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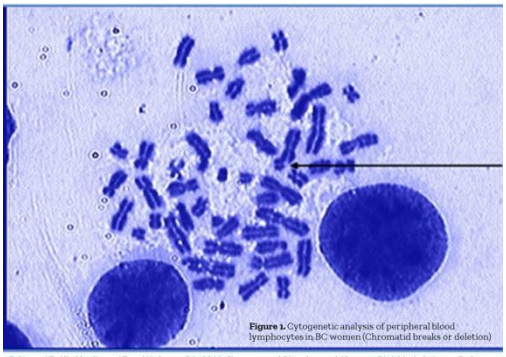
Volume 8 (1); January 25, 2018

Research Paper

Chromosomal Disorders and Aberrant DNA Methylation as Early Biomarkers of Breast Cancer Risk in Young Women.

Zakirova LT, Alimkhodjaeva LT and Kadyrova DA.
J. Life Sci. Biomed., 8(1): 01-05, 2018;
pii:S225199391800001-8



Abstract
Genetic instability is an early and constant characteristic of tumor cells. Chromosomal aberrations and epigenetic anomalies are the factors leading to genomic instability. This study aimed to investigate the relationship between chromosomal disorders and aberrant DNA methylation as strong biomarkers in early diagnostics of breast cancer in young women. The research was conducted in 20 patients, and involved 15 young females with breast cancer at stages T2-4N0-3MO (histologically confirmed). Cytogenetic tests of lymphocytes of females with breast cancer (BC) revealed chromosomal abnormalities expressing as deletions, iso-locus deletion of chromosomes and gaps. Activity of the DNA methyltransferase (DNA MTase) in BC is shown to rise up to 58%, in comparison with the normal indexes. Conclusion: Cytogenetic analysis of lymphocytes in BC women has revealed chromosomal abnormalities in the form of deletions, isolation of chromosome deletions and gaps. It has been shown that in BC, the activity of DNA methyltransferase is increased by 58%, compared with the normal indexes.

Keywords: Chromosomal aberrations, DNA methyltransferase, Breast cancer, Early diagnosis, Iso-locus deletion, Epigenetic control, DNA methylation, Histone modifications

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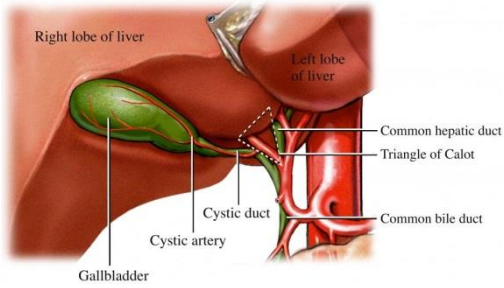
Research Paper

Endoscopic Interventions in Patients with External Biliary Fistulas Caused by Iatrogenic Injuries of Biliary Tracts.

Turakulov UN.
J. Life Sci. Biomed., 8(1): 06-09, 2018;
pii:S225199391800002-8



Abstract
The aim of this study was to evaluate the frequency and severity as well as to define the best treatment option for patients with iatrogenic injuries. The article presents the analysis of surgical treatment of 49 patients with external biliary fistulas (EBFs) caused by iatrogenic injuries of anhepatic bile-ducts. The causes of strictures and external biliary fistulas formation were intra-operative injuries during cholecystectomy, gastric resection and echinococcectomy. Successful results were achieved in 43 (87.6%) cases using endoscopic transpapillary elimination of external biliary fistulas. Endoscopic manipulations promote the relief of clinical manifestations of CBD cicatrical stricture and provide the choice of the optimal reconstructive surgery.

Keywords: Hepatic Bile Ducts, External Biliary Fistula, Iatrogenic Injuries, Cicatrical Strictures, Diagnostics, Treatment.

[Full text-PDF] [XML]

Research Paper

Antioxidant Activity of Protein Fractions Derived from *Acrochaetium sp.* (Rhodophyta) Enzymatic Hydrolysates.

Windarto S, Nursyam H, Hsu J-L, Lee M-Ch.
J. Life Sci. Biomed., 8(1): 10-18, 2018;
pii:S225199391800003-8

Abstract
Natural antioxidants are helpful in the prevention of human diseases. The objective of this study is to isolate the potential protein fractions from *Acrochaetium sp.* as an antioxidant. Fractions were obtained by proteolytic digestion using α-chymotrypsin, pepsin, trypsin, thermolysin individually and in combination of two enzymes, then centrifuged using 3 kDa molecular weight cut-off (MWCO) ultrafiltration membrane and fractionated by reversed-phase high performance liquid chromatography (RP-HPLC). The 2,2-Diphenyl-1-picrylhydrazyl free radical (DPPH) assay was used to measure the antioxidant activity. Result showed that thermolysin hydrolysate and the combination of trypsin-thermolysin hydrolysates had the highest antioxidant activity compared to the other hydrolysates with IC_{50} value of 1.48±0.92 mg/mL and 1.37±0.84 mg/mL after fractionated using 3 kDa MWCO ultrafiltration membrane. Fractionation using RP-HPLC resulted fraction 7 obtained from thermolysin hydrolysates showed the highest antioxidant activity with IC_{50} value 0.58±0.56 mg/mL and fraction I obtained from trypsin-thermolysin hydrolysates showed the highest antioxidant activity with IC_{50} value 0.38±0.33 mg/mL. The protein fractions from *Acrochaetium sp.* hydrolysates as antioxidant still has not been reported previously, therefore it can indicated as a potential therapeutic source for reducing oxidative stress.

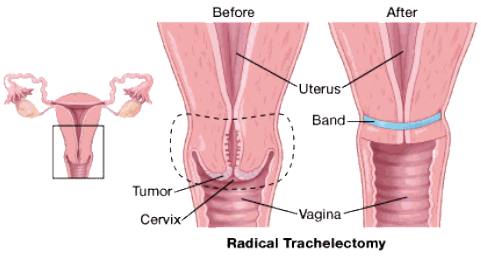
Keywords: Acrochaetium sp., Antioxidant, DPPH, Enzymatic hydrolysates, Fractions, RP-HPLC

[Full text-PDF] [XML]

Research Paper

Modified Radical Abdominal Trachelectomy in Cervical Cancer in Young Women.

Navruzova V.S.
J. Life Sci. Biomed., 8(1): 19-23, 2018;
pii:S225199391800004-8



Abstract
A modification of traditional fertility-sparing abdominal radical trachelectomy (ART) has been developed to reduce the opportunity for intra-operative injuries to occur through better management of the surgical field. The technique is similar to the standard abdominal radical trachelectomy. The ART modification developed by us enables to perform total or partial resection of the affected part of the uterine cervix after total mobilization of the cervix and excision of the upper and middle parts of the vagina. We have performed 204 modified fertility-sparing ARTs for CC women of reproductive age (27 to 37 years) at the early stage of the disease (T1A, T1B). On average the surgery lasted 140 ± 28.7 min, blood loss was 420 ± 50 ml. Epithelization of the uterine stump after surgery lasted 5 - 8 weeks. No intra-operative injuries of the nearby organs occurred. The follow-up period has lasted for 42 months. Oncological outcomes. No patient had CC recurrence and metastasis (till 42 months after the first surgery).

Keywords: Cervical Cancer, Squamous Cells, Dynamic Monitoring, Fertility-Sparing Surgery, Abdominal Radical Trachelectomy, Quality of Life

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Chromosomal Disorders and Aberrant DNA Methylation as Early Biomarkers of Breast Cancer Risk in Young Women

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ABSTRACT

Genetic instability is an early and constant characteristic of tumor cells. Chromosomal aberrations and epigenetic anomalies are the factors leading to genomic instability. This study aimed to investigate the relationship between chromosomal disorders and aberrant DNA methylation as strong biomarkers in early diagnostics of breast cancer in young women. The research was conducted in 20 patients, and involved 15 young females with breast cancer at stages T2-4N0-3MO (histologically confirmed). Cytogenetic tests of lymphocytes of females with breast cancer (BC) revealed chromosomal abnormalities expressing as deletions, iso-locus deletion of chromosomes and gaps. Activity of the DNA methyltransferase (DNA MTase) in BC is shown to rise up to 58%, in comparison with the normal indexes. Conclusion: Cytogenetic analysis of lymphocytes in BC women has revealed chromosomal abnormalities in the form of deletions, isolation of chromosome deletions and gaps. It has been shown that in BC, the activity of DNA methyltransferase is increased by 58%, compared with the normal indexes.

Original Article
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Keywords
Chromosomal aberrations,
DNA methyltransferase,
Breast cancer,
Early diagnosis,
Iso-locus deletion,
Epigenetic control,
DNA methylation,
Histone modifications,

INTRODUCTION

Breast cancer (BC) is the most common form of malignancy in women and the leading cause of female cancer death. Despite the fact that BC is more common at the age of 55-65 years, recently the worldwide trend has developed towards BC higher incidence in young women. The highest morbidity is reported at the age of 32 - 38 years, i.e. during the active reproductive period [1-4].

The problem of early diagnosis of the tumor development, primary BC prevention, i.e., anticipation of conditions that lead to functional and morphological prerequisites to the onset of dysplasia viz mammary gland precancer, remains an urgent one. To solve the problem of early diagnosis, some reliable and simple methods of the tumor detection at preclinical stages are needed.

Genetic instability is an early and permanent hallmark of tumor cells [5]. Such disorders of the genetic apparatus of cells as chromosomal aberrations and epigenetic abnormalities are the factors leading to genomic

instability. Chromosomal abnormality is one of early genetic disorders resulting in the induction of the cell genome instability and, as a consequence, its malignant transformation. The pattern of neoplastic cells methylation changes significantly in comparison with normal cells; total demethylation is accompanied by an increase in the activity of DNA methyltransferase DNA MTase and local hypermethylation of CpG islands. The mechanism of local hypermethylation is not completely clear. Apparently, the important role in this process is played by an increase in methyltransferase activity [6, 7]. The interrelation of genes, chromosomal and epigenetic disorders in induction of genomic instability in the development of BC is of great importance [8].

MATERIAL AND METHODS

During the research, DNA samples obtained from peripheral blood leukocytes of BC patients (15 females) were used. The blood of BC patients was received at the Department of Mammalogy of the National Center for Cancer Research of the Ministry of Health (MoH) of Uzbekistan. As a control, DNA from peripheral blood leukocytes taken from healthy donors was used (10 donors). Generally accepted clinical and morphological prognostic criteria were evaluated: the tumor histological type, tumor receptor status, HER2/neu expression. All of them were studied using the biopsy material.

Ethical approval

The review board and ethics committee of National Center for Cancer Research under the MoH Tashkent, Uzbekistan AND Bioorganic Chemistry Institute named after A.S. Sadyko. Tashkent, Uzbekistan. Academy of Sciences, Tashkent, Uzbekistan approved the study protocol and gave permission.

Extraction of eDNA from serum / plasma

One ml of peripheral blood taken from the ulnar vein was transferred to plastic tubes with Na_2-EDTA sprayed. The blood was centrifuged at 40 °C sequentially at 1500 rpm for 10 minutes, at 3000 rpm for 15 minutes, at 5000 rpm for 15 minutes. After centrifugation, 400 µl of blood serum were taken from the tubes and transferred to new sterile tubes. The serum was pretreated with the RNA (100 µg/ml), incubated at 37 °C for 1 h, then resuspended with proteinase K (50 µg/ml), incubated for 1 hour at 37 °C. After enzymatic treatment, the blood serum was added to 200 µl of lysis buffer (100 mM Tris- HCl, pH 8.0; 25 mM EDTA, pH 8.0; 0.15 M NaCl; 0.7 M β-Mercaptoethanol; SDS) to a final concentration of 2%. The lysis was carried out in the cold for 3 minutes (on ice). The aliquots were deproteinized for 15 minutes in 1.5 ml phenol / chloroform mixture (1: 2) followed by 15 min. centrifugation at 5000 rpm at 40 °C. The supernatant was transferred to new test-tubes, 1/10 volume of 3M sodium acetate pH 5.2 was added, as well as 2.5-volume of cooled 96% ethanol, and the tubes were left overnight at -200 °C. The denatured eDNA preparations were centrifuged at 5000 rpm for 30 min at 40°C. The eDNA precipitate was washed in 1 ml with cooled 70% ethanol for 15 minutes, and then centrifuged at 13.000 rpm for 15 min at 40 °C, then it was dried in vacuum desiccator for 15 minutes and dissolved in 300 µl of TE buffer, pH 8.0 and stored at -200 °C. The eDNA aliquots were analyzed in 2% agarose gel containing 0.5 µg/ml ethidium bromide. Electrophoresis was conducted for 1 hour at 100V; the gel was photographed in UV rays.

Cultivation of lymphocytes

The culture medium consisting of (per vial): 6 ml of RPMI 1640 medium with glutamine (PANECO, Russia), 1 ml of fetal bovine serum (produced in France-Germany), 40 µg/ml gentamicin and 20 µg/ml mitogen - phytohemagglutinin (PHA DifcoP) was added to 0.8 ml of whole blood. The prepared cell culture was incubated in thermostat at 370°C and was periodically shaken gently (1-2 times per day). This procedure prevents excessive agglutination of erythrocytes. The cultivation time under the experimental conditions was 72 hours. The cells were fixed for 72 hours after the initiation of cultivation. Two hours prior to fixation, colchicine (0.4 µg/ml) was injected into the culture medium that destroyed the spindle microtubules and prevented chromosome divergence. In consequence, the cellular mitosis stopped at the metaphase stage. The cultured cells suspension was poured into centrifuge tubes, centrifuged at 1000 rpm for 7 minutes. Then, the supernatant was removed, the precipitate was shaken, 7 ml of hypotonic solution 0.075 1M KCl pre-heated to 37 °C were added. After that the tubes were again placed in thermostat for 20 minutes. Hypotonic treatment is carried out for the best spread of chromosomes of lymphocytes. After the end of hypotonic treatment of the cells, they were fixed in three stages. At the first stage, the cell suspension, after treatment in KCl hypotonic

solution in thermostat, was centrifuged at 1000 rpm for 7 minutes, and then the supernatant was removed, leaving about 0.5 ml of hypotonic solution above the cell precipitate. After precipitate shaking, 8 ml of freshly prepared cold fixative solution, consisting of 3 parts of ethanol and 1 part of glacial acetic acid, were added to the cell suspension, and then placed into refrigerator (at 60 °C) for 20 min. Then, in the same way, the second stage of fixation was carried out. After this, the third and last stage of fixation was carried out similarly to the second stage. After the last fixation, the cells were pelleted by centrifugation (1000 rpm p/m) for 7 minutes and re-suspended in a small volume of the fresh fixative (0.5 ml). The suspension of the cultured cells was applied on wet cold glass slides by dropping. To do this, the cell suspension was dropped from 35 to 40 cm height with a Pasteur pipette onto the surface of the slides, which were then dried in the air. The preparations were stained with 4% Romanovsky-Giemsa stain. The analysis was carried out at the metaphase stage.

Determination of the methylating enzyme activity

The incubation mixture (130 µl) contained 5 µg of DNA, 20 µl of 3HSAM, 5 µl of methyltransferase, 50 µl of phosphate buffer containing 10 mM Na_2HPO_4 pH 7.5. The samples were incubated for 18 hours at 37 °C. The DNA samples were precipitated in 10% ice-cold TCA and applied to pre-moisten 5% TCA CF/C filters. The filters were washed with 50 ml of 5% TCA and 40 ml of ethanol. Radioactivity of precipitates on the filters was counted in Mark III liquid scintillation counter. With an increase in the amount of the enzyme in the incubation mixture, the amount of DNA proportionally increased. To control the maximum level of DNA methylation, the reaction was carried out till reaching the plateau. The methylated DNA was incubated for 40 min. at 600 °C in 0.5 NaOH solution, the DNA was centrifuged for 20 min. in "Beckman" centrifuge, and the DNA was precipitated.

RESULTS AND DISCUSSION

The research has revealed some specific features of cytogenetic disorders causing the development of genomic instability in BC. For this purpose, cytogenetic analysis of peripheral blood lymphocytes of young women with BC was performed. Cultivation of lymphocytes was carried out by the modified method of McGregor and Marley [9].

In the cell cultures at the metaphase stage in healthy women, the rearrangement was found in 0.47 - 0.56%. A slight increase in the incidence of these disorders was observed in two patients (0.66 and 0.88%, respectively). Other patients showed from 1.06 to 3.44% (almost 7 times higher than the controls).

The following types of aberrations were found: terminal single deletions occurred in 7 of 8 females (it did not occur in healthy women); isolation of chromosome deletions occurred in 4 of 8 patients; gaps were found in 3 patients.

Table 1 presents the results of cytogenetic analysis of peripheral blood lymphocytes of women with BC. They include single deletions, isolation of chromosome deletions, and gaps. The table demonstrates that the number of common chromosomal rearrangements in women suffering from BC is much higher than in healthy women.

Summarizing the results of cytogenetic analysis, it can be assumed that the occurrence of cells with chromosomal aberrations, i.e. cells with stable disorders in chromosomes in the form of deletions and insertions, is considered a sign of tumor formation process. Genetic disorders result in genomic instability that leads to acceleration of malignant processes and development of a neoplasia process.

In order to understand the cause of simultaneous hypermethylation and hypomethylation of DNA in breast cancer, we have studied the activity of DNA methyltransferase in DNA of normal and tumor cells. To that end in view, eDNA was isolated from the blood plasma of healthy women and women with BC.

For methylation, the eDNA molecules were treated with DNA methyltransferase enzyme. The change in the activity of methyltransferase in BC was revealed (Figure 2) shows the curves reflecting changes in normal activity and those ones in BC. The Figure demonstrates that in BC, methyltransferase activity increases by 58% compared with the norm. Molecular mechanisms of enhanced expression of DNA methyltransferase in tumor cells have not been elucidated. Apparently, this can be a compensatory response of the cell to general demethylation. The increase in methyltransferase activity significantly affects both the profile of DNA methylation and local hypermethylation.

Table 1. Types of chromosomal aberrations in young women with breast cancer

Patients	Number of metaphases studied	Metaphases with rearrangements %	Types of chromosomal aberrations		
			Single terminal deletions, %	Isolation of deletions without fusion ,%	Gaps, %
1.	196	2.55 ± 1.01	1.53 ± 0.69	1.02 ± 0.72	-
2.	250	0.8 ± 0.56	0.8 ± 0.56	-	-
3.	174	3.44 ± 1.4	2.87 ± 1.2	0.57 ± 0.06	-
4.	156	2.56 ± 1.2	1.28 ± 0.9	0.64 ± 0.06	0.64 ± 0.06
5.	151	0.66 ± 0.07	-	0.66 ± 0.07	-
6.	166	3.01 ± 1.34	2.41 ± 1.2	-	0.6 ± 0.06
7.	188	1.06 ± 0.75	1.06 ± 0.75	-	-
8.	193	2.6 ± 1.1	2.7 ± 1.03	-	0.56 ± 0.05
9. (control)	212	0.47 ± 0.05	-	0.47 ± 0.047	-
10. (control)	178	0.56 ± 0.06	-	-	0.56 ± 0.06

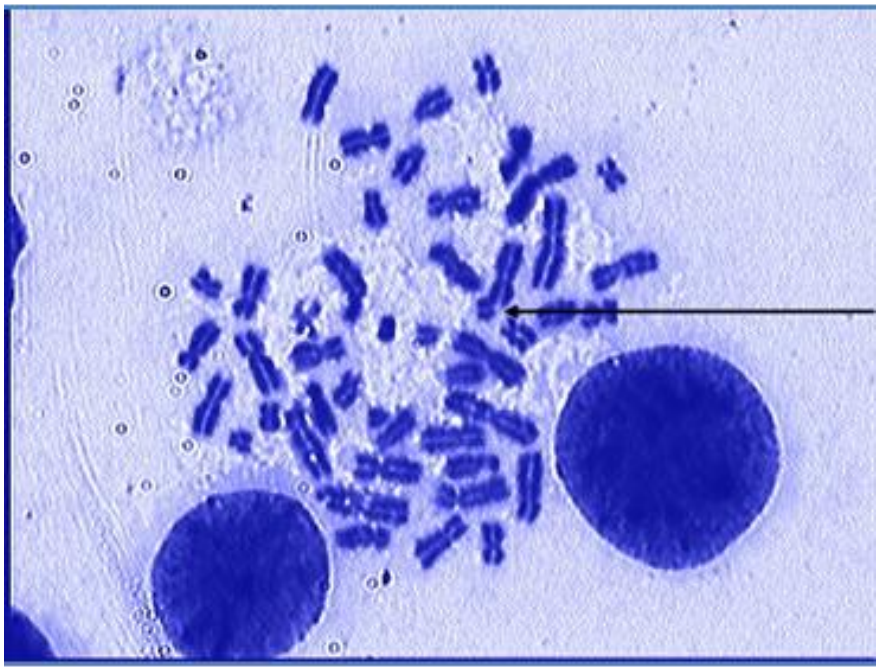

Figure 1. Cytogenetic analysis of peripheral blood lymphocytes in BC women (Chromatid breaks or deletion)

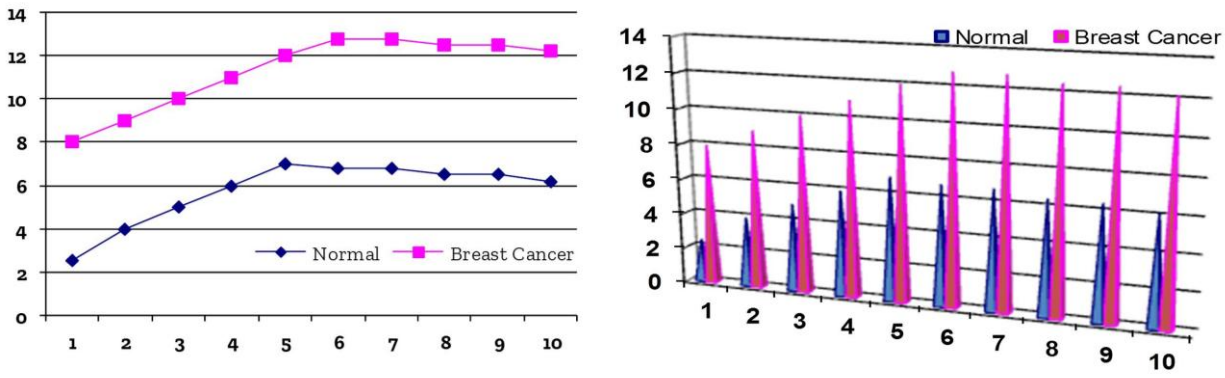

Figure 2 (A and B). Activity of DNA methyltransferase in healthy donors and BC women

CONCLUSION AND RECOMMENDATION

Cytogenetic analysis of lymphocytes in BC women has revealed chromosomal abnormalities in the form of deletions, isolation of chromosome deletions and gaps. It has been shown that in BC, the activity of DNA methyltransferase is increased by 58%, compared with the norm.

Determination of molecular markers allows us to identify a group of patients with an increased risk of suffering, early BC. But, it is not subject to preventive chemotherapy, and to assess the sensitivity to a particular type of systemic therapy, and for the purpose of individualization. Also, the importance of molecular markers can be used to develop new modern drugs, acting as a target for these molecules. Molecular-biological markers, determined in tumor tissue, make it possible to characterize the tumor with respect to: sensitivity to hormone therapy and targeted therapy, as well as a tendency to invasion and metastasis.

DECLARATIONS

Authors' Contributions

All authors contributed equally to this work.

Acknowledgements

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Competing interests

The authors declare that they have no competing interests.

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Endoscopic Interventions in Patients with External Biliary Fistulas Caused by Iatrogenic Injuries of Biliary Tracts

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ABSTRACT

The aim of this study was to evaluate the frequency and severity as well as to define the best treatment option for patients with iatrogenic injuries. The article presents the analysis of surgical treatment of 49 patients with external biliary fistulas (EBFs) caused by iatrogenic injuries of anhepatic bile-ducts. The causes of strictures and external biliary fistulas formation were intra-operative injuries during cholecystectomy, gastric resection and echinococcectomy. Successful results were achieved in 43 (87.6%) cases using endoscopic transpapillary elimination of external biliary fistulas. Endoscopic manipulations promote the relief of clinical manifestations of CBD cicatrical stricture and provide the choice of the optimal reconstructive surgery.

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Keywords
Hepatic Bile Ducts, External Biliary Fistula, Iatrogenic Injuries, Cicatrical Strictures, Diagnostics, Treatment.

INTRODUCTION

Materials of numerous international scientific conferences of hepatopancreatobiliary surgeons which have been hold for recent years attest the relevance of diagnostics and treatment issues of external biliary fistulas (EBF) [1-4]. The questions about errors, dangers, complications and prevention of EBF were always acute and topical. No one, even the most qualified surgeon cannot be totally guaranteed against errors and complications [1, 2, 5-7, 9]. The acuity of EBF is caused by the disease duration and by the development of serious complications such as obstructive jaundice, purulent cholangitis, biliary cirrhosis, portal hypertension and hepatic failure. In majority of cases such type of patients are performed recurring surgeries but the lethality remains high – up to 8-40 % [2]. The main causes of EBF occurrence are surgeries on biliary tracts, liver, stomach and duodenum. Iatrogenic injuries of biliary tracts lead to EBF formation in 40.7 – 43.1% of cases. Residual choledocholithiasis, stenosis of major duodenal papilla or their combination lead to EBF in 25.9 – 26.4% of cases [4-7, 8].

Iatrogenic injuries are a predominant factor of hepaticocholedoch cicatrical strictures formation and they are the causes of EBF development in 82-98% of cases [6]. The frequency of bile ducts injuries makes up 0.2-2.8% from the general quantity of surgeries on the biliary system and lethality after reconstructive-restorative interventions amounts 15-50% [3, 8, 10]. A particularly difficult task is to restore an adequate natural passage of bile at high EBF, at the level of the lobar hepatic ducts. As a rule, it is connected with gross violations of topographic and anatomical relationships in the hepatobiliary zone, massive inflammatory, adhesive and cicatrical processes, a severe general condition of patients caused by recurrent purulent cholangitis [1, 4].

Surgical treatment of patients with EBF has achieved a significant success, but complications associated with stenosis of bioliodigestive and biliobiliary anastomosis are developed in 4.5-25% of cases after reconstructive operations on the biliary tracts [4]. The choice of surgeries for this pathology is a matter of debates and the results cannot be considered as satisfactory ones. An intent study of possibilities of new method in the biliary tracts surgery - endobiliary stenting - has been still going on. The problem of further improvement of diagnostic methods and surgical treatment of EBF after iatrogenic injuries of hepaticocholedoch remains relevant. A comparative analysis of the reconstructive surgeries results at EBF is an important help for further improvement of diagnostic methods and surgical treatment of this pathology.

Objective of this investigation is to improve the surgical treatment results of the EBF after iatrogenic injuries of anhepatic biliary tracts with the use of endoscopic technologies.

MATERIAL AND METHODS

Endoscopic treatment as an independent method has been attempted in 49 patients with iatrogenic cicatrical strictures and external biliary fistulas. The important place in the diagnostics of intraoperative injuries, in cicatrical strictures and EBF belongs to endoscopic retrograde pancreatocholangiography (ERPHG). Polypositional fistulography is also informative in recognition of the pathology and at the choice of tactics for its elimination. We performed 49 ERPHG. A subsequent endoscopic papillosphincterotomy (EPST) was performed in 41 patients. Control ERPHG after EPST was performed in 34 patients. Patients with EBF on the background of bile ducts cicatrical stricture were performed bougienage of the stenotic segment by biopsy forceps in the closed and open forms in the combination with the local diathermocoagulation of hard-to-cure scar segment. Then they were performed stenting of the cicatrical strictures area.

Ethical approval

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

RESULTS AND DISCUSSION

The causes of cicatrical strictures and fistulas formation according to our observations were: bile ducts injuries and their inadequate drainage after cholecystectomy (90.2%), the stomach resection (7.0%) and complications of echinococcectomy (2.8%). In total we performed 49 attempts of endoscopic correction of adequate bile passage through anhepatic bile ducts in patients with stenosis and external biliary fistula (Table 1).

Table 1. An efficiency of bile ducts strictures endoscopic treatment subject to their site level

Stenosis level	Treatment		Treatment		Total	
	abs	%	abs	%	abs	%
Distal part	18	41.9	2	33.3	20	40.8
Middle part	19	44.2	2	33.3	21	42.9
Proximal part	6	13.9	2	33.3	8	16.3
Total	43	87.6	6	12.2	49	100

Endoscopic treatment was performed in 43 patients. In 6 patients we did not manage to insert a conductor through the stenosis zone; in 2 patients it was not possible to install instruments (for the probing) through the conductor or to introduce endoprosthesis.

EPST was performed as a stage of preparation for reconstructive surgery in 22 patients with a combination of cicatrical stricture with stenosing papillitis of the major duodenal papilla and residual calculus which were located below the stricture. 23 endoscopic transduodenal stentings of the external bile ducts stenotic area after primary surgical interventions were particularly singled out. Hepaticocholedoch strictures which were the cause of the EBF were found in all cases. We used the classification of E.I.Galperin at describing the level of hepaticocholedoch stricture [1].

In 6 cases the stricture was located in the confluence zone and had a critical nature which was consisted of the obstructive jaundice progression. The direct bilirubin in these patients was from 200 to 300 μmol/l. In 2 patients we observed initial signs of a hepatic failure in the form of encephalopathy, a decrease of albumin levels

below than 30 g/l, a decrease in the prothrombin index below than 82%. We rated the patient's condition as a second class according to the Child-Pugh scheme. The severity of patient's condition besides the obstructive jaundice, in 63.3% of cases was stipulated by purulent cholangitis, hepatic failure. A partial stricture of hepaticocholedoch was revealed in all cases.

In 19 cases the obstruction was located in the fusion zone of the bile duct with the bladder duct. Such position of the defect, in our opinion, is most typical, especially at the stage of the laparoscopic method mastering. All patients in this group were diagnosed promptly and appropriate interventions were performed. In 18 cases an obstruction to the bile outflow was located in the distal part of the choledoch. Exactly in such cases doctors of general surgical departments encounter the problem of recurring interventions. The content of direct bilirubin in these patients was from 300 to 390 μm. They were timely hospitalized to our hospital before the development of liver failure. The endoscopic method including the probing of the stenotic segment in combination with local diathermocoagulation of the hard-bouging cicatricial segment we managed to restore the patency of the hepaticocholedoch and to perform stenting of the stenotic segment and it led to patients' recovery and their discharge after 6-8 days.

The efficiency of drainage was estimated by endoscopic criteria, clinical condition of patients and also by laboratory indicators. Improvement of general condition, appearance of appetite, staining of feces and reduction or disappearance of skin itching were clinical signs of an effective drainage.

During the first three days the activation of patients, a decrease or complete termination of the bile secretion from the external fistula, normalization of the temperature, a decrease in the intensity of icteric staining of the skin and urine were objectively noted. There was a decrease in the level of total and direct bilirubin, normalization of alkaline phosphatase levels, enzymes in laboratory indicators.

Our observations showed that endoscopic treatment was effective almost in all patients with distal stenosis of the bile duct, in 19 from 21 patients with stenosis of the hepaticocholedoch middle part, the effectiveness of endoscopic treatment had been almost the same for these 2 groups of patients. In the treatment of proximal stenosis the success has been achieved in 6 from 8 cases and it differs both from the results of distal stenosis treatment and from the results of middle third stenosis drainage. Thus, the prospect of endoscopic treatment are determined by the localization of the cicatrical process and are less effective in patients with proximal strictures of common bile ducts.

In order to prevent the incrustation of the drainage tube a constant intake of deoxycholic acid drugs was prescribed. It should be noted that complications associated with stents of external bile ducts were not observed. The stents were extracted during duodenoscopy at different periods (from 6 to 10 months). It must also be remembered that endoscopic manipulations can cause a number of complications: duodenal injury, hemorrhage, exacerbation of cholangitis, pain syndrome and pancreatitis. Acute pancreatitis was developed in 18.4% of patients and it was stopped by conservative drugs and in 10.2% - hemorrhage from the EPST zone after endoscopic interventions. Hemorrhage was stopped by the electrocoagulation method [10]. There were no mortality outcomes in this group. Two months after the discharge 12 patients addressed with the signs of restenosis and they were performed a repeated endoscopic dilation. In 7 patients the biliostents independently emerged into the lumen of the intestine after 3 months, but the bile passage remained satisfactory. There is a stable remission at a small extent of stricture and with a greater extent of stricture and the CBD restriction had been noted by the 6^{th} month which required recurring interventions. In 9 patients with a stricture length more than 0.5 cm endoscopic manipulations were ineffective and they were performed reconstructive surgeries. Only 1 from 13 patients who were undergone balloon dilatation without biliostents had a relatively stable improvement, the rest of them were performed double or triple repeated dilation without any effect. They were performed reconstructive surgeries six months later.

CONCLUSION

Thus, the surgery of the external biliary fistula presents great difficulties. The choice of reconstructive and restorative surgeries depends on many factors. The level of fistula, its shape and direction, the cause, the nature of concomitant pathology are distinguished among them. Complex preoperative diagnostic results based on which the surgeon can carefully weighs indications or contraindications to some method of intervention are considerably significant. The use of transduodenal biliostenting makes it an alternative to the complex reconstructive interventions and creates the prospects for improving the treatment results of such complicated pathology as external biliary fistulas. The efficiency analysis of endoscopic treatment of anhepatic bile ducts

iatrogenic strictures showed that regardless to the impressive number of unsatisfactory results, this technique had the advantage of being a stage treatment and under certain conditions provided the treatment of purulent cholangitis and obstructive jaundice. This circumstance is very important at determining further surgical tactics in patients with severe degree of obstructive jaundice and purulent cholangitis. Endoscopic manipulations promote the relief of clinical manifestations of CBD cicatrical stricture and provide the choice of the optimal reconstructive surgery.

DECLARATIONS

Authors' Contributions

All authors contributed equally to this work.

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Competing interests

The authors declare that they have no competing interests.

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Antioxidant Activity of Protein Fractions Derived from *Acrochaetium sp.* (Rhodophyta) Enzymatic Hydrolysates

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ABSTRACT

Natural antioxidants are helpful in the prevention of human diseases. The objective of this study is to isolate the potential protein fractions from *Acrochaetium sp.* as an antioxidant. Fractions were obtained by proteolytic digestion using α-chymotrypsin, pepsin, trypsin, thermolysin individually and in combination of two enzymes, then centrifuged using 3 kDa molecular weight cut-off (MWCO) ultrafiltration membrane and fractionated by reversed-phase high performance liquid chromatography (RP-HPLC). The 2,2-Diphenyl-1-picrylhydrazyl free radical (DPPH) assay was used to measure the antioxidant activity. Result showed that thermolysin hydrolysate and the combination of trypsin-thermolysin hydrolysates had the highest antioxidant activity compared to the other hydrolysates with IC_{50} value of 1.48±0.92 mg/mL and 1.37±0.84 mg/mL after fractionated using 3 kDa MWCO ultrafiltration membrane. Fractionation using RP-HPLC resulted fraction 7 obtained from thermolysin hydrolysates showed the highest antioxidant activity with IC_{50} value 0.58±0.56 mg/mL and fraction I obtained from trypsin-thermolysin hydrolysates showed the highest antioxidant activity with IC_{50} value 0.38±0.33 mg/mL. The protein fractions from *Acrochaetium sp.* hydrolysates as antioxidant still has not been reported previously, therefore it can indicated as a potential therapeutic source for reducing oxidative stress.

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***Acrochaetium sp.*, Antioxidant, DPPH, Enzymatic hydrolysates, Fractions, RP-HPLC**

INTRODUCTION

The key cause of the pathogenic disorders and various chronic diseases is oxidation. The oxidative reaction is not only deteriorates the quality of food products, but also lead to various chronic diseases such as hypertension, cancer and Parkinson's disease. Cellular damage is caused by the high level of oxidative stress due to significant imbalance between the antioxidant defense system and free radicals [1, 2]. Free radicals attacks on protein, lipids and nucleic acids which lead to weakening of the antioxidant enzymes and lipid peroxidation [3]. The easiest way to prevent these diseases from human body is consume vegetables, seed, and fruits to increase the antioxidant capacity in human body. An antioxidant is a substance which inhibits oxidation of the substrate at low concentration compared to that of an oxidizable substrate [4]. Antioxidants are widely applied to medicine, chemical industries, and important food additive which are mainly used to prevent the oxidation of

fats and also avoid nutrition of food damaging, browning and fading by capture and neutralize the free radicals [5].

Currently, synthetic antioxidants such as butyl hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ) and propyl gallate (PG) are added to food products to retard lipid oxidation, thus inhibit the generation of reactive oxygen species (ROS). The synthetic antioxidants must be used under strict regulation due to their potential health hazards and when compared to natural antioxidants, natural antioxidants are more favored in the present life because of their pure nature, high security, non-toxicity and have strong antioxidant capacity [6, 7]. Therefore, there is an interest in developing natural antioxidants.

Recently, more studies have been carried out to find antioxidant in various natural products, such as seed of pea [8], chickpea [9], peanuts kernels [10] and corn [11]. Several studies about marine organism as antioxidant also have been carried out, such as yellow stripe trevally [12], muscle of ornate threadfin bream [13], pacific hake [14], aquatic species [15], capelin [16], muscle proteins of harp seal [17] and rhodophyta [18].

Marine algae are sustainable resources in marine ecosystems and mostly used as a source of food and medicine. Algae biomass has been used for centuries as food and medicine. Major compounds in algae are polysaccharides, phenolic and phlorotannins, protein, peptides and essential amino acids, lipids, terpenoids and steroids, vitamins and minerals [19, 20]. Algal biomass and algae-derived compounds have a very wide range of potential applications for nutrition and health products. Some algae are considered as rich sources of natural antioxidants. Macroalgae have received muchattention as potential natural antioxidants and there has been very limited information on antioxidant activity of macroalgae [21]. Among macroalgae, the antioxidant activity of *Acrochaetium sp.* used in this study is rarely reported. *Acrochaetium sp.* is a rhodophyta which distribute in Taiwan, South America, Atlantic Islands, Indonesia and Africa [22].

Currently, there is attention to the function and bioactivities of protein and its hydrolysates from food sources that may be used as an alternative source in the prevention of some diseases. Besides, food proteins have been known as bio-molecule that plays an important role in human improvement with their well-known nutritional values [23]. Peptides derived from food proteins can be a great source of antioxidants due to its aromatic rings, excessive donor electrons and appropriate hydrophobic character [24]. Enzymatic hydrolysis is the most reliable and an effective method to produce peptides with functional properties [25].

In this study, *Acrochaetium sp.* protein isolate was hydrolyzed using single (α-chymotrypsin, pepsin, trypsin, thermolysin) and in combination enzymatic processes. The aims of this study were to generate *Acrochaetium sp.* protein hydrolysates, fractionate the hydrolysates using RP-HPLC and evaluate the potential antioxidant activity of these samples using DPPH assay.

MATERIAL AND METHODS

Sample Preparation

Salt, sediment, and organic debris from *Acrochaetium sp.* were removed using fresh water. Algae were carefully rinsed with freshwater and dried at 40 °C for 2 h and ground to obtain a powder with a particle size lower than 1 mm and finally stored at 4 °C in plastic bags for further analysis.

Protein extraction, digestion, and ultrafiltration

The dried powder of *Acrochaetium sp.* was dissolved in 20% of trichloroacetic acid (TCA) for 12 h at 4 °C. The TCA was removed using acetone and the pellet was lyophilized. The dried protein then was hydrolyzed by α-chymotrypsin (37 °C), pepsin (37 °C), thermolysin (60 °C) and trypsin (37 °C) for 16 h. *Acrochaetium sp.* was also digested by various combinations of enzymes, for each enzyme was incubated for 3 h. The reaction was stopped by heating the mixture and then fractionated into < 3 kDa MWCO. The filtrate was collected and lyophilized for further analysis.

Fractionation of *Acrochaetium sp.* Protein Hydrolysate by RP-HPLC

Acrochaetium sp. protein hydrolysate was eluted by 5% acetonitrile (ACN) and 0.2% FA in deionized water and fractionated by reverse-phase high performance liquid chromatography (RP-HPLC, Hitachi Chromaster, Tokyo, Japan). The mobile phase of buffer A (5% ACN and 0.1% TFA in deionized water) and buffer B (95% ACN and 0.1% TFA in deionized water). Twenty microliters of < 3 kDa hydrolysates was loaded at a flow rate of 1 mL/min. Absorbance of the fractions was monitored at 214 nm.

DPPH Radical Scavenging Assay

DPPH radical scavenging assay was measured according to Yu et al. [26]. Fresh DPPH solutions (0.1 mM DPPH in purified ethanol) were prepared daily. The samples, which comprised 100 µl samples with 100 µl of DPPH solution in a 96-well plate, was mixed and incubated for 30 min in the dark at room temperature. The absorbance was measured by using ELISA at 517 nm (A_S). Ethanol was used as the blank (A_b), and distilled water was used as the control (A_C). The DPPH radical scavenging activity was calculated according to the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left[1 - \frac{As - Ac}{Ab}\right] x\ 100\%$$

where A_b is the absorbance of the blank, A_S is the absorbance of the sample solution, and A_C is the absorbance of the control.

Statistical Analysis

Data was expressed as the mean ± standard deviation (mean ± SD). The analysis was done by using one way ANOVA in SPSS 16.0 (Chicago, SPSS Inc.) followed by post-hoc Duncan's test and accepted at the $P<0.05$ level to identify the significant differences among treatments.

RESULTS AND DISCUSSION

Generation of free radicals and lipid peroxidation often occur in biological and food systems. In biological systems, antioxidants as part of the defense mechanism can prevent oxidative damage [27] and free radical generation by pro-oxidative from environment such as air pollutant, ultraviolet radiation, and cigarette smoke [28]. Recently, there is increased interest in naturally bioactive compounds as alternatives to synthetic substances, even these naturally compounds often show lower activity than the synthetic substances, but they are nontoxic and do not leave any residues [20]. As reported by Margaret et al. [29], bioactive peptides can be released by enzymatic proteolysis of food proteins, therefore pancreatic enzymes; chymotrypsin and trypsin have been used for derivation of bioactive peptides.

Enzymatic hydrolysis is the most effective method to produce peptides with functional properties; in this study we used several proteases individually and in combination. As shown in Figure 1, thermolytic hydrolysate of *Acrochaetium sp.* possessed the highest scavenging of DPPH radicals than other proteases (57.40%). These results are consistent with the previous studies suggested that thermolysin is specifically catalyzes peptide bond containing hydrophobic and aromatic amino acid, which potential as antioxidant peptide [30]. In this study, we also used the combination of two enzymes and resulted the combination of thermolysin-trypsin had the highest scavenging of DPPH radicals compared with other combination of different enzymes, as shown in the Figure 2. Besides thermolysin catalyzes peptide bond containing hydrophobic and aromatic amino acid, the using of trypsin also contribute the releasing of amino acids (2-20 residues) which formed antioxidant peptides and immobile in parent protein [24].

Bioactivity of protein hydrolysates is mainly affected by the molecular weight of the peptides. The molecular weight of hydrolyzed protein is one of an important factor in producing protein hydrolysates [31]. The thermolysin hydrolysate and thermolysin-trypsin hydrolysate was fractionated by ultrafiltration with molecular weight cut-off (MWCO) membranes of < 3 kDa. The IC_{50} values of the thermolysin hydrolysate were 1.83±0.95 mg/mL (> 3 kDa) and 1.48±0.92 mg/mL (< 3 kDa) (Figure 3). The thermolysin-trypsin hydrolysate showed the IC_{50} values of 1.70±1.03 mg/mL (> 3 kDa) and 1.37±0.84 mg/mL (< 3 kDa) (Figure 4). Ultrafiltration membrane system was used to separate the hydrolysates into defined molecular weight ranges. It holds well in purification of simple peptides from various crude protein hydrolysates [32, 33]. The isolated peptide fractions showed higher antioxidant activity than the hydrolysate [34]. This indicated that the peptide generation plays an important part in antioxidant potential of proteins. Purification step will affect the IC_{50} value, it indicated that the lower and more purified molecule has higher inhibition rate, more purified the molecule, and the IC_{50} will be decreased.

RP-HPLC involves the separation of molecules on the basis of hydrophobicity. The separation depends on the hydrophobic binding of the solute molecule from the mobile phase to the immobilized hydrophobic ligands attached to the stationary phase. RP-HPLC (detected at 214 nm under an UV-vis detector) was further used to fractionate the antioxidant peptides and the *Acrochaetium sp.* was separated into 12 fractions (fraction 1-12 for the thermolysin hydrolysate and fraction A-L for the thermolysin-trypsin hydrolysate) as shown in the Figure 5

and 7. Each fraction was collected; freeze dried, and determined its antioxidant activity. As shown in Figure 6, fraction 7 exhibited the highest DPPH free radical scavenging activity with the inhibition (57.29%) and among 12 fractions for thermolysin-trypsin hydrolysate of *Acrochaetium sp.*, fraction I showed the highest DPPH free radical scavenging activity (64.54%) (Figure 8). Furthermore, the IC_{50} value was tested for fraction 7 and fraction I. Fraction 7 from *Acrochaetium sp.* hydrolysate using thermolysin had IC_{50} value of 0.58±0.56 mg/mL and in the other hand; fraction I from *Acrochaetium sp.* hydrolysate using thermolysin-trypsin had IC_{50} value of 0.38±0.33 mg/mL (Figure 9). These results showed higher antioxidant activity compared by the other marine organisms, such as *Theragra chalcogramma* (1.3 mg/mL) [35], *Thunnus tonggol* (5 mg/mL) [36], *Gadus morhua* (2.5 mg/mL) [37], and *Navodon septentrionalis* (10 mg/mL) [38].

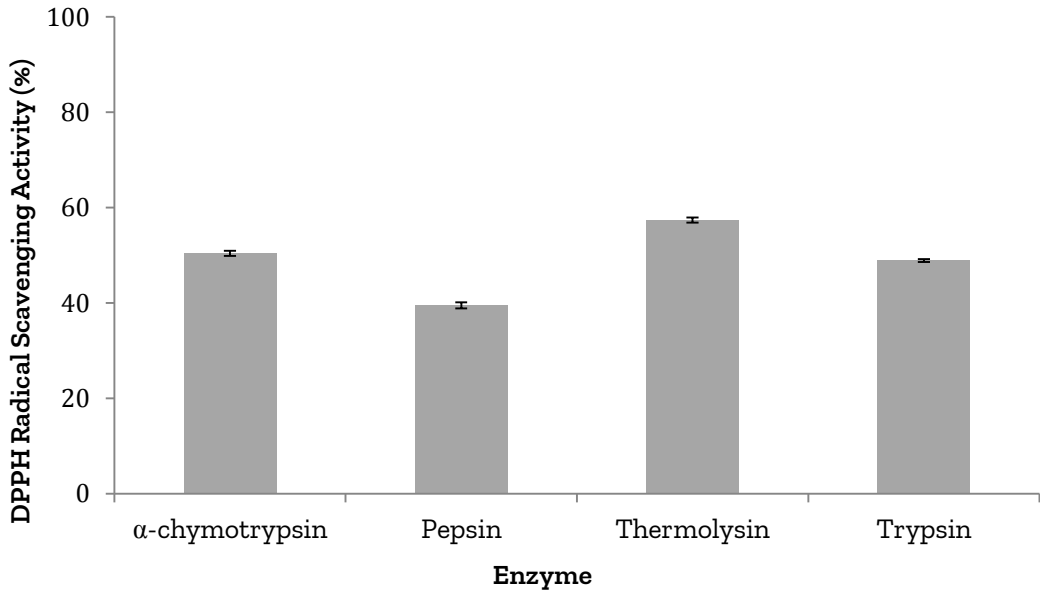

Figure 1. DPPH radical scavenging activity (%) of *Acrochaetium sp.* enzymatic hydrolysate using different single enzyme.

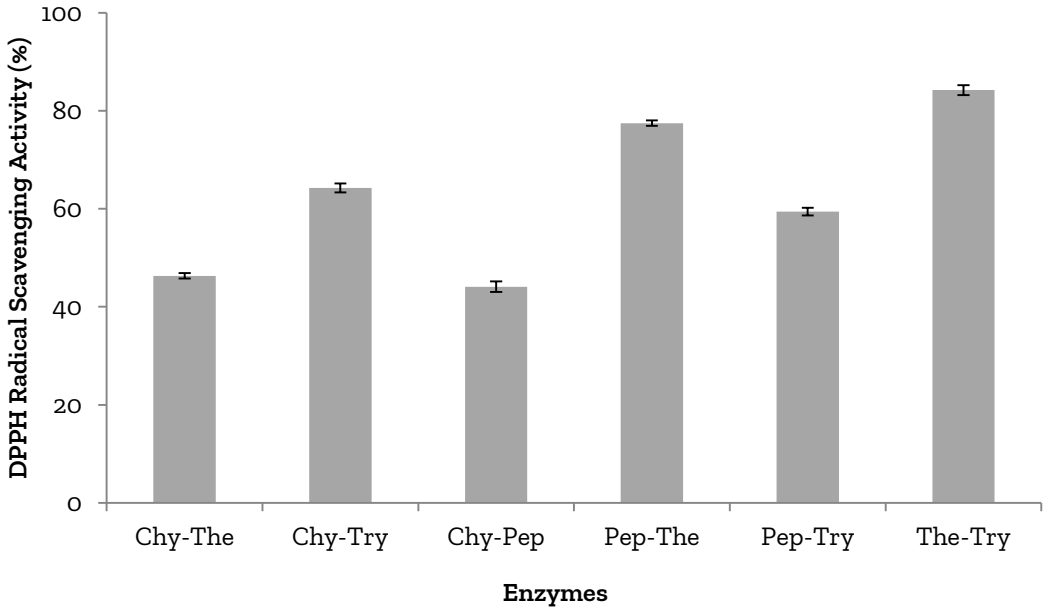

Figure 2. DPPH radical scavenging activity (%) of *Acrochaetium sp.* enzymatic hydrolysate using different combination of two enzymes (Chy: α-chymotrypsin; Pep: Pepsin; The: Thermolysin; Try: Trypsin).

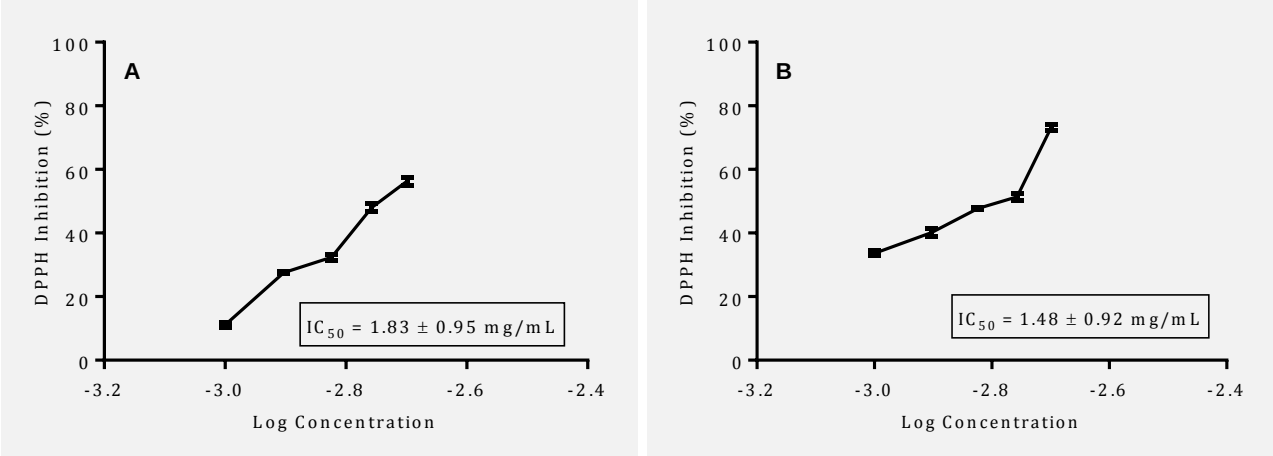

Figure 3. (**A**) IC_{50} value of *Acrochaetium sp.* hydrolysate using thermolysin (> 3 kDa) and (**B**) (<3 kDa).

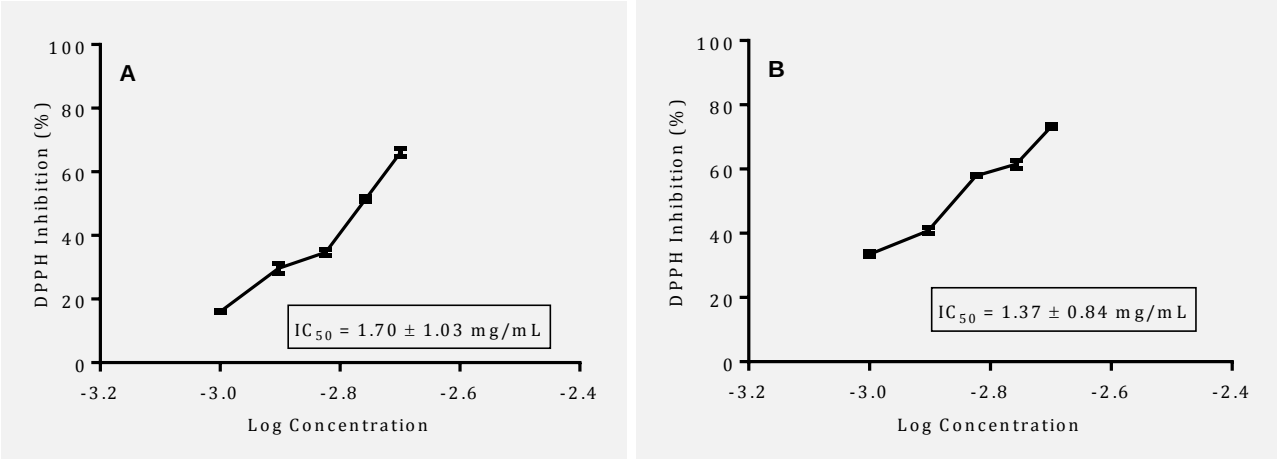

Figure 4. (**A**) IC_{50} value of *Acrochaetium sp.* hydrolysate using thermolysin-trypsin (> 3 kDa) and (**B**) (<3 kDa).

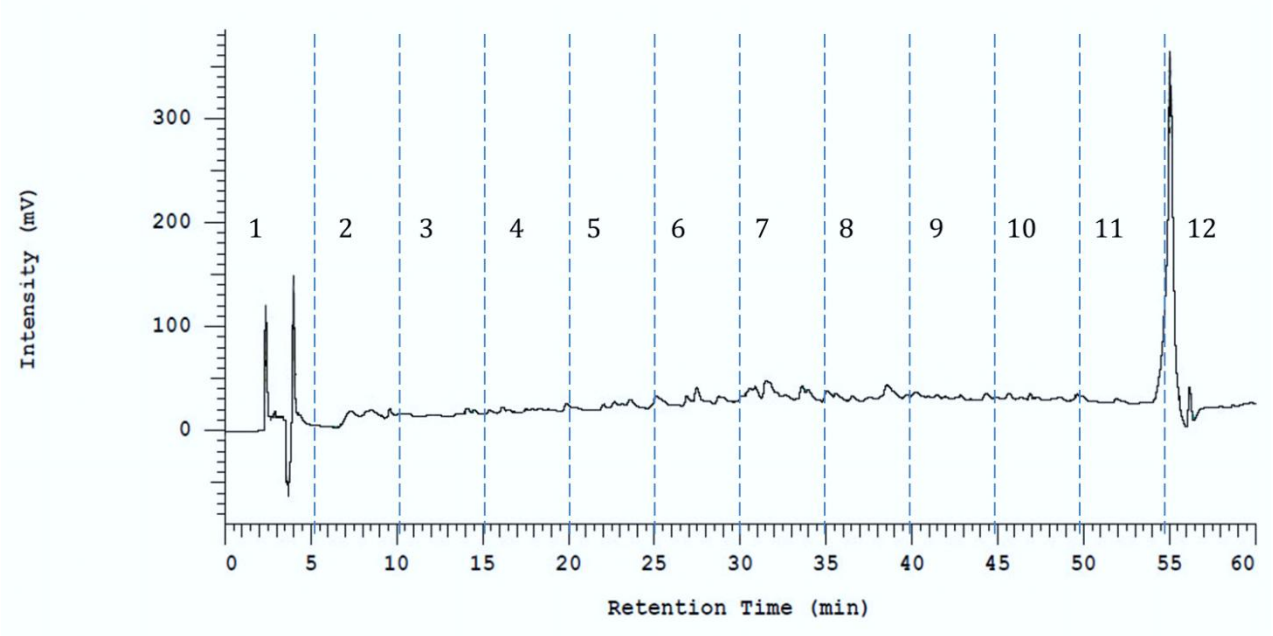

Figure 5. RP chromatogram of thermolysin hydrolysate of *Acrochaetium sp.*

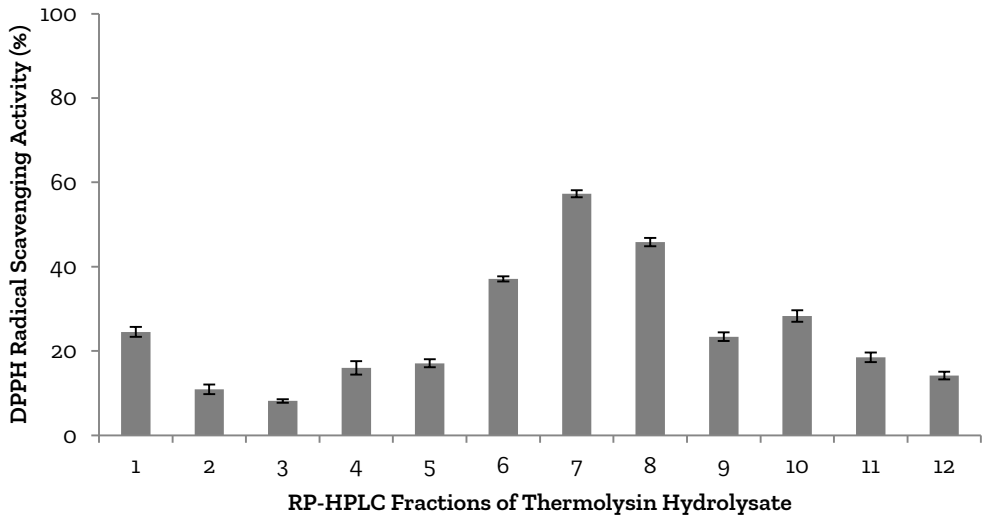

Figure 6. DPPH radical scavenging activity (%) of *Acrochaetium sp.* fractions using thermolysin.

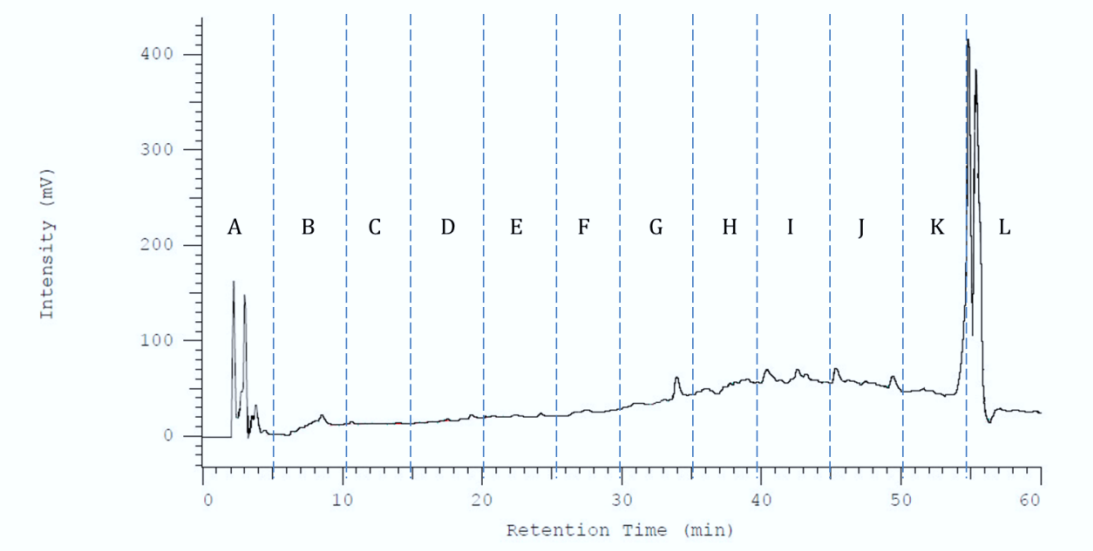

Figure 7. RP chromatogram of thermolysin-trypsin hydrolysate of *Acrochaetium sp.*

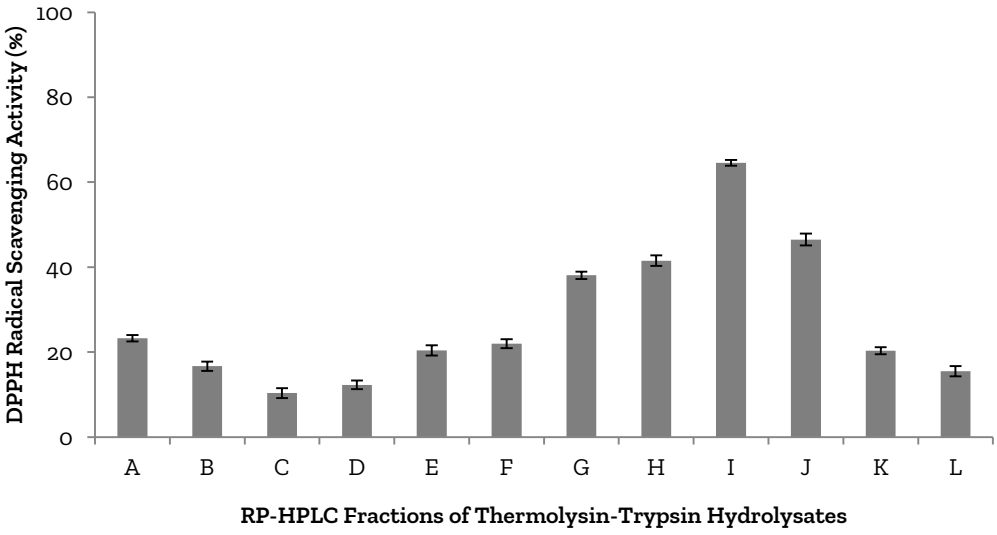

Figure 8. DPPH radical scavenging activity (%) of *Acrochaetium sp.* fractions using thermolysin-trypsin.

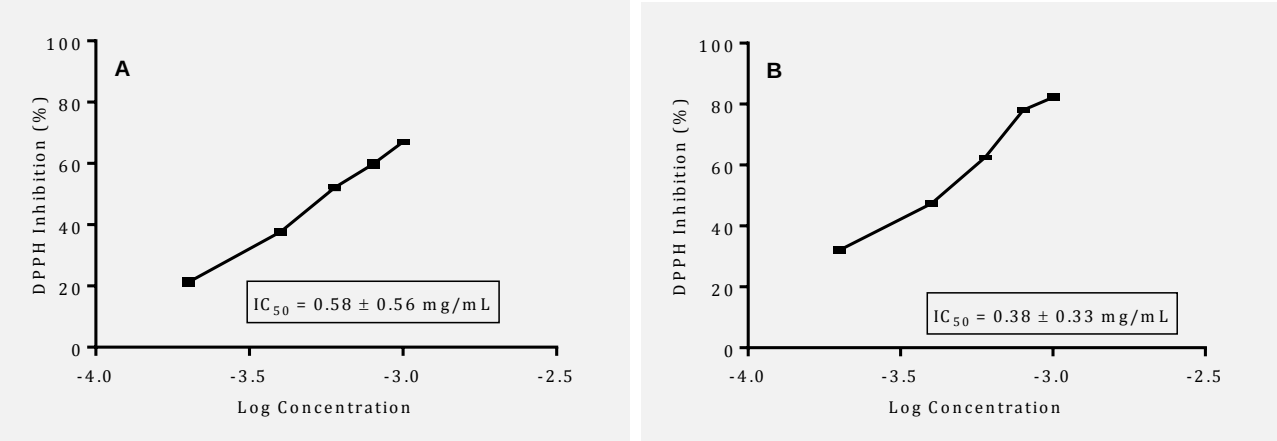

Figure 9. (**A**) IC_{50} value of fraction 7 from thermolysin hydrolysate and (**B**) IC_{50} value of fraction I from thermolysin trypsin hydrolysate.

CONCLUSION

The bioactivities of *Acrochaetium sp.* as antioxidant used in this study is rarely reported. Peptide fractions showing highly antioxidant, and it obtained from the enzymatic hydrolysates using thermolysin and thermolysin-trypsin, respectively. Fraction obtained by ultrafiltration showed an antioxidant higher than the whole hydrolysate. Fractionation using RP-HPLC resulted fraction 7 from *Acrochaetium sp.* hydrolysate using thermolysin had IC_{50} value of 0.58±0.56 mg/mL and fraction I from *Acrochaetium sp.* hydrolysate using the combination of thermolysin-trypsin had IC_{50} value of 0.38±0.33 mg/mL. Due to increasing concerns about the safety antioxidants, *Acrochaetium sp.* protein hydrolysates represent a novel source of natural antioxidant hydrolysates and antioxidant peptides. Further works such as the identification of the peptide from the fraction using LC-MS/MS, simulated gastrointestinal simulation and antioxidant activity are also suggested.

DECLARATIONS

Authors' Contributions

All authors contributed equally to this work.

Competing interests

The authors declare that they have no competing interests that might have influenced the performance or presentation of the work described in this manuscript.

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Modified Radical Abdominal Trachelectomy in Cervical Cancer in Young Women

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ABSTRACT

A modification of traditional fertility-sparing abdominal radical trachelectomy (ART) has been developed to reduce the opportunity for intra-operative injuries to occur through better management of the surgical field. The technique is similar to the standard abdominal radical trachelectomy. The ART modification developed by us enables to perform total or partial resection of the affected part of the uterine cervix after total mobilization of the cervix and excision of the upper and middle parts of the vagina. We have performed 204 modified fertility-sparing ARTs for CC women of reproductive age (27 to 37 years) at the early stage of the disease (T1A, T1B). On average the surgery lasted 140 ± 28.7 min, blood loss was 420 ± 50 ml. Epithelization of the uterine stump after surgery lasted 5 - 8 weeks. No intra-operative injuries of the nearby organs occurred. The follow-up period has lasted for 42 months. Oncological outcomes. No patient had CC recurrence and metastasis (till 42 months after the first surgery).

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INTRODUCTION

Cervical cancer (CC) is known to be the second most common malignancy in women worldwide [1]. According to WHO, some 550,000 cases of cervical cancer (CC) are registered worldwide each year, and over half of these women die from the disease [2]. In 2012 in the Republic of Uzbekistan, 1,323 patients with cervical cancer were registered and 623 of them died [3]. Recently, the CC incidence among women of reproductive age has increased. The disease more often develops at the age of 28-45 years. In our country, about 60% of CC cases are revealed at stages 1-2.

According to the Globocan 2012-data [4], nearly 83% of annual CC deaths are reported in low-income countries. However, over 25 000 new CC cases are registered annually in the EC countries and 11 000 ones were registered in the USA. In Uzbekistan, CC led in the 2003-list of female malignancies and death-causes (5.8% and 3.9%, respectively) [5]. The CC burden is mainly associated with the lack of women's screening [6]. According to Jemal et al. [6], Asians have higher cervical cancer incidence and mortality rates than U.S. whites, but lower rates than their counterparts in Latin America.

Traditional antineoplastic treatment for early-stage CC enables to save life of most patients; however, it leads to irreversible loss of fertility that greatly deteriorates the life quality of young women who have not previously realized their reproductive function. Physiological and psychological impact of infertility caused by

conservative treatment is extremely negative. Besides, most young women in this group suffer from depressions of different severity, stress and sexual dysfunction due to unrealized reproductive function [7, 8, 9, 10].

The characteristics of CC morphogenesis and carcinogenesis, high survival rates at early stages of the disease and an increase in the number of reproductive-age patients in Uzbekistan are motivating oncogynecologists to improve the quality of life for CC young women by retaining their fertility. It necessitates further development and implementation of organ-preserving surgery, i.e. abdominal radical trachelectomy (ART).

Cervical cancer is mainly characterized by the local spread of the neoplastic process. Most often the tumor involves the upper part of the vagina, parametral tissue and sacro-uterine ligaments. As our experience shows, the tumor spreading to the upper parts of the uterine is observed less often (13-15%). In 28-34% of patients, the tumor is localized in the lower part of the cervical channel, in 15% it develops in the middle part of the cervix and in 2% - in its upper part. The middle and lower thirds of the vagina are affected at later CC stages and were seldom observed.

CC metastasis development depends on the histological structure of the tumor. According to the findings of different researchers, in squamous cell CC, metastases incidence to the ovaries varies from 0.2% to 2.2%, while in adenocarcinoma it reaches 2-10%. The necessity of ovaries resection is disputable because the risk of metastasis development in this period is not high. Lymphogenic metastases in CC involve parametral, obturator, iliac, sacral, presacral, lateral and aortal lymph nodes. The most significant CC predicting factors influencing selection of treatment tactics are tumor size, invasion depth, parametral tissue infiltration, metastases in regional lymph nodes.

Objective of the research: was to develop an organ-preserving surgical technique resulting in improvement of quality of life for young CC patients through better management of the surgical field.

MATERIAL AND METHODS

Traditionally radical abdominal trachelectomy (ART) consists of total or partial resection of the uterine cervix, upper third part of the vagina, the tissue around the uterine cervix and vagina, uterovesical, sacrouterine and cardinal ligaments as well as general common, internal and external ileac vessels. The fertility-sparing ART modification developed by us enables to perform total or partial resection of the affected part of the uterine cervix after total mobilization of the cervix and excision of the upper and middle parts of the vagina. The modification provides better management of the surgical field that helps avoid accidental intra-operative injuries of the nearby organs and tissues.

In the Gynecology department of the NCRC of the Uzbekistan MoH, 204 modified fertility-sparing ARTs have been performed for CC women of reproductive age (27 to 37 years) at the early stage of the disease (T1A, T1B). The research was conducted in 2012-2015.

The eligible patients were examined clinically and instrumentally. The carefully collected history of the patients included the information on their genital and extragenital diseases and conditions. The objective gynecological examination determined the tumor type, spread of cervical tumor to loco-regional lymph nodes, condition of vaginal walls and parametral tissue. Histological investigation of the tissue samples taken from the affected area showed that most of 207 women involved in the study had squamous cell cervical cancer: 89 of them had non-keratinizing squamous cancer; 189 patients had keratinizing squamous cell cancer, and 7 ones had adenocarcinoma.

Abdominal radical trachelectomy includes partial or total hysterectomy, resection of the upper third of the vagina, pelvic tissue around the cervix and vagina, the vesico-uterine, sacro-uterine and cardinal ligaments, and the common, internal and external iliac vessels. ART is known to differ from radical hysterectomy with resection of the appendages; it not only preserves the uterus, ovaries and fallopian tubes, but the patient's reproductive function as well.

Ethical approval

The written informed consent was obtained from each patient involved in the research. The review board and ethics committee of National Center for Cancer Research under the MoH Tashkent, Uzbekistan.approved the study protocol.

Selection of patients.

The criteria of patient selected for the modified fertility-sparing ART were as follows:

- Fertility age
- Desire to preserve reproduction function
- The size of the tumor is < 2 cm
- Squamous cell tumor
- The upper third part of the uterine cervix is not affected
- No signs of metastases into the regional lymphatic nodes
- Stage T1aN0-1M0 (IA1 with invasion to the vascular space, stages IA-IB)

Criteria of patient's exclusion from the study group:

- Signs of infertility
- Malignization of the lymph nodes and margins revealed by urgent biopsy
- no opportunities for dynamic observation

Description of the technique

The modified ART was performed under general combined anesthesia and started with midline laparotomy followed by setting several wound dilators to improve the operative field view. A thorough revision of the abdomen and pelvic organs was made to explore the abdominal and pelvic cavities. The presence of adhesion signs developed after various previous interventions in this area is of particular importance, since it may be accompanied by functional or organic changes of different character.

A thorough examination of the topographic-anatomic structure of the uterus with the appendages and assessment of the vessels condition, surrounding organs and tissues, retroperitoneal space and ureters were made visually and by palpation. If there was some free liquid in the small pelvis or side channels, it was aspirated to perform urgent cytological tests. The condition of the ovaries was examined to reveal the presence of cysts or solid cystic formations. If necessary, the cysts were resected after an urgent histological examination performed within the surgery. We examined the condition of the parietal and visceral peritoneum to reveal tumor dissemination or any other morphological changes. On completion of the abdominal revision in Trendelenburg position, the intestinal loops were placed to the upper part of the abdomen and isolated from the small pelvis. The uterine fundus was stitched with Z-shaped silk suture and fixed with forceps to ensure free movement of the uterus during the operation, if needed. This procedure was performed to avoid any injury of the ovaries, fallopian tubes and uterine vessels.

The first stage of the surgery was dissection of pelvic lymph nodes that made possible to follow the principles of radical surgical treatment for CC in order to avoid the loco-regional spread of the malignant process.

The round ligaments on both sides were dissected alternately to obtain the access to the iliac region. Lymphodissection was performed around the common, external and internal iliac vessels up to the obturator fossa, around the obturator nerve, uterine cervix and the upper third of the vagina. During dissection particular attention was paid to careful coagulation and ligation of the lymph vessels in order to reduce postoperative lymphorrhea. After lymphadenectomy, the obturator area on both sides was gradually filled with gauze drapes soaked with 96% ethanol. After dissection of the lymph nodes with no signs of metastases, the second stage of the surgery began. It meant complete or partial removal of the cervix (depending on the location and size of the initial lesion) and included resection of the upper third of the vagina, paracervical and paravaginal tissue, cardinal, sacro-uterine and vesico-uterine ligaments. At this stage, the main task was not only to preserve the uterine corpus, ovaries and fallopian tubes, but also to retain vessels that adequately supply these organs. Thereby, special attention was paid to careful assessment of the uterine and ovarian vessels.

After sharp and blunt dissection of the peritoneum and the vesico-uterine folds the posterior wall of the bladder was separated from the anterior wall of the uterine cervix to the beginning of the middle third of the vagina. Carefully controlling the ureters on the both sides, the back leaf of the peritoneum covering the back leaf of the cervix was resected. The lateral leaves of the broad ligament were cautiously excised avoiding any injury of the ureters taken by tourniquets. The uterine vessels were carefully exposed. At the level of the uterine isthmus, the ascending and descending branches of the uterine vessels were carefully separated; the latter ones were cut and ligated on both sides.

The ureters were exposed sharply, starting from the area above the pelvis inlet to the place of decussation with the uterine vessels. The uterine cervix was moved proximally; the uterine vessels were moved laterally, while the bladder was moved down. Under strict visual control of the ureters' position, the vesico-uterine ligaments were cut anteriorly and the recto-uterine ligaments were cut posteriorly, ligated and fixed by forceps.

The back leaf of the peritoneum was separated from the posterior wall of the vagina by blunt dissection, thus, moving back the anterior wall of the rectum at a safe distance. The cardinal ligaments were cut and ligated on both sides and fixed by forceps. The uterine cervix was resected clipping the paravaginal tissue and vaginal tube on the line of the upper and middle thirds of the vagina with excision of all sections. The soft tissues, held by forceps, were stitched and ligated. The vaginal walls were fixed by six ligatures on forceps.

After these manipulations the uterus with the upper third of the dissected vaginal wall was carefully hold in hands and the cervix resection started. The level of cervical resection in each case was determined individually. It was performed perpendicularly to the uterus axis depending on the tumor parameters. The adequacy of the cervix removal was assessed by urgent histological examination of the margins. Thereafter, the residue of the uterus corpus was gradually sutured with eyeless needles and Vicryl threads, and then it was fixed to the middle third of the vaginal tube. If necessary, in order to reduce the vaginal lumen after an adequate juxtaposition with the uterus corpus, the vaginal walls were stitched using side sutures. Blood supply of the remaining part of the uterus and appendages was monitored. On completion of the reconstructive stage and revision, the restoration of the round ligaments integrity began after removal of gauze material from the obturator fossae. When the integrity of anterior and posterior leaves of the peritoneum had been restored, the abdominal cavity was separated from the small pelvis anatomically.

At the final stage of the surgery, a Z-shaped suture was ligated. The adequacy of blood supply to the uterus and appendages was re-assessed; the iliac-obturator area remained nonperitonized to provide lymph outflow and prevent lymph cyst formation. The Douglas' pouch was drained by silicone drains. The anterior abdominal wall was sutured in layers after revision and sanitation.

RESULTS AND DISCUSSION

Abdominal radical trachelectomy in CC women of reproductive age presupposes urgent histological examination of the margins and excision of the lymph nodes. If tumor cells were revealed, the surgery was performed by the standard method, i.e. by extended hysterectomy without appendages and transposition of the ovaries.

On average, the surgery lasted 140 ± 28.7 min with 420 ± 50 ml. blood losses. Epithelization of the uterine stump after surgery lasted 5 - 8 weeks. Application of tampons with ointment in the granulating area and periodic gentle bougienage of the cervical canal are necessary procedures at this stage.

Gynecological outcome

The menstrual cycle in the patients recovered 1 - 3 months after the surgery; one patient complained of amenorrhea at the 5th month after the surgery that is probably due to insufficiency of supplying vessels. Lymphatic cysts developed in two patients in the post-operative period. The pathological focus was eliminated in one patient after the conservative therapy and in the second patient after the puncture and extraction of the cyst content.

The dynamic monitoring of the patients has shown that their subjective state is adequate, no pathological changes in their gynecological and general status were revealed by cytological examination of the smears taken from the uterine stump and vaginal walls. Ultrasound examination of the abdomen and pelvis, X-ray examination of the lungs were performed; the levels of sex hormones and CA 125 as well as blood phosphorus and calcium were determined when it was necessary.

Oncological outcomes

No patient had CC recurrence and metastasis (till 42 months after the first surgery).

Obstetric outcomes

Postoperatively, the patients expected restoration of their reproductive function, but it has not occurred yet because of a short period of time after the surgery.

Hereby, we have presented the results of our experience of the ART modification. We are going to conduct further the research to evaluate both subjective and the objective statuses of young patients, study their reproductive behavior and quality of life, as well as late results.

CONCLUSION

Modified abdominal radical trachelectomy provides better management of the surgical field and help avoid injuring the nearby organs. ART in women of reproductive age with early-stage cervical cancer requires urgent histological examination within the surgery. ART provides a chance for young CC patients to improve their quality of life and preserve (possible) fertility.

DECLARATIONS

Authors' Contributions

All authors contributed equally to this work.

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Competing interests

The authors declare that they have no competing interests.

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2. Karen KS, Otto CM. 2007. Pregnancy in women with valvular heart disease. Heart. 2007 May; 93(5): 552–558.
3. Doll MA, Salazar-González RA, Bodduluri S, Hein DW. Arylamine N-acetyltransferase 2 genotype-dependent N-acetylation of isoniazid in cryopreserved human hepatocytes. Acta Pharm Sin B, 2017; 7(4):517-522.

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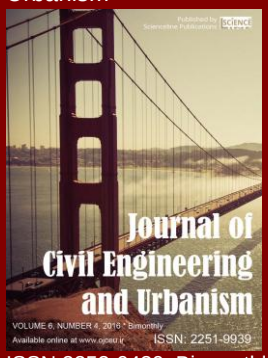

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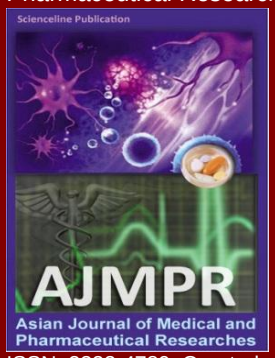

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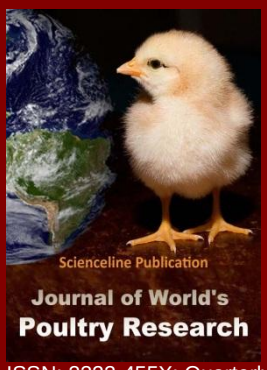

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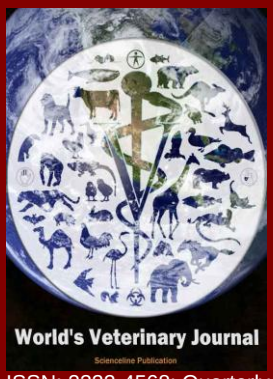

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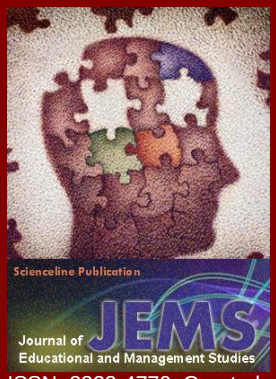

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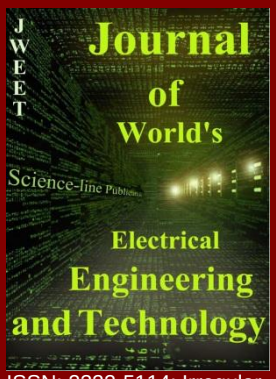

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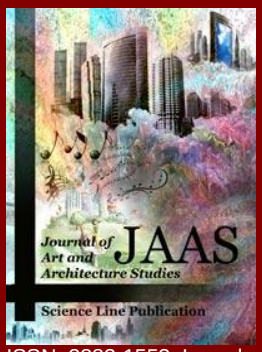

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