both CT and MR imaging is the detection of metastasis in normal-sized lymph nodes, with comparable accuracy reported (13). Distant metastases may be evaluated with either CT imaging or MR, with MR imaging superior to CT for detection of bone marrow involvement.

Squamous Cell Carcinoma

Primary squamous cell carcinoma of the bladder is relatively rare tumor, comprising 2% to 8% of all bladder cancers in the Western world, with rates varying widely according to geographic location. In parts of the world where schistosomiasis (bilharziasis) is endemic, it is a major health problem, accounting for over 50% of bladder cancers (14). Risk factors in nonbilharzial regions include chronic irritation from indwelling catheters, bladder calculi,chronic infection,but also cyclophosphamide ,smoking and intravesical bacillus Calmette-Gue´rin has also been implicated in the pathogenesis of squamous carcinoma of the bladder. Tumors are high grade and locally aggressive with muscle invasion in 80% (15). There is a predilection for the trigone and lateral bladder, and the tumor may occur in bladder diverticula as well . At cystoscopy, squamous carcinoma is a large, often ulcerated, infiltrating mass. The imaging findings in squamous carcinoma are nonspecific. Tumors may appear as a single enhancing bladder mass or as diffuse or focal wall thickening (16). In contrast to urothelial carcinoma, squamous carcinoma is sessile rather than papillary, and pure intraluminal growth is not seen. Bladder wall thickening and calcification, from chronic inflammation or infection with *Bilharzia*, may coexist and complicate the diagnosis. Muscle invasion is present in 80% of cases and extravesical spread may be extensive,involving surrounding organs and the abdominal wall (16).

Adenocarcinoma

Adenocarcinoma represents less than 2% of all bladder neoplasms. The mean age at presentation is 55 years, and its presentation can be as a primary (2/3 are nonurachal and 1/3 urahal) or as secondary appearing, metastases.

Urachal adenocarcinoma is characterististically located at the dome of the bladder in the midline or slightly off midline, and occur as characteristic soft-tissue mass with calcifications, and occurs equally often in men and women, wheares nonurachal adenocarcinoma is three times more common in men and ussualy appears at the bladder base represent like diffuse bladderthickening at CT.

Adenocarcinoma is usually associated with bladder exstrophy and a persistent urachus, but there are more risk factors for this type of bladder neoplasms which include intestinal metaplasia from chronic mucosal irritation,after urinary diversions such as enterocystoplasty and has an increased prevalence in pelvic lipomatosis because of associated cystitis glandularis (17).

The main symptoms are haematuria in 90% of cases and irritative symptoms in 50%. Mucus may be secreted in the urine in 25% of patients with urachaladenocarcinoma (18).

Adenocarcinoma is the most common histologic type of secondary bladder neoplasms. The bladder can be directly invaded by adjacent pelvic neoplasms, most commonly in the colon, prostate, and rectum (19). It is extremely important to distinguish primary from secondary adenocarcinoma because of different treatment options.

At CT, 84% of adenocarcinomas are present as a mixed, solid and cystic tumor because of mucin which is very common finding in these tumors.CT is the most sensitive modality for calcification, which is present in 72% of cases ,more commonly peripheral than stippled (20,21). Urachal carcinoma (Fig. 4) can be intraluminal, but the bulk of tumor is outside the bladder in 88% of cases (20).

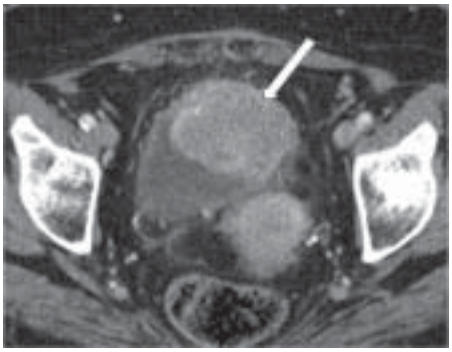

Fig. 4. Contrast enhanced CT scan of adenocarcinoma of the urinary bladder-urahal carcinoma

In contradistinction to urothelial carcinoma, extravesical spread is very common, with bladder wall invasion in 92% of cases and metastases in 48% (20,21).

At MR imaging, the location of urachal carcinoma is best demonstrated on sagittal images. On T2-weighted images, focal areas of high signal intensity from mucin are highly suggestive (22). The solid portions of the tumor are isointense to soft tissue on T1-weighted images and enhance with intravenous contrast material.

Most tumors are high grade and have diffusely invaded the bladder muscle at diagnosis (1).

Leiomyosarcoma

Sarcomas of the bladder are rare, and account for less than 1% of all malignant bladder tumors (23). Sarcomas are mesodermal in origin and undergo variable degrees of differentiation into striated muscle, smooth muscle, or connective tissue. Leiomyosarcomas are the most common histologic type of sarcoma occurring in the bladder in adults .Leiomyosarcoma is the most common nonepithelial malignant bladder tumor in adults. Sarcomas of the bladder are rare, and account for less than 1% of all malignant bladder tumors (23). Sarcomas are mesodermal in origin and undergo variable degrees of differentiation into striated muscle, smooth muscle, or connective tissue.

Leiomyosarcomas are the most common histologic type of sarcoma occurring in the bladder in adults .An increased prevalence is seen after radiation therapy or systemic chemotherapy with cyclophosphamide for another neoplasm.Main symptom is hematuria and some of them have urinary obstruction. The age range is wide at 25–88 years with a male-to-female ratio of 3:1 (24). Eighty percents of leiomyo-sarcomas are high grade at presentation, although both high-grade and low-grade tumors can behave aggressively with local recurrence and distant metastases.It arise in the sub mucosa, but usually grows intra or extravesical.

At CT and MR imaging is very difficult to distinguish leiomyoma from leiomyo-sarcoma.They are smooth, invasive masses with a mean size of 7cm (24),but because of necrossis which is common in leiomyosarcomas,they present heterodensity on CT which tend to be poorly circumscribed, relatively low signal intensity on T2-weighted MR images (25). Consequently, they are more hetero-geneous on T2-weighted images and demonstrate nonenhancing areas secondary to necrosis (Fig. 5)

Rhabdomyosarcoma

5% of all rhabdomyosarcomas occur in the bladder and prostate. Rhabdomyo-sarcoma is the most common bladder tumor in patients under the age of 10 years, with a mean patient age of 4 years; it affects boys more than girls in a ratio of 3:1 (1). It is exceedingly rare in adults. Children present with hematuria, dysuria, retention, or urinary tract infection. Rhabdomyosarcoma can manifest as a diffusely infiltrative lesion or as masses, which can be polypoid and “grapelike” (sarcoma botryoides). These tumors often involve the bladder base, and when large, are difficult to differentiatefrom those originating from the prostate gland.

At MR imaging, rhabdomyosarcoma has low signal intensity on T1-weighted images and high signal intensity on T2-weighted images with heterogeneous enhancement (25). Multiple grapelike intraluminal masses are highly suggestive of botryoid rhabdomyosarcoma. Rhabdomyosarcoma may be locally invasive, and a significant extravesical component may be present.

Conclusions

There is a significant overlap in the clinical features and radiological findings of bladder tumors,and only biopsy give a definitive diagnosis. Multiphase CT and MR imaging has a sensitivity and specificity of over 90% for the diagnosis of bladder cancer in patients with hematuria. MR imaging has been shown to allow more accurate staging of bladder carcinomas than CT because of its high soft-tissue contrast resolution which allows clear differentiation between blad-der wall layers, but they are not specific to recognize and make a clear diagnose for the type of the tumor .

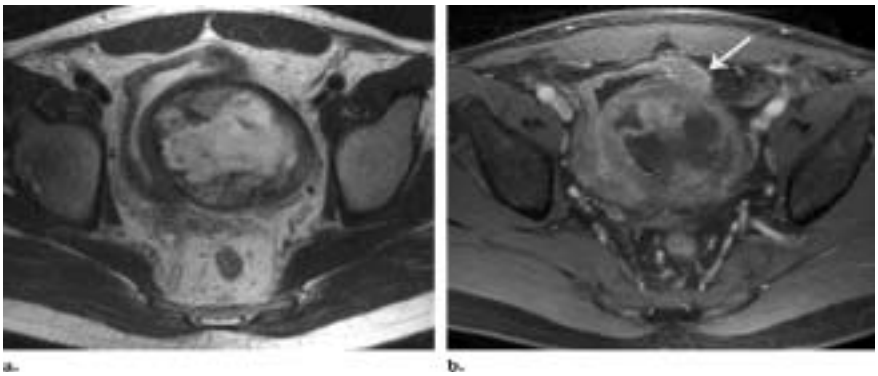

Fig. 5. Leiomyosarcoma- (a) Axial T2-weighted MR image shows a large heterogeneous mass within the bladder wall. (b) Axial gadolinium-enhanced fat-suppressed T1-weighted MR image shows irregular enhancement of the mass

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VALUE OF BREAST MICROCALCIFICATIONS IN ASYMPTOMATIC WOMEN

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Abstract

Objective: The aim of this study was to evaluate the microcalcifications in breasts on mammography in asymptomatic women.

Materials and methods: The cases of 28 women with microcalcifications in breast were analyzed. Microcalcifications were also classified according to the BI-RADS classification. We used mammomat Hologic, hard film copy, and perforated compressive plate for wire localization of microcalcifications. After wire localization patients were referred to surgical treatment. Pathohistologic results were a golden standard for establishing the final diagnosis. There were no complications after wire localization.

Statistical analysis of data was done using the statistical program SPSS, ver. 13.0.

Results: A total of 28 women were analyzed in this study. Their age ranged from 40 to 70 years; mean age 51.89 years. The microcalcifications were presented in the right and in left breast without prevalence of the side.

According to the BI-RADS classification for mammography we found BI-RADS 3 in one woman (3.57%), BI-RADS 4 in 22 women (78.57%) and BI-RADS 5 in 5 women (17.86%).

After surgery, pathohistology results were as following: 17 patients (60.7%) with benign findings and 11 (39.29%) with a malignant disease. DCIS was found in 6 patients (21.43%).

Conclusion: All breast microcalcifications should be carefully analyzed, especially in asymptomatic patients with no other evidence of persisting disease (such as palpable mass, hypoechoic mass or other signs). For all microcalcifications classified BI-RADS 4, we suggest wire localization before surgery and pathohistological evaluation.

Key words: *mammography, breast, microcalcifications, wire localization, breast cancer*

Introduction

Breast cancer is heterogeneous in terms of histology, imaging and outcome. Mammography has brought to light a new spectrum of breast cancer cases dominated by non-palpable preclinical tumors and microcalcifications.

Early detection of breast cancer is important in reducing morbidity and mortality in asymptomatic population (1). Evaluation of microcalcifications in breast lesions on a mammography as a diagnostic tool is a major assessment criterion for early detection of breast carcinoma. The morphology and the distribution of calcifications have important relation with histology of the lesions (2). Microcalcifications can be divided into benign, intermediate concern, and higher probability of malignancy according to radiological morphology (3).

Aim: To present the value of microcalcifications in early detection of breast cancer in asymptomatic women.

Material and methods

We prospectively evaluated mammographies of 28 asymptomatic women with microcalcifications. Their age ranged from 40 to 70 years; with mean age of 51.89 years.

All mammograms were done on Mamommat Hollogic, analogue machine and hard film images, which were later analyzed. The routine mammograms include a craniocaudal view and an oblique view.

In analyzing mammograms, breast composition is very important. The breast composition is a volume of attenuating tissue in the breast (3). Breast composition should be described using the following patterns:

1. The breast is almost entirely fat (<25% glandular);
2. There are scattered fibroglandular densities (approximately 25-50% glandular);
3. The breast tissue is heterogeneously dense, which could obscure detection of small masses (approximately 51% - 75% glandular);
4. The breast tissue is extremely dense (>75% glandular).

Microcalcifications in breasts are described according to the BI-RADS classification and a description of calcifications should include their morphology and distribution. Calcifications associated with malignancy (and many benign calcifications as well) are usually very small and often require the use of a magnifying glass to be seen well.

There are the following types of microcalcifications:

1. Benign calcifications;

2. Intermediate concern: amorphous or indistinct and coarse heterogeneous microcalcifications (larger than 0.5 mm);
3. Higher probability of malignancy: fine pleomorphic calcifications (smaller than 0.5 mm in diameter) and fine linear (smaller than 0.5 mm in width).

Also the distribution of calcifications could be:
a) Diffuse or scattered;
b) Regional - they are scattered in large volume >2cc;
c) Grouped or clustered (at least five calcifications occupy a small volume <1cc of tissue);
d) Linear - arrayed in a line;
e) Segmental.

We also report localization of microcalcifications in breast quadrants: superolateral quadrant, margins of proximal quadrant, margins of distal quadrant and inferolateral quadrant.

The classification of breast calcifications is based on the assessment of the morphology and the distribution, as recommended by the American College of Radiology, in the Breast Imaging Reporting and Data System (BI-RADS) (4). The BI-RADS calcifications are divided into typically benign, intermediate concern calcifications and higher probability of malignancy. We use the BI-RADS reporting system for radiologic interpretation. The BI-RADS is a quality assurance tool designed to standardize mammography reporting, reduce confusion in breast imaging interpretation and facilitate outcome monitoring. According to the BI-RADS classification on mammography:
BI-RADS 0 - need additional imaging evaluation or prior mammograms;
BI-RADS 1 - negative. There is nothing to comment on;
BI-RADS 2 - benign finding;
BI-RADS 3 - probably benign finding (<2% malignant);
BI-RADS 4 - suspicious abnormality (2-95% malignant);
BI-RADS 5 - highly suggestive of malignancy (>95% malignant);
BI-RADS 6 - known biopsy-proven malignancy.

We analyzed BI-RADS 3 (occasional cases when there was breast carcinoma in the family or the women had breast carcinoma), BI-RADS 4 and BI-RADS 5. BI-RADS 0, 1, 2, 6 were excluded from the study. BI-RADS 3 was included only in cases with breast cancer on the opposite side.

When microcalcifications were present, the next step was localization with wire marker Bard hook wires using compressive perforated plate with mammographic control. The patient has to be positioned comfortably in a chair at the mammography machine. In sterile conditions the tip of the needle must rest in the location that the surgeon needs to find in order to remove the right place. The needle is removed, living wire in place on control mammogram in two projections. When the wire is finally positioned, it is secured in place with tape. The final mammograms in CC and ML/LL are performed to show the surgeon where the tip of the wire lies in relation to abnormality that is to be removed. Hook wire localization assists the surgeon in removing of breast microcalcifications. Following this procedure, specimen were radiographed in all our cases and sent for pathohistological analysis.

Statistical analysis of data obtained during the study was done with the statistical program SPSS, ver. 13.0 for Windows. The results of the study are presented with descriptive statistics (mean, standard deviation, minimum and maximum) and with distribution of frequency (absolute and relative numbers). The validity of the diagnostic test mammography has been compared with the pathophysiologic results as a golden standard.

Results

A total of 28 patients were analyzed in this study. Their age ranged from 40 to 70 years; mean age 51.89 years. Microcalcifications were present in the left and right breast without prevalence of the side. According to breast composition, scattered fibroglandular densities were found in 12 (42.86%) cases and heterogeneous densities were also found in 12 (42.86%) cases. Very dense breast with more than 75% of glandular tissue was found in 3 (10.71%) patients of our series and with less than 25% of glandular tissue in 1 (3.57%) patient. No breast surgery had 89.2% of patients, while 10.6% underwent breast surgery for malignant diseases. When we analyzed localization of microcalcifications in the breast quadrants, we noticed prevalence in superolateral quadrant (64.2%), margins of proximal quadrants (7.1%), margins of distal quadrants (17.8%) and inferolateral quadrants (3.5%).

Accepting the BI-RADS classification for mammography, we found BI-RADS 3 in 1 woman (3.57%), BI-RADS 4 in 22 (78.57%) and BI-RADS 5 in 5 women (17.86%) (Table 1).

Table 1. Distribution of patients according to BI-RADS classification for mammography

BI-RADS mammography	Number	%
3	1	3.57
4	22	78.57
5	5	17.86
Total	28	100

Table 2. Distribution of patients according to patohistologic results

Benign results	Number	%
Mastopathy	7	25.00
Sclerosis adenosis	5	17.86
Fibroadenoma	2	7.14
Intraductal papiloma	2	7.14
Fat necrosis	1	3.57
Total	17	60.71
Malignant results	**Number**	**%**
Intraductal carcinoma	4	14.29
DCIS	6	21.43
Lobular carcinoma	1	3.57
Total	11	39.29
Sub total	28	100

According to the BI-RADS classification the microcalcifications on mammograms were as follows: 52% intermediate microcalcifications (amorphous and indistinct - 35.7% and coarse heterogeneous microcalcifications - 17.8%), and 10 (36.71%) microcalcifications with higher probability of malignancy.

There were 17 patients (60.71%) with benign findings and 11 (39.29%) with malignant diseases. Six patients (21.43%) had DCIS (Table 2).

Discussion

Mammography is a method of choice for assessment of microcalcifications within the breast lesions or tissue. Mammography is highly sensitive but only moderately specific in microcalcification detection and evaluation (5). For symptomatic cancer, which may present as a mass lesion, detection of calcification is not crucial to the diagnosis, as the malignancy can be confirmed by other diagnostic modalities. In the non-palpable lesions the detection and characterization of the microcalcifications are important. Microcalcifications are particularly important for ductal carcinoma in situ (DCIS), which is the preinvasive stage of breast cancer. The incidence of calcifications in ductal carcinoma in situ has been reported within the range of 42 to 72% (6). DCIS is the most common malignancy presented with microcalcifications. In the serious of 304 breasts carcinoma

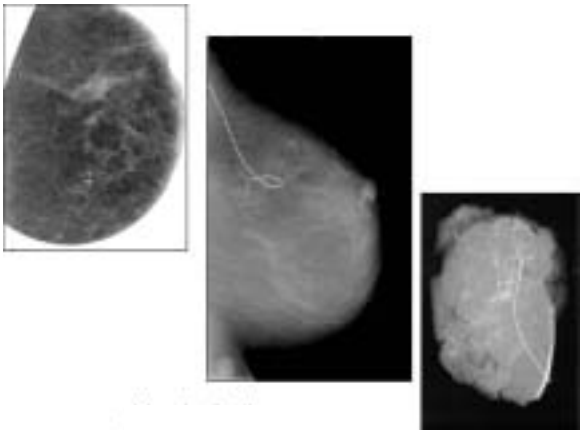

Fig. 1. Adenosis scleroticans

manifested by mammographic microcalcifications, the final pathologic diagnoses of pure ductal carcinoma in situ (DCIS) was confirmed in only 65%; of them, DCIS with focus of invasion was found in 32% and invasive carcinoma only in 4%. The median age of patients was 54 years (range 29 - 86 years) (7). We found DCIS and microcalcifications in 21.43% of patients. Pleomorphic microcalcifications were present in 5 women (17.86%) and fine linear microcalcifications in 5 cases (17.86%).

Microcalcifications associated with cancer very often have a similar morphology to those produced by benign process (8).

In general, microcalcifications, when they are diffuse and not segmental in distribution, are less worrisome as this indicates a diffuse process, typical in fibrocystic changes that involve the whole breast. Grouped or clustered microcalcifications may be indicative of either benign or malignant lesions, whereas the higher density of calcification is suggestive of a more active or proliferative process resulting in increased secretion density (8). In our study, grouped or clustered microcalcifications were found in 28 cases. Benign pathohistologic findings were present in 17 (60.71%) and malignant in 11 (39.29%) cases.

The incidence of carcinoma was 29.1% out of 189 patients (9). Invasive lobular carcinoma was detected in 3.1% in a series of 294 patients (10). In our study lobular carcinoma was discovered in only 1 woman or 3.57%.

Mammography is very sensitive in detection of microcalcifications, but benign calcifications cannot always be distinguished from those indicating malignancy, and the specificity of mammography remains low. Only 20%-35% of the cases will prove to be cancerous after hook wire guided surgical biopsy (11).

We found mammography BI-RADS 3 in one woman who had breast cancer on contralateral side. The

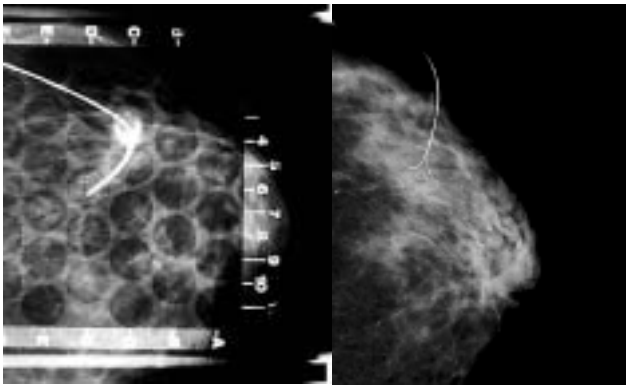

Fig. 2. Ductal carcinoma in situ (DCIS)

postoperative finding was benign (mastopathy). Sensitivity for benign microcalcifications was 100%. Mammography BI-RADS 5 was found in 5 patients (5/28) and all had proven to have breast cancer. BI-RADS 4 were 22 patients, 16 (72.73%) of them had benign disease including 6 patients sclerosans adenosis. Malignant disease was proven in 6 patients or 27.27% in patients with BI-RADS 4.

Christos Markopoulos et al. reported histological findings in 240 patients with microcalcifications who underwent hook wire localization under mammographic guidance. Malignant lesions were found in 45% (108/240) patients and benign in 55% (132/240). The most common malignant lesion was DCIS (21.3%), and lobular carcinoma was detected in 3.7%. Benign lesions were presented with mastopathy (25%), sclerosing adenosis (15.2%), and fibroadenoma (2.3%) (12).These results were very similar with ours: DCIS - 21.43%, lobular carcinoma - 3.57%, mastopathy - 25%, sclerosing adenosis - 17.86%, fibroadenoma - 7.14%.

Currently, no imaging modalities other than mammography have an accepted role in detection and characterization of microcalcifications (13).

However, analysis was difficult in 40-50% of the cases where the features of calcifications and their cluster could be classified only as indeterminate or equivocal (14). Sclerosing adenosis usually presents with calcifications and it can mimic breast cancer.

Conclusion

This study has confirmed that microcalcifications in asymptomatic women are important in early detection of breast cancer although sometimes it is difficult to classify them. The classification is especially important in the early stage of carcinoma and we recommend patohistological evaluation of microcalcifications in BI-RADS 4 category.

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VALUE OF ULTRASOUND IN DIAGNOSTING AND FOLLOW UP OF THE PATIENTS WITH BLUNT ABDOMINAL TRAUMA COMPARED WITH COMPUTED TOMOGRAPHY AND OPERATIVE FINDINGS

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Abstract

Objective: To emphasize the role and value of ultrasound (US) and computed tomography (CT) in expeditious and correct radiological evaluation of blunt abdominal trauma (BAT).

Methods: This paper is a prospective study conducted over a 3-year period, during which 60 patients with anamnesis for blunt abdominal injuries (BAT) were examined from the total of 60 patients, 39 (65%) were men, 21 (35%) were women, with an average age limit of 2-81 years and an average age of 22,7 years. An US was carried out on all patients immediately after admission, as well as more US exams during the first 24 hours. CT exam was carried out on 44 patients.

Results: All patients had abdominal pain. 21 had defans, 17 patients had symptoms from associated injuries to other organs and systems, and 12 patients had a clinical presentation of hemorrhagic shock. The diagnostic methods which are used depend on the hemodycamically stability of the injured person.

Out of 60 BAT patients who underwent an US exam, in 56 (93%) of the cases free abdominal fluid was present. In 45 of the cases CT was done, and free fluid was diagnosed in 44 (98%) of the cases. Parenchimal abdominal injury was diagnosed with US in 51 (85%) of the cases, and in 59 (98%) with CT.

Conclusions: Negative US finding does not exclude the parenchymal organ injury. In hemodynamically instable patients, an important role plays the application of US, which is fast and simple to perform method which detects the parenchymal abdominal lesion and presence of free fluid (hematoperitoneum), in the abdominal spaces. CT, especially after intravenuos contrast material application, has a leading role in diagnosing of the parenchymal injuries in BAT, in determining the place and the injury expanding. CT diagnosis enables further treatment to be determined.

***Key words* :** blunt abdominal trauma, imaging, ultrasound, computed tomography.

Introduction

Blunt abdominal trauma (BAT) is an injury of the parenchymal abdominal and retroperiotoneal organs, to the digestive tube, and to the vascular branch when force is applied to the abdominal region. Injuries often occur from traffic accidents, fall from a great height, blow with a blunt object to the abdominal area, from a fight, and from sport contacts [1]. Most frequent casualties are persons aged 15-45, although BAT can occur regardless of gender and age. The male gender dominates, with adult ratio of 3:1, and children ratio 2:1 [2]

As a parenchymal organ, most frequently subject to injury is spleen, followed by liver, kidney, pancreas, mesentery blood vessels, and intestines [3]. An isolated injury to the spleen occurs in 50% of the cases, while a blunt liver injury is associated with an injury of the spleen in 45% of the cases [4567] . Liver trauma makes up 15-20% of the BAT, and is the second most frequently injured organ. However, 50% BAT mortality rate occurs as a result of liver injury.

The clinical finding is often insufficient, and has lower sensitivity in intraperitoneal injuries as a result of BAT [8]. Although a physical exam is not entirely certain, with a diagnostic accuracy between 45% and 50%, it is nevertheless a starting diagnostic procedure in treating BAT patients [9]. The patient who is conscious and hemodynamically unstable should be immediately examined.

In our everyday practice, we use US not only to locate free fluid in the patients with BAT, but also to detect parenchymal lesions, to determine the degree and severity of the damage, to plan further treatment, as well as continuous follow up of BAT patients who are subject of conservative treatment.

Purposes

A complete radiological evaluation of BAT, and to emphasize the role and value of ultrasound and computed tomography (CT) in expeditious and correct BAT diagnosis, which will reduce the number of invasive methods such as diagnosting peritoneal drainage (DPL) and diagnostic laparotomy.

To determine the value and role of primary and repeated US in follow up of the BAT patients.

To assess the value of US and CT in determining the level of injury of parenchymal organs in case of BAT,

continuous follow up of the injury, as well as treatment planning.

Materials and methods

This is a prospective study conducted over a 3-year period, during which 60 patients with medical history for blunt abdominal injuries (BAT) were examined. This also included patients with injury to parenchymal organs of the abdomen and retroperitoneum. Patients with injury of abdominal blood vessels, intestines were excluded from the study.

Out of 60 patients, 39 (65%) were male, 21 (35%) were female, with an average age limit of 2-81 years and mean age of 22,7 years. The men-women ratio was 1,8:1 in favor of the men. The patients were divided into five groups, according to gender and age: up to 10 years of age – 15 (20,5%) patients, 6 male and 9 female; 11-20 years of age, 15 (20,5%) patients, 10 male and 5 female ; 21-30 years of age, 15 (33%) patients, 8 male and 7 female; 31-40 years of age, 8 (29,1%), 8 patients, 6 male and 2 female; and over 40, 7 (6,3%) patients, all male.

According to cause of the injury, patients were divided into 4 groups: 1. traffic accidents; 2. fall; 3. physical fight, and 4. other.

An US was carried out on all patients immediately after admission, as well as more US exams during the first 24 hours, in order to confirm or exclude the existence of trauma to the parenchymal organs. With conservatively treated patients, more US exams were carried out to monitor the condition during the treatment. During each US exam, the abdomen, retroperitoneum, and the small pelvis are explored with a 3,75 MHz probe.

CT was done in 44 of the cases, to confirm the US result or to determine the presents of a traumatic injury of parenchymal organs that was not seen on US. The interval from the US to the CT was not longer than 2-6 hours. CT was done without and with intravenous contrast material application.

Injury score was done by using the Organ Injury Scaling Committee (OISC) of the American Association for the Surgery of Trauma (AAST) [12] which, in 1987, provided a universal assessment system for injury to parenchymal organs, and in 1994 revised the assessment system for spleen injury.

The severity of the parenchymal lesion of the spleen was evaluated according to the AAST scale (table 1); the severity of the parenchymal lesion of the liver will be evaluated according to Moore (table 2), and the severity of the parenchymal lesion of the kidney will be evaluated according to the AAST scale (table 3).

In terms of the clinical state that was assessed by a surgeon and an anesthesiologist, according to the scale for hemodynamical instability (HIS) by J. Wayne Meredith, patients were divided into two groups: group I:

Table 1. Severity of the parenchymal lesion of the spleen according to AAST:

1 degree	subcapsular hematoma smaller than 1 mm, smaller than 10% of the surface or laceration with 1 mm depth
2 degree	Subcapsular hematoma > 10% and < 50% of the surface; laceration 1-3 mm depth, hematoma with diameter < 2 mm.
3 degree	Subcapsular hematoma > 50% of the surface, laceration > 3 mm depth, intraparenchymal hematoma with diameter > of 2 mm.
4 degree	Ruptured intraparenchymal hematoma with active bleeding or laceration that includes hylar or segmented blood vessels with an avascular zone > 25% of the spleen
5 degree	Fully crushed spleen, avulsion from hilus

Table 2. Severity of parenchymal lesion of the liver according to Moore:

1 degree	Subcapsular hematoma smaller than 1 mm, smaller than 10% of the surface, laceration with depth of up to 1mm
2 degree	Subcapsular hematoma of 3 mm, 10-15% of the surface, intraparenchymal hematoma with diameter < 2mm, laceration 1-3 mm.
3 degree	Subcapsular hematoma > 50% of the surface, ruptured subcapsular hematoma with active bleeding > 3 mm, intraparenchymal hematoma with diameter > 2mm, laceration > 3 mm depth
4 degree	Ruptured intraparenchymal hematoma > 10 mm with active bleeding, laceration 25-50% of the liver lobe
5 degree	Laceration > 50% liver lobe, injury to the hepatic blood vessels
6 degree	Liver avulsion

Table 3. Severity of the parenchymal lesion of the kidney according to AAST:

1 degree	minor injury: renal contusion, intrarenal and subcupsular hematoma, minor laceration with a limited perinephric hematoma without affecting the collector system or medulla, small subsegmental corticar coronary thrombosis
2 degree	Major injury: major renal laceration through the cortex that reaches the medulla or the collector system, with or without urine extravasation, segmentation renal coronary thrombosis
3 degree	Catastrophic injury: multiply renal lacerations, vascular injury that includes the vascular petal
4 degree	Urethral pelvic injury: avulsion – complete transection, laceration – incomplete severing

hemodynamically stable patients and group II: hemodynamically unstable patients.

The amount of free fluid in the abdomen was determined with the method by M.S. Huang. The following spaces are relevant: perilienal, perisplenic, the Morrison space, paracolic spaces, and rectovesical or rectovaginal recess.

The patients from this group were divided into three subgroups, according to the US finding:

subgroup I: the presence of free fluid in the abdomen, with no visible injury to the parenchymal organs,

subgroup II: where there is one injured organ

subgroup III: where two or more organs are injured.

The accuracy for detection of parenchymal lesions is much higher if the time from the injury till the US examination is as short as possible

In relation to the assessment made about the level of severity of the injuries, the patients were divided into two groups: group I: applied conservative treatment and group II: applied operative treatment.

Results

All patients had abdominal pain. 21 had defans, 17 patients had symptoms from associated injuries to other organs and systems, and 12 patients had a clinical finding of hemorrhagic shock.

The most frequent cause of the injury was from traffic accidents, in 35 (58,3%) patients, 12 (20%) patients had fall injuries, 11 (18,3%) patients sustained injuries from a fight, one patient (1,7%) was injured during a sporting event (soccer), and one patient (1,7%) was injured from a kick by a horse's hoof to the ribcage and the abdomen.

Hemodynamically stable patients were 48 (80%) and hemodynamically unstable patients were 12 (20%).

The diagnostic methods used depend on the condition of the patient, whether they are hemodynamically stable or hemodynamically unstable. Aside from the conventional radiological methods such as plain film of the abdomen, ribcage, extremities, and head, special emphasis was given to the imaging diagnostic methods such as the US or/and CT.

During the initial US exam carried out on 60 patients immediately after admission, free fluid was detected in the abdomen in 56 patients, and the other 4 patients had normal US findings.

Eight patients had 1500 mL, 14 had 1000 mL, 18 had 500 mL, and 4 had between 250 mL and 500 mL.

Four patients that were with normal findings of the initial exam, with persistent pain in the abdomen, were reexamined with US after 2 hours and the finding was identical to the initial one. Four patients were examined again six hours after their admission, and a small amount of free fluid was found in Douglas space. CT was done in 4 patients, and there were positive findings in 3 cases; two were with spleen hematoma, one with liver hematoma, and one was without CT signs for trauma to the parenchymal organs, only minimal amount of free fluid in the Douglas space was present.

From the other 56 patients with a positive finding of free fluid in the abdomen, 17 patients were polytraumatized, 2 with injury to two organs, and 39 with an injury to a parenchymal organ. All patients with polytrauma had an injury to the parenchymal organ, along with an injury to other organs and systems.

Out of 60 patients with BAT, 12 were hemodynamically unstable and were immediately taken to the operating room, after only US examination which was done immediately after being admitted to the intensive care unit. CT was done on the other 48 patients, along with the US exam.

Thirty four patients were operated on, while 26 were conservatively treated.

From the total number of 17 polytrauma patients, 7 had liver injury (Fig. 1), 9 had spleen injury (Fig. 2), 2 had kidney injury (Fig.3), and 2 had pancreas injury. Two patients had injuries to 2 or more parenchymal organs.

Out of 60 patients with BAT, 16 (26,6%) polytrauma patients died.

In 18 patients that underwent an US after admission and it was then repeated after 6 hours, a

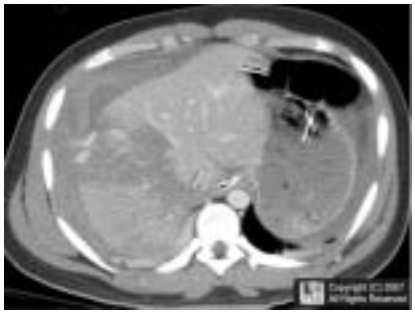

Fig. 1. CT image of liver laceration with formation of intraparenhimous hematoma and free fluid in the abdomen.

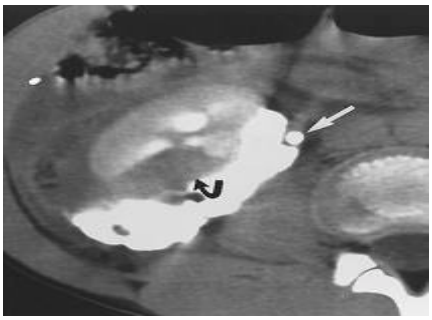

Fig. 3. CT image of kidney laceration with perirenal hematoma.

parenchymal lesion of the liver was detected. 2 patients had the subcupsular hematoma type, 5 had the laceration type, 4 had parenchymal hematoma, 3 had a deep laceration with intraparenchymal hematoma, and 1 had a ruptured spleen. A contrasted CT exam was carried out on all 18 patients. The US finding in relation to traumatic lesion was confirmed in all patients, but the difference is in determining the level of the injury's severity which is determined according to the Organ Injury Scaling Committee (OISC) of the American Association for the Surgery of Trauma (AAST) (Table 4, 5).

The final decision on which patients will be conservatively treated and which will be operatively treated, was made by the surgeon.

Table 4. Division according to the localization and degree of the traumatic injury of the parenchyma organs according to AAST and Moore, detected with US at admission.

Localization	Degree						Summary
	I	II	III	IV	V	VI	
Liver		3	7	1			11
Spleen	1	5	7				13
Pancreas							
Kidney	1	3	1	1			6

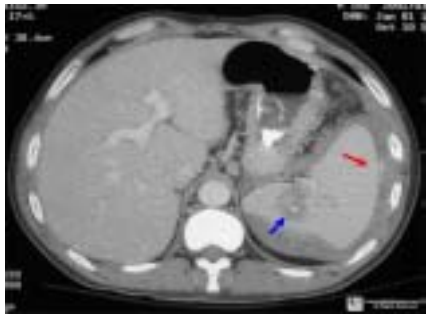

Fig. 2. Spleen trauma a/ US image of spleen trauma visible laceration with intraparenhimous hematoma b/ CT image of a traumatic spleen lesion with intraparenhimous hematoma and perilineal and perihepatal fluid in the abdomen.

Since the BAT occurrence mechanism can cause injury to other organs and systems, the patients were divided into two groups:

group I: with an injury to the abdominal or retroperitoneal parenchymal organs we have 43 (71,6%) patients and

group II: with an injury to the abdominal parenchymal organs and injuries to the locomotory system, to the chest or craniocerebral injuries (polytraumatized) we have 17 (28,3%) patients.

From the 60 BAT patients who underwent an US, 56 (93%) of them had been diagnosed with a free fluid in the abdomen. 45 of them also underwent a CT, and the free fluid was diagnosed in 44 (98%). A Student proportions test analysis revealed that there is no statistically significant difference in relation to diagnosing free fluid in the abdomen between both diagnostic methods (p= 0,2409). Two methods are required to determine free fluid in the abdomen.

From the 60 BAT participants, injury to the parenchymal organs in the abdomen is diagnosed in 51 (85%) with US and in 59 (98%) with CT. The Student proportions test analysis revealed that there is a statistically significant difference in relation to diagnosing injury to parenchymal organs in the abdomen between

Table 5. Division according to the localization and degree of the traumatic injury of the parenchyma organs according to AAST, detected with CT.

Localization	Degree						Summary
	I	II	III	IV	V	VI	
Liver	4	4	7	1			16
Spleen	5	6	9				20
Pancreas	1	2					3
Kidney	2	4	1	1			8

both diagnostic methods (p = 0, 0119). (p = is smaller than 0,05 which means that CT is a significantly more sensitive method).

BAT of the spleen can result with sub capsular hematoma, intraparenhimous hematoma, laceration, fragmentation with autospleenectomia.

Discussion

The WHO[16] data vividly show that according to the frequency, BAT is a rate of 25 in 100.000 inhabitans.

In our country, BAT is with a rate of 27 in 100.000 inhabitans, and the ratio between men and women is 2,5:1. As a cause for death, BAT in the Republic of Macedonia is on the ninth place in men, the women are on the 14th place. The mortality rate is 25 in 100.000 inhabitans, men 35 in 100.000 inhabitans, women 21 in 100.000 inhabitans.

In our series of examinees, the ratio of men towards women is 1,8:1, data found in the Republic Institute for Health Protection.

The sex distribution varies from 2:1 to 3:1 between men and women, depending on the age, but still they are never equal.

BAT most frequently appears in younger population [12, 13], in our study, according to the age distribution, most frequent are patients from 11 to 30 years (33%), followed by patients above 30 years (27%) and 22% were up to 11 years. Only 3,1% are older than 70 years. This corresponds with the data from the Republic Institute for Health Protection and WHO, as well as with the literature data [10, 11, 12].

In our study there were patients who did not have particular outstanding symptoms, but only the pain in the area of abdomen. Among the most frequent symptoms and clinical manifestations, were pain in 80%, defans in 17,7% and hemodynamic instability 6,3%. As to the symptoms, most world authors quote that those patients do not have clear symptomatology, while the others have symptoms same as in our group[5, 6, 10, 13] .

A very specific characteristic of the parenchymal abdominal organs is that they are well vascularized and their traumatic injury leads to abundant bleedings that are life threatening for the patient [3, 4, 5, 6]. The signs and symptoms of the parenchyma organs injuries are connected with blood loss, irritation of the peritoneum, sensitivity in the upper abdomen. Due to the blood loss, patients with BAT, can manifest in shock condition, hypotension and hematocrit drop [3, 4, 5, 6].

Patients with BAT can have accompanying injuries such as pelvis and extremities fractures, ribs fractures and craniocerebral injuries, because most frequently those patients are polytraumatic especially during traffic accidents or fall from greater heights [3, 4, 5, 6, 13].

Besides the clinical laboratory findings, an important role in diagnosing BAT, have US and CT.
US has to determine whether or not there is an injury to the parenchymal ograns as well as to assess the degree of injury according to the OIS system. The patients who undergo conservative treatment are continuously monitored by appling US examinations in determine time periods. This is especially important since modern views on treating BAT advocate for the parenchymal organ to be preserved as much as possible. The removal of injured organ is recommended only as the final choice. This is especially important for the young population. CT with contrast media is applied with hemodynamically stable patients and should reveal whether patients with medical history for BAT have: free fluid in the abdomen, and to determine the amount, it is determined according to Federie et al.; an injury to the parenchymal organs; to determine the severity of the injury according to the OIS system and to recommend further treatment (conservative or operative).

The normal finding on the plain film of thorax and abdomen do not exclude the parenchymal organ injuries. The most frequent finding connected with spleen injury is fracture of the left lower ribs. The ribs fracture is present in 44% of the patients with spleen rupture, so they should be further radiological examined, with US and CT [6, 13]. The ribs fracture is connected with the injury of the upper front right part of the liver in 33% of the patients [4, 5, 6, 13].

The US diagnostic ability depends on the skill and experience of the examiner, with more skillfulness, greater is the exactness of the US finding in determining the free fluid in the abdomen and the spleen parenchyma lesions [2, 3, 13]. US has a limited value in detecting vascular injuries and is not always in a condition to show injuries of the pancreas, suprarenals, intestines, mesenterium and the diaphragm. The negative US finding does not exclude the parenchyma organs injury. The differentiation of the sub capsular with the perispleenic blood collection, is difficult. Smooth collection found at the rim of the parenchyma organ, is probably a sub capsular hematoma. To be differentiated, the extra capsular blood is usually with an irregular shape, Mass effect is present in both cases and the sub capsular blood collection misshapes the parenchyma organ contour.

In hemodinamically instable patients, an important role plays the application of US, which is a very fast and simple method which detects the parenchyma lesion of the abdominal organs and fluid presence / hematoperitoneum/, in the abdominal space [20, 21].

CT, especially after intravenuos contrast, has a leading role in diagnosing the parenchyma injuries in BAT, in determining the place and the injury expanding, but first of all the CT finding enables determining of the further treatment, which is necessary for healing the trauma consequences. The contrast marks the abdominal organs parenchyma and enables a clear demarcation of traumatic lacerations and ruptures, as well as traumatic hematomas

[12, 13, 14, 23]. CT is with a high sensitivity, specificity and exactness in detecting the trauma and also comprehensiveness of the abdomen injury in traumatic patients [17, 18, 19]. This method can also give details for a trauma existence also in other organs, such as the thorax, lungs, spine and pelvis as well as for eventual existence of craniocerebral injuries,

CT cannot be applied in hemodinamically instable patients because of the vital threatening and urgent need for a surgical treatment. So, CT should be preferred in hemodinamically stable patients, able to collaborate in the course of the examination [15 16 17 22].

The use of US and CT in patients with BAT reduces the need for explorative laparotomy for a diagnostic aim, as well as laparatomy for a therapeutic aim, because they give an assessment also for the injury degree.

Conclusion

US as a fast, low cost and easily acceptable method for the examination of exclusively serious patients, has a great value and a diagnostic accuracy in 98% in the detection of a free fluid in the abdomen and 72% is the accuracy for detection of the traumatic lesions of the parenchymal organs.US achieved a high accuracy in determining the injury degree of the parenchymal organs, but still significantly lesser, compared with CT, which is uncontested in the assessment of the gravity of the parenchyma organs injury.

CT has high percent of sensitivity and specificity in examination of BAT. CT high diagnostic accuracy was confirmed for the detection of a free fluid in the abdomen, detection of traumatic lesions of the parenchymal organs and for the assessment of the injury degree.

Both image methods, US and CT, are used for the planning of treatment /conservative or operative/, as well as for the follow-up of patients in the course of the conservative treatment.

By using the imaging diagnostic methods and the original algorithm for BAT, death rate should prevented and also the unnecessary laparotomias, the number of DPL and diagnostic laparoscopy will be decreased, and also the number of patients who will have a partially or completely preserved organ which was injured, will be increased.

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INFLUENCE OF TYPE OF SURGICAL CORRECTION ON THE INCIDENCE OF PIN INFECTIONS IN PATIENTS TREATED BY ILIZAROV METHOD

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Abstract

Objective: The aim of this study was to evaluate the influence of the type of surgical correction on the incidence of pin infections, and to present the measures to minimize them in patients treated by the method of Ilizarov.

Material and methods: The analysis was made on 26 patients treated by the method of Ilizarov at the University Clinic for Orthopaedic Surgery in Skopje from 2006 to 2011. Patients were divided in three groups according to the type of surgical correction. The first group consisted of 9 patients who underwent a lengthening procedure. In the second group, 13 patients were treated with fixation, compression or deformity correction, and the third group included 4 patients with a compressive arthrodesis. In all patients, only wires have been used. Pin infections were graded using the Saleh-Scott classification system.

Results: In 16 patients (61.53%), sings of pin infections were detected. Infection rate was assessed as a percentage of the number of pin sites as well as a percentage of the number of patients. The total pin infection rate was 14.92%. The subgroup of patients with treated fractures presented the lowest incidence of pin infections (7.4%) whereas the subgroup of post-traumatic non-unions presented the highest incidence (21.21%). All the infected sites were graded as grade 1 or 2 according to the Saleh-Scott grading system.

Conclusion: Pin infections were more common in patients undergoing lengthening procedures. Nevertheless, the difference in the incidence between three groups of patients with various surgical corrections has not shown a statistical significance.

Key words: pin tract infections, incidence, Ilizarov method

Introduction

The use of the method of Ilizarov is laden with numerous complications such as injuries on nerves and blood vessels tendons or muscles, over tensioning of wires and cut-through phenomena, breaking or loosening of wires and bolts, sinking of olives on the points of contact with bone etc. Some of the complications and side effects in the treatment are obvious once the process of lengthening, compression and/or correction of deformities on segments and extremities begins, such as disturbance of normal vascularisation of the segment, distortion in bone axes, premature bone consolidation or poor bone formation, limitation in the range of passive and active motions in the neighbouring joints due to contractures, subluxations or luxations, chondrolysis, transitory or permanent damage of joint structures, bone infections, skin cicatrices on the corticotomy site as well as on the pin sites etc. Among the most common complications are the so-called pin infections. They are often associated with external fixation and are demonstrated as a superficial or deeper infection on the treated segment or even as an osteomyelitis. Some of the signs of pin infection are erythema, exudation, pin loosening, elevated local temperature, pain etc. The incidence of pin infections varies in different institutions and in some reports reaches to 50% [1]. The consequences of pin infections can range from trivial to severe. Nevertheless, most of them respond well to local pin care and oral antibiotics, excluding the cases of initial failures in insertion of pins and wires, inappropriate angles between the wires on each ring, inadequate tensioning of wires, inadequate wire and pin dimensions etc.

The purpose of this study was to determine the influence of different types of surgical correction on the incidence and severity of pin infections, as well as to present the measures to minimize them in patients treated by the method of Ilizarov.

Material and methods

In this retrospective study, 26 patients treated by the method of Ilizarov at the University Clinic for Orthopaedic Surgery in Skopje during the period from 2006 to 2011 were analyzed from the point of view of the incidence of pin tract infections. The age distribution ranged from 8 to 61 years with a mean age of 32.65 and SD 17.43. Sixteen of the patients were males and 10 females. Patients were divided in three groups according to the type of surgical correction using the method of Ilizarov.

The first group was consisted of 9 patients undergoing a lengthening procedure because of segmental shortening. Eight of them were with congenital shortening whereas in one patient the abbreviation was caused by poliomyelitis. Seven of the lengthening procedures were performed on lower leg, and 2 of them on the femoral region.

The second group was represented by 13 patients who were treated with fixation, deformity correction or compression on the segment using the Ilizarov method. Four of them were with fractures and 9 with non-unions (5 infected and 4 posttraumatic). The segmental distribution in this group was following: 7 lower legs, 3 arms and 3 femoral regions.

The third group was consisted of 4 patients treated with a compressive arthrodesis by the Ilizarov method; two of them on the knee joint, and the other two on the ankle joint. The knee arthrodeses using the apparatus of Ilizarov were performed after a failure in revision total knee arthroplasty because of septic loosening. The first compression arthrodesis of the ankle joint was performed in an adult female and was combined with a correction of severe inveterated deformity as a consequence of non-treated equinovarus deformity in childhood, and the second was performed because of an inadequately treated comminuted fracture of distal tibia.

The significance of the differences between the analyzed incidences of pin infections in the three different groups of patients was determined using the Pearson's chi-squared test as a valuable statistical test for independent samples. Statistical significance was defined as a p-value < 0.05.

In all patients included in this study, no half-pins but only wires have been used. Intraoperatively, the wires were inserted using a power drill at a slow speed with a removal of the drill once the wire exited and use of a mallet to finish the contact between the wire and the ring on the opposite side. Once fixation was bolted and nuts were placed to fix wires on the apparatus, they were consequently tensioned using a wire tensioner. At the end of the operative procedure, gauzes with betadine in form of squares were placed on each pin site and an occlusive dressing was used around. 48-72 hours postoperatively, the whole region around the pin sites was left uncovered. An additional use of gauzes was necessary only in rare cases of exudates on the pin sites. The condition of each pin site was recorded daily, and a crust removal was performed if necessary. Pin site care was accomplished by the local use of bacitracin-neomycine antibiotic spray which meant not merely a local prevention of occurrence of pin site infection, but also helped in maintaining of dry pin sites. A single preoperative parenteral dose of Ceftriaxon, which was continued postoperatively for 72 hours was usually sufficient in the general antibiotic treatment of the patients. The use of oral antibiotics was needed only in cases of development of pin site infection after the patients were discharged (Fig. 1). Since no skin tension was detected in our patients, there was no need of release of skin or subcutaneous tissue. In the period of lengthening patients were seen on a daily basis whereas in the stage of bone consolidation and remodelling patients were followed-up weekly or once in a period of two weeks, till the removal of the external frame.

Infected sites were graded according to the Saleh-Scott classification system (1992):

Grade 0–No problems
Grade 1–Responds to local treatment, increased cleaning, and massage
Grade 2–Responds to oral antibiotics
Grade 3–Responds to intravenous antibiotics or pin releases
Grade 4–Responds to removal of the pin
Grade 5–Responds to local surgical curettage
Grade 6–Chronic osteomyelitis

Fig. 1. Pin infection in a patient treated with lengthening of left femur using the Ilizarov method (grade 2 according to Saleh-Scott classification system)

Results

A total number of 26 patients with 201 wires were analyzed in this study. Since a transfixing wire has two pin sites, 402 pin sites were registered. Infection rate was assessed as a percentage of the number of pin sites as well as a percentage of the number of patients. Of the 26 patients analyzed in this study, 16 (61.53%) presented with sings of pin tract infection. The number of pin infections in each patient varied from zero to seven with a mean of 2.3 infections per patient. Sixty of 402 wire sites became infected; each wire having two sites of possible infection and hence the total pin infection rate was 14.92%. The majority of pin infections occurred in the stage of bone consolidation (68.75%).

Patients were divided in three groups, according to the type of surgical correction using the method of Ilizarov.

In the first group including patients who underwent limb lengthening procedure, 6 of 9 patients (66.66%) presented with pin infection, or 21 pin sites were infected out of 140 (15%) (Table 1).

Table 1. Incidence of pin infections in patients with limb lengthening

Number of patients	Number of patients with pin infections	%
9	6	66.66
Number of pin sites	Number of infected pin sites	%
140	21	15

The second group was consisted of 13 patients with fractures and non-unions treated with fixation, compression and/or correction of bone axes. The following percentage was obtained: 8 of 13 patients demonstrated sings of pin infection (61.54%) which means 34 of 206 pin sites (16.5%) (Table 2).

Table 2. Incidence of pin infections in patients with fixation, compression or correction

Number of patients	Number of patients with pin infections	%
13	8	61.54
Number of pin sites	Number of infected pin sites	%
206	134	16.5

In addition, in the subgroup of 4 patients with post-traumatic non-unions, (2a) 3 presented with a pin infection, (two cases of post-traumatic non-union of the arm and one of the lower leg) and the percentage of the infected pin sites was 21.21% (14 of 66 sites).

Table 2a. Subgroup with post-traumatic non-unions

Number of patients	Number of patients with pin infections	%
4	3	75
Number of pin sites	Number of infected pin sites	%
66	14	21.21

In the other subgroup (2b), 4 of 5 patients with infected non-union demonstrated pin infection (80%) with 16 infected sites out of 86 (18.6%).

Table 2b. Subgroup with infected non-unions

Number of patients	Number of patients with pin infections	%
5	4	80
Number of pin sites	Number of infected pin sites	%
86	16	18.6

In the third subgroup consisting of 4 patients with fractures, pin infection was registered on 4 sites in one patient out of the whole number of 54 (7.4%) (2c).

Table 2c. Subgroup with treated fractures

Number of patients	Number of patients with pin infections	%
4	1	25
Number of pin sites	Number of infected pin sites	%
54	4	7.4

In the third group with compressive arthrodesis performed in 4 patients, 2 patients showed signs of pin infection, whereas the percentage of the number of infected pin sites was 8.93%, respectively (5 of 56 sites) (Table 3).

Table 3. Incidence of pin infections in patients with compressive arthrodesis

Number of patients	Number of patients with pin infections	%
4	2	50
Number of pin sites	Number of infected pin sites	%
56	5	8.93

As previously mentioned, infection rate was assessed as a percentage of the number of pin sites as well as a percentage of the number of patients. According to statistical data, pin infection has been registered more frequently in patients undergoing lengthening procedure for various reasons, compared to the other two groups of patients, e.g. the group with fixation and compression on the segment as well as the group consisting of patients who underwent a compressive arthrodesis using the Ilizarov method. Nevertheless, the difference in the incidence of pin infections between the three groups of patients showed no statistical significance. (Pearson Chi-square: $p>0.05$). The percentage of infected pin sites showed no statistically significant difference between the three groups either.

All the infected sites were graded as grade 1 or 2 according to the Saleh-Scott grading system. The occurrence of pin infections has not required a change of the method of stabilization neither has required an exchange or removal of a pin due to prolonged infection. Most pin infections were mild and could be successfully managed by local treatment and administration of oral antibiotics only.

Discussion

Pin infections are one of the most common complications in limb lengthening and external fixation using the Ilizarov method. Although the initiators of the technique did not report a significant rate of pin infections [2, 3], in many studies the infection rate presents a serious problem in distraction osteogenesis [4, 5, 6, 7]. Since the pin infections can often impair the normal process of weight bearing and can cause further complications ranging from trivial to severe, one has to minimize the possibility of their onset and development. The effectiveness in minimizing of pin infections depends on taking care of some prerequisites involved in different protocols such as: proper insertion of wires, type of drilling while inserting the pins, dressing around the pin sites at the end of the surgical procedure, and the postoperative regimen of cleaning, covering and additional pin site care combined with general antibiotic treatment where necessary [8].

In our operative technique, irrigation with continuous drilling at low speed was used. Once the point of the wire was visible on the opposite side, a mallet was used to complete the contact between the wire and the ring at that level. Thereby, the possibility of necrosis usually caused by insertion of wires was minimized. Squares of gauze with betadine and dry dressing were placed around the pin sites at the end of the operative procedure. With some modifications in the operative technique and some difference in the postoperative regimen, the protocol used in our treatment was similar to the British consensus method. A total of 26 patients with 201 wires and 402 pin sites were analyzed in this study. Infection rate was assessed as a percentage of the number of pin sites as well as a percentage of the number of patients. Of the 26 analyzed patients, sings of pin tract infections were detected in 16 (61.53%). The number of pin infections in each patient varied from zero to seven with a mean of 2.3 infections per patient. Sixty of 402 wire sites became infected, each wire having two sites of possible infection, and hence the total pin site infection rate was 14.92%. Some of the reports indicate an increase of the incidence of pin infections in the latter lengthening stages [9, 10]. In this study, the majority of pin site infections occurred in the stage of bone consolidation (68.75%). All the infected sites were graded as grade 1 or 2 according to the Saleh-Scott grading system [11]. The occurrence of pin infections has not required a change of the method of stabilization neither has required an exchange or removal of a pin due to prolonged infection. Most pin infections were mild and could be successfully managed by local treatment and administration of oral antibiotics only.

Three groups of patients were analyzed and no statistical difference between the three groups was detected. The percentage of infected pin sites was under 10% only in the third group with patients who underwent a compressive arthrodesis. The percentage of the number of patients with pin infection in this group was 50% (two of four cases).

Conclusion

Although pin infections are more common in patients undergoing lengthening procedures, the difference in the incidence between the three groups of patients with various surgical corrections using the Ilizarov method, has not presented a statistical significance. The vast majority of cases with pin infections respond well to local pin care and oral antibiotics. However, in some cases pin infections can be generated either by initial failures in insertion of pins and wires, inappropriate angles of insertion between the wires on each ring, inadequate tensioning of wires, inadequate wire and pin dimensions, or by failures in preconstruction of the apparatus prior to its surgical application on the segment. In such cases, reoperation with proper positioning of the elements or sometimes even a change in the method of stabilization on the segment is required.

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TYPE 1 DIABETES MELLITUS – REVIEW OF THE IMMUNE MECHANISMS AND THE GENETIC BACKGROUND

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Abstract

Type 1 diabetes mellitus results from autoimmune destruction of the pancreatic beta-(â) cells and becomes clinically apparent after a period of varying length, during which autoimmune destruction reduces the counts of the insulin producing beta cells in the pancreatic islets to a level at which blood glucose levels can no longer be maintained in a physiologic range. T1D is defined by absolute insulin deficiency, an abrupt onset of symptoms, proneness to ketosis and dependency on exogenous insulin to sustain life. Type 1 DM is typically diagnosed in childhood, adolescence, or early adulthood in 90% of the cases, making the T1D the most common metabolic disease of childhood. Many factors contribute to the pathogenesis of autoimmune diabetes. During the last 30 years genetic studies have significantly advanced our knowledge of genetic susceptibility factors for T1D, making it one of the most studied complex genetic diseases to date. The aim of this review was to summarize the current knowledge of involvement of immune mechanisms and genetic background of the autoimmunity of the T1D.

Key words: T1D; Insulin-dependent diabetes mellitus; Type 1 diabetes; autoimmune diabetes; genetic susceptibility;

Introduction

Diabetes mellitus (DM) is a heterogeneous group of disorders characterized by high blood glucose levels (hyperglycemia), with the classical symptoms of polyuria, polydipsia, and polyphagia, caused by insufficient insulin secretion or functionality which can cause serious health complications including ketoacidosis, kidney failure, heart disease, stroke, and blindness. DM affects over 230 million people worldwide with estimated global prevalence of 5.1% [1]. There are two type forms of diabetes, type 1 diabetes (T1D, previously known as insulin-dependent diabetes or IDDM) and type 2 diabetes (T2D, previously known as non-insulin-dependent diabetes or NIDDM) [2].

Type 1 diabetes mellitus (T1D) (OMIM-222100), is defined by absolute insulin deficiency, an abrupt onset of symptoms, proneness to ketosis and dependency on exogenous insulin to sustain life [2, 3]. T1D accounts for 5-10% of the total cases with DM worldwide. Previously referred to as a juvenile-onset diabetes, type 1 DM is typically diagnosed in childhood, adolescence, or early adulthood in 90% of the cases, making the T1D the most common metabolic disease of childhood [2]. In 95% of the patients with T1D evidence of an autoimmune etiology is found [4]. Characterized by a cellular mediated autoimmune disruption of the insulin-secreting beta-cell of the pancreatic islets of Langerhans, this subtype has been called type 1A diabetes (T1AD). The other subtype is the idiopathic form of non-immune islet beta-cell loss, named type 1B diabetes (T1BD) [5]. In this review, we will focus on subtype 1A, and for simplicity it will be referred to as type 1 diabetes T1D.

Despite the differences between type 1 and type 2 DM, the costs of the two conditions are often combined. In a study that focused on type 1 only, Tao et al. estimated that in the United States, type 1 DM is responsible for $14.4 billion in medical costs and lost income each year [6].

Internationally, rates of type 1 DM are increasing. In Europe, the Middle East, and Australia, rates of type 1 DM are increasing by 2-5% per year [7]. The incidence of type 1 DM is extremely variable throughout the world with the incidence in the range from 1.2/100 000 to 60/100 000 in Finland [8, 9]. Republic of Macedonia is known as a “cold spot” of childhood diabetes with an incidence of 5.4 /100 000 children per year [10, 11].

If present trends continue, it is predicted doubling of the new cases of type 1 diabetes in European children younger than 5 years. The prevalence under age of 15 is predicted to rise from 94 000 in 2005, to 160 000 in 2020. The prevalence of type 1 DM is highest in Scandinavia (approximately 20% of the total number of people with DM) and lowest in China and Japan (less than 1% of all the people with diabetes) [12].

The chronic autoimmune disorder, Type 1 diabetes, becomes clinically apparent after a period of varying length, during which autoimmune destruction reduces the

counts of the insulin producing beta (â) cells in the pancreatic islets to a level at which blood glucose levels can no longer be maintained in a physiologic range.

Numerous studies have confirmed more than 50 loci conferring susceptibility for T1D [13, 14]. Many of these genetic loci are associated with other autoimmunity diseases [15-17]. Thus, there is no surprise why other (organ-specific) autoimmune diseases occur with increased frequency in patients with T1D [18]. Analyzing the genes in the overlapping autoimmune diseases has shown that those genes encode pro-inflammatory mediators such as cytokines, antigen processing and presenting molecules, T cell activation pathway molecules, cell adhesion molecules, and molecules related to natural killer cell that mediate cytotoxicity [19, 20].

The triggers for the autoimmune attack on the beta cells in T1D are not fully understood but it is now widely accepted that both environmental and genetic factors contribute to it.

The aim of this review was to summarize the current knowledge of involvement of immune mechanisms and genetic background of the autoimmunity of type 1 diabetes.

The roll of the immune system in beta-cells destruction

It is clear that the body's own immune system attacks the beta-cells in the islets of Langerhans of the pancreas. The cell destruction is thought to result mainly from the action of T-lymphocytes, the key players in autoimmune disease development. The beta cell autoantibodies that characterize type I diabetes may not be responsible for cell destruction. Instead, these antibodies are thought to signal a T-cell mediated immune response that sets the stage for beta cell destruction [21]. Anti-islet T cells, both CD4+ and CD8+ T cells, have been identified in type 1 diabetic patients as well as in the animal models [22]. However, the T-lymphocytes are not acting alone. They are helped in initiating the response by antigen-presenting cells (APCs) such as dendritic cells and macrophages. Apparently, this process is helped by B-lymphocytes as it has been confirmed by the level of produced autoantibodies, involving the whole immunological army [5]. In addition, the initial immune response results in impairment of â-cell function, progressive destruction of â-cells, and consequent development of type IA diabetes. The process is menacing and may evolve over several years, with the overt expression of clinical symptoms becoming apparent only when most â-cells (>80%) have been destroyed [23].

The normal T-cell activation occurs when their specific T-cell receptor (TCR) recognizes the specific antigen peptide presented with the molecules from major histocompatibility complex (MHC), by APCs. This interaction between TCR and a co-receptor (CD4+ or CD8+) with antigen/MHC complex on APCs provides only the first but specific signal for activation [24]. This signal alone is not able to induce full T-cell activation, however, it initiates T-cell anergy (loss of T-cell immune competence) or apoptosis. A second, non-specific signal, is provided through the interaction of one or multiple T-cell surface receptors with corresponding ligands on APCs. According to the resulting T-cell response, these signals can be divided into 'positive' (activating) leading to T-cell activation or 'negative' (inhibitory) co-stimulatory signals, limiting T-cell survival and increasing T-cell apoptosis [25, 26].

Co-stimulatory pathways mediated by the B7:CD28 family has been shown to play key roles in regulating T-cell activation and are promising therapeutic targets [27, 28]. These pathways not only provide critical positive second signals that promote and sustain T-cell responses, but they also increase critical negative second signals that downregulate T-cell responses. These negative signals limit, terminate, and/or attenuate T-cell responses and they appear to be especially important for regulating T-cell tolerance and autoimmunity. The B7-1/B7-2:CD28/CTLA-4 pathway is the best characterized T-cell costimulatory pathway, but it is complex because of the dual specificity of B7-1 (CD80) and B7-2 (CD86) for the stimulatory receptor CD28 and the inhibitory receptor CTLA-4 (CD152) [29]. CD28 delivers signals important for T-cell activation and survival, whereas CTLA-4 inhibits T-cell responses and regulates peripheral T-cell tolerance [30]. Researchers have found two new pathways in the B7:CD28 superfamily: one involves ICOS (inducible co-stimulator) and ICOS ligand, the other involves the PD-1 (programmed death-1) receptor and its ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC) [31 - 33]. Two additional B7 homologues, B7-H3 and B7-H4 (B7x, B7S1), also have been identified, indicating that there are still additional pathways within the B7:CD28 superfamily to be characterized. Disruption at any level of this mechanism of regulating the T-cell activation leads to generating the autoreactive T cells, named diabetogenic, as key players in the autoimmune attack of â-cells [34]. Moreover, a series of autoantigens have been identified in type I diabetes, which initiate the humoral immunity to produce antibody against different peptide of the beta-cell [35]. However, these autoantibodies in the patients do not mediate the â-cell destruction, instead serve as markers of that destruction. Importantly, transfer of anti-islet specific CD4+ or CD8+ T cells induces diabetes in immuno-incompetent recipient Non-Obese Diabetic (NOD) mice. In contrast, antibodies do not transfer the disease.

CD8+ T cells can directly kill â-cells that express Major Histocompatibility Complex (MHC) class I, through perforin/granzyme secretion [36].

Autoimmunity in T1D has typically been identified by the presence of autoantibodies to islet and/or beta-cell antigens, which in addition to their presence at the time of diagnosis, can often be detected long before the disease becomes clinically evident [84]. Autoantibody production appears in advance (months to years) of the metabolic changes of type 1 diabetes and can be used to predict disease. Among a list of T1D-associated autoantibodies that actually have more than two dozen members are islet cell autoantibodies (ICAs), autoantibodies to glutamic acid decarboxylase (GADAs), insulin autoantibodies (IAAs), and autoantibodies to transmembrane tyrosine phosphatase (IA2As), as well as those against the ZnT8 molecule (ZnT8As). Although these are the five most prevalent and best characterized, the potential for other autoantibody/autoantigen combinations remains [37].

Many studies suggest that GAD may be a key antigen stimulating the early autoimmune response. GAD responsive T cells are found in the periphery of humans with diabetes and those at high risk for disease [38, 39]. However, intriguing studies using GAD anti sense transgenic mice provide indirect evidence that the level of GAD expression in beta cells may contribute to disease susceptibility, suggesting that abnormal levels of GAD expression might be an important factor in some cases of diabetes [40].

The roll of the environmental factors in the autoimmune process

Numerous environmental factors are implicated in T1D disease development in genetically susceptible individuals. The proposed environmental factors that can trigger an autoimmune process involve nutrition and viruses.

Viral infections are considered to be the major environmental factors predisposing to T1D. *Rotaviruses*, *adenoviruses*, *retroviruses*, *reoviruses*, *cytomegalovirus*, *Epstein-Barr virus*, *mumps virus* and *rubella virus* are the ones that have been implicated in T1D pathogenesis but the most risk ones are human enteroviral (HEV) intestinal infections [41]. Coxsackie virus and echo virus serotypes of HEV infections, are highly cytolitic and can cause â-cell cytolysis and activate innate and adaptive immune system but can also activate autoreactive T cells [42]. Coxsackie viruses have been of particular interest because of a homology between the virus and the target antigen glutamic acid decarboxylase 65 (GAD65), both negative and positive studies have been reported [43, 44].

Nutritional factors were also suggested to have influence on the immunopathological processes. Nutritional factors that have been investigated include cow's milk, breastfeeding, wheat gluten, and vitamins D and E [45, 46].

Antibodies to milk proteins and T cell responses to these proteins were reported to be increased among children with T1D [47]. This includes a molecule ICA69 with some homology to bovine albumin [48]. Very recently, cross-reactivity between the beta-cell-specific protein (insulin) and bovine alfa-casein has been noted and holds interesting potential for molecular mimicry [49].

As previously discussed, the highest incidence of T1D worldwide occurs in Northern Europe, suggesting that low serum concentrations of vitamin D may not only be associated with T1D but perhaps cause for development of the disease [50]. Recently, there has been appealing evidence on the "nonclassic" role of vitamin D in many autoimmune diseases including T1D. In type 1 diabetes mellitus (T1D), it plays an immunomodulatory role through the vitamin D receptor (VDR) present on pancreatic and immune cells. Specific VDR allelic variants have been associated with T1D in many countries. In fact, retrospective studies of vitamin D supplementation during pregnancy or infancy showed a lower incidence of T1D.

$1,25(OH)_2D_3$ plays an immunomodulatory role in the prevention of T1D, through the vitamin D receptor (VDR) expressed in antigen presenting cells, activated T cells, and pancreatic islet â-cells [51]. At the level of the pancreatic islets, $1,25(OH)_2D_3$ decreased *in vivo* and *in vitro* proinflammatory chemokine and cytokine expression (e.g., IL6), which are implicated in the pathogenesis of T1D making beta-cells less chemoattractive and less prone to inflammation. This results in decreased T cell recruitment and infiltration, increased regulatory cells, and arrest of the autoimmune process [52]. Increased maternal consumption of vitamin D during pregnancy has also been associated with decreased risk of islet autoimmunity in the offspring [53]. Increased use of vitamin D supplementation during infancy has been associated with reduced risk for childhood T1D [54]. A meta-analysis of the results of observational studies suggests that the risk of T1DM is 29% reduced in those who were supplemented in childhood with vitamin D compared to those who were not [55].

The Genetic background of Type 1 Diabetes

Autoimmune diabetes is only rarely caused by mutational defects in a single gene. These monogenic forms are typically accompanied by multiple other autoimmune conditions due to the disruption of common

regulatory pathways. One such example is found in the IPEX syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked), in which mutations in the *Foxp3* transcription factor lead to the dysfunction of regulatory T cells (Tregs) and wasting multi-organ autoimmunity [56, 57]. Another example is autoimmune polyendocrine syndrome type 1 (APS-1, or APECED for autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy). Mutations in the transcription factor *AIRE* (autoimmune regulator) lead to severe autoimmune conditions, and ~20% of the cases develop T1D [58]. Deficiencies in *AIRE* inhibit the expression of peripheral molecules, for example, insulin, in the thymus. This reduced expression interferes with thymic deletion, because it allows autoreactive T cells to escape into the periphery [59, 60].

These monogenic forms are rare and represent a small minority of T1D cases. However, in general, T1D is considered as a complex genetic trait, not only do multiple genetic loci contribute to susceptibility, but environmental factors also play a major role in determining risk.

During the last 30 years genetic studies have significantly advanced our knowledge of genetic susceptibility factors for T1D, making it one of the most studied complex genetic diseases to date [35]. The ability to identify individuals at high risk for type 1 diabetes using genetic and/or autoantibody markers has been a long-standing goal of the diabetes research and clinical community and a critical element in T1D prevention strategies [61, 62].

Here we review the major findings from this large body of data and consider possible developments in the future. With the prospect of these genome-wide studies, it is relevant to ask how many genetic risk factors are still expected to be involved in T1D.

HLA-associated genes

Although a large number of genetic variants associated with T1D have been identified by genome-wide association study analyses, the major genetic determinants remain specific alleles at the HLA class II and, to a lesser extent, class I loci [63]. The first major genetic risk locus was detected as early as 1974 by Nerup *et al*. followed by Cudworth and Woodrow, who detected an association with the HLA system. The consistent evidence of *HLA* gene contribution to the disease was provided by linkage analyses and further confirmed by association analyses. *HLA* genes contribute with approximately 40% of genetic risk to T1D development [64].

The *HLA* region is a cluster of genes located within the major histocompatibility complex (MHC) on chromosome 6p21. It is considered today that the *HLA* region class II has the strongest input in the development of T1D, which has been deeply investigated in the past decades [65]. APC carry HLA class II molecules that bind main T1D autoantigens such as preproinsulin, insulinoma associated antigen 2, glutamic acid decarboxilase (GAD) and zinc transporter (ZnT8), present them to thymocites in the thymus. Through the negative selection, strongly self-reactive thymocites die by apoptosis that eliminates 98% of thymocytes. Only 2% of thymocytes that have low affinity migrate in the periphery as mature T cells where they develop into CD4+ and CD8+ T cells. CD4+ T cells are helper cells to CD8+ T killer cells in the processes of destruction of pancreatic islet â cells. It is believed that the most important autoantigen in the onset of T1D is preproinsulin, whose N-terminal signal peptide and the peptidase cleavage site is recognized by CD8+ T killer cells [34]. The proteins encoded by *HLA* class I, *HLA-A* and *HLA-B* exert smaller effects in the pathogenesis of T1D. Proteins encoded by class I *HLA* genes are expressed on nucleated cells, often in the pancreatic insulin-producing â cells. These proteins present antigens directly to the CD8+ T killer cells [10]. *HLA DR4* and *DR3* class II haplotypes are of particular importance in the T1D development [66]. It is considered that haplotypes of high risk for T1D are *DRB1*0401-DQA1*0301-DQB1*0302* and *DRB1*0301-DQA1*0501-DQB1*0201* (often expressed using the old serological designation as *DR3/DR4* or *DQ2/DQ8*) [67, 68]. However, it was observed that some *HLA* haplotype combinations have protective association with T1D. It is believed that haplotype *DRB1*1501-DQA1*0102-DQB1*0602*, which was found in about 20% of the general population and even less than 1% of patients with T1D has protective effect [69]. In T1D at least one allele of DR3 or DR4 is found in 95% in Europeans, and individuals with both DR3 and DR4 are particularly susceptible to T1D [70].

Here is described the actual process of “false” recognition of the antigen in carriers of the susceptible HLA haplotype [71].

Distribution of *HLA* susceptible and resistant haplotypes in certain populations can explain the variability in the incidence of diabetes throughout the world (R LaPorte, DIAMOND project, J. Dorman, M. Kocova for Macedonia, Illonen J,J. Immunology). But in the last few years is observed a rising incidence of T1D in children with resistant haplotypes, which can be explained by increased impact of environment on children with lower-risk HLA class II genes [72].

Non-HLA associated genes

The insulin (INS) gene. Preproinsulin has emerged as the most important autoantigen in childhood-onset T1D [73], a widely held conclusion that is underpinned by the long-known and very strong association of a promoter polymorphism of the insulin structural gene (INS) [74]. The *INS* gene, located on chromosome 11p15.5, has been designated as *IDDM2*. Positive associations have been observed with a non-transcribed variable number of tandem repeat (VNTR) in the 5' flanking region, or deletion of the gene itself [75-77]. The class I alleles of the *INS VNTR*, which increase risk of type 1 diabetes, have been associated with lower insulin mRNA and protein expression in the thymus, compared with the dominant protective class III alleles. Insulin production in the thymus is closely linked to the expression of the Autoimmune Regulator (AIRE) transcription factor whose role is to participate in the transactivation of a wide range of self-antigens necessary for T-cell negative selection [78]. The consensus model posits that low levels of thymic insulin facilitates the escape of insulin-reactive thymocytes which enter the periphery and at some point later in life are activated to recognize â cell insulin [59]. Recent work has demonstrated that AIRE can bind the VTNR1 class allele promoter with a marked reduction in insulin mRNA production [79]. Indeed AIRE disruption leads to a number of other autoimmune diseases.

HLA and insulin (*INS*) regions account for approximately 60"70 % of the familial aggregation of type 1A diabetes.

Cytotoxic T lymphocyte antigen-4 (CTLA-4). In 1996 *CTLA4* (cytotoxic T lymphocyte antigen 4, also called CD152) gene located on chromosome 2q33 was confirmed as another T1D susceptibility gene [80]. CTLA4 protein is a co-stimulatory receptor on the cell surface of CD4+ T cells. It binds B7 ligands of APCs (already mentioned costimulatory pathways) that activate main component of the co-receptor, CD28. In the same time intracellular part of the CTLA4 interact with intracellular domain of CD3 receptor and initiates phosphorylation of several downstream target molecules. Consequently, this leads to activation of T cells after their binding to HLA molecules on APCs [69]. Reduction of the CTLA4 protein in CD4+ T cells causes disruption in the regulation of the peripheral self-tolerance and the prevention of autoimmunity increasing the susceptibility to T1D as well as to other autoimmune disease [13, 50]. SNPs have been described in the human *CTLA-4* promoter region and in exon 1.The A49G polymorphism in exon 1 causes substitution of an alanine with a threonine in the signal sequence of CTLA4 protein that leads to incorrect glycosylation of mutant protein and reduction of its expression on T-cell surface. Conversely, C318T polymorphism of the *CTLA4* promoter gene region is protective inducing higher promoter activity and increased amount of CTL4 protein on the T cell surface [69].

PTPN22 gene. A relatively new member, protein tyrosine phosphatase non-receptor 22 (*PTPN22*), a gene found on chromosome 1p13 that encodes lymphoid protein tyrosine phosphatase (LYP) was found to be associated with susceptibility to type 1 diabetes in 2004 [81]. Protein tyrosine phosphatases such as LYP are responsible for preventing spontaneous T cell activation and they have the ability to prevent the response to antigen by dephosphorylating and inactivating T cell receptors [82]. It has been demonstrated that a single nucleotide polymorphism (SNP) in the *PTPN22* gene can lead to susceptibility to autoimmune diseases such as type 1 diabetes because of a decrease in negative regulation of hyper-reactive T cells. The first complete resequencing of the human *PTPN22* gene was carried out in 2005 [83]. This sequence was further analysed for polymorphisms associated with type 1 diabetes and a SNP at 1858 bp in codon 620 was found. Two alleles referred to as 1858C and 1858T were identified and the 1858T variant was shown to occur more often in type 1 diabetes populations: 30.6% of people with type 1 diabetes compared with 21.3% healthy controls [84]. Other autoimmune diseases such rheumatoid arthritis, systemic lupus, and Graves disease have been similarly linked to HLA class II, CTLA-4, and PTPN22 genes and establish a connection between these genes and immune system dysregulation [85].

The most recent genome-wide association (GWA) study focusing on T1D found that over 50 loci affect risk of T1D (see Table 1), including newly identified coding regions for immunoregulatory molecules. Allelic variation in the interleukin (IL)-2 receptor-á gene (IL2RA) region accounts for another genetic risk factor implicated in T1D. The alpha chain of the IL-2 receptor complex (IL2Rá, CD25) is an essential molecule expressed on T cells upon activation and on natural Tregs at baseline. Several regions contain new candidate genes of possible relevance to T1D (*IL10*, *IL19*, *IL20*, *GLIS3*, *CD69* and *IL27*) [13]. Most of the listed genes mediate the immune response, some exert their functions in the process of destruction of pancreatic â cells and some have a dual role [13]. Additional functional studies provided evidence of causality of several genes within established loci, such as several cytokines and their receptors (*IL10, IL2, IL27, IL7R, CCR5, SH2B3, IL18RAP*), immunomodulatory molecules (*IFIH1*, *TLR7-TLR8*, *TAGAP)* and other types of proteins (*PTPN2*, *GLIS3*). However, for the majority of associated regions the most likely causal gene still needs to be identified [50]. There are more than 300 candidate genes that are in LD with

Table 1. Chromosomes and SNPs associated with T1D according to T1D|base

	Chromosome	Markers (SNP)	Probable Associated Gene
1.	1p13.2	rs2476601, rs6679677	PTPN22
2.	1q31.2	rs1323292, rs1359062, rs2760524, rs2816316	RGS1
3.	1q32.1	rs3024505	CD55, IL10
4.	2p23.3	rs478222	
5.	2q11.2	rs10865035, rs1160542, rs11676922, rs9653442	AFF3
6.	2q24.2	rs1990760	IFIH1
7.	2q32.3	rs13426947, rs3821236, rs6752770, rs7574865, rs7582694	STAT4
8.	2q33.2	rs11571302, rs3087243	CTLA4
9.	3p21.31	rs11711054, rs2097282, rs333	CCR5
10.	4p15.2	rs10517086, rs874040, rs932036	
11.	4q27	rs13119723, rs13132308, rs17388568, rs2069762, rs2069763, rs4505848, rs6822844	IL2, IL21
12.	MHC	rs9268645	HLA-DQB1, HLA—DRB1, HLA-B, HLA-A
13.	6q15	rs10806425, rs11755527	BACH2
14.	6q22.32	rs9388489	CENPW
15.	6q23.3	rs10499194, rs13192841, rs17264332, rs2327832, rs6920220	TNFAIP3
16.	6q25.3	rs1738074, rs182429	TAGAP
17.	6q27	rs924043	
18.	7p 15.2	rs10486483, rs7804356	SKAP2
19.	7p12.2	rs10272724	IKZF1
20.	7p12.1	rs4948088	COBL
21.	9p24.2	rs7020673	GLIS3
22.	10p15.1	rs11594656, rs12251307, rs12722495, rs2104286, rs7090512	IL2RA
23.	10p15.1	rs11258747, rs2387397, rs947474	PRKCQ
24.	10q22.3	rs1250550, rs1250552, rs1250558	ZMIZ1
25.	10q23.31	rs10509540	RNLS
26.	11p15.5	rs689, rs7111341	INS-IGF2, INS
27.	12p13.31	rs4763879	CD69
28.	12q13.2	rs2292239	ERBB3
29.	12q14.1	rs10877012, rs703842	CYP27B1
30.	12q24.12	rs3184504, rs653178	SH2B3
31.	13q22.2	rs539514	
32.	13q32.3	rs9585056	GPR183
33.	14q24.1	rs1465788, rs194749	
34.	14q32.2	rs4900384	
35.	14q32.2	rs941576	DLK1
36.	15q14	rs16967103, rs17574546, rs7171171	RASGRP1
37.	15q25.1	rs3825932	CTSH
38.	16p13.13	rs12599402, rs12708715, rs12708716	CLEC16A, DEXI
39.	16p13.13	rs12928822, rs529866	
40.	16p11.2	rs151181, rs4788084, rs8049439	IL27
41.	16q23.1	rs7202877	
42.	17q12	rs12946510, rs2290400, rs2872507, rs8067378	GSDMB, ORMDL3
43.	17q21.2	rs7221109	
44.	18p11.21	rs1893217, rs45450798, rs478582	PTPN2
45.	18q22.2	rs727088, rs763361	CD226
46.	19p13.2	rs2304256	TYK2
47.	19q13.32	rs425105	
48.	19q13.33	rs281379, rs516246, rs602662	FUT2
49.	20p13	rs2281808	
50.	21q22.3	rs11203203, rs3788013	UBASH3A
51.	21q22.3	rs760426	AIRE
52.	22q12.2	rs2412970, rs2412973, rs5753037, rs713875	
53.	22q12.3	rs3218251, rs3218253	IL2RB
54.	22q12.3	rs229527, rs229541	IL2RB, C1QTNF6
55.	Xp22.2	rs5979785	TLR7, TLR8
56.	Xq28	rs2664170	

T1D associated genetic regions. The main focus of current research is to identify causal risk genes and to understand how they influence the disease [13, 50].

Application of the immunology and genetic insights in the treatment and prediction of T1D

While significant advances have been made in the clinical care of T1D with resultant improvements in quality of life and clinical outcomes, much more needs to be done to improve care of, and ultimately find a cure for T1D.

Recently, several population studies attempted to stratify children at birth according to their predisposition for T1D development by examining their *HLA* genotypes and insulin gene polymorphisms [86]. Denver, Germany and Finland studies showed that children with the high risk *HLA* genotypes or polymorphisms within the insulin gene have about 50% higher risk of developing T1D-specific antibodies by the age of 5 [87, 88]. The application of preventive therapy would be focused on those individuals who have the highest genetic predisposition for the T1D development.

So far, genetic prediction for T1D is modest and it is still not reaching criteria required for a targeted disease prevention strategy [89]. Over 85% of T1D patients do not have positive family history of T1D, however there is a 6% of disease clustering among siblings. The pattern of inheritance seems very complicated, and disease development further depends on the triggers from the environment [87].

Since disease onset rapidly reduces the beta cell population, early detection of the disease or even pre-clinical detection would greatly enhance patient outcomes. Immunotherapy techniques have the potential to restore the proper immune system balance preventing further beta cell death and increasing insulin production with the subsiding of beta cell inflammation [90, 91]. A favorable window of opportunity must be selected for immunotherapy techniques to have the greatest impact, especially since patients may have lost as much as 80% of their beta cell mass by the time of clinical diagnosis of type 1 diabetes [92]. Late treatment after further cell loss may not allow for sustainable insulin production levels to maintain euglycemia and therefore would only be a benefit if used in combination with regenerative medicine or beta cell replacement.

In the past couple of years, the most exciting development in the area of tolerance induction has been made with the antigen-specific vaccines [93]. A GAD vaccine (Diamyd®, Diamyd Medical AB, Stockholm, Sweden) with aluminum hydroxide (alum) as adjuvant has been produced and is now being investigated in phase-3 trials. Injected GAD65 is processed by antigen-presenting cells to provide peptide fragments recognized by T cells. This results in a Th1/Th2 shift consisting of induction and proliferation of a subset of GAD65-specific regulatory T cells. These specific T cells down-regulate antigen-specific killer T cells that would otherwise attack the beta cells. Three phase-3 trials of the GAD65-alum vaccine are underway in the U.S and Europe [94]. Very recently, Wherrett *et al*. reported that two or three doses of subcutaneous GAD-alum across 4 to 12 weeks does not alter the course of loss of insulin secretion during 1 year in patients with recently diagnosed T1D [95].

Conclusion

Immunology and genetic insights in the pathogenesis of T1D have increased the knowledge of the process of destruction of pancreatic â cells. However, the implementation of this knowledge in the treatment and prevention of T1D has been less successful than expected. Further research should be oriented towards the ligands and other molecules involved in the immune process that can be modified in order to protect pancreatic beta-cells. Immunotherapy techniques hold the promise of finding a true cure.

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INSULIN, INSULIN RESISTANCE AND ANTHROPOMETRIC PARAMETERS IN OVERWEIGHT AND OBESE WOMEN

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Abstract

Insulin resistance together with obesity, hypertension and dyslipidemia is part of the cluster that represents the metabolic syndrome. The impaired insulin sensitivity has been reported to be linked with obesity; however the importance of the fat distribution has also been emphasized in the development of this disorder.

The aim of our study was to determine to association of serum insulin and insulin resistance assessed by HOMA with anthropometric parameters in obese and overweight women. Also the correlation of insulin and insulin resistance (HOMA-IR) with the subcutaneous and visceral abdominal fat was evaluated.

The study included 41 overweight and obese women, aged 20- 60 years. The anthropometric parameters like body mass index (BMI), waist, hip and thigh circumference were measured with standard methods. Abdominal subcutaneous fat tissue (SFT) and visceral fat tissue (VFT) were determined by ultrasonography. Insulin values were measured with radioimmunoassay and the insulin resistance was assessed by the HOMA2 method.

The results of our study showed strong correlation of insulin and HOMA-IR with BMI (r 0.36, $p<0.01$). The median and mean values of these parameters were much higher in subjects with obesity grade 2 and 3. The insulin concentrations and HOMA-IR also showed good correlation with waist and hip circumference and with the subcutaneous fat tissue ($p<0.05$).

The results of our study point to the fact that in addition to the total body fat accumulation, the role of the subcutaneous fat tissue in the development of insulin resistance in overweight and obese Macedonian women should not be underestimated.

Key words: insulin resistance, subcutaneous fat, visceral fat

Introduction

Insulin resistance is a disturbance in the glucose homeostasis in which a greater than normal amount of insulin is required to elicit a quantitatively normal response [1]. Insulin resistance together with obesity, hypertension and dyslipidemia is part of the cluster that represents the metabolic syndrome. The impaired insulin sensitivity has been reported to be linked with obesity in many studies, although the increased body fat is not always associated with insulin resistance [2, 3, 4]. The majority of studies report the link between the accumulation of visceral fat and development of insulin resistance. However there are reports that determined the role of subcutaneous fat tissue as equally important, and in some ethnic groups even more pronounced association between insulin resistance and subcutaneous fat was found [5, 6, 7].

The aim of our study was to determine to association of serum insulin and insulin resistance assessed by HOMA with anthropometric parameters in obese and overweight women. Also the correlation of insulin and the insulin resistance with the subcutaneous and visceral abdominal fat determined by ultrasonography was evaluated.

Material and methods

The study included 41 overweight and obese women, aged 20- 60 years. The study group was selected from the participants in the project MONODIET at the Institute of Pathophysiology and Nuclear Medicine, at the Medical Faculty in Skopje. The presence of endocrinologic disorder, pregnancy, lactation, hormonal or antilipidemic drugs were exclusion criterium.

The weight of the participants was obtained with digital scale with precision of 0.1 kg, with light indoor clothes, without shoes. The hight was measured with a stadiometer to the nearest 0.5 cm.Waist circumference was measured with nonelastic plastic band over the umbilicus with subjects in a standing position. Hip circumference was measured between the anterior iliac spine and major trochanters. The thigh circumference was measured in the proximal end at the level that showed the largest circumference.The circumferences were measured to the nearest 0.5cm.Waist to hip ratio (W/H ratio) and waist to thigh ratio(W/T ratio) was calculated from the results of the measured circumferences, and body mass index from the hight and weight.

The ultrasound measurements were performed at the University Clinic for Gastroenterohepathology at

medical faculty in Skopje. The measurements were performed with 3,5 MHz ultrasound probe one centimeter above the umbilicus without applying pressure to the abdominal wall. The distance between the aorta and the interior wall of musculus rectus abdominis was defined as representative of the amount of VFT, while the distance from the skin to the exterior wall of m. rectis abdominis was defined as SFT.

According to the BMI value the subjects were divided in two groups. The group A consisted of women with BMI bellow 35kg/m^2 and group B of women with BMI greater than 35kg/m^2.

Blood samples were collected in the morning in the fasting state for the assessment of insulin levels. Insulin was determined by competitive radioimmunoassay with commercially available kit (Insulin CT RIA, CIS bioassays).The glicemia was determined with enzymatic photometric method with GOD-PAP.

The HOMA model was used to estimate the insulin resistance from fasting plasma insulin and glucose concentrations. Computer model HOMA 2 was used [8, 9] and the HOMA-IR parameter was calculated as a reciprocal value of the percent of insulin sensitivity.

The statistical analysis of the data was performed with Spearman rank correlation and with Mann Whitney U test. The probability level <0.05 was considered statistically significant.

The study was approved by the ethical committee of the Medical Faculty at the University “Sv. Kiril I Metodij “in Skopje.

Results

The analyzed group consisted of 41 women aged from 20 to 60 years (mean 42.37 +/- 10.05). The mean value for BMI ranged from 25.3 to 52.6 kg/m^2 (mean 32.8+/-6.03 kg/m^2). The values of the anthropometric parameters are presented in table 1.

Table1. Mean values for age, anthropometric parameters and abdominal fat depots

	Mean	Minimum	Maximum	Std.Dev.
age	42,3784	20,00000	60,0000	10,51547
BMI (kg/m2)	32,8486	25,30000	52,6000	6,03651
WC(cm)	103,1216	82,00000	132,0000	10,39324
HC(cm)	116,3514	99,00000	141,0000	11,22922
TC(cm)	66,3919	53,00000	87,0000	8,04081
W/H ratio	0,8855	0,78000	1,0200	0,05098
W/T ratio	1,5662	1,24000	2,0400	0,16988
SFT(cm)	2,6216	0,80000	5,8000	1,15977
VFT(cm)	5,2595	2,20000	9,7000	1,76139

Insulin levels ranged from 10.7? ìU/ml l to 72. ì U/ml (mean 32.9+/-13 ?ì U/ml). The mean value in the group A was 27.3+/-9.3?ì U/ml, and the median was 26.6 ?ì U/ml. The mean in the group B was 41.68+/-15.19? ìU/ml with median 37.7 ?ì U/ml. The Mann Whitney U test showed statistically significant difference between the groups (p< 0.001).

The insulin resistance (HOMA-IR) assessed by HOMA 2 ranged from 1.4 to 7 with mean value 4.01+/-1.1. The mean HOMA-IR in group A was 3.4+/-1.1and the median was 3.2, while in group B the mean was 4.9+/-1.2 and median 4.7. The difference between groups A and B was significantly different with p<0.001.

Spearman rank order correlation analysis was performed to assess the correlations between HOMA-IR and various anthropometric indices. Significant correlation was found between HOMA-IR and BMI, waist circumference, hip circumference and abdominal subcutaneous fat tissue. The similar results were obtained for the correlations of insulin with anthropometric indices and the two abdominal fat depots. The values for Spearman r are presented in the table 2.

Table 2. Spearman Rank Order Correlations for insulin and HOMA-IR with anthropometric parameters. Marked correlations are significant at p< 0.05

	HOMA-IR	insulin
BMI	**0,36**	**0,36**
WC	**0,37**	**0,37**
HC	**0,41**	**0,33**
TC	0,23	0,27
W/H ratio	0,007	0,02
W/T ratio	0,1	0,02
SFT	**0,34**	**0,38**
VFT	0,21	0,29

The scatterplots for the correlation of HOMA-IR with BMI, WC, HC and SFT are presented in Fig.1, 2, 3 4 respectively.

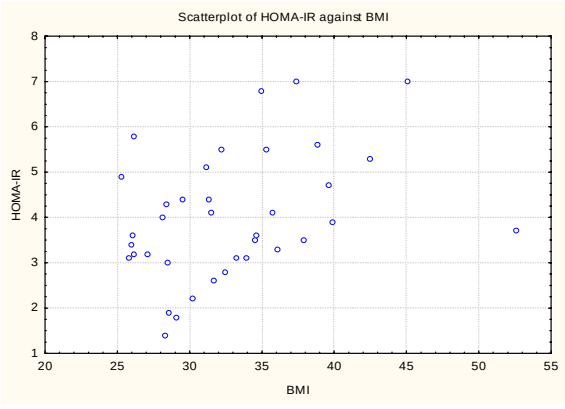

Fig. 1. Correlation between HOMA-IR and BMI

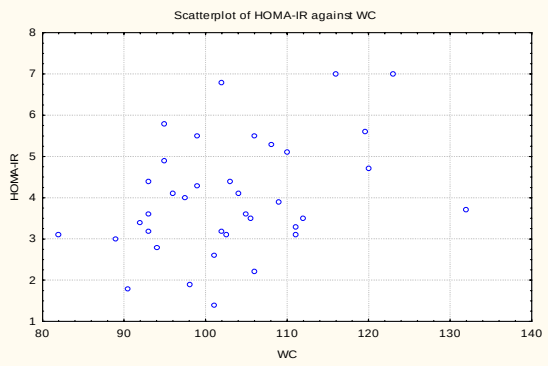

Fig. 2. Correlation between HOMA-IR and WC

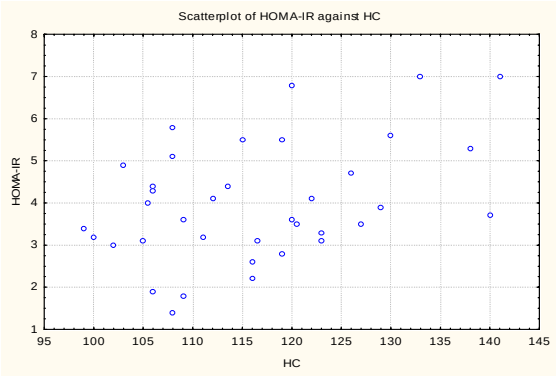

Fig. 3. Correlation between HOMA-IR and HC

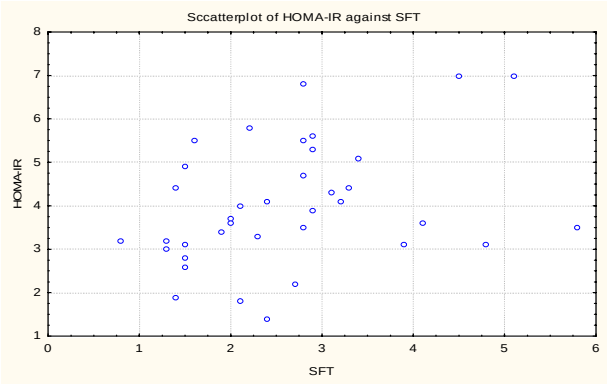

Fig. 4. Correlation between HOMA-IR and SFT

Discussion

Since 1988 when Reaven introduced the "syndrome X" [10] insulin resistance and its association with obesity and abdominal obesity has become the subject of intense research. It is widely accepted that insulin resistance is very frequent in obesity and that it is well correlated with parameters that approximate general adiposity like BMI [11, 12, 13]. Our study also reported positive correlation between BMI and insulin levels and HOMA-IR (r-0.36, $p<0.05$). The levels of circulating insulin and the values for HOMA-IR were much higher in the group of women with BMI above 35kg/m^2 ($p<0.01$). These results are in favor of higher insulin resistance in subjects with obesity grade 2 and 3, compared with overweight and subjects with obesity grade 1.

The association of insulin resistance with different patterns of body fat distribution has also been extensively researched with majority of the studies reporting a strong association of abdominal visceral fat with the grade of insulin resistance [14, 15, 16]. Our study showed correlation with waist circumference, but didn't provide any evidence on the association between the HOMA-IR and insulin and other parameters that reflect visceral fat accumulation like waist to hip ratio and waist to thigh ratio. Although the waist circumference is reported to be good indicator of risk for insulin resistance [17, 18], the majority of the studies report equal usefulness of WC and BMI in the estimation of the risk for metabolic syndrome and the related risk for cardiovascular diseases [11, 12]. Our study also demonstrated correlation between HOMA-IR and insulin with hip circumference as an anthropometric parameter that reflects the amount of peripheral fat tissue, which supports the thesis that the amount of total fat is important in the determination of insulin sensitivity in our group of women. The association of the HOMA-IR and insulin with hip circumference is also in concordance with the correlation of these parameters with subcutaneous fat in our study reflecting the influence of subcutaneous fat on insulin sensitivity. Our study did not demonstrate association of the abdominal visceral fat tissue with insulin and HOMA-IR, despite the good correlation of these parameters with WC. These findings might reflect the shortcomings of ultrasound measurements of the fat depots in contrast with the CT determination which represents the gold standard, or the fact that the majority of the participants in our study (over 70%) had increased visceral fat depots. This might be in concordance with the study of Stefan et al that reports weak predictive effect of visceral fat in regard to insulin resistance in obese subjects, while in normal weight and overweight subjects visceral fat had impact on the insulin resistance [19]. The correlation of subcutaneous fat with insulin resistance in our study was very intriguing considering that the subcutaneous fat tissue is reported as protective in regard to insulin resistance in some studies [14]. However there are studies that report the impact of the both fat depots on the development of insulin resistance [7, 13, 20, 21]. These conflicting results might be attributed to the anatomy of the subcutaneous fat tissue. The subcutaneous fat tissue is divided by a fascial plane in two compartments: the superficial adipose layer and deep layer. These two

compartments have different histology and different physiology. The study of Kelly et al. gives interesting insight in the pathophysiology of subcutaneous fat tissue [22]. This study using the CT managed to distinguish between the two different compartments of abdominal subcutaneous fat tissue. The authors have found that the insulin stimulated glucose utilization was similar for the visceral and deep subcutaneous fat tissue. They postulated that the deep layer possess the metabolic profile of the visceral fat tissue, while the superficial layer has the protective role in regard to metabolic syndrome.

The role of ethnic differences in the pattern of body fat distribution also has to be taken in account when the effect of the fat depots on insulin sensitivity is researched. There are many studies that report different body fat distribution attributed to ethnic origin [23, 24]. Also recent studies provide insight in different proportion of deep and superficial fat layer in respect to ethnicity. Kohli et al found higher amount of deep subcutaneous fat in South Asians compared with Europeans [25]. The inflammatory biomarkers derived from adipose tissue also can show ethnic dependent patterns. Caroll et al. report higher inflammation in African –American women regardless of the lower amount of VAT compared with Caucasians [26]. The findings from this study are complementary with the findings of the study by Tulloch-Reid et al. that reports stronger association of subcutaneous fat tissue rather than the visceral fat with insulin resistance in African –American women [6]. All these studies point to the fact that both fat depots might influence the development of insulin resistance in some ethnic groups.

Conclusion

The results of our study demonstrated clear association of the insulin and HOMA –IR with BMI. These parameters were in favor of greater insulin resistance in women with obesity grade 2 and 3. The WC and HC showed good correlation with insulin and HOMA-IR, and with the abdominal subcutaneous fat tissue. This study results point to the fact that in addition to the general body fat accumulation , the role of the subcutaneous fat tissue in the development of insulin resistance in Macedonian women should not be underestimated. However the influence of different fat depots on insulin resistance is complex issue influenced by many hereditary and environmental factors that needs further clarification.

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ASSESSING THE RISK OF SURGERY IN CIRRHOTIC PATIENTS

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Abstract

Nowadays, patients with cirrhosis more frequently undergo surgery now than in the past, in part because of the long-time survival of these patients. These patients are at increased risk of morbidity and mortality that is related to their underlying liver disease. Careful preopartive risk assessment, patient selection and management of various manifestations of advanced disease might decrease morbidity and mortality from surgery in patients with cirrhosis. Assesment of surgical risk provides a basis for discussion of risks and benefits, treatment decision making and for optimal management of patients for whom surgery is planned or necessary.

Three factors essentially determine the extent of surgical risk: degree of decompensation reflected by Model for End-Stage Liver Disease (MELD) and Child-Turcotte-Pugh (CTP) score, whether surgery is performed as an emergency or elective procedure, and the type of surgery.

The CTP and MELD scores provide reasonable estimation of perioperative mortality but do not replace the need for careful preoperative preparation and postoperative monitoring for detection of early complications. Medical treatment for specific manifestations of liver disease, including ascites, jaundice, encephalopathy and renal dysfunction, should be optimized preoperatively, or, if necessary, managed in the postoperative period.

Key words: cirrhosis, CTP classification, MELD score, surgery, risk assesment

Introduction

Surgery is performed in patients with cirrhosis more frequently now than in the past, in part because of the long-time survival of these patients. It is common for patients with liver disease to undergo surgery; as many as 10% of patients with advanced liver disease require a surgical procedure other than liver transplantation in the final two years of their life.(1)

Patients with compromised liver function are prone to decompensate due the stress of both anesthesia and surgery, and in spite of significant improvements in surgical and intensive care, perioperative mortality and morbidity remain high. Secondary to the loss of hepatic reserve capacity and because of other systemic derangements that are result of hepatic dysfunction, patients with liver disease have an inappropriate response to surgical stress. These patients are subjected to an increased risk of infection, bleeding, postoperative liver decompensation, including coma or death. The scale of the risk depends upon the severity of liver disease, the type of surgical procedure and presence of comorbid conditions. Therefore, the decision to perform surgery in these patients must be heavily weighed.(2.3) Appropriate preoperative evaluation and management of patients with liver disease is fundamental for counseling and selection of patients for surgical treatment, and might reduce the risk of surgery and improve outcomes for these patients.

Operative risk assessment in patients with cirrhosis

The magnitude of perioperative risk correlates with the degree of hepatic decompensation. Surgical risk assessment is less relevant if emergency surgery is required to prevent death. On the other hand, the vast majority of decisions are made in the setting of semi-urgent or elective procedures for which there is time for risk assessment, optimization of the patient's medical status, and consideration of alternative approaches.

Two risk stratification schemes have been used to estimate the perioperative risk of patients with cirrhosis: the Child-Turcotte-Pugh (CTP) score and Model for End Stage Liver Disease (MELD) score.

The Child-Turcotte-Pugh score

CTP score was initially designed to estimate the chances of survival after portosystemic shunt surgery, but since the 1970s it has been used for assessing perioperative morbidity and mortality in patients with cirrhosis.The score includes five parameters, three of them being objective (serum bilirubin, albumin and prothrombin time), and two subjective (presence of ascites and encephalopathy).(4) The score ranges from 5 to 15, and patients are further classified into three CTP classes, A (CTP score 5-6), B (CTP score 7-9) and C (CTP score 10-15).

The studies that led to this standard have all been retrospective and limited to a small number of highly selected patients, but the results have been remarkably consistent. Two of the most important studies, conducted in a time interval of 13 years, reported nearly identical results: mortality rates for patients undergoing surgery were 10% for those with Child class A, 30% for those with Child class B, and 76-82% for those with Child class C cirrhosis.(1,5) Child class also correlates with the frequency of postoperative complications, which include liver failure, worsening encephalopathy, bleeding, infection, renal failure, hypoxia and intractable ascites.

Even in patients with Child class A cirrhosis, the risk of perioperative mortality is increased when there is an associated portal hypertension. Postoperative morbidity in such patients, and possibly in patients with Child class B and C cirrhosis, may be reduced by preoperative placement of a transjugular intrahepatic portosystemic shunt.[6]

There are several other factors which can increase the perioperative risk above and beyond the Child class. Urgent surgery is associated with a higher mortality rate than non-urgent surgery: 22% versus 10% for patients in Child class A; 38% versus 30% for those in Child class B and 100% versus 82% for those in Child class C.[5]

A general consensus is that elective surgery is well tolerated in patients with Child class A cirrhosis, acceptable with preoperative preparation in patients with Child class B cirrhosis (except those undergoing extensive hepatic resection or cardiac surgery), and contraindicated in patients with Child class C cirrhosis.[7]

The Model for End-Stage Liver Disease score

The Model for End-Stage Liver Disease scoring system was developed from univariate and multivariante analyses of clinical and laboratory variables. Originally designed to predict survival after placement of transjugular intrahepatic portosystemic shunt to control bleeding varices[8], the MELD score has also been shown to predict short-term survival in patients with cirrhosis more reliably than the Child-Pugh system. The MELD score is now accepted for assesing priority for liver transplantation in patients with cirrhosis recently utilized to predict perioperative mortality.[8,9] The MELD score is a linear regression model based on a patients's serum bilirubin and creatinine levels and international normalized ratio (INR). The score is calculated from a validated predictive equation, as follows: (3.8 x ln bilirubin value) + (11.2 x ln INR) + (9.6 ln creatinine value), where bilirubin and creatinine values are expressed in milligrams per deciliter (mg/dL) and ln represents natural logarithm. The etiology of liver failure was originally an element of the MELD, but this criterion was subsequently excluded from the equation because it was proved prognostically insignificant.

This score has several distinct advantages over the Child classification: it is objective, based on quantitative parameters and does not rely on arbitrary cutoff values. Each one-point increase in the MELD score makes an incremental contribution to risk, thereby suggesting that the MELD score increases precision in predicting postoperative mortality.[7]

In general, the MELD score compares well to CTP score. However, some favor the MELD score over the CTP score as a more objective predictor of postoperative mortality, [9,10,11] especially for classifying patients along a continuum of values instead of into 3 discrete groups.

A number of studies have examined MELD score as a predictor of surgical mortality in patients with cirrhosis. A retrospective study analyzing 140 patients with cirrhosis who underwent surgery reported that the MELD score was the only statistically significant predictor of 30-day mortality. A 1% increase in mortality for each one-point increase in the MELD score from 5 to 20 and a 2% increase in mortality for each one-point increase in the MELD score above 20 was observed.[12] Another retrospective, multivariate analysis of 772 patients with cirrhosis undergoing multiple types of major surgeries, showed that the MELD score, but not the CTP score, predicted increased mortality at 30 and 90 days, 1 year, and over a long-term.[13]

More recently, the MELD score has been adapted with additional clinical factors, creating the "integrated MELD" score (iMELD): MELD + (0.3 x serum sodium [mEq/L]) + 100. In a retrospective analysis of 190 patients with cirrhosis, was demonstrated that the iMELD score had better prognostic strength compared with the MELD or CPT score.[14]

MELD score and Child class are not mutually exclusive and may complement each other, but the MELD score is probably more precise single predictor of perioperative mortality.[13]

Operative risk associated with specific types of abdominal surgery

Most of the data on the risk of surgery in patients with cirrhosis are presented in studies of abdominal surgery. Open abdominal surgery appears to carry much higher risk than laparoscopic surgery. Reported mortality rates range from 20% to 54% for various types of open abdominal surgery, with multiple factors associated with mortality.[15] Data vary considerably, however, most of the studies found that the mortality rate was higher in patients with one or more of the following: elevated bilirubin, prolonged prothrombin time, decreased albumin, encephalopathy, ascites, portal hypertension and emergency surgery. Emergency surgery has been associated with a higher morbidity and mortality (50% vs. 18%) as compared to elective surgery .[1,5,16]

Gallstone disease. Prevalence of gallstone in a cirrhotic patient is higher than in general population, reaching 7-28%. Data exist about the prevalence and incidence of significant complications of gallstone disease in patients with chronic liver disease. In one study,[17] 17% of admitted patients with chronic liver disease had cholelithiasis and in 22% of this group cholelithiasis caused cholecystitis, obstructive jaundice, biliary pain and all patients underwent a cholecystectomy. In the other 78%, cholelithiasis was asymptomatic, 20% of this group died from liver failure, and 14% had radiographically demonstrated stones that were not operated on and they had no complications on follow-up.The study confirmed the high incidence of gallstone disease in patients with cirrhosis, and complications of gallstone disease requiring an emergency operation were associated with a higher morbidity and mortality.[17] In a case-control study of patients who underwent cholecystectomy, a MELD score

e"8 had a sensitivity of 91% and specificity of 77% for predicting 90-day postoperative morbidity.[18] Generally, laparoscopic cholecystectomy is acceptable for patients with Child class A cirrhosis and selected patients with Child B cirrhosis without portal hypertension.[19] On the other hand, in patients with Child class C cirrhosis, cholecystostomy rather than cholecystectomy is recommended, but when surgery is inevitable, an open rather than laparoscopic approach is recommended.[20,21]

Obstructive jaundice. Retrospective analysis of 373 patients with obstructive jaundice identified three risk factors for perioperative mortality: low hematocrit (less than 30%), an initial serum bilirubin level greater than 200 µmol/L and malignant cause of biliary obstruction. The mortality rate was 60% when all three were present vs only 5% when none were present.[22] Malignant biliary obstruction was associated with a significantly higher operative mortality rate (26.1%) than benign biliary obstruction (3.7%). Patients with biliary obstruction also carry an increased risk of infection, gastrointestinal bleeding, disseminated intravascular coagulation, delayed wound healing and renal failure.[22]

Contemporary management of obstructive jaundice reduces the need for direct surgical intervention in most cases. Endoscopic or percutaneous biliary drainage is always preferable for surgery of benign etiologies and malignant causes that are not amenable for curative surgery. Although endoscopic sphincterotomy is associated with an increased risk of bleeding in cirrhotic patients, morbidity and mortality rates are low even in patients in Child class C cirrhosis.[23]

Gastric surgery. Peptic ulcers affect 8-20% of cirrhotic patients.[24] The mortality in emergency surgery for complications of ulcer disease such as bleeding or perforation in cirrhotic patients is significant, ranging from 23% to 64%. CTP class and presence of ascites determine mortality in these patients.[25] Laparoscopic suture of the perforated ulcer combined with proton pump inhibitors and endoscopic haemostatic procedures for bleeding ulcers have reduced the need for resectional surgery and reduced the emergency mortality rate.

Surgical treatment of gastric cancer in cirrhotic patients resulted in morbidity rate of 20-26% and mortality of 0-10%.[26,27] Another study showed mortality of 23% and morbidity of 56%, significantly higher in patients with ascites and low serum albumin.[28] The data of these studies suggest that gastric surgery is safe for CTP class A and CTP class B patients.

Colorectal surgery.Reported data of colorectal surgery (mainly diverticular disease and colorectal cancer) in patients with cirrhosis showed mortality rate of 13-23% and morbidity rate of 45-51%.[29] The factors predictive of perioperative mortality were ascites, elevated serum biliribin, low prothrombin level and emergency surgery. In the emergency situation in cirrhotic patients, procedures as colonic stenting in cases with intestinal obstruction and endoscopic management in patients with bleeding should be preferred to surgery.[5,30,31]

Colectomy in patients with inflammatory bowel disease complicated with cirrhotic primary sclerosing cholangitis is associated with a high early postoperative morbidity rate. The data suggest that strong attention should be paid to avoid or reduce pelvic sepsis, especially after ileal pouch-anal anastomosis, because pelvic sepsis is associated with higher mortality and morbidity.[32]

Pancreatic surgery.In a study that was aimed to estimate the overall prevalence of pancreatic surgery in cirrhosis, 35 patients were included; 17 underwent surgery for chronic pancreatitis, 3 for acute pancreatitis, 14 for malignant tumors and 1 for benign tumor.[33] Overall reported morbidity was 51% and mortality was 20%. All three patients who had emergency procedures died, and other deaths occurred in patients where gastrointestinal tract was opened. These findings suggested that whenever possible endoscopic and radiologic treatments should be preferred in cirrhotic patients with inflammatory disease or tumor of the pancreas. One case-control study[34] compared outcomes in 32 patients with cirrhosis vs matched controls undergoing pancreatic resection surgery. Complications were more frequent (47 vs 22%; p=0.035) in patients with cirrhosis, and there were also significantly more reoperations (34 vs 12%; p=0.039) in the cirrhotic group. The authors concluded that pancreatic surgery was associated with an increased risk of postoperative complications in patients with liver cirrhosis, and was therefore not recommended in patients with Child B cirrhosis. However, in Child A cirrhotic patients the mortality is however, comparable to non-cirrhotic patients. Due to the demanding medical efforts that these patients require, they should be treated only in high-volume centers. Based on another study[35] analyzing four patients with pancreatic tumors and compensated liver cirrhosis who successfully underwent pancreaticoduodenectomy a conclusion can be drawn that pancreaticoduodenectomy is not contraindicated in patients with cirrhosis and should be performed in expert hepatobiliary centers.

Hepatic resection. Patients with cirrhosis undergoing resection for hepatocellular carcinoma or other liver tumors are at increased risk for hepatic decompensation compared to those undergoing other types of operations.[36] Surgical resection of localized hepatocellular carcinoma in patients with cirrhosis raises concerns about the adequacy of residual reserve, especially in those who have advanced cirrhosis and portal hypertension. These patients have increased rates of perioperative complications, long-term hepatic decompensation, and death following resection, and thus patient selection is critical.[37]

In the past, cirrhosis was considered to be a contraindication to hepatic tumors resection since mortality rates exceeded 50%. More recently, the perioperative mortality rate for hepatic resection has decreased, although postoperative morbidity rates are still

as high as 60%.[38,39] The improvements in outcomes have been attributed to better patient selection, earlier tumor detection, better preoperative preparation, intensive intra- and postoperative monitoring, and improved surgical techniques. Post-resectional liver failure has been defined as a prothrombin index of less than 50% (INR>1.7) and serum bilirubin greater than 50 μmo/L (2.9 mg/dL), the so-called "50-50" criteria. When these criteria are met, the postoperative mortality is 59%, compared with 1.2% in patients not meeting these criteria.[40] Risk stratification based on the Child class and MELD score has allowed more appropriate selection of patients for hepatic resection, thus leading to lower mortality rates. In an analysis of 82 cirrhotic patients who underwent hepatic resection, the perioperative mortality rate was 29% in patients with MELD score e"9 but 0% in those with MELD score d"8.[13] In contrast, the other study showed limited ability of the MELD score to discriminate between risk groups. Overall, no prospective studies, as yet, have proven the usefulness of preoperative risk assessment with either CTP or MELD score. In this contex, CTP class and American Society of Anesthesiologists physical status classification were better predictors of morbidity and mortality than the MELD score.[41]

Preparing a cirrhotic patient for surgery

It is very important to identify and adequately treat morbid conditions that often coexist in patients with cirrhosis undergoing surgery in order to ensure safe operative and postoperative course. Cardiovascular and nutritional performance, fluid and electrolyte balance need to be optimized in order to decrease perioperative death and complications after surgery. Essential issues are treatment of manifestations of acute liver function deterioriation including encephalopathy, acute renal failure, coagulopathy, respiratory distress syndrome and sepsis.[2]

It is accepted that converting a Child C patient to Child B preoperatively could help survival after surgery.[42]

Patients with autoimmune hepatitis on steroids treatment should receive stress-dosed steroids before surgery. In patients with Wilson cirrhosis D-penicillamine can impair wound healing and in these patients the dose should be decreased for 1-2 weeks pre- and postoperatively. Patients with cirrhosis and history of alcohol abuse are at increased risk of other complications, including poor wound healing, delirium, infections and bleeding. Unless in urgent surgery, patients with alcoholic hepatitis should have medical treatment and be stabilized, or should undergo, less invasive alternative procedures in an emergency situation.[2]

Postoperative monitoring

Postoperatively, patients with cirrhosis need to be monitored for the development of signs of hepatic decompensation, including encephalopathy, coagulopathy, ascites, worsening jaundice and renal dysfunction. When any of these conditions develop, supportive therapy should be promptly initiated.The prothrombin time is the single best indicator of hepatic synthetic function. Hyperbilirubinemia can indicate worsening of liver function, although it can be elevated for other reasons, including blood transfusions, resorption of extravasated blood or infection. Renal function must be monitored closely. In case of renal dysfunction, the cause should be determined and treatment initiated. Hypoglycemia may occur in patients with decompensated cirrhosis and serum glucose levels should be monitored closely. Careful attention should be paid to the assessment of intravascular volume, which is often difficult to assess in the setting of extravascular volume overload. Intravascular volume maintenance is essential for preservation of hepatic and renal perfusion. On the other hand, infusion of too much crystalloid may lead to acute hepatic congestion, pulmonary edema, ascites, peripheral edema and wound dehiscence.[2,4,5,15]

Conclusion

The perioperative morbitity and mortality following surgery in cirrhotic patients are significant. Therefore, the decision to perform surgery in these patients must be heavily weighed. Appropriate preoperative evaluation and management of patients with liver disease are fundamental for counseling and selection of patients for surgical treatment, and might reduce the risk of surgery and improve outcomes in these patients. Strict assessment of these patients for co-morbidities and risk stratification in the preoperative period is essential. It is very important to identify and adequately treat comorbid conditions that often exist in patients with cirrhosis undergoing surgery in order to ensure safe operative and postoperative course. Surgical risk assessment is less relevant if emergency surgery is required to prevent death. On the other hand, the vast majority of decisions are made in the setting of semi-urgent or elective procedures for which there is time for risk assessment, optimization of the patient's medical status, and consideration of alternative approaches.

Three factors essentially determinate the extent of surgical risk: degree of decompensation (higher MELD and CTP score), whether surgery is performed as an emergency procedure or electively, and the type of surgery.

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FOLLICULAR LYMPHOMA– CAN WE CURE IT?

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Abstract

There are several challenges encountered by the hematologists treating patients with follicular lymphoma. Follicular lymphoma is the most common type of indolent B-cell lymphoma and it is characterized by the responsiveness to initial chemotherapy, followed by repeated relapses, which means that follicular lymphoma is a disease usually sensitive to therapeutic agents, but paradoxically, most often it remains incurable. In the last few decades there has been a significant improvement in the treatment of patients with follicular lymphoma as a result of introducing the target therapy anti-CD20 antibody - rituximab. The treatment has been improved and today there is a significantly better treatment outcome (higher rate of achieved complete remission, more durable time to progression, prolonged disease-free survival and better quality of life because of the prolonged period without therapy). There are new questions that need to be answered: Is there a need to rethink or redefine our goals of treating patients with follicular lymphoma? Is "cure" of patients with follicular lymphoma an achievable goal in the era of advanced hematology nowadays?

Key words: follicular lymphoma, rituximab – anti-CD20 antibody, maintanence therapy

Introduction

Follicular lymphoma is the second most common lymphoma in adults, accounting for about 20%-30% of all cases of non-Hodgkin lymphoma (NHL) and it is the most frequent form of indolent non-Hodgkin B cell lymphoma.[15]

Most patients have advanced disease at diagnosis and cannot be cured with currently available therapy and hence follicular lymphoma is usually considered an incurable disease. A better understanding of the biology of the disease and the development of novel target agents are challenging prior to dogmatic concept regarding the standard therapeutic approach to follicular lymphoma.

The scale of therapeutic options is wide and ranges from a watch-and-wait policy to aggressive alternative, such as hematopoietic stem cell transplant. The important issue for the experienced clinician treating patients with follicular lymphoma is to balance the efficacy of the treatment options against both toxicities and compliance. The other important issue is the optimal time to start therapy and there are still controversies regarding which end points are to be achieved. The most used system for risk stratification of the patient is FLIPI score which includes: age (>60 years), Ann Arbor stage (III - IV), hemoglobin level <12g/dl, LDH>upper limit of normal and 4 nodal sites of disease. Based on these clinical characteristics, patients with follicular lymphoma can be categorized in three risk groups with different 5 and 10 year-survival rates: "low-risk" (0-1 adverse factor present); "intermediate risk" (2 factors) and "high risk" (> 3 factors). The 5 and 10 year-survival rates between risk groups are: 90.6 and 705 for low-risk, 77.6% and 50.9% for intermediate and 52.5 and 35.2% for high risk patients.

Because indolent NHL has been considered incurable, it has historically been treated with a conservative approach, with initially mild chemotherapy, reserving more aggressive treatments until later stage of the disease. The patients with follicular lymphoma have been treated with a single-agent chemotherapy or combination chemotherapy but without result regarding prolonged duration of response or improvement in overall survival beyond what could be obtained by initial "watchful and waiting" strategy. This approach is now changing because more effective treatments such as R-chemotherapy and maintenance have been shown to improve overall survival. Today we are aware that rituximab is effective either as a single agent or in combination with chemotherapy in the management of relapsed or de novo follicular lymphoma. This therapeutic approach has changed the historic treatment paradigm in follicular lymphoma patients today.

The basic guidelines for navigating the treatment options for patients with follicular lymphoma are: tumour characteristics (rate of tumour growth; tumour bulk etc); patients' characteristics (co- morbidities, patients' wishes, "goal of therapy" etc.) and clinical/laboratory characteristics (FLIPI score etc). Ideal upfront treatment is the one with the highest rate of possibility to achieve complete remission of the disease, hopefully achieving the longest disease-free survival. Patients with responsive

disease survived significantly longer than those in whom only partial response or less could be achieved. Thus, the primary goal of the treatment is to achieve complete remission. Today standard upfront therapeutic option is R-chemotherapy, monoclonal antibody – anti-CD20 antibody (rituximab) combined with chemotherapy in regiments like R-CVP, R-CHOP, or FC-R.[3,4,6,10] Including monoclonal antibody in the first line therapy in combination with chemotherapy has revolutionized the treatment of patients with follicular lymphoma and has significantly improved the overall survival in these patients subset.

Historically, follicular lymphoma is characterized by the responsiveness to initial chemotherapy, followed by repeated relapses, which means that follicular lymphoma is a disease usually sensitive to therapeutic agents, but paradoxically, most often it remains incurable. Recurrent relapses are main reason for that, suggesting that most probably a few follicular lymphoma cells escape treatment. After the initial relapse, both the response rate and relapse-free survival decrease steadily, resulting in a median survival of 4-5 years after the first relapse.[7] Thus, the question arises: Can we cure the patient with follicular lymphoma if we prevent relapses? There is an emerging need of treatment to improve induction regimens and maintenance treatment defined as a continued treatment beyond induction therapy. There have been attempts to use chemotherapy as a treatment beyond induction demonstrated efficacy, but the ability to continue to deliver chemotherapy in full dosage was limited by myelosupression and by patient acceptance.[14] Therefore, the hematologists have been looking for a strategy to extend the duration of remission without significantly increasing toxicity, or to prolong as far as possible the time patients remain disease-free. Chemotherapy did not fulfill these criteria because of its toxicity, and rituximab has emerged as an attractive drug for maintenance therapy. There are several arguments in favor of rituximab. The main one is safety of the product. Rituximab is associated with only minimal acute toxicity and no major long-term cumulative toxicity. Rituximab targeted CD20 antigen was recognized by immunologists as an ideal antigen for monoclonal antibody therapy.[16,17] CD20 antigen usually persists on residual or recurrent lymphoma cells allowing retreatment. Patients have good compliance to rituximab treatment, because of its infrequent administration in an outpatient setting. This is possible since rituximab has long half-life, still maintaining long-term drug exposure which is the mainstay to control residual malignant cells and delay disease recurrence.[8, 9, 12]

Rituximab is a chimeric human murine monoclonal antibody that binds avidly to the CD20 antigen, a transmembrane phosphoprotein, which is expressed on almost all malignant B-cells. Proposed mechanisms of action of rituximab include direct induction of apoptosis, complement –mediated lyses and antibody-dependent cellular cytotoxicity and has a delayed therapeutic effect as well as a potential "vaccinal" effect. Today, we know that rituximab can sensitize lymphoma cells to a range of chemotherapeutic agents.[2] For the first time, rituximab was approved and used in 1997[11] in patients with relapsed indolent lymphoma. The results showed that objective response rate was 48% and the monoclonal antibody had no serious adverse effects. Today rituximab maintenance[18] is used in patients who achieved complete remission (or at least partial response) after induction treatment (chemotherapy or immunochemotherapy). Rituximab maintenance therapy can be safely administered for periods of up to two years and from patients' point of view prolongs the period in which they are free from disease-related symptoms.

In the rituximab era a question arises on the role of hematopoietic stem cell transplantation in patients with follicular lymphoma. Hematopoietic stem cell transplantation is indicated for those patients whose disease relapses within 2-3 years.[13] Patients who experience early relapse can be treated with the second-line chemotherapy plus rituximab followed by a high-dose chemotherapy and autologous stem cell transplantation. Allotransplantation is recommended for those patients in whom relapse occurred after autologous transplantation.

Despite the significant improvement in treatment of patients with follicular lymphoma there is still a high percentage of patients who fail to respond or relapse after initial remission, most probably as a result of intrinsic or acquired resistance. An important lesson learned from the clinical use of rituximab in the treatment of patients with follicular lymphoma is that the rational design of therapy for B cell lymphoma based on targeting specific unique target on neoplastic cells can be achieved. There is an ongoing story and a lot of attempts to recognize another surface antigens present on the surface of the lymphoma cell and target them with monoclonal antibody such as aletuzumab, epatuzumab, galixamab. Also, there are attempts to define pathways in lymphoma cells and use them for novel therapeutic strategies to improve antitumor activity such as bcl2 inhibitors, hypomethilating agents, proteasome inhibitors, immunotoxins, etc.

Conclusions

Having in mind that follicular NHLs are slow-growing, low-grade lymphomas, and complete remissions or partial remissions can be obtained, follicular lymphoma is still considered incurable because of a clinical course

characterised by a high relapse rate. Thus, we can shortly define the goals of the therapy as follows: prolong progression-free survival and keep patients in remission as long as possible with maintaining quality of life (QoL) and overall survival. Early treatment with rituximab produces the best response.[18]

As haematologists working in the era of the 21st century, we are close to possibility of curing patients with follicular lymphoma. We are satisfied with the possibility with rituximab maintenance. This therapy is beneficial for patients with follicular lymphoma because it prolongs the period in which they are free from disease-related symptoms; they can resume working and avoid the feeling of passively waiting for relapse.[1]

The greatest challenge for the hematologists treating patients with follicular lymphoma today is how to best stream-line the evaluation of a large number of effective therapies in order to determine their optimal use and sequencing in order to continue the improvement of both the quantity and quality of life of patients with follicular lymphoma not only today, but also for future generations of lymphoma patients.

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B b	B b	W w	Nj nj
V v	V v	O o	O o
G g	G g	P p	P p
D d	D d	R r	R r
\ |	G g	S s	S s
E e	E e	T t	T t
@ ‘	Zh zh	] }	K k
Z z	Z z	U u	U u
Y y	Dz dz	F f	F f
I i	I I	H h	Kh kh
J j	J j	C c	Ts ts
K k	K k	^ ~	Ch ch
L l	L l	X x	Dzh dzh
Q q	Lj Lj	[{	Sh sh
M m	M m		

On the basis of ISO Recomandation R-9-1968 International List of Periodical Title Abbreviations (1970)

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