

COMBINATION OF METFORMIN AND HYPERBARIC OXYGEN THERAPY INCREASED eNOS CONCENTRATION

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ABSTRACT

Metformin is the first line drug of type 2 diabetes patients. Its improves Insulin sensitivity as mechanism of action via activation of adenomonophosphate activated protein kinase (AMPK), reduces hepatic glucose product, and increases endothelial Nitric Oxide Synthase (eNOS). Chronic hyperglycemia induces Nitric Oxide (NO) dysfunction, whereas, NO is important vasodilator in human body. Due to of NO dysfunction, cells become hypoxia to ischemic then cell apoptotic, included cells beta pancreatic. This study aim to find new management in optimisation of Metformin therapy in patients with type 2 diabetes, especially in the improvement of blood glucose into 'normoglycemia' and eNOS. Hyperbaric oxygen (HBO) therapy, 2.4 ATA oxygen, which is given 3x30 minutes with 5 minutes intervals with air for 10 times within 10 days continually, reduces directly blood glucose, improves NO function and inhibits cytokines released. Therefore, HBO therapy is considered as an adjuvant treatment in type 2 DM beside Metformin. This study was conducted by a clinical pre and post experiment without control. The ten men's correspondents in the study were chosen randomly. Blood glucose and eNOS concentration were examined before, during and after the combination treatment of Metformin and HBO therapy were given. The concentration of eNOS was increased significantly and blood glucose reduced significantly after the combination treatment of Metformin and HBO therapy. In conclusion, HBO therapy works synergism with Metformin in diabetes type2 therapy, especially by improving Metformin therapy in reduced blood glucose and raised eNOS.

Keywords: type2 diabetes, metformin, HBO, eNOS and blood glucose

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INTRODUCTION

Metformin, the Biguanides derivative, is the first line oral anti diabetic (American Diabetes Association 2006, PERKENI 2007) used for the treatment of type 2 diabetes. It is an effective hypoglycemic drug that also improves lipid profiles and reduces cardiovascular risk. The Diabetes Prevention Program has recently shown that similar to diet and exercise, Metformin treatment reduces the risk of developing diabetes in glucose-intolerant individuals. Whereas most studies have shown that the glucose-lowering effects of Metformin are secondary to a decrease in hepatic glucose production via its molecular site of action remains unclear, AMP-activated protein kinase (AMPK) (Kiem et al. 2008, Darmansyah 2009). In addition to its insulin sensitizing effects, Metformin has also been shown to have direct vascular effects (Bailey 2007). Most importantly, Metformin has been shown to improve vascular functions and to dramatically reduce

cardiovascular end points and mortality for type 2 diabetic patients in large-scale clinical trials (Wiernsperger 2008).

Nitric oxide (NO) plays an important role not only in physiological conditions such as vasodilation, inhibition of platelet aggregation, and regulation of gene transcription but also in atherosclerosis development (Coleman 2001, Thammem et al. 2008). NO is synthesized from L-arginine by a family of 3 NO synthases (NOS): neuronal (nNOS), inducible (iNOS) and endothelial (eNOS). Under oxidative stress conditions, caused by atherosclerotic risk factors—such as cholesterol overloading, oxidized LDL, smoking, diabetes mellitus, etc—eNOS will be uncoupled to produce superoxide. Thus, eNOS can produce both NO and superoxide, exerting atheroprotective and proatherogenic effects, by which it modulates gene transcription (Zeng et al. 2008). Metformin, improves vascular endothelial functions and reduces

cardiovascular events in patients with type 2 diabetes via AMP-activated Protein Kinase (AMPK) (Davis et al. 2006).

Despite the long history and success of metformin as a treatment for type 2 diabetes, there is a cumulative incidence at 5 years study in UK, that the failure of Metformin monotherapy is 21% out of 4360 patients with type 2 diabetes. Even, to solve the problem of Metformin monotherapy failure, patients were given combination of with either other oral anti-diabetics drugs, such as the Thiazolidinediones derivative – Rosiglitazone, the Sulphonylureas derivative – Glibenclamide or Insulin analogues. The progress of diabetes complication such cardiovascular events were still happened (Khan et al. 2006).

HBO therapy involves the intermittent inhalation of 100% oxygen in chambers pressurized at 2.4 Atmosphere Absolute (ATA). The benefit of HBO is based on the premise that raising tissue oxygen levels will enhance glucose metabolism and NO production (Wang 2003, Gill 2004, Richard 2004, Mathieu 2006). Thus, HBOT is considered as an adjuvant treatment in patients with type 2 diabetes and Metformin monotherapy failure, especially in improving eNOS concentration.

MATERIALS AND METHODS

The one group pre – post without control design

A total of 10 Indonesian men (n = 10 people) with type 2 diabetes were selected randomized among 30 subjects recruited in the context of a study on Metformin monotherapy failure at the Department of Internal Medicine, during March to May 2009, in Naval Hospital Dr. Ramelan Surabaya to represent as subjects in this study.

The age ranged from 50 to 65 years and type 2 diabetes on the basis of more than 7% in HbA1 according to American Diabetes Association criteria. All subjects fulfilled the following inclusion criteria: 1) absence of any acute or chronic inflammatory diseases as determined by a leukocyte count or clinical signs of infection, 2) no medical history of hypertension (i.e., systolic blood pressure was less than 140 mmHg and diastolic blood pressure was less than 85 mmHg, 3) no clinical evidence of either cardiovascular or peripheral artery disease, 4) no thyroid dysfunction and other endocrine dysfunction (i.e., cushing syndrome), 5) no contra indications for both Metformin or HBO therapy, 6) do diet and exercise for diabetes, 7) no Insulin therapy and 8) no anti scavenger (i.e., Ca²⁺ supplement,

Vitamin E) within last 6 months. The study was approved by the ethics committee of the Medical Faculty University of Airlangga, No.20/EC/KEPK/FKUA/2009. All subjects gave written informed consent before taking part in the study.

Interventional study

Ten subjects were given Metformin 500 mg 4 times a day during meal and HBO therapy 2.4 ATA O₂, 10 sessions within 10 days with 2 days without HBO therapy. One HBO session took around 2 hours therapy with 3 activities, 1) 10 minutes adaptation for the change of the atmosphere pressure at the sea level, 2) breath with 100 percent Oxygen, 3 times 30 minutes with 5 minutes interval, 3) 10 minutes adaptation for back to the normal atmosphere pressure. All based line blood sample were collected three times within the treatment of HBO periods for measuring blood glucose, eNOS concentration and liver – renal function. First measurement was taken before the subjects entered HBO chamber (pre test). Second measurement was taken after the HBO therapy day 5th (durantee test) and the last measurement was taken after the subjects come out from HBO chamber day 10th (post test).

Assays, measures of blood glucose and liver – kidneys function

Samples were using serum, that took from 30 minutes clotting whole blood then centrifuged for 15 minutes at approximately 1000 x g, then homogenized and measured with “fortex mixer” equipments. eNOS ELISA development and measurement of eNOS concentrations from endothelial cells. Samples used red blood cells, which need to be lysised. The gene-encoding human eNOS was amplified from the human p-eNOS cDNA library by PCR, standard E. coli-expressed eNOS was used as an immunogen for generating human eNOS – specific monoclonal and polyclonal Abs. For making recombinant eNOS proteins in RD5K cells and E. coli, the portion of the gene encompassing presumed mature polypeptides, Leu21 through Lys414, was amplified and digested with appropriate restriction enzymes and then cloned into peNOS (R&D Systems, Inc. Minneapolis, USA). Standards and samples are pipetted into the wells and any p-eNOS present is bound by the immobilized antibody. An enzyme-linked polyclonal antibody specific for p-eNOS is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of p-eNOS bound in the initial step. The color development is stopped and the intensity of the color is measured.

Specificity

This assay recognizes recombinant and natural Human total p-eNOS. No significant cross-reactivity or interference was observed.

Statistical analyses

Data are shown as means SE, unless stated otherwise. Before statistical analysis, normally distributed parameters were logarithmically approximate a normal distribution (Kolmogorov-Smirnov test). The following statistical tests were used: paired Student's t test or paired samples test. Statistical analysis was performed using SPSS version 14.0 (Chicago, IL). P values of 0.05 were considered to be statistically significant.

RESULTS

Hemoglobin A1C was measured as confirmation due to the chronic hyperglycemia conditions. Mean of Hemoglobin A1C was 8,02% with standard deviation 0,388 (Figure 1). It may consider giving monotherapy Metformin only if the Hemoglobin A1C is less than 8% (Darmansyah 2009).

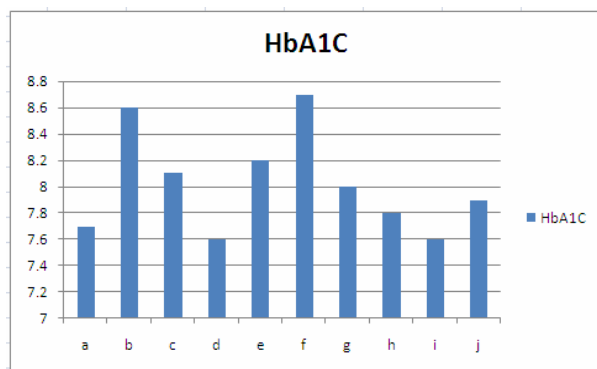


Figure 1 Mean of Hemoglobin A1C's subjects in this study.

A total of 10 Indonesian men completed a combination Metformin and HBO therapy program. The treatment effect was confirmed by a significant reduced fasting blood glucose in all subjects (Table 1 and Figure 2), and also significant improvement in eNOS concentration (Table 2 and Figure 3). Hepatic function was evaluated by SGOT and SGPT during combination Metformin and HBO therapy were given. It needed to evaluate because of Metformin's mainly work was in the liver. Metformin therapy was considered to stop if hepatic function increased (Goodman & Gilman 2006). Result of hepatic function was no significant raised (Table 3 and Figure 4).

Renal function was evaluated by BUN and Creatinine Serum during combination Metformin and HBO therapy were given. It needed to evaluate because of Metformin's mainly excretion was in renal. Metformin therapy was considered to stop if there is impaired in renal function (Goodman & Gilman 2006). Result of renal function was no significant raised (Table 4 and Figure 5).

Table 1. Paired t-Test for Fasting Blood Glucose (FBG)

	Paired t-Test	Sig.	Mean
Pair 1	FBG_I - FBG_II (pre – durantee HBO)	.000	55.2
Pair 2	FBG_II - FBG_III (durantee – post HBO)	.011	26.7
Pair 3	FBG_I - FBG_III (pre – post HBO)	.000	81.5

Sig. = significant < 0,05 ; Resource: SPSS version 14.0

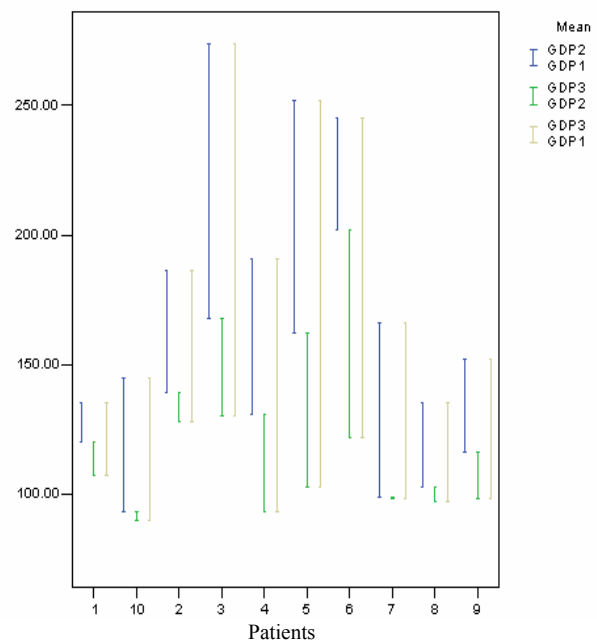


Figure 2. The chart of reducing (fasting) blood glucose. Series 1 = 1st measurement; Series 2 = 2nd measurement; Series 3 = 3rd measurement (source : SPSS 14.0)

Table 2. Paired t-Test for eNOS concentration

	Paired t-Test	Sig.	Mean
Pair 1	eNOS_I – eNOS_II (pre – durantee HBO)	.193	51.94
Pair 2	eNOS_II – eNOS_III (durantee – post HBO)	.031	489.70
Pair 3	eNOS_I – eNOS_III (pre – post HBO)	.017	541.67

Sig. = significant < 0,05 ; Resource: SPSS version 14.0

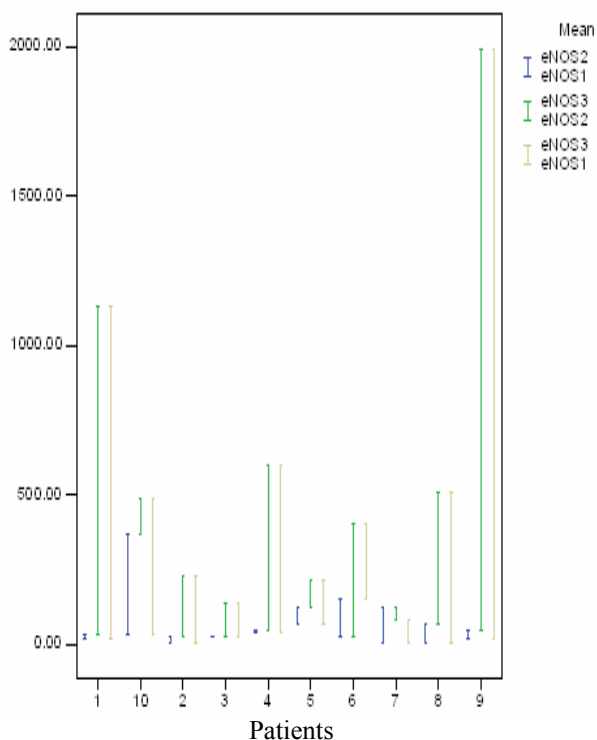


Figure 3. The chart of improvement eNOS concentration. I = 1st measurement; II= 2nd measurement; Series III = 3rd measurement (source : SPSS 14.0)

Table 3. Paired t-Test for SGOT and SGPT

	Paired t-Test	Sig.	Mean
Pair 1	SGOT_I – SGOT_II (pre – durantee HBO)	.061	1.40
Pair 2	SGOT_II – SGOT_III (durantee – post HBO)	.015	1.20
Pair 3	SGOT_I – SGOT_III (pre – post HBO)	.893	.10
Pair 4	SGPT_I – SGPT_II (pre – durantee HBO)	.541	.30
Pair 5	SGPT_II – SGPT_III (durantee – post HBO)	.560	.30
Pair 6	SGPT_I – SGPT_III (pre – post HBO)	.443	.60

Sig. = significant < 0,05 ; Resource: SPSS version 14.0

Table 4. Paired t-Test for BUN dan Creatinine Serum (SK)

	Paired t-Test	Sig.(1 tailed)
Pair 1	BUN_I – BUN_II (pre – durantee HBO)	.771
Pair 2	BUN_II – BUN_III (durantee – post HBO)	.479
Pair 3	BUN_I – BUN_III (pre – post HBO)	.364
Pair 4	SK_I – SK_II (pre – durantee HBO)	1.00
Pair 5	SK_II – SK_III (durantee – post HBO)	1.00
Pair 6	SK_I – SK_III (pre – post HBO)	1.00

Sig. = significant < 0,05 ; Resource: SPSS version 14.0

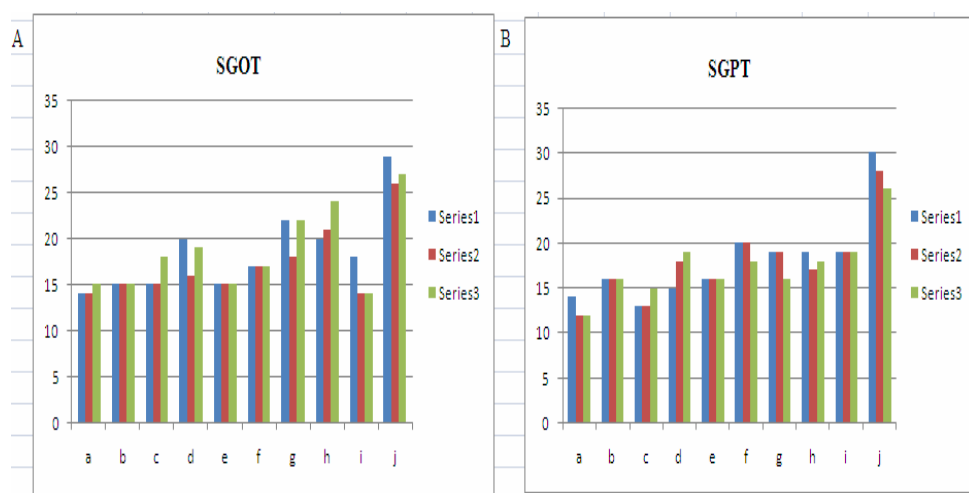


Figure 4. Chart (A) result of SGOT evaluation; (B) result of SGPT evaluation. series 1 = 1st measurement of SGOT and SGPT, series 2 = 2nd measurement of SGOT and SGPT, series 3 = 3rd measurement of SGOT and SGPT.

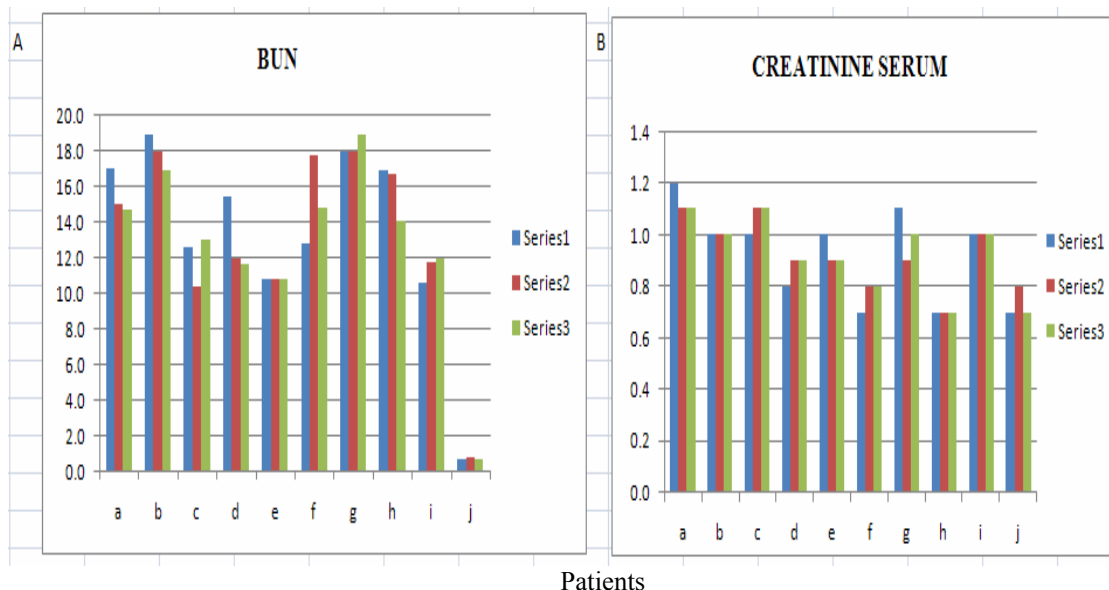


Figure 5. Chart (A) result of BUN evaluation; (B) result of SK evaluation. series 1 = 1st measurement of BUN and SK, series 2 = 2nd measurement of BUN and SK, series 3 = 3rd measurement of BUN and SK

Neither the adverse reaction of Metformin or HBO therapy were not complained during the term of combination treatment, Metformin 500mg 4 times a day and HBO 2.4 ATA 10 times.

DISCUSSION

In our study, the fasting blood glucose was nearly in "normo-glycemia" condition after the combination therapy of Metformin and HBO given. Monotherapy Metformin is able to reduce fasting glucose blood around 60-80 mg/dl (Goodman & Gilman 2006, Katzung 2008, Dipiro 2009). HBO therapy 2.4 ATA 10 times can reduce glucose blood around 73 mg/dl (Wijayatno 2000). Moreover, the combination therapy of Metformin and HBO are able to control the fasting glucose blood around 81,5 mg/dl. It presumable Metformin and HBO therapy had synergism effect in reducing hyperglycemia condition. HBO therapy has effect as same as exercise so that able to activate AMPK, thus glucose was able to use as ATP resource. However, specific mechanism of action combination Metformin and HBO therapy remains to be determined.

Furthermore, eNOS concentration was raised significantly after the combination therapy of Metformin and HBO given. Whenever eNOS concentration is raised, it will improve the NO released. NO has a lot of function to protect vascular endothel, thus, it will be a new hope to prevent patients with diabetes from cardiovascular events (Storey et al. 2001,

Hamilton et al. 2007, Calvert et al. 2008) The mechanism of increasing eNOS concentration by giving combination therapy of Metformin and HBO presumable via AMPK activation.

The other synergism effect of the combination therapy of Metformin and HBO is HBO therapy reducing plasma lactate levels, the adverse effect of Metformin, by improving cell's oxygen in aerob glucose metabolism. Moreover, the combination therapy of Metformin and HBO also work in erection ability due to the NO production. NO production persists in the penis initiating stimulus, enabling continued relaxation and full penis erection (Musicki 2006, Zeng et al. 2008).

CONCLUSION

In the therapy for type 2 diabetes, hyperbaric oxygen therapy works synergistically with Metformin, particularly by improving Metformin therapy in reducing blood glucose and in increasing eNOS. It is a new hope to prevent micro and macro angiopathy's complication in patient with chronic hyperglycemia caused by type 2 diabetes.

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