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Hyperbaric Oxygen in Animal Model of Diabetes Nephropathy: Analysis of Blood Glucose, Proteinuria and Kidney Tissue Necrosis Cells

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ABSTRACT

Diabetic nephropathy (DN) is a microvascular complication of diabetes mellitus (DM) and is the main cause of 20 to 40 times higher mortality compared to diabetes without nephropathy. Therefore, the author wants to prove the effect of hyperbaric oxygen therapy (HBO) on changes in blood glucose levels, proteinuria and kidney tissue necrosis cells in DN animal models. This study used 27 male white rats Rattus Norvegicus strain Wistar, weighing 170 - 220 grams, aged 8-12 weeks, healthy and active, divided into 3 groups, namely the normal rats group (G0), the DN rats without HBO group (G1) and the DN rats with HBO group (G2). Making a DN model with Streptozotocin (STZ) induction 75 mg / kgBW intraperitoneally in a single dose. HBO was performed in a 2.4 ATA pressurized air chamber by inhaling 98% O2 for 3 x 30 minutes interspersed with inhaling normal air for 2 x 5 minutes for 5 consecutive days. The results showed a significant decrease in blood glucose levels $p = 0.000$ ($p < 0.05$). In proteinuria levels, there was an insignificant decrease $p = 0.077$ ($p > 0.05$) in G2 compared to G1. Repair of kidney tissue damage was also indicated by a decrease in necrotic cells by 45.45% in G2 compared to G1. These results prove that HBO can repair kidney damage in DN model mice, so HBO is expected to be used as an additional therapy in cases of diabetic nephropathy.

Key words: Hyperbaric Oxygen, Diabetes Nephropaty, Blood Glucose, Proteinuria, Kidney Tissue Necrosis Cells.

INTRODUCTION

Diabetic nephropathy (DN), better known as Diabetic Kidney Disease (DKD), is characterized by proteinuria in the form of microalbuminuria or macroalbuminuria and decreased kidney function.[1] DN is one of the microvascular complications of diabetes mellitus (DM).[2] The main criteria for ND include decreased kidney function, diabetic retinopathy, proteinuria, and decreased Glomerular Filtration Rate (GFR).[1] Its characteristics are a clinical syndrome characterized by persistent albuminuria and progressive decline in kidney function as well as a typical pattern of glomerular disease.[3] The incidence of DM reaches 1.5 million every year.[4] The prevalence of DN follows the incidence of DM. In 2014 the World Health Organization (WHO) estimated that 422 million people in the world suffered from diabetes (8.4%). In 2017, the prevalence of diabetes reached 8.9% to 11.1% in Indonesia. Mortality occurs almost 20 to 40 times higher in patients with ND compared to diabetes patients without nephropathy.[5] DN is the main cause of morbidity and mortality in type 1 and type 2 diabetes.[1]

The use of hyperbaric oxygen therapy (HBOT) is a method of treating patients by placing them in a high-pressure room to breathe using a mask, using 100% oxygen with a pressure of more than 1 Absolute Atmosphere (ATA) for a certain period of time.[6] HBOT the has mechanical and physiological effects. The mechanical effect is related to Boyle's law, where existing gas bubbles shrink with increasing pressure and are eliminated by collapse or expulsion from the lung. Physiological effects are related to tissue Hyperoxia.[7] The primary effect or direct effect of the mechanism of action of HBOT is to improve hypoxic conditions by increasing oxygen delivery and pressure.[8] Based on the feasibility study, this research can be used for scientific information in developing knowledge about the effects of HBOT on reducing blood glucose, proteinuria and kidney tissue necrosis cells in animal models of DN. It is hoped that HBOT can prevent disability and death, improve health and quality of life so that it can be used as adjuvant therapy along with established standard therapy.

MATERIALS AND METHODS

The ethical feasibility of the research was submitted to the Research Ethics Commission of the Indonesian Navy Marine Health Institute Drs. Med. R. Rijadi Sastroepanoelar Phys, Surabaya on the date with ethical certificate No. 08/EC/LKS/V/2024.

The research design used true experimental research. The research design was a post-test control group design. The design description was made schematically as follows:

The number of experimental animals using the formula from Steel and Torrie, namely a total sample size of 27 animals divided into 3 groups, each group of 9 mice, namely the negative control group or normal rats (G0), the positive control group or DN rats without HBO therapy (G1) and the treatment group or DN rats with HBO therapy (G2). The

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population used was white rats Rattus Norvegicus Galar Wistar. The sampling technique used in this study was simple random sampling. There were inclusion, exclusion, and drop out criteria in the research sample. The inclusion criteria were the type of animal used in the study was a male Wistar rat, the age of the experimental animal was around 8-12 weeks, the weight of the experimental animal was 170-220 g, during the study the physical condition of the experimental animal was healthy as indicated by bright eyes, smooth fur, agile movement, good appetite, good body anatomy and no defects, body weight did not decrease by more than 10% during the adaptation period. The exclusion criteria were that the experimental animal was sick during the adaptation period. The drop out criteria were that the experimental animal died during the research process.

The research variables were the independent variable (the independent variable was the administration of HBO therapy, the dependent variable was blood sugar levels, proteinuria and the number of kidney tissue necrotic cells. The control variables were health status, food, maintenance place (cage), induction technique and dose, and evaluation time.

Acclimatization of experimental animals was carried out for 7 days at the Biochemistry Laboratory, Hang Tuah Medical Faculty, Surabaya. The creation of DN animal models was carried out by Streptozotozin (STZ) induction. STZ induction, the animal's body weight was weighed. Weighing was carried out using a digital scale. Mice were induced using STZ 75 mg/kgBW intraperitoneally, then the mice were put into a cage and left for four days with food and tap water. On the third day, the mice's blood glucose levels were checked to confirm DM. On the fourth day, the mice's proteinuria was checked to confirm DN.

Blood sampling for glucose examination was taken from the lateral tail vein of the mouse as much as 1 ml. Taking blood specimens using this method must be taken carefully so as not to cause permanent damage to the tail or amputation. The experimental animal was put in a holding container, then the rat's tail was cut and the blood was taken, Do not squeeze the tail because it caused damage and contamination of tissue fluid. Blood glucose levels were measured using the glucose oxidase biosensor method read using the GlucoDr screen after 11 seconds. Normal random sugar levels range from 80 – 120 mg/dL.

0.5 L of urine was collected by placing the rat in a cage. The mice were placed in the cage for 1 day, then took a urine container and put the urine into a tube for laboratory examination. Proteinuria levels were measured using the dipstick depth method using a URS-10T brand urine strip with a sensitivity of 0.16-0.3 g/L. Normal levels of protein in urine range from 0.15–20 g/L.

HBO therapy was given after the mice in G2 were declared DN. Therapy was carried out in a high-pressure air chamber of 2.4 ATA for animals made of steel. In each therapy session, the experimental animals inhaled 98% O_2 for 3 x 30 minutes interspersed with inhalation of normal air for 2 x 5 minutes. Therapy was carried out for 5 consecutive days. HBO therapy was carried out at a room humidity of 50% and a room temperature of around 25-26°C.

In histopathological examination of kidney tissue, mice were anesthetized using 0.5 mg/kgBW ketamine. After that, the mice were waited for 5 to 10 minutes until there was no response to pain. Rat kidneys were taken to observe changes in the histopathological appearance of the kidneys by making histological preparations of the kidney organs and stained with Hematoxylin Eosin (HE). Hematoxylin Eosin Histology Staining Procedure Deparaffinize the dried preparation in xylol 3 times (10-15 minutes each). Then put it in 96% alcohol 2 times (for 5 minutes each), then wash it with running water until the alcohol is gone. After that, put it in hematoxylin paint for 7-10 minutes and wash it with running water until it doesn't fade. Decolorize by dipping twice in HCl and washing again with running water. Then soak it in water for a while until the color turns blue, then put it in eosin paint for 3-5 minutes and wash it with running water. The next step was to put it in alcohol solution 1, then into alcohol solution 2 and wash it with running water. The preparation was then pressed with paper, wiped with cotton and then placed in xylol. The preparation was pressed with paper and wiped again with cotton. Mounting was carried out and a laboratory number was given. Then the necrosis cells were observed under a light microscope with 400x magnification. After the mice were operated on, the mice were buried.

Data Processing and Analysis was carried out by collecting data in a controlled and controlled environment. Data were analyzed statistically using Statistical Package for the Social Sciences (SPSS) software version 22.0.

RESULTS

Data analysis began with a descriptive test, then a normality test was carried out using the Shapiro-Wilk test because the sample was less than 50. The results of the normality test had a significance of $p > 0.05$ in each group, indicating that three groups of data were normally distributed. Then continued with the homogeneity test with the Lavene test, the results of the variation of inhomogeneous data were obtained $p < 0.05$. Because the data was not homogeneous, it will be continued with a non-parametric test, namely the Kruskal Wallis test.

In blood sugar levels, the Kruskall Wallis Test found a significant difference $p = 0.000$ ($p < 0.05$) in G0, G1 and G2. In group G1, the average blood glucose value was 312.667 ± 0.015 higher than in G2 which had an average of 177.222 ± 6.155. Mann Whitney test, there was a significant decrease in blood glucose levels $p = 0.000$ ($p < 0.05$) in group G2 compared to G1.

In proteinuria levels, the Kruskall Wallis Test found a significant difference $p = 0.000$ ($p < 0.05$) in G0, G1 and G2. In group G1, the average proteinuria value was 8.444 ± 2.897 higher than in G2 which had an average of 3.700 ± 2.068. Mann Whitney test, there was a decrease in proteinuria levels, although not significant $p = 0.000$ ($p < 0.05$) in group G2 compared to G1. The average results, standard deviation, minimum value, maximum value, significance results of blood glucose and proteinuria levels in the research group can be seen in Table 1.

In histopathological examination, the data obtained were semi-quantitative data analyzed using the risk analysis method. The percentage of kidney damage in mice was calculated using the formula of the number of necrotic cells divided by the total number of cells, then multiplied by 100%.

H&E staining results showed a normal kidney picture in G0. G0 showed a normal cell structure with a normal-sized cell nucleus located in the center (green arrow). There was a glomerulus (yellow line) bordered

Table 1: Blood Glucose and Proteinuria.

Parameter	Group	Mean ± SD	Minimum Value	Maximum Value	p
Blood Glucose (mg/dL)	G0	116.444 ± 1,827	107	123	
	G1	312.667 ± 9,015	275	354	0.000
	G2	177.222 ± 6,155	146	202	
Proteinuria(g/L)	G0	0.15 ± 0.000	0.15	0.15	
	G1	8.444 ± 2.897	1.00	20.00	0.000
	G2	3.700 ± 2.068	0.30	20.00	

Data are shown as mean ± SD (standard deviations); $p<0.05$ was considered statistically significant; G1= control group; G2 = DN + non-HBO group; and G3 = DN + HBO group.

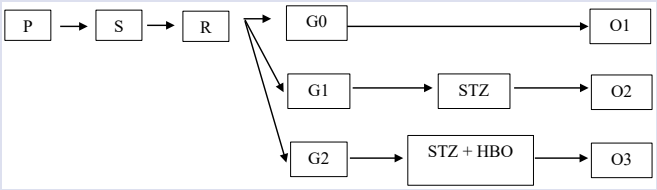

Figure 1: Research Design. Description: P: Population. S: Sample. R: Randomization. G0: Control group without streptozotozin induction. G1: Control group induced by streptozotozin. G2: Treatment group induced by streptozotozin and given HBO therapy. O1: Observation on G0. O2: Observation on G1. O3: Observation on G2 post HBO therapy. HBO: Given 100% oxygen therapy at a pressure of 2.4 ATA for 3x30 minutes with a 2x5 minute interval of normal air in a closed room for a period of 5 consecutive days.

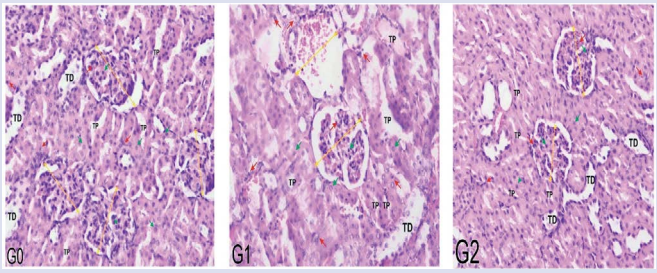

Figure 2: The expression of necrotic cells in the G0, G1 and G2. TD: distal convorted tubulus; TP: proximal convoluted tubules; Yellow line: glomerulus; Green arrow; normal cell; Red arrow: necrotic cells.

by a spatium capsulare at the edge. Around the glomerulus there were distal convoluted tubules (TD) and proximal convoluted tubules (TP). From the findings of this study, necrotic cells (red arrows) were found around 8.9% of the total with necrotic cells in the glomerulus around 8.88% and in the tubules there were around 9.1% necrotic cells.

In G1 showed normal cell structure with normal sized cell nucleus and located in the middle (green arrow). There is glomerulus (yellow line) with limited by spatium capsulare on the edge. Around the glomerulus there were distal convoluted tubules (TD) and proximal convoluted tubules (TP). From the findings of this study, necrotic cells were found (red arrow) around 58.5% of the total with necrotic cells in the glomerulus around 68.19% with congestion found in one of the preparations in the glomerulus and in the tubule there were around 57.15% necrotic cells.

In G2, it showed normal cell structure with normal sized cell nucleus located in the middle (green arrow). There was glomerulus (yellow line) with a border with spatium capsulare on the edge. Around the glomerulus there were distal convoluted tubules (TD) and proximal convoluted tubules (TP). From the findings of this study, necrotic cells (red arrow) were found as much as 22.74 percent of the total with necrotic cells in the glomerulus around 23.69 percent and in the tubules there were around 22.45 percent necrotic cells.

So, from the research results, it was found that there was a decrease in the number of necrotic cells by 45.45% in the group of DN model experimental animals that were given HBO therapy compared to the DN model experimental animals that were not given HBO therapy. The expression of necrotic cells in the research group can be seen in Figure 2.

DISCUSSION

Diabetic nephropathy (DN) is the most common cause of end-stage renal disease (ESRD). DN has characteristic renal histopathology such as glomerular basement membrane (GBM) thickening, mesangial expansion, nodular glomerular sclerosis, and tubulointerstitial fibrosis.[3] Prolonged hyperglycemia causes renal endothelial damage, inflammation, and oxidative stress, resulting in metabolic and hemodynamic disturbances that lead to impaired renal function. At the same time, fibrotic and sclerotic changes occur that worsen glomerular and tubular injury. At the macro level, the reciprocal interaction between the systemic circulation and the renal microvasculature will cause a destructive cycle that drives the progression of the disease to become more severe.

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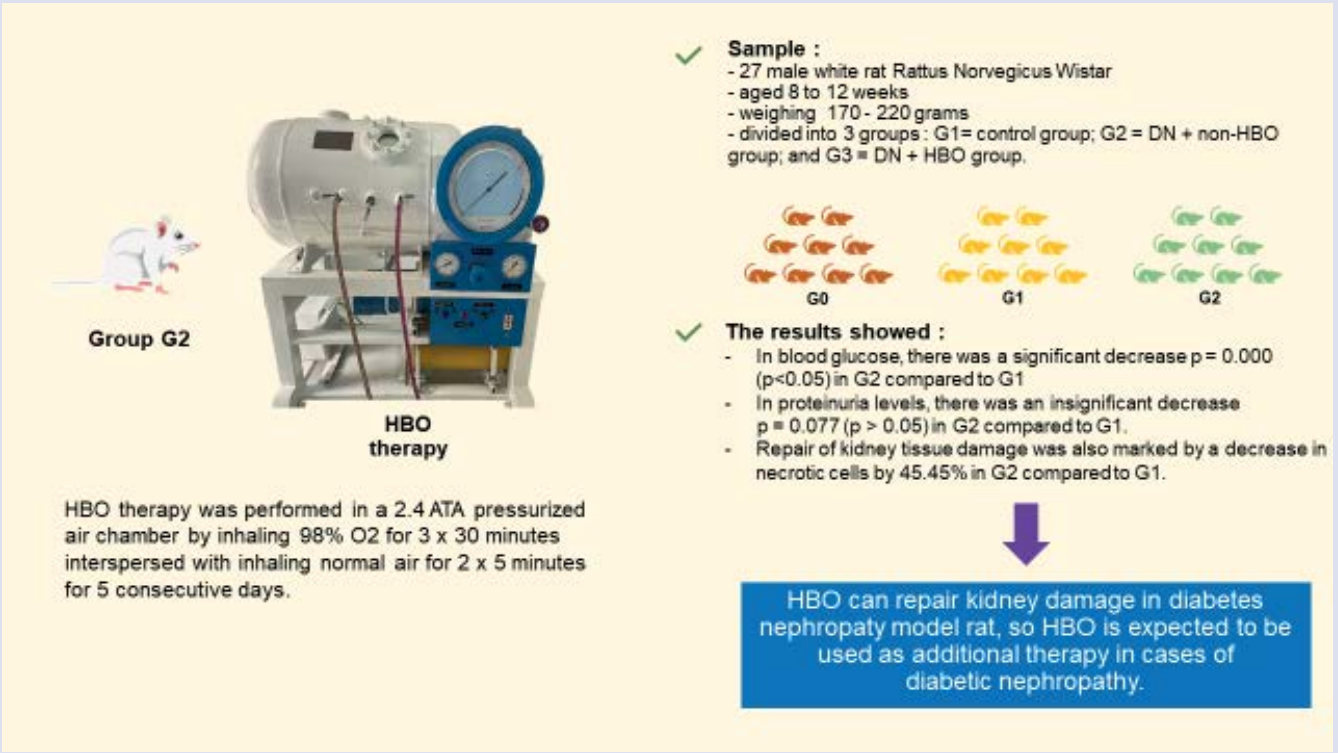
GRAPHICAL ABSTRACT



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