POSTER PRESENTATION

5. FREE PAPERS

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Diabetic nephropathy in autopsied patients with diabetes mellitus

KEYWORDS: Diabetes mellitus; Diabetic nephropathies; Kidney tubules

INTRODUCTION

Recent research on mortality in diabetic population has shown that the mortality is higher in diabetics than in other people, because of the presence of vascular complications. The mortality is 2 to 5 times higher in diabetics in comparison with non-diabetic population. The average life in diabetics is shorter. The causal factors of the majority of deaths in diabetics are vascular and renal diseases. Renal disease is a frequent complication of Diabetes mellitus. The most common form of renal disease is diabetic glomerulosclerosis, which occurs in approximately 40% of patients with type 1 diabetes and in 15-30% of patients with type 2 diabetes after 10 years of duration of the disease. Two thirds of patients with diabetic glomerulosclerosis develop renal failure requiring either dialysis or renal transplantation. Diabetic nephropathy is a common term for the clinical and morphological manifestations of the renal disease in diabetes. The most important renal lesions associated with diabetes are vascular. They include microangiopathy and macroangiopathy. Both are part of a generalized diabetic vascular disease. Renal microangiopathy involves arteriolar and glomerular capillaries. Kimmelstiel and Wilson for the first time described diabetic glomerulopathy in 1936. Diabetic microangiopathy (atherosclerosis) is present in the kidney together with its sequelae: infarctions and ischemic atrophy. Diabetic micro and microangiopathic alterations may be found separately or together, or in association with pyelonephritis. The aim of this study is the pathomorphological (autopsy and histopathological) study of the autopsied patients with diabetes mellitus, their complications and the causes of death.

MATERIAL AND METHODS

We analyzed 12 autopsied diabetic patients (1998 - 2002) at the Institute of Pathology in Skopje. We used standard autopsy protocols and histological sections. All slides were stained with standard HE. The slides from renal tissue were stained with PAS hematoxylin and silver methenamine-hematoxylin. We also used immunohistochemical staining with the immunofluorescent technique. The patients were divided in 2 groups: 5 patients with type 1 and 7 with type 2 diabetes. Seven were woman and 4 men. The age was from 49 - 79 years (average: 60 years).

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RESULTS

Correlations between clinical and pathological findings in all autopsied patients are presented in table 1.

Table 1. List of clinical diagnoses compared with histopathological findings in autopsied patients

Clinical Diagnoses	No.	Histopathological findings	No.
DM type 1	4	Diabetic nephropathy	12
DM type 2	6	Chronic pyelonephritis	5
Renal failure	3	Papillary and tubular necrosis	2
Myocardial infarction	3	Myocardial infarction	7
Dilated cardiomyopathy	1	Hypertrophic cardiomyopathy	10
Congestive heart failure	4	Generalized arteriosclerosis	10
Arterial hypertension	3	Arteriosclerosis of coronary arteries	7
Coma hyperglycemica	1	Pancreatic fibrosis and lipomatosis	12
Metabolic coma	1	Pulmonary oedema	8
Bronchopneumonia	2	Bronchopneumonia	5
		Other	10

Diabetic nephropathy was found in all patients.

Gross appearance: The kidneys were normal in size in all patients, except in those with nodular nephrosclerosis where a reduction in size was present. The subcapsular surface was granular. On the cut surface, a normal architecture was noted. The arteries were prominent, because of the thickening of their wall due to arteriosclerosis.

Table 2. Histopathological findings of diabetic kidney disease

Glomerulosclerosis nodularis (Kimmelstiel – Wilson)	3	Acute pyelonephritis	2
Glomerulosclerosis diffusa	4	Chronic pyelonephritis	5
Glomerulosclerosis diffusa and nodularis	3	Tubular necrosis	1
Glomerulosclerosis nodularis and exudativa	1	Papillary necrosis	1
Glomerulosclerosis diffusa and exudativa	1	Arteriolar and arterial atherosclerosis	10

Microscopical findings: The glomerular structure showed a broad histological spectrum: from normal to diffuse, and from nodular glomerulosclerosis to totally occluded glomeruli. In the glomeruli, we found a widening of the mesangial areas with increased mesangial matrix, clearly identifiable by PAS and silver methenamine staining. The nodules were round or oval, moderately PAS positive. On periodic acid silver methenamine stainig, they display a concentric fibrillar layering. Exudative glomerulosclerosis with "capsular drop" were also seen. Deposits of PAS positive material in the arteriolar walls have also been present. Diabetic glomerulosclerosis was associated with PAS positive arteriolar hyalinosis. The afferent and the efferent arterioles were both affected. Arterioscleroric changes may be found in the intrarenal vessels as an expression of diabetic macroangiopathy. Tubulointerstitial lesions with tubular atrophy and interstitial fibrosis were also found. The infiltration with mononuclear cells indicated the presence of pyelonephritis. Using a direct immunofluorescent technique for antihuman IgG, IgM and C3 complement, we found immunoglobulin deposits in the thickened capillary and arteriolar walls.

DISCUSSION

Diabetic glomerulosclerosis is a frequent complication of diabetes mellitus resulting in the renal failure. Patients with nephropathy frequently develop other vascular diseases, hypertension, resulting in an increased risk of early mortality. Diabetic glomerulosclerosis may be found in 3 separate pathomorphological varieties: diffuse, nodular and exudative. Diffuse glomerulosclerosis is more frequent than nodular glomerulosclerosis. Exudative glomerulosclerosis is less frequent ("fibrin cap" and "capsular drop"). Nodular and diffuse glomerulosclerosis are sometimes separate histological lesions, but they

can also be concurrent. These lesions are frequently associated with arteriolar and arteriosclerosis. Pyelonephritis is quite commonly associated with renal diabetic vasculopathy.

CONCLUSION

All these alterations can not always be in correlation with the clinical picture of the diabetic renal disease.

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Cytologic diagnosis of ameloblastoma - Report of two cases

KEYWORDS: Ameloblastoma; Needle biopsy; Cytology

INTRODUCTION

Ameloblastoma is a rare tumor of the jaw arising from odontogenic epithelium. Preoperative diagnosis is made in the light of clinical and radiologic findings which sometimes may not be characteristic. One of the possibilities to narrow the list of differential diagnoses is fine - needle aspiration biopsy. In order to use cytological method successfully , the reliable cytologic indicators must be defined.

CASE REPORTS

In two patients with ameloblastoma of the mandible, imprints were made from the surgically removed material, air - dried and stained by May - Grunwald Giemsa method. We observed the cellularity, morphology of cells and the background of the preparations. The imprints were hypercellular. In the background, the amorphous material was found as well as the bare nuclei and spindle and stellate cells with regular, oval nuclei. Beside single cells, two types of cell groupings were observed. The first kind were cohesive groups of ameloblast - like cells with palisading on the edges. The cells were cylindrical, with ill - defined borders of basophilic cytoplasm, regular, oval nuclei with finely distributed chromatin and visible nucleoli. The groups of squamous epithelial cells were also seen. The cytoplasm of these cells was polygonal, sharply defined, vacuolized and the nuclei were regular, with evenly distributed chromatin. In some cells, spherical intracytoplasmic bodies were found.

CONCLUSION

In the imprints of ameloblastoma we found characteristic morphologic features which is in accordance with the data from the literature (1,2). We suggest that the oral surgeons should consider the utilization of fine - needle aspiration biopsy in the preoperative diagnosis of ameloblastoma.

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Histopathologic parameters as prognostic factors in colorectal carcinoma (CRC) - Retrospective analysis of the five-year period (1990-1994) on the material from the Department of GIT tumours, Clinic of Oncology Clinical Centre Niš

KEYWORDS: Colorectal neoplasms; Prognosis; Staging

INTRODUCTION

Colorectal carcinoma is the third most common malignant tumor in many countries worldwide. These are mostly adenocarcinomas, which may be, according to the growth pattern, exophytic, ulcerative, stenosing or circumferent. Microscopically, vegetative, ulceroinfiltrative and scirrhous forms are distinguished. Histologically classified (WHO), malignant tumors of the large bowel may be epithelioid (adenocarcinoma, mucinous adenocarcinoma, signet-ring cell carcinoma), carcinoid tumors (argentine, non-argentine, mixed forms), non-epithelioid (leiomyosarcoma), hematopoietic and lymphoid neoplasms, secondary tumors, tumor-like lesions, as well as epithelial abnormalities of the precancerous type. Histopathologic parameters required for planning the specific oncologic therapy, significant for the prognosis and outcome of CRC, are Dukes and TNM stage of the disease, resection borders, histologic grade, vascular and perineural invasion, lymph node invasion, immunologic response to the primary tumor, reactive lymph nodes and invasion of adjacent organs. Other significant factors are nuclear morphology, cell cycle parameters, tumor ploidy, oncogene expression (c-fos, c-myc, Ha-RAS, Ki-RAS), loss of tumor suppressor gene (p53), increased epidermal growth factor receptor expression, collagen type IV in tumor matrix, CEA and CA 19-9 presence/absence and preoperative values of serum proteins. The retrospective analysis of the clinical material was conducted aiming to answer the following questions: 1. Which histologic parameters an oncologist has in the moment of therapeutic decision-making after surgery in CRC patients? 2. Are there all the relevant data so that adequate decision on postoperative treatment could be made? 3. Are there differences in 5-year survival rates related to the disease stage and other histopathologic parameters obtained from the pathologist?

PATIENTS AND METHOD

In the period of five years (January 1990-December 1994) at the Department of GIT tumors, Clinic of Oncology, Clinical Centre Niš, 147 patients with CRC were managed, out of which 53 (36%) with colon carcinoma and 94 (64%) with rectal carcinoma. The disease stage was determined based on the histopathology and Dukes classification, as well as diagnostic proceedings necessary to establish the tumor burden. All those with an operable disease were surgically treated. The treatment instituted was decided at the Council for GIT tumors, according to the treatment protocols of the Institute of Oncology and Radiology of Serbia. A number of parameters was utilised in monitoring of the treatment response, encompassing regular clinical examinations, laboratory tests, abdominal and small pelvis ultrasound, endoscopy with biopsy, chest radiographs and CT of the suspected organs as required.

RESULTS AND DISCUSSION

Most of the patients treated had rectal carcinoma (94/147; 64%). The most common colonic cancer site was the sigmoid region (18/53; 34%). In 137 the malignancy in question was adenocarcinoma, while 10 (6.8%) patients did not have histopathologic findings though they were surgically managed. In the group observed, 1/3 of the cases did not have Dukes classification (51/147; 34.7%), while 49/147 (33.3%) had Dukes B, 28/147 (19%) had Dukes C, 7/147 (4.8%) had Dukes C and just 2 patients (1.4%) Dukes A. For the cancer of the colon, the average survival for female patients was 71 months (minimum 4; maximum 120) and for men 49 months (minimum 3; maximum 120), which was statistically significant ($p<0.05$). Five cases were lost for follow-up. Of those with colon cancer, 17/23 (74%) women survived 5 years; the corresponding proportion for men was 10/25 (40%) ($p<0.05$). Out of 94 patients with rectal cancer, 14 were lost for follow-up. The average survival for women was 49 months (minimum 4; maximum 120) and for men 41 months (minimum 5; maximum 120). Five-year survival for women was 40% and for men 33%. Out of 13 patients with Dukes B_1 11 (85%) lived 5 or more years and 21/36 (57%) Dukes B_2 cases lived 5 years. For Dukes C, the 5-year survival was registered in 36% of the patients; for Dukes D none of the patients survived 5 years (16 months on the average; minimum 10, maximum 25).

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Morphological features of contralateral ovary in unilateral ovarian carcinoma

KEYWORDS: Ovarian Neoplasms; Contralateral; Pathology

INTRODUCTION

Malignant tumors originating from the surface epithelium-stroma of the ovary account for about 80-90% of ovarian malignances (1,2). Most likely they arise from epithelial inclusion cysts (3-6). Epithelial inclusion cysts thus represent a precursor lesion of ovarian carcinoma, especially when their number is increased or their epithelium displays some degree of atypia (10. The present study deals with the morphological feautures present in contralateral ovaries from patients with unilateral ovarian carcinomas derived from the surface epithelium-stroma, as compared with the ovaries of patients without ovarian pathology.

MATERIAL AND METHOD

The morphological features in contralateral ovaries from 20 patients with unilateral ovarian carcinoma (including 6 borderline tumors) were compared with the ovaries of 20 patients without ovarian pathology.The histological sections of each case were stained with hematoxyline-eosin. The histological features considered were the ovarian size, presence or absence of cortical invaginations and epithelial inclusion cysts (number, size, and presence of metaplasia and atypia), presence or absence of the cortical stromal hyperplasia, presence or absence of psammoma bodies, cortical thickness, presence or absence of surface papillae. The cortical thickness and size of cortical invaginations and epithelial inclusion cysts were mesured with a calibrated ocular micrometer. To compare the values of each variable, Fisher's exact test and nonparametric Kruskal-Wallis test for numeric variables were applied.

RESULTS

Ovarian carcinoma was located in the right side in 12 patients and in the left side in 8 cases. Six lesions were borderline tumors (4 serous, 1 mucinous, and 1 seromucinous). Of the 14 infiltrating carcinomas, 9 were serous, 3 mucinous, and 2 endometrioid cell type. The histological grade was 1 in 10 cases and 2 in 4 patients. In the ovaries from women with contralateral ovarian carcinoma, cortical invaginations and epithelial inclusion cysts were often seen (85%, 17 ?f 20). Its higher incidence was statistically significant ($p<0,001$) in comparation with the normal group (30%, 6 of 20). Statistically significant differences ($p<0,05$) were found in the number of invaginations, being more numerous in the cancer group. The number of cortical invaginations and the number of inclusion cysts correlated in the cancer and normal group ($p<0,05$). Focal surface epithelial atypia was only seen in 4 cases of the cancer group. Surface papillae were more frequently seen in the cancer group (50%, 10 of 20). The examined groups exhibited no differences regarding the ovarian size, cortical thickness, stromal hyperplasia, psammoma bodies and the presence of metaplasia.

DISCUSSION

It is thought that most ovarian carcinomas arise from the surface epithelium-stroma or from epithelial inclusion cysts, most likely as the result of the effect of exogenous or endogenous factors such as estrogens or other unknown influences (3-7). Increased ovarian surface epithelial cell division, to repair the wound created by a follicular rupture, seems to play a role. Epithelial inclusion cysts arise from cortical invaginations of the surface epithelium which have lost their connection with the surface (3-7). The epithelium lining the cysts is usually composed of a layer of flat to cuboidal cells as in the ovarian surface. Previous studies have demonstrated an increased number of epithelial inclusion cysts in the women with contralateral ovarian cancer (6). Epithelial inclusion cysts seem to have greater potential to undergo neoplastic changes if compared with the surface epithelium. According to our observations, the step preceding the formation of epithelial inclusion cyst, that is, cortical invagination, is also more frequent in patients with ovarian neoplasia, and it should also be regarded as a precursor lesion.

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Steatohepatitis - how to reach the accurate diagnosis?

KEYWORDS: Fatty liver; Hepatitis;Liver biopsy; Morphometry

INTRODUCTION

Nonalcoholic Fatty Liver Disease (NAFLD) is defined as a constellation of clinical conditions characterized by predominantly macro vesicular steatosis (1). By definition, NAFLD occurs in subjects who do not consume enough alcohol to cause a liver damage. The histological spectrum of NAFLD includes a fatty liver alone or nonalcoholic steatohepatitis (NASH). Once considered to occur in obese, middle-aged women, it is now proven that it occurs in subjects of all races and both genders, and even in those who are not obese (2). NASH can be associated with the progressive hepatic fibrosis and is an important cause of cirrhosis of the liver (3). Despite its common occurrence, the pathogenesis of NASH is unknown. NASH represents only a stage in the spectrum of nonalcoholic fatty liver disease and is defined histologically by the presence of steatosis along with necroinflammatory activity, mostly of lobular distribution, regardless of the presence of Mallory hyaline or fibrosis, features which are indistinguishable from those of alcoholic hepatitis. It should be differentiated from simple, uncomplicated steatosis, which, in most patients, follows a relatively benign course, and also from the steatosis with or without hepatitis produced by well-known causes such as hepatotoxic drugs, rapid weight lost, and gastrointestinal surgery, among others. It can be extremely difficult to reach the diagnosis of NASH in the absence of correct clinical data. The exclusion of excess alcohol intake is notoriously difficult and requires a combination of a good history supported by an appropriate laboratory investigation, such as desialylated transferrin (a method rarely present in laboratories). According to medical statistics nowadays and some of our conclusions formed in performing liver biopsies, we propose a methodological approach which can quickly guide us to the etiology of NAFLD. The aim of this study is a histomorphologic investigation of liver biopsies from patients with mild laboratory test abnormalities and steatosis complicated by necroinflammatory changes. A pilot study of morphometric characteristics of lipid droplets in NASH and alcoholic hepatitis was also performed.

MATERIALS AND METHODOLOGY

One pathologist (P.A.) blinded to patients' clinical condition and biochemical data, scored each biopsy on a scale 0-4 for steatosis, injury/ inflammation and fibrosis according to the criteria of Dixon (4). The diagnosis of NASH was based on steatosis and also on the following three factors: 1) Necroinflammatory foci with mononuclear cells and or/ neutrophils; 2) Ballooning of the hepatocytes with or without Mallory's hyaline, and 3) Pericellular fibrosis (5).

Four of these biopsies were morphometrically analysed regarding the lipid droplet features. The examinations were done using an Olympus BX 50 microscope. The microscopic images were digitalized by a SONY CCD - IRIS/ RGB camera, with the lens of 0.5h magnification. The digitalized signal was caught by the Olympus Micro Image 128 Capture Kit v.3.2. that is situated in a Pentium 266 MHz computer. We used the Olympus Micro Image - Image Analysis Software v.3.0.1. The microscopic images were analyzed as *TIF computer files. Three images of all biopsies were digitalized under the 40x objective. The separation of objects of interest was done automatically by assigning the range of colours in the field of interest. On the occasion of quantification of the features of lipid droplets, the following morphometric parameters were estimated: 1) diameter, 2) perimeter and 3) area (polygon). All values were compiled by the computer using appropriate software. The obtained values were statistically analysed using Student t-test.

RESULTS

Among 32 biopsies, 7 (22,5 %) had NASH, 5 were the result of adverse drug effects, 18 had alcoholic hepatitis and for two cases no relevant etiological factor was found. The mean age of patients was 34.7 years. F : M ratio was 1.5 :1 in NASH. According to the score system, the patients were classified into the groups 1 and 2, and there were none with marked fibrosis or cirrhosis. Among histomorphologic parameters, the presence and distribution of Mallory's hyalin was most variable. Morphometric analyses of lipid droplets showed statistically significant differences ($p < 0,05$) for all parameters tested in NASH and alcoholic hepatitis, and were more pronounced in the NASH group.

DISCUSSION

NAFLD comprises 7-9% of all liver biopsies in large series. According to relatively small number of cases that we had, these results confirm the opinion that relatively mild clinical symptoms aren't paid the required medical attention. Morphometric analyses confirm statistically significant differences of morphological presentation of steatosis in NASH and alcoholic hepatitis. As far as we know, these are the first results of this kind in this field.

CONCLUSION

In the cases of fatty changes, necro-inflammatory changes and pericellular fibrosis in the liver biopsy and the presence or absence of Mallory's hyalin, if the testing of desialylated transferrin is not possible, morphometric analysis of lipid droplets can help in reaching the definite diagnosis of NASH.

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Gastrotoxic effects of Indomethacin and Nimesulide in rats: Histopathological analysis

KEYWORDS: Non-steroidal anti-inflammatory drugs; Indomethacin; Nimesulide

INTRODUCTION

Indomethacin and nimesulide belong to the group of non-steroidal anti-inflammatory drugs (NSAIDs). These drugs inhibit the activity of cyclooxygenase (COX), a key enzyme involved in the synthesis of prostaglandins (PGs) which play an important role in the acute inflammation (1). There are two isoforms of COX: COX-1, a constitutive COX involved in the biosynthesis of the cytoprotective PGs in many organs, and COX-2, the so-called inducible COX that is activated in the inflammatory process and is included in the synthesis of proinflammatory PGs (2). Non-selective NSAIDs due to the inhibition of COX-1 activity may produce harmful effects in many organs, primarily in the stomach and kidney. Because of that gastrotoxicity and nephrotoxicity are their main adverse effects. Selective COX-2 inhibitors have lower incidence of these adverse effects (3). Gastrotoxicity is a consequence of an action of NSAIDs on normal gastric mucosal defence known as a "gastric mucosal barrier". PGs synthetised in the gastrointestinal tract are the most important factors included in this defence mechanism (4). The aim of this study was to establish the difference in the intensity of histopathological changes in rat gastric mucosa after a single administration of indomethacin, a non-selective COX-inhibitor of 'older" generation, and nimesulide, a selective COX-2 inhibitor, both given in an absolutely ulcerogenic dose (a dose causing any changes in gastric mucosa in all treated animals).

MATERIALS AND METHODS

The experiment was performed on the 18 male Wistar rats, weighing 200-250 g. All animals were divided into three groups: (1) control (DMSO 1 ml/kg *po*), (2) indomethacin (25 mg/kg *po*), and (3) nimesulide (25 mg/kg *po*). Indomethacin and nimesulide were dissolved in DMSO before administration. The animals were sacrificed at the 4h after the treatment. The stomach samples were fixed in 10% neutral formalin, dehydrated in graded alcohol, xylol and paraffin wax. The paraffin sections, 5 μm thick, were stained by hematoxylin and eosin (HE) methods.

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RESULTS

Histological analysis in all segments of the stomach of rats treated with indomethacin revealed diffuse epithelium deficit in the *tunica mucosa*. In these areas, epithelial and glandular cells were wholly necrotic. The majority of these ulcerations were enlarged to the *tunicae muscularis*. A large number of necrotic epithelial and glandular cells, neutrophile granulocytes, erythrocytes and fibrins, so-called "ulcerous fluid" were present at the bottom of the ulceration crypts filled with detritus consisted of necrotic gland cells. In the preserved parts of the stomach wall intensive edema, hyperemia and hemorrhage were found. Also, many glands with denuded basal membranes were present. The blood vessels of *laminae propriae tunicae mucosae*, *tunicae submucosae* and *tunicae muscularis* were congested with thickened walls, and near them a large number of mononuclear cell infiltrations was present, too. Histological analysis of the stomach of the animals from the third group (i.e. those treated with nimesulide) demonstrated the partial loss of epithelial cells of *laminae epithelialis tunicae mucosae*. On the surface of the changed epithelium detritus, composed mainly of desquamated epithelial cells, mucus and some neutrophile granulocytes, was present. Gastric glands were dilated with glandular detritus at the bottom. The blood vessels of *laminae propriae mucosae* were also dilated, but without thickening of the walls or cell infiltrations. In the control group all stomach samples had normal histological structure.

CONCLUSIONS

Our results demonstrate that single administration of nimesulide produces significantly less pronounced gastrotoxic effects than indomethacin given in the same dose. It means that selective COX-2 inhibitors are significantly weaker ulcerogenic agents than COX non-selective drugs.

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Novel therapeutic combination for the treatment of T-2 toxin poisoning in rats: A histopathological study

KEYWORDS: T-2 toxin, Poisoning, Therapy, Pathology

INTRODUCTION

Trichothecene mycotoxin, T-2 toxin, is one of the most important secondary metabolites produced by *Fusarium* fungi. T-2 toxin is a potent inhibitor of protein synthesis in eucaryotic cells, especially in tissues with high mitotic index. Its active metabolites are secreted into the bile and reabsorbed thus contributing to the overall toxicity of T-2 toxin. After po use, activated charcoal (AC), a well-known adsorbent, can bind the active T-2 toxin metabolites in the intestines, thus stopping their enterohepatic recirculation. Amifostine (WR-2721) is currently one of the most promising cytoprotectors, as a donor of SH-groups to enyzmes inhibited by T-2 toxin. Previous experiments with cytoprotector amifostine and AC administered separately showed good protective effects against histopathological alterations induced by T-2 toxin.The aim of this study was to investigate possible gastroenteroprotective effects of these antidotes in rats poisoned by T-2 toxin.

MATERIAL AND METHODS

Forty female Wistar rats 4-6 weeks old, weighing 200-250 g, were used in these experiments. The animals were poisoned with a single sc injection of 1 LD_{50} of T-2 toxin (0.18 mg/kg). The rats were randomly allocated to five groups (n=8). Their treatments were: (1) control group, (2) T-2 toxin 0.18 mg/kg *sc*, (3) T-2 toxin and AC 1 g/kg *po*, (4) T-2 toxin and amifostine 50 mg/kg *im* and (5) T-2 toxin, AC 1 g/kg *po* and amifostine 50 mg/kg *im*. T-2 toxin that was used in these experiments was produced in laboratory conditions from *Fusarium sporotrichoides* fungi. T-2 toxin was preliminarily tested in animals in order to obtain its LD_{50} value. It was thereafter used in the current experiment in a single dose of 0.18 mg/kg *sc*. The animals were sacrificed after the end of the day 1, 3, 5 and 7 of the study. The gut samples were fixed in 10% neutral formalin for 15 days. After the process of fixation they were dehydrated in graded alcohol, xylol and paraffin wax. Finally, 2 μm thick paraffin sections were stained by hematoxylin and eosin (HE) and periodic acid-Schiff's (PAS) methods.

RESULTS

In the gastrointestinal tract T-2 toxin caused diffuse epithelium deficit, erosions, ulcerations, hyperaemia, transmural oedema, atrophy of intestinal villi and cystic deformation of the stomach and small intestine glands. The most intensive changes were seen in *tunica mucosa* of ileum that covers the lymphatic tissue of rats sacrificed on the day 7 after the treatment. The epithelial cells of villi intestinales were totally desquamated. Atrophic villi intestinales were rounded, finger-like, dendritic or papillomatous. In these areas Lieberkühn's crypts were enlarged and filled with necrotic glandular cells debris. Depletion of lymphocytes was found in the lymphoid follicles of the Payer's patches. Macroscopic examination of T-2 toxin poisoned rats protected with AC and amifostine, showed segmental epithelium deficit of *lamina epithelialis tunicae mucosae*. Some villi intestinales of the small intestine were rounded and finger-like. A small number of the Lieberk¸hn's crypts was enlarged and filled with mucus fluid and desquamated cells. These changes were only seen in the group of animals sacrificed on the day 7 after the treatment.. They were not present in the small intestine of the poisoned animals protected with AC and amifostine. All segments of the intestinal wall were normal. The most interesting and the most intensive histopathological findings were present in a base of the intestinal glands. The majority of these glands contained mitotic cells. Single macrophages were seen in *lamina propria tunicae mucosae*.

CONCLUSIONS

These results imply that AC, as an absorbent of the active T-2 toxin metabolites, that are subject to enterohepatic recirculation and amifostine, a sulfhydryl group donor, afford a significant protection against T-2 toxin poisoning in rats.

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Ewing's sarcoma presenting as a solitary bone cyst

KEYWORDS: Ewing's sarcoma; Solitary bone cyst; Pathology; Case report

INTRODUCTION

Ewing's sarcoma is the fourth most common primary malignant bone tumor. It is a highly malignant small cell neoplasm, referred as Ewing's sarcoma since 1929, and was first recognized by Lucke in 1866. The tumor is most often solid, gray-white moist and glistening. The solitary bone cyst is a benign lesion and represents a unilocular intramedulary cavity filled with clear or sanguineous fluid. It is lined by a fibrovascular connective tissue membrane. It is quite unusual for Ewing's sarcoma to present itself as a solitary bone cyst and there are only few reports, in the English language literature, describing these two lesions in an association. We report a case of Ewing's sarcoma presenting as a cystic lesion in the proximal tibia of a 14 years old boy.

CASE REPORT

A 14 years old boy has been feeling pain and cracking sensation during the last 6 months, especially while playing football. A radioloculent lesion was noted on plain radiographs and the boy was treated with analgesics and immobilization. Six months later, he was brought to the Clinic of Orthopedic Surgery because of the existing pain, becoming more and more severe. Another radiograph revealed an elliptical lytic lesion, centrally located in the proximal part of the tibia. There was sclerosis. The only remarkable sign was a suspect moth-eaten pattern of bone destruction, and a discrete onionskin periosteal reaction at the level of the distal part of the cyst. A CT and scintigraphy were made and an open biopsy was performed in order to get the definite diagnosis. On CT, the cortex was thinner in the central part of the cyst and there was an irregular cortical destruction. The bone scan showed a focus of increased isotope uptake at the site of the lesion. During the surgical procedure a cystic lesion was found filled with sanguineous fluid. There was a small thickening in the distal part of the cystic wall, containing a gray - white friable tissue. It was thoroughly curetted and sent for microscopic examination. The histological analysis revealed broad sheets and large nests of uniform small round cells with scanty cytoplasm and indistinct cell borders. The cells were PAS and MIC2 positive and reticulin fibers were absent, so the diagnosis of Ewing's sarcoma was established. The histological slides were reviewed at AK Hospital in Wienna in order to get another opinion, which was identical with ours. The chest X-ray finding was normal, with no evidence of pulmonary metastasis. At present, the boy is under chemo treatment expecting a planned surgery.

DISCUSSION

It is well known that textbooks of orthopedic surgery do not include Ewing's sarcoma in the differential diagnosis of solitary bone cysts, or vice versa, but there are few reports of bone cysts associated with Ewing's sarcoma and other malignacies. Proximal humerus is the site more typical for solitary bone cysts while the tibia is more likely to be involved by Ewing's sarcoma. The reported patient had the lesion in the site more typical for Ewing's sarcoma. According to the literature, about 1% of solitary bone cysts may show irregular cortical destruction and 2% show a moth-eaten pattern of bone destruction, as it was in our case. Ewing's sarcoma is presented by benign radiographic features in less than 2% of the cases and with sharp endosteal margins in 10%. The cyst-like presentation of Ewing's sarcoma has previously been described in a few cases and this report is intended to turn the attention to this association in order not to misdiagnose a malignant bone tumor.

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Atypical apocrine cells in fine-needle aspirates of the breast

KEYWORDS: Breast neoplasms; Apocrine carcinoma; FNAC; Differential diagnosis

INTRODUCTION

Metaplastic apocrine cells are commonly present in fine-needle aspirates (FNA) of solid and cystic lesions in fibrocystic breasts. The nuclear/cytoplasmic ratio (N/C) is low and the cells are generally large due to abundant granular cytoplasm. Typically, the nuclei are eccentric with smooth round borders, uniformly distributed chromatin and single uniform macronucleoli (1). Occasionally, the cytologic features of apocrine cells are atypical and may present diagnostic difficulties (2). Although apocrine carcinoma of the breast is a relatively rare occurrence, it must be a diagnostic consideration when apocrine cells display markedly atypical features (3,4). The present study was undertaken to determine the significance of atypical apocrine cells occurring in breast aspirates. The cytologic features of atypical apocrine cells identified in three breast FNA suspected for apocrine carcinoma were evaluated and confirmed on tissue specimens by tru-cut and excisional biopsies of the same cases.

MATERIALS AND METHODS

Cytologic preparations from three FNAs of female breast, done at Guy's Hospital London, between 1991-93, were identified that contained atypical apocrine cells suspicious of carcinoma. FNAs were obtained using 23 gauge needle. The aspirates were then spread onto slides and rapidly air-dried. The staining was performed by Papanicolaou and MGG technique. There were usually at least two slides from each patient. The tissue specimens obtained by tru-cut and excisional biopsies were submitted for routine diagnosis and stained with hematoxylin eosin. The examined cytologic features included: the overall cellularity, the size of apocrine cells and their nuclei (compared with the size of a red blood cell), the degree of cellular and nuclear pleomorphism, the cytoplasm and cell border appearance, the N/C ratio, nuclear outline, chromatin pattern and nucleoli.

RESULTS

The atypical apocrine cells were present in cohesive groups accompanied by variable numbers of single atypical apocrine cells. Cytopathologic features of atypical apocrine cells identified in three breast FNAs are reported in Table 1.

Table 1. Cytopathologic features

Case No	Cell Size*	N/S ratio	Nuclear size*	Nuclear outline	Chromatin pattern	Cell.&nucl. pleomorph.	Nucleoli	Histologic diagnosis
1.	4-8	<1/2	3-4	round	granular	minimal	usually single	atypical apocrine metaplasia
2.	<20	<1/2	≤8	very irregular	very irregular	marked	multiple irregular	apocrine carcinoma
3.	8-16	1/2	6-8	irregular	irregular, granular	moderate	multiple irregular	apocrine carcinoma

*Times the size of red blood cell

The subsequent histologic examination of tru-cut and excisional biopsies demonstrated apocrine carcinoma in two patients and atypical apocrine metaplasia in one patient (case1).

DISCUSSION

Diagnostic criteria for apocrine carcinoma in breast FNA include a highly cellular specimen composed predominantly of atypical apocrine cells occurring in variably cohesive groups and as single cells. The features that indicate apocrine differentiation are abundant granular cytoplasm and well defined cell borders. Both the cells and the nuclei should be enlarged and pleomorphic. The nuclei may have irregular borders and typically have irregularly distributed chromatin. The nucleoli are prominent and vary in number and configuration (3). Histologically, apocrine breast cancers are composed of large eosinophilic cells with abundant granular cytoplasm, prominent nucleoli and apical snouts (4,5). Although apocrine carcinomas are usually classified as ductal neoplasms, apocrine differentiation may be associated with lobular, medullary, or adenoid cystic breast cancers, and it was realized that this type of differentiation is detectable both in in situ and in invasive carcinomas (4).

CONCLUSION

The diagnostically important features are: (1) atypical apocrine cells as the only or the predominant cell type; (2) atypical apocrine cells occurring in variably cohesive groups and singly; (3) cellular and nuclear enlargement and plemorphism; (4) irregular chromatin distribution; and (5) multiple pleomorphic nucleoli. When these features are present in cellularly adequate FNAs of breast, apocrine carcinoma should be suspected.

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Histological degrees and distribution of inflammatory processes of placentas

KEYWORDS: Placenta; Inflammation; Grading, Pathology

INTRODUCTION

The histopathological diagnosis of placental inflammation is very important because it is associated with an increased risk of perinatal morbidity and mortality and it primarily depends on the presence of neutrophilic infiltration of the placenta, membrane, and umbilical cord. Current available data describe that there is a correlation between placental inflammation, represented by chrioamniotis, and amniotic fluid of placental microbiology. The actual incidence and clinicopathologic significance of acute placental inflammation in pregnancies have not, however, been fully documented.

MATERIALS AND METHODS

A total of 20 placentas were randomly selected at the Institute of Pathology, Medical Faculty of Skopje in 2001 year. The cases were divided into 3 groups:1) term placentas with vaginal delivery [group I; n=6] 2) preterm placentas with vaginal delivery [group II; n= 1] 3) abortive material [group III; n= 13]. After gross examination, the placentas were fixed in 10% neutral formalin with the entire portion of membrane fixed in rolls. Formalin-fixed placentas were routinely processed and stained with hematoxylin- eosin. After the light microscopic examination for the presence of neutrophilic reaction in the amnion, chorion- decidua, chorinic plate, and umbilical cord, the degree of inflammation was graded according to the criteria used by Shurin et al.(6): 0 = no infiltrates; 1+ = average one to three leukocytes per high power field (hpf); 2+ = mild- four to 15 leukocytes per hpf; 3+ = moderate to severe - more than 15 leukocytes per hpf. The types of inflammation were divided into deciduitis, chorioamnionitis and funisitis, intervilitis and villitis.

RESULTS

Among 20 cases analyzed, acute chorioamnionitis was the most common inflammatory feature and was found in 20 cases (100%), while deciduitis was present in 12 (60%), villitis 3 (15%), intervillitis 4 (20%), funisitis 2 (10%). In group I all had chorioamnionitis, deciduitis was found in five cases, two had intervillitis and 1 villitis. In group II that comprised only one case, there was chorioamnionitis. In group III all had chorioamnionitis, deciduitis was present in 6 cases and villitis in 2 cases. The degree of inflammation was graded according to the criteria used by Shurin et al.: 0 - no such cases in our study; 1+ - no cases; 2+ - nine cases; 3+ - eleven cases. Conatal pneumonia as a complication in addition to the inflammatory process of the placenta was found in 4 cases whereas in 2 cases there was funisitis.

DISCUSSION

The accurate and timely diagnosis of acute placental inflammation has extreme clinicopathologic significance because it is known to be associated with intrauterine infection which usually occurs mainly via an ascending route. This type of infection is one of the major risk factors of preterm delivery, perinatal morbidity and mortality.

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Immunofluorescent studies on formalin-fixed paraffin-embedded renal biopsies

KEYWORDS: Kidney; Immunofluorescence; Pathology

INTRODUCTION

Renal biopsy is of paramount importance in clinical nephrology. Light microscopy, immunofluorescence (IF) and immunohistochemistry analyses of the renal specimens, as well as electron microscopy evaluation for selected cases, have become integral part of the efforts of every diagnostic center. The diagnostic techniques and algorithms have been developing constantly and the results have contributed to the management of the patients with renal disease, clarification of the etiology, pathogenetic mechanisms and prognosis of certain entities. The problems of adequate sampling and handling of the kidney biopsy specimens, as well as the necessity of further investigations and reproducibility of the sections, have implied the necessity of developing alternative methods of tissue preparation and staining. The aims of this study were to summarize the results from the light microscopy (both on standard and semi-thin cuts) and IF analyses and, secondly, to make qualitative comparison of IF signals on formalin-fixed paraffin-embedded kidney sections and on frozen sections from the same kidneys.

MATERIAL AND METHODS

We present a series of 50 renal biopsies performed at the Institute of Pathology in the period from November 2000 to February 2002, obtained from the Clinic of Nephrology. The series consisted of biopsy specimens from 32 male and 18 female patients. Most of the patients were adult (44) and only 6 were pediatric patients. The obtained needle biopsies were divided into 3 parts: one for frozen section analysis, one for histological procedure (including IF analyses) and the third part for electron microscopy. The frozen section analyses were performed at the Clinic for Nephrology and we used their results in our comparative study. Other two procedures were done at the Institute of Pathology.The specimens taken for histological analyses were manually proceeded through shortened fixation procedure in formaldehyde, and stained with the standard histochemical panel: HeEo, PAS, Trichrom - Goldner, Silver-methenamine Jones and Congo-red (where indicated).The sections intended for IF study were applied to silanized slides, underwent enzymatic digestion of the tissue with pronase and were incubated with the following fluorescent antisera: IgA, IgG, IgM, C1q, C3, fibrinogen, kappa and lambda light chains (the last two were used only where indicated). The biopsy specimens taken for electron microscopy analysis were fixed in glutaraldehyde, embedded in DURCUPAN and proceeded to semi-thin sections, which were analyzed after toluidine blue staining on light microscope under immersion.

RESULTS

In our study, a total of 15 histopathological entities affecting different parts of the kidney were diagnosed, and the distribution of the cases among them is shown in the table below. A special interest of our study was the congruence between the IF analyses done on frozen section and on formalin-fixed paraffin-embedded tissue sections. A total of 7 were excluded from the comparative analysis due to unsuccessful stainings (3 frozen sections and 4 paraffin-embedded tissue sections) and for 10 we did not have access to the respective clinical (hence frozen section results) history.

Table 1. Histopathological fitures

Renal compartment, No. / %	Histopathological diagnosis	No. of cases
Glomerular diseases 25 (50%)	Membranous	7
	Membranoproliferative	4
	Focal segmental	4
	End stage	3
	Extracapillary	2
	Minimal change	2
	Rapidly progressive	1
	Resolving	1
	IgA nephropathy	1
Tubulointerstitial diseases 3 (6%)	Acute tubulointerstitial nephritis	3
Vascular diseases 4 (8%)	Nephrosclerosis	4
Systemic diseases affecting kidney 8 (16%)	Amyloidosis	4
	Lupus erythematosus	2
	Diabetes mellitus	2
Tubulointerstitial and glomerular affection 3 (6%)	Chronic glomerulonephritis and acute tubulointerstitial nephritis	3
Description / unclassified 7 (14%)		7

The reminder of 33 biopsies were qualitatively compared for the results between both methods of IF: 19 (58%) had identical findings on all slides stained with different antisera, 11 (33%) showed only slight discrepancies between the results of both methods and the rest 3 cases (9%) had completely different findings. The detailed comparison of the cases with certain discrepancies between the results of both methods revealed that they were partially due to the differences in the number of present glomeruli and surrounding tissue (different parts of the kidney biopsy specimens were taken for both methods). Most of them were due to discrepancies in stainings for complement fractions (both C1q and C3), mostly giving negative results on paraffin-embedded tissue sections. Further analyses of the last 3 cases revealed focal distribution of the lesions in the affected kidneys, which explained the incongruent results of the two methods in these cases.

DISCUSSION

IF analyses with formalin-fixed paraffin-embedded tissue give many opportunities not possible with frozen sections. One of the main advantages is the possibility of using the same tissue for light and IF analyses, hence avoiding different fragments of the kidney biopsy being used for different methods. The tissue morphology preservation is much better in paraffin-embedded tissue and further sections and analyses are reproducible even after many years. Tissue antigenicity is preserved for long periods of time. On the other hand, the sensitivity of the paraffin-embedded tissue is lower for complement fractions, which was the case in our series as well. Therefore, even though IF analyses performed on frozen sections still represent the gold standard, the above described method of IF performed on formalin-fixed paraffin-embedded renal biopsies is an alternative and helpful tool to rely on when

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frozen material is not available due to the scarcity of core biopsy, when glomeruli are absent in frozen sections, or the frozen tissue is destructed .

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Concomitant presence of malignant melanoma and adenocarcinoma in the rectum: A case report

KEYWORDS: Melanoma; Colorectal neoplasms; Multiple primary neoplasms; Pathology

INTRODUCTION

Sinchronous or metachronous development of carcinomas in various organs is a characteristic of many hereditary syndromes (1). In colorectal cancer pathology, the focus of many researches represents Hereditary Non-Polyposis Colorectal Cancer (HNPCC). The data indicate that Malignant Melanoma (MM) patients may be at higher risk for having a noncutaneous invasive cancer compared with the general population (2,3). But, none of the variables of MM, such as the location of the primary tumor and the level of invasion or stage, were predictive for the second cancer (2).

CASE REPORT

Having in mind that survival of MM patients with a concomitant second primary cancer is worse, we have decided to report our patient who has been living four years after two operations. It is a 49 years old male with MM on the skin in the femoral part of his right leg. Striking variations in pigmentation appearing in shades of black, red dark and gray were observed. Irregularity of the borders of the pigmented lesion with macular and raised areas corresponding to nodular aggregates of malignant cells in the vertical phase of growth; the descending of these cells into the reticular dermis was marked. In contrast to high nuclear polymorphism and mitotic index, any nodal metastasis wasn't discovered (TNM=T3N0M0). Both nodular and diffuse dense arrangements of B lymphocytes occurred peri-tumorally. MM was removed by a surgical resection. The cancer in the rectum, histologically established as adenocarcinoma (G2) was resected after one month (TNM=T2N0M0). Now, four years after two operations, the patient is alive and in good health.

DISCUSSION

The development of multiple primary cancers of hereditary genesis is described within Lynch-II, Muir-Torre and Gardner syndromes (1). Extracolonic lesions include endometrial, breast, ovarian and gastric cancer,

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small intestinal cancer, transitional cell carcinomas of the pelviureter, glial neoplasms of the central nervous system and sebaceous adenoma (1). However, HNPCCs show at least a 60% predilection of the proximal colon including coecum, ascending colon, hepatic flexure and transverse colon. Our patient had the cancer in the rectum in the absence of the family history. Tumor Infiltrating Lymphocytes (TIL) may be evident in adenocarcinoma: and the majority of tumor infiltrating (intra-epithelial) lymphocytes are CD3+ T cells and most in turn are cytotoxic (CD8+) T cells. The heightened immune response observed in cancers has been associated with the improved survival rates (1). Finally, we suggest that, in addition to the tumor thickness (4), the host immune response to the tumor must also be included into a revised melanoma staging system..

CONCLUSION

Patients with MM may be at a higher risk for having extracutaneous cancer. The risk of the second cancer in the absence of the family history in MM patients is consistent with a polygenic model of carcinogenesis. A good clinical outcome is based on the good host immune response.

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Ovarian Sertoli-Leydig cell tumor. Case report

KEYWORDS: Ovarian neoplasms; Sertoli cell tumor, Leydig cell tumor, Pathology

INTRODUCTION

We report a case of the Sertoli-Leydig cell (SLCT) tumor in an old female (66 years of age) with virilization symptoms (deepening of the voice, facial acne, temporal recession of hair and hirsutism). She was subjected to a surgical procedure including unilateral salpingoophorectomy. Macroscopically, the tumor was hard, solid, golden-yellow in appearance, weighing 650gr. Microscopically, the well-differentiated tumor contained tubules composed of Sertolitroll cells and Leydig cells interspersed with stroma. The IA staged tumor was confined to the right ovary. Fourteen months after the surgery, the patient is still free of tumor and hirsutism.

CASE REPORT

The woman, 66 years of age with virilization symptoms (deepening of the voice, facial acne, temporal recession of hair and hirsutism) and with abdominal pain was subjected to a surgical procedure including unilateral salpingoophorectomy. Formaldehyde-fixed sections from the tumor were processed to paraffin wax cut at 4(m. The sections were stained with HE, PAS and van Gieson techniques.

RESULTS

Macroscopically, encapsulated tumor had golden-yellow appearance, hard consistency, solid structure and the weight of 650 gr. Microscopically, the well-differentiated tumor exhibited cords, trabecula and tubules with the characteristics of Sertoli-Leydig cell differentiation in different areas interspersed with stroma. Scattered islands of large cells with abundant eosinophilic granular cytoplasm with central round nuclei (Leydig cells), as well as isolated Reinke crystals were found everywhere. The tumor was confined to the right ovary, at the stage IA. A conservative surgery has appeared to be the treatment of choice, since the patient is still free of tumor and virilization symptoms, 14 months after the surgical removal.

DISCUSSION AND CONCLUSION

SLCTs are rare sex-cord stromal tumors of the ovary composed of undif-

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ferentiated gonodal stromal cells, Leydig cells and Sertoli cells, with the latter forming structures resembling fetal testicular tubules. The histogenetic basis of morphological male differentiation patterns in females is controversial (1). The morphological appearance of these tumors varies more widely than that of any other ovarian tumor except for the teratomas. Histologically they are now classified into 5 categories: well-differentiated, intermediately differentiated, poorly differentiated, heterologous and retiform (2). We have concluded that these tumors have a good course and favorable prognosis when confined to the ovaries.

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Acquired middle ear cholesteatoma: Clinical and histopathologic features

KEYWORDS: Cholesteatoma; Pathology; Diagnosis

INTRODUCTION

Cholesteatoma is a sac of stratified squamous keratinized epithelium filled with accumulated keratin growing in the foreign place - in the middle ear cavity or other pnumatized regions of the petrous bone. The simple term is "the skin in the wrong place". It is characterized by progressive hyperproliferative locally invasive growth and migration of keratinized squamous epithelium in the middle ear cavity with keratin debris accumulation and bone resorbtion. This lesion is clinically manifested by otorrhea, hearing loss, tinnitus, dizziness, earache or facial nerve paralysis. Cholesteatoma is composed of the three components: cystic content of desquamated keratin, matrix and perimatrix or lamina propria (1). This lesion is the excellent medium for bacterial growth. By continuing and permanent antigene stimulation the chronic inflammation is developed, resulting in activation of local immune reaction with accumulation of inflammatory cells, hyperproliferation of cholestatoma matrix keratinocytes and increased production of keratin. The aim of the study is to investigate the influence of a bacterial infection onto the appearance of a cellular infiltrate in cholesteatoma perimatrix or in the submucosal tissue, and to determine whether the cellular infiltrate belongs to the immune reaction of the cholesteatoma tissue, or it is the result of the secondary bacterial infection. By standardized pathohistological analysis in 22 specimens of middle ear cholesteatoma the degree of inflammatory reaction and cellular infiltrate in the cholesteatoma perimatrix was studied and compared to the existence of clinically manifested secondary bacterial infection of the middle ear by otomicroscopical examination or by surgery.

RESULTS

The secondary infection was absent in 7 patients, while it was clinically manifested as middle ear secretion, granulations, polyp or wet macerated cholesteatomatous debris in 15 patients. Histopathologically, cholesteatoma was confermed in all patients. Of the group with the secondary infection, a mild inflammatory reaction was found in 4 patients, while in 11 patients there

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was a pronounced inflammatory infiltrate. In patients without a clinically manifested infection, a mild inflammatory reaction was confermed in 5 patients while pronounced inflammatory infiltrate was noted in 2 patients.

DISCUSSION AND CONCLUSION

Bacterial infection, chronic inflammation and local immune reaction are responsible for the hyperproliferative behaviour of the cholesteatoma resulting in disturbed interaction between the matrix and perimatrix cells with increased production of some citokines and growth factors leading to enhanced degradation of extracellular matrix components and production of proteolitic enzymes (3,4). Inflammatory reaction in chronic otitis media with cholesteatoma is very similar to the cell-mediated immune response with predominating mononuclear inflammatory cells. The cellular infiltrate in the lamina propria is mostly composed of T cells, plasma cells, macrophages, fibroblasts and less frequently of mast cells. In the cholesteatoma epithelium the increased number of the Langerhans cells was found. Some investigators have showed that the inflammation with or without infection is the main factor in the bone resorbtion, but cholesteatoma epithelium can directly or indirectly enhance the bone resorbtion in the chronic otitis media with cholesteatoma (5). The presence of the immunologically active immune cells could be responsible for the induction and maintanance of the disturbed growth of cholesteatoma epithelium. But the cholesteatoma epithelium with keratin can at the same time provoke the inflammatory reaction. We found a more intense cellular infiltrate in the patients with the bacterial infection. However, a pronounced inflammatory reaction in the absence of a bacterial infection was noted, indicating that the cholesteatoma epithelium induced the immune response of the host, with the cascade sequence of cellular and molecular events leading to the agressive growth of the cholesteatoma with the bone destruction. In the conclusion, it is very important to examine the cholesteatoma tissue pathohistologically and to prove the presence of an inflammatory infilitrate in the perimatrix. as well as to determine whether it is the result of the immune reaction, or the secondary infection, or inflammation. Further detailed studies by immunocytochemical and immunohistochemical essays are necessary in order to explain better the invasive behaviour and reccurrence potential of the acquired middle ear cholesteatoma.

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Reactivity of aortocoronary grafts (human saphenous vein and human internal mammary artery) to pharmcological agents

KEYWORDS: Aortocoronary grafts; Pharmacological agents

Spasm of arterial and venous bypass grafts can occur both during harvesting and after the graft is connected (1). To select the best pharmacological agent to prevent or reverse vasoconstriction in a graft requires an understanding of the reactivity of that particular type of graft to vasoconstrictor and vasodilator agents. The pharmacological reactivity of bypass grafts has been documented through extensive studies of isolated vessels in the organ bath. In this review we summarize the current state of knowledge of the reactivity of arterial and venous grafts to vasoconstrictor and vasodilator agents. The mechanism of graft spasm is still unclear. In general, vasospasm could be the response of a vessel to many stimulants. These stimulants may be physical (mechanical stimulation or temperature changes) or pharmacological (nerve stimulation or exogenous and endogenous vasoconstrictor substances). There are basically two types of vasoconstrictors that are important spasmogens in arterial grafts. Type I (endothelin-1 (ET-1), thromboxane A_2 (TxA_2), prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and α-adrenoceptor agonists) are the most potent and strong vasoconstrictors. They strongly contract arterial and vein grafts even when endothelium is intact (2). Elevated plasma levels of ET-1 or TxA_2 have been found during cardiopulmonary bypass (1,2). Increased ET-1 levels may result in direct constriction of the venous smooth muscle, or may potentiate the constricting effects of other mediators such as 5-hydroxytryptamine (5-HT) (3). Therefore, these two vasoconstrictors are prime candidates as spasmogenic agents for arterial grafts during CABG surgery (1). $PGF_2\alpha$ has been suggested to contract smooth muscle through FP receptors (2). This prostaglandin is strong but less potent vasoconstrictor than ET-1 or TxA_2 in the HIMA. This can be reflected by a significantly higher half maximum effective dose (EC50) for this vasoconstrictor than for ET-1 or TxA_2 in the HIMA (2). Noradrenaline, nonselective adrenoceptor agonist, phenylephrine and methoxamine, α1-adrenoceptor agonists, induced concentration-dependent contraction in HIMA and HSV (2). The HIMA has been shown to be an α1-

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adrenoceptor predominant vessel. Therefore, circulating catecholamines may contract the artery mainly through an α1-adrenoceptor mechanism (1,4). In contrast, in HSV α-adrenoceptor vasoconstriction is caused by α1- and α2-receptors (4). The sensitivity of this vasoconstrictor is lower than either ET-1 or U46619 (stable TxA_2 mimetics) in the HIMA (2). Type II vasoconstrictors, such as 5-HT, only induce a weak vasoconstriction when endothelium is intact. However, those vasoconstrictors probably play an important role in vasoconstriction and spasm of arterial grafts if endothelium is lost by surgical handling (2). In the HIMA histamine induced relaxations in rings with but not without endothelium. In contrast, in HSV, histamine caused only weak or absent endothelium-dependent relaxations. The reason for that might be the fact that production of nitric oxide and prostacyclin is lower in veins than arteries (5). Today in the aim of prevention and reversion of the graft spasm, various vasodilator agents such as papaverine, nitrovasodilators, and calcium antagonists are used. Phosphodiesterase inhibitors, TxA_2-antagonists, and potassium channel openers (PCOs) should be considered too as potential new vasodilator agents. The response to some dilator agents depends on the nature of vasoconstriction that is mediated through receptors or ion channels. This is particularly important in the case of calcium antagonists. When the HIMA is contracted by depolarising agent such as K^+, nifedipine or other calcium-channel antagonist are very effective in either preventing or reducing the contraction. However, in the case of contraction mediated by membrane receptors, such as ET-1-, TxA_2-, or a-receptors, calcium antagonists such as nifedipine are less effective. The effect of vasodilator may depend on whether it is given before or after a vasoconstrictor. Some dilator agents are ineffective if applied before the constrictor stimulus but will be effective if applied to already contracted vessels. This is especially important for glyceryl trinitrate (GTN) in the HIMA. Here, GTN may effectively reverse an already established contraction, but it has little efficacy to prevent contraction if used before the contraction is initiated, by K^+ or ET-1-, TxA2-, or α-agonists (1). A large body of evidence in vitro is available showing that pretreatment with various PCOs (levcromakalim, cromakalim, KRN4884) depresses significantly the maximal contractile response to potential spasmogens, such as noradrenaline, U46619, serotonin, angiotensin II, but does not affect those of ET-1 and K^+ in isolated human blood vessels used as bypass grafts (6). Precontracted vein rings showed a concentration-dependent relaxation to all PCOs tested (7). Therefore, these results provide evidence that PCOs may have therapeutic value in the prevention of spasm of bypass graft. Further on, short review of our prior results will be presented. Our study was designed to examine the response of the HSV and HIMA without endothelium to serotonin, histamine, and phenylephrine as provocation agents of vasoconstriction. These agents caused concentration-dependent contractions in all vessels tested. The EC_{50} values for serotonin, histamine and phenylephrine of HSV were as follows: $3.66 \pm 0.21 \times 10^{-6}$, $4.44 \pm 0.12 \times 10^{-5}$ and $5.9 \pm 0.25 \times 10^{-6}$. In HIMA EC_{50} values for the same vasoconstrictors were: $4.52 \pm 0.23 \times 10^{-6}$, $6.18 \pm 0.10 \times 10^{-6}$ and $4.27 \pm 0.27 \times 10^{-6}$. In HSV segments, the order of sensitivity to the substances expressed by their EC_{50} values was: serotonin = phenylephrine > histamine ($p<0.05$). The HIMA had equal sensitivity to serotonin, histamine and phenylephrine ($p>0.05$). Compared with HIMA, HSV showed decreased sensitivity to histamine ($p<0.05$) (8).

In the second study we investigated the effects of pinacidil and levcromakalim, two PCOs, on HIMA obtained from patients undergoing CABG. Pinacidil and levcromakalim induced a concentration-dependent relaxation of the precontracted arterial segments ($pEC50 = 5.77 \pm 0.05$ and 6.89 ± 0.03, respectively). The difference between the pEC50 values was statistically significant ($P<0.01$). In the HIMA, levcromakalim was 13 times more potent than pinacidil (9). These results could be very useful for removing perioperative and postoperative vasospasm of venous and arterial aortocoronary bypass grafts.

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