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Clinical Article

Analysis of Fluid in Craniopharyngioma-Related Cysts in Children: Proteins, Lactate and pH

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Summary

The purpose of this study was to evaluate the possible role of blood-brain barrier disruption in cyst formation in craniopharyngioma. Fifteen samples of cyst fluid and 14 samples of blood serum were collected from 14 patients with cystic forms of craniopharyngiomas and studied biochemically regarding total protein, albumin, immunoglobulins G and M contents, lactate and pH. Analysis of the data obtained for cyst fluids according to Felgenhauer and comparing them to those obtained for the corresponding blood sera led us to prove the hypothesis of blood-brain barrier impairment in patients with cyst formations in craniopharyngioma. We have also revealed an elevated lactate content and decreased pH in cyst fluids compared with blood sera. Thus the pathogenesis of craniopharyngiomal cyst appears to be much more akin to those described for cysts accompanying other brain tumours than it was believed earlier.

Keywords: Cranioharyngioma; brain cyst; blood-brain barrier; lactate.

Introduction

Craniopharyngiomas are polymorphous intracranial tumours which commonly show cystic, solid or mixed patterns. Aspiration of the tumour cyst content often results in symptomatic improvement, but after the aspiration the fluid and subsequent symptoms usually recur. The recurrence of the fluid suggests its active production by the tumour or by the invaded tissue or its passive accumulation through disruption of the blood-brain barrier (BBB) and leakage of protein into the cyst. It seems to be proved that cysts associated with gliomas and some other brain tumours such as ependymomas, hemangioblastomas and metastases have a pathogenesis bearing upon blood-brain barrier impairment [7]. However there are only few publications concerning research into the fluid contents of craniopharyngiomal cysts [3, 12, 15].

The aim of this study is to measure the concentrations of lactate and of some proteins in cyst fluid of craniopharyngiomas and to compare these data to those described for the other brain tumour cysts.

Patients and Methods

Fifteen samples of cyst fluid and 15 samples of blood serum were collected from 14 patients aged from 3 to 15 years with cystic craniopharyngiomas. There was one patient with two independent cysts. Aspiration of cyst fluid was performed during surgery. In the case of normal viscosity and transparency of the fluid, the pH was measured immediately with a Ciba-Corning 280 Blood Gas Analyzer and the fluid was frozen at $-20\,^{\circ}C$. Turbid fluids were centrifuged at 3000 g for 10 min. and the supernatants were also stored at $-20\,^{\circ}C$ until used (less than 2 months).

There were also 3 patients from whom several samples of cyst fluid were taken from an Ommaya reservoir followed by the replacement of aspirated fluid by an equal volume of saline. These specimen were deproteinated with 10% TCA (1 : 2 v/v) and stored at $-20\,^{\circ}C$ for less than two months.

The concentration of lactate in protein-free cyst fluid was measured manually using a Biocon "Lactate-PAP" kit (Germany). Total protein in serum and cyst fluid was measured by the biuretic method and albumin (Alb), immunoglobulin G (IgG) and immunoglobulin M (IgM) were measured by the turbidimetric method with a Cobas INTEGRA700 analyzer (Roche, Switzerland) according to recommendations of manufacture. The measured concentrations of proteins were expressed as the ratio Q = [serum concentration]/[cyst fluid concentration]. A Felgenhauer gradient curve was constructed by plotting the Q values against the hydrodynamic radius (R = 3.58 nm, 5.34 nm and 12.1 nm for serum albumin, IgG and IgM respectively) of the correspondent protein in semilogarithmic co-ordinates [2].

Results

Table 1 presents the Q-ratios for Alb, IgG and IgM from which Felgenhauer curves are plotted. Protein gradient curves for 15 samples of cyst fluid are shown

Table 1. *Q-Ratios and Indexes for Serum Proteins in Patients with Cyst Craniopharyngiomas*

N	$Q_{albumin.}$	Q_{IgG}	Q_{IgM}	IgG index	IgM index
1	0.80	0.63	0.95	1.27	0.84
2	0.76	0.36	0.78	2.11	0.97
3	1.59	1.18	1.70	1.35	0.94
4	1.58	1.16	1.56	1.36	1.01
5	1.71	1.18	1.70	1.45	1.01
6	1.13	0.96	1.07	1.18	1.06
7	0.93	0.68	1.58	1.37	0.59
8	0.67	0.48	0.77	1.4	0.87
9	1.42	1.33	2.34	1.07	0.61
10	1.05	0.83	1.29	1.27	0.81
11	0.85	0.69	1.24	1.23	0.69
12	2.65	2.49	3.08	1.06	0.86
13	1.37	0.72	2.61	1.9	0.52
14	0.70	0.51	3.28	1.37	0.21
15	0.65	0.37	1.36	1.76	0.48
Mean	*1.19*	*0.90*	*1.69*	*1.41*	*0.76*
SD	*0.54*	*0.54*	*0.79*	*0.30*	*0.24*

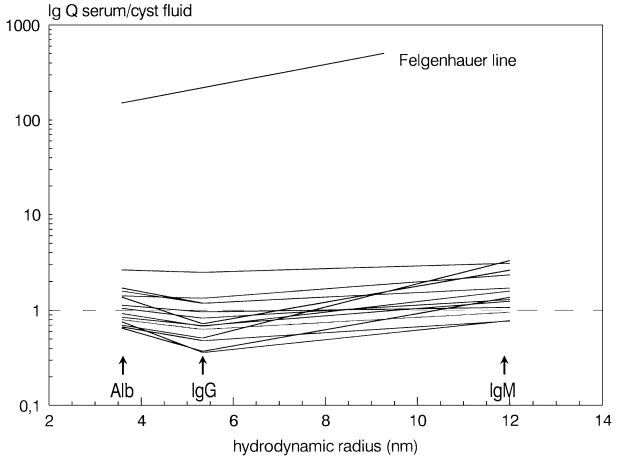

Fig. 1. Semilogarithmic plot of Q-ratios for proteins studied against their molecular hydrodynamic radius. The normal protein gradient curve (Felgenhauer line) represents an intact blood-brain barrier

Table 2. *Lactate Concentration and pH in Cyst Fluid and Blood Serum of the Patients with Cyst Craniopharyngiomas*

N	Cyst fluid		Blood serum lactate (mmol/l)
	lactate (mmol/l)	pH	
1	4.3	7.30	1.3
2	10.2	6.95	1.4
3	10.6	ND	1.7
4	10.9	ND	2.7
5	4.2	ND	1.9
6	5.9	7.15	1.1
7	8.5	ND	2.7
8	3.2	ND	1.0
9	5.5	7.28	1.3
10	13.0	ND	0.1
11	5.9	7.35	0.3
12	7.1	7.06	0.7
13	3.5	ND	0.3
14	7.6	7.28	2.8
Mean	*7.2*	*7.20*	*1.4*
SD	*3.1*	*0.14*	*0.9*

ND Not determined.

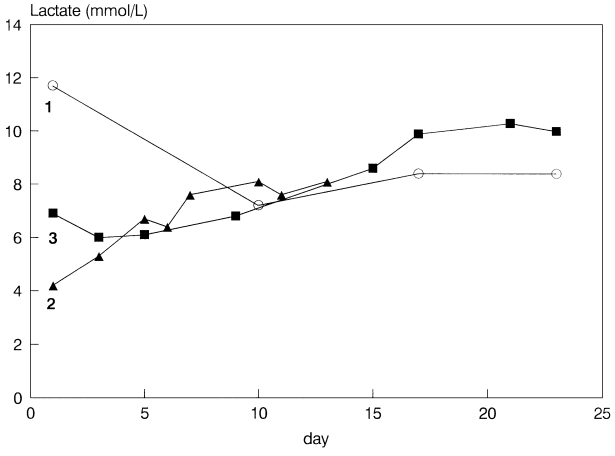

Fig. 2. Time courses of lactate contents in cyst fluids of 3 patients. Each line marked by the corresponding patient number. All samples of cyst fluid were taken from an Ommaya reservoir

in Fig. 1. All these curves are much lower than the normal plasma/CSF curve reported by Felgenhauer [2]. These data strongly support the hypothesis of blood-brain barrier impairment for patients with craniopharyngioma cysts. In addition, Table 1 presents IgG and IgM indexes reflecting local synthesis of these proteins [9, 14]. The mean IgG and IgM indexes are 1.41 ± 0.3 and 0.76 ± 0.24 respectively. Thus IgG local production was found in 13 of 15 cyst fluid samples.

Table 2 presents the data of pH values and lactate concentrations measured in cyst fluid and blood serum, collected from 14 patients with craniopharyngiomas. All the measured lactate concentrations in cyst fluid were much higher than the serum blood level ($p < 0.001$). Moreover, there is no correlation between lactate contents in cyst fluid and blood serum ($r = 0.11$, $p > 0.05$). With the exception of one sample (N 11), pH values in cyst fluid were lower than the normal blood pH level of 7.32.

Time courses of lactate concentration in cyst fluids of 3 patients, obtained from Ommaya reservoirs are shown in Fig. 2. "Washing out" of cyst cavities in 2 cases (2 and 3) led to the increase of lactate content, and in the case of extremely high lactate concentration

(N 1) to the decrease of this parameter. All free curves reached a plateau at about 8–9 mmol/L.

Discussion

It is well known that the large cysts frequently found in some intracranial tumours can cause neurological symptoms and signs regardless of the size of the tumour. Thus, the study of mechanisms of cyst formation seems to be very important. At present the pivotal role of BBB disruption in the formation of cysts in gliomas is well proved by several research groups [1, 6, 7, 10]. Very interesting data regarding serum proteins, lactate and pH in cyst fluids accompanying primary and metastatic brain tumours were published by P. Lohle *et al.* in 1998 [8]. The study was made mainly in glioma-associated cysts. The authors excluded craniopharyngiomas as tumours with lining of neural epithelium because they believed that these cysts are not the result of blood-brain barrier disruption [7]. Indeed it is known that the peripheral epithelium in craniopharyngiomas is the actively proliferating component, which probably plays a role in tumour cyst formation. It is also known that craniopharyngiomas are hormonally dependent [5, 13] and secretorily active tumours [3, 4].

One year later J. Vaquero *et al.* [15] reported that the formation of cysts in craniopharyngiomas also related to the expression of vascular endothelial grow/permeability factor (VEG/PF) by tumour cells, as it was shown previously for gliomas. So we would like to compare our data obtained in craniopharyngiomas with those published by Lohle. We have found that the protein gradient curves of craniopharyngioma-associated cyst contents shown in Figure 1 are much below the normal plasma – CSF curve, described by Felgenhauer and in most cases even further below the corresponding curves, described by Lohle for glioma-associated cyst fluids. The first portions (from Q albumin to Q IgG) of 13 from 15 curves have a reversed slope indicating the local synthesis of IgG. The mean value of IgG index obtained in our study differs significantly from that calculated from the data published for gliomas (1.41 ± 0.30 and 0.81 ± 0.14 respectively, $p < 0.001$).

We also found elevated lactate levels in cyst fluids of craniopharyngiomas, which did not correlate with serum lactate levels (Table 2). These results are in good agreement with the data of the proton magnetic resonance spectroscopy, reported by L. Sutton *et al.* [11]. They have found that all craniopharyngiomas showed a dominant peak at 1 to 2 ppm, consistent with lactate or lipids, with trace amounts of other metabolites. In contrast to gliomas, pH in craniopharyngiomal cyst fluids was shown to be slightly below the normal blood level corresponding to elevated lactate concentration. Repeated partial removal of cyst fluid did not lead to the decrease of lactate concentration to the level determined in blood. Time courses of lactate in cyst fluids of three patients with Ommaya reservoirs implanted into the cyst cavity are shown in Fig. 2. In the first case extremely high lactate level of 12 mM decreased. Contrarily, in the second and third cases lactate concentrations increased reaching in both cases approximately the same level of 8–9 mM, which could be considered as an equilibrium lactate level in craniopharyngiomal cyst fluids.

This study confirms that in spite of major histopathological differences between epithelial and glial-cell-lining tumours the mechanisms of cyst formation and enlargement are similar.

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Comments

This is a study of 15 children who underwent surgery for cystic craniopharyngioma. The authors aspirated the fluid and then analyzed it for various chemicals including protein, lactate and pH. They concluded that the cystic fluid most probably comes from a defective blood brain barrier around the tumor rather than from cellular debris and exudate from tumor cells themselves.

This is clearly different from what most neurosurgeons (including myself) have believed over the years.

I am not certain at this point whether this will make any difference in the clinical management of craniopharyngiomas, but it is clearly a different concept than most neurosurgeons have believed about cystic craniopharyngiomas.

J. T. Hoff

The message of the authors is that craniopharyngioma cysts do not appear different from cysts of other intracranial tumors testing the proteins, lactate and pH of the cyst fluid. However, craniopharyngiomas are more complex lesions than presented in this report. Relying on their findings, the authors cannot conclude that cysts associated with craniopharyngiomas have a pathogenesis similar to those associated with other intracranial lesions, like gliomas. There are some major histopathological differences which should be taken into consideration and should be discussed by the authors (epithelial- vs. glial-cell-lining). It has been postulated that the peripheral palisaded epithelium is the actively proliferating component in craniopharyngiomas, which is probably playing an important role in the pathogenesis of the cysts and its content. Therefore, it has to be discussed whether the recurrence of the cyst is not only due to the disruption of the blood-brain-barrier but is also as the result of active fluid production by the cyst wall. One has also to keep in mind, that craniopharyngiomas are hormonally dependent, since hormonal influences may stimulate their growth. Studies have demonstrated the presence of estrogen and progesterone receptors in human craniopharyngiomas. These results provide evidence that the messenger RNA of these hormones can be produced by human craniopharyngiomas and that this is translated into biologically active receptor protein. Proliferation and active fluid production may be the effect of hormonal influences on the cyst wall and this is certainly a matter for further debate. The secretory activity of craniopharyngiomas is also proved by the presence of hCG, which has been considered to be specific for germ-cell tumors, in the cyst fluid. This cannot be the result of disruption of the blood-brain barrier. The conclusion should be that the pathogenesis of the cyst is multifactorial. In addition to the hormonal influences and the secretory activity of the lesions, the postulated disruption of the blood-brain barrier may also play an important role in formation and enlargement of craniopharyngioma cysts.

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