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C O N T E N T S

Articles

Articles

Relationship Between Salivary and Serum Activity of Beta-Glucuronidase and Multiple Risk Factor for Colorectal Cancer

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Abstract

This study is directed to clarify the role of beta-glucuronidase (GUSB) in the occurrence and development of colorectal cancer (CRC). This issue is closely related to life of intestinal microflora, since we know about the beta-glucuronidase of bacterial origin, to play an important role in carcinogenesis along with its own enzyme.

The study included the results of determining the specific activity of beta-glucuronidase in the blood serum and saliva. We include to research 24 volunteers who did not have or had from one to four risk factors for CRC. Additionally, we performed the fractionation of blood serum and saliva proteins to establish the possibility of native and bacterial enzyme detection.

The study showed the greatest GUSB activity in blood serum and saliva to be a finding in smokers, as well as in the presence of several (three to four) risk factors for CRC. GUSB activity is also correlated with the presence of risk factors such as age and obesity. The peaks of GUSB activity, according to the results of gel chromatography, confirm the fact that bacterial forms contributed to the formation of the overall activity of this enzyme in blood serum and saliva.

The obtained results can be used to create diagnostic algorithms aimed at screening for increased risk of CRC in humans based on salivary GUSB analysis.

Keywords: beta-glucuronidase, blood, saliva, colorectal cancer, oncogenesis.

1. Introduction

Colorectal cancer (colorectal carcinoma, CRC) is one of the most common human cancers with a tendency to increase the number of diseases and deaths in many countries of the world. More than one million people suffer from CRC every year, in developed countries the death rate from this disease is almost 33 % (Cunningham et al., 2010; Siegel et al., 2019). Morbidity rates for men are higher than for women with an approximate 1.4-fold and 1.5-fold difference in morbidity (23.6 vs. 16.3 cases per 100,000 people per year) and mortality (10.8 vs. 7.2 deaths per 100,000 people per year), respectively. These indicators related to the fact that men were affected by environmental factors more than by genetic factors compared to women, so a CRC inheritance is of 45 % for women and 28 % for men (Graff et al., 2017; Ma et al., 2017).

The risk of developing CRC increases after the age of 50, and 90 % of cases are registered in people older than this age. In addition to age, the family history, obesity, diabetes, sedentary

lifestyle, smoking, high alcohol consumption, eating high-fat, and low-fiber foods are the main risk factors for CRC (Waszkiewicz et al., 2012, Shuwen et al., 2017).

In terms of ethnic differences, Africans and peoples of African descent are the most susceptible to the disease, while Asian and Pacific islanders are the least susceptible. This is true for both morbidity (43.2 vs. 28.8 cases per 100,000 people per year) and mortality (18.6 vs. 9.9 deaths per 100,000 people per year). Genetic factors may play a role in this difference, for example, several single-nucleotide polymorphisms associated with CRC risk were found in Africans (Ashktorab et al., 2015; Wang et al., 2017).

To date, for more than 50 years of studying the participation of intestinal microflora in the development of gut tumors, many of evidences have been provided for intestinal microorganisms to play a key role in carcinogenesis. Among the microbiota representatives, such species as *Bacteroides, Escherichia, Fusobacterium* and *Porphyromonas* play an important role in CRC occurrence and progression (Tarashi et al., 2019; Thomas et al., 2019). Some specific bacteria, exampling *Bacteroides fragilis, Streptococcus bovis, Escherichia coli, Enterococcus faecalis, and Streptococcus gallolyticus*, are very closely related to CRC (Zeller et al., 2014; Dashnyam et al., 2018; Tarashi et al., 2019). Recent human studies have identified new strains of bacteria involved in CRC carcinogenesis, including bacteria of genera *Parvimonas, Peptostreptococcus, Porphyromonas*, and *Prevotella* (Leystra, Clapper, 2019; Wirbel et al., 2019; Yachida et al., 2019).

Beta-glucuronidase (beta-D-glucuronide-glucuronohydrolase, CF 3.2.1.31, GUSB) is a lysosomal exoglycosidase stimulating the cleavage of beta-D-glucuronides to form D-glucuronic acid, as well as a transglycosylation reaction. This enzyme participates in the degradation of glycosaminoglycans of cell membranes and extracellular matrix of many tissues, including the intestinal mucosa. Tumorous and chronic inflammatory diseases of liver are typical reason to GUSB increase, but high ulcerative colitis, pancreatic, intestinal, breast and cervical carcinomas, and myocardial infarction may accompany by high GUSB activity in serum. Excessive GUSB activity may be a major factor in the etiology of CRC (Muzny et al., 2012; Naz et al., 2013; Sun et al., 2019).

The serum GUSB activity in humans is determined by the intake of its own enzyme from tissues and the activity of intestinal bacteria (Han et al., 2018). Evaluation of the activity of lysosomal hydrolases in serum and urine, according to several studies, can be used in the screening diagnosis of intestinal tumors in the early stages of the disease (Chojnowska et al., 2011; Häuselmann, Borsig, 2014).

Saliva (oral fluid) is a complex filtrate of blood plasma, and it also contains components derived from the mucous membranes, gingival fissures, and dental plaque, including microorganisms and their products (Bhardwaj et al., 2013).

Saliva can change its composition very significantly and properties after exposition of a various stimuli, so the determination of some biomarkers in saliva becomes an important part of laboratory diagnostics to predict not only oral diseases, and also pathology of other tissues and organs (Syndergaard et al., 2014; Ebersole et al., 2015) The diagnostic capabilities of these methods are still far from being exhausted.

The aim of the study was to establish Parallels between the activity of beta-glucuronidase in the blood serum and saliva of healthy volunteers and individuals at high risk of developing intestinal tumors.

2. Materials and methods
Characteristics of the surveyed group

Blood and saliva samples were obtained from 24 individuals (12 men and 12 women) aged 21-60 years who applied for a scheduled medical examination in connection with their current or upcoming professional activities (Table 1).

The exclusion criterion was the presence of a burdened anamnesis for the main kinds of somatic diseases (cardiovascular pathology, respiratory diseases, diabetes, autoimmune disorders, and oncology), or mental disorders. The group also did not includes pregnant or nursing women. Due to the need to study saliva, the subjects must have functionally formed dentitions, fillings and individual crowns are allowed, but the presence of removable orthopedic structures, diseases of the oral mucosa and pronounced periodontal pathology was not allowed.

Table 1. Gender and age characteristics of the surveyed group

Age	Surveyed individuals		
	Number	Including	
		Female	Male
20-29 years	4	2	2
30-39 years	5	3	2
40-49 years	8	4	4
50-60 years	7	3	4
Total number	24	12	12

For each surveyed person we additionally noted the presence or absence of following risk factors for CRC development (Shuwen et al., 2017): age over 50 (7 people), male gender (12), obesity (11), and smoking (10 people). As a result, we found 11 people with at least one risk factor (46 %), and 6 people with three or four risk factors (25 %). The latter were considered separately as individuals with a serious risk of CRC.

Sampling and sample preparation

Blood serum was provided as scrap material for routine biochemical examination of patients (glucose, cholesterol, total protein). To collect saliva, patients were informed about the purpose and conditions of the study, the methods used. This information we included in the text of the voluntary informed consent to medical procedures.

Non-hemolized blood serum was centrifuged at room temperature in the mode of 3200 g for 10 min.

When taking saliva, we additionally took into account a number of requirements, primarily the stability of the results, as well as minimal invasiveness for the patient and simplicity (Syndergaard et al., 2014). Before sampling patients avoided smoking and exercise within two hours, rinsed their mouths twice with drinking water, removed the remaining water with a clean cloth, and then collected saliva through a sterile funnel into a plastic test tubes with a lid (Postnova et al., 2011).

In parallel, the protein concentration was determined using Coomassie dye according to the method of Marion Bradford (Bradford, 1976). The studies were carried out on a SmartSpec Plus spectrophotometer (Bio-Rad, USA) at a wavelength of 595 nm.

Determination of GUSB activity in serum and saliva

To determine the enzymatic activity of SB, we used a Merck KGaA commercial kit (Germany) in accordance with the manufacturer's instructions. The principle of the method is that in the presence of GUSB activity, p-nitrophenyl-β-D-glucuronide is hydrolyzed to p-nitrophenol as a colored compound and glucuronide. The color intensity is proportional to the activity of the enzyme and it is determined spectrophotometrically at a wavelength of 405 nm (Marciniak et al., 2006).

In parallel, we determined the protein concentration using Coomassie dye by Marion Bradford method (Bradford, 1976). The research was performed on a SmartSpec Plus spectrophotometer (Bio-Rad, USA) at a wavelength of 595 nm.

The enzyme activity in the test sample was calculated using the formula:

$$A = K \times E / Pt,$$

where: K is the coefficient calculated from the calibration graph, E is the optical density of the test sample measured against the corresponding control sample, and Pt is the protein concentration (g/l).

The results were expressed in micromol/s×g of protein.

Fractionation of serum and saliva proteins

To carry out gel chromatography we used ENrich SEC 650 column (Bio-Rad, USA) filled with Sephadex G-200. The separated substances were eluted with a borate buffer (pH = 8.80). As an

internal control, for the construction of the calibration curve, a joint injection of a solution containing aliquots of purified human hemoglobin (molecular weight 68 kDa) and colored "blue" dextran (molecular weight 2000 kDa) were used. After identification of peaks on the obtained chromatograms we digitized and characterized its.

Methods of mathematical processing of results

Statistical analysis was performed using the software package Statistica 12.0 (StatSoft Inc., USA). Previously, based on the Kolmogorov-Smirnov and Shapiro-Wilk criteria, the hypothesis of normality of the distribution was rejected, and therefore nonparametric criteria were used for the analysis. In this regard, we used nonparametric criteria. Distribution in the samples was expressed as a median and the interval between the first and third quartile (Me [Q1÷Q3]), the Mann-Whitney criterion (critical significance level $p < 0.01$) was used to analyze the differences between the two research methods, and the Craskell-Wallis criterion for multiple groups ($p < 0.01$) was used to compare the results between several groups. Descriptive statistics were supplemented with Spearman correlation analysis (critical confidence level $p < 0.05$).

3. Results and discussion
GUSB activity in blood serum and saliva

Table 2 shows the results of determining the activity of GUSB due to the absence or presence of risk factors for CRC. As can be seen from the data, we did not find significant differences in GUSB activity in the blood serum and saliva of the examined individuals.

Table 2. Specific activity of GUSB in blood serum and saliva in subjects of the study group and in subgroups of risk expressed as Me [Q1÷Q3]

Group/subgroup	GUSB activity, μM/s × g of protein		P
	Serum	Saliva	
Risk-free group (n = 7)	0.09 [0.05 ÷ 0.12]	0.07 [0.04 ÷ 0.10]	n. r.
Men (n = 12)	0.14 [0.09 ÷ 0.18]	0.11 [0.06 ÷ 0.16]	< 0.01
Over 50 years old (n = 7)	0.18 [0.14 ÷ 0.25] *	0.14 [0.11 ÷ 0.21] *	< 0.01
Obesity (n = 11)	0.18 [0.13 ÷ 0.22] *	0.07 [0.05 ÷ 0.11]	< 0.01
Smoking (n = 10)	0.25 [0.15 ÷ 0.32] *	0.09 [0.06 ÷ 0.14]	< 0.01
High risk (n = 6)	0.22 [0.15 ÷ 0.29] *	0.12 [0.09 ÷ 0.17]	< 0.01

* – significant differences compared to the risk-free group

In the blood serum the GUSB activity in men and in persons over 50 years of age was 2.0 times higher, compared to the value in persons without risk factors. The highest values were found in smokers (2.8 times more than in the risk-free group) and in the presence of several risk factors (2.4 times more). In saliva the GUSB activity was on average 1.8 times lower than in blood serum. The differences between the risk-free subgroup and those over 50 years of age and those with multiple risk factors for CRC, however, were found to be significant.

To assess the involvement of the studied enzyme in the formation of risk for CRC incidence, we performed a Spearman correlation analysis. Table 3 shows the results of this analysis. As can be seen, eight positive dependencies were found between risk factors and indicators of GUSB activity in serum and saliva, six of them were weak ($r < 0.5$) and two ones were moderate ($0.5 < r < 0.8$). The differences in this case were significant ($p > 0.05$). The relationship between

age and GUSB activity was the most significant, the gender factor did not give such a high dependence.

Table 3. Rank correlations between the values of GUSB activity indicators in blood serum and saliva and the presence of individual risk factors (R)

Indices	Risk factors			
	Over 50 years old (n = 7)	Men (n = 12)	Obesity (n = 11)	Smoking (n = 10)
GUSB activity in serum	0.582 *	0.288	0.064	0.116
GUSB activity in saliva	0.566 *	0.119	0.006	0.009

* – significant differences compared to the risk-free group

Results of activity determination in individual protein fractions

As a result of gel chromatography of 8 samples of blood and saliva, we isolated 2 large fractions in the range of molecular weights: from 225 to 350 kDa (fraction 1) and from 450 to 700 kDa (fraction 2). The 1st fraction approximately includes human GUSB, and the fraction 2 GUSB of bacterial nature.
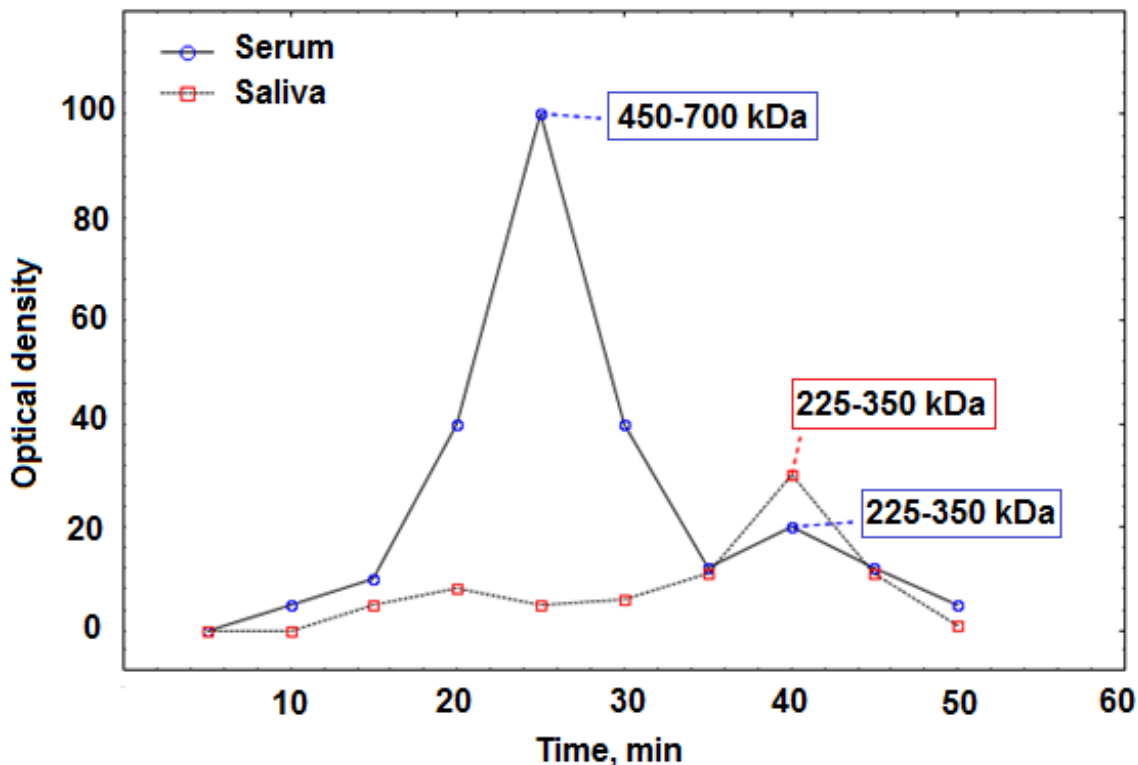
Fig. 1. Chromatogram of GUSB isolation from blood serum and saliva

The Figure 1 represents chromatograms, and we see that there is a peak of activity in the blood serum, which presumably should include GUSB of a bacterial nature. The second peak is found both on the chromatograms of blood serum and saliva samples, and it is presumably associated with the activity of Human GUSB (Liu et al., 2014; Uroy et al., 2019).

At the same time, the second fraction of the blood serum had a high peak in the range of 450-700 kDa, and when the saliva was separated, the 2nd fraction was not detected. The specific GUSB activity was reliably detected in the 2nd fraction of blood serum and amounted to 0.392 µmol/s×g of protein, which is 6 times less than in the 1st fraction. An important point is that in the 2nd fraction after saliva separation, the specific activity of GUSB was practically not detected. This may be due to the fact that there are almost no bacterial enzymes in saliva, since

they have a higher molecular weight, and it is more difficult to penetrate the plasma filtrate, which is the basis of the protein composition of saliva (Zefferman, 2015; Hudson et al., 2017).

4. Conclusion

In this study, we determined the activity and specific activity of GUSB in blood serum and saliva, as well as the differences in these indicators in different groups of people who are not at risk, as well as with a high risk of CRC. In this regard, we can say that the main cause of changes in the activity and specific activity of GUSB is a factor such as Smoking, while changes are observed in both saliva and blood serum. Next in terms of influence on the specific GUSB activity are two factors: age and obesity, but it is important that smoking is the most powerful factor that affects the GUSB activity. The results of correlation analysis confirm the fact about the GUSB activity in blood serum and saliva to depend on the presence of risk factors for CRC, such as age and smoking.

Fractionation of serum and saliva proteins indicates that the main form of GUSB associated with variation in activity due to the presence or absence of risk factors for CRC is Human form of GUSB.

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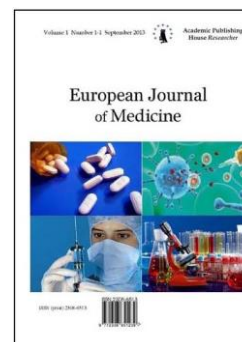
Impact of Diabetes Among People Infected with COVID-19

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Abstract

This is a brief review aimed to characterize the novel coronavirus (SARS-CoV-2) for a better understanding of the COVID-19 in people with diabetes, and its management. We searched for articles in PubMed and Google Scholar databases for articles related to this subject.

The clinical spectrum of COVID-19 is heterogeneous. Older age, diabetes, and other comorbidities are reported as significant predictors of morbidity and mortality. Chronic inflammation, increased coagulation activity, immune response impairment, and potential direct pancreatic damage by SARS-CoV-2 might be among the underlying mechanisms of the association between diabetes and COVID-19.

Human pathogenic coronaviruses (severe acute respiratory syndrome coronavirus (SARS-CoV and SARSCoV-2) were bind to their target cells through angiotensin-converting enzyme 2 (ACE2), which is expressed by epithelial cells of the lung, intestine, kidney, and blood vessels. The expression of ACE2 is substantially increased in patients with type 1 or type 2 diabetes, who are treated with ACE inhibitors and angiotensin II type-I receptor blockers (ARBs).

These data suggest that ACE2 expression is increased in diabetes with ACE inhibitors and ARBs increases ACE2 expression. Consequently, the increased expression of ACE2 would facilitate infection with COVID-19. We therefore hypothesize that diabetes treatment with ACE2-stimulating drugs increases the risk of developing severe and fatal COVID-19.

Caution should be taken to potential hypoglycemic events with the use of chloroquine in diabetic patients. In conclusion: It is difficult to extract specific conclusions based on currently limited evidence. Therefore, further researches are needed to identify the real relationship between diabetic patients and COVID-19.

Keywords: COVID-19, SARS-CoV-2, diabetes, diagnosis, treatment.

1. Introduction

The relationship between peoples with diabetes and coronavirus disease (COVID-19) caused by SARS-CoV-2 is still limited and unclear. Therefore, the understanding the nature of this relationship is very important to enabling patients and healthcare professionals to adopt the right choice about how to manage peoples with diabetes during the COVID-19 pandemic.

Diabetes as one of the most common comorbidities lead to higher mortality has been reported (Yang et al., 2020: 533).

COVID-19 is rapidly increasing globally.

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However, risk factors for the severity and prognosis of COVID-19 are poorly understood. Such information is critical to identify high risk patients and to facilitate planning (Matsushita et al., 2020: 4).

A meta-analysis study revealed that there are some risk factors associated with COVID-19 such as cardiovascular disease (CVD)(sever COVID-19 with patients with CVD compared without CVD: RR=3.58 [2.06-6.21]), age (Older age was consistently associated with severe COVID-19 with RR >~5 in >60-65 vs < 50 years), gender (severe COVID-19 in men than in women, with a pooled crude relative risk estimate of severe COVID-19 between men and women of 1.70 (95 % CI 1.52-1.89), current smoking (the association across categories of current smoking (odds ratio 2.84 [1.57-5.14]), hypertension (a positive association of hypertension with severe COVID-19, with the pooled relative risk estimate of 2.74 (95 % CI 2.12-3.54), and diabetes (a positive association between diabetes and severe COVID-19, with a pooled relative risk estimate of 2.81 (2.01-3.93) (Matsushita et al., 2020: 8).

However, results have been inconsistent. Few studies concerned for the confounding between age and sex when they evaluated other risk factors. For example, some studies reported that hypertension is a risk factor of severe COVID-19, but it is well known that hypertension is more common in older adults (Benjamin et al., 2020: 67).

This observation, together with the fact SARS-CoV-2 uses angiotensin-converting enzyme 2 as an entry to human body (Zhang et al., 2020: 588), has raised a concern about continued use of renin-angiotensin system increase among some clinicians and researchers (Esler, Esler, 2020: 782; Fang et al., 2020: 21).

Diabetes was seen as a risk factor for mortality in patients infected with Pandemic Influenza A 2009 (H1N1), Severe Acute Respiratory Syndrome (SARS) coronavirus and Middle East Respiratory Syndrome-related coronavirus (MERSCoV) (Yang et al., 2006: 625; Schoen et al., 2019: 964; Song et al., 2019: 11).

2. Results and discussion
Diagnosis
Diagnostics can play an important role in the containment of COVID-19, enabling the rapid implementation of control measures that limit the spread through case identification, isolation, and contact tracing (Udugama et al., 2020: 3830).

The symptoms expressed by COVID-19 patients are nonspecific and cannot be used for an accurate diagnosis because of Many of these symptoms could be associated with other respiratory infections (Udugama et al., 2020: 3830). There are several markers have been used for detection such as IgG, IgM, levels of C-reactive protein and D-dimer as well as levels of lymphocytes, leukocytes, and blood platelets (Guan et al., 2020a). The using of these biomarkers could lead to inaccurate diagnosis because of they are also abnormal in other illnesses (Udugama et al., 2020: 3830). However, the real-time fluorescence (RT-PCR) remains till now the standard method for diagnosis of COVID-19. Long et al. (2020) reported that RT-PCR may produce initial false negative results therefore they suggest that negative RT-PCR results should be isolated, and RT-PCR should be repeated to avoid misdiagnosis. Due to the shortage of kits and false negative rate of RT-PCR, the Hubei Province, China temporarily used CT scans as a clinical diagnosis for COVID-19 (Yang, Yang, 2020).

In recent years, smartphone components (e.g., camera, flashlight, and audio jack) have been used for the readout of diagnostic assays in place of conventional laboratory equipment (Malekjahani et al., 2019).

Treatments patients with COVID-19
Development of therapeutics and vaccines is underway, but there is no approved therapeutics or vaccines for the treatment of COVID-19 (Udugama et al., 2020).

The treatment is symptomatic, and oxygen therapy represents the major treatment intervention for patients with severe infection. Mechanical ventilation may be necessary in cases of respiratory failure refractory to oxygen therapy, whereas hemodynamic support is essential for managing septic shock (Cascella et al., 2020).

Although no antiviral treatments have been approved, several approaches have been proposed such as lopinavir/ritonavir (400/100 mg every 12 hours), chloroquine (500 mg every

12 hours), and hydroxychloroquine (200 mg every 12 hours). Alpha-interferon (e.g., 5 million units by aerosol inhalation twice per day) is also used.

The drug Chloroquine is approved to treat malaria and rheumatoid arthritis. It has been tested in vitro against a number of viruses, including SARS, and found to inhibit growth (Touret, de Lamballerie, 2020). This drug has been tested against SARS-CoV-2 – the cause of COVID-19 and found "highly effective," although the evidence is still limited and need larger controlled trials to determine accurately the effectiveness of chloroquine as a treatment for COVID-19 (Gao et al., 2020; Wang et al., 2020).

Reported that there are three effects could help to stimulate the body's ability to fight off covid-19: firstly: the virus is initially taken up into an intracellular 'compartment' which is typically acidic but Chloroquine could alter the acidity of this compartment, which can interfere with the ability of viruses to escape into the host cell and start replicating. Another possibility is that chloroquine may alter the ability of the virus to bind to outside of a host cell, and lastly, chloroquine has subtle effects on a wide variety of immune cells as this drug is used sometimes in autoimmune conditions (Mahase, 2020).

Kaletra drug which is a combination of two antiviral drugs—lopinavir and ritonavir—normally used to treat HIV. It was suggested for treatment patients with COVID-19 but the results from China depend on a randomized controlled trial obtained the non-benefit of its using (Cao et al., 2020). Also, there are other drugs have been used and need to test for patients with COVID-19 using a clinical trial such as: Interferon β 1a (SNG001), Remdesivir, Tocilizumab (Actemra), and Favipiravir (Avigan) (Chen et al., 2020; Financial Times, 2020; USFDA, 2020).

The reliable results about COVID-19 in patients with diabetes are limited at present. The results also are inconsistent. Peng (2020) reported that Diabetes was present in 42.3 % of 26 death cases due to COVID-19 in Wuhan, China. Another study in 140 patients with COVID-19 in Wuhan, China, results showed that the diabetes was not a risk factor for severe disease course (Zhang et al., 2020). Whereas, another study in 150 patients (68 deaths and 82 recovered patients) in Wuhan showed that a total of 63 % (43/68) of death cases had underlying diseases (Ruan et al., 2020). Analysis of 11 studies out of 217 articles regarding laboratory abnormalities in patients with COVID-19 did not clearly describe the rate of patients with laboratories abnormalities, the results, including increased values of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), lactate dehydrogenase (LDH) and D-dimer. They did not mention raised blood glucose or diabetes as predictor of severe disease (Lippi, Plebani, 2020). On the other hand, a report published by Chinese Centre for Disease Control and Prevention showed that the overall case-fatality rate (CFR) was 2.3 % (1023 deaths among 44 672 confirmed cases) whereas, the CFR was elevated among those with diabetes (7.3 %) (Wu, McGoogan, 2020).

A meta-analysis study including 1527 patients in China showed that the most prevalent comorbidities with COVID-19 were hypertension (17.1 %, 95 % CI 9.9 – 24.4 %) and cardio-cerebrovascular disease (16.4 %, 95 % CI 6.6 – 26.1 %), followed by diabetes (9.7 %, 95 % CI 6.9 – 12.5 %). In this study, patients with diabetes or hypertension had a 2-fold at risk of severe COVID-19 disease (Li et al., 2020)

Limited data were existed regarding the glucose metabolism and development of acute complications of diabetes (e.g., ketoacidosis) in patients with COVID-19.

Infection of SARS-CoV-2 in those with diabetes possibly caused higher stress conditions, lead to greater release of hyperglycemic hormones, e.g., glucocorticoids and catecholamines, and then resulted to increased blood glucose levels (Wang et al., 2020).

Diabetes is a chronic inflammatory condition characterized by multiple metabolic and vascular abnormalities that can affect response to pathogens (Knapp, 2013). Hyperglycemia and insulin resistance promote increased synthesis of glycosylation end products (AGEs) and pro-inflammatory cytokines, oxidative stress, in addition to stimulating the production of adhesion molecules that mediate tissue inflammation (Knapp, 2013; Petrie et al., 2018). This inflammatory process may compose the underlying mechanism that leads to a higher propensity to infections, with worse outcomes thereof in patients with diabetes (Knapp, 2013).

The results of the *In vitro* studies showed that pulmonary epithelial cells exposure to high glucose concentrations significantly increases influenza virus infection and replication, thus, the hyperglycemia may enhance viral replication *in vivo* (Kohio, Adamson, 2013).

Animal studies involving SARSCoV reported that older age was related to defects in T-cell and B-cell function and excess inflammation markers. Thus, T2DM alone or in association with older age, hypertension and/or CVDs might contribute to a deficient control of SARS-CoV-2 replication and more prolonged proinflammatory response, potentially leading to poor outcomes (Zhou et al., 2020).

The fundamental component of cross-species transmission is viral entry into the host cells, particularly for the coronaviruses (CoVs). After exposure of the host to the virus, all CoVs, bind to cells by express specific receptors through a Spike protein. After that the host-cell protease cleaves the spike to be able to enter the cell and replicate (Letko et al., 2020). The angiotensin-converting enzyme 2 (ACE2) has been identified as one of the main receptors for both SARS-CoV (51) and SARS-CoV-2 (Letko et al., 2020). ACE2 is widely expressed on the respiratory tract, heart, kidneys, intestines, cerebral neurons, endothelium of arteries and veins, immune cells and pancreas (Song et al., 2019). A Chinese study compared 39 SARS-CoV patients without previous diabetes, who did not receive steroid treatment, with 39 matched healthy siblings and showed that 20 of the 39 SARS-CoV patients developed diabetes during hospitalization. Since immunostaining for ACE2 was strong in the pancreatic islets, it was suggested that SARS-CoV might have damaged islets and caused acute insulin dependent diabetes mellitus (Yang et al., 2010). Although further evidence is needed to confirm that pancreatic damage may also be present in COVID-19 patients, possibly contributing to worse outcomes in subjects with diabetes (Hussain et al., 2020).

The data from 1590 laboratory-confirmed hospitalized patients across mainland China between December 11th, 2019 and January 31st, 2020 showed that the most prevalent comorbidity was hypertension (16.9 %), followed by diabetes (8.2 %). After adjusting for age and smoking status the hazards ratio (HR) of diabetes was 1.59, (95 % CI 1.03–2.45) and patients with two or more comorbidities were more commonly seen in severe cases than in non-severe cases (40.0 % *versus* 29.4 %) (Guan et al., 2020b).

Some studies reported that the prevalence of diabetes in people infected with the virus area slightly lower (Fadini et al., 2020; Li et al., 2020).

A meta-analysis (12 studies) from 2,108 Chinese patients with COVID-19 reported a diabetes prevalence of 10.3 % (Fadini et al., 2020) was similar to the national prevalence of 10.9 % reported by Wang et al. (2013). An equivalent prevalence was reported in Italy among 146 patients with confirmed SARS-CoV-2 infection at the University Hospital of Padova. The prevalence of diabetes in these patients was 8.9 % (mean age 65.3 year) and 11.0 % (mean age 65) for the same location in 2018 (Longato et al., 2020).

Treatment of patients with diabetic infected by COVID-19

The patients with diabetes infected by COVID-19 could be treated with chloroquine which was reported as a potential broad-spectrum antiviral drug (Hussain et al., 2020). Although the efficacy and safety of chloroquine for COVID-19 treatment still unclear, a recent study confirmed that the drug was highly effective in controlling SARS-CoV-2 infection *in vitro* (Wang et al., 2020).

The results of more than 100 patients included in a Chinese clinical trial showed that chloroquine was superior to the control group and revealed improvement without severe side effects (Gao et al., 2020).

Although, some studies reported that hydroxychloroquine improves glycemic control in patients with diabetes (Gerstein et al., 2002; Rekedal et al., 2010). The underlying mechanism of hydroxychloroquine's hypoglycemic effect is unclear (Rekedal et al., 2010).

However, a study contributed that the chloroquine will increase the C peptide response and lead to improve the pancreatic β-cell function (Rekedal et al., 2010). The increasing in the insulin accumulation has also been reported as possible effects of hydroxychloroquine in animals' models (Emami et al., 1999).

A Chinese Centre for Disease Control and Prevention report reported that from 72,314 cases, the overall case – fatality rate (CFR) was 2.3 % (1023 deaths among 44,672 confirmed cases) whereas the CFR was 7.3 % for diabetes.

In another study included of 191 patients from Wuhan Pulmonary Hospital I China showed that a total of 137 were discharged and 54 died. The patients who had a comorbidity was 91 (48 %), with hypertension being the most common (58 [30 %] patients), followed by diabetes (36 [19 %] patients and coronary heart disease (15 [8 %] patients and found that diabetes was associated with significantly higher odds of death (OR 2.85, 95 % CI 1.35 to 6.05) (Zhou et al., 2020).

In a retrospective case study included of 1,590 laboratory-confirmed hospitalized patients in China found that after adjusting for age and smoking status, diabetes significantly increased risk (hazard ratio 1.59, 95 % CI 1.03–2.45). 34.6 % of severe cases were in patient with diabetes compared to 14.3 % in non-severe cases (Guan et al., 2020).

3. Conclusion

COVID-19 is spread rapidly, therefor, the early diagnosis, and management represent the better control ways of the disease. Diabetes is significant predictors of morbidity and mortality in patients with COVID-19. Future research is urgently needed to provide a better understanding regarding potential differences in genetic predispositions across populations, underlying pathophysiological mechanisms of the association between COVID-19 and diabetes, and its clinical management. At the moment, the therapeutic strategies to deal with the infection are only supportive, and prevention in order to reducing the transmission in the community is our best weapon (Cascella et al., 2020).

Declaration of competing interest
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The Effect of Sulphated Cellulose on Haemostasis

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Abstract
Background: Experimental studies and analyses of new compounds with different mechanisms of action on systemic haemostasis are relevant for the identification and development of potential pharmacological preparations. Objective: The modified sulphated polysaccharides with anticoagulant and antithrombin activity were studied for haemostasis. Methods: Platelet-rich plasma was obtained by centrifugation at 200 g for 10 minutes. The remaining citrate blood was further centrifuged at 1500 g for 10 min to obtain platelet-poor plasma. The antithrombin activity of the compounds was evaluated in vitro by their effect on the recalcification time, thrombin and prothrombin time of rabbit and human blood plasma stabilized with a 3.8 % sodium citrate solution in the ratio 9:1. Results: The results showed that the anticoagulant activity of the studied sulphates increased with an increasing degree of sulphation. Sulphated polysaccharides showed strong anticoagulant activity *in vitro*. The experimental results showed a significant increase in the coagulation time of blood plasma in tests for prothrombin and thrombin time. Conclusion: These properties of these components are of particular interest, and further detailed studies of the physicochemical characteristics and mechanisms of action of these molecules should be performed, which will eventually allow them to be used as heparin-like drugs.
Keywords: modified sulphated polysaccharides, activated partial thromboplastin time (APTT), prothrombin time, anticoagulant, thrombin time, platelet aggregation, ADP.

1. Introduction

Currently, modified sulphated polysaccharides, which are a complicated group of macromolecules possessing a wide spectrum of biological characteristics, have attracted increased attention. Along with antiviral, antimicrobial, and anti-inflammatory properties, these molecules also have anticoagulant properties (Alekseenko et al., 2007; Chen et al., 2009; Hayashi et al., 2008). Cellulose is the most widely available compound, is physiologically related to polysaccharides in human and has a well-known structure, which has been extensively studied. The basic materials for the synthesis of cellulose are various structural modifications of cellulose: a cotton cellulose fibre with a degree of polymerization from 1000 and higher or other pulp forms obtained from cotton, wood, and other raw materials (Athukorala et al., 2008). To increase the anticoagulant activity, researchers have introduced strong electronegative groups into cellulose, such as sulphate, phosphate, carboxylic, and amine groups, indicating that the mechanism of action is associated with electrostatic complex formation between blood proteins and cellulose

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(Wang et al., 2005). Fan. L et al. also noted that with the anticoagulant activity increased with the introduction of many sulphate groups in the starch molecule (Fan et al., 2013).

Anticoagulants are used, inter alia, for the prevention and treatment of thrombus in humans (Geerts et al., 2008; Ageno et al., 2014). Anticoagulants with indirect and direct types of action are distinguished (Molteni et al., 2014). The mechanism of action of direct anticoagulants is associated either with the direct inhibition of the activity of thrombin or factor Xa or with the activation of the plasma inhibitor antithrombin (Vo et al., 2014).

The anticoagulant drugs that are currently being used do not satisfy the clinical requirements. Due to their indirect effects, indirect anticoagulants and heparin preparations are limited. One of the most common side effects of all modern anticoagulants is bleeding (Levi et al., 2012; Tosetto et al., 2013; Liotta et al., 2013). Along with preparations of heparin and anticoagulants with direct action, modern inhibitors of thrombin/factor Xa for oral and parenteral use are administered (Ganetsky et al., 2014; Limdi et al., 2013; Roskell et al., 2013; Scaglione, 2013). Therefore, the development of new anticoagulants based on compounds with different chemical structures and low haemorrhagic activity is needed.

Thus, the search for new chemical compounds for the development of drugs possessing anticoagulant activity is important. In vitro experiments can be used to determine the specific activity of the compounds, select an treatment, provide data for in vivo experiments, evaluate the pharmacodynamics, and determine the dosage of the anticoagulants needed in experimental animals.

Experimental research and analysis of new compounds with different mechanisms of anticoagulant action are needed for the identification and development of potential pharmacological preparations. Investigation of the bond structure and the activity of cellulose sulphate will allow the most active polysaccharides to be obtained.

The purpose of the study is to investigate the heparin-like activity of various chemically modified sulphated polysaccharides in systemic haemostasis.

2. Material and methods
2.1. Experimental biological part
Plasma for research was used without platelets. Platelet-rich plasma was obtained by centrifugation at 200 g for 10 minutes. The remaining citrate blood was further centrifuged at 1500 g for 10 min to obtain platelet-poor plasma. The antithrombin activity of the compounds was evaluated in vitro by their effect on the recalcification time, thrombin and prothrombin time of rabbit and human blood plasma stabilized with a 3.8 % sodium citrate solution in the ratio 9:1 (Menshikov, 1987). To isolate platelets used 150 μM NaCl, 2,7 μM KCl, 0,37 μM NaH_2PO_4 ' $2H_2O$,1 μM $CaCl_2$, 5 μM glucose, 10 μM HEPES. In coagulation tests, sulfated compounds were used at a dose of 10-50 μg / ml. Thrombin (1 unit) was used as a control. Prothrombin activity was tested by the Quick method with thromboplastin (Quick). To determine the effect of compounds on thrombosis, it was evaluated by their effect on known haemostasis tests. Thrombin (1 unit) was also used as a control. For experiments using sulfate cellulose 1 mg/6 ml of H_2O. The effect of anticoagulants on the coagulation of human and rabbit plasma in vitro was evaluated using the following tests: activated partial thromboplastin time (APTT) (Scaglione, 2013), prothrombin time (PT) (Rosenberg, 1977) thrombin time (TT) (Teien, 1975), and the ReaClot – Heparin (NPO Rename Russia, Moscow) tests (Yin, 1973) for analysis of the effects on fibrinogen polymerization (for thrombin and buffer, Cypress Diagnostics, Belgium). All coagulation tests (with human plasma) were performed on a single channel coagulometer (CYANCoag, Belgium.CY003, SN:5400439). For evaluation of the anticoagulation potential of the compounds, the effective concentrations in the APTT, PT, TT, and ReaClot tests, which were found on the abscissa of the curves showing the dependence on the concentration of the anticoagulant, were graphically determined. We detected a 2-fold increase in plasma clotting time compared with that of the control, which had no anticoagulants.

2.2. Experimental chemical part
In this work, various modified sulphated polysaccharides (MSPs) with different molecular masses and linear compounds obtained via homo- and heterogeneous sulphation, were used.

The modified sulfated polysaccharides studied in this research was cellulose sulfates with different molecular parameters that synthesized, purified and characterized as described in

(Muhitdinov et al., 2017; Muhitdinov et al., 2019). The information regarding the cellulose sulfates is included in the revised manuscript.

The molecular structure of the cellulose sulfates are added into the manuscript. The molecular structure of the cellulose sulfates is as follow (Figure 1):
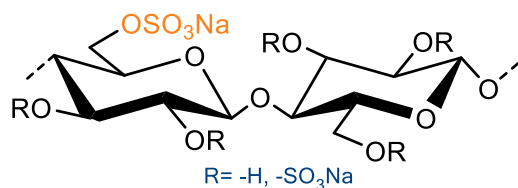

R= -H, -SO₃Na

Fig. 1. Molecular structure of the cellulose sulfates

The polysaccharide sulfates used in this study were purified. Sulfate content of the samples were evaluated by elemental analysis and ^{13}C NMR spectroscopy methods. The sample BOS-122 is depolymerized, low molecular weight cellulose sulfate degree of polymerization (DP) and degree of substitution (DS) values of 59 and of 2.01, respectively. The sample GSC-14 is cellulose sulfate having DP and DS values of 724 and 1.98, respectively.

2.3. Statistical analysis
The measurements were made using a universal spectrometer (USB-2000). Statistical significance of differences between control and experimental values determined for a number of data using a paired t-test, where the control and the experimental values are taken together, and unpaired t-test, if they are taken separately. The value of P <0.05 indicated a statistically significant differences. The results obtained are statistically processed to Origin 7,5 (Origin Lab Corporation, USA).

3. Results and discussion
In this study, the effect of various MSPs on blood coagulation in laboratory tests, as well as the comparison of the anticoagulant effect to that of heparin, was studied. The effects of the studied compounds on the plasma clotting time in the APTT, PT, TT, and ReaClot tests were determined.

When the samples were supplemented with citrate human plasma in vitro, the time of appearance of a fibrin clot in the APTT, TT, PT and ReaClot-Heparin tests was increased; thus, the compounds are anticoagulants with direct action; BOS-122 SC and SC GSC-14 antithrombin activity reached 2.9 ± 1.1 and 7.6 ± 0.6 mcg/mL, respectively, and showed efficacy in the tests at concentrations not substantially different for those of unfractionated heparin (heparin) and were equal to 6.3 ± 0.1 and 7.6 ± 0.5 μg/ml, respectively (Figure 2 A, B, C).

In addition, it was noted that the anti-factor Xa activity among the most promising compounds was several times less than the antithrombin activity of these compounds and was similar to that of heparin (Linhardt et al., 2012).

We showed that the more complicated the coagulation test is, the more SC is required to achieve the same efficiency. Consequently, the effect of the SCs is not excluded at the stages preceding the coagulative transformation of fibrinogen, when the sulphated components are consumed. In contrast, their concentrations in different tests would be equal or at least comparable. This reduction is enhanced by the fact that, according to the electrocautery data, SCs extended the period before the formation of the fibrin clot.

To resolve this problem, we used a technique that allows us to isolate the coagulation of fibrinogenic coagulation from the overall cascade of coagulation reactions. This method is based on the fact that pulmonary donor plasma is released from fibrinogen by soft heat denaturation (56 °C, 3 minutes). If fibrinogen is added to such defibrinated plasma, followed by SC, and then mixed, SC can influence any plasma coagulation stage. If, however, in a defibrinated plasma, the activation of the coagulation cascade is first activated, and then, after forming thrombin, fibrinogen and SC are added, only the latter can influence the coagulation of fibrinogenic transformation.
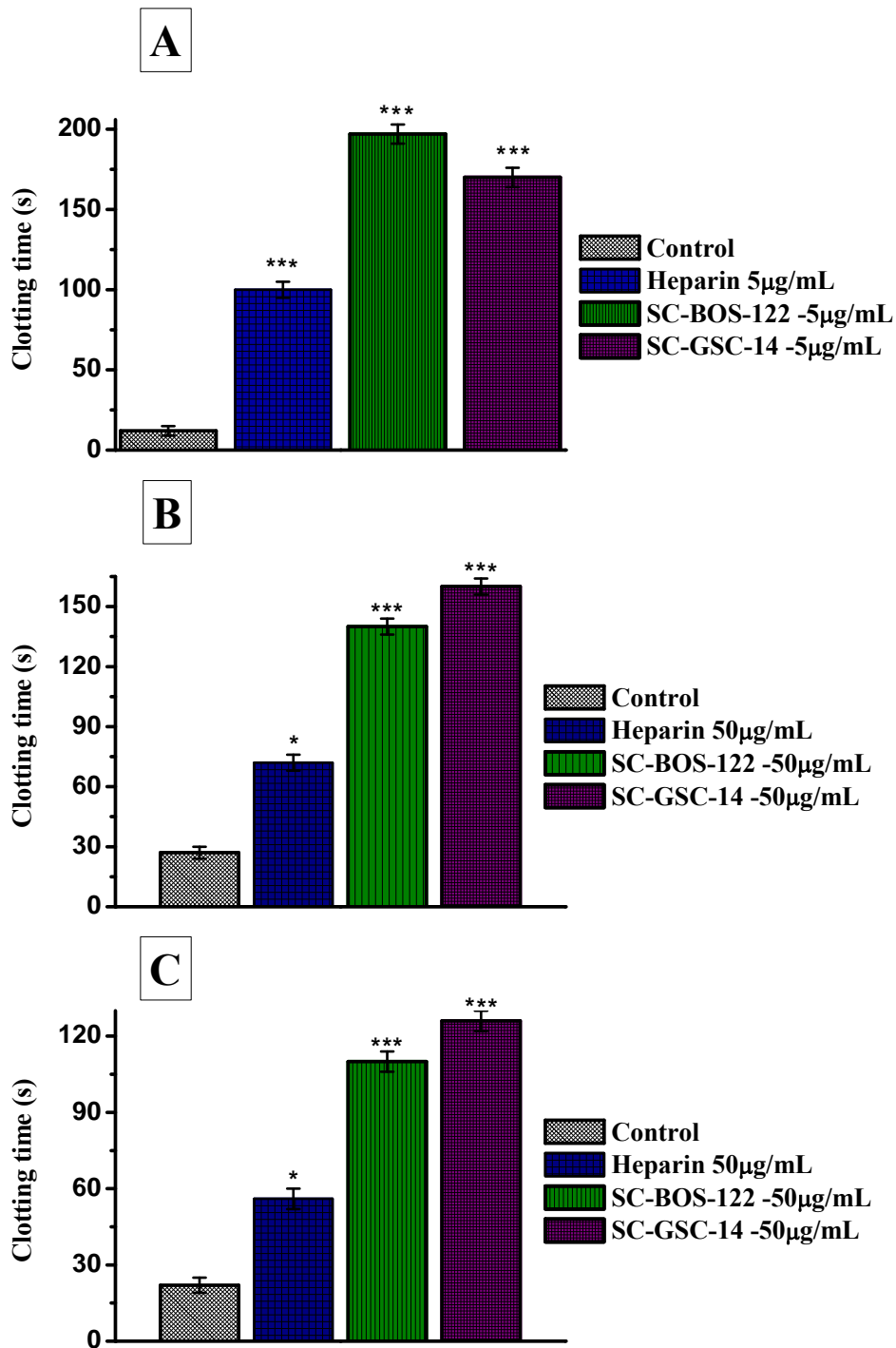
Fig. 2. Anticoagulant activity of SC BOS-122 and SC GSC-14 in (A) APTT, (B) TT, and (C) PT versus heparin *- $P < 0.05$; **- $P < 0.01$; ***- $P < 0.001$. (n=6)

The results of this experiment showed that the inhibitory efficiency in such cases as those described above was almost equal to that when using both BOS-122 and GSC-14. Therefore, the initial assumption about SCs was confirmed at the level of coagulation of fibrinogen transformation, and the differences in the effects of SCs on coagulation were observed at the stage of fibrinogen transformation.

To confirm this conclusion, we determined the effectiveness of inhibition of the interaction between thrombin and the fibrinogen of the SCs and their additive effects compared to the expected effect. The expected (theoretical) effect was significantly lower (on average by 70 %),

indicating the synergistic effect of the SC. These data also indicate that the mechanism of the studied SCs on the coagulative transformation of fibrinogen is different: in the case of an identical mechanism, we would observe additive effects based on the deficiency of the SC in the system or antagonism due to the supersaturation of the SCs.

More information about the differences in the mechanism of action of the SCs was obtained by observing the coagulation of fibrinogen transformation with a nephelometer with an automatic recording of the stages of this process.

SC BOS-122, compared with the control, on average always delayed the formation of oligomers by 9.7 % and increased the time required for the formation of a fibrin clot by 81 %. Since the aggregation of protofibrils is almost instantaneous, SC BOS-122 mainly inhibits the formation of relatively mature oligomers. In contrast, GSC-14 SC predominantly blocked monomer and oligomer assembly; no critical increase in the aggregates was observed with a spectrometer. However, the formation of a fibrin clot increased by an average of 70 % compared to that of the control (Figure 3).
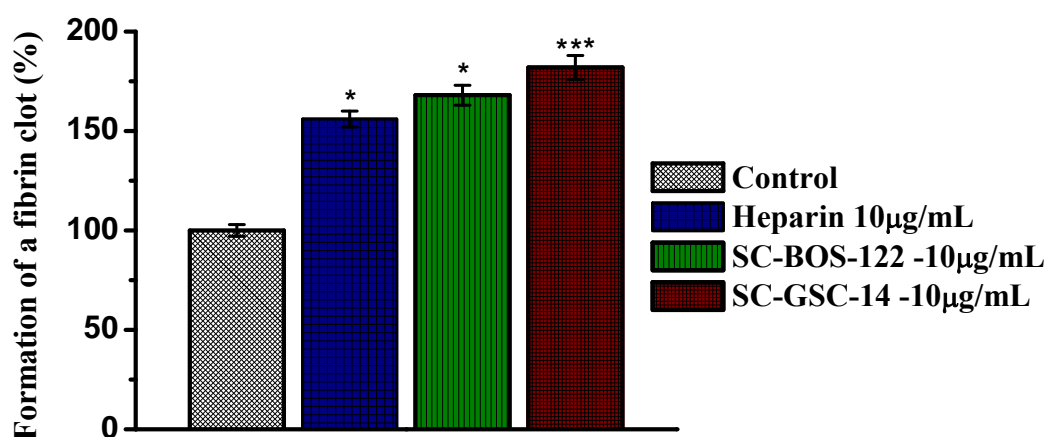

Fig. 3. The effect of SC BOS-122 SC and SC GSC-14 on the formation of fibrin clots in human blood. *- $P < 0.05$; **- $P < 0.01$; ***- $P < 0.001$. (n=6)

To study the effect of SC BOS-122 on the anticoagulant activity of rabbit plasma, we intravenously injected the test compound at various doses into the marginal ear vein. After the plasma was collected at different time intervals after SC administration, the clotting time was determined in the APTT/ReaClot-Heparin tests, and the plasma Xa was determined compared with that with heparin.

To evaluate the effectiveness of the anticoagulants in practice, clinics have used the rate of double the plasma clotting time or human blood counts in some coagulant tests compared with the readings before drug administration (Ng et al., 2003). *In vitro*, we showed that the effective concentrations of the studied compounds (APTT, PT, TT, ReaClot) decreased with an increase in the degree of sulphation; similar findings for sulphates of polysaccharides from various types of vegetation have been found in multiple works (Almedia-Lima et al., 2010; Nasirov et al., 2020; Cipriani et al., 2009; Nadjimova et al., 2020; Khoshimov et al., 2015; Luo et al., 2013; Maas et al., 2012; Silva et al., 2012).

For monitoring of heparin therapy, blood clotting time, plasma clotting time in the APTT test, activated blood clotting time, plasma antithrombin and aXa activities in the plasma are determined. APTT is the most widely used test for determining the degree of anticoagulation after administration of heparin at therapeutic doses (Kleinjan et al., 2013; Marlar et al., 2013).

In the 1970s, the anticoagulant effect of the APTT range (1.5–2.5 times that of the control) was established to reduce the risk of recurrent thrombosis in patients When the concentration of heparin in plasma was 0.3 aXa U/ml, the average time in the APTT test was 48 to 108 seconds, depending on the laboratory test method. Therapeutic levels of heparin (0.3 - 0.7 aXa U/ml) in modern tests of APTT demonstrate ratios of 1.6-2.7 and 3.7-6.2 times (Baglin et al., 2006).

In this work, a definite increase in plasma clotting in the APTT and ReaClot-Heparin tests were performed after an increase in the dose of SC (3, 5 and 7 mg/kg) or heparin (0.75 and 1 μg/kg) after intravenous administration. The time of action (for APTT and ReaClot-Heparin) after the introduction of heparin at doses of 0.75 and 1 mg/kg reached 72 minutes and 113 minutes, respectively. The greater prolongation of the anticoagulant effect of SC BOS-122 compared with that of heparin was associated with higher doses. To achieve the same effect at the time of plasma coagulation in the APTT test (15 minutes after the injection), SC BOS-122 required a 7 times greater concentration than heparin.

The maximum plasma aXa activity was noted in the 5th minute after the administration of SC BOS-122 and heparin into the blood, which coincides with the literature on the intravenous administration of the direct anticoagulant heparin (James, Coller, 2012). The complete disappearance of the aXa activity in the rabbit plasma was observed with the introduction of only heparin at doses of 0.75 and 1 μg/kg after 170 and 110 minutes, which is consistent with the data on the plasma coagulation time in the APTT and ReaClot-Heparin tests.

We observed a naturally greater antithrombin activity in the rabbit plasma compared with the aXa activity after the introduction of SC BOS-122 at different doses. This finding is expected since the specific activity of SC BOS-122 is greater than the aXa activity.

To neutralize the anticoagulant effect of heparin, in clinical practice, researchers administer protamine sulphate (Suryanarayan, Schulman, 2014). Consistent administration of sulphate protamine for sulphate cellulose or unfractionated heparin (in the same doses) caused a reduction in the clotting time of plasma in the APTT/ReaClot Heparin-aXa tests in rabbits and reduced plasma activity. Therefore, 15 minutes after the SC and protamine sulphate (PS) were injected, the plasma coagulation time in the APTT test depending on the dose was 2.3 - 4.4 times shorter than that with the administration of only SC; for heparin and SP, this difference was 15 and 6 times for 15 minutes, depending on the dose. An analysis of the plasma 15 minutes after the SC and SP were injected with the ReaClot-Heparin test showed that the coagulation time was 1.5-2.0 times lower, depending on the dose; for heparin and PS, this difference was 15 minutes, 1.9 and 3.4 times, depending on the dose. The effect of PS on heparin has long been known (Montalescot, 1990).

With the introduction of heparin and the subsequent neutralization of its effect in the 15th minute after administration, we noted an 8-fold decrease in antithrombin activity. The subsequent maintenance of protamine sulphate led to a decrease in the plasma activity after the administration of BOS-122 SC by an average of 2 times compared to the results without treatment.

The introduction of an antidote after the administration of SC BOS-122 or heparin led to a decrease in the anticoagulation activity in the rabbit plasma. Therefore, in the 6th minute of aXa, plasma activity with the introduction of SC BOS-122 and SP at doses of 3, 5, and 7 mg/kg on average decreased almost 3 times compared with the introduction of SC BOS-122 only, with the disappearance of plasma aXa activity after 160 minutes. With the introduction of PS for heparin aXa, plasma activity decreased 13 times.

In vitro experiments showed that for neutralization of the anticoagulant effect of SC BOS-122, depending on the concentration, the addition of protamine sulphate at a ratio of 1 to 10 (by weight) to the anticoagulant may be needed. In our animal experiments, we showed that for neutralization of the anticoagulant activity of BOS-122 SC, it may be sufficient to use protamine sulphate and the anticoagulant at identical doses.

We conducted studies on the influence of SC BOS-122 and SC GSC-14 (at equal concentrations of 10–100 μM) on the aggregation function of thrombocytes. ADPs (2.5 and 10 μg/ml) and adrenaline (Tonogen Solution) were used as inducers, since the results of the aggregation intensity values, caused by the above inductors, are the most satisfactory, and the extent of the aggregation, caused by the exposure, is also determined by the application of adrenaline (Avenarius, Deinhardt, 1980).

Under these conditions, both studied celluloses inhibited the aggregation of thrombocytes, but their mechanism of action, as well as their effect on the coagulation of fibrinogen, was significantly different.

Thus, when using ADP with a concentration of 2.5 μg/ml SC BOS-122 and SC GSC-14 at 10-100 μM, the first wave of aggregation, which is characterized by the formation of a clot under the influence of an inductor, was not changed, but the same symptoms were observed. The maximum magnitudes of the second wave of aggregation for SC BOS-122 and SC GSC-14 were

similar and were lower than the control value by 31 % and 38 %, respectively. SC BOS-122 and SC GSC-14 shortened the time necessary to reach the maximum value of the second wave but showed a difference in intensity of 62.5 % and 37.5 %, respectively. In addition, SC BOS-122 activated disaggregation, and SC GSC-14 did not affect this process (Figure 4A).
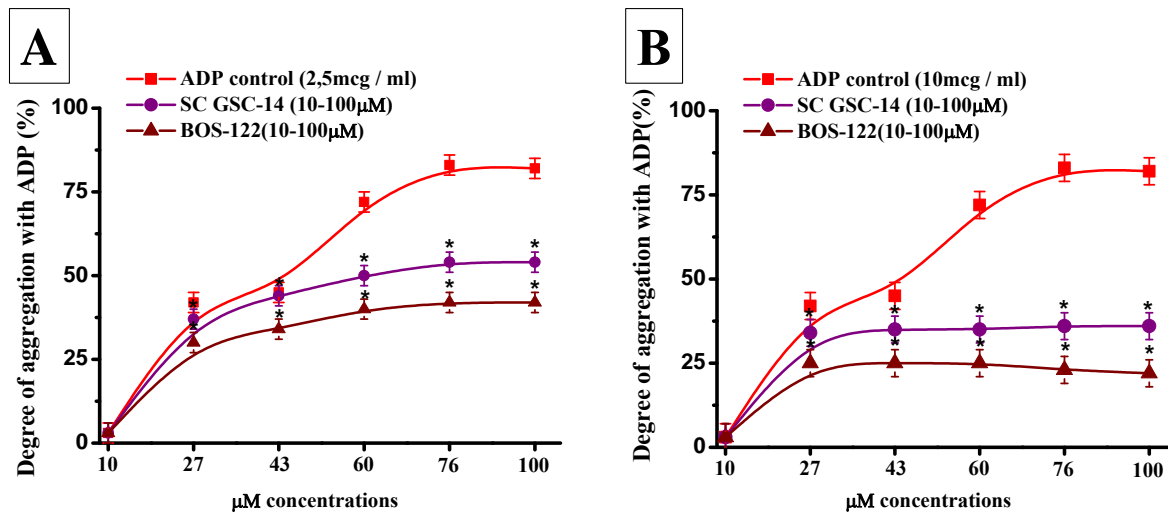

Fig. 4. The effect of sulphated cellulose on platelet aggregation with ADP 2.5 μg/ml (A) and 10 μg/ml (B). *- P < 0.05; **- P < 0.01; ***- P < 0.001. (n=6)

Significant differences in the mechanism of antiaggregant action were observed with an increase in the concentration of the inducer to 10 μg/ml. In this case, control of the first wave of aggregation was not observed. This finding was not observed in the presence of SC GSC-14, but the first wave of aggregation appeared in the presence of SC BOS-122.

The maximum values of the second wave of aggregation for SC BOS-122 and SC GSC-14 were lower than the control value by 44% and 32%, respectively. SC BOS-122 did not significantly change the time of occurrence of the maximum value in the second wave, and SC GSC-14 increased it by an average of 13 % (Figure 4B).

When used as an adrenaline inducer, SC GSC-14 smoothed the first aggregation wave, reduced the maximum value by a second, and lengthened its maximum time by 12.7 %. In contrast, SC BOS-122, without changing the maximum value of the first wave of aggregation, doubled the time required for its achievement, reduced the maximum value of the second by 50.4 % and lengthened the time of its maximum by 50.6 %.

When deciphering the mechanism of restriction of blood plasma clotting activity, we first used an objective method (determination of plasma coagulation with a CYANCOag coagulometer) and found that the SC had pronounced anticoagulant activity. In this case, pulverized donor plasma was used as a substrate, and BOS-122 SC and GSC-14 as anticoagulants were combined and added. In this case, some differences in the influence of carriers on blood plasma recalcification were revealed, which are especially reflected in the change in time before the start of coagulation, as well as in the rate of retraction and fibrinolysis.

Differences in the effect of the inhibitors on coagulation of fibrinogen transformation were found by another objective method: a USB-2000 spectrometer. SC GSC-14 had little effect on the early stages of the formation of protofibrils, and it predominantly slowed the autopolymerization of relatively mature oligomers. SC BOS-122, in contrast, had a pronounced inhibitory effect on the early stages of polymerization. With their joint influence on the coagulation of fibrinogen, a synergistic effect was observed, which confirmed the aforementioned differences in the mechanisms of action.

Thus, the current findings on the anticoagulant activity of these molecules in the final phase of coagulation showed the predominant influence of SC BOS-122 and SC GSC-14 on self-polymerizing fibrin.

We identified only those indicators that integrally reflect the total coagulation activity of the blood plasma (recalcification time), the speed of the interaction between thrombin and fibrinogen (thrombin time) and autopolymerization of the subject.

The properties of these components are of particular interest, and further detailed studies of the physicochemical characteristics and mechanisms of action of these molecules are needed, which will eventually allow them to be used as a heparin-like drug.

4. Conclusion

Some differences in the effects of SC BOS-122 and SC GSC-14 on the recalcification of blood plasma were revealed, especially the change in time before the start of coagulation, as well as in the rate of retraction and fibrinolysis.

The anticoagulant activity of directly acting cellulose sulphates, with a molecular mass of 21,500 kDa (sulphation degree of 2.01), is mediated through an interaction with antithrombin. The anticoagulant activity of the sulphate molecules studied increased with an increasing degree of sulphation. The maximum antithrombin activities of SC BOS-122 and SC GSC-14 reached 2.9 ± 1.1 and 7.6 ± 0.6 µg/ml, respectively.

Certain indicators generally reflect the total coagulation activity of blood plasma (recalcification time), the rate of interaction between thrombin and fibrinogen (thrombin time) and the autopolymerization of monomeric fibrin (time of self-scavenging of fibrin in plasma).

5. Acknowledgements

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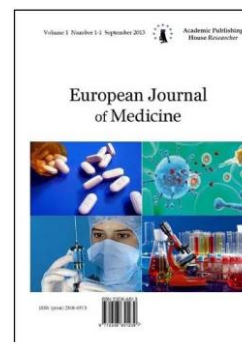
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Spatio-Temporal Morphological Changes of Structures in Infrahyoid Triangles in Human Fetuses During Fetal Period of Human Ontogenesis

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Abstract

Infrahyoid triangles in anterior region of neck contain crucial structures for human body like trachea, thyroid gland, nerve plexuses and magistral blood vessels. Infrahyoid muscle flaps are often used for reconstructive surgeries that is why the topography of their innervation is important for successful dissection and minimization of postsurgical complications. That is why the aim of our study was to examine topographical peculiarities of structures in infrahyoid triangles in human fetuses during late intrauterine prenatal development (PND) that would provide clinical practitioners like pediatric and oncologic surgeons with additional anatomic data.

We have examined 18 specimens of human fetuses (4-9th month of PND, 82,0-311,0 mm of parieto-coccigeal length (PCL)). All material was obtained and studied at Chernivtsy Regional Pathologists Office (Ukraine) in accordance with bilateral collaboration with the Department of Histology, Cytology and Embryology of BSMU. To reach the aim of the research we have used classic morphological methods (anthropometry, morphometry, layered dissection) as well as injection of the blood vessels (in 6 specimens by the means of dies) and three-dimensional reconstructioning. The study was performed in accordance with the provisions of the Declaration of Helsinki on ethical issues of studies conducted with humans (1964-2008).

We have seen that in fetal period of human PND (80,0-310,0 mm of PCL) ansa cervicalis, that provides innervation for infrahyoid structures, is formed by anastomosis of the superior and inferior roots (C2 and C3) that are merging with internal carotid artery and internal jugular vein. The infrahyoid muscles receive nourishment predominantly from the superior thyroid artery, that is a branch of external carotid artery (91 %) or rarely bifurcation (3 %) and either as a branch of common carotid artery (6 %). Specific feature of infrahyoid triangles during fetal period of PND is the fact that it highly relies on bony boundaries such as mandible, sternum and clavicle that haven't reached their mature morphology at 3rd month of PND.

Investigations of topographical peculiarities of structures in infrahyoid triangles in fetal period of PND are important for successful reconstruction surgeries in postnatal and adult age.

Keywords: prenatal development, neck morphology, fetuses, embryology, triangles of the neck, human.

1. Introduction

Medical embryology is aimed to study normal human morphogenesis, which gives a basis for further investigations of possible pathological conditions during and after intrauterine development (O'rahilly, Müller, 2010: 74). Studies that are devoted to the problem of

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embryological sources, differentiation and topographical changes of neck structures in human fetuses during prenatal development (PND) play an outstanding role in fundamental anatomical researches (O'rahilly, Müller, 2010: 75; Naimo et al., 2015: 177; Belle et al., 2017: 162). Such investigations complement existing data on human PND and moreover, give useful data for medical practitioners such as maxillofacial surgeons, aesthetic specialists and pediatricians (Frisdal, Trainor, 2014: 416). Infrahyoid region includes crucial blood vessels, a system of muscles that is often used in reconstruction surgeries and is a place of regular development of congenital malformations (Rasool, Hassan, 2017: 330). That is why the aim of our research was to investigate topographical and developmental features of structures in the infrahyoid triangles in anterior and lateral portions of neck in human fetuses during late intrauterine development and describe the course of blood vessels, nerve plexuses and muscles' topography.

2. Materials and methods

We have examined 18 specimens of human fetuses (4-9th month of PND, 82,0-311,0 mm of parieto-coccigeal length (PCL)). All material was obtained and studied at Chernivtsy Regional Pathologists Office (Ukraine) in accordance with bilateral collaboration with the Department of Histology, Cytology and Embryology of Bukovinian State Medical University (BSMU). All specimens were obtained from ectopic pregnancies or spontaneous abortions, and no part of the material gave indications of possible malformation. Approval for the study was granted by the Ethics Committee of BSMU on the question of studies that involve human materials. To reach the aim of the research we have used classic morphological methods (anthropometry, morphometry, layered dissection) as well as injection of the blood vessels (in 6 specimens by the means of dies). The study was performed in accordance with the provisions of the Declaration of Helsinki on ethical issues of studies conducted with humans (1964-2008) and is a part of the scientific research of the Department of Histology, Cytology and Embryology at BSMU.

3. Discussion

The anatomy of the neck is better understood by dividing it into regions with triangular shape. The anterior triangle of neck (AT) in human fetuses (81,0-310,0 mm PCL) is limited by the sternocleidomastoid muscle laterally (that already has developed into a mature muscular structure that spreads from the mastoid process to the clavicle), by the mandible superiorly and by the midline anteriorly (vertical line from the midpoint of the mandible that reaches the midpoint of the sternal incision). Specific feature of AT during fetal period of PND is the fact that it highly relies on bony boundaries such as mandible, sternum and clavicle that haven't reached their mature morphology at 3rd month of PND (Figure 1), which influences sized and topographical location of infrahyoid triangles (Begnoni et al., 2018).

Infrahyoid group of muscles include paired omohyoid, thyrohyoid, sternothyroid and sternohyoid that already have precise features of attachment points and fasciae coverings. It is worth to mention that at late fetal period (end of the 9th month of PND) a few neck spaces can be already distinguished. For instance, carotid space that is composed of two areas that extend from the base of the skull to the aortic arch: supra- and infrahyoid portions of neck and mediastinum. Carotid space contains internal jugular vein (IJV), common carotid artery (CCA), internal carotid artery, cranial nerves, lymph nodes and vagus nerve. In the infrahyoid region of neck, carotid space is bordered by anterior cervical space in the front, retropharyngeal space medially and by perivertebral cervical space posteriorly.
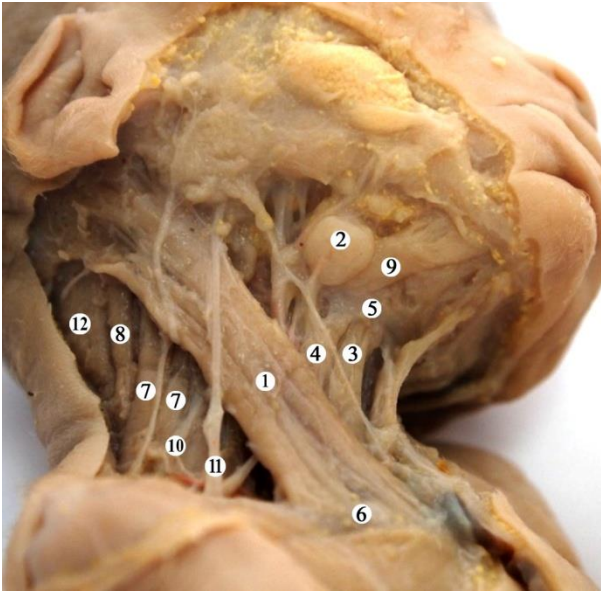
Fig. 1. Superficial structures in anterio-lateral right projection in human fetus (195,0 mm of PCL). Macrospecimen. Magnification 4^x:
1 – sternocleidomastoid muscle; 2 – submandibular gland; 3 – sternohyoid muscle; 4 – omohyoid; 5 – hyoid bone; 6 – clavicle; 7 – scalene muscles; 8 – levator scapulae; 9 – digastric; 10 – cervical plexus; 11 – branches of brachial plexus, 12 – deltoid muscle.

In late fetal period of human PND (271,0-310,0 mm PCL) CCA runs behind the sternocleidomastoid muscle and medially to the IJV. The IJV in fetuses has descend course, along with the lateral wall of pharynx and posteriorly to internal carotid artery. Topographical interconnection between the CCA and the vagus nerve is variating in the late fetal period (311,0-346,0 mm PCL): it changes the direction from a ventral course on intermediate one between the artery and vein (Figure 2). This is the final dorsal course that is tending to have a define adult features.
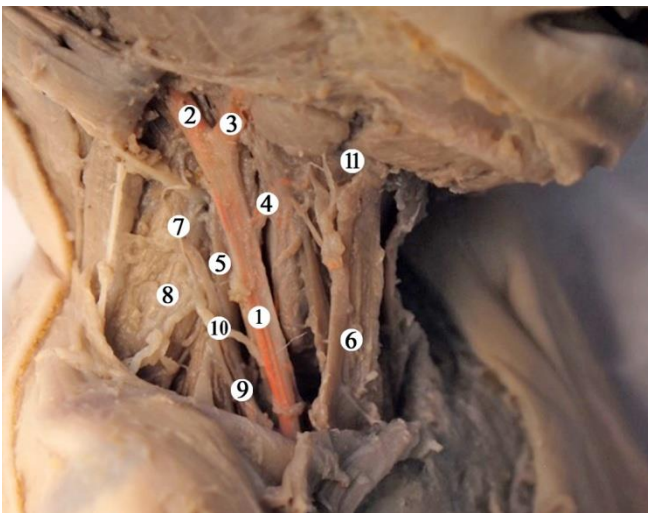
Fig. 2. Right lateral neck structures in human fetus (210,0 mm of PCL, male) after the dissection of platysma and sternocleidomastoid muscles. Macrospecimen. Magnification 5^x:
1 – common carotid artery; 2 – internal carotid artery; 3 – external carotid artery; 4 – superior thyroid artery; 5 – vagus nerve; 6 – sternohyoid muscle; 7 – branches of ansa cervicalis; 8 – cervical plexus; 9 – suprascapular artery; 10 – accessorial nerve; 11 – hyoid bone.

The hyoid bone (HB), that plays an important role in maintaining the commutative spatial relationship of the bones in the upper body of human fetuses, has U-shape form and is located under mandible. The HB is the bound of infrahyoid triangles, which extend from the body or horns of the hyoid downwards to the inferior border of neck and anterior borders of trapezoid muscles (starting from 80,0 mm of PCL). The HB, which is derived from the posterior portion of neural crest in human fetuses (Rodríguez-Vázquez et al., 2011: 145), divides anterior neck triangle into supra- and infrahyoid regions. Infrahyoid triangles contain such important organs like larynx, hypopharynx, cervical trachea, esophagus, thyroid and parathyroid glands. The body of the hyoid is shaped slightly curved, anterior surface is convex exhibiting a small eminence in the midline. Moreover, lateral borders of body in HB are somewhat thickened and rounded. The greater horns are joined with the body by a dense mesenchymal tissue. Connection of the lesser horn with the body and the junction between the body and the greater horn are formed by a dense stripe of closely packed cells (fetuses 85,0 mm of PCL). The prenatal morphogenesis of HB is always connected with transformation of the branchial arches (de Bakker et al., 2018: 1830). The origin of the greater horns is considered to be of the lateral cartilages of the third visceral arch, the lesser horn originates from the distal part of Reichert's cartilage (Kadir et al., 2015: 45). Superior and inferior belly of omohyoid muscle together with sternocleidomastoid subdivide infrahyoid region on smaller triangles: carotid, omotracheal, omoclavicular and omotrapezoid (Figure 3).
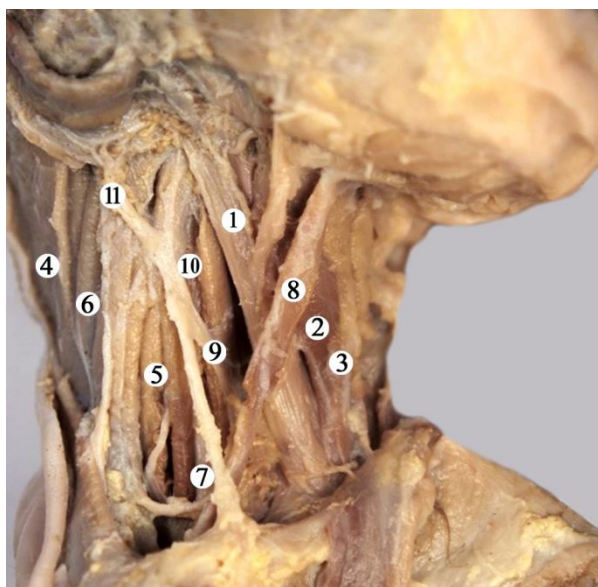

Fig. 3. Profound structures of the neck in human fetus (163,0 mm of PCL, male), anterio-lateral right view. Macrospecimen. Magnification 5^x:
1 – internal jugular vein, 2 – sternothyroid, 3 – sternohyoid, 4 – trapezoid muscle, 5 – scalene muscles, 6 – levator scapulae muscle, 7 – transverse cervical artery, 8 – superior belly of omohyoid, 9 – superior laryngeal artery, 10 – vagus nerve, 11 – great auricular nerve.

The infrahyoid group of neck muscle receive nourishment predominantly from the superior thyroid artery (STA), that is a branch of external carotid artery (91 %) or rarely bifurcation (3 %) and either as a branch of CCA (6 %) (Figure 2). The sternohyoid muscle gets the blood supply from the branches of STA: superior and inferior, that are subdivided on smaller twigs (Figures 2, 3). We have observed a few cases in the middle prefetal stage (42,0-53,0 mm PCL) where the inferior branch of the STA was absent. The upper portion of the sternothyroid muscle is nourished by the STA branched twig, number of which vary from 2 to 3. It was found a variant of a common arterial trunk arising from the STA that gives branches to infrahyoid muscles like sternothyroid and sternohyoid.

Innervation of the infrahyoid structures is provided by ansa cervicalis (Kikuta et al., 2019: 222) that is formed by the means of two twigs fusion: the superior and inferior ones (C2 and C3). The superior root was observed to have ascending topography next to the lateral portion of the IJV. The terminal twigs of the nerve trunk were found next to sternothyroid and sternohyoid muscles.

We have seen two types of superior root topography: medial and lateral subtypes. The inferior root has begun from the C2 and C3. The fusion between hypoglossal nerve and ansa cervicalis was observed to have classical curved shape (fetuses 250 – 310 mm of PCL). It is important to mention that we have seen varieties in nerve course: in medial position to the IJV the topography of ansa cervicalis in total may change and to be formed between IJV and CCA. Such peculiarities during reconstructive surgeries are important for successful outcome (Yuan, Gao, 2018: 373; Som, Laitman, 2017: 242).

4. Conclusion

In conclusions, it should be noted that topography of infrahyoid triangles in late fetal period of PND is characterized by mature demarcation of their borders that are represented by infrahyoid muscles and contain fascial spaces. Vascularization of infrahyoid muscles is provided by branches of external carotid artery that have a variable course. The HB horns have reached their mature morphology, but ossification has not been completed. HB plays a key role for attachment of infrahyoid muscles and margination of spaces in anterior region of neck.

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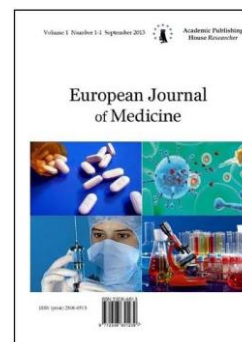
Feeling Elated: A Grounded Theory Study of Rhinoplasty

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Abstract
Background
Rhinoplasty is the most common cosmetic surgery in Iran while more than half of that is not necessary. Because of lacking the qualitative studies in this field, the authors prompted to conduct this research. The aim of this study was to explore the experience of people who undergo Rhinoplasty in order to prevent unnecessary operation.
Methods
Sixteen participants were recruited based on the theoretical sampling from the people who referred to ENT or aesthetic clinic and by the people who knew these individuals who underwent Rhinoplasty more than one year ago. Grounded Theory was applied to analyze the data.
Results
All participants experienced a kind of elation so that the "feeling elated" constituted the core concept of the analysis. This feeling was reported to be experienced by all individuals through two main ways including "removing perceived defect" and "having expected beauty". Furthermore, the main process of Rhinoplasty took place in three stages including preoccupation, inquiry, and operation.
Conclusions
The present study had some recommendations to the professionals so that some individuals who prefer the Rhinoplasty should be referred to the psychologist based on some criteria which are mentioned in this study. According to our findings, usually, people from the HEB group need to psychological consultations rather than surgery.
Keywords: grounded theory, Iran, rhinoplasty, qualitative study.

1. Introduction

Cosmetic Rhinoplasty refers to any type of plastic surgery for nasal reconstruction or cosmetic purposes (Zahiroddin et al., 2008). It is the most common cosmetic surgery in Iran so that it has the first rank in the world with about 180 cases per 100000 populations (Loghmani et al., 2017). Low cost and advanced procedures of the surgery (Motamedi et al., 2016), Islamic dress code which is resulted in only face exposure (Mianroodi et al., 2012) and some psychosocial problems such as body dysmorphic disorders, dissatisfaction with body image, low self-esteem and so on (von Soest et al., 2009) are the main reasons for such action which has been mentioned in previous studies. Studies show that cosmetic surgeries are on the upswing while 60 percent of applicants do not need to undergo aesthetic surgery (Yarmohammad Touski., 2015).

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The primary stimulus for individuals to undergo rhinoplasty is more satisfied with their own appearance and improving their psychosocial functioning (von Soest et al., 2009). Several studies have demonstrated that rhinoplasty may improve quality of life (QoL), self-esteem, body image and social interactions (Esteves et al., 2017). A study showed that cosmetic surgery improves body image and self-esteem but it does not have any effect on psychological problems (von Soest et al., 2009). A preliminary study showed that the severity of body image distress may negatively impact on satisfaction with the outcome of the surgery (Di Mattei et al., 2015). A qualitative study in Iran focused on paradigm models to explain conditions, processes, and consequences of Rhinoplasty among the participants (Pournasiri, 2016) but it did not present a deep understanding from the studied phenomenon to explore the core concept so that it focused only on some general categories. Findings from some studies show that there is no relationship between Rhinoplasty and socio-demographic factors except the gender that shows females have more intend to undergo Rhinoplasty because of some reasons like beauty, stay in fashion, show off and so on (Mianroodi et al., 2012).

Iran is located in the Middle East with approximately 80 million inhabitants according to the last census in 2016 (Soltaninejad, 2017). Based on religion and custom, Iranian people should cover their bodies and hair and only can expose their face. Accordingly, some studies argue that women in Iran are more sensitive on their faces which is resulted in reduced preoccupation on the other parts of the body and more intention to cosmetic surgery on the face (Zakeri et al., 2017). Although it seems that Rhinoplasty is necessary for some cases, the high prevalence of that indicates that most operations are not necessary which is resulted in later frequent operations and or a lot of side effects (Yarmohammad Touski., 2015).

Although there have been several quantitative studies of Rhinoplasty and its role in increasing self-esteem, improving body image, quality of life and social interactions (von Soest et al., 2009; Di Mattei et al., 2015; Moghadam et al., 2018; Naraghi et al., 2016) there is a lack in qualitative studies especially in Iran (Pournasiri, 2016). Achieving a deep understanding of the phenomenon from the subjective perspective of the participants and exploration of underlying trends is the main purpose of grounded theory while the current studies show a gap in this field. They mentioned only a series of categories in a static state without a comprehensive expanding the properties and dimensions of the categories in the form of a process.

The aim of this research is to provide a deep understanding of the Rhinoplasty phenomenon. Therefore, the research questions are: how is the experience of individuals who undergo Rhinoplasty? What are the mechanisms and process of Rhinoplasty? How can prevent unnecessary Rhinoplasty?

2. Materials and methods

The study was conducted in the state of Kerman which is located in the southeast of Iran. Theoretical sampling was applied among individuals who referred to ENT or aesthetic clinics or by introducing medical specialists and people who knew such individuals. Inclusion criteria included people who underwent Rhinoplasty for more than one year. Constructing grounded theory by observing maximum and minimum contrast and variation in the sample ensured both developments of properties and dimensions of categories and identification and a better understanding of the phenomenon's processes (Charmaz, 2014).

First of all, a woman who was eligible according to the inclusion criteria was interviewed based on a semi-structured interview by using an interview guide. The interview was done in a calm room without any disturbing factor by a trained interviewer. The interviewer played no role in the treatment process, nor was she a member of data analysis. Each interview initiated with a narrative question such as, "would you please tell me about your experiences with Rhinoplasty?" the interviewer allows the participant to explain her thoughts, feelings, believes and everything else. Then, other questions were posed according to the interview guide. The interview was audiotaped and transcribed in order to start data analyzing. Getting a general understanding of the manuscript required several times reviewing. The initial coding was conducted by using gerunds in order to stick to the data and identify processes. After that, the primary categories and processes and also new questions were derived from the data. Based on this, the characteristics of the next interviewee were identified and some new questions required asking the first participant again. To explore latent aspects of the phenomenon an interview was done with a case that had not well-

proportioned nose but she did not intend to undergo Rhinoplasty. As the interview proceeded, constant comparative method and asking questions from the data (Glaser, Straus, 1967) established analytic distinction and helped to detect the main properties and dimensions of each category. MAXQDA software helped the author to analyze and edit qualitative data. Thus, the primary categories with the same content were further categorized to form a set of relevant topics. In a focused coding process, most analytic or frequent codes were used to facilitate the axial coding process. Here, the core concept and most relevant categories in a dynamic sense were derived from the initial codes gradually. The relating core concept, main categories, and sub-categories in a coherent dynamic sense took place in the axial coding phase. The interviews continued until data saturation, development of main properties and dimensions of the phenomenon as well as variations and processes (Corbin, Straus, 2014). Finally, saturation occurred after 16 interviews. The arithmetic mean of interviews was 48 min duration.

Trustworthiness

Deviant case analysis and using referential adequacy ensured credibility. A transparent description of research steps confirmed conformability. Supporting of interpretations and findings by the data by using a researcher not involved in the research process approved dependability.

Ethical considerations

The ethics committee of Kerman University of medical sciences approved the study with the reference number of 96001040. Interviewees participated in this study voluntarily and verbal information was given to them according to the interview guide. Voice of the participants was recorded with the permission of them and the interviewer explained to participants that they can exit from the study any time they want. Also, the interviews were performed using a number-based identification method.

3. Results

All 16 participants met the inclusion criteria as formulated. The socio-demographic characteristics of the participants are shown in Table 1.

Table 1. Socio-demographic characteristics of the participants

Participant number	gender	age	Marital status	Employment status	Income in year (€)	Type of surgeon	Time since Rhinoplasty (years)
1	Female	38.0	Married	employed	7500	ENT	1.0
2	Female	22.0	Single	unemployed	0.0	ENT	1.5
3	Female	33.0	Married	employed	1750	ENT	5.0
4	Female	32.0	single	employed	3000	Cosmetic surgeon	6.0
5	Female	25.0	Single	employed	3125	ENT	3.5
6	Male	24.0	Single	employed	2500	Cosmetic surgeon	1.5
7	Male	28.0	Married	employed	5000	ENT	1.5
8	Male	20.0	Single	unemployed	0.0	ENT	1.5
9	Male	24.0	Single	employed	8750	Cosmetic surgeon	4.0
10	female	24.0	Single	employed	2500	-----	----
11	male	36.0	Married	unemployed	1800	ENT	3.0
12	male	21.0	Single	unemployed	0.0	Cosmetic surgeon	2.0
13	female	46.0	Married	employed	3200	ENT	12.0

14	female	23.0	Single	unemployed	0.0	Cosmetic surgeon	3.0
15	female	32.0	Married	employed	2600	ENT	6.0
16	male	25.0	Single	unemployed	0.0	Cosmetic surgeon	2.0

Most of the individuals who underwent Rhinoplasty experienced a kind of elation resulted from a change in their face which was named, "feeling elated". This feeling was reported to be experienced by all individuals through two main ways including "removing perceived defect" and "having expected beauty". Furthermore, the main process of Rhinoplasty took place in three stages including preoccupation, inquiry, and operation. In figure 1, the experience of Rhinoplasty has been shown.
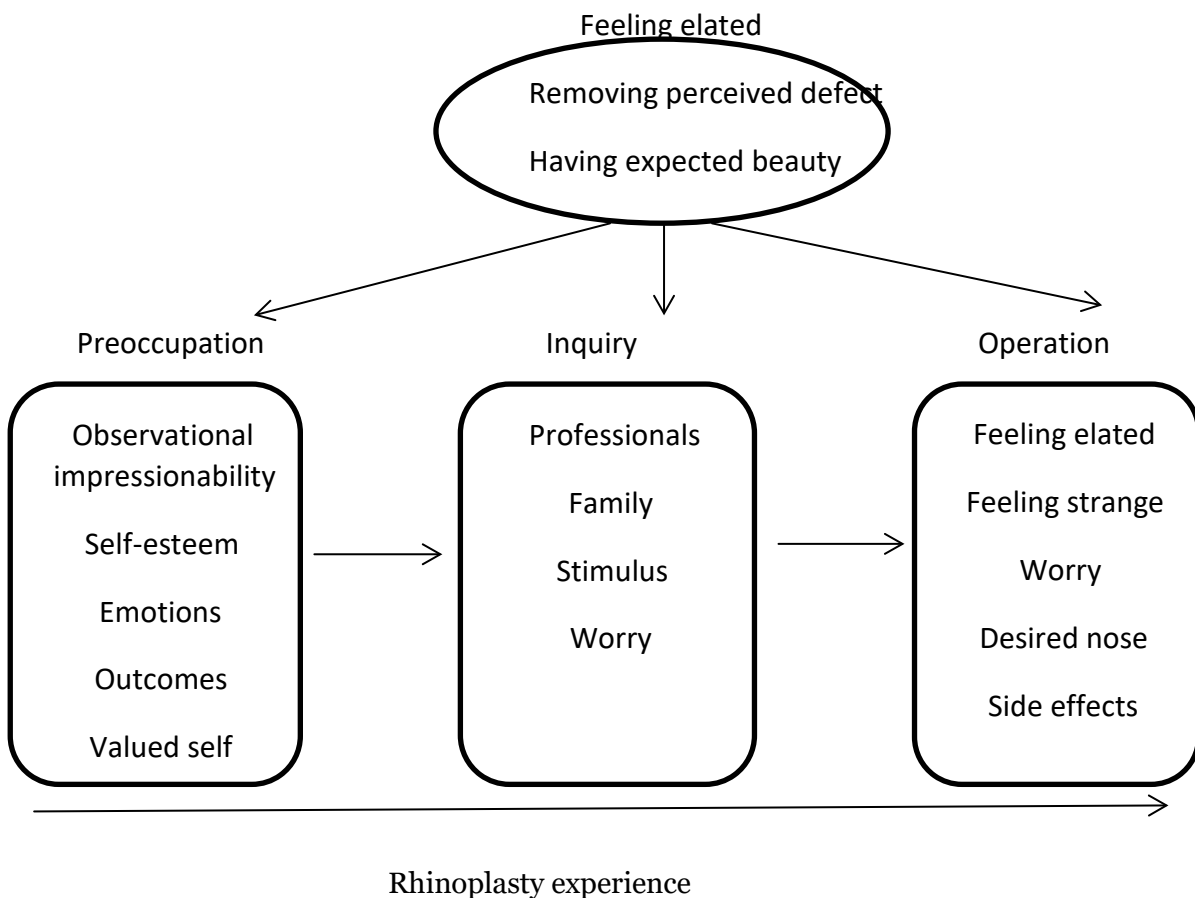

Fig. 1. The experience of Rhinoplasty

Preoccupation stage
This stage has some properties which are different between the two aforementioned groups. Those are listed below.
Observational impressionability
Both people who intend to remove a defect that had been perceived in their nose (RPD) and people who intended to have expected beauty (HEB) are impressed by observing the frequencies and results of operations that have been done by others. The participant Nr. 5 stated, "In recent years, there is a lot of Rhinoplasty in our country thus, I had a sense that I should do Rhinoplasty. That is so. It means the effect of people who did Rhinoplasty was not a few."
Self-esteem
One of the main reasons that occupied the mind of individuals to undergo Rhinoplasty was a lack of self-esteem. Approximately all individuals in the two groups had some complaints regarding

the lack of self-esteem and social interactions. It seems that the men who underwent the Rhinoplasty had lower self-esteem in comparison with the women because all men from two groups indicated the lack of self-esteem while it was more evident among the women who intended to have expected beauty. The participant Nr. 3 expressed, "I did not like my nose form and it had been caused that my self-esteem goes down."

Emotions

Associating emotions with beauty was one of the important distinguishing factors between two groups so that the HEB group associates the beauty with some emotions such as feeling happiness or depression while the RPD group has not such attribution. A man participant declared, "My nose had affected my morale so that I felt depressed. Every time I thought about my nose. I was so irritable. If anyone joking with me, I would get upset soon."

Outcomes

A participant was not going to undergo Rhinoplasty despite not having a well-proportionate nose. One of the main reasons for her was the outcomes of the operation. She said, "I do not think about my nose a lot. If people give me a comment about my nose and doing operation maybe I thought about that for a moment and say to me, no, for a nose, it does not worth undergoing anesthesia… I think about how people are satisfied to do an elective operation despite a lot of risks. I never accept this risk."

Blemishing valued self

It is related to the participant who did not intend to do Rhinoplasty. The participant Nr. 10 stated, "I coped with my face. I like myself rather than change it… I think people who undergo Rhinoplasty are like several fruits that transplant to each other and finally, it is changed into strange fruit… I like myself and I like to stay such without any change."

Inquiry stage

Professionals and people who have already experienced Rhinoplasty

People who want to undergo Rhinoplasty usually inquire about the type of surgeon (ENT or plastic surgeon), quality of operation, financial costs, the shape of the nose (fantastic or natural), probable side effects, anesthesia situation, satisfaction after the operation and so on. Some of the people are ambivalent about doing the operation and the statements of the surgeon encourage them to do that. The participant Nr. 4 stated, "I felt fear and ambivalent about the operation. When I came to the doctor he told me that your nose is not so bad but if you want to do Rhinoplasty, I do it for you and I change it to the form that you intend. The form of doctor's statements was very effective on my decision so that I encouraged doing Rhinoplasty."

Concerning the shape of the nose, the HEB group usually intends to have a preconceived or fantastic nose while the RPD group wants to have a natural nose so that the defects are only removed. The participant Nr. 1 said, "When I went to the doctor I told him that pulls the tip of my nose up. I like the fantastic nose."

Regarding the type of surgeon, quality of operation and financial costs, the author could not find any difference between the two groups so that all participants had approximately a lot of inquiries about these issues.

Family

People who want to do Rhinoplasty sometimes have some challenges with their families. These challenges appear, especially in the inquiring stage. Some families disagree with the operation and declare that it is not a necessary operation and the side effects are more than advantages. But the interesting finding in this part is the differences between the two groups in the face of family satisfaction. The opinion and satisfaction of the family are important for the RPD group and it is important to persuade and make their families agreeable with themselves. While in the HEB group the counseling with the family and getting their opinion is not one of their priorities. A participant Nr. 9 stated, "The … my family told me that for the boy every nose is good. Do not think so much about that. Operation is dangerous… when I found that after 18 years old can do the operation, I gathered my money and with the satisfaction of myself did the operation… In fact, I did not inquire the opinion of my family while I wanted to do Rhinoplasty."

Stimulus

It seems that the RPD group needs stimulus, accompanying or support for doing operation while the HEB group usually does the operation without any support. It is more evident by the

women in comparison with the men. Usually, the women from the RPD group seek support or stimuli like the family, friends, surgeon and so on to do the operation. The participants Nr. 15 expressed, "for a while, I was thinking about the Rhinoplasty but I was ambivalent about that. When I spoke with my family and they did not oppose me, I decided to do Rhinoplasty."

Worry

Although some worries exist in the preoccupation stage, most worries happen in the inquiry stage when the people encounter the comments of the professionals, family and other people who have already experienced Rhinoplasty and or observing some side effects of the people who underwent the Rhinoplasty. One of the considerable findings is regarding the differences between the two groups in terms of worry. Usually, the worries of the RPD group are more than HEB groups in terms of allergy, anesthesia side effects, duration of anesthesia, breathing problems while they worry of HEB group is more about the shape of the nose and immediate side effects such as inflammation, bruise and so on. Because of these worries, the duration of inquiry by the RPD group is more than the HEB group. The participant Nr. 12 declared, "...I was worried about breathing problems and allergy because I heard from a doctor maybe I encounter to some side effects like allergy or breathing problems after operation..."

Operation stage

Feeling elated

It was a ubiquitous statement of the people who underwent Rhinoplasty. Although some participants declare that it is not the shape that they expect or had some complaints about the side effects, they had a satisfaction feeling about the change in their faces. They acknowledged that the shape of their nose is better than before. The participant Nr. 8 expressed, "When I saw myself in the mirror, it was so good. I was satisfied with myself... I liked myself much more than before. I felt so happy..."

Feeling strange or different

It points to the feeling that some participants mentioned regarding the change in their faces. Some of them declared that they had a strange feeling for a few weeks after surgery. This feeling was mentioned much more among the RPD group in comparison with the HEB group. The participant Nr. 3 stated, "When I saw myself in the mirror for the first time I felt my face has changed completely... I felt strange... but this feeling removed gradually. When I saw my prior photos say to me (e) this is me?"

Worry

Like the worries before surgery, there were some worries after surgery. They were often about changes in the shape of the nose or some side effects in the future. According to the statements, it seems that the RPD group is more worried about future side effects and changing the shape of the nose in comparison with the HEB group. The participant Nr. 16 said, "I hope my nose is the same and no change happens in that because I saw a lot of noses that have changed after several years. I hope that does not happen."

Desired nose

People, who intend a special form of the nose, state their dissatisfaction more probable from the nose shape after surgery. Sometimes they do several surgeries on their nose to get an expected nose. Our results show that the people from the RPD group usually are satisfied with the operation but some of the people from the HEB group are not satisfied and intend to do more operation in order to get the desired nose. The participant Nr.1 who did the second operation on his nose declared, "I was crying after the first surgery that why my nose is not fantastic. I did not regret the operation but I intended to be more fantastic. The doctor and my friends gave me consolation that after a few months it gets better... But it was no such a thing that I wanted. Again I asked the doctor and persisted on the repeated operation."

Side effects

All participants had some complaints about short term or long term side effects and doing intensive care during recovery. They included feeling pain, feeling choked, breathing difficulties, headache, bruising, edema, feeling numb in the nose, feeling hanging in the nose, feeling weak and lethargic and so on. The participant Nr. 6 acknowledged, "Generally, it does not worth it. After several months some pains still exist. It is hard. You have something that if you did not operation, you did not have them. For example, I feel numb in my nose. Although my downward nose and breathing problem has been removed, I felt healthier if I did not operate. I always feel hanging in

my nose. The sense that I do not say how is it. You feel something has been attached to your nose and it is changing."

4. Discussion

To our knowledge, it is the first study that explores two main groups that undergo Rhinoplasty and introduce some properties for them. As our results showed although some of the participants were not completely satisfied with their nose shape, feeling elated and a kind of satisfaction was the ubiquitous feeling among all participants because apart from the nose shape they experienced a change in their face towards improving. In line with our findings, several studies confirmed that feeling elated and positive mood is the predominant consequence of Rhinoplasty (Von Soest et al., 2009; Vali-allah et al., 2016; Fathi azar et al., 2014). Also, our findings are inconsistent with a lot of studies that explored that Rhinoplasty has a considerable effect on self-esteem (Hosseinzadeh et al., 2016; Naraghi et al., 2016). Regarding the lower self-esteem of men who undergo Rhinoplasty in comparison to women which was mentioned in our study, a study showed a significant difference between men and women so that the men had lower self-esteem (Hosseinzadeh et al., 2016). A study that investigated the role of social and familial factors in Rhinoplasty showed that mass media especially satellite, family and being recommended by others who underwent Rhinoplasty were the most important factors in stimulating individuals to do Rhinoplasty. It is in accordance with our findings that indicated observation had a primary effect on deciding to do Rhinoplasty (Yarmohammad Touski, 2014; Memarian et al., 2014).

The most striking finding in the current study was identifying two main groups (HEB and RPD) who underwent Rhinoplasty. Distinguishing between these two groups mostly happened through differences between their emotions and the similitudes that they applied. HEB group mostly likened the Rhinoplasty to some emotional statements such as a gift from God and intended to attribute the improvement in moral status to the beauty and changing in their face. The linked psychological problems to beauty while the studies indicated that the level of psychological problems does not change after surgery (Von Soest et al., 2009). A more interesting finding was about preoccupation. The person who did not intend to undergo Rhinoplasty did not have any preoccupation regarding that and the preoccupation of the RPD group was significantly less than the HEB group so that a participant likened the Rhinoplasty to a half-finished work that must be finished. Also, it seems that people who consider changing in face equal to change in self-concept and blemishing valued self does not intend to do Rhinoplasty. It is inconsistent with the studies showed that the Rhinoplasty has no effect on self-concept (Gulbas, 2013; Vali-allah et al., 2016). An explanation is that studies compared the participants in terms of pre and post-operation while in our study the person who believed Rhinoplasty can affect his self-concept did not intend to undergo Rhinoplasty. Further studies in a quantitative manner should investigate this issue.

One of the findings of the present study was the need for support and accompanying of professionals or family by the RPD group in comparison with the HEB group. One reason is because of ambivalent feeling and the degree of worries that is more by the RPD group. In fact, they need to feel confident about their decision and outcomes of the surgery. Thus, they resort to the professionals and their families to get support and accompanying them. It is clear that the RPD group is more worried about the surgery and its side effects. Apart from the amount of worry, the type of that also is different between groups. So that the RPD group is more worried about side effects that are not necessarily related to the nose and predominantly related to the other part of bodies such as breathing problems, anesthesia, and allergy. On explanation is that they are more intending to see Rhinoplasty in a logical way. That is why the duration and type of inquiry are more prominent by them.

All in all, based on the results of the present study professionals and families can prevent unnecessary operations considering the following points. Also, there is a need for psychological consultation instead of Rhinoplasty especially for individuals from the HEB group.

First, the attribution of beauty to the mood is a key point. Whatever this attribution is more powerful, the professional like surgeon should be more cautious about doing surgery. Secondly, having a special form of the nose also is an important issue so that these people also are individuals with a high-risk operation in terms of satisfaction after surgery. This addresses some statements so that some people declare that they want to be like a specific person or having a fantastic nose. Third, people who feel ambivalent about the operation usually need to support, stimulus and

accompanying from the professionals, friends, and family. In fact, to these people, if they do not have any comorbid problems like breathing problems, fractures and so on, it is better to be given awareness about side effects and professionals try to dissuade them from the operation. Forth, attention to the type of worries is important. Usually, the kind of worries by the people who are more proper for the operation is not just on the nose. They have some worries about breathing problems, kind, and duration of anesthesia, allergy and long term side effects while the worries of people who are risky are usually concentrated on the immediate and short term side effects such as edema, bruising, headache and so on.

One of the limitations of the present study is about transferability. As the current study conducted in a confined social and cultural network with a small sample size, a similar project with a qualitative method in different societies and cultures should be conducted to prove the transferability of the findings. Also, five of the 16 participants were recruited via the medical staff. Although this limits the transferability and credibility of the findings, by observing the heterogeneity in the sample size, the authors tried to increase credibility.

5. Conclusion

Results of the study demonstrated that the Rhinoplasty has a three-stage process including preoccupation, inquiry, and operation. The core concept was feeling elated which is made through removing the perceived defect (RPD) or having expected beauty (HEB). According to our findings, usually, people from the HEB group need to psychological consultations rather than surgery.

6. Conflicts of interest

The authors report no conflicts of interest.

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