GASTROINTESTINAL SYSTEM PATHOLOGY

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Islet amyloid polypeptide (IAPP, amylin) in dexamethasone treated rats

ABSTRACT

Amyloid deposits are present in more than 80% of individuals with type 2 diabetes and can be used as a marker for non-insulin-dependent diabetes mellitus (NIDDM). Dexamethasone is a higly potent glucocorticoid. Glucocorticoid treatment results in several metabolic perturbations, many of which are also found in type 2 diabetes. The aim of this study was to investigate by immunocytochemistry possible changes in the amylin deposits in the islets of Langerhans in rats treated with dexamethasone. Male Wistar rats were treated with 2mg/kg dexamethasone intraperitonealy during 12 days. The control group received an equal quantity of saline. Bouin fixed and paraffin embedded specimens were cut to 5 μm thickness and mounted on chrome-alum coated slides. Peroxydase- antiperoxydase (PAP) method was used for determination of amylin in the islets of Langerhans. Standard stereological equations were used to calculate volume density and area of islets of Langerhans. The results obtained in this study revealed that all islets of Langerhans had strong immunopositivity of amylin. This strong reaction was especially observed only in the central parts of the islets. In the controls, strong immunopositivity was observed in several cells at the periphery of the islets. The result indicated that strong immunopositivity of amylin in the rats treated with dexamethasone was the results of accumulation of amyloid deposits in the b cell.

KEYWORDS: Dexamethasone; Amyloid; Islets of Langerhans

INTRODUCTION

Islet amyloid polypeptide (IAPP, amylin), consisting of 37 amino acid residues, is the major protein component of amyloid deposits found in human insulinoma, pancreatic islets of the diabetic cat, and pancreatic islet of type 2 diabetic patients (1,2). Secretion of IAPP occurs together with insulin from the same β cell secretory granules of the pancreatic islets (3). Dexamethasone is a highly potent glucocorticoid. Glucocorticoid treatment results in several metabolic perturbation, many of which are also found in type 2 diabetes (4). The aim of this study was to investigate possible changes in the islet of Langerhans and immunocytochemically detection of amylin in rats treated with dexamethasone.

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MATERIALS AND METHODS

Twenty male Wistar rats, 2 months old, were randomly divided into two groups of ten rats each.The rats were injected daily with 2 mg /kg of dexamethasone intraperitoneally (group E), the control group received an equal quantity of saline (group C). The experiment lasted for 12 days. The rats were fasted owernight and killed by the exposure to dietylether, after wards the specimens from the pancreas were promptly excised and weighed in the air. For immunocytochemistry, the specimens were fixed in Bouin's solution, dehydrated and embedded in paraffin. Sections were cut to 5 μm thickness and peroxydase-antyperoxydase (PAP) method was used for determination of amylin in the β cells. Standard stereological equatations were used to calculate the volume density, total volume and the area of islets of Langerhans.

RESULTS

Strong immunocytochemical reaction of amylin was observed in all islets of Langerhans in dexamethasone treated rats, involving not only β cells, but also almost all cells in the islets. In the controls, strong immunopositivity of amylin was not found, except in some cells at the periphery of islets. The clear ring of non β cell was observed in control rats, contrary to the E group of rats. Namely, in the E group of rats, only few non β cells could be observed. Stereological investigations showed that volume density of the islets was significantlly increased (0.005±0.0005 mm0 in controls, v.s 0.011±0.0014 mm0 in experimental rats, p (0.001), as well as the area of islets of Langerhans (5931±934 mm2 in controls v.s. 9377 ± 910 mm^2 in experimental rats, $p<0.01$).

DISCUSSION

In the rat, amylin is secreted from the β cells together with insulin, in a molar basis at a rate approximately 1-5% of the insulin one (5). Weak immunocytochemical reaction of amylin was found in all β cells of control rats and proved that those endocrine cells normaly cosecrete amylin with insulin. Our immunocytochemical and stereological investigations showed that dexamethasone caused hypertrophy of islets of Langerhans due to hypertrophy and hyperplasia of β cells. This is in accordance with earlier literature data that dexamethasone increased the area of b cells, as well as hyperplasia of the islet cells (6,7).

CONCLUSION

Our results also revealed that almost all islet endocrine cells showed immunoreactivity of amylin suggesting that the other non β cells were decreased in number.

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Pathohistological changes in the small and large intestine of rats acutely intoxicated with T-2 toxin

ABSTRACT

Trichotecene mycotoxin, T-2 toxin, is one of the most cytotoxic fungal metabolites produced by Fusarium species, such as: F.trincictum, F. poae, F. sporotrichoides and F. roseum. T-2 toxin can causes damage to the cells of intestinal crypt epithelium and lymphoid tissues. Female Wistar rats were included in the study and pathohistological analysis was based on the haematoxilin and eosin (HE) and periodic acid-Schiffës (PAS) methods. In a small intestine of female Wistar rats 1LD-50 T-2 toxin (0,18 mg/kg sc) causes a diffuse epithelium deficit, hyperemia, transmural edema, atrophy of villi intestinales and cystic deformation of small intestine glands. Described changes were the most intensive on tunica mucosa of ileum which covers lymphatic tissue of rats sacrificed third on fifth day of application by T-2 toxin. Pathohistological analysis in all segments of rats large intestine on the third and fifth day of application of T-2 toxin didnot show any changes. Intensity of appearing changes in a small intestine of rats intoxicated by T-2 toxin depends directly on intensity of toxic activity.

KEYWORDS: T-2 Toxin; Rats; Intestine, Small; Intestine, Large; Histocytochemistry

INTRODUCTION

T-2 toxin, a trichotecene mycotoxin, is one of the most important secondary metabolites produced primarily by Fusarium species. This cytotoxic mycotoxin was isolated from fungi, such as: F.trincictum, F.sporotrichoides, F.poae and F.roseum, that were attended in corn and other grains (1,2). If

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people and animals were ingested by food poisoned with T-2 toxin they would be acutely intoxicated with pathohistological changes on many organs (3). Since, T-2 toxin is a potent inhibitor of protein synthesis in eucharistic and animal cells (4), especially in a tissue with high mitotic index, it's effects will be more evident in the cells of intestinal crypt epithelium and lymphoid tissue (5,6).

MATERIALS AND METHODS

The investigation was carried out on twenty four female rats, weighting between 200 and 250 gr. Wistar rats were poisoned by T-2 toxin, which was isolated from F.sporotrichoides (7,8). T-2 toxin was applicated one time in a dose of 0,18 mg/kg sc (1 LD-50). Surviving rats were registrated after 24 hours. The experiment lasted six days and animals were sacrificed after the end of third and fifth day of application by T-2 toxin. The small and large intestine samples were fixed in 10% neutral formalin for 3 to 5 days. After the process of fixation they were dehidreted in graded alcohol, xylol and paraffin wax. Finally, 5 μm thick paraffin sections were stained by haematoxilin and eosin (HE) and periodic acid-Schiffës (PAS) methods.

RESULTS

Macroscopic examination of the control animals and of those treated by T-2 toxin showed no changes. Histological analysis of all segments of rats duodenum and jejunum sacrificed on third day of application by T-2 toxin, were found segmental epithelium deficit of villi intestinales. Some villi were rounded and edematous. Therefore, all segments of intestinal wall were congested and edematous in all animals. The most interesting and the most intensive changes were seen on tunica mucosa of ileum which covers the lymphatic tissue. Epithelial cells of intestinal villi were wholly desquamated on the same places. Atrophied villi intestinales were rounded, fingerlike, dendrite or papillomatous. In this areas Lieberkuhn's crypts were enlarged and filled with detritus consisted of necrotic gland cells. Depletion of lymphocytes was found in the lymphoid follicles of the Peyerës patches. The small number of eosinophil granulocytes and lymphocytes were present in the lamina propria. T-2 toxin, in a small intestine of female rats sacrificed on fifth day of experiment, caused hyperemia and edema and diffuse epithelium deficit of villi intestinales. The large number of villi intestinales was atrophied, deformed and fusioned. The Liberkuhn's crypts were cystic enlarged and filled with necrotic epithelial cells. A large number of mitotic cells was present in a base of the same intestinal glands. This change appeared in a segment of the intestine crypt epithelium and gland, they were both necrotic. Single macrophages were seen in the lamina propria. Described changes were most intensive in the ileum. Pathohistological analysis of all segments of the large intestine and in rats of the third and fifth day of application by T-2 toxin did not show any changes.

DISCUSSION

In this study, a single dose of T-2 toxin (0,18 mg/kg sc) 1 LD-50, caused pathohistological changes only in a small intestine of rats. Other authors (9), reported that T-2 toxin caused the same changes in a large intestine. In a small intestine of all experimental animals segmental atrophy of villi intestinales was found. The most intensive atrophy of villi intestinales seen in the ileum (10). The large number of Liberkuhn's crypt was enlarged. Described changes were most intensive on tunica mucosa of ileum which covers Peyer's patches. Lymphatic tissue of Peyerës patches was not discovered in the necrotic lymphocytes (11). Pathohistological changes in a small intestine of rats, on the fifth day of application by T-2 toxin, were same in character and more intensive. The small group of macrophages was detected in the lamina propria of villi intestinales. This villi were edematous, atrophied and deformed. The most intensive changes were seen in the ileum which was found to have a diffuse epithelium deficit. The large number of Liberkuhnës crypts was cystic enlarged with exposed basal membrane. The normal Liberkuhn's crypts had a lot of mitotic cells (12). These results revealed that the described changes were irreversible, since the actively dividing crypt epithelial cells were necrotic. Moreover, the actively dividing cells of Liberkuhn's crypts are the basic cells for all epithelium cells of the tunica mucosa.

CONCLUSION

Intensity of appearing changes in a small intestine of rats intoxicated by T-2 toxin depends directly on intensity of toxically activity.

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β-cells in dexamethasone treated rats

ABSTRACT

Dexamethasone is a higly potent glucocorticoid. Treatment with dexamethasone results in several metabolic perturbations, many of which are found in type 2 diabetes. The aim of this study was to investigate by immunocytochemistry the changes on the β-cells in experimentaly induced diabetes type 2 in rats. Male Wistar rats were treated with 2mg/kg dexamethasone intraperitonealy during 12 days. Bouin fixed and paraffin embedded specimens were cut to 5 μm thickness and mounted on chrome-alum coated slides. Peroxydase - antiperoxydase (PAP) method was used for determination of insulin secreting β cells in the islets of Langerhans. Pancreatic and plasma insulin levels were determined using radioimmunoassay (RIA). Plasma and urine glucose levels were also determined. Weak immunopositive reaction for insulin was obtained in the central parts of the islets of Langerhans. At the periphery of those islets strong immunopositive reaction was found, as well as in the very small islets. Plasma glucose levels were significantly decreased in dexamethasone treated rats, as compared to the control group. Plasma and pancreatic insulin levels were significantly increased, as well as glucose level in the urine. The results indicated that weak immunopositivity was due to hypersecretion of insulin from the islets of Langerhans.

KEYWORDS: Dexamethasone; Islets of Langerhans; Rats

INTRODUCTION

The etiology of non-insulin-dependent diabetes mellitus (NIDDM) can be regarded as a complex interaction of genetic predisposition, β-cell dysfunction and insulin resistance. It is frequantly characterized both by an inadequate release of insulin in response to glucose (1) and a reduced sensitivity of peripheral tissues to insulin (2). Dexamethasone is a highly potent glucocorticoid. Glucocorticoid treatment results in several metabolic perturbations, many of which are found in NIDDM. Dexamethasone has thus been employed as an experimental model for type 2 diabetes in rats.

MATERIALS AND METHODS

Twenty male Wistar rats, 2 months old, were randomly divided into two groups of ten rats each. The rats were injected daily with 2 mg/kg of dexamethasone intraperitoneally (group E), the control group received an equal quantity of saline (group C). The experiment lasted for 12 days. The rats were fasted owernight and killed by exposure to diethylether, after which specimens from the pancreas were promptly excised and weighed in the air. For the immunocytochemistry, the specimens were fixed in Bouin's solution, dexydrated and embedded in paraffin. Sections were cut to 5 μm thickness and peroxydase - antyperoxydase (PAP) method was used for determination of insulin in the β-cells. Pancreatic and plasma insulin levels were determined by radioimmunoassay (RIA). Plasma and urine glucose level were also determined.

RESULTS

Weak immunocytochemical reaction of insulin was observed in the central parts of islets of Langerhans in the experimental group of rats. At the periphery of those islets a strong immunopositivity was found, as well as in the very small islets. Plasma glucose level was significantly decreased in dexamethasone treated rats, as compared to the control group (Table 1). Plasma and pancreatic insulin levels were significantly increased, as well as the glucose level in the urine.

DISCUSSION

Our investigations showed that dexamethasone caused hypertrophy of islets of Langerhans due to hypertrophy and hyperplasia of β-cells. The decreased plasma glucose level was probably a result of increasing insulin secretion which is in accordance with our immunocytochemical findings suggesting the depletion of granulae. The finding that some β-cells showed strong immunopositivity confirmed the existance of a subpopulation of those cells (3) which was probably not included in hypersection of insulin.

Table 1. Correlation between p53, Ki-67 and hormone receptor status

	C	E	P
Plasma insulin (mIU/L)	46.33 ± 11.74	154.38±37.05	p<0.050
Pancreatic insulin (mIU/g)	10.50 ± 1.93	28.10±3.44	p<0.001
Plasma glucose (mmol/L)	4.45 ± 0.22	2.66±0.44	p<0.001
Glucose in urine (mmol/L)	-	22.70±4.47	

CONCLUSION

The results indicated that weak immunopositivity was due to hypersecretion of insulin from the islets of Langerhans.

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Histochemistry of epithelial mucins in the stomach of patients with pernicious anemia

ABSTRACT

The aim of the work is the examination of qualitative and quantitative featurs of epithelial mucins in the stomach of patients with pernicious anemia. During the period from l990 to 1999, 158 patients with pernicious anemia and 3o patients with dyspeptic syndrome were examined.Laboratory sections of our patients were stained with AB-PAS. During a histochemical examination of the gastric mucosa it was seen that, in chronic atrophic gastritis with a range and a type of metaplasia,both the quantity and quality of mucin were changed. In atrophic gastritis of the first degree a quantity of neutral mucins was decreased, while in atrophic gastritis of the second and the third degree with intestinal metaplasia a quality of mucins was changed: acid mucins were increased, while at the same time neutral mucins were decreased. In patients with mature intestinal metaplasia a quantity of acid mucins was increased, while in dysplastic intestinal metaplasia acid and neutral mucins were present very slightly. In pernicious anemia with chronic atrophic gastritis associated with pyloric metaplasia, acid mucins were nor present, while the neutral ones were present in a great quantity in the type of chronic atrophic gastritis of the second degree, or they were present in an average quantity with the atrophic gastritis of the third degree, but always inside foci of pyloric metaplasia. These changes in the mucin content are of the great importance in the genesis of gastric cancer.

KEYWORDS: Anemia Pernicious; Gastritis, Atrophic; Gastric Mucosa; Metaplasia; Epithelium; Mucins; Histocytochemistry

INTRODUCTION

Epithelial mucins have very important role in the mucous barrier and help the transition nutrients through the gastrointestinal tract. In addition, epithelial mucins protect the gastric epithelium of mechanical, chemical and the other damages. The aim of the work is the examination of qualitative and quantitative features of epithelial mucins in the stomach of patients with pernicious anemia (PA).

MATERIALS AND METHODS

During the period between l99o and l999 in the Clinic of Hematology in Niš, l58 patients with pernicious anemia were examined (both sexes, between 33 and 79 years) and 3o patients with dyspeptic syndrome (both sexes, between 38 and 78 years). The group of patients with dyspeptic syndrome was without a patohistologic lesion in the gastric mucosa with normal blood and marrow findings represented in the control group. Laboratory sections of our patients were stained with AB-PAS, AB pH 2,5. Neutral mucins were stained red and acid mucins blue.

RESULTS

Epithelial mucins were studied in the surface and foveolar epithelium, as well as inside the foci of intestinal and pyloric metaplasias. Among the patients with pernicious anemia; a significant correlation between the degree of atrophic gastritis and histochemical mucin content, was observed; the same correlation was also found between the degree of atrophic gastritis and distribution and the type of intestinal metaplasia.

Namely, hyposecretion of the epithelial mucin was present in pernicious anemia with atrophic gastritis without metaplasias.

A qualitative mucin lesion, characterised by a secretion of sialomucin (intestinal type of mucin) was found inside the foci with intestinal metaplasia. The same type of mucins also showed hyposecretion, when dysplastic intestinal metaplasia was found. Glands of pyloric metaplasia always contained neutral mucins. Compared with the control group of patients, a significant difference in quantity and quality of gastric epithelial mucins, were observed.

DISCUSSION

The lesion of gastric epithelial mucins is conspicuons in patients with the pernicious anemia. The secretion of sialomucin may by linked in several ways to gastric carcinogenesis. It may signal the presence of a carcinogenic microenviroment, e.g. high gastric pH. In addition, a sialomucin decrese, an intercelular adhesion and inhibit known mechanisms which control the celular growth and differentiation were registered as well.

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Lymphocyt immunophenotype in helicobacter pylori associated chronic antral gastritis

ABSTRACT

It is well known that Helicobacter pylori induces a chronic-active inflammation of the gastric mucosa. The aim of this study was to determine an immunophenotype of the lymphocyte infiltrates in the lamina propria. The frozen sections of gastric mucosa were studied by immunohistochemistry using antibodies to CD3, CD4, CD8, CD20, CD30, and CD45. Specific antibodies showed CD4 and CD8 positive lymphocytes. Our observations demonstrated that infection with H. pylori is associated with marked T-cell infiltrates in lamina propria.

KEYWORDS: Helicobacter pylori; Lymphocytes; Gastritis; Immunophenotyping

INTRODUCTION

Helicobacter pylori is a widespread infectious agent resulting in gastritis and some infected individuals develop peptic ulcer, maltomas and gastric cancer. In spite of sufficient evidence of a marked systemic and local immune response against H. Pylori, most infected individuals cannot eliminate the bacterium, leading to a persistence of chronic inflammatory changes. Histopathologic examination of the diseased gastric mucosa shows a marked association between the density of bacterial colonization and intensity of cellular infiltrates.

MATERIALS AND METHODS

Biopsy specimens of the gastric antrum were obtained from patients undergoing gastroduodenoscopy for various clinical indications. Frozen sections were processed by immunoalkaline phosphatase (APAAP) using antibodies to human CD3, CD4, CD20, CD30, CD45 and anti-H. pylori.

RESULTS

With anti-H. pylori antibody immunostaining, microorganisms were identified attached to the brush border of the gastric foveolar epithelial cells or within the superficial mucus. The lymphoid follicles were detected within lamina propria. Lymphocytes showed intense staining with CD3, CD4, CD8, CD45 antibodies; only small numbers of lymphocytes were CD20 positive. T suppressor and T helper cells were evidented in the epithelium as well as in the lamina propria.

DISCUSSION

Helicobacter pylori infection has been recognized as a causative agent of chronic active gastritis that predisposes the mucosa to duodenal and gastric ulceration. H. pylori infection generally persists over the life-time in association with gastric inflammatory response, implying that local mechanisms are ineffective at clearing infection (1). Studies have shown that gastric T lymphocytes are increased during H. pylori infection. H pylori expressed several molecules, which may interfere with antigen presentation and T-cells activation (2). Direct evidence now exists to support the hypothesis that the immune-inflammatory responses observed during infection with H. pylori do induce auto-immune-mediated damage to the gastric epithelium.

CONCLUSION

Our observations demonstrated that infection with H. pylori is associated with marked T-cell infiltrates in lamina propria.

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Effects of porta-caval shunt (PCS) on rat pancreatic islets: a immunohistochemical, ultrastructural and

ABSTRACT

Some endocrine consequences of PCS have been reported in the rat including abnormalities in glucose homeostasis and in plasma levels and secretion patterns of pancreatic hormones. In the present study, we investigated effects of PCS on endocrine pancreas of the rats, eight weeks after surgery. The pancreatic islets were studied immunohistochemically, morphometrically, and ultrastructurally. Moreover, plasma/serum levels of insulin, glucagon, somatostatin, and glucose were determined. The results showed immunocytochemical and ultrastructural signs of an impairment of the secretory activity of B cells, and a normal secretory pattern of A, D and PP cells. In addition, normal plasma levels of insulin and somatostatin, as well as hyperglucagonemia, and hypoglycemia were observed. The results suggest that the functional deterioration of the liver due to the diversion of the portal blood in the systemic circulation, is responsible for islet cell changes.

KEYWORDS: Pancreas; Portacaval Shunt, Surgical; Immunohistochemistry; Microscopy, Electron; Pancreatic Hormones

INTRODUCTION

The rat with PCS can be regarded as an experimental model mimicking the conditions that occur in patients with liver failure, especially due to cirrhosis, and with portal-systemic shunt (3). Some endocrine consequences of PCS have been reported in the rat, including abnormalities in glucose homeostasis and in plasma levels and secretion patterns of pancreatic hormones (1,4). In the present study, the pancreatic islets were examined immunohistochemically, morphometrically and ultrastructurally, in rats with surgically constructed end-to-side porta-caval anastomosis.

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MATERIALS AND METHODS

The adult male Wistar rats were divided in two groups: 1) control (C) group, n=11, and 2) PCS rats, n=27. End-to-side PCS was performed according to the technique described by Lee and Fisher (2). All the animals were killed 8 weeks later. Immunohistochemical studies of Bouin-fixed, paraffin-embedded sections were performed by the DAKO LSAB+method. The battery of antibodies used included neuron-specific enolase (NSE), insulin, glucagon, somatostatin, pancreatic polypeptide and amylin. Computer-assisted morphometry was carried out on immunostained sections by a method employed previously by as for similar purposes (2). The specimens for electron microscopic examination were processed routinelly prior to viewing with an Opton 109 electron microscope. The basal serum/plasma levels of insulin, glucagon and somatostatin, as a pancreatic content of these hormones were determined by radioimmunoassay (RIA). Basal plasma glucose was measured by the glucose oxidase method.

RESULTS

Body weights were not significantly different between the groups eight weeks after operation. The liver weight was significantly lower, but the pancreatic weight was significantly greater in PCS than in C group (1.12±0.38 vs. 0.77±0.27 g, $p<0.01$). In rats with PCS compared with controls, the serum insulin was slightly reduced, plasma somatostatin was not significantly changed, and plasma glucagon was markedly increased.(555±97 vs. 196.5±44 pg/ml, $p<0.01$). Moreover, pancreatic glucagon content was increased (2280±53.7 vs. 1256±59.4 pg/g, $p<0.01$), while insulin content was significantly reduced (10.2±7.3 vs. 14.6±6.9 mIU/g, $p<0.01$). Also, in rats with PCS basal plasma level of glucose was significantly lower (4.02+1.08 vs. 5.19±1.06 mmol/l, $p<0.01$).

No signs of an islet cell damage, inflammatory reaction, and de novo formation of endocrine cellls out-side the islets could ever be detected. The morphometric analysis carried out on the immunostained islets of PCS rats showed a significant increase in cell density of each immunoreactive NSE, insulin and amylin, and no significant differences in the cell densities of immunoreactive glucagon, somatostatin and PP (Table 1).

In addition, the islets from the rats with PCS showed a majority of B cells with very intense both insulin- and amylin- immunoreactivity. On electron microscopic examination, most B cells in PCS rats contained rather numerous beta granules, mainly of mature type with electron-dense cores, a moderately developed endoplasm, and a Golgy apparatus divided in small areas scattered througout the cytoplasm. On the other hand, in the control islets, several B cells were rather poor in secretory granules, the egrastoplasm was usualy well developed, and the Golgy apparatus occupied a single, large area near the nucleus.

Table 1. Table 1. Morphometric analysis of endocrine cells of islets of langerhans in pcs and control rats

	C (n=11)	PCS (n=27)	p
Number of endocrine cells / μm^2 of islet of Langerhans			
NSE-immunoreactive	13.836±760	16.320±970	<0.01
Insulin-immunoreactive	7.086±860	9.132±1113	<0.01
Glucagon-immunoreactive	4.869±320	4.998±418	NS
Somatostatin-immunoreactive	890±98	1.070±95	NS
PP-immunoreactive	1.000±101	1.130±85	NS
Amilin-immunoreactive	6.982±954	7.237±1.1770	<0.05

DISCUSSION

The current findings indicate that, following PCS, there was hypertrophy of the pancreas. This observation suggests that PCS may raise the sensitivity of pancreatic CCK-A receptors to CCK (5). Our results also indicated that there are impairment in B cell function in rats with PCS (increased storage of insulin in B cells and depressed synthesis of secretory proteins) which is due to the

persistent hypoglycemia. In spite of the decrease of insulin secretion, the serum levels of the hormone are not reduced. This is probably a consequence of a diminished extraction of insulin by atrophic liver cells. In rats with PCS there were the marked accumulation of amylin in B cells, as well as the increased number of amylin-immunoreactive cells. Later observations need further investigations. The elevated plasma levels of glucagon in the PCS rats are associated with structural features of A cells, indicating a normal secretion pattern. Hence, hyperglucagonemia does not seem to be caused by hypersecretion by islet A cells but, rather, by reduced catabolism of glucagon by the liver. The coexistence og hyperglucagonemia and hypoglycemia is probably related mainly to the functional impairment of the liver that, being almost completely devoid of glycogen, is no more able to adequately respond to glycogenolytic stimuli. Our results are similar to those in previous studies (1, 3).

CONCLUSION

The results suggest that the functional deterioration of the liver due to the diversion of the portal blood in the systemic circulation, is responsible for islet cell changes.

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A morphological and ultrastructural changes of hepatocytes following PCS in the rat and their influence on the IGF-I synthesis

ABSTRACT

The aim of the current study was to examine the pathohistological and ultrastructural changes of the liver tissue on the experimental model of a chronic liver disease (end-to-side portacaval shunt) and a derangement in the insulin-like growth factor-I (IGF-I) synthesis. The surgical procedure end-to-side PCS was performed in Wistar rats. The liver hystology and ultrastructural patterns of hepatocytes were examined. Moreover, immunohistochemistry was used for examined liver IGF-I-immunoreactivity. Radioimmunoassay for IGF-I and growth hormone (GH) was used. In rats with PCS, eight weeks after the operation, the liver atrophy was observed. Findings of liver histology showed glycogen reduction, degenerative and microvesicular fatty changes, and atrophy of hepatocytes. Ultrastructural lesions in the hepatocytes showed reduction and fragmentation of the rough endoplasmic reticulum. Basal level of total IGF-I was significantly reduced ($p<0.01$), while basal GH serum concentration was significatnly increased ($p<0.05$). Also, we found significantly decreased ($p<0.05$) liver IGF-I contents in the PCS group. The present study suggests that end-to-side PCS causes liver atrophy and that a morphological and ultrastructural changes of hepatocytes partially explain a decrease in IGF-I synthesis.

KEYWORDS: Portacaval Shunt, Surgical; Insulin-Like Growth Factor I; Liver + ultrastructure

INTRODUCTION

The end-to-side PCS diverts the portal blood flow from the liver, i.e. hepatofugal blood flow, and leads to a significant decrease of the total liver

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blood flow, decreased hepatic arteriolar resistance and hypoxia, caused atrophy and functional impairment of liver cells (2,4).

MATERIALS AND METHODS

Adult male Wistar rats, weighing 180-250 g at the beginning of the experiment, were used. The rats were divided in three groups: 1) control (C rats, n=11), 2) sham operated (SHAM rats, n=17), and 3) experimental group (PCS, n=27). End-to-side PCS was performed according to the technique described by Lee and Fisher (1). The basal serum levels of GH and IGF-I were determined by radioimmunoassay, as well as the liver contents of IGF-I. The liver sections were analyzed by microscopic examination after hematoxylin-eosin, Massoǹs trichrome, PAS and Perl̀s staining. Liver IGF-I immunohistochemistry was carried out with polyclonal antibody to human IGF-I. For electron microscopy examination, the small liver pieces were fixed in a cold 3% glutaraldehyde in 0,2 M cacodylate buffer with 0,2 M sucrose and post-fixed in 1% osmium tetroxide. Ultrathin sections were cut and stained with uranyl acetate followed by lead citrate and examined by Philips III electron microscope.

RESULTS

Body and liver weights: body weights were not significantly different between the groups the eight weeks after operation. The liver weight was significantly lower in PCS rats than in control rats (5.12±1.22 vs. 6.94±1.34 g, respectively, $p<0.01$). The same was observed when liver weight was expressed as a percentage of body weight (2.15±0.25 vs. 2.95±0.48 %, respectively, $p<0.01$). Concentrations of IGF-I and GH. PCS rats showed significantly reduced ($p<0.01$) basal concentrations of total IGF-I (485+61.3 mg/l) compared to both control (658+57.3 mg/l) and sham operated rats (697+51.6 mg/l). Also, we found significantly decreased ($p<0.05$) liver IGF-I contents in the PCS group (69.7+10.1 vs. 91.3+8.3 vs. 87.6+11.2 ng/g wet weight for PCS, C, and SHAM, respectively). Fasting basal GH serum concentrations were higher ($p<0.05$) in rats with PCS than in those without PCS (0.0807+0.02 vs. 0.078+0.027 vs. 0.0795+0.0795+0.019 mIU/l for PCS, C and SHAM, respectively). Liver histology, electron microscopy and immunohistochemistry. Liver histology in rats with PCS showed glycogen reduction and sinusoidal dilatation around the hepatic vein. Kupffer's cells were filled with haemosiderin. The hepatocytes surrounding the portal space had degenerative and microvesicular fatty changes. Atrophy of hepatocytes in other parenchymal zones was presented. Apoptopic hepatocytes were seen more frequently in rats with PCS. Ultrastructural characteristics of hepatocyte cell lesions of rats with PCS in comparasion to the C and SHAM rats, are depicted in Table 1.

Immunohistochemical staining of the liver revealed large populations of hepatocytes containing IGF-I in the control, as well as in sham operated rats. However, expression of IGF-I immunoreactivity in hepatocytes of PCS rats was lower than in C and SHAM rats.

DISCUSSSION

Table 1. Summary of characteristics of hepatocyte cell lesions of rats with PCS at the end point of the experiment

Alterations in cell organelles, lipid and glycogen inclusions	PCS group (n=27)	SHAM group (n=14)	C group (n=11)
Mitochondrial aberrations	+	-	-
Pycnotic euchromatic nuclei	+/++	-	-
RER fragmentations	++	-	-
Poyribosome depletion	++	-	-
Glycogen depletions	+++	-	-
Lipid inclusions	++	-	-

Liver atrophy caused by PCS, is manifested by a significant reduction of the liver weight (3,5). The development of liver atrophy in rats with PCS was a consquence of the liver blood flow reduction (3) or a results of deprivation of hepatotrophic substances normally present in the portal blood. Our findings of the liver histology in rats with PCS showed glycogen reduction in hepatocytes and sinusoidal dilatation around the hepatic vein, degenerative, as well as microvesicular fatty changes. Apoptotic hepatocytes were seen in rats with PCS. Ultrastructural examination of the hepatocytes of rats with PCS at the end of our experiment showed reduction and fragmentation of rough endoplasmic reticulum with destroyed and dilated cisternae and fewer polysomes accompanied with smooth endoplasmic reticulum proliferation. The mitochondria were round to elongated, with prominent cristae. Moreover, a significant decrease in glycogen particle content and an increase of the number of small lipid droplets were noted in PCS rats. These hepatocyte cell lesions, together with pathohystological change in the liver which are similar to the findings of other autors (1,4,5) cause metabolic and endocrines abnormalites, i.e. a disturbance of glucose homeostasis, reduction of IGF-I concentrations in serum and liver tissue, among others. In the present study, GH was increased in the the serum of rats with PCS, while serum concentration of IGF-I, as well as liver IGF-I content was reduced. Moreower, immunohistochemical stainig of the liver clearly revealed the less expression of IGF-I-immunoreactivity in hepatocytes of PCS. Similarlly, in patients with cirrhosis low concentations of „free" IGF-I are the resuts of a reduced hepatocellular synthesis (6).

CONCLUSION

The present study suggests that end-to-side PCS causes liver atrophy and that a morphological and ultrastructural changes of hepatocytes partially explain a decrease in IGF-I synthesis.

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Immunohistochemical localization of sensitive afferent nerve fibers in the gastric mucosa of patients with helicobacter pylori (HP) infection

ABSTRACT

It is well known that afferent senzory neurons might participate in gastric defense mechanisms and contribute to the healing of gastric lesions. The aim of the present study was to investigate the immunohistochemical localization of CGRP and SP in the human gastric mucosa obtained by endoscopic biopsies from patients with Hp infection. Biopsies were taken from the antral gastric mucosa in 68 patients with Hp and 28 patients without Hp infection. Immunohistochemistry was carried out according to the streptavidin-biotin complex method using an anti-PGP 9,5, synaptophisin, CGRP and SP antibodies. Intensive CGRP staining was more marked in the specimens collected from Hp uninfected stomach compared to those from the portions with Hp infection. Moreover, CGRP-containing nerves were absent in 67% patients with Hp infection, especially in cases with severe granulocyte activity and inflammation. Regarding the distribution and density of SP-immunoreactive nerve fibers, the intensity of staining as well as nerve density was higher in Hp infected subjects than that obtained from non-infected ones. In light of our present finding that the CGRP-immunostaining was lower and SP-immunostaining was more intense in Hp infected patients compared to those without infection, it is suggested that those disturbances might have pathophysiologically relevant implications on restorative processes in injured stomach mucosa during Hp infection.

KEYWORDS: Calcitonin Gene Related Peptide; Substance P; Gastric Mucosa; Nerve Fibers; Helicobacter pilory

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INTRODUCTION

Capsaicin sensitive afferent neurons contain a number of bioactive peptides, including CGRP and tachikinins, SP and neurokinin A. It is well known that these neurons might participate in gastric defense mechanisms through local release of CGRP and contribute to the healing of gastric lesions. In addition, these neurons have been implicated in the regulation of the adherence, migration and activity of leukocytes. SP is devoid of protective action and has been found to exaggerate a mucosal damage by degranulation of mast cells (1-3). However, it remains to be determined whether inflammatory processes in course of Helicobacter pylori infection in the stomach are also under the control of afferent neurons. The aim of the present study was to investigate the immunohistochemical localization patterns of CGRP and SP in the human antral gastric mucosa obtained by endoscopic biopsy from patients with or without Helicobacter pylori infection.

MATERIALS AND METHODS

The study population consisted of 61 Helicobacter pylori (Hp) infected subjects with antral or pangastritis and 28 patients without Hp infection (21 with antral or pangastritis and 7 healthy subjects). Four antral biopsy specimens were obtained. Hp colonization was determined using either rapid urease test or histological examination of Genta-stained specimens. Sections were stained immuno-histochemically using an anti-PGP 9.5, synaptophysin, SP and CGRP antibodies (Table 1) and LSAB+ Dako kit. Immunoreactive sites were visualized by 3-amino-9-ethylcarbazole substrate chromogen. For electron microscopy examination small tissue specimens were taken from the pyloric gland area and fixed in a mixture of glutaraldehyde (1%) and formaldehyde (3%) in 0,075 M sodium phosphate buffer. The specimens were postfixed in 1% osmium tetroxyde and embedded in Epon 812. Ultrathin sections contrasted and examined in Philips CM 10 electron microscope.

RESULTS

Generally, our results showed that most nerve fibers are located in deeper portions of the mucosa and that there is no difference in PGP 9.5-immunoreactive fibers density in the lamina propria of the mucosa of the antrum between examined groups of subjects. Similar results were found for the sinaptophysin-immunoreactive nerve fibers. No detectable changes were found in the ultrastructure of the nerve processes in the antral mucosa between examined subjects. However, intensive CGRP staining was more marked in the specimens collected from Hp uninfected stomach compared to those from the portions with Hp infection. Moreover, CGRP- containing nerves were absent in 67% patients with Hp infection, especially in cases with severe granulocytes activity and inflammation. Regarding the distribution and density of SP-immunoreactive nerve fibers, the intensity of staining, as well as nerve density, was higher in Hp infected subjects than that obtained from non-infected ones.

Table 1. Antisera used for immunohistochemistry

Antibody	Type	Immunogen	Dilution	Source
PGP 9.5	Polyclonal	PGP 9.5 isolated from bovine brain	1 : 100	DAKO A/S, Denmark
Synaptophysin	Monoclonal	Presynaptic vesicles from bovine brain	1 : 20	DAKO A/S, Denmark
SP	Polyclonal	Synthetic substance P	1 : 2000	Serotec, UK
CGRP rat	Polyclonal	CGRP rat, conjugated to BSA	1 : 100	Pennisula laboratories Belmont, CA, USA
Human	Polyclonal	CGRP human, conjugated to BSA	1: 100	Pennisula laboratories Belmont, CA, USA

PGP 9.5 – Protein gene product 9.5; SP – Substance; CGRP – Calcitonin gene-related peptide; BSA – Bovine serum albumine

DISCUSSION AND CONCLUSION

There have been many studies showing that capsaicin- sensitive neurons

might participate in a gastric defense mechanisms trough a local release of CGRP and SP (1-3). It was reported that CGRP stimulates the proliferation of human endothelial cells, mediated the mucosal hyperemia responses associated with acid back-diffusion and by facilitating acid disposal in the mucosa. Also, recently there are many reports that sensory neurons confer protection against injury via mechanisms that are unrelated to gastric blood flow as well as that those peptides are involved in regulation of inflammatory processes. In light of our present finding that the CGRP-immunostaining was lower and SP-immunostaining was more intense in Hp infected patients compared to those without infection, it is suggested that those disturbances might have pathophysiologicaly relevant implications on restorative processes in injured stomach mucosa during Helicobacter pylori infection.

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Adenoid cystic carcinoma of esophagus as an indicator of the embrional origin of esophageal epithelium

ABSTRACT

Adenoid cystic carcinoma develops from the ductal and the myoepithelial cells of different glands, whose epithelium develops completly or partly from the ectoderm. It is one of the rarest esophageal tumors. In the last five years it was reported only in two cases, with the present case as the third one. The possibility of its development in the esophagus is relevant in the elucidation of the embryonal origin of the epithelial components of this organ. In a 73-year-old male patient in the distal portion of the esophagus and the proximal part of the body of the stomach a tumorous tissue was found, with pathohystological characteristics of an adenoid cystic carcinoma, reaching the mesenterial connective and adipose tissue, with perineural infiltration and metastases into regional lymph nodes. The classical embryology data testify that the esophageal epithelium and the epithelium of the lower respiratory tract (beeng one of the important places of occurrence of adenoid cystic carcinoma), develops from the endodermal layer as a superficial layer of the primitive gut. The initially highly cyllindrical esophageal epithelium, after transitory proliferation and obliteration, and subsequent recanalization of its cavity, changes into a stratified squamous epithelium in a process of metaplasia or descent of oral epithelium, itself of ectodermal origin. The occurrence of adenoid cystic carcinoma with myoepithelial cells originating from ectoderm, is an indirect proof that the definitive esophageal epithelium is formed by descending of the oral cavity epithelium and that it is of ectodermal origin.

KEYWORDS: Carcinoma, Adenoid Cystic; Esophageal Neoplasms; Neoplasms, Germ Cell and Embrional

INTRODUCTION

Adenoid cystic carcinoma is a tumor which develops from ductal and myoepithelial cells of different glands. Myoepitelial cells are of ectodermal ori-

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gin, so adenoid cystic carcinoma can be present only in organs whose glandular epithelium develops completly or partly from the ectoderm (1). Adenoid cystic carcinoma appears with different frequency in salivary glands, especially in small ones, in the glands of the respiratory system including sinonasal glands, pharingeal, tracheal and laryngeal glands, offen in sweat glands, rarely in lacrimal and mammal and esopageal glands, vulva and uterine cervix glands. The classical embryology data testify that the superficial and glandular esophageal epithelium and the epithelium of the lower respiratory tract including larynx, trachea and lungs develop from the endodermal layer as a superficial layer of the primitive gut. The striated musculature of the upper part of the esophagus originates from the ectomesenchyma of the branchial arches (arising itself from the ectoderm of the neural crest), whereas the rest of the tissue, including the connective tissue of the mucous and submucous membrane, the adventitia and the smooth musculature of the lower esophageal parts, is of mesodermal origin (2,3). Adenoid cystic carcinoma of esophagus is a very rare tumor; according to our knowledge, in the last five years it was found only in two cases (4,5), with the present case as the third one. The predominance of the myoepitehlial component in adenoid cystic carcinoma occurs in all mentiond organs and opens a new possibility in the explanation of the embryologic origin of esophageal epithelium in consideration of ectodermal origin of myoepithelial cells.

PATIENT AND METHODS

A 1.5 cm long resection of the esophagus, 10 cm long resection of the stomach and the spleen of a 73-year-old male patient with a clinical diagnosis of Tumor partis distalis oesophagi were obtained and pathohistologically analysed. Since the spleen resection showed no pathologic changes connected with the basic disease, only the resections of the esophagus and stomach were further analysed. The histologic sections were stained according to the standard HE, PAS alcian and Gomori methods and light microscopically analysed.

RESULTS

Macroscopically, the resection of the distal part of the esophagus was a ring-like section with a partly preserved mucosa having a thickened wall without clearly visible stratification due to the presence of a foreign tissue of a radiant whitish color. In the stomach resection, the mucosa was mostly preserved, partly hemorrhagic. The other layers of the gastric wall showed an impaired structure because of the foreign tissue, which, without clear demarcation, entered also into the surrounding loose connective and adipose tissue. The pathohistologic analysis points to the presence of a tumorous tissue composed of the myoepithelial cells having mostly cribriform distribution, with cystical spaces filled with strongly eosinophilic, PAS-positive material. Some of this spaces were enclosed by duct lining cells. The tumorous tissue was present in the form of small accumulations in the esophageal mucosa, but it completely occupied the submucosa and pervaded the muscular layer of the esophagus; it stretched through the submucosa to the stomach, spread in the entire gastric mucosa, infiltrated into the muscular layer of the stomach, and reached the mesenterial connective and adipose tissue, with multiple perineural infiltration, and metastases and infiltration into regional lymph nodes.

DISCUSSION

During the development of the esophagus, in the initially highly cylindrical epithelium, endodermal cells proliferate and the epithelium undergoes pseudostratification, with transitory obliteration of the lumen and, finally, recanalization by the end of the embryonal period. The esophaeal glands are formed by invagination, proliferation and differentiation of the surface epithelium. The definitive esophageal epithelium is stratified squamous without keratinization, whose occurrence could be explained by the process of metaplasia of pseudostratified epithelium into stratified squamous one, as influenced by mechanical factors of swallowing of amniotic water, or by the descending of the stratified squamous epithelium of the oral cavity toward the esophagus after opening of the buccopharingeal membrane. These two possibilities are considered equally liable in embriology, although the development of adenoid cystic carcinoma as a tumor of the cells of ectodermal origin supports the second hypothesis. The appearance of adenoid cystic carcinoma in different organs of the respiratory system proves the participation of ectodermal germinative layer in the process of development of its glandular epithelium, also. With respect to the etiopathogenesis of this tumor, very interesting is the coincidence of adenoid cystic carcinoma of the skin and of minor salivary glands, and Schwann cell differentiation of myoepithelial cells within an adenoid cystic carcinoma (6,7).

CONCLUSION

The possibility of development of adenoid cystic carcinoma in the esophagus, as a tumor of well-differentiated neoplastic ducts surrounded by neoplastic myoepithelial cells (the latter one of ectodermal embrional origin), despite its low frequency, indirectly testifies that the definitive stratified squamous epithelium of the esophagus is formed by descending of the epithelium of the oral cavity, and that it is, accordingly, entirely or only partly of ectodermal origin.

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Impact of preservation fluid on early graft function in liver transplantation

ABSTRACT

The type of preservation fluid was found to be rather relevant for the succes of the liver transplantation, especially for the post-operative graft function. The aim of our study was to investigate the correlation of the type of preservation fluid and total ischemic time (TIT) with the liver function in the early post-operative period in patients with liver transplantation. Our group consisted of 3 women and 6 men, at the average age of 36 (22-55) years. HTK solution was used in 7 and UW solution in 2 patients. Average TIT for HTK solution was 294.56 min and for UW solution it was 222.5 min. Total bilirubin, alkaline phosphatase, SGOT, SGPT and GGT were measured before and on 0th , 7th , and 14th day after liver transplantation. We found higher levels of total bilirubin, alkaline phosphatase and GGT during the observed postoperative period in HTK group in comparison with UW group. Results showed that liver transaminases, SGOT and SGPT were higher in UW group in comparison with HTK group during the postoperative period. The observed differences were not statistically significant. Comparison of the parameters before and after the liver transplantation demonstrated a smaller increase of transaminases in HTK group after liver transplantation. We also found strong correlation between TIT and the increase in SGPT (r=0,593, r2=p,352) and GGT (r=0,493, r2=0,243) postoperatively and a weaker correlation for SGOT (r=0,23, r2=0,05). Both solutions were effective in the liver preservation although they differ in the influence on various parameters of liver fuction. Total ischemic time was found to be a significant predictor of postoperative liver function.

KEYWORDS: Liver transplantation; Organ preservation; Organ procurement

INTRODUCTION

Development of new surgical techniques and new immunosuppressive drugs enabled organ and tissue transplantation in a large number of patients (1). The lack of cadaveric organ donors and defined national donation program represent the most important barriers for the full development of transplants in Yugoslavia (2). Until 1995 organ donation program in Yugoslavia was limited to bone marrow, cadaveric and living-related kidney transplantation (3). At the Cardiovascular Institute „Dedinje“, Belgrade in the period July 1995-December 1999, 12 liver, 7 heart and one pancreas transplantation have been performed. Transplantations were enabled by establishing the organ transplantation center in CVI „Dedinje“ and a successful co-operation with 3 clinical centers where multiorgan procurement was introduced (4,5). It is well known that besides immunosuppresive drugs, introduction of special preservation fluids enabled a succesful organ transplantation. The type of preservation fluid influences significantly the graft function and together with cold and total ischemia time is crucial for ischemia/reperfusion injury. The aim of our study was to investigate the correlation between the type of the preservation fluid and total ischemic time (TIT) and the liver function in the early post-operative period in patients with liver transplantation.

PATIENTS AND METHODS

In our study we included 9 patients, 3 women and 6 men, at the average age of 36 (22-55) years, transplanted in CVI ìDedinjeî from 1995 to 1998. We used two most common preservation fluids: HTK (Bretschneiders) solution and UW (University of Wisconsin) solution for liver preservation. HTK solution was used in 7 and UW solution in 2 patients. Average TIT for HTK solution was 294.56 min and for UW solution was 222.5 min. Total bilirubin, alkaline phosphatase, SGOT, SGPT and GGT were measured before and on 0th , 7th , and 14th day after liver transplantation. The statistical analysis was done using a linear correlation.

RESULTS

We found higher levels of total bilirubin, alkaline phosphatase and GGT during the observed postoperative period in HTK group in comparison with the UW group. The obtained results showed that liver transaminases, SGOT and SGPT were higher in UW group in comparison with the HTK group during the postoperative period. The observed differences were not statistically significant. Comparison of the parameters before and after liver transplantation demonstrated a smaller increase of transaminases in the HTK group after liver transplantation. We also found a strong correlation between TIT and an increase in SGPT (r=0,593, r2=p,352) and GGT (r=0,493, r2=0,243) postoperatively and weaker correlation for SGOT (r=0,23, r2=0,05).

DISSCUSION

The protective effect of the HTK (histidine-tryptophan-ketoglutarate) organ preservation fluid is based on a low electrolyte content which allows a high buffer concentration at iso-osmolarity. This is different from the University of Wisconsin preservation fluid (UW) the main components of which are impermeants (raffinose and lactobionate) and a colloid (hydroxyethyl starch). Several experimental studies showed good preservation of liver function after the usage of both solutions in liver preservations (6). A prospective, randomized study of Erhard and al. (30 month period, 60 patients) showed that hepatocellular injury (SGPT, SGOT) was more significant in the HTK group but both solutions were appropriate for liver preservations (7). Our study revealed that in early post-operative period SGOT and SGPT were higher in the UW group but the difference was not significant. Our experience showed that both solutions were effective in the liver preservation although they differ in the influence on various parameters of the liver fuction. The total ischemic time was found to be a significant predictor of the postoperative liver function.

CONCLUSION

Both solutions were effective in the liver preservation although they differ in the influence on various parameters of liver fuction. Total ischemic time was found to be a significant predictor of postoperative liver function.

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Macrophage heterogenity in normal and Crohn's disease terminal ileum mucosa

ABSTRACT

The aim of this study was to analyze heterogenity of human intestinal macrophages in 10 surgical samples of normal terminal ileum mucosa and 20 surgical samples of Crohn's disease patients as well as its relationship with local inflammatory and repair mechanisms. A panel of antibodies working in paraffin-embedded tissue was employed and included CD68, HLA-DR, S-100 protein and factor XIIIa. Hystologically normal areas within each of the examined sections showed conservation of accessory cells in a manner indistinguishable from that seen in normal small intestine. There was, wide variation in the number of cells staining with the different antibodies in inflammed and ulcerated areas. Immunohistochemical analysis showed that relative proportions of the accessory cells stained with antibodies appeared to be strongly influenced by localized changes in the development of the healing and repair process. This study confirmed hypothesis that subpopulations of intestinal macrophages are lakly to have different functional roles. Phenotypic changes during inflammation may be induced by mediators of inflammation or may represent a recently recruited population of cells.

KEYWORDS: Crohn Diseases; Macrophages; Intestinal Mucosa; Immunohistochemistry

INTRODUCTION

Mucosal lymphoid tissue in gastrointestinal tract performs two roles, the provision of immunological tolerance to dietary antigens and immunity to pathogens. It is recognized that accessory cells have a critical role in modulating these events (1). There is a large population of these cells in the normal intestinal mucosa where they represent the major antigen presenting cell population capable of determining the type of T cell responses that develop to luminal antigens (2). In active inflammatory bowel disease there is an increase in the mucosal macrophage population, derived from circulating monocytes. These recruited macrophages are phenotypically different from the resident population of cells and play a major role in mediating the chronic mucosal inflammation seen in patients with ulcerative colitis(UC) and Crohn's disease (CD). Macrophages also appear to be important during res-

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olution of inflammation and repair of the intestinal mucosa that occurs during disease remission (3). In this research the histological characteristics of the macrophages on the terminal ileum mucosa of normal intestine and Crohn's disease were analyzed. We and the others have recently applied a panel of antibodies working on paraffin - embedded tissue directed against differing macrophage populations to examine the nature of mucosal infiltrates in normal ileum mucosa and in CD patients.

MATERIALS AND METHODS

Formalin-fixed paraffin-embedded resection specimens from 10 non-inflammed terminal ileum (obtained from right hemicolectomy specimens removed for carcinoma of the colon) and 20 terminal ileum of CD patients were examined. The diagnosis of CD had been established by endoscopic, radiological and histological criteria in each case. All CD samples for this study were taken from areas of transmural chronic inflammation. The sections were stained with peroxidase-antiperoxidase (PAP) technique using the next antibodies (DAKO production): CD68, S-100 protein, HLA-DR and factor XIIIa (F XIIIa).

RESULTS

Two macrophage populations were identified on dome areas of helty terminal ileum mucosa, one positive for HLA class II only had the second positive for S-100 protein, CD68 and HLA-DR. S-100 protein positive cells were restricted to the most superfitial parts of the dome. Villous tip macrophages stained positively for CD68 and HLA-DR only, while deeper cryptal and submucosal populations expressed positivity for F XIIIa, CD68 and HLA-DR but were negative for S-100 protein. Germinal centre macrophages were positive for CD68, HLA-DR and negative for F XIIIa and S-100 protein. T zone dendritic cells stained positively for S-100 protein and with HLA-DR. these cells did not label with CD68 and F XIIIa. Sections from terminal ileum of CD patients showed classical histological features: focal ulcers, deep fissures, transmural inflammation. Granulomas had been found in 8 patients. Hystologicaly normal areas in all specimens showed conservation of accessory cells as weel as seen in normal small intestine just described. The intensity of accessory cells infiltration stained with the antibodies in inflammed areas was strongly influenced by localized changes during healing and repair process. The macrophages distribution inside the non-inflammed group mucosa was subepithelial, while in the illness group, it reached all the mucosa that was concentrated on the basis of ulcers and all along the fissures. There was a general increase in the number of macrophages in inflammed mucosa in relation to normal mucosa. On the CD, granulomas stained strongly with CD68 antibody and CD68 positive cells facilited the identification of the microgranulomas, sometimes unnoticed in the hematoxiline-eosine. S-100 protein positive dendritic cells were identified in inflammed but intact mucosa, near to ulceration and in proximity to epithelium. In almost all sections we have found S-100 protein positive nerve bundles. It was in agreement with neuronal hypertrophy which is one of the features of CD. In areas of active scaring there were numerous F XIIIa positive dendritic cells. Those cells were also numerous in muscularis and serosa, but were absent from granulomas and from germinal centres in the lymphoid tissue. F VIIIa positive dendritic cells were localized at the base of the crypts. Intensity of epithelial HLA-DR expression corelated with severity of local inflammation. Particulary intense expression of this antibody was on ulcer-associated lineage-type epithelium. Dendritic cells, particulary in areas of ulcearation also expressed HLA-DR antigen.

DISCUSSION

Earlier studies have shown that there is an increase heterogenity in the macrophage cell population in inflammatory bowel disease but limitations of sampling because of use of the frozen section and some overlap in the patterns of reactivity of the used antibodies that have left some ambiguity in the interpretation of the findings (4). This study has revealed heterogenous populations of accessory cells in formalin-fixed paraffin-embedded tissue with distinctive anatomical localizations in normal and inflammed gut mucosa. We have found two cell populations in the dome areas of normal intestinal mucosa. One of them were S-100 protein positive dendritic cells. This may represent an adaptive response in an area of antigen challenge. In contrast, in CD ileum mucosa, there was a relatively small number of dendritic macrophages which were largely restricted to the inflammated, but intact mucosa. The small number of S-100 protein positive cells in Crohn'disease, suggest that this subpopulation has a relatively minor role in this inflammatory process (5). The abundance of F XIIIa positive dendritic cells within areas of scaring indicates that this population has a role in the repair process. In normal intestine, F XIIIa cells have a deeper distribution and are distant from areas of immediate antigen challenge. Abberant epithelial expression of HLA-DR antigen has been previously reported as a result of inflammation in the intestine (6). It has been postulated that this expression may contribute to MHC restricted cytotoxicity that might have a role in perpetuating the mucosal damage in inflammatory bowel disease (7). It is not, at present, possible to explain the reason for the intense up-regulation of HLA-DR on ulcer-associated lineage-type epithelium. The same result was obtained by Sarsfield (1). The CD68 did not identify the different functional status of the macrophages, but their position in the mucosa suggest that, in terms of fissures and ulcers, their mainly function should be the phagocitosis.

CONCLUSION

This study confirmed hypothesis that subpopulations of intestinal macrophages are lackly to have different functional roles. Phenotypic changes during inflammation may be induced by mediators of inflammation or may represent a recently recruited population of cells. Macrophages also appear to be important during resolution of inflammation and repair of the intestinal mucosa that occurs during disease remission.

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The present situation and the projection of malignant diseases distribution in Yugoslavia with a special regard to Belgrade up to the year 2020

ABSTRACT

The paper presents data based on a 30 year period of descriptive and analytic studies conducted in Yugoslavia. It includes geographical distribution of malignant diseases and assessment of their frequency according to gender and age and the projection up to the year 2020. The data sources are Federal Statistical Office, Population Register of Carcinoma, Hospital Cancer Register of the Oncology and Radiology Institute, Health Care Fund of Serbia, Internet. We have used common statistical procedures and rate standardization, correlation coefficient, determination of the trends in time and PC-software. Up to last census, about 10 million people live in Serbia. The total number of malignant patients is 140,000-145,000 and 19,000-21,000 deaths are registered. In Belgrade (with 16,4% of people in Serbia), there are 7,500-8,000 new diagnosed patients annually, with a total number of malignant diseases of 35,000-40,000 and 3,500-4,000 of deaths. In 2020 year, 12 million people will live in Serbia. We could expect 70,300 new diagnosed cancers and 40,000 deaths. With good preventive measures we could expect in decrease of these numbers, on 37,000 and 22,000, respectively. The presented epidemiology data regarding malignant diseases and their projection until 2020 demands new algorithms for cancer prevention and screening. The plans for some malignancy algorithms are near the end, but it is needed for them to become part of whole community, governments, and not only the part of health services.

KEYWORDS: Neoplasms + epidemiology; Yugoslavia; Medical Oncology + trends

INTRODUCTION

The paper presents data based on a 30-year period of descriptive and analytic studies conducted in Yugoslavia. These are the data about cancer statistics, nowadays and in the future. The problem is enormous. If we do not apply some measures, the cancer problem threatens to get the epidemic proportion.

MATERIALS AND METHODS

Descriptive and analytical methods with use of statistics software (SPSS) are applied. Descriptive epidemiology: mortality statistics is based on the counting of the number of deaths. It could be used in cancer with short survival period (Republic Institute for Statistics) (6). Morbidity statistics is based on registration of all cases of malignant diseases in the priod of one year. The frequency of the new cases is compared to all existing cases - frequency of the existing patients. (Source of data are vital statistics, population cancer registries, hostal cancer registries, and the annual reports of clinics and hospitals) (4,6).

Analytic epidemiology: The results of the anamnestic studies (case-control studies), conducted in hospitals for leading malignant diseases are also used for this paper (17).

RESULTS

Up to the census from 1991, 9,776,625 people have been registered to lie in Serbia; 16,4% people live in the capital city-Beolgrade. There are small differences in the age distribution compared to Europe data: a smaller number of youngsteres (age 0-19) and higher number of the older population (more than 60) (1,4). The natality rate is decreasing in most Serbia regions for a long time. Exceptions are the southern regions, where this rate showed enormous increase until 1995 (13). The mortality rates (standardized 1:100,000) in Serbia are: cardiovascular diseases 528,2; malignant diseases 198,7; undefined causes of death 72,6; respiratory diseases 52,7; injuries and intoxication 50,0; digestive diseases 28,7; other 16,1 (1,2). Until 1995, a number of 29,000 to 31,000 new cancer patients were registered annually. The total number of malignant patietns is 140,000-145,000 and 19,000-21,000 deaths are registered. In Belgrade (with 16,4% of people in Serbia), there are 7,500-8,000 new diagnosed patients annually, with a total number of malignant diseases of 35,000-40,000, and 3,500-4,000 of deaths (2,3,16). Malignant diseases mortality rates have an increasing time trend ($y=118.7+6.7x$). The average standardized mortality rates of Serbia regions show geographical variations due to way of life, culture, diet (1)… Malignant diseases in Belgrade are presented with 20%; all other diseases with 80%. The number of patients with malignancy and deaths had increased for 40% in the last ten year on the territory of Belgrade (6,16). Results of other studies show that there are many more possibilities for decreasing number of lungs, liver and stomach cancer on the level of prevention. The number of breast, cervix and colon cancers could decrease with better methods of screening, and the number of Hodgkin disease, testis cancer and leukemia could be decreased with satisfactory therapy methods (9,10,20,21).

In 2020 year, there will be 12 million people living in Serbia. From 32,000 new diagnosed malignancies (data for 1997), 70,300 new diagnosed malignancies are expected in 2020. There were 20,000 deaths due to malignancies (in 1997), and 40,000 deaths are expected in 2020 (5,18). If good preventive measures are applied the projected number of 70,300 new diagnosed patients should decrease on 37,000. Also, with good preventive measures, instead 40,000, we would expect 22,000 deaths in 2020 (4,5,18).

DISCUSSION

Smoking, infection and diet are risk factors for some „usual" malignancies. We have calculated that 388 people (from 1,945) would not get bladder, pancreas or kidney cancer if they stopped smoking. This is decrease of 20%. By smoking cessation, 5,664 people (from total 7,080) would not get lung, larynx or oropharynx caner. The decrease is much greater - 80%. Is it worth

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it?

What could infection prevention and appropriate diet do? With effective prevention and infection therapy 146 people (from 730) would not get bladder cancer. Also, with same measures 4,320 people (from total 5,400) would not get stomach, cervix, and liver cancer, and non Hodgkin lymphoma. Appropriate diet should decrease the number of liver, prostate and oropharynx cancer for 705 cases (from 2,820) and stomach, colon, breast and esophagus cancer for 5,407 (from 7,210) (18). In addition, cost benefit is measured in 100 millions dollars (5). As of the etiology of malignant diseases, the causes are considered to be multifactorial. They could be exogenous and endogenous. The exogenous factors are cancerogens (chemical elements, compounds), physical agents (radiation), biological agents (viruses), and their combinations. Endogenous factors, psychostress in the first place, physiological individual balance through nervous system, endocrine system, causes numerous metabolic disorders. The increase of lung cancer in men, but also in women is expected in the next 10 years (18). The same situation is with colon, breast, prostate, liver, CNS, bladder, pancreas, uterine body, ovary cancer and also with leukomia.

CONCLUSION

The presented epidemiology data regarding malignant diseases and their projection until 2020 demands new algorithms for cancer prevention and screening. The plans for some malignancy algorithms are near the end, but it is needed for them to become part of whole community, governments, and not only the part of health services.

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The epidemiological data about colorectal cancers

ABSTRACT

Colon and rectum cancers are the most frequent cancers of the gastrointestinal tract. They are on the third place after lung and prostate cancers in men and on the second place in women after breast cancer. Incidence and mortality rates are increasing. The data sources are Federal Statistical Office, Population Register of Carcinoma, Hospital Cancer Register of the Oncology and Radiology Institute, Health Care Fund of Serbia, Internet. We have used common statistical procedures and rate standardization, correlation coefficient, determination of the trends in time and PC-sofrware. The population of Serbia is about 10 million. There are about 2,700 and 3,000 new diagnosed colorectal cancers per year. The incidence rate is increasing and the highest rate is recoreded on territory of Belgrade and Vojvodina (1,800-1,900 colon cancer and 900-1,000 rectum cancers). There are 700-800 deaths of colon cancer per year (the trend is increasing) and 830-860 deaths due to rectum cancer. The highest standardiazed mortality rate is in Vojvodina. Incidence rates have been increasing in Serbia. North and Eastern European countries. The appearance of these cancers has been connected with high fat and protein intake, and low vegetable, fruit and fiber intake. Considering above data we suggest organizing of long-term primary and secondary prevention in the purpose of decreasing mortality and incidence colorectal cancer rates.

KEYWORDS: Colorectal Neoplasms + epidemiology; Yugoslavia

INTRODUCTION

Colon cancers are among the most common cancers in Serbia and Western European countries (after breast and lung cancers). Incidence and mortality are high in north and west parts of the European Community (1). The similarity of male-female geographic distribution is not striking as it is with stomach cancer (6). The lowest rates are in southern countries, i.e. in Italy, Spain, Portugal and Greece (Mediterranean diet). The aim of this paper is to perceive epidemiological situation on colorectal cancer in Serbia.

MATERIALS AND METHODS

Descriptive and analytical methods with use of statistic software (SPSS) are used. The data base was formed on mortality statistics of Federal Statistical Office, and on morbidity statistics of Population Register of Carcinoma, Hospital Cancer Register of the Oncology and Radilogy Institute, Health Care Fund of Serbia, Internet.

RESULTS

Morbidity statistics: The number of new diagnosed colon cancers is 1,800-1,900 per year, and 900-1,000 for rectum cancers. The standardized incidence rates for colorectal cancers are 44-44,8 in Central Serbia, 61,1 in Belgrade, and 61,5 in Vojvodina and 7,1 in Kosovo (the data for Kosovo are not reliable because of the irregulations during the last census). The time trend is increasing for all rates (2). Mortality statistics: The number of colon cancer deaths is 736-770 per year, and 842-856 for rectum cancers. The standardized mortality rates for colon cancers are 6,5 for Central Serbia, 9,5 for Vojvodina and 0,7 for Kosovo (the data for Kosovo are not reliable). The standardized mortality rates for rectum cancers are 7,9 for Central Serbia, 12,0 for Vojvodina and 1,2 for Kosovo (the data for Kosovo are not reliable). Mortality rates for rectum cancer are especially increasing after age of 45. The mortality time trend is increasing (2,3).

Gender: The rates (mortality, morbidity) for colorectal cancers are similar for male-female distribution (2).

Age: This cancer is the most frequent in elderly. The incidence rate increases after 50 years, with the peak in 80's. The medium age when colorectal cancer is diagnosed is 67 (4).

Anatomy: Some differences for colorectal cancer frequency are recorded regarding the colon segments. In the last three decades cancers of the right colon are becoming more frequent. So, the number of rectum cancers is decreasing (from 40-50% to 16%, and for the righ colon cancers from 12-15% to 27%). The distribution of cancers is> 27% for right colon, 10% for transverse colon, 36% for descending and sigmoid colon, 16% for rectum and 3% for anal cancers (2,4).

Survival: The five-year survival is between 26 and 29% for the colon cancer, and 31-70% for the rectum cancer. Like other cancers, if disease is diagnosed earlier, the five-year survival will be longer (3).

Etiology: The colorectal cancer in multi etiology disease (7,9). on the basis of the case-control studies the most common risk factors are poliposis, adenomas, dietary habits, holecystectomy, hereditary factors, inflammatory diseases, obesity, cigarette and alcohol abuse (8).

DISCUSSION

Colorectal cancers are on the second place with 13% of all cancers in EU (1), and in Serbia they participate with 11,3% of all cancers. In Serbia, the incidence and mortality time trend are increasing (2). The therapy results are not satisfying. The best five-year survival is with rectum cancer - if it is diagnosed on time. But, the colorectal cancer, in the most cases, is diagnosed while regional metastases exists (about in 40-50%). It is recorded that there are more cancers on the right colon, in the last decade. They are not easy for diagnostics and also the symptoms become obvious in the late stage of the disease. Specific and sensitive tests for organized screening of the cancer do not exist. Opinions about hemocult test as screening method are opposite (7). The only thing left is organized and continuous primary prevention - elimination of numerous risk factors.

CONCLUSION

Incidence rates have been increasing in Serbia. North and Eastern European countries. The appearance of these cancers has been connected with high fat and protein intake, and low vegetable, fruit and fiber intake. Considering above data we suggest organizing of long-term primary and secondary prevention in the purpose of decreasing mortality and incidence colorectal cancer rates.

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Kupffer cells in viral hepatitis associated with heroin abusers

ABSTRACT

Having in the mind that Kupffer's cells are the most important category of fixed reticuloendothelial cells and that they participate in the host defense to infection, we have studied them in 14 autopsied heroin abusers with chronic active hepatitis and posthepatitis cirrhosis. Laboratory sections of liver specimens were stained with HE, Van Gieson, PAS and Gomori methods. Statistically, the significant difference in the number of KCs between the control and heroin abuser-group, has been observed. The authors have concluded that Kupffer's cells were active in heroin abusers with liver infection.

KEYWORDS: Kupffer cells; Histology; Heroin Dependence

INTRODUCTION

Kupffer's cells (KCs) are the most important category of fixed reticuloendothelial cells (1). They are thought to constitute the largest population of fixed macrophages in humans and various vertebrate species. In many diseases, an increase and sometimes a depletion of liver macrophages is known to occur (2,3). KCs are macrophages with phagocytic capacity for rather large particles. Their shape and the surface is irregular; the cells have elongeted cytoplasmic processes that can strech along or underneath the endothelium. They lie upon or are embedded in, or covered by the endothelium (4). Having in mind that KCs participate in the host to infection and that may modulate the resistance of the host to infection, it is of interest to investigate the change in numbers and distribution of KCs in normal liver and in viral hepatitis associated with heroin abusers.

MATERIALS AND METHODS

Forty intravenously heroin addicts with heroin abusing history of 2-15 years were autopsied. Ten young persons who died from traffic accident were also autopsied as a control group. Liver specimens were routinelly fixed, processed and embedded in paraffin. Laboratory sections were stained with HE, Van Gieson and Gomori methods. Morphometrical analysis of KCs was studied by using Test M 42 system. Statistical analysis of the obtained results

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was performed by using the Student t test.

RESULTS

From 40 heroin abusers autopsies, the examination of KCs has been done in 8 cases with chronic active hepatitis (CAH) and in 6 cases with chronic active hepatitis associated with cirrhosis (CAH with C). Compared with KCs in controls, marked hyperplasia and hypertrophy of these cells has been found in heroin abusers' autopsies. Statistically, the number of KCs showed a significant difference between the control and heroin addictsí group.

DISCUSSION

KCs are histochemically characterized by a high endogenous peroxidase activity and by high activities of glucose-6-phosphate dehidrogenase. In addition to their phagocytic capacity, KCs handle low density lipoproteins, and produce lymphokine mediators that direct protein synthesis by hepatocytes (5). Moreover, they produce hepatocyte-protective prostaglandins (6). KCs induce specific immunity to antigenic material that they remove from the portal blood. In some situations, however, KCs can act as antigen-presenting cells. So, in pathological conditions, such as inflammatory reactions, they can initiate I and B-lymphocyte mediated immunity (2,6). Lastly, KCs secrete two groups of factors. The first group comprises prostaglandins (in particular PGE2) and leucotrienes which play an important role in the inflammatory reaction (2). The second group contains IL-1 and IL-6, interferon and TNF-alfa. These factors play an important role in the regulation of the immune response, for instance through the activation of T lymphocytes, B lymphocytes and natural killer cells. Interferon and TNF-alfa are also directly involved in the antiviral and antitumor response. Our results about hyperplasia, hypertrophia and hyperactivity (7) of KCs in heroin addicts with hepatitis and posthepatitic cirrhosis confirm the suggestion that KCs have a very important role, not only in the antivirous and antibacterial liver infections, but also in the development of posthepatitic cirrhosis, probably via their cytokines and growth factors.

CONCLUSION

On the basis of the obtained results, we have concluded that Kupffer cells are very active in heroin addicts with chronic active hepatitis and with posthepatitic cirrhosis.

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Evaluation of radiology and histopathology in the diagnosis of Barrett's oesophagus

ABSTRACT

A form of reflux oesophagitis is now clinically, endoscopically, radiologically, and pathologically distinguished as Barrett's oesophagus. Associated with hiatus hernia, or not, the lower oesophagus is lined by gastric mucosa, either gastric fundic-type mucosa or gastric antral mucosa below the junction. A retrospective study of 20 patients with oesophageal columnar metaplasia and with symptoms of a pathological gastro-oesophageal reflux was performed. The patients were examinated by radiological, endoscopical and microscopical methods. In Barrett's oesophagus of our 20 patients, we have found: peptic oesophagitis, peptic ulcer, low grade dysplasia, high grade dysplasia and metaplastic epithelium of columnar or intestinal type associated with adenocarcinoma. Not only that biopsy specimens confirm radiological and endoscopical characteristics of Barrett's oesophagus, but they also discover precancerous lesions of the incomplete type of intestinal metaplasia and dysplasia, preventing, in that way, the development of adenocarcinoma by a surgical therapy.

KEYWORDS: Barrett Esophagus; Radiology; Endoscopy; Pathology

INTRODUCTION

In 1950 Norman Barrett described a patient with an oesophageal peptic ulcer high in the thorax with columnar epithelium below (1). Barrett's interpretation was that there was a marked oesophageal shortening and that the columnar lined segment below the stricture was a stomach-made tubular by oesophageal shortening. Barrett's anatomical interpretation was incorect and subsequently revised to acknowledge that the columnar lined segment was oesophageal, the normal squamous epithelium being replaced by columnar epithelium following the healing of severe peptic oesophagitis (2). Because that Barrettís oesophagus is a precancerous condition and its diagnosis is relatively rare, we have decided to study its radiological, endoscopical and histological characteristics.

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MATERIALS AND METHODS

A retrospective study of 20 patients with oesophageal columnar metaplasia and with symptoms of a pathological gastro-oesophageal reflux. The patients were examinated by radiological, endoscopical and microscopical methods. To confirm the endoscopic diagnosis, endoscopic biopsy material was fixed in 10% formaldehyde, routinelly processed and embedded. Laboratory sections were stained with HE, PAS and HID-AB, pH=2,5.

RESULTS

Clinical characteristics. The most frequent symptoms in our patients were induced with acid hypersecretion and both gastric content and duodenal content reflux. In one patient with Barretts oesophagus and adenocarcinoma, dysphagia was the presenting symptom.

Radiological characteristics. There is no pathognomonic radological finding for Barrettís oesophagus. This diagnosis was suggested by a benign appearing stricture of the mid or proximal oesophagus, a marker of a high squamo-columnar junction. The presence of a long stricture above the hiatus hernia is also higly suggestive of Barrett. The presence of deep oesophageal ulceration is strongly suggestive of oesophageal columnar metaplasia, but can also be due to squamous cell carcinoma.

Endoscopical characteristics. The sensitivity of endoscopic recognition of high squamo-columnar junction is heavily dependent on the endoscopist's expirience. The columnar lined segment has a velvety, salmon pink appearance wich often, but by no means always, contrasts sharply with the pale, glossy squamous epithelium. Finger-like projections of columnar epithelium may extend over several centimetres up into the oesophagus, alongside squamous mucosa. Islands of metaplastic columnar epithelium may also be seen.

Pathohistological finding. Biopsy is essential to confirm the endoscopic diagnosis of Barrett's oesophagus. In our study, endoscopically recognized columnar epithelium, was microscopically confirmed in 78% of patients. In Barrett's oesophagus of our 20 patients, we have found: peptic oesophagitis, peptic ulcer, low grade dysplasia, high grade dysplasia and metaplastic epithelium of columnar or intestinal type associated with adenocarcinoma.

DISCUSSION

The malignant potential of Barrett's oesophagus is the prime reason for the current high level of interest in this condition (3,4). This risk is the highest in patients with high grade dysplasia (5,6). If multiple foci of high grade dysplasia are present, resectional surgery should be the first management option considered, especially in the relatively young, fit patients (6). If it is elected not to undertake oesophageal resection in a patient with high grade dysplasia, at least 6-monthly histological sampling is recommended (6).

CONCLUSION

Not only that biopsy specimens confirm radiological and endoscopical characteristics of Barrett's oesophagus, but they also discover precancerous lesions of the incomplete type of intestinal metaplasia and dysplasia, preventing, in that way, the development of adenocarcinoma by a surgical therapy.

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Characteristics of gastric-type adenocarcinomas

ABSTRACT

The characteristics of gastric-type adenocarcinomas were studied in endoscopically removed hyperplastic polyps large in size and in surgically resected stomach with adenocarcinomas. Histologically sections, fixed in 10% formaldehyde, were stained with: HE, GOS, CPS III, HID-AB, pH=2,5 and Streptavidine/biotin technique (using anti-PCNA antibody). Gastric-type adenocarcinoma was composed of columnar cells with clear to slightly basophilic cytoplasm, closely resembling foveolar epithelial cells, and the nuclei were well-polarized. Cells in the upper layer were positive for GOS, and those in the lower layer were positive for CPS III. PCNA-positive cells were confined to the middle level of the normal mucosa and randomly densely distributed in the gastric-type of cancer.

KE WORDS: Stomach Neoplasms; Adenocarcinoma; Histocytochemistry; Gastric Mucosa + pathology

INTRODUCTION

Lauren (1965) advocated categorizing stomach cancer into two histological types, differing in morphology and epidemiological characteristics. Intestinal adenocarcinoma is more usual in males and older age groups. This type is prominent in high risk areas and has been decreasing its relative frequency in the US and Norway, where mortality rates have been declining (1). The studies showed that the intestinal type varies in place and time and is likely to be linked to environmental factors (2). The diffuse type of carcinoma is equally frequent in both sexes, is more common in younger age groups and has a worse prognosis than the intestinal type (3). Recent reports suggesting that adenocarcinoma of the stomach may be derived from both foveolar hyperplasia and hyperplastic polyps and that it is named the gastric type (4), oriented us to define histochemical, morphological and immunohistochemical characteristics of gastric carcinoma.

MATERIALS AND METHODS

This study was based on 10 hyperplastic polyps removed endoscopically and 10 resected stomachs with gastric cancer. The specimens were fixed in 10% formalin for 24-48 h and embedded in paraffin. The methods were as follows: Classic, HE-for histological diagnosis of polyps and carcinomas; Mucin-histochemical studies: Galactose-oxidase/Schiff stain (GOS, for mucin of the surface mucous cell type); Concanavalin A paradoxical staining for stable class III mucosubstances CPS III, for mucin of the mucous neck cell/pyloric gland type, and High-Iron Diamine/Alcian Blue (pH=2,5) stain (HID-AB), for the intestinal types of mucins. Immunohistochemical studies: PCNA (Proliferating Cell Nuclear Antigen), using Streptavidine/biotin techniques.

RESULTS

Histologically, the gastric-type adenocarcinoma was composed of columnar cells with clear to slightly basophilic cytoplasm, closely resembling foveolar epithelial cells, and the nuclei were well polarized. In hyperplastic polyp-cancers (73% gastric-type), cancer was frequently seen near the surface layers of the polyp, and the cells often showed a cribriform pattern or intraluminal papillary proliferation, with a background of edematous stroma with strong inflammatory cell infiltration. In intra-mucosal carcinoma, seen in surgically resected stomachs, villous proliferation was often observed. Histochemically, mucin cancer cells in the upper layer of cancerous glands had GOS-positive surface-mucous cell type mucin, whereas cells in the lower layer had CPS III - positive pyloric-gland cell type mucin. PCNA staining showed that the rate of PCNA-positive cell in gastric-type adenocarcinomas tended to be lower than in other histological types of differentiated type carcinomas, similar to undifferentiated type carcinomas. PCNA-positive cells tended to be localized in the middle and lower levels of cancerous glands, or randomly distributed over the entire cancer tissue.

DISCUSSION

Altough the development of cancer was previously considered to be a rare phenomenon in hyperplastic polyps, which are the most common type of gastric polyps, 2-4% of these lesions have been found to become cancerous (4-6). Kozuka with his collaborants (1977) suggests that hyperplastic polyp-cancer might result from atypical foveolar hyperplasia occuring in the polyp (7). Gastric type adenocarcinomas may arise in the gastric mucosa lacking hyperplastic polyps, when foveolar hyperplasia takes place. Stump carcinomas in humans, and gastric adeno-carcinomas in animals induced by chemical carcinogenes (8), in which intestinal characteristics are rarely seen, might also represent gastric type neoplasias. In these cancers, foveolar hype-rplasia rather than intestinal metaplasia may be the lesion predisposing the development of carcinomas.

CONCLUSION

Gastric type adenocarcinomas may arise in the gastric mucosa lacking hyperplastic polyps, when foveolar hyperplasia takes place.

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Characteristics of epithelial metaplasia in human stomach

ABSTRACT

The recent evidence suggests that gastric cancer is the end result of a series of events manifested by the chronic gastritis complex. Endoscopical and surgical biopsies from 100 patients with: more advanced stages of atrophy of chronic gastritis (20), duodenal ulcus complicated by pyloric stenosis (20), adenocarcinoma (40) and the operated stomach (20 patients). A series of progressive changes representing gradual loss of differentiation is postulated after the gastric process has reached by the stage of atrophy and various types of epithelial metaplasia: intestinal (types I and II), pyoric, ciliated and pancreatic-acinar type, confirmed by our results. So, cancerogenesis is a multi-stage process in which the key events represent changes in the microenvironment, composed by the gastric mucosa and the content of the gastric cavity.

KEYWORDS: Stomach Neoplasms; Metaplasia; Epithelium; Gastritis; Immunohistochemistry; Intestinal Mucosa + pathology

INTRODUCTION

Histochemical and immunohistochemical techniques reveal the functional divergence of carcinomas of the gastrointestinal tract and their tissues of origin. On the other hand, certain non-neoplastic disorders of differentiation, which loosely may be termed metaplasias, may reveal many of the functional alterations associated with a malignancy. Some of these metaplasias may be precancerous, whereas benign metaplastic lesions may signal the presence of a potentially carcinogenic microenviro-nment and linked indirectly with malignant transformation (1-3). Having in the mind that the role of epithelial metaplasia in the histogenesis of gastric carcinoma has been the subject of much debate (4,5) and that new data brought more light into metaplastic stomach lesions (6,7), we decided to describe them.

MATERIALS AND METHODS

Endoscopical and surgical biopsies from 100 patients with: more advanced stages of atrophy of chronic gastritis (20), duodenal ulcus complicated by pyloric stenosis (20), adenocarcinoma (40) and the operated stomach (20 patients). Formalin fixed, routinelly processed and paraffin embedded specimens were stained with HE, PAS, HID-AB, pH=2,5 and a paradoxical

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concanavalin staining for class III mucin.

RESULTS

The following types of epithelial metaplasias were found: intestinal, pyloric, pancreatic and ciliated type.

Intestinal metaplasia (IM). IM may be subdivided into complete (type I) and incomplete (type II), „colonic", forms. Colonic type is sulfomucin secreting variant of IM, and distributed in both corpus and antral mucosa. IM type I was found in 68% of adenocarcinoma, 52% of atrophic gastritis, 32% of operated stomach and in 5% of duodenal ulcer. IM type II was found in: 42% of adenocarcinomas, 37% of atrophic gastritis, 25% of operated stomach and 2% of duodenal ulcer with pyloric stenosis.

Pyloric metaplasia (PM). PM is restricted to the corpus mucosa. It starts in the distal body mucosa and gradually spreads proximally. Mucous neck cells proliferate and gradually replace the specialized chief and parietal cells. At the end, gastric glands resemble those in the atrum, and the distinction between fundic and antral mucosa on biopsy becomes impossible. PM was found in 19% of adenocarcinomas, 39% of fundic (A type) gastritis, 76% of operated stomach and 1% of duodenl ulcer.

Ciliated metaplasia (CM). In atrophic gastritis with intramucous cysts, surrounding antral ulcer-cancer, we have observed the presence of cilia on the free border of cells. The cells bearing cilia have vacuolated cytoplasm. Special histochemical stains demonstrated the absence of mucus substances. CM coexisted with intestinal metaplasia.

Pancreatic (acinar) metaplasia (PAM). In two operated stomachs because of duodenal ulcer with pyloric stenosis, the new type of metaplasia characterized by the occurence of the pancreatic acinar cells within the glandular epithelium of the antral gastric mucosa, was discovered. Acinar cells, often arranged in small acini, were located only in antral glands.

DISCUSSION

The metaplastic epithelium of the complete type has the structural and histochemical characteristics of small intestinal crypts and includes absorptive cells in varying stages of maturation, sometimes with microvilli, goblet cells and Paneth's cells. Argentaffin cells are increased in number, containing serotonin in a rich amount (8). The goblet cells of complete IM secrete mucins of N- and O-acylated sialomucin and a small amount of sulphomucin. Certain endocrine cells, normally found predominantly in small intestinal crypts (e.g. D, A, Mo, I) have been demonstrated in areas of IM (9,10). In chronic gastritis with advanced intestinal metaplasia, a full complement of small intestinal enzymes appears in the surface epithelium (3,4). Lacking differentiation in IM is manifested by a decrease in the more differentiated cells and an increase in goblet cells. This colonic metaplasia, the sulphomucin secreting variant of IM, occurs most often in assotiation with the intestinal type of gastric cancer (4). The secretion of sulphomucin may be linked in several ways to gastric carcinogenesis: it may be signal of presence of a carcinogenesic microenvironment, the adapted cell may (by its production of sulphomucin) be able to survive and proliferate in the presence of mutagen when normal cells would be destroyed and finally, neoplastic clones which are protected by the secretion of sulphomucin, may be better to resist peptic digestion (5). Due to the development of immunohistochemistry, mucin antigens (M1, M2, M3 and Sialosyl-Tn antigen) for foveolar, deep gastric mucosa (pyloric and Brunner glands) and for „pyloric metaplasia" were discovered (3). The definition of new gastric-type of adenocarcinoma in the stomach and its origin from hyperplastic polyps and foveolar or pyloric metaplasia, are based on the results of the immunohistochemical study.

CONCLUSION

So, cancerogenesis is a multi-stage process in which the key events represent changes in the microenvironment, composed by the gastric mucosa and the content of the gastric cavity.

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Patohistological indicators for predicting fibrosis in chronic hepatitis C (CHC)

ABSTRACT

The course of chronic hepatitis C is difficult to predict, even after antiviral treatment. Although, the disease is frequently asymptomatic, the development of liver fibrosis, and cirrhosis is it's major complication. There are no reliable biological or clinical indicators that can predict which patient will develop fibrosis, but if they would be found that would be of help in selecting patients for antiviral treatment. The aim of this study was to asses the patohistological features which are coexisting with greater potential for fibrosis in CHC. Forty liver biopsies, seen for the first time during the procedure of CHC diagnosis, were analysed using semiquantitative method for the degree of fibrosis and the presence of histological features common to CHC. The results show very high (up to 70%) correlation of fibrosis and number and frequency of focal lobular necrosis. It is suggest that focal lobular necrosis can be the parameter for predicting the fibrosis of the liver in CHC. The analysis of frequency and number of lobular necrosis can be a histological parameter for predicting and following the evolution of fibrosis in chronic hepatitis C.

KEYWORDS: Liver Cirrhosis; Hepatitis C, Chronic; Liver; Necrosis

INTRODUCTION

Chronic hepatitis C (CHC) is the inflammation of the liver, which is caused by hepatitis C virus and last for more than six months with the potential for progression to cirrhosis or can be joined with cirrhosis (2). The course of CHC infection, even after anti-viral treatment, is difficult to predict. Although, the disease is frequently asymptomatic, the development of liver fibrosis is common, and cirrhosis, occurring of about one in five cases (4) is the major complication in this disease. There are no reliable biological or clinical indicators that can predict which patients will develop fibrosis, but if they would be found that would be of help in selecting appropriate patients for anti-viral treatment. Histological examination of liver biopsy in CHC is useful during the diagnostic process and has critical role in evaluation necro-inflammatory changes, so it allows activity and fibrosis to be assessed. It has been suggested that fibrosis in B virus hepatitis is the result of activity and thus, histological activity can predict fibrosis and cirrhosis. The attempt in the usage of this hypothesis on CHC is inadequate, after the studies of large series of liver biopsies have shown that in most patients with CHC histological activity is mild,but cirrhosis is frequent. The analysis of histological changes, which suggests fibrosis in CHC, is the mode of investigation of fibroses and cirrhoses in this disease. The aim of this study is to assess pathohistological features which are coexisting with greater potential for fibrosis in CHC.

MATERIALS AND METHOD

We have evaluated the degree of fibrosis in the 40 liver biopsies in CHC by using semiquantitative method. We have also made the comparison and correlation to the main patohistological features present in all biopsies. Forty biopsies and five controls were analyzed after standard preparation for light microscopy. The samples were stained by HE, van Gieson, reticulin and trichrom-Mallory. For each biopsy specimen, the semiquantitative assessment of elementary features potentially present in CHC hepatitis has been performed using by a standardized questionnaire (4) Histological activity index of Knodel (periportal t bridging necrosis, lobular necrosis, inflammation and portal fibrosis scores) was separately recorded (3).

RESULTS

The mean time interval between the infection and a liver biopsy is 7,66 months (3- 36 months). For each biopsy spacimen, the principal histological feature and the degree of activity according to METAVIR and Knodel index where: fibrosis graded as F1 in 23 patients, F2 in 11 patients, F3 in 5 and F4 in one. Four patients had no activity (Ao), 17 had mild activity (A1), 18 had moderate activity (A2), and one had severe activity (A4). In 70% of our patients fibrosis was in correlation to the number and frequency of focal lobular necrosis.

DISCUSSION

The histomorphological examination of liver biopsies,on which the first biopsy has shown the presence of fibrosis not being in correlation with histological activity. That result is similar to some other reports in the literature (4) . Comparison of histological features, which was the constant finding in CHC, have shown strong correlation in frequency and number of intralobular necrosis, except in the group of drug abusers. The investigations have shown that exacerbation of CHC is followed by increased number of intralobular necrosis. The greater quantity of TGFp1 in the liver tissue at the site of lobular necrosis was also found. It is known that TGFG1 has important and/or critical rule in pathogenesis of fibrosis in chronic hepatitis and cirrhosis by stimulation of stem and Ito cells (1).

CONCLUSION

The analysis of frequency and number of lobular necrosis can be a histological parameter for predicting and following the evolution of fibrosis in chronic hepatitis C.

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Malignant pancreatic somatostatinoma with lymph node metastasis

ABSTRACT

Pancreatic endocrine tumors represent a rare, heterogeneous (ten classes) group of tumors that produce active hormone and result in distinct clinical syndromes. A 41-year-old woman had two years' history of diabetes mellitus, chronic diarrhea, vomiting and abdominal pain. Both ultrasound and computer tomography examination demonstrated a tumor in the left hypochondrium. During the operation a part of the pancreatic tail with solitary, sharply demarcated tumor 10 cm in diameter and the spleen were removed. The routine histological examination suggested a pancreatic endocrine tumor. The same tumor metastatic deposit occupied one peripancreatic lymph node. Immunohistological staining showed positivity for neuroendocrine cells' markers and somatostatin with negativity for other specific pancreatic endocrine cells' markers. Pancreatic somatostatinomas are often associated with somatostatinoma syndrome, a clinical triad including steatorrhea, diabetes mellitus and cholelithiasis. The immunohistochemistry plays a key role in the diagnosis of the specific population of endocrine tumor cells. The resection of the tumor is a recommended treatment. Our patient lives without any signs of illnes.

KEYWORDS: Pancreatic Neoplasms; Somatostatinoma; Lymphatic Metastasis

INTRODUCTION

Pancreatic endocrine tumors (PET's) are a heterogenous group of tumors that produce active hormone and result in distinct clinical syndromes. PET's can be divided on a clinical and pathological basis into ten classes, among them one of somatostatinomas (1). Somatostatinoma is a rare tumor. For the first time it was described in 1977 as of pancreatic, and two years later as of duodenal origin (2,3). The pancreatic somatostatinomas are often associated with somatostatinoma syndrome, a clinical triad consisiting of steatorrhea, diabetes mellitus and cholelithiasis. The duodenal somatostatinomas are frequently associated with von Recklinghausen's disease (4). We report a case of malignant pancreatic somatostatinoma with lymph node metastasis.

CASE REPORT

In 1997 a 41-year-old woman was for the first time addmited at the Internal Department of our hospital and examined for mucosanguineous chronic diarrhea, vomiting, loss of the body weight and diffuse adbominal pain. Coproculture was negative. The blood glucosa showed pathological levels and the patient was registred as a new case of diabetes mellitus and treated with a diet and oral antidiabetic drugs. Family history was negative for endocrine disorders. The second hospitalisation was in 1999 with similar symptoms. The ultrasound examination revealed a solid tumor mass 10 cm in diameter, located between the spleen and left kidney. Irrigoscopy and intravenous urography was normal. Computer tomography scan demonstrated a tumor in the left hypochondrium between the spleen, upper pole of the left kidney and behind the stomach. The tumor was sharply contoured, it measured 92x80 mm. The great part of the tumor showed hyperdensity with small scattered areas of hypodensity. Both ultrasound and computer tomography examination suggested that the tumor was probably of pancreatic origin. During the operation the surgeron found a tumor at the pancreatic tail. The tumor, part of the pancreatic tail and the spleen were removed. The postoperative course was normal. The operation wound healed by first intention. The patient had no previous symptoms of illness. The blood glucosa level normalized.

RESULTS

Gross pathology: The tumor was a demarcated, "encapsulated", round mass sized 10x9.5x9 cm. Its surface was nodular. The section disclosed a firm, nearly uniformly homogeneous cut surface, yellow-greyish in color, with small haemorrhagic and necrotic areas. The spleen showed no gross abnormalites. The surgeron resected a small peripancreatic lymph node 6 mm in diameter. Microscopical examination: The tumor samples were fixed in formalin and embedded in paraffin. Approximetly 5 mm-thick section stained with haematoxilin&eosin and periodic acid-Schiff methods. For immunohistological analysis the following antigens were localized using their respective antibodies: cytokeratin, NSE, chromogranin, synaptophysin, insulin, glucagon, gastrin, somatostatin, PP, IAPP, estrogen and progesteron. Microscopically, the tumor was composed of solid nests and gyriform structures, ribbons and festoons of small, relatively uniform cuboidal cells with finely granular amphophilic cytoplasm. The nucleus was centrally located with a visible nucleolus. The nuclear pleomorphism was mild to moderate in degree. The mitotic index was sparse. The stroma of the tumor was highly vascular with abundant hyaline material that separates the tumor cells. No psammoma bodies were found among the tumor cells. The cytoplasm of tumor cells were PAS negative. The tumor cells showed diffuse positivity with a group of immunohistochemical markers specific for endocrine pancreatic cells and their tumors (NSE, chromogranin, synaptophisin). The second group of markers had allowed the mapping of the specific endocrine population of pancreatic cells and their tumors. Among specific markers, our tumor showed positivity for somatostatin and negativity for other markers. The histologically same tumor metastatic deposit occupied the resected peripancreatic lymph node. Our final diagnosis was malignant pancreatic endocrine tumor - somatostatinoma - with lymph node metastasis.

DISCUSSION

PET's are rare neoplasms. However, by a careful examination of the pancreas carried out at necropsy small PET's are found in over one per cent of all cases. Most of these tumors produce no ill effects (5). The histological examination of HE section allows the simple diagnosis of endocrine cell tumors of the pancreas. The immunohistochemistry plays a key role in specific diagnosis of mapping of the specific population of endocrine tumor cells. Delta cells tumor, somatostatinoma is generally seen as nonfunctioning at the clinical level because of the fact that somatostatin is an inhibitory hormon. However, as a result of the inhibitory properties, the patient may be present with diabetes, cholelithiasis, steatorrhea, indigestion, hypochlorydia and

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occasionaly anemia (6). The histological recognition of the malignant potential of these tumors can be very difficult. Such diagnosis must only be made where there is indisputable evidence of infiltration or metastasis, such as in our case. The long term course of malignant PET's with metastasis may be relatively good (7). Recently, at the consensus conference, a resection of the tumor was the proposed treatment (8). Our patient has been living six months without any signs of the disease.

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Malignant lymphoepithelial lesion of the parotid gland

ABSTRACT

Malignant lymphoepithelial lesion (MLEL) is a tumor of the salivary glands which develops from benign lymphoepithelial lesion (BLEL). The malignant transformation of the BLEL is rare and it takes place in the course of many years, usully as malignant alteration of the lymphoid component in Malt lymphoma. There are a few literature date on malignant transformation of the epithelial components into squamos carcinoma with spindle or pleomorphic cell and, even rarely, into adenocarcinoma. This is why we chose to report the present case. This is a case report of a 75-year-old-man patient with clinical sings of a painless englarment of the left parotid salivary glands, wich was first noticed 24 year before. The englared gland was surgically removed. Macroscopically, it had a changed structure, with firmed areas of white-greyish color. The pathohistologic analyses showed the presence of a lymphoepithelial lesion with multifocal regions of malignant alteration of the epithelial componenet towards squamous carcinoma with spindle shaped cells. The material also contained the regional lymph nodes with tumorous deposits having the same characteristics as the epithelial component of the tumor. The authors report an interesting and rare case of a parotid gland tumor manifested as a malignanat lymphoepithelial lesion with a transformed epithelial component. The occurrence of metastatic deposits in regional lymph nodes and the perineural spreade of the tumor testifies to its malignanacy and its sudden aggressive bahavior after more than two decades of benignanty.

KEYWORDS: Parotid Neoplasms; Salivary Gland Neoplasms; Parotid Gland + pathology; Epithelial Cells + pathology

INTRODUCTION

Malignant lymphoepitelial lesion (MLEL) is a very rare tumor of the salivary glands with a somewhat higher incidence in the population of Canada, Alaska and Eskimos (1). It is mostly localized in the area of parotid gland, but it can also be found in the other large and small salivary glands. The tumor usully develops by malignant transformation of benign lymphoepithelial lesion (BLEL) and can be manifested as a MALT lymphoma if the lymphoid component is malignant altered or, rarely, as an anaplastic or poorly differentiated planocellular carcinoma if the epithelial componenet undergoes malignant changes. There are some litrature date on the cases of transformation into adenocarcinoma as well (2). The aim of the paper was to report a case of

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MLEL with the transformation of the epithelial component into a planocellular carcinoma of spindle-cell.

CASE REPORT

The authors report a case of a 75-year-old man patient with a slow and long-term (24 year), painless englaring of the parotid gland without sings of malignanacy. At tle Clinic for Maxillofacial Surgery in Novi Sad, the whole parotid gland was removed together with the regional lymph nodes. The material was sent to the Departmant of Pathology and Hisotlogy of the Novi Sad for pathohistologic analysis. It was macroscopically described as a roundish, lobulated, whitish, homogenous tissue with a surrounding connective tissue. From macroscopically chosen samples, standard histologic preparations was made and stained with haematoxylin and eosin and pathohistologically analyzed. Light microscopy lead to the diagnosis of a lymphoepithelial lesion with a multifocal malignanat alteration of the epithelial componenet, and the formation of fields of tumorous spindle epithelial cells with low degree of atypia; they were surrouded by a rich lymphous tissue of usual histologic properties. The normal acini were almost completely replace by the tumorous tissue and found only in the subcapsular part of the gland. In the parotid gland capsule, a perineural infitration was found. In the regional lymph nodes, there were tumorous masses whose characteristics were indentical to the MLEL epithelial component.

DISCUSSION

The basis of development of MLEL or a poorly differentiated carcinoma with lymphoid stroma is a benign lymphoepithelial lesion (BLEL) wich occurs more often in women in the sixth or seventh decade of life (3). The changes are usully localized in the parotid gland, but can be found in the other large or small salivary glands as well (2,4), The pathogenesis of BLEL is unknowen. It is considered to develop due to changes in the ductal epithelium of the large periferial ducts (5,6), with a consequent accumulation of mature lymphocytes around the canals and formation of epimyoepithelial islets (5). The malignant transformation of BLEL can progress in two direction: towards a malignant alteration of lymphoid componenet and the formation of MALT lymphoma, or, very rarely, by a malignant alteration of epimyoepithelial islets, towards anaplastic carcinoma or poorly differentiated planocellular carcinoma with spindle cells, like in our case. In investigating MLEL in relation to BLEL, there was a rise of the antibody titer of the Epstein- Barr virus, the expression being p53, and a proliferative cellular activity (1,4,7,8), but they were not examined in our case.

CONCLUSION

This is a case of an ineteresting and rare tumor of the parotid gland, wich was manifested as a malignant lymphoepithelial lesion with the transformation of epithelial componenet. The presence of the metastatic deposits in the regional lymph nodes and the perineural spread of the tumor confirm its malignant nature and explains its suddenly aggressive behavior after its long-term benign development.

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Coeliac disease manifested by constipation and endocrine disfunctions

ABSTRACT

A case of a 17-year old patient, with 8-month history of constipation, anorexia, amenorrhea and cachexia, is presented. The absence of typical gastrointestinal symptoms and adolescent period of life were the main causes for a significant delay in the diagnosis of coeliac disease. The diagnosis of coeliac disease has been suggested during endoscopically examination and proved by micromorphological and histochemical analysis.

KEYWORDS: Celiac Disease; Constipation; Endocrine Glands + physiopathology; Histochemistry

INTRODUCTION

Coeliac disease, or Gluten-sensitive enteropathy (GSE) is defined as a condition in which there is an abnormal jejunal mucosa that responds morphologically to the treatment with a gluten-free diet (1). The condition mainly involves proximal small intestine and is usually most severe proximally and less severe distally. It may have its onset either during early childhood, and thus some authors restrict the term coeliac disease to childhood sprue, or during adulthood. Clinically, it is characterized by steatorrhea, diarrhea and loss of body weight due to malabsorption of food, water and minerals. Our aim is to report a case with constipation, increased loss of body weight and endocrine disfunction.

MATERIALS AND METHODS

Our patient is a 17-year old girl, with a history of anorexia, loosing her body weight (18 kg for 8 month) and amenorrhea. The absence of characteristic gastrointestinal symptoms (steatorrhea and diarrhea) and the adolescent period are the reasons for neurological diagnosis #anorexia nervosa#. After her admission to the hospital, gastroduodenoscopy was done and duodenal biopsies from II and III segment were taken. Formalin-fixed and paraffin embedded specimens were stained with HE, PAS, HID-AB, pH=2,5 and Massonís argentaffin reaction for an identification of EC cells.

RESULTS

Clinical characteristics were: marked cachexia, anorexia, constipation and amenorrhea. Duodenal mucosa was endoscopically atrophic. Decreased serum levels of LH (0,2 mIU/ml) and Estradiol (27 pq/ml) were detected.

Micromorphological characteristics: histopathologic evaluation of the intestinal mucosa was based on the following parameters: mean height of villi, epithelial characteristics, lymphocytic infiltration of epithelium, inflammatory changes of the lamina propria, crypt depth, and number of mitotic figures in the crypts. The following classes were known: class I (normal mucosa), class II (partial villous atrophy), class III (subtotal villous atrophy) and class IV (total villous atrophy). Our case was classified into class III. By histochemical study regarding the mucins of the small and large intestine we found goblet cells with HID positive (brown-black) and AB-positive (blue) mucosubstances. EC cell hyperplasia has not been observed.

DISCUSSION

GSE may have its onset, either during early childhood, and thus some authors restrict the term coeliac disease to childhood sprue, or during adulthood. Clinically, it is characterized by steatorrhea, diarrhea and weight body loss, due to malabsorption of food, water and minerals (2-4). Gluten, the protein moiety of wheat barley and rye and probably also present in oats, is thought to bind to a receptor on the surface intestinal epithelial cells and to sensitize the immunocytes between the epithelial cells and in the lamina propria. Sensitization is associated with the generation of lymphokines and antibodies to gluten, as well as activation of lymphocytes, resulting in domage to the epithelial cell to which the gluten is attached. The exact mechanism of gluten toxicity is not known, but evidence suggests that cell-mediated immunity to gluten may be involved (2,3). Both atypical clinical picture and adolescence of our patient are the reasons for a significant delay in the diagnosis of coeliac disease.

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Buying time till liver transplant

ABSTRACT

The outcome of portal hypertension caused by surgically non-correctable abnormalies is considered fatal without liver transplant. A girl, age 5 years, well nourished, mildly jaundiced, with moderately distended abdomen, cavernous umbilical veins, liver 3.5 cm and spleen 6 cm below costal margin was referred to hospital. Since infancy she had pale stools and mild jaundice occasionally and for one year enlarged abdomen and dilated umbilical veins. Results: Liver function tests were abnormal. Image techniques (liver US, portal system Doppler, magnetic resonance, splenoportography, hepatic venography and cavography revealed liver nodularity, enlarged caudate lobe (30x50mm), dilated portal vein (PV) to 15x12mm and lienal vein (LV), patent umbilical vein, v.cava inferior (VCI) compressed for around 90% in a 55mm long prestenotic portion, posteriorly displaced, with slow flow (7-8cm/sec.), lienal pulp pressure 40cm H20, reversed flow in PV, stenotic hepatic veins and collateral circulation. Liver biopsy showed bile duct paucity and fibrosis. Two episodes of bleeding, severe anemia and thrombocytopenia prompted operative treatment. Patch graft to stenotic VCI, taken from girl's mother vena saphena, and spleno-renal shunt were performed, resulting in VCI dilatation by around 70% and lienal pulp pressure drop (8cm H20). Till 8yrs. the girl was doing well, with very slow enlargement of spleen and deepening of jaundice. Now, at 10yrs. she suffers severe chronic liver deasease. Low protein diet and lactulose keep blood amonia moderately increased and despite substitution, hypoalbuminaemia and hypopotassaemia persist. The girl is on the waiting list for liver transplant, as her only cure.

KEYWORDS: Hypertension, Portal; Child; Liver transplantation

INTRODUCTION

Portal hypertension (PH), a sporadic problem in children, irrespectively of the etiology, is a serious medical challenge. The underlying cause is governing the severity of its progression and unless the basic abnormality is surgically correctable, which is rare, the outcome is fatal without liver transplant.

CASE REPORT

A girl, 5 yrs. old, a refugee from Bosnia (village near Bihać), was referred to paediatric gastroenterology for splenomegaly. Her previous history was documented with only one medical report and information gathered from her grandparents (father killed in the war, mother suffering of a mental depression) was poor. At the age of 6 weeks, she was hospitalized for jaundice, at the Paediatric Clinic in Zagreb (no discharge list available). Occasionally, she had pale stools and mild jaundice. At the age of 4.5 years, she was hospitalized at the Paediatric Department in Banja Luka. The discharge list stated only the diagnosis (Cholestasis intrahepatalis. Cirrhosis hepatis). The war in Bosnia forced the family to flee from the region and after several months long refugee journey, settle near Novi Sad. At that time, the girl had enlarged abdomen and dilated umbilical veins for around a year, but was physically very active. She was referred to hospital for splenomegaly.

RESULTS

On admission, age 5 years, the weight was 19 kg (50 pct.) and height 116 cm 75 pct, jaundice was mild, abdomen moderately enlarged, umbilical veins cavernous, practically non-firm liver 3.5 cm, and firm spleen 6 cm below the costal margin. Liver function tests were abnormal. Liver, spleen US and portal system Doppler revealed hypoechogenic liver nodules (15-20 mm), enlarged caudate lobe (30x50 mm), dilated portal (PV) and lienal vein (LV), recanalised umbilical vein (fast flow, 25cm/sec.), vena cava inferior (VCI) compressed and posteriorly dysplaced by hypertrophied caudate lobe, slow flow of 7-8 cm/sec, the spleen 16.6 cm. On magnetic resonance VCI was dilated (22x11 mm) in a 55 mm long prestenotic portion, as PV (15x12 mm). Splenoportography, hepatic venography and cavography revealed: lienal pulp pressure of 40 cm H20, reversed flow in PV, stenosis of distal VCI for about 90% and of hepatic veins, collateral circulation through gastric coronary veins with oesophageal and fundic gastric varices. Liver biopsy showed disarranged lobular structure due to fibrosis with formation of lobules and pseudolobules; bile ducts significantly reduced in number. Deepening jaundice, two episodes of bleeding, severe anemia, thrombocytopenia of around 30x109/L and gigantic spleen, resulted in operative treatment at the age of 5.5 years. Stenotic VCI was dilated with patch graft created from vena saphena obtained from the girl's mother, during a simultaneous operation. End to side right renal and lienal vein anastomosis (central spleno-renal shunt) was also created. The immediate result of the operation was: VCI diameter increased for about 70%. On control, splenoportography, hepatic venography and cavography trans-stenotic flow was fast with gradient of 6 cm H20 and lienal pulp pressure drop to 8 cm H20. For nearly two years, she was doing well, with very gradual deepening of jaundice and enlargement of the spleen, which was significantly decreased in size after the operation. At the age of 8 years, the girl was physically still very active, attending school. Her weight was 30 kg (75 pct.) and height 138 cm (95 pct.), bone maturation was significantly accelerated, the liver was 4.5 cm and spleen 5 cm below costal margin, bilirubin 180 (mol/L and platelets around 80x109/L. Now, at the age of 10 yrs. the girl suffers from a severe chronic liver deasease with cachexia (weight 34 kg, 50 pct. and height 148 cm, 97 pct.), deep jaundice (bilirubin around 300 (mol/L), prominent finger and toe clubbing, recurrent, bilateral knee joint transsudation, firm spleen 7 cm below the umbilicus, firm liver 3 cm below costal margine. Hypoprotein diet and lactulose keep her blood amonia at a moderately increased level. Serum albumin and potassium are low despite substitution. She does not attend school any more and is capable to walk only short distances. ERCP showed abnormal bile ducts in both lobes, right hepatic duct with irregular lumen and ramification and deformed branches, bile ducts in the left lobe dilated (Caroli's like). Upper GI endoscopy did not confirm oesophegadel varices, yet. Osteodensitometry of L2-L4 is consistent with osteomalacia.

DISCUSSION

Operation and subsequent death of the patient's younger sister with jaundice, suggest similar or the same cause of the liver disease. Syndromic bile duct paucity (Alagille syndrome) is excluded in this girl due to lack of any other physical abnormalities. Idiopathic, non-syndromic bile duct paucity is a possibility while Caroli's disease is not very likely - left lobe bile duct dilatations could be secondary due to fibrosis. Budd-Chiari syndrome resulting from vena

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cava inferior stenosis is undoubted. However, the basic underlying cause is questionable, including not only the bile duct, but also a vascular congenital abnormality.

CONCLUSION

A set of unfavourable circumstances - low educational level of the patient's family ignoring her symptoms and a 5 months long refugee travel to final settling place in Vojvodina postponed the diagnosis, but probably would not change the outcome which is inavitabely fatal without liver transplantation. Over two past years, efforts to organise the liver transplant on humanitarian basis abroad have failed. The girl is now on the waiting list for the liver transplant in Yugoslavia.

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Feline infectious peritonitis (FIP) - the first cases diagnosed on our section material

ABSTRACT

Our report describes the first cases of The Feline Infectious Peritonitis (FIP) diagnosed over the last two years on our section material. Positive reaction to coronavirus antigens has been obtained on the tissues of three cats (the two of which were domestic females, while the third one was Persian male). The two cats have shown the effusive form of the disease whereas the third cat has shown granulomatous form of the disease. Granulomas on the liver, pleura, kidneys and omentum were composed of fibrinoid-necrotic component with lymphocyteÒmonocyte infiltrate and numerous angioblasts and fibroblasts. Though typical for infectious peritonitis, pathoanatomical and histopathological findings are not always sufficient for obtaining an accurate diagnose of the disease. This explains the necessity to identify antigens of coronavirus, as it has been performed in our study, using peroxidase antiperoxidase (PAP) immunohistochemical method.

KEYWORDS: Feline Infectious Peritonitis; Immunohistochemistry; Histocytochemistry

INTRODUCTION

Feline infectious peritonitis (FIP) is caused by a coronavirus closely related to a transmissible gastroenteritis virus (TGEV) of swine, canine coronavirus (CCoV) and human coronavirus (229E) (3). Recently FIP has been more frequently diagnosed with domestic cats, though some wild species of felidae are susceptible to the disease as well (1,7). Over the past two years, the first cases of FIP have been diagnosed in our section material. The disease occurs in both effusive and non-effusive forms with changes in the area of abdominal cavity, such as aggregating of a golden-yellow gelatinous exudate followed by fibrinous material on peritoneum and in some cases with granulomatous changes in abdominal cavity as well. Histological structure of these granulomas is typical in samples stained with hematoxylin-eosin (HE), though immunohistochemical confirmation is necessary for a definite diagnosis of virus antigens.

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MATERIALS AND METHODS

Organs of three cats (two domestic females aged 2 and 5 years and a Persian male aged 4) with pathoanatomical findings suggestive of FIP were taken after autopsy for pathohistological and immunohistochemical studies. Samples of the liver, spleen, kidneys, mesenterial lymphnodes and mesentery were fixed in 10% neutral formalin and after the usual procedure embedded in paraffin. After xylol deparaffinization and rehydration in a series of alcohol, the 3-5mm thick tissue sections were stained by hematoxylin-eosin (HE). The same series of sections was subjected to immunohistochemical study by peroxidase-antiperoxidase (PAP) method. To demonstrate coronavirus antigen, sectinos were treated with target unmasking fluid (TUF; Dianova GmbH, Hamburg, Germany) for 10 min. at 96∞C. After pretreatment, 10% rat serum in TBS was applied for 10 min at room temperature prior to the monoclonal mouse antibodies. Slides were then incubated for 12-16h at 4∞C with the primary antisera: mouse anti-corona virus (FCV3-70; 1:100 in TBS; Kipar, Institute for Veterinary Pathology Giessen, Germany). For the mouse mAbs, rat anti-mouse IgG (1:100 in TBS, Dianova, GmbH) and mouse PAP complex (1:500 in TBS; Dijanova GmbH) were applied. Incubations were performed at room temperature for 30min. each. Between each incubation step slides were washed with Tris-buffered saline (TBS, 0,1M Tris-HCl with 0.9% NaCl, pH 7,6). Endogenous peroxidase was blocked by incubation with 0.3% hydrogen peroxide in methanol at room temperature for 30min.Visualization of PAP reaction was achieved with diaminobensidine (DAB/0,1M imidazole-HCl, pH=7,1) for 10 minutes. The samples were then counterstained with hematoxylin and coversliped. Negative controls for mAbs were incubated with normal rat serum.

RESULTS

The studied cats (two domestic females and a Persian male) were in their mature age (2, 5 and 4 years, respectively). Macroscopically, the abdominal cavity of all three cats revealed gelatinous golden-yellow exudate and fibrin deposits on the parietal peritoneum and omentum. In two cats necrotic lymphadenitis of the mesenterial lymphnodes was noted, and in one (Persian, aged 4) grayish-white submiliary nodules were seen on the liver. The third cat (domestic, aged 2) had fibrino-granulomatous pleuritis. This animal had a co-infection with feline leukemia virus (FeLV) which was previously evidenced by immunohistochemical methods. Histological structure of granuloma in the liver, kidneys, omentum and pleura was similar. They were composed of fibrinoid-necrotic, granulocyte, lymphocyte and monocyte components and multiplied angioblasts and fibroblasts. On the mesentery lymphnodes and in the spleen follicular hyperplasia was noted. Immunohistochemical PAP method revealed that corona virus antigens were expressed in the macrophage cytoplasm and, less commonly, in necrotic fields. Granuloma found in the liver contained also some positive plasma cells expressing a lower amount of the viral antigens. Negative controls did not show any positive staining.

DISCUSSION

In the studied material the exudative form was prevailing in two cats (a domestic cat aged 2 and a Persian cat aged 4) while the proliferative form manifested as granulomatous pleuritis and granulomatous peritonitis was described in one animal (a domestic cat, aged 2). Histologic structure of the granuloma with fibrinoid-necrotic fields, lymphocyte-monocyte infiltrate, angioblasts and fibroblasts corresponded to the description of a granuloma reported by other authors (5,6). Expression of the viral antigens was noted predominantly in the macrophage cytoplasm and exudate itself. In our study the coronavirus antigens were evidenced on formalin-fixed and paraffin-embedded samples. The histopathological findings in spontaneus and experimental feline infectious peritonitis (FIP) have been described in several previous reports on effusive and non-effusive FIP (2, 4). Although usually characteristic both macroscopic and histological alterations were not always indicative of FIP. Therefore, differential diagnose can sometimes not be readily exscluded by routine post-mortem histopathology, and the diagnosis of FIP has to be confirmed by immunohistological demonstration of coronavirus antigen within the lesions.

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Prognostic factors in colorectal carcinoma

KEYWORDS: Colorectal Neoplasms; Prognosis

Morbidity and mortality due to colorectal carcinoma (CRC) are increasing in both sexes. The aim of the work was to establish valid prognostic factors regarding the outcome of the disease. Sixty-eight patients treated for CRC were included in the analysis. The anatomic localization, stage of the disease, macroscopic appearances and microscopic characteristics of the lesions were analyzed. Colorectal carcinoma was localized in the left colon and rectum in 76% of cases. It was present in the right colon in the form of exophytic, cauliflower-like tumorous mass, and in the transversal and left colon as an annular, infiltrative-ulcerous lesion. In the rectum, it was present in both macroscopic forms. At the time of surgery, it most frequently was in B and C stage of the disease. A differentiated type of adenocarcinoma was diagnosed in 87% of the cases, and non-differentiated-anaplastic carcinoma in 13%. Extracellular mucus was detected in 18%, and in 4%, a signet ring cell carcinoma was found. Although CRC of the right colon was detected later, its prognosis was better. Exophytic tumors had better prognosis than infiltrative/ulcerous tumors. Pathohistologically, non-differented types and signet ring carcinomas had worse prognosis. Extracellulary present colloid had no prognostic significance. The more advanced stage of the disease, the worse the prognosis.

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Echinococcosis in the region of Banjaluka

KEYWORDS: Echinococcosis + epidemiology; Biopsy

Echinococcus is a Cestode which has three different forms: E.granulosus, E.multilocularis, E.Vogeli. When a larv-form of Echinococcus gets intoa human or animal body, it produces an illness in different forms cystic, alveolar, polycystic hydatid disease, depending of the Echinococcus form. The material of makcroscopic end microscopic resarch consists of 54 radically removed cysts of Echinococcus located in olivers organs, in the period from 1989 - 1999 in the Banjaluka region. Three cases of Echinococcus are registertd on the arerage per a year. The of cases were detected in the year 1997 (17 cases - 31%). Most of the cases were registrated in age group from 37 to 46 years (10 cases - 18.56%), more often in women (35 cases - 64.81%).The illness is mostly located in the liver (33 cases - 61.11%), lungs (12 cases - 22.22%). Scolex was found in 56%of the cases. All cases had a chronic nonspecific inflammatory infiltrate and 5% of the cases had a granulomatous infiltrate. This illness occured mostly in Banja Luka (43%) and Mrkonjić Grad (9% cases). Echinococcosis appears in the biopsy material of Banja Luka majoriti of 3 cases per year, with an increasing tendency few years.

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Gastric mucosa in patients with chronic renal failure

KEYWORDS: Kidney Failure, Chronic; Gastric Mucosa; Gastritis; Uremia; Helicobacter pylori

The objective of the study was to evaluate gastric histology and Helicobacter pylori status in patients with chronic renal failure undergoing maintenance hemodialysis treatment. Upper endoscopy with gastric biopsy was performed in 23 uremic patients under hemodialysis with dyspepsia and gastrointestinal bleeding. Two antral and corpus biopsies were subjected to histopathologic assesment. H. pylori infection was detected histologically by Giemsa method. A control group comprised a series of gastric biopsies of 62 consecutive non-uremic patients with dyspepsia. Histological examination of the gastric mucosa of the uremic patients showed gastritis in 18/23 (78%) patients; active chronic gastritis was established in 16 cases, and H. pylori infection in 15 of them. In the control group gastritis was found in 71% of the cases with H. pylori infection in 72%. H. pylori infection in 72%. H. pylori infection was encountered only in active chronic gastritis in both groups. There were no significant differences in the incidence of gastritis ($p>0,05$) and H. pylori infection ($p>0,05$) between the patients with chronic renal failure and the control group. However, some histological abnormalities of the gastric mucosa were seen in uremic patients. Cystic dilatation of the fundic and antral glands, parietal cell hydropic swelling and coagulative necrosis were frequently noted. Fibrosis of the lamina propria and muscularis mucosae, and peculiar posthemorrhagic changes in propria were present in a few cases. The prevalence of histologically proven gastritis and H. pylori infection was not different in uremic and non-uremic dyspeptic patients. The prevalence of H. pylori infection in uremics is rather the matter of the infection per se, than it is specifically associated with the higher gastric urea content.

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Adenocarcinoma of the appendix

KEYWORDS: Appendiceal neoplasms; Adenocarcinoma

Adenocarcinomas of the appendix are very rare. They are probably found in 0.01% or less of all operated appendixes. Most tumors induce symptoms of an acute appendicitis. The adenocarcinoma of the appendix is macroscopically small and occasionally it is detected incidentally during histological observation of the appendix. The histological features of an appendiceal carcinoma are those of a large bowel cancer in general and neoplasmas are mostly well differentiated adenocarcinomas. We present the adenocarcinoma of appendix in a 59 years old female patient. The disease began as the acute appendicitis. We have made the diagnosis with many macroscopical cuts, taken from various places of the appendix, and with three histological staining methods H&E, PAS and Grimelius. Many young pathologists have not seen this rare carcinoma. We have therefore presented our case, giving the hints for making the diagnosis.

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Epitheloid leiomyosarcoma of the small intestine

KEYWORDS: Leiomyosarcoma; Intestine Small

Epitheloid leiomyosarcoma (malignant leiomyoblastoma) of the small intestine is a rare neoplasma, which occurs with patients at an early age. It is histomorphologically caracterised in these malignant forms the cells are less mature, as it is evidenced by the less abudant cytoplasm and greater degree of pleomorfism in mitotic activity . The cells may be arranged in sheets, in small whorls around blood vessels, in a pseudoalveolar pattern. The author presented a case of a 35 years old patient, surgical treated because of acut pain of the abdomen . Solitar tumors are excided , dimensions 150 x 100mm et 10mm, which are soft and contain areas hemorrhage et necrosis. Apart from the standard HE staining the obtained surgical material was also reviewed histochemicali (PAS , PAS and Reticulin) and imunohistochemically (Aktin, Desmin, S l00 and F VIII). A number of ultrastructural studies of leiomyoblastomas have appeared, although it is not always clear whether these tumors were considered benign or malignant. The tumor has varied ; some showed all the caracteristics of smooth muscule cells (thin myofilaments, cytoplasmatic and subplasmalemal dens bodies, basal lamina and intercellular junction). The patient died ten months after the surgery.

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Warthin's tumor: clinical significance and pathohistological features

KEYWORDS: Salivary Gland Neoplasms; Adenolymphoma

Warthin's tumor (papillary Cystadenoma Lymphomatosum) is a relatively rare benign entity, but the second most common salivary gland neoplasm. It arises almost always in the parotid gland (virtually restricted to it), predominantly in males, usually in the fifth to seventh decades of life. About 10% are multifocal, and 10% bilateral. Seven patients with this tumor were studied in order to determinate its pathohistological features, clinical significance, diagnostic dilemmas and possible histogenesis. Surgical specimens were fixed in 10% formaldehyde, routinely processed and embedded in paraffine. Laboratory sections were stained with HE and AB-PAS. Out of seven patients, 5 were males and 1 female. In all of them, the tumor was localized in one parotid gland, 3,5-6 cm in diametar, movable, palpable, encapsulated, round or oval. The clinical diagnosis was different - from „Tumor of parotid gland" to „Lymphoma". The transection reveals a pale, gray surface, punctuated by narrow cystic spaces filled with mucinous or serous section. Microscopically, these spaces are lined by a double layer of epithelial cells resting on a dense, lymphoid stroma, sometimes with germinal centers. The double layer of lining cells is quite distinctive, with a surface palisade of columnar cells, having an abundant, granular cosinophilic cytoplasm, imparting an oncocytic appearance, resting on a layer of cuboidal to palygonal cells. Frequently, the spaces are narrowed by polypoid projections of the lymphoepithelial elements. Secretory cells are dispersed in the columnar cell layer and occasionally, there are foci of squamous metaplasia. Warthin's tumor (sometimes also called adenolymphoma) because of its shape and localization, is often confused with a malignant lymphoma. The dense lymphoid stroma sometimes is bearing germinal centers which are frequentlly misconstructed to imply a metastasis of adenocarcinoma. But as tumor is movable, and there is no evidence of cellular or nuclear polymorphism (in the glandular component) and mitoses and, first of all, of a subcapsular sinus, those are the most important criteria for the diagnosis of Warthin's tumor. Having the interesting architecture, many authors are encouraged to believe that this tumor arises from absent inclusions of the lymphoid tissue into the parotid gland (this histogenesis has long been disputed). There are sporadic reports of malignant Wathin's tumors. The diagnostic dilemma, being always on a pathologist's mind may be, on the basis of our results, resolved. The absence of cellular and nuclear polymorphism in the glandular component of the tumor, as well as the absence of the subcapsular sinus, are, in our opinion., the most important features that support the diagnosis.