INVESTIGATION OF IVS14+1G>A POLYMORPHISM OF DPYD GENE IN A GROUP OF BOSNIAN PATIENTS TREATED WITH 5-FLUOROURACIL AND CAPECITABINE

Timur Cerić[1*], Nermina Obralić[1], Lejla Kapur-Pojskić[2], Draženka Macić[2], Semir Bešlija[1], Anes Pašić[1], Šejla Cerić[3]

[1] Oncology Clinic, University of Sarajevo Clinics Centre, Bolnička 25, 71 000 Sarajevo, Bosnia and Herzegovina

[2] INGEB - Institute for Genetic Engineering and Biotechnology, Gajev trg 4, 71 000 Sarajevo, Bosnia and Herzegovina

[3] Nuclear Medicine Clinic, University of Sarajevo Clinics Centre, Bolnička 25, 71 000 Sarajevo, Bosnia and Herzegovina

[*] Corresponding author

Abstract

Adverse drug reactions still pose an important clinical problem. Dihydropyrimidine dehydrogenase (DPD) is an enzyme that regulates 5-FU quantities available for anabolic processes and hence affects its pharmacokinetics, toxicity and efficacy.

There are several studies describing a hereditary (pharmacogenetic) disorder in which individuals with absent or significantly reduced DPD activity may even develop a life-threatening toxicity following exposure to 5-FU. The most common mutation is known as the DPYD*2A or as the splice-site mutation (IVS14 + 1G A) leading to creation of a dysfunctional protein. An objective behind the study was to ascertain existence of the IVS14+ 1G A mutation among the population of Bosnia and Herzegovina. Our research has undeniably attested to existence of one heterozygote for the DPYD gene mutation, i.e. one heterozygote for IVS14 + 1 G > A, DPYD*2A mutation.

KEY WORDS: pharmacogenetics, Dihydropyrimidine dehydrogenase, DPYD2A mutation

Introduction

Adverse drug reactions still pose an important clinical problem. Therein, it was estimated that in the last decade alone they caused over 100,000 deaths annually in the United States. As such, these reactions were the fourth leading cause of deaths in the U.S., immediately after hearth diseases, malignancies and strokes (1). There is a direct link between therapeutical effects and toxicity of 5-FU and the drug's anabolic process related to nucleotides. In turn, this may inhibit activities of thymidylate synthetase or incorporate 5-FU into RNA and/or DNA. More than 85% of the 5-FU dosage administered to human subjects was degraded through catabolic pathways, meaning that only app. 15% of the dosage was left to the anabolic process. In the past ten years it became clear that the dihydropyrimidine dehydrogenase (DPD) is an enzyme that regulates 5-FU quantities available for anabolic processes and hence affects its pharmacokinetics, toxicity and efficacy. The 5-FU anabolism leads to creation of 5 fluoro2'-deoxyuridine-5'-monophosphate (FdUMP), which is a cytotoxic product of a multilevel activation pathway of the 5-FU. FdUMP acts as an inhibitor of the thymidylate synthetase (TS), thus causing an intracellular accumulation of the deoxyuridine monophosphate (dUMP) and a reduction in a level of deoxythymidine monophosphate (dTMP). Finally, this induces an arrest of a DNA synthesis. The initial and rate-limiting enzyme in the catabolism of the 5-FU is dihydropyrimidine dehydrogenase (DPD) that catalyses a 5-FU reduction into 5,6-dihydrofluorouracil (DHFU). Then on, DHFU is degraded down to fluoro-β-ureido propionic acid (FUPA) and flouro-β-alanine (FBAL) (2). There are several studies describing a hereditary (pharmacogenetic) disorder in which individuals with absent or significantly reduced DPD activity may even develop a life-threatening toxicity following exposure to 5-FU. Here we need to consider the non-functional catabolic pathway due to a DPD inability to metabolise. Many studies, of which the most important are the ones by Van Kuilenburg and Johnson, have indicated that a high rate of the 5-FU toxicity is attributable to the reduced DPD activity (3). Van Kuilenburg has found that 59% of oncological patients with 5-FU toxicity have also shown signs of reduced DPD activity, while Johnson (4) saw this frequency to be 43%, thereof app. 12% having profound and app. 13% partial DPD deficiency. These patients are likely to develop an unanticipated toxic reaction subsequent to being treated with the 5-FU. When given in standard doses and coupled with altered pharmacokinetics, 5-FU may lead to severe toxic reactions like mucositis, granulocytopenia, neuropathy, diarrhea and even death in the worst-case scenario. A cause to this toxicity seems to be in a decreased drug clearance hence resulting in an extended 5-FU exposure. DPD-deficient patients exhibit a normal phenotype until such time they have been administered with the 5-FU. It has been estimated that nearly 3-5% of the general population have the DPD activity below 95% (<0,064 nmol/min/mg for frozen samples) of the lower limit for the normal population (5). It has been reported that 55% of patients with reduced DPD activity suffers from the grade 4 neutropenia, as opposed to 13% of patients with normal DPD activity (P = 0,01) (6). Moreover, the toxicity occurs much earlier with regards to patients with low DPD activity (10,0 ± 7,6 versus 19,1 ± 15,3 days, P < 0,05). The patients suffering from the severe 5-FU toxicity have exhibited more than 30 mutations in the DPYD gene to include one G to A mutation in the 5'-splice recognition site of intron 14 that resulted in a 165-bp deletion that correspond to exon 14. This mutation is known as the DPYD*2A as per defined nomenclature or as the splice-site mutation (IVS14 + 1G A) leading to creation of a dysfunctional protein (7). The mutation was not only the first one to be analysed, but also the most frequently observed of all mutations in the population studies (app. 52%) (8).

Materials and Methods

The study was approved by the local ethics committee, and written informed consent was obtained from each patient. This experimental research was designed as a prospective, open and translation study. The study was performed among patients of the Oncology Clinic of the University of Sarajevo Clinics Centre treated in the period from 2006-2007. An objective behind the study was to ascertain existence of the IVS14 + 1G → A mutation among the population of Bosnia and Herzegovina. A test group observed in the subject-matter research consisted of 50 subjects – patients at the Oncology of the University of Sarajevo Clinics Centre that have undertaken chemotherapy and been treated with 5-FU or Capecetabine in the period from 2006 to 2007. Therein, we have done a genomic analysis of DPYD gene for these patients. These patients were also divided into two subgroups regarding presence of toxicity grade 3 or 4 or without toxicity after administration of 5FU. All genotype analysis were conducted on samples of the patients' peripheral blood. The blood samples were

taken by means of a venipuncture of a cubital vein that appears full and stored in vacutainers (BD biosciences) of 6ml with Na-EDTA anticoagulant. This was preceded by a written and informed consent by patients to participate in this study. The blood samples were refrigerated for a short period of time on 4°C (not beyond 48 hours) prior to their extraction. This was necessary as to preserve the quality and quantity of the isolated DNA. The DNA samples were stored on a temperature of –20°C until we moved onto the next process step in our analysis. Determining a target DPYD gene structure was done by the Institute for Genetic Engineering and Biotechnology in Sarajevo (INGEB) by means of the following methods and procedures: A genotyping methods applied in the study relied on the PCR technology. We have amplified a genotyping-relevant sequence by applying an adequate primer pair subsequent to which we subjected it to an appropriate gene expression profiling (an automatic or a manual fragment analysis). Genomic DNA was extracted from leucocytes with the standard techniques and actual genotyping was done by means of the PCR-RFLP. The DPYD exon 14 and its flanking 5' donor intronic region was amplified with the PCR.

Primers used in the amplification of the exon 14 and its marginal regions (including newly formed *Ndel* restriction sites) are:

F: 5' ATC ″AGG ACA TTG TGA CAT ATG TTT C 3'
R: 5' CTT GTT TTA GAT GTT AAA TCA CAC ATA 3'

In our study, we have applied a method of highly specific typisation of the DNA sequence previously replicated during the PCR process. The PCR method followed the above outlined primer sequences as to ensure genotyping of *DPYD2A** polymorphisms in the DPYD gene. Relevant PCR products were tested by an agarose gel electrophoresis and then treated with the restriction enzyme Ndel (endonuclease) in an appropriate buffer solution on 37°C. During the RFLP processing, we have incubated 14ml of the PCR products overnight on 37°C with 3U endonuclease Ndel. Restriction fragments were separated by the 3% agarose gel electrophoresis stained with ethidium bromide. The genotyping, i.e. assessment of the size of restriction fragments, was done on a visual basis by identifying the wild-type allele (IVS14+1G) by means of splicing 198 bp with the PCR product to 181 bp and 17 bp fragments. Conversely, a mutant allele produced three fragments subsequent to Ndel digestion: 154 bp, 27, bp and 17 bp. Relevant results were recorded by taking photographs of gels under an UV light.

Results

The human DPD gene (*DPYD*) is present as a single copy gene on the chromosome 1p22 and consists of 23 exons.

		w/o toxicity		*Toxicity*			*Total*	
Age		*No .25*	*Percentage*	*No .25*		*Percentage*	*No. 50*	***Percentage***
	Median	62,8		59,8				
	Range	42-77		41-75				
Sex								
	Male	14	56	13		52	27	**54**
	Female	11	44	12		48	23	**46**
Diagnosis								
	Rectum						18	**36**
	Colon						23	**46**
	Ventricle						3	**6**
	Pancreas						1	**2**
	Breast						3	**6**
	Gall Bladder						2	**4**
Therapy								
	5-FU						42	**84**
	Capecetabine						8	**16**
Toxicity				**Gr 3**	**Gr 4**			
	Diarrhea			9	3		12	**38,7**
	Neutropenia			8	5		13	**41.9**
	Mucositis			5	1		6	**19,35**
Mutation				1		4	1	**2%**
Lethal outcome				2		8	2	**4%**

TABLE 1. Overview of patients characteristics

Table 1. provides an overview of all patients who participated in our study, their respective characteristics and responses to treatments vs. the subject-matter mutation.

Toxicity and lethal outcome

A toxic events analysis was done in accordance with the Common Toxicity Criteria (CTC). We have factored in only those toxic events that could be interpreted by means of either laboratory tests or clinical examinations. There were also other cases like a neurological toxicity, but we have not taken it into consideration as we were unable to draw a clear line between this toxicity and the 5-FU. We have described three types of toxicities exhibited by our patients: diarrhea, neutropenia with a resulting leukopenia and mucositis (to include stomatitis as well) (Chart 1.) Majority of cases, i.e. 13 subjects, had neutropenia, which is nearly 42% of all symptoms, while remaining subjects had diarrhea and mucositis. Not one of these events had a statistical significance over the other two. While the grade 3 mostly involves diarrhea, the grade 4 neutropenia, so it is evident that the only 2 lethal outcomes occurred with patients with the grade 4 neutropenia. This is to say that this occurrence bears a statistical significance of p=0,021505 according to the Fisher's exact test. During our research, we had two lethal outcome cases that belonged to the group of subjects with toxicity subsequent to the 5-FU treatment. Therein, the significance level p was 0,244897. Both of these cases occurred after the grade 4 neutropenia, so this event bears a statistical significance of p= 0,021505 according to the Fisher's exact test.

*IVS14 + 1 G > A, DPYD*2A MUTATION*

Our research has undeniably attested to existence of one heterozygote for the DPYD gene mutation, i.e. one heterozygote for IVS14 + 1 G > A, DPYD*2A mutation.

We have determined existence of the heterozygote for the subject-matter mutation with the patient No. 28 of our test group. This patient was 39 years of age when she was diagnosed with a breast cancer with a liver metastasis as per relevant x-ray and CT scan imaging. After convening a consultation team, it was opted against conducting any surgery due to the stage of the illness. Instead, it was decided to start with a chemotherapy protocol with Capecitabine. Seven days after beginning the Capecitabine treatment, i.e. during the first cycle of treatment, the patient was admitted as an emergency case by our Emergency Department after being administered with a Capecitabine dosage of 1,250 mg / m2 twice a day. Attending physician's exam and relevant laboratory tests have indicated to leukopenia and neutropenia – grade 4 (L 0,61 109/l), as well as the grade 4 mucositis (oral cavity was covered with white coating and crusts) and the grade ¾ diarrhea (over 10 liquid stools within 24 hours). The patient was prostrated, hypotensive and exhibited signs of hepatic insufficiency. She was then treated with GSF (Neupogen), antibiotics (Amoxicillin, clavulanic acid and Ciprofloxacin), intravenous solutions and other symptomatic therapy. After 4 days, despite all measures of supportive therapy in accordance with the NCNC Guide, the patient fell in a comatose state. In a matter of hours, exitus letalis occurred.

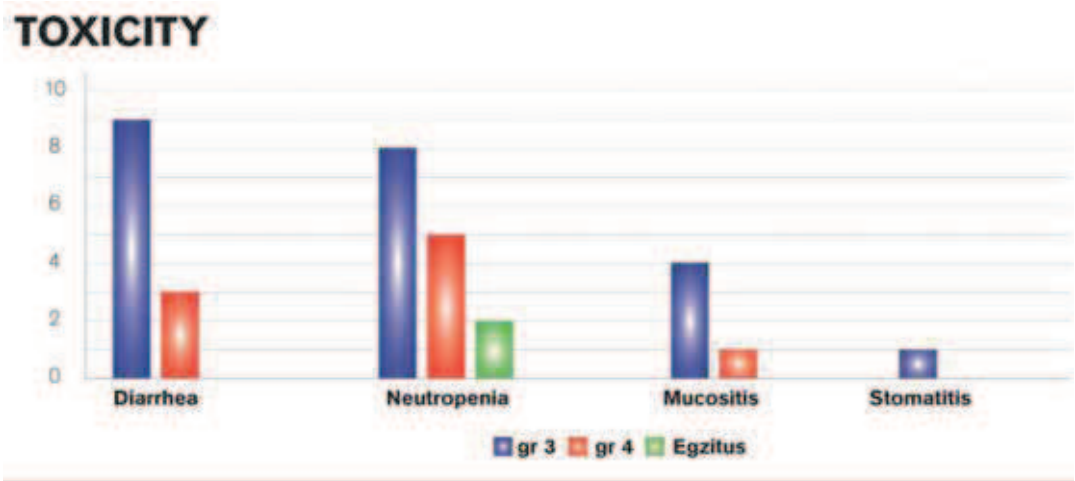

CHART 1. Overview of toxicity types

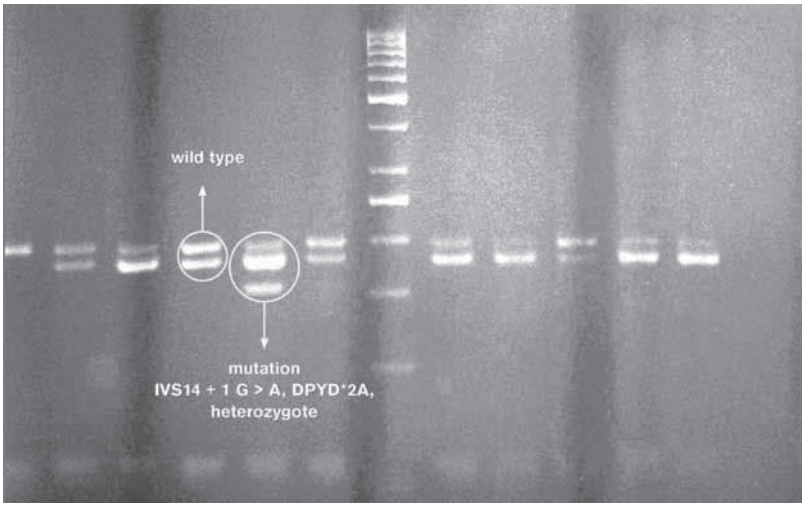

FIGURE 1. *DPYD*2A mutation*

Find below is an UV photograph (Figure 1.) taken after staining the 3% agarose gel with ethidium bromide. The image displays wild-type DPYD in lane 4 and heterozygote mutation in lane 5. The latter is IVS14 + 1 G > A, DPYD*2A that appeared after restriction, while the wild-type allele was identified by splicing 198 bp with a PCR product on 181 bp and 17 bp fragments. Conversely, the mutant allele produced three fragments after Ndel digestion: 154 bp, 27, bp and 17 bp.

Discussion

Although it was synthesised over 40 years ago, the 5-FU remains one of the most prescribed cytostatic agents in treatment of various malignant diseases. Adverse drug reactions still remain one of the most significant clinical problems. This, coupled with the meta-analysis results involving 1,219 patients treated with the 5-FU, indicate that grades 3 and 4 toxicities occur with 31-24% of patients and have a lethality rate of 0,5% (9). Although we are well aware now that many human illnesses are caused by gene mutations, there is a lesser awareness of a fact that known gene variances can affect a patient's response to certain drugs (10,11).

The importance of the DPD deficiency and severe 5-FU-related toxicity is of even greater significance considering the wide range of the 5-FU administration. The meta-analysis covering a group of over 1,200 patients suggests that more than 30% of patients treated with the 5-FU experienced a major toxic event prompted by this medication. A frequency of low DPD enzyme activity in the general population was initialy evaluated to be somewhere between 3-5%. However, further studies have proven a significant variability between different ethnic groups (Table 2). The human DPD gene (*DPYD*) is present as a single copy gene on the chromosome 1p22 and consists of 23 exons. The Table 2. provides an insight into results of certain studies (ours included) that dealt with determining relevant prevalence rate with patients of different ethnic groups. As we can see, none of the neighboring countries, has had anything published regarding this type of research on patients administered with the 5- fluorouracil, as far as we know. Hence, this makes our study that more important as it practically substantiates the only example of the DPYD 2A mutation in the region. In Bosnia and Herzegovina, more precisely in the Federation of Bosnia and Herzegovina, there were 1,303 patients treated with the 5-FU during the Y2008, while in the same period there were 657 patients administered with Capecetabine (source: FB&H Solidarity Insurance Fund). To certain extent, these figures apply to our study as well, as we do not have specific information on the 5-FU-related toxicity. This is to say there is no exact method of monitoring these patients and recording of toxic events, so we can only speculate on the issue at the country level. Given these numbers, i.e. considering that in the Y2008 alone there were altogether 1,960 patients treated with the 5-FU and Capecetabine and considering that our study indicated to 2% mutation frequency, this means that there are over 39,2 patients every year that is prone to a severe toxic response and possibly lethal outcome subsequent to the 5-FU administration. The root cause here is the DPD gene mutation. Another fact to consider is that exon 14-skipping accounts for nearly 50%

Country	*Sample*	*Number of heterozygous individuals*	*Mutation frequency*	*Mutant allele frequency*
Poland	252	*1*	*0,44%*	*0,22*
Portugal	73	1	1,37%	0,68
Germany	267	3	1,12%	0,56
Scotland	72	1	1,39%	0,69
Scotland	37	1	2,7%	1,35
Turkey	200	3	1,5%	1,25
Japan	104	0	0,0%	0,0
Bosnia and Herzegovina (the subject-matter study)	50	1	2%	1%
Holland	1357	24 ZO	1,77%	0,88
Finland	90	2 ZO	2,22%	1,11
Japan	50	0 ZO	0,0%	0,0
USA (African Americans)	105	0 ZO	0,0%	0,0%
Taiwan	300	0 ZO	0,0%	0,0%
Turkey	250	0 ZO	0,0%	0,0%
Germany	157	0 ZO	0,0%	0,0%
Egypt	247	0 ZO	0,0%	0,0%
USA (Caucasians)	95	0 ZO	0,0%	**0,0%**
USA (African Americans)	95	0 ZO	0,0%	0,0%

TABLE 2. Prevalence of IVS14+1G> A DPYD mutation in persons from different countries (12)

of the DPD deficiency. Therefore, the aforesaid number of patients would have been much greater even based solely on changes in one gene in the 5-FU metabolism.

Another interesting information here is that all heterozygous patients with proven mutations in all mentioned studies, have had the grade 4 neutropenia. If we take the findings of our study and compare it against the grade 4 neutropenia alone (wherein relevant toxicity rate was above 90% for patients with the DPYD 2A* mutation and ended in a lethal outcome) and considering that it indicates that 20% of our test group with the grade 4 neutropenia symptoms had the subject-matter mutation, then our results are very similar with such studies. Of course, we take due note of the earlier mentioned limitations imposed on this research. In conclusion, during our study, the lethal outcome occurred with two patients suffering from the grade 4 neutropenia. It is pertinent to note here that the said mutation (IVS14+1G>A), in any case, does not represent a sole cause of death in patients developing the life-threatening toxicity after the 5-FU administration. So far, the analysed gene displayed over 70 mutations and other metabolic pathways that may have caused the said lethality. The deficient DPD activity is a pharmacogenetic syndrome with a possible fatal outcome following the 5-FU therapy. Although molecular defects of DPYD leading to the deficient DPD activity can be root causes of the 5-FU syndrome, actual DPD regulatory mechanisms have not been clearly outlined. This calls for highly specific techniques for screening of the entire DPYD gene and for measuring DPD activity. Resultantly, this would enable us to draw clear conclusions of the relationship between the genotype and phenotype of the pharmacogenetic syndrome in question.

Certainly, this is only a speculative statement on our part, as more elaborate and definite conclusions can be reached only after conducting a greater scale of research. Still, it provides us with indicative information.

Even major countries have not yet solved a matter of routine screening of patients, but there is an increasing tendency of such tests. This is rooted in a pharmaco-economic aspect as treatment costs for patients experiencing severe toxicity have already proven to be much greater than screening costs. Needless to say, new screening methods are reducing inherent costs even further down.

Conclusion

Our study has indicated to the presence of the DPD mutation on the exon 14 (IVS14+1G>A). This is the first time such a case was reported with respect to the Bosnian population. (DPYD*2A frequency is nearly 2%).
Furthermore, we attest to IVS14+1G>A DPYD mutation being responsible for a significant proportion of the life-threatening toxicity in the 5-FU administration. This is based on a sample of four patients suffering from the CTC-defined grade 4 myelosupression after being treated with the 5-FU.

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EPIDEMIOLOGY AND ETIOLOGY OF OBESITY IN CHILDREN AND YOUTH OF SARAJEVO CANTON

Sniježana Hasanbegović[1*], Senka Mesihović-Dinarević[1], Mustafa Cuplov[2], Amra Hadžimuratović[2], Hajra Boškailo[3], Nevenka Ilić[3], Azemina Njuhović[4], Nermina Čengić[1], Emina Bajramović[3], Šejla Brković[2]

[1] Paediatric Clinic, University of Sarajevo Clinics Centre, Patriotske lige 81, 71000 Sarajevo, Bosnia and Herzegovina

[2] Ministries of Health, Sarajevo Canton, Reisa Džemaludina Čauševića 1, 71000 Sarajevo, Bosnia and Herzegovina

[3] Health Care Centres, Sarajevo Canton, Vrazova 11, 71000 Sarajevo, Bosnia and Herzegovina

[4] Ministry of Education and Science, Sarajevo Canton, Reisa Džemaludina Čauševića 1, 71000 Sarajevo, Bosnia and Herzegovina

* Corresponding author

Abstract

The aims of the study were to estimate the prevalence of excessive weight in infants and school-age children in Sarajevo Canton, to isolate the main causative agents and to propose a strategy for its efficient prevention. The methods included anthropometry and originally designed questionnaire. Calculated body mass index was classified according to the criteria proposed by Centre for Disease Control and Prevention (CDC). The research included 3608 students from elementary and secondary schools from Sarajevo Canton. Nearly 1/5 of subjects had excessive body weight while 12,49% of students were malnourished. Elementary school lower graders had the highest grade of excessive weight, while the secondary school students exhibited the lowest grade of excessive weight. During school hours, about 42,47% of students were fed on bakery produces and snacks. Non-sparkling, thickened juices are frequently consumed beverages (20,65%), second only to water (51,82%). 58,15% of children consume sweets on daily basis. This is even more prominent among secondary school students (80,85%). Only 1/3 of students practice sports on daily basis, while 8,51% of them rarely engage in sports. Elementary school lower grade students had the lowest level of activity while the secondary school students were the most active. As many as 27,56% students spend two hours or more sitting by the computer or TV set.

The most significant mediators of excessive weight gain are sedentary life-style, frequent consumption of sweets and thickened juices and unsuitable nutrition during school hours. Continuous preventive and therapeutically activities must be undertaken among as wide population as possible.

KEY WORDS: obesity, children, youth

Introduction

Obesity epidemic is one of the most important health problems of modern age. Over the past two decades, obesity prevalence in European countries has tripled. About 50% of adults have excessive weight while 1/3 of European population is obese. Even, 20-30% of European children and adolescents are overweight (1, 2). Statistical data by USA Centre for Disease Control and Prevention (CDC) also testify that the overweight population has tripled over the past two decades. Also, 16% of children and adolescents between 6 and 19 years of age are overweight (3, 4, and 5).

Approximately 60-85% overweight children grow up into obese adults, who lead to earlier and more frequent development of chronic non-infectious diseases: hypertension, early atherosclerosis, Diabetes mellitus Type 2, orthopaedic, endocrine and psycho-social deviations (6, 7). Although hereditary and hormonal factors may be significant in overweight development in children, excessive food consumption and low physical activity are undoubtedly the major causes (8, 9).

Sedentary time spent with TV set and computer coinciding with the consumption of calories-rich food and sweet beverages in the long term leads to an imbalance between energy intake and expenditure. The result of such imbalance is excessive body weight. Body mass index BMI > p 95 is considered obese (5, 10, 11).

An alarming trend of obesity epidemic expansion, and increase in prevalence in young population in particular, represent a problem of major economical and social consequences for every community (12, 13).

European charter on counteracting obesity adopted in 2006 proposes global measures for obesity prevention in all European countries. Precise epidemiological data on the number of obese children and youth and information of their eating habits and activities are prerogative for designing efficient action plan for the prevention of obesity development in each country (14, 15).

Aims of the study

The aims of the study were:
- estimate the prevalence of excessive body weight in children and youth in Sarajevo Canton,
- delineate major causes of this condition and to propose the strategy for its efficient prevention.

Materials and Methods

The study was conducted during 2008 and 2009. It included representative sample of Sarajevo Canton elementary and secondary schools, which were randomly selected. The number of subjects per school class (elementary 1-8 and secondary 1-4) was balanced. The students were requested to fill in a questionnaire. The questionnaire was originally designed for this study and includes questions that pertain to eating habits (frequency, quantity and types of consumed food), consumption of liquids, and physical activity (frequency and intensity). The questionnaire addressed subjects' social status regarding the total number of family members that are sustained from the same source per employed individual. The questionnaires for elementary and secondary schools contained the same questions presented in the form suitable for the participants' age. Anthropometric measurements: body height, body weight, measured in all subjects. The height was measured using vertical stat meter, the values were expressed in centimetres (cm) and rounded at 0,5 cm. Body weight was measured using digital balance, the values were expressed in kilograms (kg) and rounded at 0,5 kg. The study was conducted by 2 teams which included 2 physicians and a certified nurse. The subjects participated in the study on voluntary basis. The data entered into database were anonymous. The statistical data processing was performed according to the age groups: I-IV and V-VIII grades of elementary school and I-IV grade of secondary school.

BMI was used to estimate the nutritional status. It was calculated automatically according to the formula: BMI = body weight (kg) / height $(m)^2$

Nutritional status was derived automatically based on CDC criteria (16):
- BMI < 5 percentile indicates malnourishment.
- BMI < 85 and > 5 percentile indicates normal nutritional status
- BMI > 85 a < 95 indicates higher body weight,
- BMI > 95 percentile indicates obesity.

Results

The total of 3608 students (2329 students from 10 elementary schools and 1270 from 6 secondary schools from Sarajevo Canton) filled in the questionnaire and were examined anthropometrically.

Table 1 presents an overview of the total number of subjects and breakdown according to school age and gender.

Grade School	Total students	M	F
I-IV Elementary	1077	546	531
V-VIII Elementary	1252	680	572
I-IV Secondary	1279	531	748
Schools Total	3608	1757	1851

TABLE 1. Analysis of the examined student group
According to school level, grade and gender

Table 2 presents the data on BMI distribution among the examined students according to the school grade and gender.

Grade School	BMI classification			
	Malnourished (%)	Ideal weight (%)	Excessive weight (%)	Obese (%)
I-IV Elementary	T: 20,86 M: 7,72 F: 13,14	T: 55,26 M: 28,68 F: 26,58	T: 12,28 M: 7,47 F: 4,81	T: 11,58 M: 6,77 F: 4,81
V-VIII Elementary	T: 9,16 M: 4,65 F: 4,51	T: 69,80 M: 37,98 F: 31,82	T: 13,07 M: 7,49 F: 5,58	T: 8,00 M: 3,69 F: 4,31
I-IV Secondary	T: 6,76 M: 1,31 F: 5,45	T: 80,43 M: 34,15 F: 46,28	T: 9,55 M: 5,18 F: 4,37	T: 3,24 M: 1,47 F: 1,77
Schools Total	T: 12,49 M: 4,63 F: 7,86	T: 68,74 M: 33,60 F: 35,14	T: 11,86 M: 6,71 F: 5,15	T: 6,86 M: 1,32 F: 5,54

TABLE 2. BMI classification of students according to the school grade and gender

The quality of nourishment during school hours in the examined students and in various school age groups is presented in Table 3.

Grade School	Home-made sandwich (%)	School provided sandwich (%)	Bakery produces (%)	Snacks (%)	Do not eat at school (%)
I-IV Elementary	30,69	44,63	5,04	17,68	1,98
V-VIII Elementary	16,57	24,62	42,67	10,96	5,27
I-IV Secondary	2,70	41,09	42,01	8,89	5,37
Schools Total	16,65	36,78	29,90	12,57	4,20

TABLE 3. Quality of nourishment during school hours

Table 4 presents distribution of beverages consumption during the day among the student groups.

The frequencies of sweets consumption among students are presented in Table 5.

Grade School	Water (%)	Non-sparkling juice (%)	Milk (%)	Sparkling beverages (%)
I-IV Elementary	50,88	23,19	23,37	2,6
V-VIII Elementary	54,94	21,71	12,45	11,87
I-IV Secondary	49,35	16,77	13,19	20,72
Schools Total	51,82	20,65	16,43	11,05

TABLE 4. Types of beverages consumed during school hours

Grade School	Sweets consumed on daily basis (%)	Sweets consumed rarely or occasionally (%)
I-IV Elementary	30,89	59,11
V-VIII Elementary	64,53	33,47
I-IV Secondary	80,85	19,15
Schools Total	58,15	31,24

TABLE 5. Distribution of sweets consumption frequencies

Table 6 presents the distribution of the degree of physical activity among students that participated in the study.

Grade School	Active on daily basis (%)	Active in sports classes only (%)	Active 2-3 times weekly (%)	Rarely(%)
I-IV Elementary	19,92	39,05	40,36	0,00
V-VIII Elementary	36,42	27,56	24,52	11,50
I-IV Secondary	46,89	29,19	18,08	5,65
Schools Total	31,07	31,93	27,65	8,51

TABLE 6. Distribution of the degree of physical activity among the students

Table 7 presents the distribution of the time that students spend with computer or TV set (higher grades of elementary school and secondary school students).

Grade School	< 1 hour (%)	1-2 hours (%)	2-3 hours (%)	> 3 hours (%)
I-IV Elementary	23,60	36,62	18,51	20,89
V-VIII Elementary	13,81	30,56	27,74	27,96
I-IV Secondary	18,70	33,59	23,12	24,42
Schools Total	31,07	31,93	27,65	8,51

TABLE 7. Distribution of the time spent with computer or TV set

Discussion

This is the first study in the Federation of Bosnia and Herzegovina that presents data on the frequencies of obesity among students of elementary and second-