study. Despite the many indications for corticosteroid use, it was wiser to limit its use because of its severe side effects.[5]

It was not clear from this study nor from other previous studies, however, whether the association between corticosteroid use was related to the way the corticosteroid were used: oral, topical, injection etc. It was also not clear yet, whether it was chronic low dose use or intermittent high dose use that was worse for the bone.[5] Our study observed that habitual coffee consumption was also risk factor for fracture. Liu *et al*.[6] in their systematic review reported their support for overall harm of coffee intake in increasing the risk of fractures, especially for women.

Hallstrom *et al.*[7] reported that men consuming 4 cups of coffee or more per day had 4% lower BMD at the proximal femur ($p = 0.04$) compared with low or non-consumers of coffee. This difference was not observed in women. However, their study also observed no evidence of a higher rate of any fracture (HR = 0.99; 95% CI 0.98 - 1.00 per 200 ml coffee) or hip fracture (HR = 0.97; 95% CI 0.95 - 1.00 per 200 ml coffee) with increasing coffee consumption. A study in rats observed negative interference of coffee consumption on the material and structural bone properties, diminishes trabecular and cortical bone density, and hence making bones more fragile and likely to fractures.[8] Human physiological studies and controlled balance studies show a clear but only a very small depressant effect of caffeine on intestinal calcium absorption, and no effect on total 24-h urinary calcium excretion.[9]

The epidemiologic studies showing a negative effect may be explained in part by an inverse relationship between consumption of milk and caffeine-containing beverages. Low calcium intake is clearly linked to skeletal fragility, and it is likely that a high caffeine intake is often a marker for a low calcium intake. The negative effect of caffeine on calcium absorption was small enough to be fully offset by as little as 1–2 tablespoons of milk. All of the observations implicating caffeine-containing beverages as a risk factor for osteoporosis were performed in populations consuming substantially less than optimal calcium intakes. There was no evidence that caffeine had any harmful effect on bone status or on the calcium economy in individuals who ingested the recommended daily allowances of calcium.[9]

Visual accuity was also associated with increase risk for fractures. Studies observed, it was not the accuity of the visus that was important, rather than, it was the ability to differ contrast and sense of three-dimension, i.e. the ability to perceived depth, that was an important risk for falling, and hence, risk for fracture. The impairment of the three-dimension perception was especially dangerous when the patient ascends or descends stairs. They lacked the ability to perceived the depth of the steps. What was worse, due to lesser daily activity, visual

inaccuity in older people was also seldom recognized.[10]

Sensory impairments affect older adults' ability to interact with and navigate safely in their environment, as demonstrated by the high prevalence of sensory impairment in this sample of hip fracture patients as compared with the general population of community dwelling older adults.[10] Cox *et al.*[11] observed, in femoral neck fracture patients in Glasgow, bilateral visual impairment (binocular visual acuity worse than 6/12) was found in 239 of 518 patients (46%). Of this group, the principal causes for visual deficit were untreated cataract (49%), macular degeneration (21%), uncorrected refractive error (17%), and glaucoma (3%). The visually impaired group were more likely to have symptomatic visual complaints (58 vs 26%), however, were less likely to be under optometric care (71 vs 85%). A higher proportion of the group with visual impairment lived in areas of social deprivation (40 vs 26%).

This study observed that in general, habitual use of slippers was associated with increased risk for fracture. Use of slippers decreased semato-sensoric accuity of the soles, decreased the ability to perceived sliperiness of the floor, hence increased the risk for slipping. Slippers, socks or any unstable footwears were not advisable to wear. A prospective cohort study in Boston, however, observed that wearing stable shoes was protective for indoor fall compared to walking barefoot or wearing socks only or slippers or walking in shoes with inadequate fixation (that is, no laces, straps, or buckles), increased heel height, and reduced contact area of sole.[12,13]

Engagement in routine sport was protective for fracture. Beside a proxy for better general health, active life was associated with higher bone density, hence, lower risk for fractures. Subjects who were only able to walk in short distances had 7-8 times higher risk for fractures, while those who were only able to walk few steps or bed-ridden had 11 times higher risk for fracture.[3] Our findings support encouregement for active life and engagement in routine sport activity to lessen the risk for fracture. In line with this findings, subjects who had poor self reported health had higher risk for fracture, 8 times higher than those who reported good health, and for times higher than those who reported moderate health, a clear doses dependent association.[14,15]

CONCLUSSION

This study observes that corticosteroid use, habitual coffee consumption, visual acuity disorder and habitual use of slippers are risk factors for fractures, while engagement in routine sport is a protecting factor. It is necessary to educate people, especially to those who had already have the inevitable risks, to avoid these life styles' risks and to have regular sport activity.

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Prognostic factors affecting the mortality of burn injuries patients in Dr. Sardjito General Hospital, Yogyakarta, Indonesia

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ABSTRACT

About two million people suffer from burn injuries in the United States each year, with 100,000 hospitalized in the burn unit. Around 1000 patients suffer from severe burn injuries, with each year average of 300 deaths. Improvements in the understanding of the prognostic factors affecting burn injuries over the past decades have led to advances in medical and surgical treatment. However, comprehensive data on the factors affecting burn injuries in Indonesia have not been available, yet. The aim of the study was to investigate the prognostic factors affecting the mortality of 2nd and 3rd burn injuries patients in Dr. Sardjito General Hospital, Yogyakarta. This was a cross-sectional study conducted within the period of 2007-2011 using secondary data from the Department of Medical Records. Chi-square and logistic regression analysis were used to evaluate the correlation between the prognostic factors and the mortality. A p value < 0.05 (95% confidence interval) was considered to be significant. A significantly correlation between age, burn injuries percentage, arrival time, inhalation trauma, hemoglobin level, albumin level, creatinine level, hematocrit level and the patient's mortality was observed in this study ($p<0.05$). However, the cause of burn injuries and leukocyte count had no correlation with the patient's mortality ($p>0.05$). Furthermore, patients with albumin level < 3.5 mg/dL, burn injuries percentage $>50\%$, inhalation trauma and hospitalized in 24 hours after the incident were at 22.98, 7.65, 3.0 and 4.59 times higher risk of mortality, respectively ($p<0.05$). In conclusion, albumin level, burn injury percentage, inhalation trauma and time of arrival are prognostic factors affecting the mortality of the burn injuries patients.

ABSTRAK

Sekitar dua juta orang menderita luka bakar di Amerika Serikat setiap tahun, dengan 100.000 dirawat di unit luka bakar. Sekitar 1.000 pasien menderita luka bakar parah, dengan rerata mortalitas 300 jiwa pertahun. Perbaikan dalam pemahaman faktor prognostik mortalitas akibat luka bakar selama beberapa dekade terakhir bermanfaat memajukan perawatan medis dan bedah. Namundemikian, data komprehensif tentang faktor prognostik yang mempengaruhi mortalitas akibat

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luka bakar di Indonesia belum tersedia. Penelitian ini bertujuan untuk mengkaji faktor prognostik yang mempengaruhi mortalitas pasien luka bakar derajat 2 dan 3 di Rumah Sakit Umum Pusat Dr. Sardjito, Yogyakarta. Penelitian ini merupakan penelitian potong lintang yag dilakukan selama periode 2007-2011 menggunakan data sekunder dari Departemen Rekam Medik. Analisis Chi-kuadrat dan regresi logistik digunakan untuk mengkaji hubungan antara faktor prognostic dan mortalitas. Nilai p < 0,05 dengan taraf kepercayaan 95% dianggap berbeda nyata. Terdapat hubungan nyata antara umur, persentase luka bakar, waktu kedatangan, trauma inhalasi, kadar hemoglobin, kadar albumin, kadar kreatinin, kadar hematokrit dan mortalitas ($p<0,05$). Namun demikian, penyebab luka bakar dan angka leukosit tidak berhubungan mortalitas pasien ($p>0,05$). Selanjutnya, pasien dengan kadar albumin $<3,5$ mg/dL, persentase luka bakar $> 50\%$, trauma inhalasi dan waktu kedatangan dalam 24 jam setelah kejadian mempunyai risiko mortalitas secara berturut-turut 22,98, 7,65, 3,0 dan 4,50 lebih tinggi ($p<0,05$). Dapat disimpulkan, kadar albumin, persentase luka bakar, trauma inhalasi dan waktu kedatangan merupakan factor prognostik yang mempengaruhi mortalitas pasien luka bakar.

Keywords : burn injuries – mortality – prognostic factors – tertiary care center - Indonesia

INTRODUCTION

Burn injuries are one of the most common traumas faced by medical professionals. Severe burn injuries show high morbidity and disability compared to other injuries.[1] In the United States, about 1.1 million people suffer from burn injuries each year and should receive emergency medical attention. About 45,000 people with the burn injuries need to be hospitalized, and around 4,500 people died. In Indonesia, a study performed in a hospital in Makassar reported that in a period of 5 years, 102 burn patients were treated at burn care, with mortality as much 9.2%. The highest degree of burns found was degree II a-b with 36 cases (46.7%). More than 90% of burn complications can be prevented, but the prevention and management of burn complications still require long-term solutions.[2]

Burn injuries might affect all aspects of the patient both physically and psychologically. Burn injuries might also affect all ages, from infants to elderly, and constitute problems in both developed and developing countries. The pain and suffering caused by burn injuries are not limited to the time it happened. Visible physical injuries and invisible psychological injuries take a long time in the healing process and often lead to chronic disability.[3] Severe burn injuries can also lead to death. A study showed that from 980 patients treated, 62 (6.3%) of them died. In addition, positive correlations between age, degree, type of burn injuries, burn injuries percentage and death were reported.[4]

In Indonesia, comprehensive studies concerning prognostic factors affecting mortality of hospitalized burn injuries patients especially who suffer 2nd and 3rd degree have not been reported, yet. The aim of this study was to investigate the prognostic factors

affecting the mortality of hospitalized 2nd and 3rd-degree burn injuries patients in Dr. Sardjito General Hospital, Yogyakarta, Indonesia.

MATERIALS AND METHODS

Subjects

This was a cross-sectional study to investigate the prognostic factors affecting mortality of 2nd and 3rd-degree burn injuries patients in Dr. Sardjito General Hospital, Yogyakarta, Indonesia within the period of 2007 to 2011. This study used a non-probability purposive or judgmental sampling method. The data were secondarily collected from the Department of Medical Records, Dr. Sardjito General Hospital, Yogyakarta, Indonesia, from November 2011 to February 2012. The population of this study were all hospitalized 2nd and 3rd degree burn injuries patients in Dr. Sardjito General Hospital, Yogyakarta, Indonesia, from January 2007 to October 2011.

Protocol of the study

One hundred and ninety eight burn injuries patients, not included burn injuries victins of mount Merapi's eruption, were selected in the study. Among them, only 119 patients met the inclusion and exclusion criteria. The inclusion criteria were all hospitalized 2nd and 3rd degree burn injuries patients in Dr. Sardjito Hospital, Yogyakarta, Indonesia, from January 2007 to October 2011. The exclusion criteria were: (a) burn injuries victims of Mount Merapi's eruption, (b) refusal of admission to inpatient treatment, (c) patients with other severe trauma, (d) patients with other serious co morbidities, (e) patient with incomplete medical records, (f) patient with medical record that did not contain the required data.

The burn injuries were classified into 1st until 3th degree based on the depth of the burn. Patients were considered suffer from 1st degree burn injury if the injury was limited to the upper layers of the epidermis. However, if the deeper dermal layers involved in the burn injury patients were considered suffer from 2nd degree burn injury. In this 2nd degree, the superficial was much more painful, while the deeper burn was less pain and has a feeling of blunt pressure. Patients were considered suffer from 3rd degree burn injury if all layers of the dermis involved, so that the skin was hard, dark, dry, painless, and had typical burn scar.[5]

All factors that might lead to mortality of the 2nd and 3rd degree burn injuries patients were evaluated such as the causes burn injuries, burn injuries percentage, time of arrival, inhalation trauma, hemoglobin level, leukocyte count, albumin level, creatinine level, and hematocrit level.

Statistical analysis

Data were presented as frequencies or percentages and analyzed using SPSS 15.0. Univariate and bivariate analysis using Chi-square test, and multivariate analysis with logistic regression equation were performed to describe and evaluate the relationship between the prognostic factors and the mortality of burn injuries patients. A p value < 0.05 (95% confidence interval or 95% CI) was considered to be significant.

RESULTS

The characteristics of the burn injuries patients are presented in TABLE 1. The highest number of burn victims was female with the ages mostly adults. Most of the patients involved in this study were live. The

most common cause of burns was fire with the highest percentage of the wound was ≤ 50%. Patients came to health services within ≤ 24 hours. The majority of patients did not experience inhalation trauma. From the laboratory test, the Hb level of most patients was ≥ 10 g/dL. Most patients were having total leukocyte count ≥ 12.000/mm3, the highest albumin level > 3.4 g/dL and creatinine level in the group < 1.5 g/dL. Highest hematocrit level in the group was <41%. The academic background, most patients were from senior high school. Most patients were referrals from other health facilities.

TABLE 1. Characteristic of the burn injuries patients

Characteristic of patients	Frequency (n)	Percentage (%)
Sex		
● Women	71	59.7
● Man	48	40.3
Total	119	100
Age		
● Infant	25	21.0
● Children	11	9.2
● Adult	75	63.0
● Elderly	8	6.7
Total	119	100
Death		
● Yes	28	23.5
● No	91	76.5
Total	119	100
Causes of burn injuries		
● Fire	47	39.5
● Hot water	27	22.7
● Electrical	34	28.6
● Hot oil	8	6.7
● Other	3	2.5
Total	119	100
Burn injuries percentage		
● > 50 %	20	16.8
● ≤ 50 %	99	83.2
Total	119	100
Time of arrival		
● > 24 hours	30	25.2
● ≤ 24 hours	89	74.8
Total	119	100
Inhalation trauma		
● Trauma +	14	11.8
● Trauma -	105	88.2
Total	19	100
Hemoglobin level		
● < 10 g/dL	11	9.2
● ≥ 10 g/dL	108	90.8
Total	119	100
Leukocyte count		
● ≥ 12.000/mm3	88	73.9
● < 12.000/mm3	31	26.1
Total	119	100
Albumin level		
● < 3.4 g/dL	43	36.1
● > 3.4 g/dL	76	63.9
Total	119	100
Creatinine level		
● > 1.5 g/dL	11	9.2
● ≤ 1.5 g/dL	108	90.8
Total	119	100
Hematocrit level		
● > 41 %	31	26.1
● ≤ 41 %	83	73.9
Total	119	100
Academic background		
● No school	28	23.5
● Elementary school	29	24.4
● Junior high school	26	21.8
● Senior high school	31	26,1
College/university	5	4.2
Total	119	100
Referral		
● Yes	78	65.5
● No	41	34.5
Total	119	100

A significantly correlation between age, burn injuries percentage, arrival time, inhalation trauma, hemoglobin level, albumin level, creatinine level, hematocrit level and the patient's mortality was observed in this study ($p<0.05$). However, the cause of burn injuries and leukocyte count had no correlation with the patient's mortality ($p>0.05$) as shown in TABLE 2.

TABLE 2. Multivariate and bivariate of variables and mortality

Variables	Mortality (+) n (%)	Mortality (-) n (%)	Total n (%)	p	RR (CI 95%)
Age					
• Infant	9 (36)	16 (64)	25 (100)		
• Children	0 (0)	11 (100)	11 (100)		
• Adult	15 (20)	60 (80)	75 (100)	0.027	
• Elderly	4 (50)	4 (50)	8 (100)		
Total	28 (8.4)	91 (91.6)	119 (100)		
Causes of burn injuries					
• Fire	17 (36.2)	30 (63.8)	47 (100)		
• Electrical	7 (20.6)	27 (79.4)	34 (100)		
• Hot water	3 (11.1)	24 (88.9)	27 (100)	0.087	
• Hot oil	1 (12.5)	7 (87.5)	8 (100)		
• Other	0 (0)	3 (100)	3 (100)		
Total	28 (23.5)	91 (76.5)	119 (100)		
Burn injuries percentage					
• > 50%	17 (85)	3 (15)	20 (100)		7.65
• ≤ 50%	11 (11.1)	88 (88.9)	99 (100)	0.001	
Total	28 (23.5)	91 (76.5)	119 (100)		(4.254-13.756)
Arrival time					
• > 24 hours	17 (56.6)	13 (43.3)	30 (100)		4.585
• ≤ 24 hours	11 (12.4)	78 (87.6)	89 (100)	0.001	
Total	28 (23.5)	91 (76.5)	119 (100)		(2.428-8.657)
Inhalation trauma					
• Yes	8 (57.1)	6 (42.9)	14 (100)		3.0
• No	20 (19.0)	85 (81)	105 (100)	0.004	
Total	28 (23.5)	91 (76.5)	119 (100)		(1.645-5.472)
Hb level					
• > 10 g/dL	6 (54.4)	5 (45.5)	11 (100)		2.678
• ≤ 10 g/dL	22 (20.4)	86 (79.6)	108 00)	0.02	
Total	28 (23.5)	91 (76.5)	119 00)		(1.390-5.159)
Leukocyte count					
• > 12x10^3/mm^3	23 (26.1)	65 (73.9)	88 (100)		1.620
• ≤ 12x10^3/mm^3	5 (16.1)	26 (83.9)	31 (100)	0.259	
Total	28 (23.5)	91 (76.5)	119 (100)		(0.675-3.892)
Albumin level					
• < 3.4 g/dL	26 (60.5)	17 (39.5)	43 (100)		22.977
• ≥ 3.4 g/dL	2 (26.3)	74 (73.7)	76 (100)	0.001	
Total	28 (23.5)	91 (76.5)	119 (100)		(5.73-92.132)
Creatinine level					
• > 1.5 g/dL	9 (81.8)	2 (18.2)	11 (100)		4.651
• ≤ 1.5 g/dL	19 (17.6)	89 (82.4)	108 (100)	0.001	
Total	28 (23.5)	91 (76.5)	119 (100)		(2.837-7.623)
Hematocrit level					
• > 41%	15 (48.4)	16 (51.6)	31 (100)		3.275
• ≤ 41%	13 (15.6)	75 (84.4)	83 (100)	0.001	
Total	28 (23.5)	91 (76.5)	119 (100)		(1.763-6.087)

A significantly correlation between albumin level [p = 0.001; Ep (B) = 22.977; 95% CI (5.73 to 92.132)], burn injuries percentage [p = 0.001; Ep (B) = 7.65; 95% CI (4.254-13.756)], inhalation trauma [p = 0.004; Ep (B) = 3.0 95% CI (1.645 to 5.472)], time of arrival [p = 0.001; Ep (B) = 4.585; CI 95% (2.428 to 8.657)] and the patient's mortality was observed in this study. Patients with albumin level < 3.5 mg/dL, burn injuries percentage >50%, inhalation trauma and who hospitalized in 24 hours after the incident of burn injury were at 22.98, 7.65, 3.0 and 4.59 times higher risk of death, respectively.

DISCUSSION

Burn injury is a major cause of morbidity and mortality compared to the other injuries. It can lead to social, physical and psychological impairment of the patients. The burn injury could be caused by thermal contact from a hot object, hot liquids (scalds) and fire, electrical burns, chemical burns and friction burns. Without prompt treatment, the burn injury results in longer length-of-stay in the hospital and a substantial cost treatment. The most common age group in the incidence of burn according to our cases is children. The child's exploratory and curious behavior and inability to understand the hazard is assumed to be the indirect cause of the cases. A pediatric burn injury can be more severe compared to adults because of their skin is thinner. They are also more vulnerable to burn injuries due to the bigger ratio of body surface area and body weight, and also the function of kidney, liver, and immune system are still immature.[6,7,8]

On the other hand, burn injury on the elderly patients who are having comorbid diseases and degenerative processes may worsen the prognosis. Our study showed no statistically significant correlation between the causes of burn injuries and mortality rate. However, prevous study showed a correlation between causes of burn injuries and mortality rate.[4] Further study with a bigger number of samples may be needed.

Patients with a larger percentage of burn injuries are less likely to survive. In this study, 17 of 20 patients with more than 50% burn injuries percentage died. Previous study showed that extensive burn injury correlated with patient mortality, where the more extensive the burn, the greater the mortality rate.[4] Initial treatment before receiving hospital care (the quality of pre-hospital care), competent team, assessment of patients with primary survey and secondary survey, management of life-threatening injuries, adequate intravenous fluid administration, adequate transportation, assessment and appropriate initial treatment will reduce the mortality in patients with burn injuries.[9] Inhalation trauma had a significant impact on patients survival in which this is one of the factors that increases the risk of mortality in burn injuries. There are three components in inhalation trauma, the edema in the upper respiratory tract, acute respiratory failure and carbon monoxide intoxication. In general, the diagnosis of inhalation trauma is based on the patient history and clinical presentation. Facial or nasal hair burns, soot deposition of the face or stridor breathing sound are possible signs of inhalation trauma. Burn injuries effect to the respiratory tract will result in the development of edema after 12 to 24 hours after injury. Inhalation trauma becomes the most common cause of death in patients with burn injuries.[10] However a study reported by Hu *et al*.[11] found no correlation between inhalation trauma and mortality. This discrepancy may be due to their low overall mortality rate and the patients having burn in a high percentage of total body surface area (%TBSA). This may have decreased the impact of inhalation injury on mortality.

Burn injuries can reduce the level of hemoglobin. Capillaries are damaged due to exposure to high temperatures. Damaged blood cells may cause anemia in burn injuries patients.[1] In the early stage of burn injuries, there is a destruction of red blood cells equal to the severity of skin damage. In extensive burn injuries, the loss of red blood cells is as much as 8-12% of the total circulating red blood cells per day in 5 to 7 days after injury. This situation is due to hemolytic process caused by heat and the presence of micro-thrombus in the area of necrosis, clearance by the reticulo-endothelial system, blood sampling for laboratory tests and wounds treatment.[12] Leukocytes help the body to fight infections and diseases as part of the immune system. An increasing number of leukocytes in the acute phase of burns is a normal reaction of the bone marrow due to the external stimulation, such as burns.[13]

Burn injury may reduce albumin levels. Burn on the skin induce a strong inflammatory response and release vaso-active substance that can increase vascular permeability, making the small molecules like water and albumin become easier to leaks. Our data showed that albumin levels may be useful in predicting mortality in burn patients. Another study was showed that overall mortality risk increased 84% on the patient which albumin level was <2 g/dL. Because the most important function of albumin is to maintain osmotic pressure of at least 80% of the normal level, and its reduction could induce another complication to the patients such as malnutrition, impaired immune response, increased risk of infection, slow wound healing, decrease in body mass and inhibition of rehabilitation. These results suggest that hypoalbuminemia has a detrimental effect on patient survival. The albumin level could be used as an indicator of mortality.[14, 8]

The mortality of burn injuries patients has also increased in the presence of acute renal failure as a complication of severe burn injuries due to decreased glomerular filtration rate (GFR). Decrease in GFR due to hypovolumia, depressed myocardium, and protein denaturation. Rhabdomyolysis and free hemoglobin may result in acute renal failure.[15] Hematocrit levels will increase due to the hemo-concentration. Increased hematocrit level indicates a decrease of intravascular fluid volume. If the intravascular space is not filled again with intravenous fluids, hypovolumic shock in patients with extensive burns may occur. Skin damage from burn injuries causes loss of fluids due to excessive evaporation, the entry of fluid into the bullae on the 2nd degree burn injuries, and the fluid loss in scar of 3rd degree burn injuries.[1]

Nevertheless, there are several limitations to this study. This was a cross-sectional study at a single center, and some data collection was incomplete that makes the samples excluded. The total numbers of patients with 2nd and 3rd degree of burn injuries were not mentioned. Despite these limitations, we believe that this study can provide important information about prognostic factors affecting the mortality of patients with 2nd and 3rd degree of burn injuries.

CONCLUSION

Albumin level, burn injury percentage, inhalation trauma and time of arrival are prognostic factors affecting the mortality of the 2nd and 3rd degree burn injuries patients in Dr. Sardjito General Hospital, Yogyakarta, Indonesia.

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The correlation between occurrence of dental caries and oral health-related quality of life (OHRQoL) of elderly population in Yogyakarta Special Region

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ABSTRACT

Dental caries is the most common oral disease affecting humans. Based on the Indonesia Basic Health Research (*Riskesdas*) 2013, prevalence of dental caries in 2013 increased up to 53.2% from 43.4% in 2007. One of the two most increasing prevalence occurred in population of more than 65 years. This disease might affect oral health-related quality of life (OHRQoL) since it causes pain, physical and psychological discomfort. The aim of the study was to evaluate the correlation between occurrence of dental caries and OHRQoL in elderly population in Yogyakarta Special Region. One hundred and eighteen people aged 60 – 80 years consisting 73 female and 45 male involved in the study. The occurrence of dental caries and OHRQoL were determined using decay-missing-filling teeth (DMFT) index, whereas geriatric oral health assessment index (GOHAI) instruments, respectively. The data then were classified into very low, low, moderate and high DMFT and low, moderate and high GOHAI. Spearman's rank correlation test was conducted to determine correlation between occurrence of dental caries and OHRQoL. Mean scores of DMFT index and GOHAI were 16.61 ± 7.16 and 47.97 ± 9.03, respectively. Very low, low, moderate, and high DMFT index were experienced by 4 (3.38%), 13 (11.02%), 25 (21.19%) and 76 (64.41%) of 118 elderly, respectively. Low, moderate and high GOHAI were experienced by 71 (60.17%), 25 (21.19%) and 22 (18.64%) of 118 elderly, respectively. Spearman's rank correlation test showed that the correlation coefficient (r) was -0.263 (p = 0.004). There is a negative moderate significant correlation between the occurrence of dental caries and OHRQoL of elderly population in Yogyakarta Special Region.

ABSTRAK

Karies gigi merupakan penyakit mulut yang paling sering mengenai manusia. Berdasarkan data riset kesehatan dasar (Riskesdas) 2013, prevalensi karies gigi di Indonesia tahun 2013 meningkat sampai 53,2% dari 43,4% tahun 2007. Peningkatan prevalensi antara lain terjadi pada populasi berumur lebih dari 65 tahun. Penyakit ini kemungkinan akan mempengaruhi kualitas hidup terkait kesehatan mulut karena karies gigi menyebabkan nyeri, ketidaknyamanan fisik dan psikologis. Penelitian ini bertujuan mengkaji hubungan antara kejadian karies gigi dengan kualitas hidup terkait kesehatan mulut pada populasi lanjut usia di Daerah Istimewa Yogyakarta (DIY). Seratus delapan belas penduduk berumur 60-84 tahun terdiri dari 73 wanita dan 45 laki-laki terlibat dalam penelitian ini.

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Kejadian karies gigi dan kualitas hidup terkait kesehatan mulut masing-masing ditentukan menggunakan indeks *decay-missing-filling teeth* (DMFT) dan *geriatric oral health assessment index* (GOHAI). Data yang diperoleh kemudian diklasifikasikan menjadi DMFT sangat rendah, rendah, sedang dan tinggi serta GOHAI rendah, sedang dan tinggi. Uji korelasi Spearman dilakukan untuk menentukan korelasi antara kejadian karies gigi dengan kualitas hidup terkait kesehatan mulut. Rerata indeks DMFT dan GOHAI masing-masing yaitu 16,61 ± 7,16 dan 47,97 ± 9,03. Indeks DMFT sangat rendah, rendah, sedang dan tinggi masing-masing dialami oleh 4 (3,38%), 13 (11,02%), 25 (21,19%) dan 76 (64,41%) dari 118 lansia. GOHAI rendah, sedang dan tinggi masing-masing dialami oleh 71 (60,17%), 25 (21,19%) dan 22 (18,64%) dari 118 lansia. Hasil uji korelasi Spearman menunjukkan bahwa koefisien korelasi (r) yaitu -0,263 (p = 0,004). Dapat disimpulkan terdapat korelasi negatif signifikan sedang antara kejadian karies gigi dengan kualitas hidup terkait kesehatan mulut pada populasi lanjut usia di DIY.

Keywords: Correlation – DMFT – GOHAI – OHRQoL - elderly

INTRODUCTION

Despite advancements in oral disease science, dental caries continues to be a worldwide health concern, affecting humans of all ages. Approximately 2.3 billion (32%) people have dental caries in their permanent teeth worlwide.[1] Dental caries is one of the most common infectious diseases linked to bacteria in the dental plaque overlying the dental hard tissue. Although acid generating bacteria are the etiologic agents, dental caries has been thought of as multifactorial. It is influenced by dietary and host factors as well. In addition, the role of saliva as a defense system against dental caries is well documented. These defense systems include clearance, buffering, antimicrobial agents, and calcium and phosphate delivery for remineralization.[2]

The first and most common symptom of dental caries is toothache. This is typically an infection or irritation of the tooth pulp usually causes the pain. Tooth pain or achy feeling, particularly after sweet, hot, or cold foods and drinks are first indicator. If dental caries is more severe, it can cause eating difficulty.[3] Dental caries can also cause bad breath.[4] In highly progressed cases, an infection can spread from the tooth to the surrounding soft tissues. Complications may include inflammation of the tissue around the tooth, tooth loss, and infection or abscess formation.[3] The earliest sign of a new carious lesion is the appearance of a chalky white spot on the surface of the tooth, indicating an area of demineralization of enamel. Visible pits or holes in the teeth are strong positive indicator of tooth decay.[2] Recently, health is defined by a complete physical, mental and social well-being, not merely the absence of disease. Thereby the quality of life of a patient is taken into account. Oral health-related quality of life (OHRQoL) is defined as a multidimensional construct that reflects people's comfort when eating, sleeping, and engaging in social interaction; their self-esteem; and their satisfaction with respect to their oral health.[5] The OHRQoL is usually assessed by studying how factors such as function, pain, psychological, and social aspects affect the well-being of an individual.[6] Oral health-related quality of life is a more holistic approach in health care, in order to improve the oral related satisfaction and quality of life of patients, and not merely the eradication of disease.[7]

There has been a significant increase of elderly population in recent years. About 80%

of elderly worldwide is found in developing countries.[8] World Health Organization (WHO) predicted that the population of elderly in Indonesia will reach 11.34% or 28.8 million people in 2020.[9] Amongst 33 provinces in Indonesia, Yogyakarta Special Province is a province with the highest number of elderly that reaches up to 14.02% in 2010. Moreover, Yogyakarta Special Province is a province with the longest life expectancy as well i.e. up to 74.2 years in 2010 and will be predicted up to 75.5 years in 2035.[10] The longer life expectancy of population in Yogyakarta Special Province is contributed by following factors : (i) comfortable environment; (ii) very good social support for elderly activities; (iii) very good community care; (iv) a relative cheap of life expenditure; (v) adequate health care facilities for elderly; (iv) accessible health care facilities.[11]

Health problem of elderly varies as consequences of physiologic or pathologic processes. Becoming old someone prones to chronic diseases and acute infections. This condition is deteriorated by decreasing immune system in elderly. Elderly at least have one chronic medical disturbance, so increasing elderly population might increase percentage of chronic diseases as well.[12] It is common that polymedication is experienced by elderly. Majority of elderly at least is taking one prescribed medication.[13] Polypathology and polymedication result from aging and disease processes. Medication for systemic diseases and systemic disease itself in elderly might cause hyposalivation either with or without xerostomia. It has been reported that 80% of prescribed medication cause xerostomia.[14]

On the other hand, oral health and function deteriorate as long as getting older.[15] Poor oral health in elders is caused by edentulism, dental caries, periodontal disease, xerostomia, dysfunction of salivary gland and oral mucosal lesion including oral precancer.[8] All these findings may give badly impact for daily life of elderly that results in decreasing of oral function, self confidence and social life that eventually affect OHRQoL. Indonesia Basic Health Research (*Riskesdas*) 2013 reported that the prevalence of dental caries in Indonesia increased up to 53.2% in 2013 from 43.4% in 2007. One of the two most increasing prevalence occurred in population of more than 65 years.[16] The aim of this study was to investigate the correlation between occurrence of dental caries and OHRQoL of elderly population in Yogyakarta Special Region.

MATERIALS AND METHODS

Participants

This was an observational community-based cross sectional study involving a total of 118 elderly aged 60-84 years consist of 45 males and 73 females. Participants were recruited randomly from six community health station for elderly (*Posyandu Lansia*) i.e from three representative urban area (Wirobrajan, Sewon, Minomartani) and three representative rural area (Pundong, Moyudan, Berbah) of Yogyakarta Special Region. The characteristics of rural and urban area was determined based on the criteria published by the Indonesian National Board of Statistics in 2010. A scoring technique which corresponds with the population density, proportion of agricultural-related profession, and the existence of public-leisure facilities was used to establish the criterion.[10] Written informed consent was obtained from all participants following after receive explanation concerning the goal, the significance, and the course of the study. The protocol of the study has been approved by the Medical and Health Research Ethics Committee (MHREC), Faculty of

Medicine, Universitas Gadjah Mada and Dr. Sardjito General Hospital, Yogyakarta (KE/FK/441/EC/2016).

Procedure

The subjects were conducted clinical intraoral examination to determine DMFT index (the total number of decayed/D, missing/M and filled/F permanent teeth in an individual) using dental diagnostic instrument. Intraoral examination was carried out by four trained dentists under sufficient illumination with artificial light. Dentition status to measure the DMFT was examined using the procedures guided by the WHO Basic Oral Health Survey 2013 method. The examiners were calibrated before and during the survey, and inter-examiner reliability was assessed. According to replicated examinations of 10 patients, the Kappa value ranged from 0.75 to 0.9 which corresponds with substantial to almost perfect agreement according to the WHO Basic Oral Health Survey Method.[17]

The classification of DMFT index was very low (< 5.0), low (5.0-8.9), moderate (9.0-13.9), and high (> 13.9). The maximum score of DMFT index is 32 whereby a higher score indicates a more prevalence of dental caries.[17]

Oral health-related quality of life was determined using GOHAI. The 12-item questionnaire of GOHAI was developed to assess three dimensions of OHRQoL i.e. physical function, pain or discomfort and psychosocial function. It consists of a six point Likert scale from never, seldom, sometimes, often, very often and always with the score ranging from 0 to 5. The final score ranges from 0 to 60 whereby a higher score indicates a better OHRQoL. The classification of GOHAI score was high (57-60), moderate (51-56) and low (≤50). The higher score of GOHAI the better OHRQoL.[18]

Statistical analysis

Spearman's rank correlation test using software of SPSS of 16.0 version was applied to evaluate the correlation between occurrence of dental caries and OHRQoL.

RESULTS

Clinical intraoral examination to determine DMFT index was conducted to all subjects using dental diagnostic instrument. The results are presented in TABLE 1. Mean of DMFT index for all subjects was 16.61 ± 7.16 ranging from 2 to 32.

TABLE 1. Result of DMFT index (n=118)

DMFT index	Classification	Number of subjects	Percentage (%)
< 5.0	Very low	4	3.38
5.0–8.9	Low	13	11.02
9.0–13.9	Moderate	25	21.19
> 13.9	High	76	64.41

Oral health-related quality of life was determined based on GOHAI score as demonstrated on TABLE 2. Mean of GOHAI score was 47.97 ± 9.03 ranging from 5 to 60.

TABLE 2. Result of OHRQoL based on GOHAI measurement (n=118)

GOHAI score	Classification	Number of subjects	Percentage (%)
≤50	Low	71	60.17
51 - 56	Moderate	25	21.19
57 - 60	High	22	18.64

Statistical analysis showed that the correlation coefficient (r) was -0.265 with p=0.004. It was indicated that there is a negative moderate significant correlation between the occurrence of dental caries and OHRQoL of elderly population in Yogyakarta Special Region.

DISCUSSION

Result of DMFT index showed that majority of subjects (64.41%) had high DMFT index (≥13.9) with the mean was 16.61. This mean was much higher than the mean of DMFT index in New Delhi's elderly that was 13.8 from total of 452 participants.[19] On the other hand, from Srivastava *et al.*'s study in New Delhi as well, found that the mean of DMFT index in persons aged ≥60 years during 2006-2010 was 14.4. from total of 448 subjects.[20] The mean of DMFT in this study was higher than that in Northeast China i.e. 13.9.[21] On the other hand, dental caries prevalence among the elderly in Norway was 25.4 of 582 subjects.[22] According to *Riskesdas* prevalence of dental caries in Indonesia in 2013 increased up to 53.2% compared to 43.4% in 2007.[16] The two most increasing prevalence occurred in population of more than 65 years and in children of 12 years. The increasing prevalence occurred in persons ≥65 years was up to 14.3% compared to that of the children of 12 years that was 13.7%.

Oral and dental disease is the most disease suffered by people with the prevalence up to 61%. Dental caries and periodontal (tooth supporting tissue) disease was the two most oral and dental diseases experienced by Indonesian population.[16] These diseases are caused by dental plaque (biofilm) as a result of poor oral hygiene which leads to bacteria spreading across the tooth's surface. Biofilm accumulates in the oral cavity causes dental caries and periodontitis.[23]

Only a few countries appear to have national data on oral hygiene habits among older people. Tooth brushing remains the most popular oral hygiene practice worldwide. However, according to the country reports this practice is less frequent in developing countries than in developed countries. Meanwhile, traditional oral self-care by use of chew sticks or powder is common in developing countries. Within regions, substantial variation is reported in the percentage of older people performing regular oral hygiene.[24]

Aging is a natural and progressive process capable of producing limitations and changes in the functioning of the body making the individual more vulnerable and susceptible to chronic diseases such as osteoarthritis, osteoporosis, Parkinson's disease.[25] Severity of osteoarthritis in the hands is correlated with impaired functional ability resulting in unable to maintain proper oral hygiene that leads to plaque accumulation which increases the likehood of dental caries.[26,27] Parkinson's disease is characterized by dementia and loss of cognitive abilities.[28] Due to loss of cognitive function patients having difficulties to memorize oral hygiene practice. Besides that, in the early stages patients may present the inability to perform functions and their motor skills that makes patients have difficulty to maintain the oral health care.[29]

Other oral problem that commonly experienced by elderly is xerostomia. Xerostomia is subjective feeling of dry mouth either accompanied with hyposalivation (saliva secretion per minute <0.1 mL) or not.[30] It is estimated that about 30% of the population older than 65 suffer from xerostomia.[31] Medications and systemic disease are aggravating factors that contribute to xerostomia in the elderly.[32] Xerostomia has a variety of possible causes. In recent years, the most common cause of xerostomia is medications. Xerostomia has been associated with more than 500 medications. Xerostomia can be caused by many factors such as diseases, medications, complications of radiation-therapy or chemotherapy, dehydration, psychological conditions such as anxiety and stress, complication of chronic graft-versus host disease (cGVHD), malnutrition and

mouth breathing.[33,34] Xerostomia-associated diseases could be Sjogren Syndrome, sarcoidosis, diabetes mellitus, primary biliary cirrhosis, rheumatoid arthritis, stroke, Alzheimer's, depression, and chronic anxiety. Some medication that can cause xerogenic effects such as analgesics, antianxiety/ sedative/hypnotics, anticonvulsants, antidepressants, antihypertensives, antihistamines, bronchodilators, diuretics, gastrointestinal drugs, antispasmodics, cytotoxic drugs, skeletal muscle relaxants.[34-36] Patient with hyposalivation or xerostomia also are susceptible to oral infection including candidiasis, dental caries, periodontal disease and tooth loss.[37] Without enough saliva, oral environment cannot be maintained in optimal pH, so the mouth is colonized rapidly with cariogenic bacteria and oral self-cleansing cannot be implemented that causes bad oral hygiene[33], in turn, someone will be more susceptible having dental caries. So, the high prevalence of dental caries in this study might be contributed as well by medications consumed and diseases experienced by the subjects. In this study 19 subjects consumed antihypertensives, 8 subjects consumed analgesics/anti-inflammatory medications. Six subjects consumed antihistamines and 5 subjects consumed gastrointestinal drugs. Besides that, it was detected that 8 subjects suffered from diabetes mellitus. Osteoarthritis, rheumatoid arthritis and stroke, each was also experienced by one subject. Another cause of dental caries is poor oral hygiene since the biofilm will more accumulated in oral cavity. In this study, 51 of 118 subjects (43.22%) had poor oral hygiene that made them prone experiencing dental caries.

It was clear that majority of elderly (60.17%) had low OHRQoL that might be caused by poor oral health condition (TABLE 2). This findings supported the statements that deterioration of oral health and function go along with the increasing age of people.[15] From this result it seemed that the care towards oral health was still low in elderly in which this condition was probably influenced also by ageism concept that was believed by almost all elderly.[38] In this concept, elderly believes that deterioration of oral condition was natural process and occurs for all elderly, so it makes elderly having less effort to improve their oral condition.

To assess OHRQoL in this study was something so difficult since concept of quality of life is elusive and abstract. Quality of life can be intuitively understood however, it is very difficult to be defined. Perception of quality of life is influenced by many factors such as socio-economic condition, level of education, cultural, political, practical contexts in where the quality of life is implemented and measured.[39]

According to the result of Spearman's rank correlation test showed that there was a negative moderate significant correlation between the occurrence of dental caries and OHRQoL of elderly population in Yogyakarta Special Region. A negative correlation between the occurrence of dental caries and OHRQoL indicates that an increase score of DMFT index will bring a decline in the OHRQoL. The negative moderate significant correlation meant the more dental caries the more impact on GOHAI score by decreasing the OHRQoL. Or it can be said that the higher score of DMFT, the lower the OHRQoL of the elderly population in Yogyakarta Special Region.

As stated above, person with dental caries will have a symptom of pain. The pain is getting severe along with the more progressive caries process. When the enamel and dentin are destroyed, the cavity becomes more noticeable. Once the decay passes through

enamel, the dentinal tubules, which have passages to the nerve of the tooth, become exposed, resulting in pain that can be transient, temporarily worsening with exposure to heat, cold, or sweet foods and drinks. When the decay has progressed enough to allow the bacteria to overwhelm the pulp tissue in the center of the tooth, a toothache can result and the pain will become more constant.[3]

By understanding the chronological process of tooth decay, it was clear that dental caries will cause pain and the pain will influence the GOHAI assessment. There was three dimensions of OHRQoL i.e. physical function, pain or discomfort and psychosocial function that was assessed in GOHAI. If the dental caries was still untreated, the dental pulp will be non vital, and then the infection will spread to the periodontal tissue causing of tender to pressure. The latter will result in eating difficulty that was associated with the oral dysfunction. Dental caries becomes area of focal infection if still untreated. And it has to be extracted to prevent the spreading of the infection. Dental caries and periodontal disease is the two most oral disease that cause tooth loss. Tooth loss will impair mastication function of oral tissue. Unrehabilitated tooth loss may influence psychological condition of someone. On the other hand, dental caries can also cause bad breath that may decrease self convidence of someone.[4] In the psychosocial aspect, speech and eating difficulties can impair social interactions which may cause some patients to avoid social engagements where it affects the OHRQoL.[31] Social interactions may also be affected due to a decreased self-esteem caused by difficulties in speech and mastication.[40] All of those impacts of dental caries in turn affect OHRQoL negatively.

This is the first study conducted in Yogyakarta Special Region to correlate the occurrence of dental caries and OHRQoL in elderly population. The results of this study might be considered by Indonesian government especially in Yogyakarta Special Region to plan the better oral health management for elderly, in turn, it can increase OHRQoL. To improve the oral health or to reduce the occurrence of dental caries in elderly requires inter-professional collaboration of health personnel since dental caries is a multi-factorial disease modulated by many aspects of health and behavior not only oral ecology.

Finally, the limitations of our study should be taken into consideration. The exact mechanism of this relationship was not clarified in this study and it needs to be further explored in longitudinal studies. Since this study was a cross sectional, which was conducted on modest sample size of 118 subjects, study with larger sample sizes needs to be carried out in the future to endorse the results observed in our study. Future work with larger, more diverse populations and more complete information would be essential to complete our findings. Furthermore, as the nature of the sample size used in this study, the result generalisability might not be completely dependable.

CONCLUSIONS

In conclusion, there is a negative moderate significant correlation between the occurrence of dental caries and OHRQoL of elderly population in Yogyakarta Special Region. The higher score of DMFT, the lower the OHRQoL is observed in elderly population.

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Utilization of statins, an HMG-CoA reductase inhibitors, in Ambon District Hospital, Maluku: a retrospective study

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ABSTRACT

Non-infectious diseases, including hypercholesterolemia, are now ranked top 10 in Indonesia. Statins are inhibitors of HMG-CoA reductase, an enzyme for biosynthesis of cholesterol in the liver. Statins have been proven to reduce the risk of death due to CHD and mortality from various reasons. The objective of the study is to know the utilization of statins in peripheral area of Indonesia. This retrospective study on utilization of statins prescribed for hypercholesterolemia was taken from medical record year of 2014-2015 of a District Hospital – Dr. Haulussy, Ambon, and Maluku. We collected demographic data, pre- and treated concentrations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG), types and dosages of statins or other lipid-lowering medications given. Student t-test using was performed to check statistical differences for all cholesterol and TG differences prior to therapy versus during treatment. Eighty three respondents consisted of 26 men (31.3%) and 57 women (68.7%) with mean age 60 ± 11 years old. The pre-treatment/post-treatment values (mg/dL) of TC: 245.22 ± 51.40/224.97 ± 98.79 ($p=0.004$); LDL-C: 166.07 ± 45.36/146.00 ± 41.07; HDL-C: 54.52 ± 37.95/43.00 ± 0.00; and TG: 177.36 ± 103.25/121.00 ± 52.87, respectively. Seventy-nine patients (95.2%) were treated with statins. The dosage given were 10 mg (n = 19, 22.9%), 20 mg (n = 63, 75.9%), and 40 mg (n = 1, 1.2%). Twenty-eight patients (33.7%) had no post-treatment data of cholesterol, and 31 out of 55 patients (56%) were responders. All cholesterol levels were decreased, but responder rate was only 56%. Therefore, dosage adjustment and prerequisite cholesterol level during and post-treatment measurement should be made regularly.

ABSTRAK

Penyakit non-infeksi, termasuk hiperkolesterolemia, sekarang menduduki peringkat 10 teratas di Indonesia. Statin adalah inhibitor HMG-CoA reduktase, yaitu enzim untuk biosintesis kolesterol di hati. Statin telah terbukti mengurangi risiko kematian akibat PJK dan kematian akibat berbagai alasan. Tujuan dari penelitian ini untuk mengetahui pemanfaatan statin di daerah periferal Indonesia. Penelitian retrospektif tentang pemanfaatan statin yang diresepkan untuk hiperkolesterolemia diambil dari rekam medis tahun 2014-2015 dari Rumah Sakit Distrik - Dr. Haulussy, Ambon, dan Maluku. Kami mengumpulkan data demografi, konsentrasi pra-perlakuan dan kolesterol Total (TC),

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kolesterol low-density lipoprotein (LDL-C), kolesterol HDT, dan Trigliserida (TG), jenis dan dosis statin atau obat penurun lipid lainnya yang diberikan. Uji t dilakukan untuk memeriksa perbedaan statistik untuk semua perbedaan kolesterol dan TG sebelum terapi dibandingkan selama pengobatan. Delapan puluh tiga responden terdiri dari 26 pria (31,3%) dan 57 wanita (68,7%) dengan usia rata-rata 60 ± 11 y.o. Nilai pra-perawatan/pasca perawatan (mg/dL) dari TC: 245,22 ± 51,40 / 224,97 ± 98,79 (p = 0,004); LDL-C: 166.07 ± 45.36/146.00 ± 41.07; HDL-C: 54,52 ± 37,95 / 43,00 ± 0,00; dan TG: 177,36 ± 103,25/121,00 ± 52,87. Tujuh puluh sembilan pasien (95,2%) diobati dengan statin. Dosis yang diberikan adalah 10 mg (n = 19, 22,9%), 20 mg (n = 63; 75,9%), dan 40 mg (n = 1; 1,2%). Dua puluh delapan pasien (33,7%) tidak memiliki data pasca perawatan kolesterol, dan 31 dari 55 pasien (56%) adalah responden. Semua kadar kolesterol menurun, tetapi tingkat responden hanya 56%. Oleh karena itu, penyesuaian dosis dan pengukuran tingkat kolesterol prasyarat selama dan pasca perawatan harus dilakukan secara teratur.

Keywords: Statins, drug utilization, hypercholesterolemia, HMG-CoA reductase inhibitor

INTRODUCTION

Coronary heart disease (CHD) is substantially replacing communicable diseases in many low- and middle-income countries, including Indonesia. And dyslipidemia is one of the major risk-factors of CHD. According to the latest data metabolic disease is now ranked . The first attempt to lower the risk is by changing their lifestyles, which include reducing the body weight, low animal fat and high fibre diet, taking exercise and smoking cessation. However, these efforts could only reduce the cholesterol level up to 15%.[1]

Statins are group of lipid-lowering drugs, which have been proven by many clinical trials not only in lowering the cholesterol level but also cardiovascular diseases in primary and secondary prevention studies.[2-6] From these short and medium-term clinical trial data and long-term clinical studies, the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) established targets for LDL-C that depend on the individual risk of the patient, with the lowest LDL-C targets (<100 mg/dL) recommended for patients with CHD or other forms of vascular disease.[7,8] However, recently American College of Cardiology/ American Heart Association (ACC/AHA) in 2013 made another approach which is focused not on lowering LDL-C level per se, but make statins the linchpin of their recommendation.[9] This notion was made due to lack of clinical evidences to support titration of drug therapy to specific LDL-C and/or non-HDL-C goals. On contrary, the intensity of statin therapy did show evidences on reducing the CHD.[10] Evidence suggests that in actual practice it is often the goal of decreasing cholesterol levels is not achieved especially in developing countries. Previous studies showed that there is a huge gap on the effectiveness of statin used in clinical practice in comparison with clinical trials.[11-13]

The aim of the study was to investigate the utilization of statin in peripheral area of Indonesia. The data was gathered from a district hospital in Maluku province, Indonesia.

MATERIALS AND MethodS

Subjects

A retrospective study on utilization of statins prescribed for hypercholesterolemia was conducted by collecting data from medical record year of 2014-2015 from District

Hospital – Dr. Haulussy, Ambon, Maluku. The inclusion criteria were 1) all patients diagnosed with primary hypercholesterolemia with a value of TC ≥ 240 mg/dL or LDL cholesterol: ≥ 160 mg/dL with or without other complications (e.g. T2DM); 2) all of the above patients who were given statin-lowering cholesterol-lowering drugs (HMG-CoA reductase inhibitor); 3) statins that are used as main drugs or supplemental drugs with other cholesterol-lowering. The exclusion criteria were 1) all subjects diagnosed not with primary hypercholesterolemia; 2) subjects who were diagnosed with primary hypercholesterolemia but did not receive any statins; 3) subjects who have had not measurement of cholesterol concentration prior to treatment with statin.

Protocol of study

Patients' medical records were reviewed to select patients who fulfil the inclusion and exclusion criteria. The data collection form was prepared to collect patients' characteristics (gender and age), diagnosis, relevant laboratory value of pre- and during treatment i.e. total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG), concomitant of diseases i.e. T2DM, hypertension, chronic kidney diseases), and prescribed medications (types of statins or other lipid-lowering medications given, and dosages of statins).

Statistical analysis

Data of cholesterol profile and TG were presented as mean ± standard deviation (SD). Student t-test was performed using SPSS ver. 22 to determine statistical differences for all cholesterol and TG differences pre treatment and at the time the data collected.

RESULTS

We gathered data from eighty three respondents consisted of 26 men (31.3%) and 57 women (68.7%) with mean of age 60 ± 11 years old. Seventy nine subjects (95.2%) were treated with simvastatin, 3 subjects (3.61%) with atorvastatin and 1 patient (1.20%) with pravastatin, respectfully. The dosage given were 10 mg (n=19, 22.9%), 20 mg (n= 63, 75.9%), and 40 mg (n=1, 1.2%). Twenty-eight subjects (33.7%) had no post-treatment data of cholesterol. None of them reached the targeted LDL-C concentration of < 100 mg/dL.

It is clearly shown from TABLE 1 that the only parameter for diagnosis and for follow-up is total cholesterol and only few were measured their non-HDL cholesterol and LDL-C. Moreover, very few subjects who were followed-up to measure the effect of the treatment.

TABLE 1. Cholesterol profiles (mean ± SD) of respondents pre treatment and at the time of data collected

Cholesterol (mg/dL)	Pretreatment (n)	Treatment (n)	CI	p
TC	245.22 ± 51.4 (83)	224.97 ± 98.8 (39)	13.4-64.2	0.004
LDL-C	166.07 ± 45.4 (27)	146.00 ± 41.1 (3)	-111.4-174	0.445
HDL-C	54.52 ± 37.95 (25)	43.00 ± 0.00 (2)	-97.35 – 131	0.310
TG	177.36 ± 103.2 (28)	121.00 ± 52.87 (5)	-97.37 – 137	0.661

TABLE 2 shows that only 14.45% of subjects are diagnosed with dyslipidaemia and the rest of them having comorbidities such as hypertension, type 2 diabetes mellitus (T2DM), and chronic kidney diseases (CKD).

TABLE 2. Type of diagnosis and number of cases

Diagnosis	Number of cases	Percentage (%)
Dyslipidaemia	12	14.45
Dyslipidaemia + hypertension	38	45.78
Dyslipidaemia + T2DM	17	20.48
Dyslipidaemia + hypertension + T2DM	15	18.07
Dyslipidaemia + CKD	1	1.20
Total	83	99.98

Legend: T2DM=type 2-Diabetes Mellitus, CKD=chronic kidney disease

TABLE 3 shows cases of dyslipidaemia subjects who have had comorbid and treated with simvastatin.

TABLE 3. Cases of dyslipidaemia subjects who have had comorbid and treated with simvastatin

Medical record No.	Sex	Age	Comorbid	Type & dosage of statin – Intensity therapy
027810	F	50	T2DM, Hypertension	Simvastatin, 20 mg, MI
000166	F	51	T2DM, Hypertension	Simvastatin, 20 mg, MI
181971	F	52	T2DM, Hypertension	Simvastatin, 20 mg, MI
138527	M	51	T2DM	Simvastatin, 20 mg, MI
029457	F	53	T2DM	Simvastatin, dose n/a
006518	F	67	Hypertension	Simvastatin, dose n/a

Legend: T2DM=Type2-Diabetes Mellitus, MI = moderate-intensity statin therapy

TABLE 4 shows the percentage reduction of number of subjects who did not take routine check-up for their cholesterol level. It appears that measurements of total cholesterol, LDL, HDL and TG levels were not consistent.

TABLE 4. Percentage reduction in the number of subjects who re-examined cholesterol levels

Cholesterol	Number of subjects		% of decrease of subjects who did follows-up
	Pre-treatment	At the time of observation	
TC	83	39	53
LDL-C	27	3	89
HDL-C	25	2	92
TG	28	5	82

DISCUSSION

Total Cholesterol, LDL-C, and TG levels were decreased, but only TC was significantly decreased. However, none of these results reached the target level, and responder rate was only 56%. This inefficacy most likely caused by inappropriate of dosing, incompliant, and lack of laboratory monitoring. This was also in accordance to other studies conducted in three hospitals in Jakarta, Indonesia.[13] However, other statins, such as atorvastatin, rosuvastatin, pravastatin and lovastatin were used in those three hospitals, and it was shown also that atorvastatin was more effective compared to other statins.

TABLE 2 shows that more than 85% subjects were diagnosed not only dyslipidaemia but also with other comorbid. The most comorbid observed were subjects with hypertension (38 subjects, 45.78%), T2DM (17 subjects, 20.48%), hypertension and T2DM (15 subjects, 18.07%), and CKD (1 patient, 1.20%), respectively. However, if we observed the dosage of the simvastatin, it was shown that all subjects received the same dosage, regardless of the cholesterol concentration they had and their comorbidities (TABLE 3). Adjustment of statin dose should be done regularly according to the cholesterol levels as well as existing comorbidities and in accordance to the newest guidelines.[7,14,15] With regards to the issues on the relationship between statins and T2DM, we do not know whether there are new-onset of T2DM emerged among the subjects in our study. Some studies reported that all statins have the potency to increase the incident, but other studies mentioned that the evidence is not firm yet.[16] Although, in JUPITER study, rosuvastatin 20 mg versus placebo, has high hazard ratio (HR: 1.25, 95% CI, 1.05-1.49) and in CORONA study (rosuvastatin 10 mg versus placebo) with RR 1.13 (95% CI, 0.86-1.50). Whereas, simvastatin, as it is most prescribed statin in this hospital, has lower RR.[17] Other aspect that should be considered, what is the impact of statins on glycaemic control in subjects with DM? Due to insufficient methodological approaches and data, experts could not draw a firm conclusion on that issue. The available data suggest that, statin impacts on glycaemia control is very small (mean increase of ≈ 0.3% or less).

The use of statins in peripheral areas often could not follow the guidelines. Various factors underlie this issue. As it is shown in TABLE 3, most of the subjects did not take regular check-up for monitoring their therapy. Findings from previous research indicate that lack of medication adherence, follow-up examination of treatment outcomes by doctors, routine cholesterol checks and other clinical examinations are commonplace in health care facilities in developing countries.

Nevertheless, since 4 years, Indonesia has been running a system of national health services named *Jaringan Kesehatan Nasional-Badan Penyelenggara Jaminan Sosial* (National Health Network – Social Security Assurance Agency) that applies to the whole community. Prior to this program, the people should pay from their own pocket for any health services they received. This could be the reason why most of the subjects did not comply with the guidelines. Through this program, it is hoped that the above problems will no longer occur. Initial examination and follow-up examination can be performed regularly, so monitoring the success of therapy can be guaranteed. Therefore, a further study to compare of health service in pre- and JKN-BPJS era, with regards to metabolic diseases should be considered.

CONCLUSION

Utilization of statins in Ambon District Hospital, Maluku can decrease all cholesterol

levels were decreased. However, the responder rate is only 56%. Therefore, dosage adjustment and prerequisite cholesterol level during and post-treatment measurement should be made regularly.

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Hearing treshold before and after middle ear surgery in chronic suppurative otitis media (CSOM)

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ABSTRACT

Hearing loss is a common symptom in chronic suppurative otitis media (CSOM), often cause social communication disturbance. Canal wall up tympanoplasty and canal wall down tympanoplasty are surgery procedures for managing this disease that no response to convensional treatment. These surgery procedures should consider to hearing function impact. The aim of this study was to evaluate the difference hearing threshold between before and after middle ear surgery on CSOM patients. It was an historical cohort study conducted from January 2015 to December 2016 involving CSOM patients who underwent canal wall up tympanoplasty surgery and canal wall down tympanoplasty in The Otology Division, Departement of Ear, Nose, Throat, Head and Neck Health, Dr. Sardjito General Hospital, Yogyakarta. The inclusion criteria included basic data, diagnostics, surgery reports, and audiometry results before and 3 months postoperatively, while the exclusion criteria were not complete medical record data. Total of 64 patients with CSOM were involved in this study consisting of 32 patients who underwent canal wall up tympanoplasty and 32 patients who underwent canal wall down tympanoplasty. Significantly different in the increasing of hearing threshold between before and after canal wall up tympanoplasty surgery compared to the canal wall down tympanoplasty was observed ($p = 0.021$). In addition, surgical technique was the main factor affecting postoperative hearing threshold in CSOM patients ($p < 0.05$). In conclusion, the increasing of hearing threshold in CSOM patients underwent canal wall up tympanoplasty surgery is better than those underwent canal wall down tympanoplasty.

ABSTRAK

Penurunan pendengaran merupakan gejala umum pada penderita otitis media supuratif kronis (OMSK) yang sering menyebabkan gangguan komunikasi sosial. Timpanoplasti dengan metode *canal wall up* dan *canal wall down* merupakan tindakan bedah untuk pengelolaan penyakit ini apabila pengobatan konvensional tidak memberikan respon. Tindakan bedah ini harus mempertimbangkan efek sampingnya pada fungsi pendengaran. Tujuan penelitian ini adalah untuk mengkaji perbedaan antara ambang pendengaran sebelum dan sesudah tindakan bedah telinga tengah pada pasien OMSK. Penelitian kohort

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historik ini dilakukan dari Januari 2015 sampai Desember 2016 melibatkan pasien OMSK yang menjalani timpanoplasti dengan metode *canal wall up* dan *canal wall down* di Divisi Otologi, Departemen Ilmu Kesehatan Telinga Hidung Tenggorok-Kepala dan Leher (THT-KL), RSUP Dr. Sardjito, Yogyakarta. Kriteria inklusi adalah pasien mempunyai kelengkapan data dasar, hasil diagnosis, laporan operasi dan hasil audiometri sebelum dan 3 bulan pasca operasi, sedangkan kriteria eksklusi adalah data rekam medis pasien tidak lengkap. Total sebanyak 64 pasine dengan OMSK yang terdiri dari 32 pasien menjalani timpanoplasti metode *canal wall up* dan 32 pasien metode *canal wall down.* Terdapat perbedaan nyata perbaikan ambang pendengaran sebelum dan setelah timpanoplasti dengan metode *canal wall up* dibandingkan dengan metode canal wall down (p = 0,021). Selanjutnya terbukti, teknik bedah merupakan factor utama yang berpengruh terhadap ambang pendengaran pasca operasi pada pasien OMSK (p <0.05). Dapat disimpulkan, peningkatan ambang pendengaran pasien OMSK yang menjalani timpanoplasti metode *canal wall up* lebih baik dibandingkan dengan metode *canal wall down*.

Keywords : chronic suppurative otitis media – hearing threshold – middle ear surgery – tympanoplasty - audiometry

INTRODUCTION

Chronic suppurative otitis media (CSOM) is one of the chronic inflammatory diseases of the middle ear which is characterized by perforation of tympanic membrane and persistent or absent secretions that occur over 3 months. This disease still as problem in Ear, Nose and Throat field.[1] The CSOM can be divided into two types i.e. benign type and danger type (maligna).[2] According to other literature the CSOM can be divided into two types i.e. CSOM with kolesteatoma and CSOM without kolesteatoma. The management of CSOM is different depending on the type.[3,4]

The CSOM is a common worldwide disease especially in developing countries with low socioeconomic status with the prevalence varies from 0.5 to 30%.[5] Prevalence of CSOM surveys worldwide showed the global burden of illness from CSOM involved 65-330 million people with otorrhoea, 60% of whom (39-200 million) suffered from significant hearing loss.[2] In Indonesia, the CSOM prevalence ranges from 3.9 up to 5.6%. Data from Hearing and Illumination Health Survey conducted in seven provinces from 1994 to 1996 showed that the most common cause of middle ear morbidity was benign CSOM (3%).[6] According to the patient's medical records in Departement of Otorhinolaryngology, Head and Neck Surgery, Dr. Sardjito General Hospital Yogyakarta in 1998-1999, 40 patients with CSOM was recorded and 25 (62.5%) of them underwent mastoidectomy surgery.[7]

Chronic suppurative otitis media causes damage to some or all of the tympanic membrane and affects hearing loss with a maximum reduction of 40 dB. Tympanic membrane perforation with damage auditory ossicles may affect the hearing loss of conduction-type hearing loss by 60 to 70 dB.[5,8] The type of deafness caused by CSOM in the form of conductive deafness and deafness of the mixture with the degree of deafness depends on the involvement of the hearing bones.[9] Chronic suppurative otitis media can be managed with medical treatment and surgery. Tympanoplasty is a technique of middle ear surgery with the goal of eradication of pathological tissue and infection in the middle ear and reconstruct hearing mechanisms with or without graph and reconstruction of the

hearing bones, this surgical technique can be combined with canal wall upmastoidectomy or canal wall down with the aim of eradication of disease in the mastoid region and middle ear.[1]

Examination of pure tone audiometry is a still relevant assessment of hearing status. Audiometry can be used to assess the presence or absence of post-surgical hearing repair in the middle ear by measuring both air delivery and bone conduction at frequencies 500, 1000, 2000, 4000, 8000 Hz, for the calculation of mean hearing threshold values can be measured at frequencies 500, 1000, 2000 Hz because these frequencies represent daily conversations.[1,8] The aim of this study was to evaluate the difference hearing threshold before and after middle ear surgery between canal wall up tympanoplasty and canal wall down tympanoplasty procedures in patients CSOM.

MATERIALS AND METHODS

Subjects

This was an observational retrospective cohort study to evaluate the difference hearing threshold of CSOM patients between before and after middle ear surgery using canal wall up tympanoplasty and canal wall down tympanoplasty technique. The study was conducted over a period of January 2015 until December 2016 in the Otology Division, Departement of Otorhinolaryngology, Dr. Sardjito General Hospital Yogyakarta on CSOM patients who meet the inclusion and exclusion criteria. The inclusion criterion included basic data, diagnostics, surgery reports, and audiometry results before and 3 months postoperatively, while the exclusion criteria were not complete medical record data.

Protocol of study

All patients who presented signs and symptoms suggesting CSOM based on medical record data and underwent middle ear surgery both with canal wall up and canal wall down tympanoplasty methods by the same surgeon were identified. Thereafter, the patients were the grouped into two groups i.e. group of patients who underwent canal wall up tympanoplasty and group of patients who underwent canal wall down tympanoplasty. The audiometry was conducted following standard protocol before and 3 months after surgery. For calculation of average hearing loss four frequencies were selected i.e. 500, 1000, 2000 and 4000 Hz and assessed by air-bone conduction range (ABG). Protocol of this study has been approved by the Health Research Etics Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta (Ref: KE/FK/0196/EC/2017).

Statistical analysis

The differences of ABG at the frequency of 500, 1000, 2000 and 4000 Hz in each midle ear surgery method were analyzed using independent t-test. Furthermore, the difference of ABG between before and after surgery was analyzed using paired t-test. A p value < 0.05 was considered to be significantly different.

RESULTS

Sixty four CSOM patients consisting 32 patients undergoing a canal up wall tympanoplasty and 32 patients undergoing a canal wall down tympanoplasty procedures were included in this study. The mean age of patients undergoing the canal wall up tympanoplasty was 36.78 ± 12.21 years with the youngest aged 18 years and the oldest aged

60 years, whereas the mean age of patients undergoing canal wall down tympanoplasty was 32.88 ± 14.91 years with the youngest aged 8 years and the oldest aged 56 years (TABLE 1). No significantly different was observed in the mean age of the both groups (p=0.246). Distribution of sex in the group of canal wall up tympanoplasty were 16 (51.61%) male patients and 16 (48.48%) female patients, whereas in the group of canal wall down tympanoplasty were 15 (48.39%) male patients and 17 (51.52%) female patients. No significantly different was also observed in the sex distribution of the both groups (p=0.210).

TABLE 1. The basic characteristics of patients

Variable	Canal wall up tympanoplasty	Canal wall down tympanoplasty	p
Age (mean±SD years)	36.78 ± 12.21	32.88 ± 14.91	0.246[a]
Sex [n (%)]			
Male	16 (51.61)	15 (48.39)	0.210[b]
Female	16 (48.48)	17 (51.52)	
AC (mean ± SD dB)			
AC 500 Hz	46.25 ± 13.01	64.22 ± 13.14	0.001[a]
AC 1000 Hz	46.88 ± 1.55	66.72 ± 14.73	0.001[a]
AC 2000 Hz	46.88 ± 11.13	66.41 ± 13.33	0.001[a]
AC 4000 Hz	42.81 ± 14.59	63.13 ± 14.91	0.001[a]
BC (mean ± SD dB)			
BC 500 Hz	13.75 ± 10.63	25.16 ± 12.92	0.001[a]
BC 1000 Hz	12.19 ± 7.61	27.19 ± 11.77	0.001[a]
BC 2000 Hz	18.44 ± 8.47	30.00 ± 14.59	0.001[a]
BC 4000 Hz	15.47 ± 11.59	30.31 ± 14.69	0.001[a]
ABG (mean ± SD dB)			
ABG 500 Hz	32.5 ± 12.38	39.06 ± 14.67	0.058[a]
ABG 1000 Hz	34.69 ± 13.67	39.22 ± 18.87	0.209[a]
ABG 2000 Hz	28.43 ± 10.88	36.41 ± 10.94	0.005[a]
ABG 4000 Hz	27.34 ± 12.51	32.81 ± 11.21	0.070[a]

Note : SD: standard deviation; AC: air conduction; BC: bone conduction; ABG: air bone gap; [a]:independent t-test; [b]: chi-square;

Table 2 show the difference between air conduction (AC) and bone conduction (BC) both group of surgical methods. For the difference of air surge increase based on surgery on each frequency 500 Hz, 1000 Hz, 2000 Hz and 4000 Hz in both groups not statistically significant difference with p> 0.05 as in table 3, while table 4 showsthe air-bone gap between before and after surgery.

TABLE 2. Differences AC (mean ± SD dB) before and after surgery at each audiometric frequency

Frequency	Method	Befofe surgery	After surgery	p
500Hz	Canal wall up tympanoplasty	46.25 ± 13.01	40.78 ± 12.12	0.006
	Canal wall down tympanoplasty	64.22 ± 13.14	59.06 ± 16.19	0.001
1000Hz	Canal wall up tympanoplasty	46.88 ± 13.55	40.47 ± 10.58	0.001
	Canal wall down tympanoplasty	66.72 ± 14.73	63.28 ± 17.63	0.001
2000Hz	Canal wall up tympanoplasty	46.88 ± 11.13	39.84 ± 8.37	0.003
	Canal wall down tympanoplasty	66.41 ± 13.33	62.03 ± 16.16	0.001
4000Hz	Canal wall up tympanoplasty	42.81 ± 14.59	41.41 ± 13.27	0.515
	Canal wall down tympanoplasty	63.13 ± 14.91	60.00 ± 17.60	0.001

Note : AC: air conduction

TABLE 3. Differences in the increase in AC (mean ± SD dB) based on the type of surgical methode at each audiometric frequency

Frequency	Canal wall up tympanoplasty	Canal wall down tympanoplasty	p
500 Hz	5.47 ± 10.5	5.16 ± 10.36	0.905
1000 Hz	6.41 ± 9.86	3.44 ± 9.95	0.235
2000 Hz	7.03 ± 12.17	4.38 ± 8.4	0.314
4000 Hz	1.4 ± 12.06	3.13 ± 8.4	0.511

Note : AC: air conduction

TABLE 4. Differences ABG (mean ± SD dB) before and after surgery at each audiometric frequency

Frequency	Method	Befofe surgery	After surgery	p
500Hz	Canal wall up tympanoplasty	32.50 ± 12.38	27.19 ± 11.07	0.039
	Canal wall down tympanoplasty	39.06 ± 14.67	36.09 ± 14.01	0.142
1000Hz	Canal wall up tympanoplasty	34.69 ± 13.67	29.53 ± 10.73	0.039
	Canal wall down tympanoplasty	39.22 ± 14.87	37.81 ± 14.86	0.397
2000Hz	Canal wall up tympanoplasty	28.43 ± 10.88	21.72 ± 9.97	0.004
	Canal wall down tympanoplasty	36.41 ± 10.94	33.75 ± 11.43	0.074
4000Hz	Canal wall up tympanoplasty	27.34 ± 12.51	23.59 ± 10.49	0.021
	Canal wall down tympanoplasty	32.81 ± 11.21	30.31 ± 12.70	0.065

Note : ABG: air bone gaap

DISCUSSION

No significantly different was observed in the mean of patients age and sex distribution of the both groups. The results of this mean age and sex distribution were consistent with the results reported by Shyfakumar *et al.*[10] which showed the pateints mean was 30.14 ± 0.98 years consisting 24 (48%) male and 26 (52%) among 50 CSOM patients involved in their study. It was indicated that the CSOM

became a major problem in the productive age in the male and femal patients.

The AC and BC hearing thresholds of CSOM patients undergoing canal wall up tympanoplasty at each audiometric frequency (TABLE 2 and 4) were significantly different compared to those who undergoing canal wall down tympanoplasty (p=0.001). These suggest a more severe in hearing loss in patients underwent canal wall up tympanoplasty than in canal wall down tympanoplasty group. The CSOM patients have pathological abnormalities with the extension of pathological tissue and cholesteatoma reaching to mesotympany and hypotympanic areas so that there is damage to the circuit of hearing bone and also happened fixation of stapes foot which impact on decreasing threshold reach 60 dB or more.[2,11] Albera *et al.*[12] reported that 82% of patients with malignant/ cholesteatoma CSOM had impaired or impaired hearing bone chain. More than 78% of damage occurred in incus auditory bone and more than 45% of damage occurred in more than one hearing bone. This results in heavier conductive deafness when compared with CSOM patients in the absence of cholesteatoma in addition to the presence of cholesteatoma tissue in a nice round window region will result in impedance and phase disturbance in the oval and transparent lobes resulting in noise conduction disturbance in the cochlea.[11,13]

Other factors that may result in decreased bone conduction in patients with CSOM are influenced by: 1) sealed or obliterated round window by middle ear granulation tissue that affects the loss of phase difference between round window and oval window, 2) stiffness of hearing bone as a result of granulation tissue and cholesteatomas that envelop the hearing bones, 3) perforation and discontinuities of the hearing bone.[4]

The difference between the air-bone conductivity range in each groups of both canal wall up and canal wall down tympanoplasty is statistically significant, especially at 2000 Hz (p=0.005). The diagnosis and management of each group sample has been adjusted for clinical indications and considerations for the selection of surgical procedures. Patients with a diagnosis of chronic active suppurative otitis media of unsigned benign/ unsafe type performed canal wall up tympanoplasty procedures, while patients with chronic malignant suppurative otitis media were performed by canal wall down tympanoplasty surgery. The selection of these two surgical techniques is based on the extension of the pathologic/ cholesteatoma tissue, access to reach the tympanic cavity area, the threshold value and the presence or absence of both intratemporal and intracranial complications.[11]

The significantly differences between AC hearing thresholds on canal wall up tympanoplasty and canal wall down at frequencie 500 and 1000 and 2000 Hz was oserved ($p<0.05$). However, there is significantly differences at frequencies 4000 Hz (p=0.515). This suggests that the surgery performed on the CSOM patients accompanied by a good middle ear reconstruction with canal wall up tympanoplasty techniques and canal wall down tonnage can improve the conduction system in the auditory process. This result does not differ greatly from the previous study research in which the pre-operation airspeed threshold at frequencies 500, 1000, 2000 and 3000 Hz at 51 ± 13 dB and after surgery to 40 ± 11 dB.[14]

Significantly differences in outcome of the air-bone conduction range, especially in the canal wall up tympanoplasty group before and after surgery at all frequencies (TABLE 4) were observed ($p<0.05$). However, no significantly differences of the air-bone conduction range

in canal wall down tympanoplasty group were observed as shown in TABLE 4 ($p>0.05$). This result are similar to the previous study which reported that no significantly differences in air-bone conduction in ABG between canal wall up tympanoplasty and canal wall down tympanoplasty after surgery with a value of 10.9 dB in canal wall uptank and 13.5 dB on the wall tympanoplasty collapsed.[15]

CONCLUSION

In conclusion, canal wall up tympanoplasty in CSOM patients is better to improve hearing threshold compared to canal wall down tympanoplasty.

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Surgical outcome of scoliosis in Marfan syndrome: a case series report

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ABSTRACT

Scoliosis in marfan syndrome (MFS) manifests on 60% patients. Moreover, the scoliosis noticeable in earlier age is more progressive, refracted, and rigid compared to idiopathic adult scoliosis. The surgical correction provides notorious higher perioperative risk, whereas conservative treatment using brace is not effective to prevent progressivity of the scoliosis. In this a case report, we reported the surgical outcome of MFS scoliais patients with MFS who operated using posterior fusion instrumentation by mean of the quality of life SF-36 questioner. This was a retrospective case series involving five MFS scoliosis patients who underwent posterior fusion instrumentation with initial Cobb angle of 87.417.57° and initial kyphotic angle of 32.8 ± 14.52°. Clinical, radiological and quality of life of the patients based on SF-36 questionnaire were evaluated within 6-36 months follow up. Post-operative showed the Cobb angle become 46.2 ± 16.3° and the kyphotic angle become 21.6 ± 9.94°. No intraoperative or post-operative complications were observed. After 6-36 months follow up, the Cobb angle became 45.2 ± 17.48° and the kyphotic angle became 21.6 ± 9.94°. In addition, all patients had physical and mental health scored similar to 2 years post-surgery scoliosis scoring according to SF-36 orthopedic scoring guidelines. I conclusion, the surgical outcome of posterior fusion instrumentation in MFS scoliosis showed good correction of Cobb angle and Kyphotic angle. The quality of life of the patients based on physical and mental health questionnaire is satisfactory.

ABSTRAK

Skoliasis diderita pada 60% penderita sindrom Marfan (SM). Selain itu, skoliasis yang diderita sejak usia muda lebih progresif, bias dan kaku dibandngkan dengan skoliais dewasa idiopatik. Penatalaksanaan melalui tindakan bedah memberikan risiko perioperative lebih tinggi, sedangkan penatalksanaan konservatif dengan penjepitan tidak efektif untuk mencegah progresivitasnya. Dalam laporan kasus ini disampaikan luaran tindakan bedah pasien SM dengan skoliais dan kualitas hidupnya berdasarkan pertanyaan dalam SF-36 setelah dilakukan tindakan dengan peralatan fusi posterior. Loran kasus serial retrospektif ini melibatkan lima penerita skoliosis dengan SM yang menjalani instrumentasi fusi posterior sudut Cobb awal 87,4 ± 17,57° dan sudut kifotic awal 32,8 ± 14,52°. Kondisi klinik, hasil pemeriksaan radiologi dan kualitas hidup berdasarkan kuisionair SF-36 dievaluasi selama pengamatan 6-36 bulan. Pasca operasi menunjukkan sudut Cobb menjadi 46,2 ± 16,30° dan sudut kifotik menjadi 21,6 ± 9,94°. Tidak dijumpai komplikasi intraoperasi dan pasca operasi selama pengamatan. Setelah dilakukan pengamatan selama 6-36 bulan, sudut Cobb menjadi 45,2 ± 17,48° dan sudut kifotik menjadi 21,6 ± 9,94°.Semua pasien mempunyai skor kesehatan fisik dan mental sama dengan skor skolastis setelah 2

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tahun pasca operasi menurut petunjuk penilaian oropedi SF-36. Dapa disimpulkan luaran bedah instrumentasi fusi posterior pada pasien scoliosis dengan SM menunjukkan koreksi yang baik terhadap suduk Cobb dan kifotik. Kualitas hidup pasien berdasarkan kuisionair kesehatan fisik dan mental memuaskan.

Keywords: scoliosis - Marfan syndrome – quality of life - questionnaire SF-36 – Cobb angle

INTRODUCTION

Marfan syndrome (MFS) is one of multi systemically disorder caused by generalized collagen abnormality (FBN1; fibrillin-1) that is inherited in autosomal dominant.[1,2] Other than the excessive longitudinal growth on growth plate cartilage (hyperchondroplasia), thin and long extremity seen as *spider-like finger* (*arachnodactyly)*; the tangible sign are the facial features (dolichocephalic, enophthalmos, down slanting palpebral fissures, malar hypoplasia, retrognathia), and chest asymmetry (pectus excavatum/ carinatum).[3,4] The condition is somehow stigmatism, hamper the life insurance opportunity, as well as the psychosocial burden[2]. The diagnosis criteria have been revised for the purposes not to over diagnosed or under diagnosed it.[2,3] Genetic evaluation is not the only diagnosis tool, yet other ancillary technique is not always available and feasible for our community.

The undetected dilatation of aortic base such as aneurysm of ascending aorta might complicate the scoliosis surgery or the other way the abnormal thoracic cage would complicate the cardiac and pulmonary condition which could happened later in young adult age.[1,2,3,4] The main management for Marfan syndrome depends on which chief organ system involved.[3,4,8,9] Scoliosis prevalence in this syndrome is around 60 %. Scoliosis in MFS usually occur and being noticeable at younger age, more progressive, refractory, and rigid. It is also the main complaint of back pain in the later age.[5,6,7]

In common community, scoliosis is the disease, not just the symptom. Very seldom the underlying cause was investigated. In our former study in screening the junior high school student in Surabaya, 2.7% students indicate scoliosis and 3 neglected cases were found.[10] Most of the cases are adolescent idiopathic scoliosis (AIS); which surgery is performed when the cobb angel is more than 40°. But in MFS the early onset and progressive scoliosis is the problem to be solved.[3,4,5] The use of brace in conservative treatment is not effective for preventing the progressivity of the scoliosis.[5] Surgery by posterior fusion instrumentation is one of the technique for scoliosis correction nevertheless the collagen abnormality and anatomical deformity in MFS might complicate the perioperative risk and the correction result.[7,11,12] The purpose of this study is to evaluate the surgical outcome not only the correction by clinical and radiology but also by using SF-36 questionnaires to value the physical and mental health of the MFS patient.

CASE REPORT

This is a descriptive retrospective study of case series. The research protocol was approved by institutional ethics and review board in both 2 hospitals, Dr Soetomo General Hospital Surabaya and Surabaya Orthopedic & Traumatology Hospital. Five MFS patients with scoliosis were underwent corrective

surgery using posterior fusion instrumentation according to standardized department protocol during 2014 to 2016. All patients were managed by a single orthopedic surgeon, same instrument, and same management protocol. The MFS was diagnosed based on Revised Ghent criteria.[2] The pre-operative management was incorporating cardiac evaluation, ophthalmology evaluation, and MRI ofthe spine to evaluate the dural ectasia. None of the patients had aortic root aneurysm, ectopia lentis, or family history. But 4 out of 5 had thick myopia, the facial features, and >7/20 systemic features involvement.[2] Database of initial patient condition were recorded from medical record, including the demographic data, clinical and radio imaging data. Pre and post Cobb angel and Kyphotic angel were compared to the follow-up measurement. The SF-36 questionnaire was performed by interviewing the patient on the follow up visit. Follow up was 6-36 months.

RESULTS

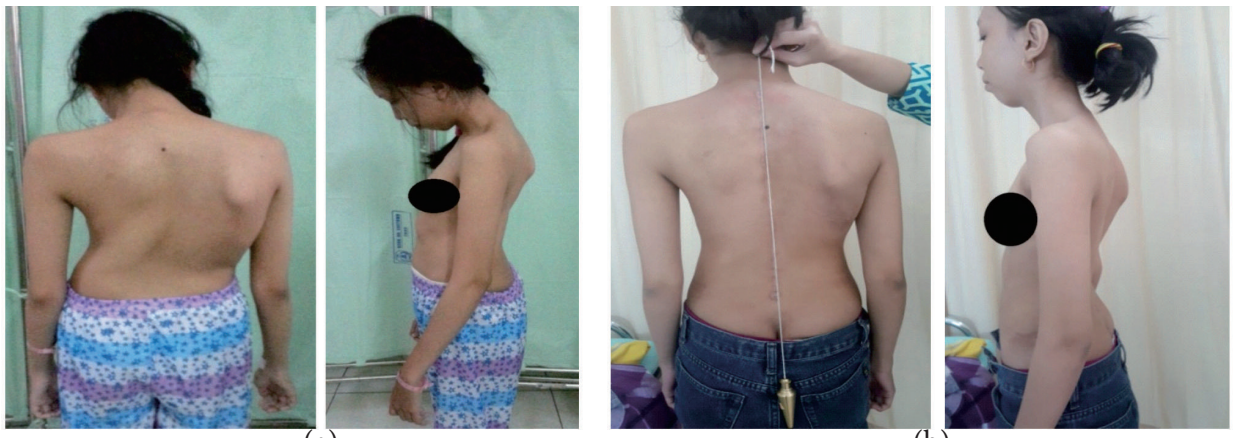
The 5 MFS patients were all female with the age range of 11–17 years (:13.6 years) at surgery; 13–18 years (:15.6 years) at the follow up, and all were without cardiac or respiratory abnormalities. All of them were came when the Cobb angel were > 70°; 2 of them were > 100° and 2 patients of double curves (RT-LL). The mean Cobb angel before surgery was (87.4±17.5)°. The initial kyphotic angel was (32.8±14.5)°. The degree of correction directly post-surgery was (41.2±1.78)° and (16.8±9.83)° respectively. The number of the segment fusion were 15(Th2-L4) – 17(Th1-L5) segments. After surgery all patients were immobilized with brace for 3-6 months.

The length of the surgery averaged (322±38.3) minutes (range:270-370 minutes). The average blood loss was (495±44.7) cc (range: 450–550 cc). There was absent of surgical complication during and after the surgery. On Follow-up after 6 – 36 months, there were almost no scoliosis nor kyphotic progression in all patients. The physical health status and mental health status from SF-36 questionnaire were good with the average of PHS 48.4 and MHS 49.

TABLE 1. Clinical, radiology, correction, and quality of life (SF-36) in Scoliosis Marfan syndrome patients

Patient	Sex	Age (years)	Curve pattern	Fusion Level	Cobb angle (°)			Kyphotic angle (°)			Op time (min)	Blood Loss (cc)	SF-36 score	
					Pre-op	Post op	FU	Pre-op	Post op	FU			PHS	MHS
1	F	18	RTL	T2-L5	102	58	58	45	16	16	340	500	45	55
2	F	16	RT	T2-L4	70	30	25	38	32	32	270	450	55	46
3	F	18	RT-LL	T1-L4	80/68	40/32	40/32	8	22	22	330	550	42	48
4	F	13	RT	T1-L4	110	68	68	33	8	8	300	450	47	50
5	F	13	RT-LL	T1-L5	75/55	35/20	35/20	40	30	30	370	525	53	46

RTL: right thoraco-lumbal; RT: right thoracal; RT-LL: right thoracal-left lumbal; Pre-op: preoperative; Post op: post-operative; Op time: operative time; FU: follow up (6-36 months); PHS: Physical Health Status; MHS: Mental Health Status



(a) (b)

FIGURE 1. Clinical appearance 18 years female with RT-LL curve treated with posterior fusion instrumentation, (a) initial pre-operative, (b) follow up post-operative after 1 year

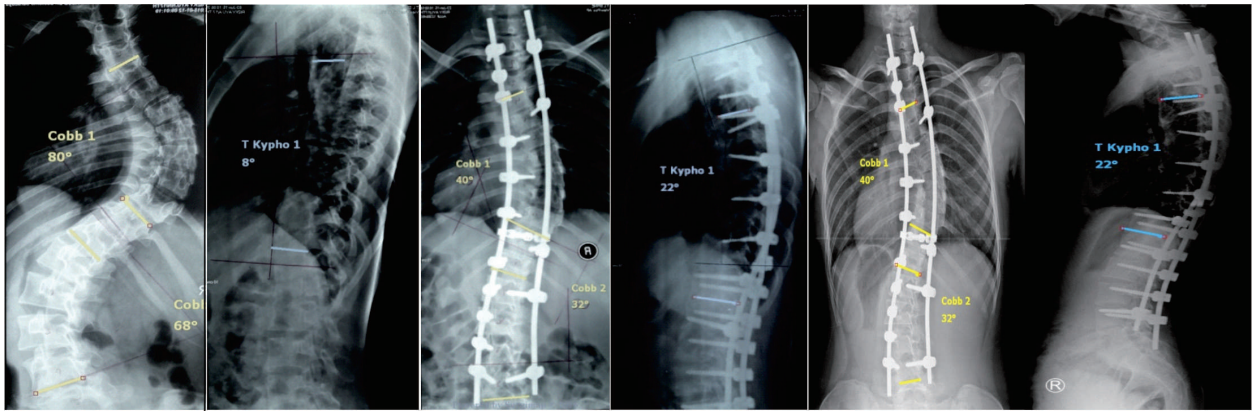


FIGURE 2. Radiological imaging of 18 years female with RT-LL curve treated with posterior fusion instrumentation, (a) pre-operative, (b) post-operative, (c) follow up after 1 year

DISCUSSION

In this study the surgical outcome is not only about the physical outcome (cobb angel and kyphotic angel) but also about the quality of life. Since the main complaint from the patient's site is the body contour, the self-esteem confident is also considered as one of the surgical outcome. The SF-36 do not have the cut-off point for good or bad result.[13] The SF-36 forms have been used often in examining orthopaedic patient populations. The brief guide from Various Orthopaedic Procedures and Conditions comparing SF-36 pre and post-surgery reported similar result of scoliosis patient, PH 42.2 to 46.4 and MH 48.9 to 50.6.[14]

Surgical outcome of a reconstruction surgery is usually concern for degree of correction. The degree of correction of the cobb angel (40–44)° and kyphotic angel (6-29) ° were comparable to study reported by Zenner et.al when using posterior spinal fusion only (44°) yet less than when using combine PSF and Anterior (57°).[11] Nonetheless blood loss and time of surgery were better in posterior spinal fusion only.[6,7,11,12] When comparing with surgery of AIS, the correction, blood loss, and time of surgery were similar and not significant.[6,15]

Surgical therapy is an effective choice of therapy for scoliosis in Marfan syndrome, bracing has been proven only success in 17% cases but eventually those cases need surgery as well, owing to the progressing curve.[5,7,11,12] The use of growing rod is a good choice, anterior release is not necessary if surgery was not postponed; when the curve has progressed rapidly which is the notorious problem in MFS.[6,7,11] Hook is not advisable due to the underlying desmogenic disorder. Before surgery MRI should be assessed for possible dural ectasia, pedicle thinning, and dysplastic lamina.[15]

The surgery must cover all the major curve including pelvis fusion involvement when necessary to avoid the re-surgery.[11,12,15] We fused 15-17 level and after up to 3 years follow up the progression was none to minimal (5°) in one case. The invention of pedicle screws gives important progression in scoliosis correction. Pedicle screws, using the strongest part of vertebral body as an anchor, provide the spine surgeon with an enhanced three-dimensional deformity correction. Pedicle screws that placed in the vertebral body have 30 % greater moment arm for applying corrective forces than posterior hooks. Posterior segmental instrumentation with a powerful pedicle screw anchor offers satisfactory correction without significant loss of curve correction even in severe deformity cases.[12] Posterior fusion with instrumentation has been widely used for the surgical treatment of scoliosis in the Marfan syndrome, particularly in the curves ranging beyond 40°–50° that tend to progress more after skeletal maturation.[7,12]

This study need to be continued with larger samples and multicenter to give orthopedic surgeons precise and merit planning in surgical management of scoliosis in MFS. Scoliosis in MFS would not be too complicated if planned in the current knowledge of underlying the multi systemically disorder as MFS.

CONCLUSION

The surgical outcome of posterior fusion instrumentation in MFS scoliosis shows good Cobb angle and Kyphotic angle correction. The blood loss, time of surgery, and surgical complication is all satisfying and comparable to other study with larger samples. The quality of life of the patients based on physical and mental health questionnaire (SF-36) is similar to other various orthopedic procedure and condition.

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Precocious puberty in McCune-Albright syndrome: a case report

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ABSTRACT

McCune-Albright syndrome (MAS) is a rare disease characterized by a triad of fibrous dysplasia, *cafe-au-lait* spots and peripheral precocious puberty. We reported a 5-year-8-month old girl with MAS who has been followed-up for 2 years and 8 months. She was referred to pediatric endocrinology clinic in our hospital for vaginal bleeding at age of 2 years 11 months. She had peripheral precocious puberty, i.e. increased estrogen level associated with very low gonadotropins, and *cafe-au-lait* spots on her face and was diagnosed as MAS. The patient was treated with estrogen receptor blocker (tamoxifen). She had no menses during the 2 years and 8 months of tamoxifen treatment. Her growth rate and bone maturation were also in normal ranges. However, at the end of tamoxifen treatment she had an episode of vaginal bleeding so that we had to change to other treatment modalities.

ABSTRAK

Sindrom McCune-Albright merupakan penyakit langka yang ditandai dengan trias displasia fibrosa, makula *cafe-au-lait* dan pubertas prekok perifer. Kami melaporkan anak perempuan usia 5 tahun 8 bulan yang telah kami amati selama 2 tahun 8 bulan. Pasien datang ke poliklinik endokrinologi di rumah sakit kami dengan keluhan perdarahan pervaginam pada usia 2 tahun 11 bulan. Ia mengalami pubertas prekok perifer yang ditandai dengan peningkatan kadar estrogen dan rendahnya kadar gonadotropin,, makula *cafe-au-lait*, dan didiagnosis dengan sindrom McCune-Albright. Pasien diterapi dengan penghambat reseptor estrogen (tamoxifen). Selama terapi, siklus menstruasi terhenti, kecepatan pertumbuhan dan maturasi tulang dalam batas normal. Namun pada akhir terapi dengan tamoxifen, pasien kembali mengalami perdarahan pervaginam sehingga kami harus mengganti dengan modalitas terapi yang lain.

Keywords : McCune-Albright syndrome - precocious puberty – tamoxifen – genetic disorders - gonadotropins

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INTRODUCTION

McCune-Albright syndrome (MAS) is a genetic disorder characterized by abnormalities in skin pigmentation, endocrine system, and bone growth due to the GNAS (guanine nucleotide binding protein, alpha stimulating*)* gene mutations.[1,2] Prevalence of the disease ranged from 1:100.000 to 1:1.000.000 worldwide.[3] Classically, this syndrome presents with triad of fibrous dysplasia, typical skin pigmentation (*cafe-au-lait*), and endocrinopathies, including precocious puberty, hyperthyroidism, Cushing's syndrome, growth hormone excess, hyperprolactinemia, hyperparathyroidism, and/or rickets or osteomalacia.

Precocious puberty was the most common endocrinopathy found in MAS. It occurs in 64-79% of girls and 15% of boys.[4] Aromatase inhibitors, e.g. testolactone, fadrazole, anastrozole, and letrozole, selective estrogen receptor modulators, e.g. tamoxifen, analogue of gonadotropin-releasing hormone (GnRH), and surgery were, so far, the suggested modalities to manage precocious puberty associated with MAS.[5] However, due to the rareness of the disease, the experience of using any of the above modalities are very limited. In this case report, we present a 2-years-8-month follow-up of tamoxifen use in a patient with peripheral precocious puberty of MAS.

CASE

A 2-years-11-month-girl, presented to pediatric endocrinology clinic at Dr. Sardjito General Hospital, Yogyakarta due to vaginal bleeding. The bleeding last for 2-3 days. On physical examination, we found breast enlargement (Tanner stage 2) and *cafe-au-lait* spots on her face. The child's height was 87.0 cm (plotted at -1.8 SD on the WHO child growth standard 2006). We observed low levels of gonadotropins, i.e. luteinizing hormone (LH) <0.1 mIU/mL (normal values: 2.4-12.6 mIU/mL, follicle-stimulating hormone (FSH) of 0.109 mIU/mL (normal values: 3.5-12.5 mIU/mL) and increased level of estradiol (635 pg/mL, normal values: 6-20 pg/mL). Bone age, assessed by Greulich-Pyle method, was equivalent to 2 years old. Pelvic ultrasound examination revealed post pubertal uterine size (2.57 x 2.67 x 1.73 cm). However, the ovaries were unvisualized. No history of pathological fracture was reported. Based on the presence of peripheral precocious puberty and *cafe-au-lait* spots on the face, the patient was diagnosed as MAS.

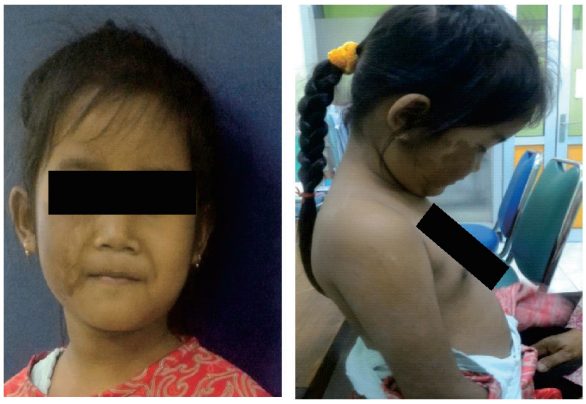

FIGURE 1. Cafe-au-lait skin pigmentation on the face

Treatment with tamoxifen (20 mg/day) was started immediately following the diagnosis. The menstrual bleeding was ceased shortly after the initiation of therapy. The serum estradiol level measured one year after the treatment declined to less than 5 pg/mL. Pelvic ultrasound examination carried out one year after the treatment showed smaller uterine size (2.71 x 2.21 x 1.20 cm). The ovaries were also unvisualized.

After 2 years and 8 months of regular treatment with single dose of tamoxifen

20 mg/day, the child had an episode of vaginal bleeding and breast enlargement, accompanied by pubic hair growth (Tanner stage 2). Evaluation of endocrine function observed low levels of gonadotropins (LH 0.1 mIU/mL, FSH 0.8 mIU/mL) and normal level of estradiol (5 pg/mL). Bone age was equivalent to 5 years old, which was still in-line for chronological age of 5 years and 8 months. On pelvic ultrasound examination, the uterine size increased compared to the last evaluation (3.79 x 2.17 x 1.96 cm), and the ovaries were still unvisualized.

The child has been growing well. Currently, at the age of 5 years and 8 months, she was 112.0 cm in height and 19.0 kg in weight (equal to -0.8 SD and -0.5 SD, respectively, on the WHO growth standard 2006), and has achieved Tanner stage of B2 and P2. The growth velocity was 7.5 cm/year during the last 2 years (95% confidence interval of growth velocity in pre-pubertal girls is 5.1-9.3 cm/year). During the observation, no other endocrinopathies that often accompany MAS (Cushing's syndrome, hyperprolactinoma, hyperthyroidism, and growth hormone excess) were observed. Thyroid ultrasound, and TSH and FT4 levels were normal. There were no bone abnormalities suggesting fibrous dysplasia. No pathological fracture was observed, but at the age of 4 years there was an open second metatarsal fracture on the left foot, coincidently due to motorcycle accident. The fracture completely healed without deformity or gait impairment after surgical treatment of open reduction and internal fixation.

DISCUSSION

McCune-Albright syndrome is characterized by a triad of fibrous dysplasia, *cafe-au-lait* spots, and precocious puberty. Other endocrinopathies such as hyperthyroidism, growth hormone excess, Cushing's syndrome, hyperprolactinemia, and rickets or osteomalacia may also occur, as well as other involvement of the liver, parathyroid, pancreas, and heart.[6] McCune-Albright syndrome is associated with mutation in the GNAS1 gene, which is mapped to chromosome 20q13.3. The protein product is involved in G-protein signaling.[2] The mutation in the GNAS gene occurs randomly during pregnancy and will result in mosaic of normal and mutated cells.[1,7] The manifestation and the severity of this disease depends on the number and the location of the cells expressing the mutated gene. The mutation influences activity of the related organ system at the level of interaction between hormones and receptor.[2]

Precocious puberty is the most common endocrinopathy found in MAS. Precocious puberty affects more girls than boys, characterized by premature vaginal bleeding. The precocious puberty was peripheral in origin, that is the hypothalamic-pituitary-gonadal axis was not active. The pathogenesis involves autonomous activation of ovarian tissue lead to estrogen hypersecretion. However, progression into central precocious puberty may also occur. [5,8]

Therapeutic options include the use of anti-estrogen (aromatase inhibitor, e.g. testolactone, fadrozole, anastrozole, andletrozole), estrogen receptor inhibitor (e.g. tamoxifen), analogue of GnRH (if there is a progression to central precocious puberty), and surgery (unilateral oophorectomy, cystectomy).[5] A multicenter study on the use of tamoxifen for precocious puberty in girls with MAS for one year has shown a decrease in vaginal bleeding episodes and significant improvements of growth velocity and bone maturation.[9]

We treated our patient with tamoxifen and observed good response during the first and second year of treatment. She had no menses, and her growth rate and bone maturation were in normal ranges. However, on third year (after 2-years-and-8-month treatment) she suddenly had new episode of vaginal bleeding and breast enlargement, accompanied by pubic hair growth, despite regular treatment with tamoxifen. In fact, the patient had good adherence to the treatment, as we evaluated using the Medication Morisky Adherence scale (MMAS-8).[10] We decided to change to other treatment modalities since, to the best of our knowledge, there is no study so far on the safety of using higher dose of tamoxifen. Hormonal evaluation after the vaginal bleeding revealed no increase in gonadotropin level, therefore, progression to central precocious puberty can be ruled out.

Nunez *et al*.[11] reported that fadrazole was not effective for the treatment of precocious puberty and causing adrenal insufficiency. Meanwhile, Feuillan *et al*.[12] reported that testolactone may be used as a therapy for precocious puberty since it leads to a decrease in ovarian volume and estrogen concentration. However, one study[13], which evaluates the long-term therapy of testolactone, shows some patients have persistent symptoms of puberty, suggesting incomplete inhibitory effects of estrogen production. Other study[14] indicates that letrozole is an effective medication for precocious puberty in MAS. Recently, Estrada *et al.*[15] shows that letrozole has good long-term effect for precocious puberty in MAS in reducing episodes of menstruation and keeping the skeletal maturation and growth velocity so that predicted adult height can be achieved.

Based on those recent studies on the long-term effects letrozole in girls with precocious puberty in MAS, we changed the medication for our patient to letrozole following the failure of two-year-treatment with tamoxifen. We started the treatment with letrozole at dose of 1.25 mg/day since March 2017, and continuing to observe the result.

CONCLUSION

The diagnosis of MAS must be kept in mind in cases with gonadotropin-independent precocious puberty. Radiologic and laboratory assessments should be performed in order to investigate the presence of accompanying endocrinological and non-endocrinological disorders. A careful clinical observation and follow up of patients with mentioned clinical presentations is necessary.

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