Antimicrobial effects of craniopharyngioma cystic fluid

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Abstract

Background Tumors are known to increase the risk of infections, more-so in the central nervous system where tumors may require insertion of surgical hardware/shunts such as in craniopharyngiomas. In contrast, our observation demonstrate that infections of surgical hardware are surprisingly scarce in craniopharyngioma cases. In this study, we explore the possibility of antimicrobial effects of craniopharyngioma cystic fluid.

Methods The antibacterial effect of samples of craniopharyngioma cystic fluid against the selected human pathogens *Escherichia. coli*, *S. aureus* and *S. pneumoniae* was determined using the agar disc diffusion method. *These results were* compared with that of streptomycin and ampicillin.

Results The samples of craniopharyngioma cystic fluid inhibited growth of gram-positive bacteria (*S. aureus* and *S. pneumoniae*) but not the gram-negative bacteria, *E. coli*. The samples showed highest zones of *S. pneumoniae* growth inhibition.

Conclusion Craniopharyngioma cystic fluid demonstrated significant antibacterial properties against gram positive bacteria. More studies need to be carried out to further elucidate this unique finding.

Introduction

Craniopharyngiomas are rare benign tumours which account for 6-9% of all childhood tumors and are the most common non-glial tumors in childhood.[3] According to the World Health Organisation (WHO), they account for 1.2 to 4.6% of all brain tumours.[3] There are two types of craniopharyngiomas namely: the adamantinomatous type and the papillary type. The adamantinomatous craniopharyngioma type usually occur in childhood whilst the papillary type occurs in middle-aged adults.[5] Craniopharyngiomas usually favour the supra sellar region.[7] They are of epithelial histology and often exhibit calcification and sometimes single large or multiple cysts that contain fluid. This fluid is yellow, viscous, proteinaceous and rich in cholesterol crystals.[12] Literature has scanty information on craniopharyngiomas, let alone the tumour cystic fluid thereof. [3,5,7,12]

Treatment of these slow growing tumours include partial to total surgical resection, radiation therapy, insertion of ventriculoperitoneal shunts in the event of hydrocephalus and insertion of an ommaya reservoir to allow interval drainage of cystic fluid. This may involve injection of sclerosing agents like bleomycin into the cystic cavity via the ommaya reservoir. [3,6,9]

Tumours are known to increase the risk of infections, possibly more so within central nervous system tumors such as craniopharyngiomas which may require the insertion of a shunt and ommaya reservoir. [10] We have observed that infections of surgical hardware are surprisingly fewer in craniopharyngioma cases. Thus, to confirm this qualitative observation, this study performs quantitative tests to elucidate the antimicrobial effects of cystic fluid in craniopharyngioma tumours.

Methodology

Purposive sampling of tumour cystic fluid was performed under sterile conditions on consecutive, histologically confirmed craniopharyngioma (adamantinomatous type) patients attending our clinic. Ethical approval was given and informed written consent was obtained from the parent/guardian for participation in the study. Two samples were collected in their numerical order (1 and 2) over a period of six weeks and were stored at 4°C until testing a week later. Patient demographics and relevant clinical history was captured.

Preparation of Test Organisms

Three bacterial species common in our hospitals, namely, *Escherichia coli* (Gram negative), *Staphylococcus aureus* and *Streptococcus pneumoniae* (Gram positive), all clinical isolates, were selected as test organisms. *E. coli* and *S. aureus* were obtained from the Cimas Medical Aid Society laboratory and *S. pneumoniae* from Lancet laboratory. The test organisms were cultured in Mueller-Hinton broth overnight at 37°C. Immediately prior to the antibacterial assay, McFarland standard was used as a reference to adjust the inoculum size of each test organism to a concentration of 1×10^6 CFU/ml using sterile broth.

Bacterial Growth Inhibition Assay

The antibacterial effect of the three samples against the selected human pathogens *Escherichia. coli*, *S. aureus* and *S. pneumoniae* was determined using the agar disc diffusion method. Briefly, an aliquot of suspension containing 1×10^6 CFU/ml of bacteria was inoculated onto Mueller Hinton Agar (Mast Group Ltd., Merseyside, U.K.) and spread evenly on the agar surface. The sterile filter paper discs (Whatman No. 1, 6 mm) were soaked in 50 μ l of each sample and then placed on the inoculated Mueller-Hinton agar plates. The discs were pressed gently to ensure complete contact with agar. Distilled water-loaded discs were used as negative controls while filter paper disks loaded with 20 μ g/ml ampicillin or 50 μ g/ml streptomycin were used as positive controls for the tested bacteria. The plates were stored at 4°C for 30 min to allow diffusion of extracts prior to incubation at $35 \pm 2^\circ\text{C}$ for 16 h. After incubation, the plates were observed for the formation of a clear zone around the discs which corresponds to the antibacterial activity of the tested extracts. The zones of inhibition (ZOI) observed were recorded as the diameter of the growth-free zones around the discs, measured in mm using a caliper. The antibacterial assay was carried out in triplicate.

Statistical Analysis

Graph-pad Prism 6 $\times$ 01 was used to analyse the data. Frequency distribution of the numerical data was examined for normality and means, $\pm$ standard deviation was used as appropriate. Parametric data was analysed using a One-way ANOVA across all groups. A p value ≤ 0.05 was considered to be statistically significant.

Results

Sample 1 was taken from a nine-year-old girl and sample 2 from an 11-year-old boy (Table 1). Both samples were from children of African descent diagnosed 1 and 3 years respectively prior to commencement of this study. Both patients had neuro-surgical hardware insertion and displayed skin erosion over the ommaya shunt (see Fig 1 and 2). On Patient 1, the exposed shunt cultured staphylococcus aureus, but the intracystic part, cystic fluid and blood culture did not grow any pathogens. The rest of the infection screen on both patients was negative. Neither of them showed any signs of infection on examination. Patient 2 reported an episode of the ommaya shunt falling-off and the mother, in ignorance picked it up from the bathroom floor and stuck it back into the patient. Due to their rural abode, they reached the health care facility a week later. Both had not received antibiotics prior to presentation.

The effects of the two samples against the growth of *E. coli*, *S. pneumoniae* and *S. aureus*, expressed as the mean zone of inhibition diameters (mm) were notable (Table 2). Samples 1 and 2 had the highest zones of inhibition of growth of *S. pneumoniae*. Both samples inhibited gram positive bacteria (*S. aureus* and *S. pneumoniae*) but not the gram-negative bacteria (*E. coli*). (see Fig 3-5)

Table I: Characteristics of patients who provided the samples

Characteristics of participants	Sample 1/ Patient 1	Sample 2/ Patient 2
Age	9	11
Sex	Female	Male
Tumour position	Sellar region	Sellar region
Ventriculo-peritoneal Shunt	present	absent
Omayya reservoir	present	present
Infection risk	Skin erosion over omayya reservoir	Skin erosion over omayya reservoir leading to extrusion of reservoir

Table II: Diameter of zones of inhibition (mm) of cystic fluid against bacteria

Inhibition zones (mm)

Sample/Extract	<i>E. coli</i>	<i>S. aureus</i>	<i>S. pneumoniae</i>
Sample 1	0×0	12×7 ± 1×2	20×0 ± 1×0
Sample 2	0×0	11×5 ± 1×3	19×3 ± 0×6
Streptomycin	11×0 ± 0×0	18×0 ± 0×0	18×0 ± 1×0
Ampicillin	0×0	13×0 ± 0×0	9×0 ± 0×0
Water	0×0	0×0	0×0

Values for zone of bacterial growth inhibition (measured as the diameter of the clear zone around the paper disc) are means of triplicates. The diameter of the paper disc (6 mm) is included. Zero (0×0) inhibition (inhibition zone diameter $\leq$ 6 mm). Statistical significance was determined by one-way variance analysis (ANOVA), $p < 0.001$

Discussion

To our knowledge, this is the first study that demonstrates antimicrobial activity within cystic fluid from craniopharyngiomas in paediatric patients of African descent. We report that the zones of inhibition of growth of *S. pneumoniae* within craniopharyngioma cystic fluid was higher than that of the commercial drug streptomycin. These results confirm an inherent anti-microbial activity of the cystic fluid. Although ampicillin is known to inhibit gram negative bacteria like *E. coli*, the clinical bacterial isolate tested in this study showed resistance to the antibiotic. *S. pneumoniae* was the most susceptible strain to the samples, followed by *S. aureus* while *E. coli* was the most resistant strain to both samples.

We also report skin erosion over the omya shunt in both patients, a feature normally associated with infection in non-craniopharyngioma patients. [1,4] There was an absence of infection in both patients. This was unexpected as we anticipated infection, especially in the patient that reported the shunt falling off. It is plausible that the cystic fluid has an inherent antibiotic activity. Why the antimicrobial effect seems to be against gram positives and not gram negatives is a mystery and would need further studies.

A limitation of our study is the small sample size. The storage of the cystic fluid albeit at 4°C may have confounded the results. Nonetheless considering the rarity of craniopharyngiomas and the infrequency of sampling of their cystic fluid, these cases, albeit few, presented a rare opportunity to interrogate further properties of this fluid. While this study may not allow bold statements to be made yet, it undoubtedly presents a unique finding.

The antibacterial activity of the tumour cystic fluid against *E.coli*, *S. aureus* and *S. pneumoniae* is of great importance as *E.coli* accounts for more than 70% of the infections of the urinary tract worldwide. [8] *S. aureus* is the most common cause of bacterial infections in abscesses of skin, joints and bones [2] while

S. pneumoniae is the most common cause of community acquired pneumonia (CAP) in children. Resistance to antibiotics have been reported for *S. aureus*, *S. pneumoniae* and *E.coli*. [8,11] Notably, the antibacterial properties of tumour cystic fluid could provide affordable treatment for a range of common infections particularly prevalent in resource-constrained settings.

Conclusion

This study reports evidence of an antibacterial property of cystic fluid from craniopharyngioma patients. This is an important property in preventing infections in these patients and may also help to select bacterial strains when infection occurs, which is of paramount importance. Further biochemical investigations into the properties of cystic fluid that makes it have antibacterial properties is urgently needed.

Declarations

Funding: No external funding was sought

Conflicts of interest: Authors declare no conflict of interest

Availability of Data Material: Raw data can be made available on request

Code availability: Available on request

Author's contribution: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Luxwell Jokonya, Tsungai Reid, Maritha Kasambala, Tariro Mduluz-Jokonya. The first draft of the manuscript was written by Luxwell Jokonya and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

Ethics approval: All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of Harare Central Hospital.

Consent to participate: Written consent was obtained from care givers of participants and assent from patients

Consent for publication: consent for publication was obtained from participants

Availability of data and materiel: All data and materiel used is available on request to the corresponding

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Data Availability

The statistical data and all raw data used in this study are available from the corresponding author upon request.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Luxwell Jokonya, Tsungai Reid, Maritha Kasambala, Tariro Mduluz-Jokonya. The first draft of the manuscript was written by Luxwell Jokonya and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

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References

1. Akdag O (2018) Management of exposed ventriculoperitoneal shunt on the scalp in pediatric patients. *Child's Nerv Syst* 34(6):1229-1233. doi:10.1007/s00381-017-3702-9
2. Blossom DB, Namayanja-Kaye G, Nankya-Mutyoba J, et al (2006) Jt Clin Res Cent 10(6):458-464. doi:10.2307/j.ctt1zkjxzm.
3. Bunin GR, Surawicz TS, Witman PA, Preston-Martin S, Davis F, Bruner JM (1998) The descriptive epidemiology of craniopharyngioma. *J Neurosurg* 89(4):547-551. doi:10.3171/jns.1998.89.4.0547
4. Hamdan AR (2018) Ventriculoperitoneal shunt complications: a local study at Qena University Hospital: a retrospective study. *Egypt J Neurosurg* 33(1). doi:10.1186/s41984-018-0008-5
5. Lilia Tena Suck M (2015) Oil Machinery Fluid of Craniopharyngioma. *Biochemical Analysis: Clinico-Pathological Correlation. Am J Clin Exp Med* 3(2):52. doi:10.11648/j.ajcem.20150302.11
6. Moussa AH, Kerasha AA, Mahmoud ME (2013) Surprising outcome of ommaya reservoir in treating cystic craniopharyngioma: A retrospective study. *Br J Neurosurg* 27(3):370-373. doi:10.3109/02688697.2012.741732
7. Müller HL (2014) Craniopharyngioma. *Endocr Rev* 35(3):513-543. doi:10.1210/er.2013-1115
8. Niranjana V, Malini A (2014) Antimicrobial resistance pattern in *Escherichia coli* causing urinary tract infection among inpatients. *Indian J Med Res* 139(JUN):945-948.
9. Olsson DS, Andersson E, Bryngelsson IL, Nilsson AG, Johannsson G (2015) Excess mortality and morbidity in patients with craniopharyngioma, especially in patients with childhood onset: A population-based study in Sweden. *J Clin Endocrinol Metab* 100(2):467-474. doi:10.1210/jc.2014-3525

10. Rolston KVI (2017) Infections in Cancer Patients with Solid Tumors: A Review. *Infect Dis Ther* 6(1):69-83. doi:10.1007/s40121-017-0146-1
11. Stanley CN, Ugboma H, Ibezim E, Attama A (2014) Prevalence and Antibiotic Susceptibility of *Staphylococcus Aureus* and Other *Staphylococcal* Infections in Pregnant Women Attending Antenatal Clinic in a Tertiary Hospital in Port Harcourt, Nigeria. *J Infect Dis Ther* 02(01):1-6. doi:10.4172/2332-0877.1000125
12. Wijnen M, Van Den Heuvel-Eibrink MM, Janssen JAMJL, et al (2017) Very long-Term sequelae of craniopharyngioma. *Eur J Endocrinol* 176(6):755-767. doi:10.1530/EJE-17-0044

Figures

Figure 1

Exposed ommaya shunt in patient 1

Figure 2

Exposed ommaya shunt in patient 2

Figure 3

Growth inhibition of *S. Aureus* by samples 1 and 2, negative control water and the positive control, streptomycin (St)

Figure 4

Growth inhibition of *S. Pneumoniae* by samples 1 and 2, positive control streptomycin (St)

Figure 5

Growth inhibition of samples 1 and 2 by E.Coli, positive control streptomycin (St) and Ampicillin (A)