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Research Report

The Role of Hyperbaric Oxygen to Platelet Aggregation in Diabetic Patients Type II (NIDDM)

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ABSTRACT

Prevalency of Diabetes Mellitus in Indonesia has tendency to be increased from year to year. Hyperbaric Oxygenation (HBO) has been used as treatment of Diabetes Mellitus's complication especially diabetic gangrene. But the effect of HBO to the rheology's disfunction especially platelet's aggregation in the patients of NIDDM was investigated. The randomized pretest-posttest design was used in this study. An experimental laboratory study was performed at Naval Health Institution in Surabaya. 32 patients of NIDDM,women, 40–75 years old, normal physic's diagnosa, normal thorak's photo, normal EKG, normal Ear Nose and Throat, Diabetes Mellitus's family's record, normal weight (BMI), blood glucose level didn't exceed 400 mg/dl (including controlled DM FBG < 120 mg/dl dan 2 H BG < 160 mg/dl), NIDDM, normal level of HbA1c (4–5,9), as long as this research they couldn't take their Oral Hipoglikemic Agent, Oral Anti Trombotic, vitamin C and vitamin E. They are divided into 2 group: group of HBO 100% O_2 2,4 ATA for 3x30 minutes with interval 5 minutes to inhalate air once a day daily for 5 days subsequently and the extraction of data (PAT) had been held before oxygenation hyperbaric therapy at the first day and the end of fifth days, in control group only giving 20% O_2 with the pressure 1 ATA for 90 minutes once a day daily for 5 days subsequently and the extraction of data (PAT) had been held before normoxia normobaric therapy at the first day and the end of fifth days. The results were significant decrease of the platelet's aggregation level especially percent of aggregation after 5 days from 76,56 ± 8,06 become 69,13 ± 6,03. Latent Periode has also decrease from 28,75 ± 3,87 become 25,75 ± 2,82. Speed of Aggregation has also decrease from 66,25 ± 3,17 become 62,50 ± 3,44. Index of Aggregation has also decrease from 0,763 ± 0,071 become 0,581 ± 0,083. Using paired t-test, it could be seen the decrease of Latent Periode (p= 0,001) and index of aggregation (p= 0,000) significantly after exposure of oxygenation hyperbaric HBO 2,4 ATA 100% O_2 3×30 minutes with interval 5 minutes inhalate air once a day for 5 days,subsequently. Speed of aggregation (p = 0,022) and percent of aggregation (p= 0,013) are nonsignificantly. The conclusion of this research is that oxygenation hyperbaric 2,4 ATA 100% O_2 3×30 minutes with interval 5 minutes inhalate air once a day for 5 days,subsequently could decrease latent periode, speed of aggregation, index of aggregation and percent of aggregation in NIDDM.

Key words: *HBO, NIDDM, HbA1c, Platelet's Aggregation (Latent Periode, Speed of Aggregation, Index of Aggregation and Percent of Aggregation)*

INTRODUCTION

The prevalence of Diabetes Mellitus patient in Indonesia has a tendency to be increased from year to year (Suyono, 1996). The latest report of McCarthy on 1994 the sum of diabetic patient in the world is 110,4 million and it would be reached 1,5 fold on 2000 (175,4 million) and 2 fold on 2010 (239,3 million). Diabetes Mellitus (DM) is endocrine disease with abnormal metabolic that has long term complication in several part of body such eye, kidney, neuron and vessel.

Diagnosis of Diagnosis DM based on symptoms, diuretic osmotic and hyperglikemia (Foster, 1998). Long term hyperglikemia would induce rheology disfunction such as platelet agregation (PA), eritrocyte, leucocyte and blood viscosity (Tjokroprawiro, 1997). Hyperbaric oxygenation

(HBO) has been used for therapy of DM complication especially gangrene diabetik in Diabetic Center of Niguarda Hospital, Milan, Italy on 1980 (Oriani, 1995).

Diabetic patient has been experienced hemorheologic disorder (pathorheology) and hyperactive platelet factor which play main role in the pathogenesis of hyperviscosity and microthrombus. Hemoreologic disorder would disturb the blood flow in micro-sirculation region such as arteriole-capillary-venule. Microsirculation region is the area of oxygen and nutrient exchange in the tissue and taking process of some waste product (Muller, 1986). Blood fluidity disorder could induce the inclination of platelet aggregation, abnormal Hb - O_2 binding, the inclination of HbA1c, erythrocyte aggregation, the decline of erythrocyte deformability, the inclination of plasma viscosity and hypercoagulability. All of the disorder above could yield ischemic and necrosis condition (McMillan, 1987; Colwell, 1980; Soeharjono, 1985; Muller, 1986).

Ditzel (1967) is showed that static vena and the destruction of capillary- vena flow in diabetic patient is caused by 3 factor, such as functional alteration (pathofisiology) vessel wall which related with redistribution flow and the leak of plasma component through venule, erythrocyte aggregation and the inclination of blood viscosity. Bridges (1965) has been found the increase of platelet viscocity and the alteration of plasma composition in diabetic patient. In the small vessel, it has been found the increase of speed among 2 liquid divide with the distance between 2 layer. Shear rate could stimulate the platelet disability. This condition would release some of mediators which activated Tromboxane-A2. The interaction of TxA_2 and *Adenosine Diphosphate* (ADP) could stimulate platelet aggregation. (Hendromartono, 1997).

HBO is therapy using 100% O_2 in the hyperbaric chamber (> 1 Atmospher Absolute (ATA). It could handle cellular hypoxia and increase O_2 supply to the destruct tissue. HBO could decrease ADP and collagen with its function as agonist of platelet aggregation (Ersoz et al, 1998). HBO 100% O_2 3 ATA for 2 hours could support the NO• regeneration (Ito, 1996). This NO• could activate *guanylate cyclase* to stimulate c-GMP production (Schmidt HH, 1993). c-GMP could inhibit platelet aggregation (Radomsky, 1993). HBO therapy is performed by inhaling the oxygen through mask, and endotrachea channel which has valsava technique previously in 10–14 session on 2,4 ATA for 3×30 minutes with 5 minutes interval inhale the air (Epsthein, 1998).

MATERIAL AND METHOD

This research has been done in Diabetes Departement Navy Hospital Dr. Ramelan Surabaya. The patient was giving the lottere. Before they become the research subject, they should fulfilled letter of agreement (inform concent) and quiz. The selected patient (who fulfilled the inclution criteria, who agree to become research subject, who has been follow the medical examination which needed for this research), has been randomized using *random number.* Sample has been taken from the population randomly and divided into 2 group such as control group (NONB): patient in normoxia normobaric (20% O_2 1 ATA) for 90 minutes once a day for 5 days subsequently and treatment group (HBO): patient in hyperoxia hyperbaric (100%O_2 2,4 ATA) for 3×30 minute with 5 minute interval inhale air once a day for 5 days subsequently. By using random sampling, then the sample was determined.

The determination of platelet aggregation has been done twice:

1. In the first day before the HBO treatment.
2. In the end of 5th day after the HBO treatment.

In the beginning of the research, we have did the randomization based on inclution criteria, (HbA1c = 4,5–5,9). Blood sample which has been taken from the patients in Diabetic Clinic of Navy Hospital Dr.Ramelan Surabaya then has been delivered to Prodia Clinical Laboratory. The blood's patient from control group (normoxia normobaric therapy (20% O_2 1 ATA) 90 minutes once a day for 5 days) and treatment group hyperoxia hyperbaric (100% O_2 2,4 ATA) 3×30 minutes with interval 5 minutes inhale air once per day for 5 days subsequently. Blood sample then have been delivered to Clinical Laboratory Catholic Hospital St.Vincentius Paulo Surabaya.

RESULT

There are several variable in this research such as age, weight, height, fasting blood glucose (mg/dl), 2 hours post prandial blood glucose (mg/dl), Hb A1c (%), HBO (oxygenation hyperbaric) 2,4 ATA 100%O_2 3×30 minutes with interval 2×5 minutes inhale air and aggregation parameter: latent period (second), aggregation speed (°), aggregation index (dh/dt), aggregation procentage (%) with collagen aggregator in NIDDM patient. The data has been processed by descriptive statistic and inferential such as normality, homogenity, anava same subject, correlation test, anakova.

The result of normality test of all variable such as age, weight, height, fasting blood glucose (mg/dl), 2 hours post prandial blood glucose (mg/dl), Hb A1c (%), in both group (NONB and HBO) is in normal distribution.Using the uji univariate (Tests of Between-Subjects Effects) and multivariate test, the data in both group is homogen.

The result of correlation test between moderator variable and dependent variable in both group (HBO and NONB) is non significant.he normality test of aggregation parameter such as Latent Period, Speed of aggregation, aggregation of index, aggregation procentage in HBO and NONB has normal distribution. Based on u nivariate test (Tests of Between-Subjects Effects) and multivariate is showed that all the aggregation parameter (laten phase, aggregation speed, aggregation index, aggregation procentage in HBO group and NONB group are homogen.

The result showed the differences of latent phase between HBO and NONB group ($p = 0.001$; $p < 0.05$) with the significant role of HbA1c ($p = 0.039$, $p < 0.05$). There is no significancy of aggregation speed between 2 groups (HBO and NONB) ($p = 0.439$; $p > 0.05$) with the influence of 2 hours post prandial blood glucose test. There is significant difference of aggregation index between 2 groups (HBO and NONB) ($p = 0.043$; $p < 0.05$). The result did not showed difference of aggregation procentage between HBO and NONB group ($p = 0.545$; $p > 0.05$). There are 2 significant variable such as latent period alteration from the beginning to the end ($p = 0.009$; $p < 0.05$) and the alteration of aggregation index ($p = 0.006$; $p < 0.05$).

DISCUSSION

Using correlation test among moderator variable such as age, weight, height, fasting blood glucose, 2 Hours Post Prandrial Blood Glucose to aggregation parameter such as latent period, aggregation speed, aggregation index, and aggregation procentage, there is no significant correlation ($p > 0.05$). This result means that all the moderator variables above do not influence the aggregation parameter such as latent period, aggregation speed, aggregation index, and aggregation procentage before and after the treatment.

Aggregation Parameter (latent period, aggregation speed, aggregation index, and aggregation procentage) in Hyperbaric Oxygenation (HBO) Group

The normal value of platelet aggregation using collagen aggregator is 35–50. There is the decrease of latent period of platelet aggregation in this group after the treatment compared with the value before the treatment (28,75 ± 3,87 become 25,75 ± 2,82). Latent period ($p = 0.001$) has been showed the significant differences ($p < 0.05$). This result mean that HBO treatment 2,4 ATA 100% O_2 3×30 minutes with interval 5 minutes inhale air once a day for 5 days subsequently could decrease latent period of platelet aggregation in NIDDM patient.

Normal value of platelet's aggregation speed of platetet with collagen aggregator is 52–80. There is a declination of platelet's aggregation speed in HBO Group after the treatment compared with before the treatment (66,25 ± 3,17 become 62,50 ± 3,44). Platelet aggregation speed ($p = 0.022$) was showed significant difference ($p < 0.05$), this means HBO 2,4 ATA 100% O_2 3×30 minutes with interval 5 minutes inhale air once a day for 5 days subsequently could decrease platelet aggregation speed in NIDDM patients.

Normal value of platelet aggregation index with collagen aggregator is 0.3–0.7. It seems the declination of platelet aggregation index in HBO group after the fifth day compared with the value from the first day before HBO treatment (0.763 ± 0.072 become 0,581 ± 0,083). This means HBO 2.4 ATA 100%O_2 3×30 minutes with interval 5 minutes inhale air once a day for 5 days subsequently could decrease platelet aggregation index in NIDDM patients.

Normal value of platelet aggregation procentage with collagen aggregator is 50–75. There was the declination of platelet aggregation procentage in HBO group in the fifth day compared with the value in the first day before the HBO treatment (76,56 ± 8,06 become 69,13 ± 6,03). There was significant difference ($p = 0,013$), it means that HBO 2,4 ATA 100% O_2 3×30 minutes with interval 5 minutes inhale air once a day for 5 days subsequently could decrease platelet aggregation procentage in NIDDM patient.

From this research, we could conclude that HBO 100% O_2 3×30 minutes with interval 5 minutes inhale air once a day for 5 days subsequently could decrease platelet latent period, aggregation speed, aggregation index and aggregation procentage. This result was linear with the research of Ito et al (1996) which stated that HBO 100% O_2 3 ATA 2 hours could support NO• regeneration and Oury et al (1992) which stated that NO• could stimulate *c-guanosine mono phosphate* (c-GMP) production. Radomsky et al. (1993) was stated that *c-guanosine monophosphate* production could inhibited platelet activation. Schmidt HH (1993) also support the theory above that NO• could activate *guanylate cyclase* to arrange *cyclic guanosine monophosphate* (*c-GMP*). NO• could inhibite the adhesion and platelet aggregation through c-GMP mechanism (Moncada S, 1993).

La Croix (1990) has been stated that the HBO exposure 100% O_2 3×30 minutes with interval 5 minutes inhale air once a day for 5 days subsequently could decrease ADP and collagen as aggregator (platelet aggregation's trigger). According to Nadler JL dan Natarajan R, (2000) Diabetes Mellitus could decrease production and action of NO• that is the interaction with *glycosylation end products*. DM could impair the production and action of NO•. DM mechanism could alter the action of NO• by decreasing the production and action of NO• such as the interaction with glycosylation end products, NO• reaction with super anion to yield peroxyinitrite lipid, increase renal production and sensitivity of NO•. The beneficial action of NO• in the blood vessel such as vasodilator of endothelium-dependent smooth muscle, inhibit the adhesion and platelet aggregation, inhibit the adhesion of leucocyte to activated endothelium, inhibit migration and proliferation of vascular smooth cell migration, decrease the oxidation macrophage to low density lipoprotein and inhibit the expression of endothelin and PDGF. Endotel cell release NO• as important regulatorfor tone blood vessel and vascular homeostasis vascular through its effect to platelet and smooth muscle cell. NO• could decrease platelet activation without systemic effect (Nong Z et al., 1997). In his research, Nong Z (1997) has been evaluated the inhalation effect of NO• to collagen which could stimulate platelet aggregation in vivo and c-GMP intrathrombocyte level and antithrombotic activity.

Parameter of Platelet Aggregation (Latent Period, Aggregation Speed, Aggregation Index, Aggregation Procentage)in NONB Group

In this NONB group, we found the declination of aggregation speed (68,19 ± 3,37 become 62,81 ± 6,52), aggregation index (0,706 ± 0,118 become 0,625 ± 0,161) and aggregation procentage (73,94 ± 9,45 become 70,44 ± 9,86) from the first day and the fifth day value (before and after treatment). This could be cause by several factors such as exercise, stress and food diet. The exercise factor is still influenced by duration, the magnification (heavy or light exercise) and the level of fitness. In DM patient, there are also several other factors such as plasma insulin level, blood glucose level and the proportion of body fluid (Sherwood L, 1994). In active muscles, the muscle need to glucose has been increased without the inclination of insulin level. This is caused by the inclination of receptor sensitivity in muscle and the addition of active insulin receptor. The active muscle is called *non-insulin dependent tissue* (Stacy P, 1986; Storlien H, 1993). Exercise for NIDDM patient is beneficial to act as glycemic control, todecrease weight, to handle atherogenic complication, disturbance of blood lipid, the inclination of blood pressure and blood hypercoagulation (Storlien H, 1993; Wolfe RR, 1998). The aim of nutrient therapy in NIDDM patient are to control glucose level, lipid level and hypertension. The declination of the weight and hypocalory diet could impair short term glycaemic level and has the potency to increase long term metabolic control (Soekardji K, 1999). Emotional stress manifestation in DM patient oftenly appear as denied attitude, obsession, angry, frustration, fear, depression, anxiety and food problem. All the disorders could destruct the blood glucose level and would cause acute and chronic complication including rheology disorder (Semiardji G, 1999).

CONCLUSION

HBO 2,4 ATA 100% O_2, 3×30 minutes with interval 5 minutes inhale air, once a day, for 5 days subsequently could decrease latent period, aggregation speed, aggregation index, aggregation procentge in NIDDM patient. There is no correlation between age, weight, height, BMI, Fasting blood glucose, 2 hours post prandial blood glucose, HbA1c as moderator variable with dengan latent period, aggregation speed, aggregation index, aggregation procentage in NIDDM patient.

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