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Hyperbaric Oxygen in a Streptozotocin-Induced Animal Model: Pancreatic Tissue Analysis, Blood Glucose and HbA1c

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ABSTRACT

OBJECTIVE: The study aims to examine the effect of hyperbaric oxygen (HBO) on the improvement of blood glucose, haemoglobin A1c (HbA1c) levels, and pancreatic apoptotic cells in a streptozotocin (STZ)- induced animal model.

METHODOLOGY: 27 white rats of the Rattus norvegicus strain Wistar were grouped into three: healthy rats (G0), diabetic rats not treated with HBO (G1), and diabetic rats treated with HBO (G2). A single intraperitoneal (i.p.) dose of 75 mg/kg body weight of STZ was used to create a DM model. HBO was performed in a pressurized chamber at 2.4 ATA by inhaling 98% O2 for three 30-minute breaths, alternating with two 5-minute breaths of normal air, for 10 successive days.

RESULTS: The outcome showed a significant decrease in blood glucose levels ($p = 0.000$, $p < 0.05$) and HbA1c levels ($p = 0.017$, $p < 0.05$) in G2 compared to G1. Improvement in pancreatic tissue damage was demonstrated by the absence of apoptotic cells in G2 compared to G1.

CONCLUSION: HBO can repair pancreatic damage in DM model mouse. HBO is expected to be used as an adjunct treatment in diabetic cases.

KEYWORDS: Hyperbaric oxygen, diabetes mellitus, apoptotic cells, blood glucose, HbA1c

INTRODUCTION

Diabetes Mellitus (DM) is a life-threatening disease. High blood glucose levels are a sign of insufficient insulin production or the body's failure to utilize the insulin it produces[1]. Previous research has shown that oxidative stress leading to cell apoptosis is a major factor in beta cell damage in type 1 diabetes mellitus (T1DM)[2]. HbA1c is a biomarker that can reflect glucose levels in DM patients and is used as a marker for monitoring DM[3].

According to data from the International Diabetes Federation (IDF), this incidence rate is predicted to continue to increase progressively, namely in 2030 to 643 million (11.3%) and in 2045 to 783 million (12.2%), with most of this increase occurring in developing countries[1]. Demographic transition, changes in habits and lifestyles and delays in diagnosis resulting in not receiving appropriate treatment result in a much higher risk of complications and thus facing a significantly higher risk of complications[4].

Streptozotocin (STZ) is the most widely used diabetogenic chemical to create mouse models of T1DM and type 2 DM (T2DM)[5]. STZ is a glucosamine-nitrosourea that is toxic to pancreatic β-cells. The STZ model with high doses of STZ is primarily used to study type 1 DM, as the latter STZ regimen creates severe and extensive β-cell necrosis with complete loss of pancreatic insulin secretion[6]. STZ induction causes oxidative stress and increased secretion of nitric oxide synthase (NOS), which is involved in the destruction and death of pancreatic islet cells[2].

Hyperbaric Oxygen Therapy (HBO) is a therapeutic approach that utilizes exposure to concentrated pure oxygen (O_2) or 100% O_2 combined with increased atmospheric pressure[6]. This research is expected to prove the benefits of HBO in controlling pancreatic apoptotic cells in DM animal models, thus serving as a basis for further research.

METHODOLOGY

The type of research is "True experimental" using a "Randomized post-test control group design only". Before conducting the research, we conducted an animal ethics feasibility test at the Naval Health Institute Surabaya, East Java, Indonesia, on February 3, 2025. After the ethics test was completed and approved, our research continued to the next stage. The creation of experimental animal models, blood analysis, and histopathology of the experimental animal models were carried out in the hyperbaric laboratory of the Faculty of Medicine, Hang Tuah University, Indonesia. Meanwhile, hyperbaric oxygen therapy was carried out in the hyperbaric laboratory of the Naval Health Institute, Surabaya, Indonesia.

Experimental group

The research animals comprised 27 white rats (Rattus norvegicus, Wistar strain). The experimental animals were male, healthy, active, aged 8–12 weeks, and weighing 170–220 grams. The experimental animals were separated into 3 groups: the normal group (Group 0/G0), the experimental group of diabetic animals without HBO therapy (Group 1/G1), and the experimental group of diabetic animals given HBO therapy (Group 2/G2). Each group was placed in a separate cage. G1 and G2 were injected with STZ 75 mg/kg BW i.p. After induction, on the third day, the blood glucose levels of the experimental animals were checked to determine whether they had diabetes.

Hyperbaric Oxygen

Group 2 was given HBO. Group 2 underwent HBO at a continuous depth of 2.4 ATA within 10 minutes. Then proceed with 98.9% O_2 inhalation for 90 minutes. Administration of 100% O_2 was divided into three sessions and two air-breathing intervals of 5 minutes. The duration of one 100% O_2 inhalation session is 30 minutes. Then, breathe in air with pressure that gradually decreases until it reaches normal levels (1 ATA) within 10 minutes. Group 2 received HBO for 10 consecutive days with 50% humidity and a temperature of 28°C.

Blood sampling and collection

Blood was drawn intracardially to measure blood sugar and HbA1c levels. In this method, the rats were anesthetized with 0.5 mg/kg body weight of ketamine. Afterwards, the rats were kept in an anesthetized state for 5 to 10 minutes until they no longer responded to pain. The rats were then surgically withdrawn, and blood was drawn intracardially using a 3 ml syringe. The rats did not survive after the blood was drawn intracardially.

Blood sugar levels

Blood glucose concentrations were determined via the glucose oxidase biosensor technique. The steps include washing and drying your hands, preparing the equipment and test strip, drawing blood from the syringe onto the strip, and then reading the results on the Gluco Dr. glucometer eleven seconds later. Reference blood sugar levels extend from 80 to 120 mg/dL.

HbA1c levels

Here are the general steps for HbA1c testing in rats. HbA1c levels were measured using the enzymatic fluorescence method. The heart collection procedure is also invasive and is generally performed in studies requiring larger blood volumes. The rats are anesthetized, and blood is drawn from the heart using a syringe. Sample Preparation: The collected blood is then processed to separate the red blood cells from the plasma. Often, red blood cells containing hemoglobin are used for HbA1c analysis. HbA1c analysis uses enzymes to measure the level of glucose bound to hemoglobin, which is then used to calculate HbA1c

levels. HbA1c measurement results are expressed as a percentage, which reflects the proportion of glycated hemoglobin. The normal range of HbA1c in rats may differ from humans and needs to be determined based on the research protocol used. HbA1c should be reported in NGSP/DCCT units (%).

Pancreas histopathology

Rat pancreas sections were taken for histopathological examination on the 10th day after administration of HBO. Pancreas tissue was fixed in 4% formaldehyde at 4°C for 24 h, and embedded in paraffin wax. The pancreatic tissue was paraffinized and then sliced to the same thickness, namely 4–6 µm, using a cutting machine, and stained using the hematoxylin and eosin (H&E) method. Then the slides were examined using a light microscope.

RESULTS

After data analysis, a significant or meaningful difference was obtained $p = 0.000$ ($p < 0.05$) from the average blood glucose levels taken from the blood of the normal experimental animal group or negative control group (G0), the type 1 DM model experimental animal that was not given HBO or the positive control group (G1) and the type 1 DM model experimental animal group that was given HBO or the treatment group (G2). In the G0 group, the average blood glucose value was 113,333±5.958, which was lower than in the G1 group, which had an average of 308,889±26,689. Non-parametric tests using the Mann-Whitney U test showed a statistically significant difference of $p = 0.000$ ($p < 0.05$) in blood glucose levels between G0 and G1. In the G1 group, the average blood glucose value was 308.889 ± 26.689, which was higher than in the G2 group with a mean of 165.111±23.476. The comparison between the G1 group and the G2 group obtained a significance of $p = 0.000$ ($p < 0.05$), so that a significant difference was found in the average blood glucose levels in G1 and G2. The average blood glucose levels in the three study groups are shown in **Figure 1**.

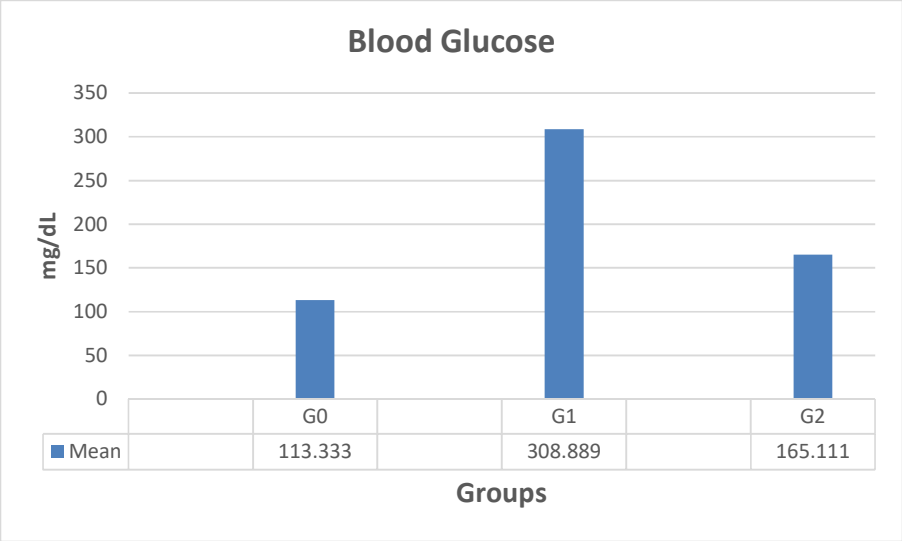

Figure 1: The average blood glucose levels in the three research groups

In HbA1c levels, data analysis revealed a significant difference in the mean HbA1c levels taken from the blood of normal Wistar rats (G0), untreated type 1 DM (G1), and HBO-treated type 1 DM (G2). In the G0 group, the average HbA1c value was 4.756±0.467, lower

than that of the G1 group, which had an average of 8.256±0.713. A post-hoc test using the Tukey (honest significant difference) technique found a significant difference between the G0 and G1 groups of 0.000 ($p < 0.05$). It can be concluded that there was a significant difference in the mean HbA1c levels between the G0 group and the positive control group (G1).

In group G1, namely the experimental animal model of type 1 DM that was not treated with HBO, the HbA1c value was significantly higher $p = 0.017$, $p < 0.05$, with an average of 8.256±0.713, while the treatment group G2 or the experimental animal model of type 1 DM that was treated with HBO, had an average of 7.433±0.543. From these results, there is an effect of administering HBO therapy on HbA1c levels obtained from the blood of STZ-induced animal models. The average HbA1c levels in the three research groups are shown in **Figure 2**.

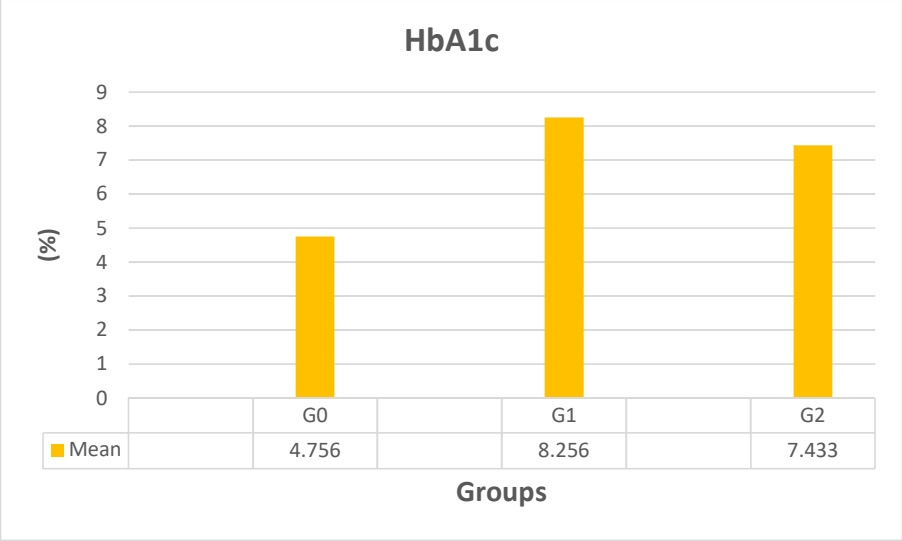

Figure 2: The average HbA1c levels in the three research groups

The histopathology results showed a normal pancreatic appearance in group G0. Group G0 shows pancreatic tissue with pancreatic islets indicated by yellow arrows. In the islets of Langerhans, there is a physiological β-cell structure with a normal-sized nucleus located in the centre (blue arrow); no apoptotic cells were found. Group G1 shows pancreatic tissue with islets of Langerhans (yellow arrow). In the islets of Langerhans, there are apoptotic cells with cell shrinkage, chromatin condensation, apoptotic body formation, and phagocytosis. There are also plasma membrane changes such as blebbing (protrusions in the membrane). Group G2 shows pancreatic tissue with islets of Langerhans (pancreatic islets) indicated by yellow arrows. In the islets of Langerhans, there is normal cell structure with a normal-sized nucleus located in the centre (blue arrow); no apoptotic cells were found. Pancreatic tissue expression in the three research groups is shown in **Figure 3**.

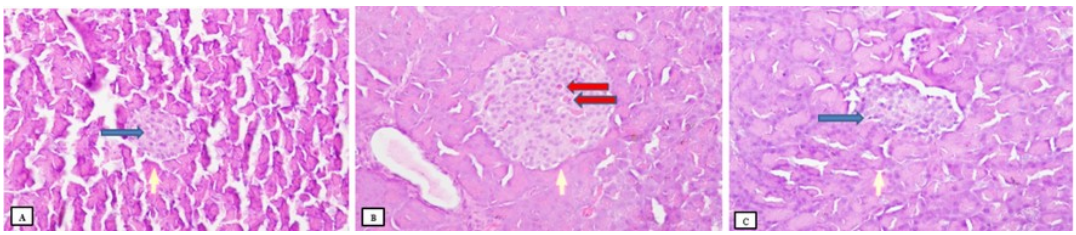

Figure 3: Expression of pancreatic tissue cells.
A: Pancreatic tissue in G0. B: Pancreatic tissue in G1. C: Pancreatic tissue in G2. Yellow arrow: Islets of Langerhans. Blue arrow: Normal β cells with normal-sized and centrally located nuclei, no apoptotic cells. Red arrow: Apoptotic cells with cell shrinkage, chromatin condensation, apoptotic body formation and phagocytosis, also experiencing changes in the plasma membrane such as blebbing (protrusions on the membrane).

DISCUSSION

Diabetes Mellitus (DM) is chronic and progressive. Persistently high blood sugar levels are influenced by insulin production and how it works[7]. Hyperglycemia causes hypoxia due to increased oxygen consumption in cells, including mitochondria. Hyperglycemia and hypoxia are inseparable and mutually influencing conditions, which can lead to excessive endothelin-1 production and the induction of apoptosis[8]. Hyperglycemia has toxic effects, damaging nearly all body cells, including the endoplasmic reticulum (ER) and pancreatic β cells, through various mechanisms. Hypoxia causes oxidative stress, which ultimately leads to inflammation, which can lead to cell damage and death[9].

Pancreatic β-cell apoptosis also plays an important role in the pathophysiology of Type 2 Diabetes Mellitus (T2DM). It is also influenced by the loss of pancreatic β-cell mass due to apoptosis, resulting in impaired insulin secretion. This lack of insulin secretion ultimately leads to chronic hyperglycemia[10]. Apoptosis is induced by hyperglycemia. In vitro apoptosis in isolated islets and insulinoma cell cultures is influenced by the balance of pro-apoptotic Bcl-2 factor proteins such as Bad, Bid, Bik, and Bax and anti-apoptotic Bcl family proteins such as Bcl-2 and Bcl-xL, as well as of caspase-mediated signalling. In the intrinsic pathway of mitochondrial membranes, apoptosis can only occur when the concentration of pro-apoptotic Bcl-2 exceeds the concentration of anti-apoptotic proteins. Fundamental DNA repair enzymes and poly(ADP-ribose) polymerase-1 (PARP1) can regulate β-cell death, replication, and insulin secretion[11].

Hyperbaric oxygen has a remarkable effect on the immune system. HBO can be administered to both healthy and diseased individuals. HBO increases dissolved oxygen in plasma and enhances oxygen transport to tissues independently of hemoglobin. Furthermore, the resulting increase in barometric pressure can stimulate anti-tumor biological activity through gene expression. This makes HBO superior to other oxygen delivery methods[12]. Oxidative stress damages beta cells. This study intended to determine the impact of HBO on beta-cell function in streptozotocin (STZ)-induced type 1 diabetic rats. By increasing oxygen tension, HBO increases dissolved oxygen in blood plasma, which more effectively delivers oxygen to pancreatic tissue, improves the microenvironment, and supports beta-cell metabolism. HBO helps lower ROS levels, reduces DNA damage, and protects cells from death. HBO reduces inflammation by reducing white blood cell (leukocyte) activation and improves vascular permeability, which is important for reducing autoimmune damage to beta cells. Endothelial (blood vessel lining) dysfunction is common in diabetes. HBO improves endothelial function, which indirectly improves blood vessel health in the pancreas, supporting healing and cell function.

HBO significantly reduced the rate of β-cell apoptosis in DM rats through the Bcl-2/caspase-3/pancreatic poly(ADP-ribose) polymerase (PARP) apoptosis pathway. Furthermore, HBO improved pancreas tissue morphology and increased hepatic glycogen storage in rats. These findings suggest that HBO improves insulin sensitivity, thus lowering blood sugar levels, by reducing the rate of β-cell apoptosis through the Bcl-2/caspase-3/pancreatic PARP apoptosis pathway. Research has focused on reducing blood sugar levels without increasing insulin secretion from the pancreas, but rather on improving insulin effectiveness. This research includes increasing insulin receptor activity and changing insulin sensitivity through upregulation of PPAR-γ (peroxisome proliferator-activated receptor-γ)[13].

Excess glucose will covalently bind to hemoglobin irreversibly, a process called glycosylation or non-enzymatic glycation. Hemoglobin gives blood its bright red color. Glycosylation and protein glycation are distinct processes involving the modification of carbohydrates to proteins. Enzymes mediate glycosylation. During biosynthesis, glycosylation attaches polysaccharides to proteins, affecting protein folding and function.

Intracellular glycolysis produces glucose, which further contributes to the formation of AGEs and cell damage.
In contrast, glycation occurs randomly in the intracellular and extracellular spaces, involving the non-enzymatic attachment of glucose to proteins. Glycation ultimately results in the formation of AGEs, which are implicated in diabetes-related diseases. In diabetes, glycation becomes disordered, leading to the accumulation of AGEs, which alter cell structure and function[14]. So if blood sugar levels increase, more glucose binds to the hemoglobin protein, resulting in a higher HbA1c value[15]. Red blood cells are between 100 and 120 days old. Therefore, HbA1c indicates the mean of blood sugar level over the past 60 days[16]. This study shows that HBO can reduce HbA1c levels over 2 months.

CONCLUSION

This study shows that HBO therapy carried out by inhaling 100% O_2 for 3x30 minutes interspersed with 2x5 minutes of rest at a pressure of 2.4 ATA for 10 consecutive days can reduce apoptosis of pancreatic B cells, thereby reducing blood sugar and HbA1c levels in STZ-induced experimental animals.

Ethical permission: Ethics clearance is granted by the Research Ethics Committee of the Indonesian Naval Health Institute in Surabaya, East Java, Indonesia. No. 02/EC/LKS/II/2025.
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Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publicly.

AUTHOR CONTRIBUTION

Harnanik T: Study design, concept, drafting, data interpretation, literature research
Sulistio CB: Study concept, data analysis
Zansen J: Data interpretation, drafting
Astarini LK: Data collection, questionnaire design, final approval

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