Short Communication

J Babol Univ Med Sci
Vol 19, Issu 12; Dec 2017. P:22-27

Investigation of Mutation in a Part of Exon 15 of APC Gene in Patients with Familial Adenomatous Polyposis in Gilan Province

M. Ahmad Sharbafi (MSc)[1], N. Ranji (PhD)*[2]

1.Faculty of Basic Sciences, Zanjan Branch, Islamic Azad University, Zanjan, I.R.Iran

2.Department of Biology, Faculty of Basic Sciences, Rasht Branch, Islamic Azad University, Rasht, I.R.Iran

J Babol Univ Med Sci; 19(12); Dec 2017; PP: 22-7

Received: Jul 20th 2017, Revised: Oct 14th 2017, Accepted: Nov 12th 2017.

ABSTRACT

BACKGROUND AND OBJECTIVE: Familial adenomatous polyposis (FAP) is a colorectal cancer caused by the mutation in the APC gene, inherited as an autosomal dominant. In patients with FAP, adenomas are formed after the age of 20, which develop malignant tumors one or two decades later. The aim of this study was to determine the mutation in a part of exon 15 of APC gene in patients with familial adenomatous polyposis in Gilan province.

METHODS: In this study, a nonsignificant mutation (c.3184C > T, p.Q1062X) was identified in a person with a classic FAP with severe polyposis.

FINDINGS: In this study one nonsense mutation (c.3184C>T, p.Q1062X) was identified in a classic FAP patient with severe polyposis.

CONCLUSION: The results of the study showed that severe polyposis was associated with a nonsignificant mutation that resulted in the production of short APC protein in a person with FAP.

KEY WORDS: *APC Gene, FAP, Mutation, PCR, Sequencing.*

Please cite this article as follows:

Ahmad Sharbafi M, Ranji N. Investigation of Mutation in a Part of Exon 15 of APC Gene in Patients with Familial Adenomatous Polyposis in Gilan Province. J Babol Univ Med Sci. 2017;19(12):22-7.

* **Corresponding author: Ranji (PhD)**
Address: Department of Biology, Faculty of Basic Sciences, Rasht Branch, Islamic Azad University, Rasht, I.R.Iran
Tel: +98 1333424080
E-mail: n_ranji@iaurasht.ac.ir

[DOR: 20.1001.1.15614107.1396.19.12.4.7]
[DOI: 10.18869/acadpub.jbums.19.12.22]

Introduction

Familial adenomatous polyposis (FAP) is a syndrome that predisposes an individual to colorectal cancer (1) inherited by the autosomal dominant pattern (2, 3) and approximately, 1 in every 5,000 to 10,000 people in each population suffers from FAP disease (4). This disease manifests itself through the formation of hundreds to thousands of adenomatous polyps in the colon and rectum during the second and third decades of life (5).

Periodic sigmoidoscopy for colorectal cancer is recommended from the age of 10 years and colectomy is recommended from the age of 20 years to prevent polyps from becoming cancerous (4). The genetic cause of FAP is the germinal mutation in the Adenomatous Polyposis Coli (*APC*) gene. Its longest exon is exon 15, which has hotspots mutations (codons 1061 and 1309) in FAP patients in this exon. In different studies were reported nucleotide deletion in these regions in *APC* gene in Iran (6) and other countries (7,8) in FAP patients. In classic FAP, adenomatous polyps usually appear at the age of 16 years and reach more than 100 polyps in colorectal, and if not treated, the occurrence of colorectal cancer at the age of 40 is inevitable (9).

Attenuated FAP (AFAP) is a mild form of familial adenomatous polyposis that appears later and with fewer polyps (10 to 100) in colorectal compared to the classic FAP (10). Considering that FAP is a dominant autosomal disease with a probability of death at young ages if left untreated, people at risk of disease need to be screened from an early age. Considering the length of the gene, it is necessary to identify the hotspots mutations in each geographical region, this study was conducted to identify common mutations in Guilan province, and thus, the APC gene mutations in FAP patients in exon 3 and a part of exon 15 were investigated by PCR sequencing method.

Methods

Patients: After being approved by the ethics committee of Lorestan University of Medical Sciences (with the code of ethics LUMS.REC.1395.80), this cross-sectional study was conducted over a period of six months on5 FAP patients from among 100 patients with colorectal cancer that had 5 to 100 polyps (AFAP) or more than 100 polyp in colorectal (classic FAP) who referred to the clinic of GI tract, liver and oncology specialists (11). After obtaining informed written consent from patients, 5 ml blood samples were collected

Genomic DNA extraction: DNA purification was carried out using the DynaBio^{Tm} Blood/Tissue DNA Extraction miniKit (Takapoozist, Tehran) according to the manufacturer's instructions. Extracted DNA was run in 1.5% agarose gel electrophoresis.

PCR sequencing: After DNA extraction, PCR master mix were prepared at a final volume of 25 µl using Golden double helix kit (Golden Double Helix Co., South Korea) and adding extracted DNA, primers(20 µM) and sterile water to a Premix solution. The primers used in this study (exon 3 and a part of exon 15) were designed using CLC main workbench v3.5 software. Primer pairs were synthesis by Macrogen Corporation (South Korea) (Table 1).

The PCR reaction was programmed with: Initial denaturation at 95 ˚C for 5 min, 30 cycles at 95˚C for 1 min, 65˚C for 1 min, 72˚C for 1 min and final extension at 72˚C for 5 min using Analytik Jena Thermal Cycler (Germany). The used primers were listed in Table 1. PCR product was studied by direct sequencing and analyzed by CLC main workbench v3.5 software compared to the reference samples available on the NCBI website (NG_008481.4).

Results

Five FAP patients were identified in this study. One patient suffered from mild FAP (48 years old) and four patients suffered from classic FAP (30 – 40 years old) (Table 2).

Sequencing of exon 3 and a part of the exon 15 of the APC gene: After assuring the accuracy of DNA extraction (Fig. 1) and the PCR reaction (Fig. 2 and Fig. 3), the results of the study were sequenced. The sequencing analysis showed that none of the patients had mutation in exon 3 (Fig. 4). In this study, in patient No. 3 with severe polyposis and familial history of FAP, a silent mutation (c.3183A > G, p.K1061K) with changes in AAA > AAG in codon 1061 (Fig. 5) and a nonsignificant mutation (c.3184C > T, p.Q1062X) with changes in CGA > TGA in codon 1062 (Fig. 6) were reported, and the nonsignificant mutation in this person resulted in the conversion of amino acid glutamine to the termination codon..

[DOR: 20.1001.1.15614107.1396.19.12.4.7]
[DOI: 10.18869/acadpub.jbums.19.12.22]

Table 1. Sequence of primers used for PCR

Primers name	Exon	Primer sequence	Length of production
APC- F1 APC- R1	Exon 3	5'-TTTTACCCTGACCCAAGTGGAC -3' 5'-CAATAAACTGGAGTACACAAGGC -3'	431 bp
APC- F2 APC- R2	Exon 15	5'-GAACAAAAGGAGATGTGGAATACTTGG-3' 5'- TTCTGTTGCTGGATGGTAGTTGCC-3'	788 bp
APC-F3 APC-R3	Exon 15	5'-CAGTGTTACCCAGCTCCTCTTCATC -3' 5'-AAAATGTGGTTGGAACTTGAGGTGT -3'	794 bp

Table 2. The pathologic information of FAP patients

Patient's number	Gender	The level of polyps	Ethnicity	Relatives of patients	The type of cancer in relatives
1	Male	Severe polyposis	Gilaki	-	-
2	Female	Mild polyposis	Gilaki	uncles and cousins	Colon Cancer
3	Female	Severe polyposis	Gilaki	Several relatives of the patient	-
4	Male	Severe polyposis	Gilaki	-	-
5	Male	Severe polyposis	Gilaki	Father and brother	Colon Cancer

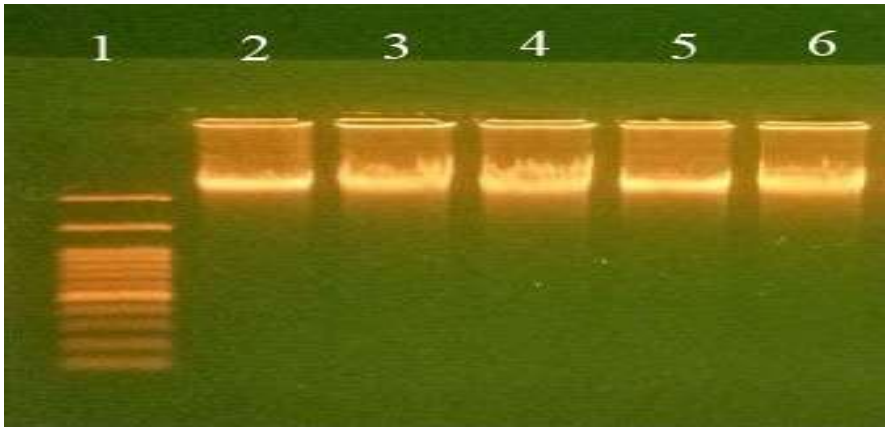

Figure 1. Electrophoresis of the purified DNA samples in 1.5% agarose gel. Sample 1 is DNA marker (100 bp). Samples 2 to 6 related to purified DNA of FAP patients

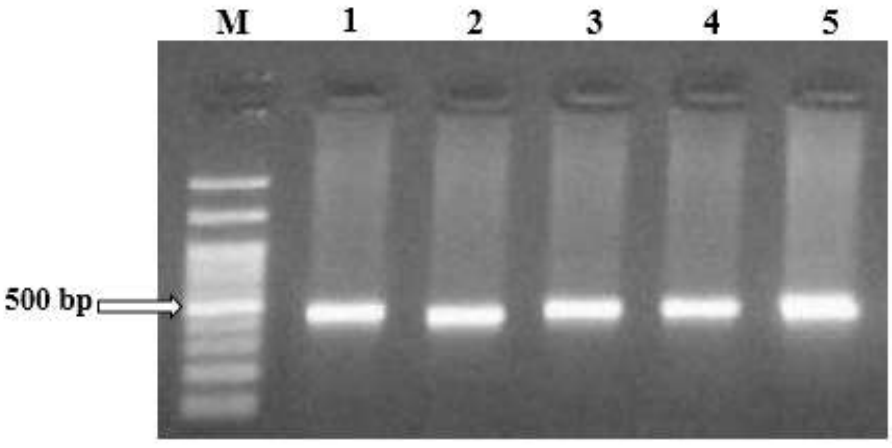

Figure 2. Electrophoresis of PCR products of exon 3 (431 bp) of APC gene in 2% agarose gel. M: DNAmarker by 100 bp length

[DOR: 20.1001.1.15614107.1396.19.12.4.7]
[DOI: 10.18869/acadpub.jbums.19.12.22]

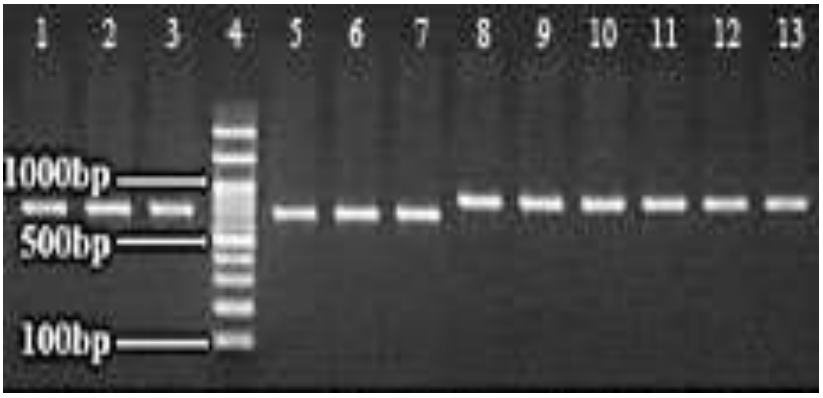

Figure 3. Electrophoresis of PCR products of exon 15by 788 bp length (samples 1 to 7) and 794 bp length (samples 8 to 13), and DNA marker (sample 4) by 100 bp length in 2% agarose gel

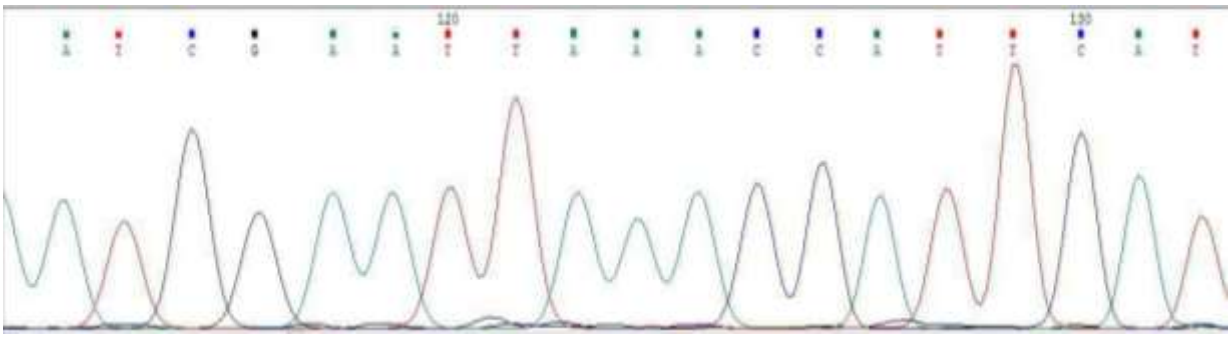

Figure 4. Electropherogram of APC gene (exon 3) related to a FAP patient (no. 1). There was no mutation in this region

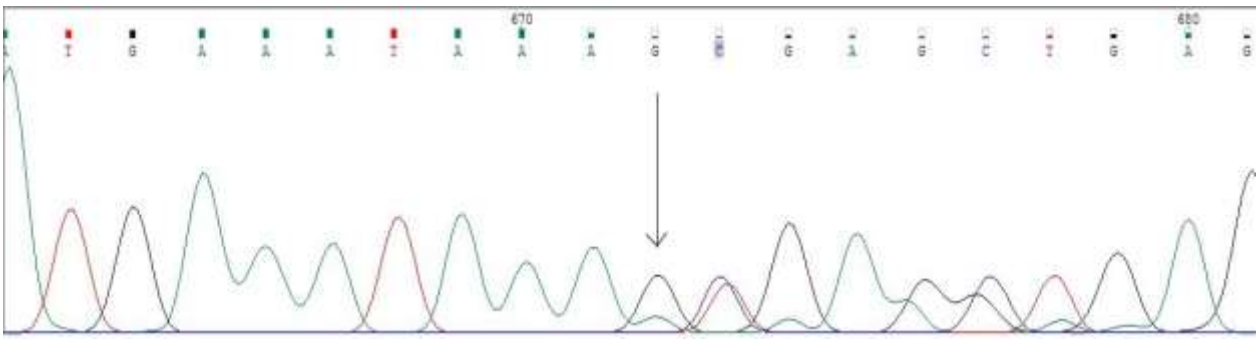

Figure 5. Electropherogram of APC gene (exon 15) related to a FAP patient (no. 3) the silent mutation (c.3183A>G) K1061K was reported in this region

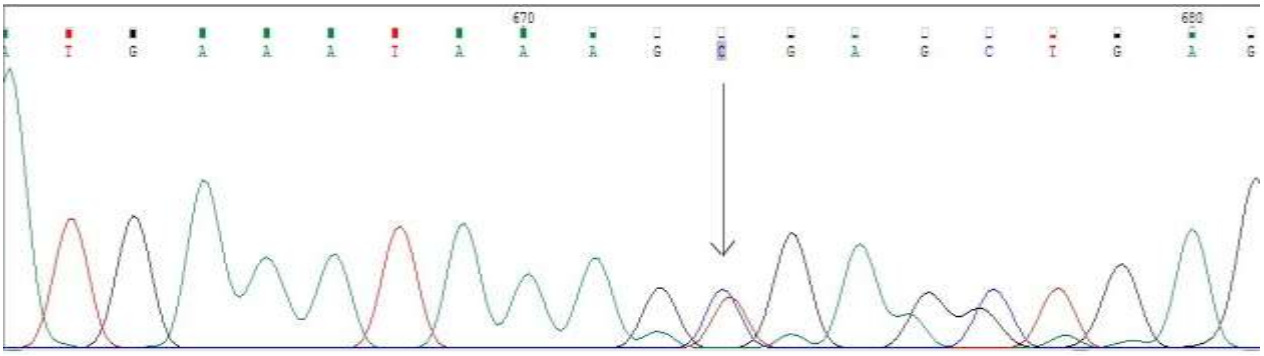

Figure 6. Electropherogram of APC gene (exon 15) related to a FAP patient (no. 3) Nonsense mutation (c.3184C>T, Q1062X) was reported in this region

[DOR: 20.1001.1.15614107.1396.19.12.4.7]
[DOI: 10.18869/acadpub.jbums.19.12.22]

Discussion

Funding of *APC* gene mutations in FAP patients in different populations may be useful as a suitable tool for predictive diagnosis of subjects at risk. In this study, five unrelated FAP patients were found and nonsense mutation Q1062X was detected in exon 15 of the APC gene in one of the FAP patients. APC mutation Analysis is time-consuming and costly due to the high length of the APC gene, thus herein we investigated hotspot region of the *APC* gene in exon 15 (12). Also, exon 3 of APC gene that studied as one of the hotspot region in AFAP patients (13), was analyzed herein.

In this study, none of the patients had mutation in exon 3 of *APC* gene. However, in AFAP patient, it is expected that mutations occur more at the 5'-end of gene (the first five exons of the gene), in alternative splicing region of exon 9 and the 3'-end of the gene (after the codon 1580) (13), no mutation was identified in the patient of this study in exon 3.

Investigation of hotspots mutations of *APC* gene in exon 15 by direct sequencing showed that one FAP patient had a nonsense mutation Q1062X (c.3184C>T). In several studies, a mutation in codon 1062 as one of the hotspots mutations was reported. Papp *et al.* reported that deletion of 5-nucleotides (c.3183_3187del5) led to nonsense mutation Q1062X in *APC* gene (14). Furthermore, Khan *et al*. found same deletion of 5-nucleotides that led to premature stop codon in codon 1062 (Q1062X) (7).

These studies reported different type of mutation compared to our study; however, the result of changes in this region in all three studies was conversation of arginine codon into stop codon. Most common mutations in APC gene has been reported are deletion of 5-nucleotides starting from the codon 1061 (c.3183-3187delACAAA) or starting from codon 1309 (c.3927-3931delAAAGA) as hotspot mutational regions (15, 16). For example, in the study of Gómez-Fernández *et al*. among nine FAP families, these two mutations were reported with high frequency (17). In the present study, nucleotide substitution in codon 1062 resulted in premature termination of translation at codon 1062, but in the abovementioned studies, deletion starting from codon 1061 resulted in the termination of protein synthesis in codon 1062, thus for first time in the world we reported Q1062 X mutation with a new reason in APC gene in a FAP patient in Iran On the other hand, the Q1062X mutation in this study result of a nucleotide substitution was led to produce APC truncated protein in a FAP patient. This patient suffered from severe polyposis and had several related patients in family. In this patient, APC truncated protein due to the absence of some 15-amino acids repeats and all seven 20-amino acids repeats could not completely bind to β-catenin and therefore cannot regulate this protein. Carcinogenesis induced by deletion of some 15-amino acids repeats (12) has been not reported. But a pathogenic reason of APC gene mutations is lack of C-terminal regions such as lack of 20-amino acids repeats (14). At least three amino acid sequences in APC are necessary to regulate the cytoplasmic level of β-catenin, and lack of all or most of them in the tumors has been observed (12).

In addition, APC truncated protein (in this study) lacks other C-terminal regions such as basic domain (microtubules binding site) and EB1 binding site, which disturbs in chromosomes segregation during cell division and causes chromosomal instabilities and aneuploid cells. The outcome of such a mutation in the germinal cells of the patients is the occurrence of adenomatous polyposis in younger ages that, if not treated promptly, there is a possibility of progression of adenomas to carcinoma and cancerous growth of the polyps in these individuals. Considering the autosomal dominant inheritance of FAP disease, it is necessary to take precautionary screening for individual at risk of this disease. In this regard, individual at risk should be screened from a childhood for gene mutation so that in the presence of mutations in PAC gene in them preventive medical interventions and experiments be performed. It should be noted that there is a relationship between the position of the mutation (genotype) and phenotype of the disease, and in this regard, appropriate therapeutic approaches can be used by identifying the mutations.

Acknowledgments

Hereby, we thank GI and liver specialists and oncologists who helped us collect the samples from patients.

[DOR: 20.1001.1.15614107.1396.19.12.4.7]
[DOI: 10.18869/acadpub.jbums.19.12.22]

References

1.Zhang Z, Liang S, Wang D, Li Y, Wang B, Jiang T, et al. A novel pathogenic single nucleotide germline deletion in APC gene in a four generation Chinese family with familial adenomatous polyposis. Sci Rep. 2017;7(1):12357.

2.Roncucci L, Pedroni M, Mariani F. Attenuated adenomatous polyposis of the large bowel: Present and future. World J Gastroenter. 2017;23(23):4135-9.

3.Ghorbanoghli Z, Bastiaansen BA, Langers AM, Nagengast FM, Poley JW, Hardwick JC, et al. Extracolonic cancer risk in Dutch patients with APC (adenomatous polyposis coli)-associated polyposis. J Med Gen. 2017.

4.Nieuwenhuis MH, Vasen HF. Correlations between mutation site in APC and phenotype of familial adenomatous polyposis (FAP): a review of the literature. Crit Rev Oncol Hematol. 2007;61(2):153-61.

5.Plawski A, Banasiewicz T, Borun P, Kubaszewski L, Krokowicz P, Skrzypczak-Zielinska M, et al. Familial adenomatous polyposis of the colon. Hereditary cancer in clinical practice. 2013;11(1):15.

6.Sadighi S, Ghaffari-Moghaddam M, Saffari M, Mohagheghi MA, Shirkoohi R. A patient with desmoid tumors and familial fap having frame shift mutation of the apc gene. Acta medica Iranica. 2017;55(2):134-8.

7.Khan N, Lipsa A, Arunachal G, Ramadwar M, Sarin R. Novel mutations and phenotypic associations identified through apc, mutyh, nthl1, pold1, pole gene analysis in indian familial adenomatous polyposis cohort. Sci Rep. 2017;7(1):2214.

8.Dodaro C, Grifasi C, Florio J, Santangelo ML, Duraturo F, De Rosa M, et al. The role of mutation analysis of the APC gene in the management of FAP patients. A controversial issue. Ann Italian Di Chirurg. 2016;87:321-5.

9.Talseth-Palmer BA. The genetic basis of colonic adenomatous polyposis syndromes. Heredit Cancer Clin Prac. 2017;15:5.

10.Half E, Bercovich D, Rozen P. Familial adenomatous polyposis. Orphanet J Rare Dis. 2009;4:22.

11.Marabelli M, Molinaro V, Abou Khouzam R, Berrino E, Panero M, Balsamo A, et al. Colorectal adenomatous polyposis: heterogeneity of susceptibility gene mutations and phenotypes in a cohort of italian patients. Gen Test Molecul Biomarkers. 2016;20(12):777-85.

12.Masjedizade A FA, Galedari H, Ranji N. A study on mutations in hotspot sites of exon 15 of apc gene in familial adenomatous polyposis patients and their relatives in Iran Khuzestan province. Sci Med J. 2009;8(1):70-8.

13.Torrezan GT, da Silva FC, Santos EM, Krepischi AC, Achatz MI, Aguiar S, Jr., et al. Mutational spectrum of the APC and MUTYH genes and genotype-phenotype correlations in Brazilian FAP, AFAP, and MAP patients. Orphanet J Rare Dis. 2013;8:54.

14.Papp J, Kovacs ME, Matrai Z, Orosz E, Kasler M, Borresen-Dale AL, et al. Contribution of APC and MUTYH mutations to familial adenomatous polyposis susceptibility in Hungary. Fam Cancer. 2016;15(1):85-97.

15.Aretz S, Uhlhaas S, Caspari R, Mangold E, Pagenstecher C, Propping P, et al. Frequency and parental origin of de novo APC mutations in familial adenomatous polyposis. Eur J Hum Genet. 2004;12(1):52-8.

16.Christie M, Jorissen RN, Mouradov D, Sakthianandeswaren A, Li S, Day F, et al. Different APC genotypes in proximal and distal sporadic colorectal cancers suggest distinct WNT/beta-catenin signalling thresholds for tumourigenesis. Oncogene. 2013;32(39):4675-82.

17.Gomez-Fernandez N, Castellvi-Bel S, Fernandez-Rozadilla C, Balaguer F, Munoz J, Madrigal I, et al. Molecular analysis of the APC and MUTYH genes in Galician and Catalonian FAP families: a different spectrum of mutations?. BMC Med Gen. 2009;10:57.

[DOR: 20.1001.1.15614107.1396.19.12.4.7]
[DOI: 10.18869/acadpub.jbums.19.12.22]