Rapid development of a salivary calculus in submandibular gland and its potential causes in a young victim following Russell's viper bite

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Abstract

Russell's viper bites are known to cause a range of haemotoxic, neurotoxic, myotoxic, cytotoxic and nephrotoxic complications. However, the impact of Russell's viper bites as well as bites from other venomous snakes on sialolithiasis has not been previously reported. Here, we present an interesting case where a Russell's viper bite induced the rapid development of a calculus in submandibular gland in a 10-year-old boy. Upon admission, the victim did not show any symptoms of swelling and/or pain around his oral cavity. He received antivenom treatment to normalise his coagulation parameters, however, on day three he developed swelling and extreme pain around his right mandibular region. An ultrasound investigation revealed the presence of a calculus in his submandibular gland, which was removed using a minor surgical procedure. The histopathological examination revealed this as a poorly calcified salivary calculus, which is composed of cell debris, mucopolysaccharides and lipids. The mechanisms behind its rapid development following a snakebite are unclear although this could be linked to excessive inflammation or modifications to the composition of saliva induced by venom toxins or other unknown factors. This report reveals an unusual complication induced by a Russell's viper bite and alerts clinicians who treat snakebites to be aware of such envenomation effects. Moreover, this will lead to novel research to explore the relationship between venom toxins and functions of salivary glands.

Key words

Snakebite envenomation; Russell's viper; salivary calculi; salivary glands; sialolithiasis; inflammation

Introduction

Sialolithiasis is the formation of calculi in salivary glands, primarily in ducts connecting the glands to the oral cavity (Delli et al., 2014; Hammett and Walker, 2021; Kraaij et al., 2014). This occurs predominantly in adults (mostly in males) who are aged between 30 and 60 years old with occurrence among children being rare (Kraaij et al., 2014). Between the three pairs of salivary glands (parotid, submandibular, and sublingual), calculi formation has been predominantly (over 85% cases) reported in submandibular glands (Grases et al., 2003; Kraaij et al., 2014), mostly in the excretory ducts (Sigismund et al., 2015). There are no precise mechanisms or causative factors established yet for the development of salivary calculi although they are broadly linked to various anatomical features of salivary glands and composition of calculi (Grases et al., 2003). For example, accumulation of bacteria, foreign bodies (Marchal et al., 2001), cellular debris (Harrison, 2009) and excessive inflammation, which triggers the infiltration of neutrophils (Schapher et al., 2020) in ducts

are likely to result in sialolithiasis. The presence of micro calculi (concrements) in the excretory duct system in submandibular glands even in many healthy, asymptomatic individuals (Sigismund et al., 2015) may act as nuclei for the deposition of various organic and inorganic molecules from saliva to ultimately form large calculi over a long period (Grases et al., 2003; Sivasubramaniam et al., 2015). Moreover, ducts in submandibular glands allow a slower salivary flow which may be affected when the salivary thickness is increased leading to the development of sludge and ultimately calculi. Similarly, increased ionic contents (specifically calcium) and modified amylase activities may induce the formation of salivary calculi (Kraaij et al., 2014; Schrøder et al., 2017).

Snakebite envenomation (SBE) has been reported to cause diverse neurotoxic, haemotoxic, myotoxic, cytotoxic and nephrotoxic complications based on the type of offending snake and the nature of their venom (Gutiérrez et al., 2017; Williams et al., 2019). Russell's viper (*Daboia russelii*) is a key species that causes numerous bites and resulting deaths and disabilities in India (Samuel et al., 2020; Vaiyapuri et al., 2013). While viper venoms generally induce haemotoxic effects, Russell's viper venom causes both haemotoxic and neurotoxic effects (Silva et al., 2017; Silva et al., 2016). In addition, Russell's viper bites are known to induce rare complications such as priapism (Senthilkumaran et al., 2021) and bilateral blindness (Subasinghe et al., 2014). While Russell's viper bite-induced swelling in parotid glands has been reported in few case reports (Deepak et al., 2013; Sasidaran et al., 2017), the impact of SBE including bites from Russell's viper on salivary calculi formation has not been previously documented. Hence, we report this unusual case of rapid salivary calculus formation in submandibular gland in a 10-year-old boy following a Russell's viper bite. This rare case is not only reporting an unusual complication of Russell's viper bite, but it also opens a new avenue to explore the impacts of venom toxins on the pathophysiology of salivary glands. Moreover, this will create awareness among clinicians who treat SBE to closely monitor the development of this type of rare complications in SBE victims to provide better care.

Case presentation

A 10-year-old boy with no previous medical conditions was brought to our emergency department following a snakebite on his left foot while walking in a field nearly two hours prior to the admittance. A tourniquet had been placed above the bite site using a banana tree fibre by the victim's father. They also brought the offending snake (killed), which was identified as a Russell's viper by a herpetologist (**Figure 1A**). Upon admission, the boy was conscious, well oriented, afebrile, and mildly anxious. Swelling was observed around the bite site although there was no excessive bleeding or tissue damage (**Figure 1B**). His initial vital signs were measured as; blood pressure - 116/72 mm Hg, heart rate - 129 beats/min, respiratory rate - 26 breaths/min, temperature - 36.9ºC, and oxygen saturation on room air - 100%. There were no obvious signs of swelling in his lymph nodes or around the oral cavity. He exhibited typical features of Russell's viper bite-induced systemic (gum bleeding), and local (swelling, developing wound and mild bleeding at bite site) envenomation effects. The 20-minute whole blood clotting time was prolonged and therefore, he received 100 mL of polyvalent antivenom (Bharat Serums and Vaccines Limited, India) intravenously according to the standard protocols. The antivenom administration was well tolerated with no signs of adverse reactions. He received another 100 mL of antivenom and four units of fresh frozen plasma to normalise his coagulation parameters over the next twenty-four hours. His other haematologic, metabolic, and biochemical parameters were within the normal limits. He was comfortable on the second day with no obvious local inflammatory changes. However, on the third day (while staying at the hospital), he complained of sudden onset of pain and swelling on the right mandibular region. On bimanual palpation, right submandibular gland was warm and tender with swollen lymph node. Oral examination has revealed a firm, tender, inflamed and erythematous duct opening with hard swelling situated near the right premolars, which was extremely painful on palpation. There was no associated discharge or bleeding from the affected area. The occlusal radiograph indicated the absence of any salivary calculi although an ultrasound scan of his right cheek confirmed the presence of a calculus measuring around 6 mm (**Figure 1C**). His salivary analysis confirmed moderately increased thickness and threads in mucus, and a few epithelial and bacterial cells. However, no crystals or major differences in biochemical parameters (including calcium levels) were noticed in saliva (**Table 1**). Under local anaesthesia, an incision was made at the ductal orifice and the calculus together with surrounding affected tissues was exposed and removed (**Figure 1D**). Absorbable sutures were placed to close the incised area and his post-operative healing was smooth and uneventful. Histopathological examination of salivary gland tissues extracted together with the

calculus displayed the infiltration of inflammatory cells and ductal dilation, and the composition of calculus as glycoproteins, cellular debris, lipids and mucopolysaccharides (**Figure 1E**). The boy was discharged on day seven, and his subsequent weekly reviews did not display any further complications.

Case discussion

While SBE is known to induce a diverse spectrum of complications in humans (Williams et al., 2019), to the best of our knowledge, its impact on sialolithiasis has not been previously reported. Moreover, sialolithiasis is commonly encountered among adults who are aged between 30 and 60 years old (Grases et al., 2003; Hammett and Walker, 2021). Hence, this report presents an interesting case of SBE (specifically Russell's viper bite)-induced sialolithiasis in a 10-year-old boy with no previous history of any medical conditions. Here, we discuss various potential reasons for sialolithiasis in this patient, although this could have independently developed without any impacts by SBE. Notably in this patient, the salivary calculus has appeared to be formed rapidly following the bite despite antivenom treatment. Similarly, the calculus could have formed already, but the area became very sensitive and extremely painful following the bite. Although occlusal radiography has been used to identify salivary calculi that are rich in calcium (Kim et al., 2016), it is not helpful in identifying poorly calcified calculi as in this case. Hence, it is important to use alternative diagnostic tools such as ultrasound and computed tomography (CT) scans, digital subtraction sialography and sialendoscopy to corroborate the presence of calculi (Goncalves et al., 2017; Hammett and Walker, 2021; Kim et al., 2016). Salivary calculi normally form at the rate of 1 mm per year, and they are noticeable only after several years (Grases et al., 2003). However, in this case, the incidence of pain with associated swelling was discovered within three days following the bite. Therefore, further investigation on the physical and biochemical nature of the calculi that are formed acutely (within a short period of time as reported in this case) and chronically (formed over a longer time as reported generally for sialolithiasis) should be thoroughly investigated to determine the mechanisms and major differences between these two methods. While the mechanisms behind the development of salivary calculi in this case are not entirely clear, here we discuss some plausible mechanisms for this incident.

The production of saliva and its delivery to the oral cavity is a tightly regulated phenomenon (Delli et al., 2014). Therefore, any adverse changes to the anatomical features of salivary glands and ducts are likely to result in sialolithiasis. For example, inflammation in glands or ducts will lead to infiltration of immune cells (specifically neutrophils) (Hammett and Walker, 2021; Marchal et al., 2001; Schrøder et al., 2017), stenosis or dilation and accumulation of minerals, foreign bodies, or cellular debris, which will result in sialolithiasis (Delli et al., 2014; Grases et al., 2003; Harrison, 2009; Kraaij et al., 2014; Marchal et al., 2001). Schapher et al. (Schapher et al., 2020) reported the impact of inflammation in salivary glands by increasing the sudden flow of neutrophils in the ducts leading to release of neutrophil extracellular traps (NETs), which subsequently induced the formation of calculi by acting as a scaffold. Although NETosis is an inflammatory response elicited by neutrophils mainly to remove the invading pathogens and cellular debris, it has been demonstrated to induce the deposition of various organic and inorganic materials in different organs in addition to salivary glands (Maueröder et al., 2015; Muñoz et al., 2019). In the present case, it was evident that inflammatory cells were infiltrated in the ducts of submandibular glands. Moreover, the accumulation of cellular debris, glycoproteins and mucopolysaccharides was noted during pathological examination of extracted calculi. The venom of the saw-scaled viper (*Echis carinatus*) has been reported to induce NETosis (Katkar et al., 2016), although no direct link to sialolithiasis has been established. Russell's viper bites are known to induce inflammatory responses and cytotoxicity due to the large proportion of phospholipase A_2 (PLA_2) present in this venom (Kalita et al., 2018). In addition, the snake venom metalloproteases (Teixeira Cde et al., 2005) and serine proteases (Menaldo et al., 2013) are able to contribute to the development of inflammation. Hence, the components of Russell's viper venom could have induced the inflammation in salivary glands resulting in infiltration of neutrophils and NETosis. The NETs released might have acted as a base for the rapid accumulation of cellular debris, phospholipids and mucopolysaccharides to develop poorly calcified calculus. However, it is unclear on why the toxic venom components have targeted only salivary glands instead of any other organs although the damage to other organs could be minimal and hence unnoticed.

The saliva secreted from submandibular glands contains higher concentration of calcium than from other salivary glands (Harrison et al., 1993). The submandibular calculi are formed with over 70% inorganic materials, mostly calcium phosphates in the form of hydroxylapatite (Grases et al., 2003; Hammett and Walker, 2021). The formation of salivary calculi has been demonstrated to occur due to accumulation and deterioration of calcium rich saliva in the ductal area (Grases et al., 2003). It was suggested that an organic molecule or particle forms a base micro calculus upon which other salts, organic and inorganic matters accumulate to form large calculi over a long time (Harrison, 2009). Indeed, the presence of micro calculi has been reported even in many healthy individuals and they can slowly induce congestion in ducts and stasis of saliva resulting in inflammation and further growth even in asymptomatic people (Epivatianos and Harrison, 1989). In addition to calcium, phospholipids and secreted glycoproteins will contribute to the development of calculi (Boskey et al., 1981; Boskey et al., 1983; Harrison, 2009). Higher concentrations of bicarbonate in saliva are also able to alter the ionic composition of saliva and induce NETosis (Leppkes et al., 2016). Therefore, all these factors could have contributed to the rapid development of calculus in the present case although the calculus was poorly calcified. Notably, in this patient, there were no major changes in their biochemical parameters in saliva including calcium and bicarbonate levels compared to the reference levels. PLA_2 present in Russell's viper venom causes cytotoxic effects (Chakrabarty and Sarkar, 2015) and these may in turn induced the accumulation of cellular debris and associated components to accumulate around the pre-existing base calculi. Acinar cells and duct cells are the major cellular components of salivary glands. Acinar cells are involved in serous saliva secretion while the duct cells secrete a more mucinous saliva (Kondo et al., 2015). The acinar cells produce an isotonic fluid that is modified into a hypotonic fluid by duct cells before its release into the oral cavity (Kondo et al., 2015). It has been reported that intracellular calcium plays a significant role in the secretion of saliva (Ambudkar, 2016). Indeed, calcium acts as a regulator of various ion pathways involved in salivary secretion. CRISPs (cysteine-rich secretary proteins) that are largely found in venoms of vipers (including Russell's viper) and elapid snakes have been reported to block calcium ion channels in various cell types (Tadokoro et al., 2020). Hence, these non-enzymatic proteins could have affected calcium channels which might have affected the salivary production and its ionic concentrations resulting in increased thickness of saliva. Therefore, studying the impact of CRISPs of Russell's viper venom may demonstrate their influence on ion channels involved in salivary production and their ultimate impact on the formation of salivary calculi. Moreover, an increase in bicarbonate content in saliva precipitates more calcium and phosphate ions leading to formation of calculi (Grases et al., 2003; Hammett and Walker, 2021). Hence, investigating the effects of Russell's viper venom components in modulating bicarbonate synthesis and maintenance will also be useful. Notably, the composition of Russell's viper venom differs based on their geographical locations (Kalita et al., 2018). Therefore, it might be possible for Russell's vipers from specific regions to induce this type of unusual complications in victims especially children.

Since Russell's viper bites are known to cause neurological complications (Kalita et al., 2018; Silva et al., 2017), it is important to determine the impact of these venom toxins on neural networks that regulate salivary secretion. Salivary neural networks involve autonomous, parasympathetic, and sympathetic stimulations (Ekström, 1989; Proctor and Carpenter, 2007). The parasympathetic signalling system in saliva secretion involves acetylcholine and noradrenaline release (Proctor and Carpenter, 2014). Both acinar and duct cells express cholinergic receptors as acetylcholine is the major neurotransmitter secreted by parasympathetic vagal fibres. Russell's viper venom consists of pre-synoptically active neurotoxic, PLA_2 (Kalita et al., 2018). Therefore, further investigation into the effects of this venom on salivary neural cells might shed light on the impact of Russell's viper bite on the salivary neural systems and their association with the calculi formation. In addition, SBE including from Russell's vipers is known to induce acute pain and long-term complications in multiple regions other than the bite site (Waiddyanatha et al., 2019). Therefore, it is possible that the calculus might have formed already, but the area was sensitised and became painful due to the direct and/or indirect actions of venom toxins.

We cannot rule out the effects of antivenom administered in this victim on sialolithiasis, as the calculi formation was discovered post antivenom treatment. Although this patient did not display any adverse reactions upon infusion of antivenom, the antigen-antibody (venom/antivenom) complexes formed might have triggered inflammatory responses specifically in salivary glands and/or ducts through inflammatory responses (de Silva et al., 2016; Morais and Massaldi, 2009).

The polyvalent antivenom used in this case is known to cause adverse reactions due to immunoglobulin complexes and impurities. Among the adverse reactions caused by antivenom, IgE-mediated and non-IgE mediated anaphylactic reactions are known to cause increased vascular permeability and mucous secretion (de Silva et al., 2016; Morais and Massaldi, 2009). Similarly, the fresh frozen plasma transfused in this patient could have triggered or contributed to this complication as it is known induce hypersensitivity reactions (Pandey and Vyas, 2012) although it was not evident in this case. These reactions could affect the rate of saliva production in submandibular glands and its composition. Hence, this aspect should be further investigated.

In conclusion, it is not entirely clear if venom components alone induced the rapid formation of poorly calcified salivary calculus in this case, or it was an aggravation of an already growing micro calculi due to excessive inflammation. There is also a potential possibility that it was due to adverse reactions to the antivenom or fresh frozen plasma. Further research into these types of unusual cases can not only help us in understanding the molecular mechanisms of venom (and antivenom) components but also enlighten us with the wide spectrum of envenomation complications which will result in revising treatment guidelines for better clinical management of SBE. This case report will assist clinicians in areas where SBE commonly occurs to be aware of such unusual clinical manifestations specifically among children.

Ethical statement

The data collection, consent form, and information sheets were approved by the Institutional Ethics Committee at Toxiven Biotech, Tamil Nadu, India (Reference number: ICMR-Toxiven Ethics 2021/1). A written consent was obtained from the patient's parents (since the patient was a minor) to collect and publish the data presented in this article.

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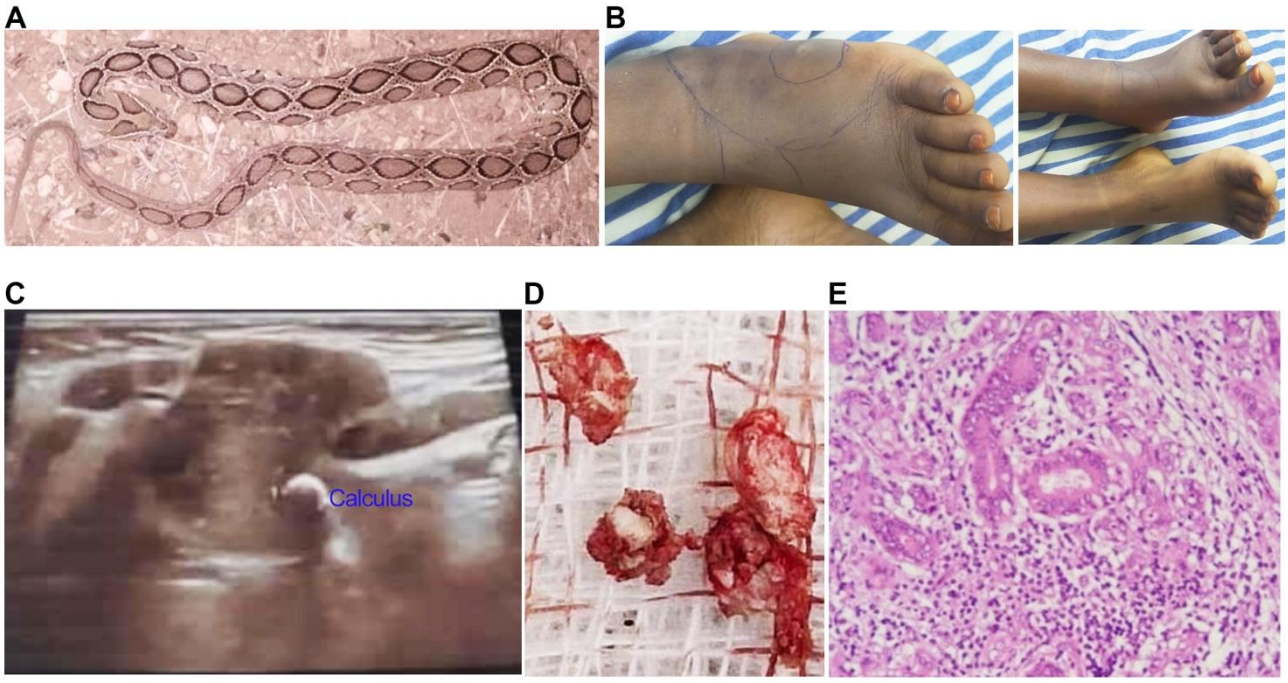
Figure Legnds

Figure 1: A, the offending snake was identified as a Russell's viper. **B**, bite area shows mild swelling and developing wound. **C**, an ultrasound scan reveals the presence of a calculus at the duct of submandibular gland. **D**, extracted calculus with affected tissues using a minor surgery. **E**, haemotoxylin and eosin staining of extracted salivary tissues demonstrates the excessive infiltration of leukocytes.

Figure 1



389 **Table 1:** Salivary analysis report

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Parameter analysed	Results	Unit	Reference range
Colour	White	-	-
Appearance	Turbid	-	-
Reaction	Alkaline	-	-
pH	7.12	-	-
Specific gravity	1.53	-	-
Cast	Mucus ++	-	-
Crystals	None	-	-
Muscus threads	++	-	-
Others	None	-	-
Sodium	130	mmol/L	135 to 150
Potassium	4.0	mmol/L	3.5 to 5.0
Chloride	112	mmol/L	98 to 107
Bicarbonate	21	mmol/L	22 to 29
Calcium	7.1	mg/dL	8.4 to 10.2
Pus cells	0	-	0-1/HPF
Red blood cells	0	-	0-1/HPF
Epithelial cells	4-6	-	2-3/HPF
Bacteria	Present	-	-

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