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Research Paper

Azygoportal Total Dissociative Procedures for Portal Hypertension Treatment; Evolution of Surgical Techniques.

Nazirov F.G., Devyatov A.V., Babadjanov A.Kh., Salimov U.R. and Khakimov D.M.

J. Life Sci. Biomed., 8 (2): 24-30, 2018;
pii:S225199391800005-8

Abstract

The aim of the study was to determine the efficiency of azygoportal collector total dissociation in patients with portal hypertension. Depending on the procedure, the patients were divided into two groups. An original method of azygoportal dissociation was performed in 63 patients (the first group). In the second group a modified version of azygoportal dissociation was performed. Patients were comparable in the main pathology and course of the disease. Edematous ascites syndrome; liver failure; insufficiency of gastro-gastral anastomosis and haemorrhagic syndrome, were observed in 28.6%; 23.8%; 11.1%; and 14.3 % of patients operated by the original method vs. 16.5%; 7.7%; 0%; and 4.4% for patients operated in the modified technique, respectively. From the results it can be concluded that, proposed modified method of azygoportal collector dissociation on a prosthesis is more effective method for hemorrhagic syndrome control, and also allows to significantly reduce the incidence of severe complications in the immediate postoperative period.

Keywords: Liver Cirrhosis, Portal Hypertension, Dissociative Operations, Ligature Transection Method, Bleeding from Esophageal Varices.

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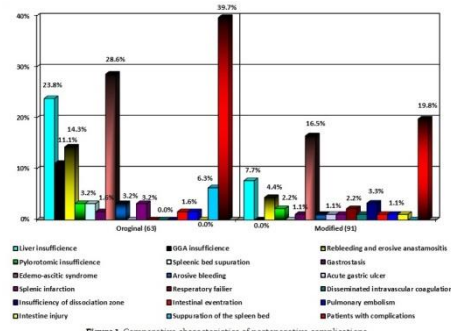


Figure 1. Comparative characteristics of postoperative complications

To cite this paper: Nazirov F.G., Devyatov A.V., Babadjanov A.Kh., Salimov U.R. and Khakimov D.M. 2018. Azygoportal Total Dissociative Procedures for Portal Hypertension Treatment; Evolution of Surgical Techniques. *J. Life Sci. Biomed.* 8(2): 24-30. www.jlsb.scribd.com

Research Paper

Prophylactic Administration of *Ginkgo biloba* Leaf Extract (EGb 761) Inhibits Inflammation in Carrageenan Rat Paw Edema Model.

Abdulrazak S., Nuhu A.A., and Yashim Z.I.

J. Life Sci. Biomed., 8 (2): 31-36, 2018;
pii:S225199391800006-8

Abstract

Acute toxicity and anti-inflammatory effect of *Ginkgo biloba* leaf extract (EGb 761) were carried out in this study. The anti-inflammatory activity was studied using the carrageenan model whereby twenty rats were randomly divided into four groups of five animals each. Groups one and two were administered the EGb 761 extract at 500 mg/kg and 250 mg/kg, respectively. Rats in groups three (positive control group) and four (non-treated control group) were given piroxicam (10 mg/kg) and normal saline (5 ml/kg), respectively. Oedema was induced by injecting 100 µl of fresh carrageenan into the right plantar surface of the hind paw of each rat 30 minutes after administration. The acute toxicity tests result showed that the extract is safe at 5000mg/kg dose. *Ginkgo biloba* leaf extract caused a significant ($P < 0.05$) decrease in the size of the paw oedema when compared to control. Of interest, EGb 761 at 250 mg/kg was as effective as, or better than piroxicam (10 mg/kg). These findings further justify the use of *Ginkgo biloba* leaf extract in both medical and ethnomedical practice and may be used in treatment of inflammation.

Keywords: *Ginkgo Biloba* Leaf Extract, Carrageenan, Rats, Paw Oedema, Inflammation

[Full text-[PDF](#)]



Red Blood Cells Morphology Monitoring to Predict Hyperfunction of Subclavian-Pulmonary Anastomosis in Patients with Fallot Tetralogy.

Ibadov R.A., Baybekov I.M., Abralov Kh.K., Strijkov N.A., Julamanova D.I., Khamdamovich I.S., Ravshanovich I.R.
J. Life Sci. Biomed., 8 (2): 37-42, 2018;
pii:S225199391800007-8

Abstract

Hyperfunction of subclavian-pulmonary anastomosis in patients with tetralogy of Fallot (TOF) is known to be a rather common condition in the early post-operative period. It resulted in development of hypervolemic pulmonary circulation and edema. Morphometry of peripheral blood cells of 81 TOF post-operative patients revealed an increase in the number of pathologically shaped red blood cells (PS RBCs) in 14 of them. Mainly these were the ones with a ridge-like structure on their surface. The thick drop express-technique (TDET) enables to evaluate the correlation of normal RBCs/ PS RBCs for 10-15 min for the entire procedure. The progressive deterioration of RBCs morphological features is suggested to be a predictor of the anastomosis hyperfunction due to changed blood rheology. In addition the correlation of normal and pathological forms of erythrocytes can be an evaluation criterion of effectiveness of patient management tactics of cardiologic intensive care.

Keywords: Red Blood Cell, Tetralogy of Fallot, Subclavian-Pulmonary Anastomosis, Thick Drop Technique, Scanning Electronic Microscopy, Cardiologic Resuscitation

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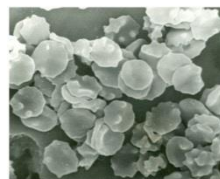


Figure 1. The blood sample of the CTOP patient. The evident domination of pathologically shaped RBCs. SEM x 1,000

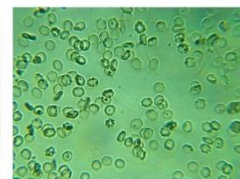


Figure 2. The blood of the same patient. Numerous echinocytes, cells with ridges and stomatocytes. TDET 10 x 60.

To cite this paper: Ibadov R.A., Baybekov I.M., Abralov Kh.K., Strijkov N.A., Julamanova D.I., Khamdamovich I.S., Ravshanovich I.R. 2018. Red Blood Cells Morphology Monitoring to Predict hyperfunction of Subclavian-Pulmonary Anastomosis in Patients with Fallot Tetralogy. *J. Life Sci. Biomed.* 8(2): 37-42. [www.jlsb.science-line.com](https://doi.org/10.2478/225199391800007)

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Azygoportal Total Dissociative Procedures for Portal Hypertension Treatment; Evolution of Surgical Techniques

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ABSTRACT

The aim of the study was to determine the efficiency of azygoportal collector total dissociation in patients with portal hypertension. Depending on the procedure, the patients were divided into two groups. An original method of azygoportal dissociation was performed in 63 patients (the first group). In the second group a modified version of azygoportal dissociation was performed. Patients were comparable in the main pathology and course of the disease. Edematous ascites syndrome; liver failure; insufficiency of gastro-gastral anastomosis and haemorrhagic syndrome, were observed in 28.6%; 23.8%; 11.1%; and 14.3 % of patients operated by the original method vs. 16.5%; 7.7%; 0%; and 4.4% for patients operated in the modified technique, respectively. From the results it can be concluded that, proposed modified method of azygoportal collector dissociation on a prosthesis is more effective method for hemorrhagic syndrome control, and also allows to significantly reduce the incidence of severe complications in the immediate postoperative period.

Original Article

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Keywords

Liver Cirrhosis,
Portal Hypertension,
Dissociative
Operations,
Ligature Transection
Method,
Bleeding from
Esophageal Varices.

INTRODUCTION

Hemorrhagic syndrome is one of the most severe and unpredictable complication of liver cirrhosis (LC) with portal hypertension (PH). The bleeding from esophago-gastric varicose (EV), is observed in 20-50% of patients with LC and clinically significant PH [1-10]. Endoscopic interventions used to control varices bleeding, due to their low invasiveness and ease of execution, are the first-line methods in treatment and prophylactics of hemorrhage. However, they remain unsuccessful in 17-37% of patients [11]. Nowadays many different surgical procedures are known and are frequently used as a second line method in the bleeding control and prophylactics. Among such methods, liver transplantation (LT), a surgical portosystemic shunting (PSS), transjugular intrahepatic portosystemic shunting (TIPS) and dissociative interventions are the most frequently used. It is known that LT is the only curative option for patients suffering from LC. At the same time deficit of donor organs is still a quite acute problem and many patients in the waiting list will not have a donor organ in time [12].

From the other hand traditional surgical PSS give a good long time results in bleeding control, but due to its complexity, its application is limited. Besides in the last decade one can observe significant decrees of surgical PSS application in contrast to TIPS. But by the opinion of many investigators and with the accordance to last studies wide popularization of the TIPS is not often reasonable due to some significant lacks of the method [6, 13, 14]. At the same time, surgical PSS which are more effective in prevention of hemorrhagic syndrome cannot be performed in all patients. A wide group of patients is out of the possibility for PSS application due to the liver decomposition or not-typical angioarchitectonics of the portal pool. In such circumstances dissociation procedures still remain as the only method choice.

Therefore aim of study was to determine the efficiency of azygoportal collector total dissociation in patients with portal hypertension.

MATERIAL AND METHODS

A comparative investigation of two azygoportal collector dissociation methods in patients with PH syndrome has been carried out. Treatment results of 155 patients who were operated at the Republican Specialized Centre of Surgery (RSCS) named after academician V.Vakhidov from 1997 to 2017 were analyzed.

With the accordance of the total dissociative method, 2 groups of patients were formed. For the patients of the 1st group, original disconnection method of azygoportal collector was performed. Dissociation of the gastroesophageal collector in the modified type was performed in patients of the 2nd group. There were 63 patients with PH syndrome in the 1st group: 40 (63.5%) of them had liver cirrhosis and 23 (36.5%) patients were suffered from extrahepatic form of PH. In 19 (30.2%) cases, the surgery was performed at the peak of the hemorrhage. Dissociation of the azygoportal collector in combination with splenectomy was carried out in 19 (11.9%) patients. The 2nd group contained 92 patients with portal hypertension. Liver cirrhosis was observed in 57 (62.6%) of patients, 33 (36.3%) – had extra hepatic form of PH. One patient (1.1%) was admitted with Budd-Chiari syndrome. In 28 (30.7%) cases, the surgery was performed at the peak of the hemorrhage. Dissociation of the azygoportal collector in combination with splenectomy was carried out in 12 (13.2%) cases. In the other 7 (7.7%) patients, the azygoportal disconnection was supplemented with the ligation of a splenic artery.

F.G. Nazirov's original method (the 1st group) [Invention №IAP 20080375]

Devascularization of the stomach is carried out after upper laparotomy up to the abdominal part of the esophagus along both parts of the stomach. The organ blood supply is kept due to right gastric and two gastroepiploic arteries. Left gastric artery is ligated and dissected out of the organ. Double circular suture is formed at the subcardial level and the ligature is tightened. Thereby two gastric cameras are formed. The next stage is the formation of anterior gastro-gastral anastomosis between the upper and the lower parts of the stomach (were formed by the ligature and transection).

The size of anastomosis camera is up to 3 cm. The important advantage of the surgery is in keeping the cardioesophageal connection and in the prevention of reflux esophagitis in the postoperative period (Figure 1). The pointed method allows achieving an effective hemostasis in patients with bleeding from EV. But this method had the number of complications associated, as a rule, with an imposition of gastro-gastral anastomosis (GGA). The complications of the nearest postoperative period are presented in the Table 1.

The most dangerous complication of the nearest postoperative period was insufficiency of GGA which had led to the development of peritonitis. Hepatic failure progresses proportionally to the level of a surgical injury and its combination with GGA insufficiency was registered in all 11 patients with those complications. Hereby, the modification of the offered method was developed at the RSCS to eliminate the most frequent and dangerous complication such as GGA insufficiency.

F.G. Nazirov's modified method (the 2nd group) [Second invention].

Surgical approach and stomach devascularization are carried out in the same extent as in the original method. Then a transversal gastrotomy up to 3 cm is carried out in the medium part of the stomach along the anterior wall. A synthetic polyvinyl prosthesis in the form of corrugated tube with the length of 2,5-3 cm and 2,5 in diameter is introduced through the formed hole to the gastric lumen. That prosthesis is set up in the lumen of the stomach's cardial part. Sewing of the stomach both parts through all the layers with capron thread № 5 is carried out from the anterior wall of the stomach and by medial wall of the intraorgan prosthesis, ligature divides the stomach to the upper 1/3 and the lower 2/3 parts. The next ligature is imposed in the same way but

it is tighten directly over the prosthesis in the direction of lesser curvature and at the same time the prosthesis is fixed with surgeon's finger which is placed in the lumen. In such way we can control the location of the prosthesis and the tension of the ligature. Then a repeated ligature is imposed near the first one. Thereby we perform a cross-clamping of intramural venous vessels. A corrugated prosthesis provides the fixing of the ligatures and blocks their displacement. A nasogastric tube is conducted through the prosthesis with the aim of decompression in the postoperative period.

Gastrotomic hole is sutured by double-row stitch. A number of sero-serous stitches are also imposed over the stomach ligature. A Heineke-Mikulicz pyloroplasty is carried out to prevent gastrostasis. The endoscopic investigation with a removal of the prosthesis is performed after 1-1.5 months and the imposed ligatures are also removed.

Ethical approval

The review board and ethics committee of Republican Specialized Center of Surgery named after acad.V.Vakhidov. Tashkent. Uzbekistan approved the study protocol and gave permission.

Table 1. The frequency of postoperative complications in patients operated by the original method

Complication	Abs. frequency	% frequency
Hepatic failure	15	23.8%
Insufficiency of GGA	7	11.1%
Hemorrhagic syndrome,(including erosive anastomosis of GGA	9	14.3%
Insufficiency of pylorotomic hole	2	3.2%
Suppuration of the spleen bed	2	3.2%
Arrosive hemorrhage	2	3.2%
Splenic infarction	2	3.2%
Gastrostasis	1	1.6%

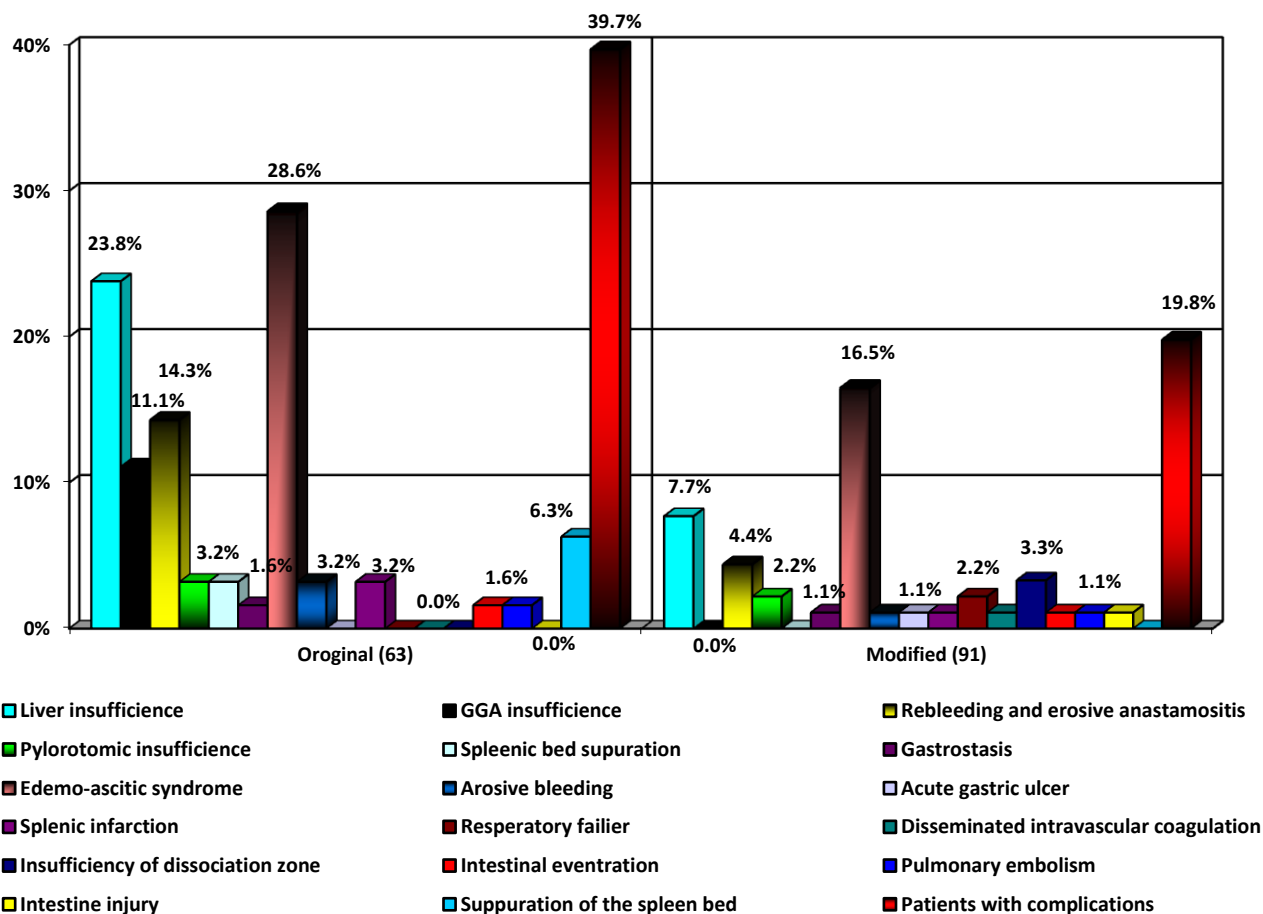


Figure 1. Comparative characteristics of postoperative complications

RESULTS

From 63 patients of the 1st group and 91 patients of the 2nd we observed a complicated nearest postoperative period in 25 (39,7 %) and 18 (19,8%) patients respectively. The structure of complications was as follows: the edematous ascites syndrome; hepatic failure; insufficiency of GGA and hemorrhagic syndrome the frequency of which made up 28.6%; 23.8%; 11.1%; and 14.3% versus 16.5%; 7.7%; 0%; and 4.4% for the 1st and the 2nd groups respectively.

Already as a result of a comparative analysis of the nearest postoperative period, it is possible to judge the degree of effectiveness of bleeding control is higher in the modified technique. Thus, the frequency of recurrence of hemorrhagic syndrome in the immediate postoperative period was 3 times higher in patients operated by the original method and amounted to 14.3% compared with 4.4% of patients operated by a modified procedure. The overall incidence of complications of the immediate postoperative period is shown in Figure 1.

In addition, an important prediction factor in the effectiveness of surgical treatment is the liver parenchymal decompensation degree. Thus, the incidence of complications in patients operated in an emergency was 2-3 times higher, the fact is explained by a higher operational risk in patients with severe parenchymal decompensation on the background of bleeding. This fact is also confirmed by the incidence of liver failure in patients hospitalized in urgent order in comparison with those who were operated in a planned manner.

Thus, , among patients hospitalized on an emergency basis in the 1st and 2nd comparison group, hepatic insufficiency in the postoperative period was observed in 42.1% (in 8 of 19 patients) against 10.7% (in 3 of 28 patients operated urgently) of patients respectively.

Liver cirrhosis

In consideration of the severity of PH syndrome course in patients with LC we have analyzed the frequency of complications development in this group of patients who were performed the original and modified methods. In the nearest postoperative period the frequency of the hepatic failure predominated in both groups and it complicated a restorative period course in 15 (38.5%) patients of the 1st group and in 7 (12.3%) patients of the 2nd group. The recurrence of hemorrhagic syndrome was in 7 (17.9%) patients (the 1st group) and in 4 (7.0%) patients of the 2nd group. The edematous ascitic syndrome rarely occurred in the group of patients who were performed the original method of surgery – 35.9% vs. 21.1%. The mentioned results are explained by the direct correlation of edematous ascitic syndrome with the rate of hepatic dysfunction. In connection with the reduction of the liver protein-synthetic function, both volume and adiphoria of ascitic syndrome are risen.

The frequency and resistance of the edematous ascitic syndrome is decreased due to significantly less traumatism of the original method of the surgery and the less rate of hepatocellular failure. In 2 (3.5%) cases of the 2nd group we registered the development of dissociative zone's failure. In 1 case the mentioned complication was developed in the patient who was performed the surgery having an active hemorrhage and a severe form of diabetes mellitus. In the second case that complication was developed in the patient with a total thrombosis of the portal vein and massive collateral circulation of cardioesophageal transition and retroperitoneal space (that case required a total devascularization of the stomach). In both cases the complication was solved by conservative procedures. There were 18 (46.2%) patients with different complications (the 1st group) and 15 (26.3%) patients in the 2nd group.

Extra hepatic form of portal hypertension

It is known that the prognosis of the disease in patients with extrahepatic portal hypertension (APH) is more favorable then in patients with a compromised liver. But according to some literary data, only in 12% of patients recanalization of the portal vein is observed in the rest of cases a clinically significant PH syndrome is formed and it is required an operative correction. The operative treatment results of the patients with the safe live function who were performed original and modified surgeries were studied. We did not observe the laboratory manifestations of hepatic failure in patients of both groups. But an occurrence of the edematous ascitic syndrome was observed in 4 (16.7%) cases of the 1st and in 3 (9.1%) patients of the 2nd groups. The recurrence of hemorrhagic syndrome was registered in 2 (8.3%) patients of the 1st group. There was no hemorrhage recurrence in the 2nd group. The postoperative period was complicated in 7 (29,2%) and 3 (9.1%) patients with APH.

The lethality of patients who undergone original and modified methods of the surgery

The patient's lethality also differed in both groups - it proves that a modified method is more effective. The lethality of the 1st group (original method) made up 10 (15.9%) cases, and in the 2nd group it was 10 (11%) patients. At the same time, even a lethality rate reaching 15.9% significantly differs from the stated rate which is typical for many other methods used in the world today. For example, by different authors data, a hospital lethality of the nearest postoperative period is observed in (35-75%) cases after a surgery offered by Boerema et al. [4] and in 20-55% - after the Sugiura's surgery [1, 2, 4, 14]. The hospital lethality after the M.D. Patsiora's surgery does not exceed 15% vs. 11% for the patients who were performed F.G.Nazirov's modified surgery. According to a comparatively low postoperative lethality which is typical for M.D.Patsiora's surgery the frequency of the hemorrhagic syndrome recurrence in the nearest postoperative period reaches 20% vs. 4.4% of F.G.Nazirov's modified surgery.

In our investigation the causes of the hospital lethality in the 1st group were: the hemorrhagic syndrome; hepatic failure; insufficiency of pylorotomic hole, corrosion hemorrhage which made up 4 (6%), 4 (6%), 1 (2%) and 1 (2%). In the 2nd group the causes of the hospital lethality were the hemorrhagic syndrome; hepatic failure; insufficiency of pylorotomic hole; corrosion hemorrhage and intestinal perforation which were observed in 3 (3.3%); 3 (3.3%); 2 (2.2%); 1 (1.1%); and 1 (1.1%) patients.

DISCUSSION

Azygoportal dissociation method in patients with LC is of a less risk of hepatic failure and encephalopathy. Dissociative procedures can be applied at the peak of hemorrhage and are easy to perform. But, in spite of a big quantity of such surgeries, almost all of them are followed by either early hemorrhage recurrence, or high operative trauma and low survival rates. As an example the frequency of hemorrhage recurrence following N. Tanner's surgery is 35-45% [1, 16]. After M.D. Patsiora's surgery this index can make up to 20% and more. Besides, in 8-14% of cases it is impossible to achieve bleeding control during the surgery [1, 16].

The M.A. Hassab's surgery which is widely-spread among the Asian-Pacific countries allows to reliably control the hemorrhagic syndrome. At the same time, a negative peculiarity of this method is a conservation of plethoric intramural veins of esophagus and stomach which also stipulates a high frequency of the hemorrhage recurrence (up to 25-34%) up to 5 years of observation [1, 17, 18].

One of the well-known and inconsistent methods of azygoportal total dissociation is the Sugiura's and S. Futagava's surgery. The method has been upgraded many times with the aim of saving hemorrhage control results on the background of operative trauma reductions and [1, 19]. Though more than 20 modifications of the surgery has been offered but still postoperative lethality remains high and can reach 50%.

The development and adoption of TIPS seemed to be a perspective method [15, 20]. But the recent wide investigations showed that this method also had serious disadvantages. A number of the late researches give significant defects of TIPS vs. porto-systemic shunting. Hosokawa et al. [21] states that a frequency of the hepatic encephalopathy was observed by them 1.5 times more frequent in patients performed TIPS vs. traditional interventions (39% vs. 26%) [21].

Shunt occlusion was developed in 26% of patients after TIPS and was not observed in patients after the surgical portosystemic shunting. But, as it is mentioned above, in spite of the advantages of surgical shunting interventions it is not always possible to perform them.

Thereby, nowadays there is no operative technique in the world which can be called "a golden standard" in the treatment of bleedings from esophageal varices. In this connection we have developed an original type of the operative intervention in our

Hereby, the results of this study allowed regarding the F.G.Nazirov's surgery as a competitive prevention and treatment method for hemorrhagic syndrome in patients with the PH in the conditions of impossibility to perform surgical shunting and at the ineffective endoscopic hemostasis.

CONCLUSION

In conclusion it can be said that postoperative complication rates and lethality, showed a significantly lower rates in the modified technique group than in any of known analogues. A modification of the original method of gastroesophageal collector dissociation allowed to reduce the frequency of such complications as

edematous ascitic syndrome; hepatic failure; insufficiency of GGA and hemorrhagic syndrome from 28.6%; 23.8%; 11.1%; and 14.3 in the original method up to 16.5%; 7.7%; 0%; and 4.4% for the modified method.

DECLARATIONS

Authors' Contributions

All authors contributed equally to this work.

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Competing interests

The authors declare that they have no competing interests.

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Prophylactic Administration of *Ginkgo biloba* Leaf Extract (EGb 761) Inhibits Inflammation in Carrageenan Rat Paw Edema Model

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ABSTRACT

Acute toxicity and anti-inflammatory effect of *Ginkgo biloba* leaf extract (EGb 761) were carried out in this study. The anti-inflammatory activity was studied using the carrageenan model whereby twenty rats were randomly divided into four groups of five animals each. Groups one and two were administered the EGb 761 extract at 500 mg/kg and 250 mg/kg, respectively. Rats in groups three (positive control group) and four (non-treated control group) were given piroxicam (10 mg/kg) and normal saline (5 ml/kg), respectively. Oedema was induced by injecting 100 µl of fresh carrageenan into the right plantar surface of the hind paw of each rat 30 minutes after administration. The acute toxicity tests result showed that the extract is safe at 5000mg/kg dose. *Ginkgo biloba* leaf extract caused a significant ($P < 0.05$) decrease in the size of the paw oedema when compared to control. Of interest, EGb 761 at 250 mg/kg was as effective as, or better than piroxicam (10 mg/kg). These findings further justify the use of *Ginkgo biloba* leaf extract in both medical and ethnomedical practice and may be used in treatment of inflammation.

ABBREVIATION

EGb 761	-	<i>Ginkgo biloba</i> leaf extract
g	-	Gram
GABA	-	γ- aminobutyric acid
IL	-	Interleukin
IL-4	-	Interleukin-4
IL-6	-	Interleukin-6
LD ₅₀	-	Lethal dose 50
mg/kg	-	Milligram per kilogram
ml/kg	-	Milliliter per kilogram
NO	-	Nitric oxide
PG	-	Prostaglandin
SEM	-	Standard error of mean
µL	-	Microlitre

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Ginkgo Biloba Leaf Extract,
Carrageenan, Rats, Paw Oedema, Inflammation

INTRODUCTION

Inflammation is the body's physiologic defense mechanism against infection, burn, toxic chemicals, allergens or other noxious stimuli [1, 2]. Diseases and disorders are manifested through inflammatory responses as the body recognizes the injury and prepares to repair the damage [3]. Endogenous mediators like prostaglandins, histamine, serotonin, bradykinin, etc. are liberated when inflammation occurs. Prostaglandins (PG) indicate and modulate the body's response to inflammation. These substances can elicit pain response which in turn causes dropped muscular activities [4].

Medicinal plants have provided biologically relevant products for centuries, and are still a source for new medicines [5]. *Ginkgo biloba* is a widely used plant in treatment of asthma, bronchitis, hearing loss, tuberculosis, cognitive dysfunction, stomach pain, skin problems, and anxiety [5, 6, 7]. *Ginkgo biloba* leaf extract (EGb 761) contains flavonoids and triterpenes as the main active ingredients, and these substances possess anti-inflammatory activity [8]. The extracts of *Ginkgo biloba* is said to have promising anti-inflammatory effect. Although it involves other mechanisms, interleukin (IL) is one of the most important in the anti-inflammatory functions of *Ginkgo biloba* [9]. Haines et al. [10] showed that the synergistic interaction of *Ginkgo biloba* leaf extract (EGb 761), astaxanthin and vitamin C suppress respiratory inflammation in asthmatic guinea pigs.

Bao et al. [11] reported that EGb 761 alleviate inflammatory reactions. This is done as a result of heightened activity of Interleukin-4, an anti-inflammatory cytokine, and inhibition of Interleukin-6 (IL-6), an inflammatory cytokine by dual activity. Using carrageenan model, Thorpe et al. [12] reported that EGb 761 has anti-inflammatory activity. Similarly, Ou et al. [13] also reported that inflammatory processes resulting from oxidized low density lipoproteins-induced oxidative stress in vascular endothelial cells were ameliorated by the administration of *Ginkgo biloba* extract.

The anti-inflammatory agents of plant origin have been the major focus of most research globally. Thus, evaluation of anti-inflammatory effects of *Ginkgo biloba* leaf extract is of great importance in the effective treatment and prophylaxis of several disease conditions in both humans and animals.

MATERIAL AND METHODS

Experimental animals and Ethical approval

Albino rats weighing an average of 180 g were acclimatized for 2 weeks prior to the experiment, fed standard diet and water was provided *ad-libitum*. All animal experimentation was done in accordance with Ahmadu Bello University Animal Use and Care Guidelines. Ethical clearance with approval number ABUCAUC/2016/015 was obtained from Committee on Animal Use and Care, Directorate of Academic Planning and Monitoring, Ahmadu Bello University, Zaria before the commencement of the study.

Experimental design

Acute toxicity study

The method of Lorke [14] with modification was used to determine the median lethal dose (LD_{50}) of the extracts in rats. This modification involves the introduction of uniform number of rats per group and the use of 18 albino rats instead of 12 for the study. In this study, 18 albino rats were randomly allocated into 6 groups of 3 rats each. The animals were starved of food *ad libitum* and water for 12 hours to avoid formation of complexes with food substances. Groups 1, 2, 3, 4, 5 and 6 were treated with the extract orally at 10, 100, 1000, 1600, 2900 and 5000 mg/kg body weight respectively. Rats were observed for 48 hours for any sign of toxicity or mortality.

Anti-inflammatory study

The method as described by Suleiman et al. [15] with modification was employed. Twenty rats were randomly divided into four groups of five animals each. Groups one and two received the extract at 500 mg/kg and 250 mg/kg, respectively. Rats in groups three (positive control group) and four (non-treated control group) were given piroxicam (10 mg/kg) and normal saline (5 ml/kg), respectively. All treatments were administered by oral route. Oedema was induced by injecting 100 μ L of fresh carrageenan into the right plantar surface of the hind paw of each rat 30 minutes after administration. The paw diameter was measured at 0, 30 minutes, 1, 2, 3, 4, 5, and 6 hours after administration.

Statistical Analysis

Data were expressed as mean \pm standard error of mean (S.E.M) and then analysed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. The analyses were done using Graphpad Prism version 5. Values of $P < 0.05$ were considered significant.

RESULTS

Acute Toxicity Study

Table 1 shows the results of acute toxicity study of *Ginkgo biloba* leaf extract (EGb 761). The extract administered at doses of 10, 100, and 1000, 1600, 2900, and 5000 mg/kg respectively did not produce any sign of toxicity or mortality. Also, *Ginkgo biloba* leaf extract (EGb 761) did not alter the behavior of the animals during the period of the study. Therefore, *Ginkgo biloba* leaf extract is considered relatively safe.

Anti-inflammatory study

Sub-plantar injections of carrageenan induced inflammation as evident in the increased paw diameter of the untreated control rats. Oedema was visible within the first 5-10 minutes of administration of carrageenan, the peak of swelling occurred approximately 2-3 hours following injection of carrageenan. *Ginkgo biloba* leaf extract produced a significant ($P < 0.05$) decrease in the size of the paw oedema as shown in Table 2. The activity of *Ginkgo biloba* leaf extract was highest at 250 mg/kg after 3 hours and was comparable to Piroxicam (standard anti-inflammatory agent; 10 mg/kg).

Table 1. Acute toxicity study of *Ginkgo biloba* leaf extract (EGb 761)

Groups	Dose/Day	Mortality (x/N)
Group 1	10 mg/kg	0/3
Group 2	100 mg/kg	0/3
Group 3	1000 mg/kg	0/3
Group 4	1600 mg/kg	0/3
Group 5	2900 mg/kg	0/3
Group 6	5000 mg/kg	0/3

*Group 1 (10 mg/kg Extract); Group 2 (100 mg/kg Extract); Group 3 (1000 mg/kg Extract); Group 4 (1600 mg/kg Extract); Group 5 (2900 mg/kg Extract); Group 6 (5000 mg/kg Extract).

Table 2. Effect of *Ginkgo biloba* leaf extract on carrageenan induced acute inflammation measured as paw size in mm (mean \pm SEM)

Items	0 hr *	0.5 hr	1 hr	2 hrs	3 hrs *	4 hrs *	5 hrs *	6 hrs *
Group A	3.32 \pm 0.18 ^a	4.89 \pm 0.23	5.71 \pm 0.25	5.99 \pm 0.20	5.94 \pm 0.30	5.23 \pm 0.39	4.48 \pm 0.17	3.72 \pm 0.19
Group B	3.00 \pm 0.14	4.64 \pm 0.40	5.14 \pm 0.32	5.97 \pm 0.38	5.60 \pm 0.56	4.60 \pm 0.35 ^a	3.92 \pm 0.25 ^a	3.55 \pm 0.17 ^a
Group C	2.46 \pm 0.18 ^b	4.36 \pm 0.22	4.72 \pm 0.18	5.52 \pm 0.09	5.13 \pm 0.16 ^a	4.91 \pm 0.16	4.02 \pm 0.20 ^a	3.47 \pm 0.13 ^a
Group D	2.55 \pm 0.13 ^b	5.02 \pm 0.11	5.65 \pm 0.25	6.67 \pm 0.48	6.83 \pm 0.49 ^b	6.16 \pm 0.30 ^b	5.30 \pm 0.30 ^b	4.34 \pm 0.21 ^b

*ANOVA: Indicates that Comparism for all groups is statistically significant ($P < 0.05$) within the same column. Tukey's test: Means having different superscript (^{a,b}) letters are significantly different ($P < 0.05$). Group A (500 mg/kg Extract); Group B (250 mg/kg Extract); Group C (Piroxicam (10 mg/kg); Group D (Normal saline (5 ml/kg)).

DISCUSSION

Acute Toxicity Study

Toxicological study is first assayed to determine the safety of drugs and plant products for human and animal use [15]. The calculated LD₅₀ of *Ginkgo biloba* leaf extract (EGb 761) was greater than 5000 mg/kg. This value falls within the practically non-toxic range [14]. Doses up to 5000 mg/kg, orally administered, did not alter the behavior of the animals during the period of the study, thus, the extract was considered relatively safe.

This finding was consistent with the outcome of a similar study carried out by Salvador [16], who reported that the LD₅₀ of standardized *Ginkgo biloba* extract administered orally to mice was 7,730 mg/kg. He

also reported no organ damage or impairment of hepatic or renal function when *Ginkgo biloba* extract was administered orally over 27 weeks to rats and mice at doses ranging from 100 to 1,600 mg/kg.

Anti-inflammatory study

Results from this study suggest *Ginkgo biloba* leaf extract possessed anti-inflammatory effect. This may be as a result of inhibition of inflammatory mediators, such as nitric oxide (NO), prostaglandins, and proinflammatory cytokines into the paw tissue, because evidence shows that *Ginkgo biloba* and its constituents suppress induction of these mediators [17].

Of interest, EGb 761 at 250 mg/kg was as effective as, or better than piroxicam (10 mg/kg). However, administration of higher dose (500 mg/kg) of the extract did not produce such or higher anti-inflammatory effect. This may not be unconnected to the reports of Ivic et al. [18] and Kiewert et al. [19] that EGb 761 contains triterpenes; ginkgolides and bilobalide, and these active components at higher doses are known antagonists at both glycine and γ -aminobutyric acid (GABA) in the body, which are neurotransmitters that are known to inhibit the activities of neurons that activate the release of inflammatory agents and regulate inflammation in the body.

Our finding is consistent with the work of Abdel Salam et al. [20] and Han [21], who reported that oral administration of *Ginkgo biloba* extract significantly reduced carrageenan induced paw oedema. Other studies have shown that treatment with *Ginkgo biloba* extract (30–120mg/kg; orally) reduced inflammation and acute colonic damage induced by acetic acid [22]. Similar studies on the anti-inflammatory properties of flavonoids, quercetin and kaempferol have also demonstrated reduced carrageenan-induced hind paw oedema in mice [23]. However, our result disagrees with the findings of Biddlestone et al. [24], who reported that *Ginkgo biloba* had no effect on paw oedema regardless of dose or duration of administration.

CONCLUSION

This study shows that *Ginkgo biloba* leaf extract (EGb 761) is practically non-toxic and is considered relatively safe. Also, the extract possessed prophylactic anti-inflammatory effect and was as effective as, or better than Piroxicam, a standard anti-inflammatory drug.

DECLARATIONS

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Authors' Contributions

AAN designed the study. SA, AAN, and ZIY carried out the experimental research, collected the data, analysed and interpreted the results. The first draft of manuscript was prepared by SA and reviewed by the rest of the authors and the final version of the manuscript was read and accepted by all the authors.

Ethics Committee Approval

This experimental research was approved by the committee on animal use and care, directorate of academic planning and monitoring, Ahmadu Bello University, Zaria. Ethical clearance with approval number ABUCAUC/2016/015 was obtained for this experiment.

Consent to Publish

Not applicable

Competing Interests

The authors declare that there is no conflict of interest.

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Red Blood Cells Morphology Monitoring to Predict Hyperfunction of Subclavian-Pulmonary Anastomosis in Patients with Fallot Tetralogy

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ABSTRACT

Hyperfunction of subclavian-pulmonary anastomosis in patients with tetralogy of Fallot (TOF) is known to be a rather common condition in the early post-operative period. It resulted in development of hypervolemic pulmonary circulation and edema. Morphometry of peripheral blood cells of 81 TOF post-operative patients revealed an increase in the number of pathologically shaped red blood cells (PS RBCs) in 14 of them. Mainly these were the ones with a ridge-like structure on their surface. The thick drop express-technique (TDET) enables to evaluate the correlation of normal RBCs/ PS RBCs for 10-15 min for the entire procedure. The progressive deterioration of RBCs morphological features is suggested to be a predictor of the anastomosis hyperfunction due to changed blood rheology. In addition the correlation of normal and pathological forms of erythrocytes can be an evaluation criterion of effectiveness of patient management tactics of cardiologic intensive care.

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INTRODUCTION

Tetralogy of Fallot is one of the most common congenital heart disorders across the world. For instance, the centers for disease control and prevention (CDC) estimate that each year about 1,660 babies in the United States are born with this pathology [1, 2]. If left untreated, TOF children face additional risks that include paradoxical emboli leading to stroke, pulmonary embolus, and subacute bacterial endocarditis [3]. In most of these children, the causes of stroke, along with thromboemboli, have been related to prolonged hypotension, anoxic polycythemia.

Most TOF infants require surgery and a lot of surgical series have reported excellent short-term clinical results since the time when the first classic Blalock-Taussig shunt between the subclavian artery and the pulmonary artery was made. Primary repair of tetralogy of Fallot is known to have low surgical mortality; however, some patients still experience significant postoperative morbidity [4, 5]. Several attempts have been

made recently to find out predictors of early post-operative complications in TOF patients depending on the surgery profile [5-10].

One of the main problems of patients, who undergone cardiosurgery, in particular the ones with congenital heart defects due to impaired blood circulation, is a considerable change in delivery of O₂ to tissues [4, 8]. Unfortunately, adequate attention has not been paid so far to the change in the hemorheology status and transfusion indicators during post-operative adaptive transformation of hemodynamics as well as to the methods of their evaluation and monitoring.

The research was focused on evaluating the efficiency of thick-drop technique of scanning electron microscopy in predicting and monitoring the hyperfunction of subclavian-pulmonary anastomosis in TOF patients at the early post-operative period.

MATERIAL AND METHODS

Eighty one TOF patients aged 1 - 22 years (mean age 8.7 ± 0.9), including 43 males (53%), 38 females (47%), have been operated in Republican Specialized Center of Surgery named after academician V.Vakhidov (Tashkent, Uzbekistan) from 2015 to 2017. In all the cases, the modified subclavian-pulmonary anastomosis (SPA) was performed. Artificial lung ventilation was carried out to the SPA patients in the standard regimes in early post-operative period. The relative predictors of intensive care unit (ICU) stay and morbidity were age and weight of the patients, while the surgery profile suggested the duration of mechanical ventilation. Hyperfunction of the anastomosis in the early post-operative period developed in 14 patients (17.3%). The median duration of their mechanical ventilation was 19 hours. The ICU stay ranged from 2 to 14 days. Five of these patients were randomly selected to form the study group; 8 patients with no SPA hyperfunction were matched by age, sex and concomitant conditions to compose the comparison group.

To monitor the RBC status, scanning electronic microscopy (SEM) was used since it enables to differentiate and count precisely normal RBCs having the shape of biconcave discocytes (D) from pathologically shaped RBCs (PS RBC). Usually, the most frequent PS RBCs found are echinocytes, i.e. RBCs with numerous processes, stomatocytes, RBCs with a ridge-like structure, and considerably changed PS RBCs or so called irreversible RBCs.

Most scanning electron microscopes are comparatively easy to operate, with user-friendly interfaces. Many applications require minimal sample preparation and data acquisition is rapid (less than 5 min/image). The thick-drop express-technique (TDET) has been elaborated at the NSCS for practical and research purposes. This technique and relevant software have been developed and patented in Uzbekistan [3, 6]. One of advantages of the technique is that it preserves the natural condition of RBCs and quickly evaluates the correlation of D/ PS RBCs (for 10-15 min).

Ethical approval

The review board and ethics committee of Republican Specialized Center of Surgery named after academician V.Vakhidov approved the study protocol and gave permission for study.

RESULTS AND DISCUSSION

The TDET enabled to monitor the RBCs morphologic condition and evaluate the hemodynamic changes in the early post-operative period of 14 TOF patients, in particular the development of hypervolemia of the pulmonary circulation and pulmonary edema. The proportion of the PS RBCs in TOF-SPA patients' blood significantly increased. Studying the RBC profile in patients with cyanotic TOF (CTOF) demonstrated that the discocyte count in the early post-operative period made 40% with 85% reference value. The most part of the rest RBCs (60%) was presented by the population of pathologically-shaped and lysed cells (Figures 1 and 2).

The echinocyte population of adult patients with CTOF was more remarkable; it included 26% of echinocytes of class I; 8% of the second class echinocytes and 5% of the third class cells. The number of stomatocytes and hydrocytes proved to be larger than in children with CTOF. It made 3% of stomatocytes of class I, while the stomatocytes of the second and third classes made 7% and 5%, respectively. The population of discocytes with a ridge-like structure was distributed as follows: small ridges were found in 1.5-2%, the medium-sized ones were found in 1-1.5%, and 0.5% of the discocytes had large ridges.

The morphological cell variability reflected differences in the physical condition and compensation-adaptation mechanism of the patients. It is worth mentioning that children elder than 10 years need to be monitored more closely due to a notable increase in the number of pathologically shaped erythrocytes before the surgery. It should be taken into consideration at the next stages of treatment, in particular during the surgery, anesthetic management and perfusion.

After the SPA-surgery the proportion of discocytes decreased while that one of PS RBCs increased; at the same time number of echinocytes increased, as well as the number of irreversibly altered RBCs. Two hours after the surgery, the proportion of pathologically shaped RSCs increased, mainly those ones with ridges and echinocytes (Figure 3). Twelve hours after the surgery the number of discocytes in the blood significantly increased with a considerable drop in the number of RSCs with ridge and echinocytes.

The TDET used to evaluate alterations in the RBC shape in TOF patients before and after the surgery enabled to estimate the discocytes/PS RBC within 15 min after the surgery and conduct rather large hemomorphologic study. The TDET evaluation of RBCs in the comparison group demonstrated significant domination of discocytes. In addition to the characteristic shapes of the biconcave discs, they had a smooth external membrane with no processes, folds and depressions (Table 1).

The TDET made to TOF patients before the surgery showed a considerable increase in the PS RBC proportion. They made 1/3 of the RBCs, 61% of them were discocytes, but RBCs with a ridge dominated (Table 1, Figure 4). Immediately after the surgery, a lot of PS RBCs were found with higher proportion of echinocytes (Table 1). Two hours after the surgery the counts of discocytes and stomatocytes tended to diminish while the number of RBCs with ridge increased (Figure 5).

When anastomosis hyperfunction has developed, the clinical changes are manifested by pulmonary edema with an increase in PS RBC count in peripheral blood and a decrease in the number of discocytes up to 49%. The RBCs with ridge composed up to 16%, and echinocytes of classes 1 and 2 made 14%. Stomatocytes, the cells with coarse echinocyte transformations, and irreversibly shaped cells were presented in relatively equal numbers: 6%, 7% and 8%, respectively (Table 1).

When post-SPA hyperfunction developed, the set of intensive therapy interventions procedures included application of the regulating cuff. It contributed to restoration of the peripheral blood RBCs shape in 120-180 minutes with the increase in discocyte count from 49% to 55%, while the number of PS RBCs decreased by 6% (Figures 6 and 7).

Twelve hours after the surgery we noted the tendency to an increase in the number of discocytes and a decrease in the PS RBC number (Table 1). The dynamics of morphological monitoring is as follows: at hours 12-15 after SPA hyperfunction development the number of discocytes in peripheral blood is increasing because of the restoration of pathologically changed erythrocytes (Table 1).

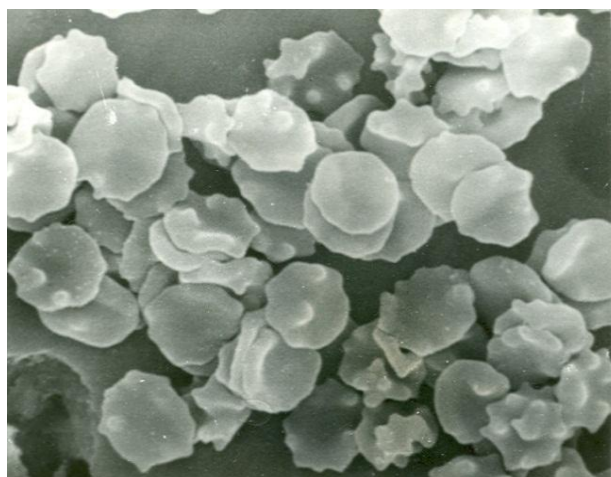


Figure 1. The blood sample of the CTOT patient. The evident domination of pathologically shaped RBCs. SEM $\times 1.000$

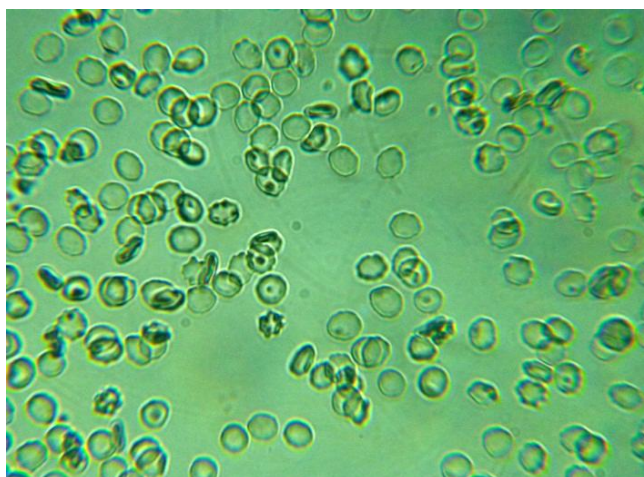


Figure 2. The blood of the same patient. Numerous echinocytes, cells with ridges and stomatocytes. TDET 10×60 .

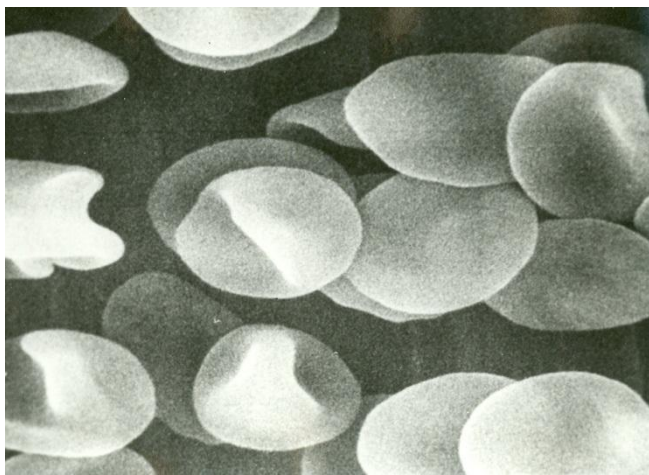


Figure 3. The RBC of the TOF patient 2 hours after the surgery: the increasing proportions of PS RBC, RBCs with ridge and echinocytes. SEM $\times 4.000$

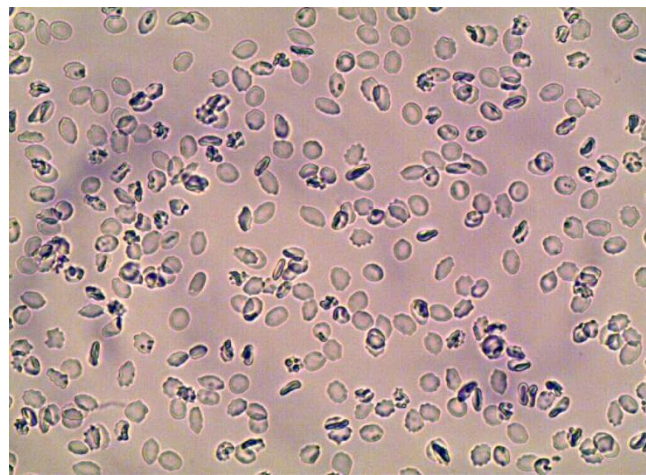


Figure 4. The RBCs of the TOF patient before the surgery: domination of PS RBCs, RBCs with ridge in particular. TDET 10 \times 40

Table 1. Dynamics of the subclavian-pulmonary anastomosis effect on peripheral blood RBCs of TOF patients (%)

Items	Comparison group n=8	Before the surgery n=10	Immediately after the surgery n=10	120 min. after the surgery n=5	Anastomosis hyper-function Pulmonary edema, n=5	120 min. after RSPA n=5	12 hr after the surgery n=5	12 hr after the RSPA n=5
Discocytes	85 \pm 1.2	59 \pm 1.1	57 \pm 2.5*	56 \pm 2.3**	49 \pm 2.9**	55 \pm 2.4 **	61 \pm 2.2***	64 \pm 2.4***
Echinocytes	2 \pm 0.1	7 \pm 0.2	10 \pm 0.6*	11 \pm 0.7**	14 \pm 1.3**	12 \pm 0.8 **	10 \pm 0.6***	9 \pm 0.5***
Stomatocytes	3 \pm 0.2	8 \pm 0.4	7 \pm 0.4*	4 \pm 0.3**	6 \pm 0.6**	5 \pm 0.4**	4 \pm 0.4***	4 \pm 0.3***
With ridge	4 \pm 0.2	19 \pm 0.2	14 \pm 0.3*	17 \pm 0.3**	16 \pm 0.5**	15 \pm 0.3 **	15 \pm 0.3	12 \pm 0.4
Echinocytes rough	4 \pm 0.3	5 \pm 0.4	6 \pm 0.4	7 \pm 0.3	7 \pm 0.4	7 \pm 0.4	5 \pm 0.3	4 \pm 0.3
Irreversible cells	2 \pm 0.1	2 \pm 0.7	6 \pm 0.5	5 \pm 0.3	8 \pm 0.5	6 \pm 0.8	5 \pm 0.4	7 \pm 0.3

*significant difference ($P < 0.05$) from the previous group; **significant difference ($P < 0.05$) from group*; *** significant difference ($P < 0.05$) from group**; RSPA= Regulated subclavian-pulmonary anastomosis.

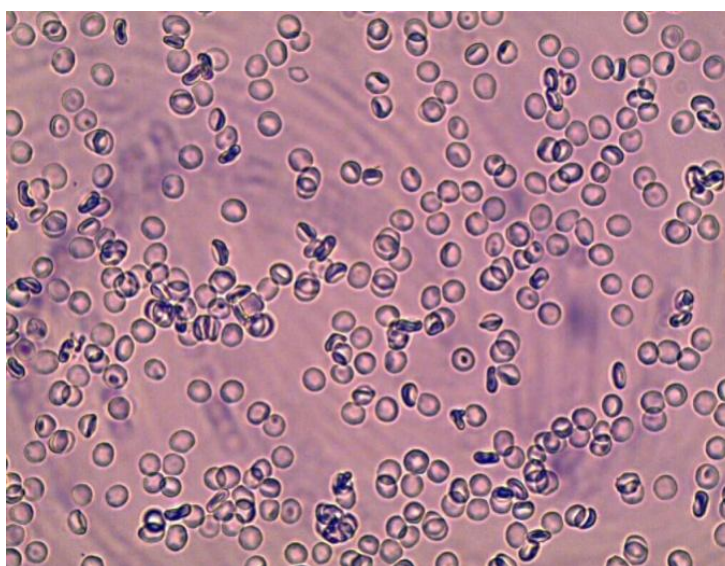


Figure 5. Two hours after SPA. A higher proportion of pathologically shaped RBCs, RBCs with ridge and echinocytes. TDET 10 \times 40

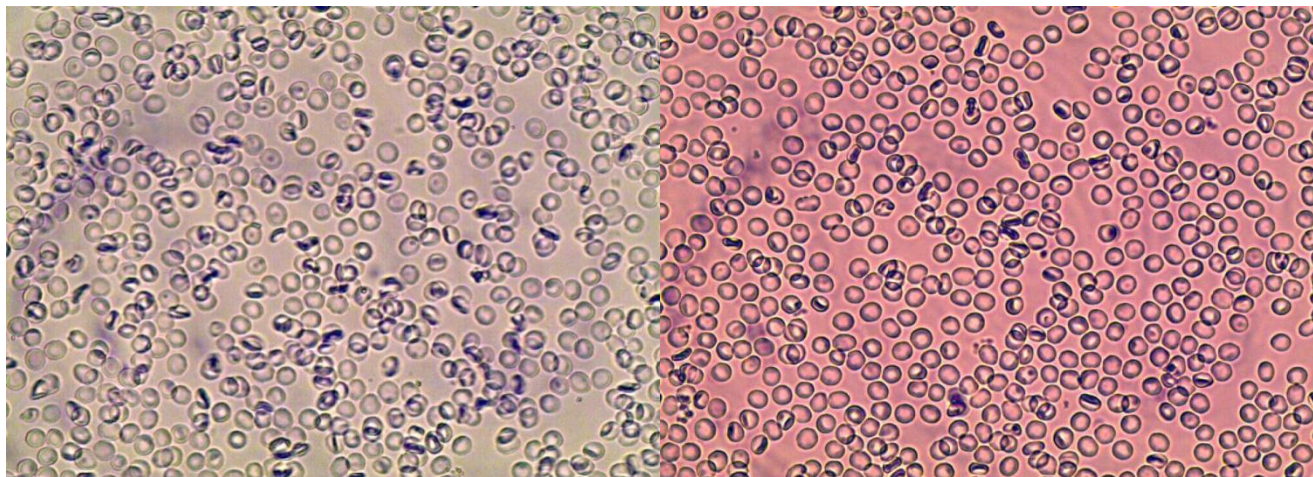


Figure 6. Two hours after application of the regulating cuff in post-SPA hyperfunction. An increase in the number of discocytes with a significantly decreased proportion of the cells with processes. TDEM 10 × 40

Figure 7. Two hours after application of the regulating cuff in post-SPA hyperfunction. The RBC count tends to normalize. TDEM 10 × 40

CONCLUSION

Morphological features of peripheral blood cells in patients with tetralogy of Fallot demonstrated that the number of pathologically shaped RBCs increased up to 41 %, these were mainly erythrocytes with ridge (up to 16 %). The early post-operative period after performance of subclavian-pulmonary anastomosis is characterized by the decrease of RBCs count up to 56-57%. The share of pathologically shaped RBCs in peripheral blood below 49 % is the morphological predictor of anastomosis hyperfunction development. The morphological monitoring of the correlation between normal and pathologically shaped erythrocytes after SPA-surgery for tetralogy of Fallot can provide the criterion of efficiency of the medical and diagnostic tactics in anastomosis hyperfunction development.

DECLARATIONS

Authors' Contributions

All authors contributed equally to this work.

Acknowledgements

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Competing interests

The authors declare that they have no competing interests.

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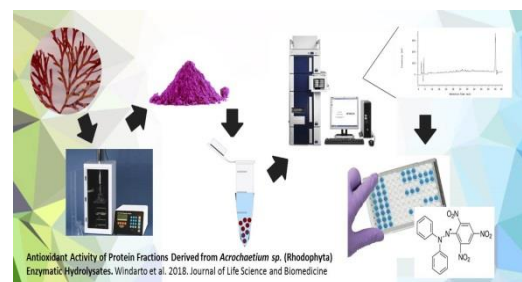
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7. Use Symbol fonts for "±"; "≤" and "≥" (avoid underline).
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9. Numbers up to 10 should be written in the text by words. Numbers above 1000 are recommended to be given as 10 powered x.
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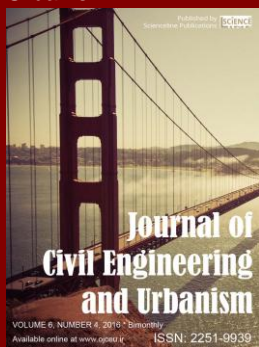
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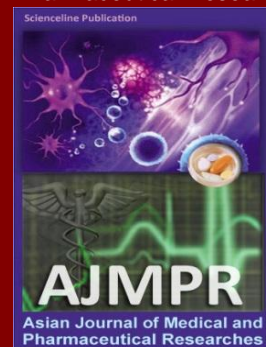
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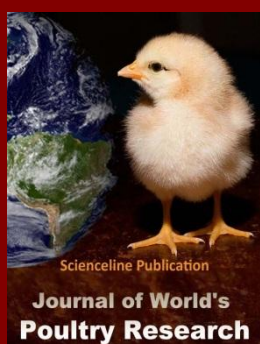
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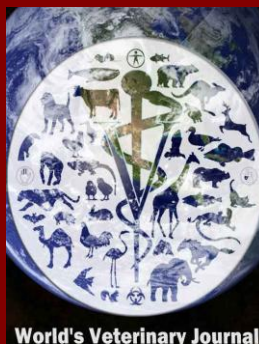
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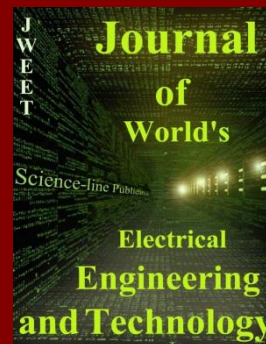
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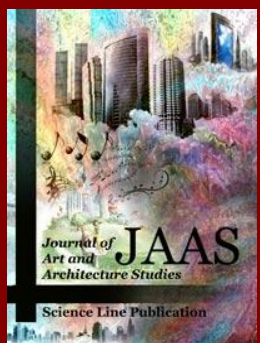
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