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Histopathological profile of fatal snake bite autopsy cases in a tertiary care center in South India

K. Sathish^{1*}, Kusa Kumar Shaha², Ambika Prasad Patra^{3†} and J. Sree Rekha^{3†}

Abstract

Background: Snake bite is a major public health problem, especially in the tropical areas of the world, which the World Health Organization aims to eradicate. According to the National Crime Records Bureau in India, where agriculture is the mainstay of employment attributes about 8660 mortalities in a year. Histopathological results are of immense help in establishing the cause of death in those snake bite cases where other circumstantial shreds of evidence are lacking.

Results: An autopsy-based prospective study was conducted on fatal cases of snake bite reported at a tertiary care center in south India over a period of 2 years. A total of 38 fatal snake bite cases were included in the study. Epidemiological data were collected and analyzed. Histopathological findings of the kidneys, heart, and endocrine glands (pituitary, adrenals, thyroid, and pancreas) were analyzed. The significant findings of the kidneys were distinct corticomedullary demarcation on the gross surface (65.8%) and congestion and acute tubular necrosis (55.3%) on histology. On the other hand, in the set of endocrine glands, adrenals and pituitary showed predominantly hemorrhagic infarction on the histological analysis (18.4% and 52.6%) respectively.

Conclusion: The results of the current study enlighten the findings such as distinct corticomedullary demarcation and acute tubular necrosis in the kidneys and hemorrhagic infarction in the endocrine glands in a case of snake bite. It highlights the need for histopathological analysis in the cases of a fatal snake bite when other circumstantial and gross features become indecisive. This can aid immensely in a medico-legal investigation of snake bite cases.

Keywords: Snake bite, Autopsy, South India, Occupational hazard, Congestion, Tubular necrosis, Hemorrhagic infarction

Background

Snake bite is one of the common causes of unnatural death ever since the evolution of mankind. The prevalence of venomous snake bites is common in tropical countries such as Africa, South and Southeast Asia, and Latin America. Snake bite is considered as a neglected tropical disease that the World Health Organization

(WHO) intends to eradicate. It constitutes a major public health problem in tropical areas of the world. The WHO estimates that around 5.4 million snake bites occur each year, causing in 1.8 to 2.7 million cases of envenoming. It is estimated that these cause deaths of 81,000–138,000 people a year and leave a further 400,000 with permanent disabilities (World Health Assembly 2018). In India, being a tropical country where farming is a major source of employment, snake bite has become a common medical emergency and an occupational hazard with the highest snake bite mortality in the world, about 45,000 deaths per annum (Mohapatra et al. 2011). According to the National Crime Records Bureau, there

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were 8660 deaths due to snake bite reported in the year 2015 of which 2230 were from south India (National Crime Records Bureau Ministry of Home Affairs 2015). Histopathology examination is usually needed to prove the grounds of death when any morbid gross changes are found in organs and tissues that could be the cause of the instability of vital signs of the patient. Vasculotoxic snake bite produces renal damage that encompasses a variety of manifestations like acute kidney injury (AKI), hematuria, and proteinuria. The mechanism of AKI is attributed to disseminated intravascular coagulation, intravascular hemolysis, and rhabdomyolysis (Li et al. 2016). Histopathologically, there are evidences supporting hemorrhage at the site of the bite, in the pituitary, and pancreas, sub-endocardial and myocardial hemorrhages (Francis et al. 1989). There are studies on histopathology of individual organs, but a combined profile of kidneys along with the heart and endocrine organs in fatal cases in an Indian scenario is lacking.

Methods

An autopsy-based cross-sectional descriptive study was conducted from January 2017 to December 2018 on deceased patients with snake bite in all age groups received at the mortuary, department of forensic medicine and toxicology in a tertiary care center in south India. This institute is a referral tertiary care hospital in south India, where patients come from the various districts of neighboring states and other parts of Southern India. Ethical clearance was obtained from the Institutional Ethics Committee. Before commencing the study, written informed consent was obtained from the legally acceptable representative of the deceased for the participation and sample collection in the study. All the cases brought to the department of forensic medicine as a snake bite for

medico-legal autopsy were included in the study. Cases in advanced stages of decomposition were set as exclusion criteria. The data on relevant epidemiological, demographic factors and variables were collected using a structured questionnaire from the police and legally acceptable representative in all the autopsy cases of those who died due to snake bite. During autopsy, gross analysis of the study samples, i.e., the kidneys, heart, adrenals, pancreas, pituitary, and thyroid, was done. These samples were then preserved in 10% formalin. The samples were then sent to histopathology laboratory to be processed and reported by routine histopathological examination. After standard tissue processing, thin sections were cut at the 5- μ m thickness with a rotary microtome and stained with hematoxylin and eosin (H&E). Special histochemical stains like Periodic acid Schiff (PAS), potassium metabisulfite, and phosphotungstic acid were performed based on the histopathological findings in H&E slides. The stains were performed as and when required when there are extensive secondary changes like hemorrhage and necrosis to identify the endocrine cells. These slides were studied under various magnifications and reported. The obtained data were tabulated and analyzed using statistical tests. All data recording and analysis were carried out using IBM PASW statistics (SPSS)—Version 19.0.

Results

Out of the total 1407 medico-legal autopsies conducted in the study period, 38 cases were due to fatal snake bite envenomation which constitutes around 2.73% of the total cases. All the 38 satisfied the necessary inclusion criteria and were incorporated into the study. Males were affected more than females, and the male/female ratio was 1.7:1. In the majority of the cases (12 cases, 65.8%), the type of snake was not identified by either the

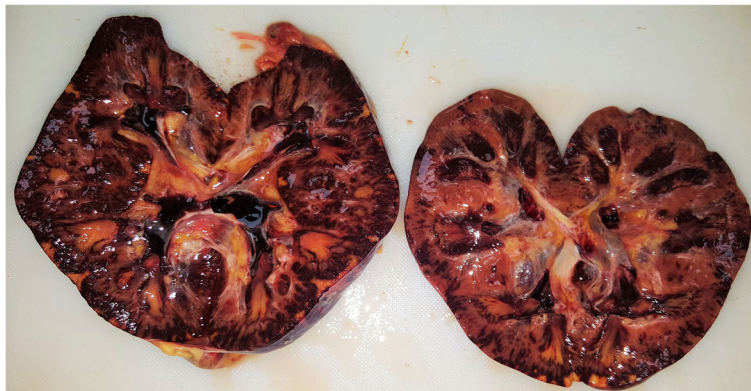


Fig. 1 Cut surface of kidneys showing medullary hemorrhage

Table 1 Findings of kidneys

S.No	Characteristics	Findings	N (%)
1	Outer surface	Congested	34 (89.5%)
2	Cut surface	Corticomedullary demarcation	25 (65.8%)
3	Glomeruli	Congested capillary loops	16 (42.1%)
4	Renal tubules	Acute tubular necrosis	21 (55.3%)
5	Renal interstitium	Congestion	26 (68.4%)
6	Evidence of glomerulonephritis/chronic kidney disease	Present	3 (7.9%)

victim or the bystanders. Among the identified cases, the most common offender was viper amounting to 31.6% of cases (25 cases) followed by cobra causing 2.6% of the deaths.

Kidneys

Kidneys showed variable degrees of congestion on the outer surface in most of the kidneys (89.5%). On the cut section, kidneys showed distinct cortico-medullary demarcation and cortico-medullary hemorrhage in 25 (65.8%) cases (Fig. 1). Interstitial congestion was the predominant feature noted in the majority of the cases (68.45), and congested capillary loops were found in 16 (42.1%) cases. Acute tubular necrosis was demonstrated in 21 (55.3%) cases which substantiated to be the significant finding in all cases it was present (Table 1) (Fig. 2). Around 67% of cases in the group of acute tubular necrosis was applied with tourniquet at the time of arrival to the hospital. This illustrates that the association between the first aid given prior to hospitalization (tourniquet) and the histopathology of renal tubules on autopsy were statistically significant ($p < 0.05$). The frequency of renal tubular damage

is more in cases applied with a tourniquet compared with those who do not.

Heart

The most common gross autopsy finding in the heart was epicardial hemorrhage alone in 15 cases (39.5%) followed by combined epicardial and sub-endocardial hemorrhages in 12 (31.6%) cases. Two cases showed narrowed coronary arteries along with hemorrhage, all of which were atherosclerotic origin presented as incidental finding in above 50 years of age. All the cases of Russell's viper bite had features of hemorrhage with the combination of epicardial and sub-endocardial hemorrhage. In the one case of cobra bite, there was evidence of sub-endocardial hemorrhage. In cases of bite by an unidentified snake, epicardial and sub-endocardial hemorrhage were common (64% cases). Intact heart was observed only in the unidentified snake group (28% cases) compared to others. This shows that the association between the type of snake and the gross findings in the heart on autopsy were statistically significant ($p < 0.05$). The heart was histologically unremarkable in 31 (81.6%) cases. Congestion in the epicardium and myocardium was present in two cases. Only one case showed coagulative necrosis of the ventricles.

Endocrine glands

Gross features

Among the cases studied, in 68.4% of cases, adrenals were intact and congested, and 31.6% of cases were hemorrhagic on both cortex and medulla (Fig. 3). Hemorrhagic features were more common in the pituitary (76.3%) (Fig. 4). Both the pancreas and thyroid predominantly showed congestion on the gross section (Table 2).

Histopathological features

In the histopathology of adrenals, seven (18.4%) cases showed hemorrhagic infarction on biopsy, followed by 12 cases (31.6%) that showed congestion of

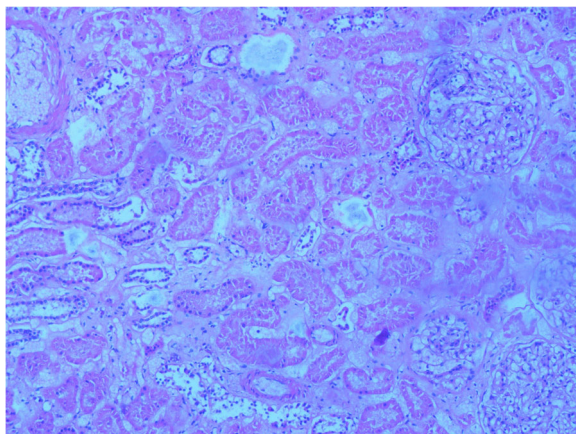


Fig. 2 Section from the kidney showing focal acute tubular necrosis. H&E stain, $\times 200$

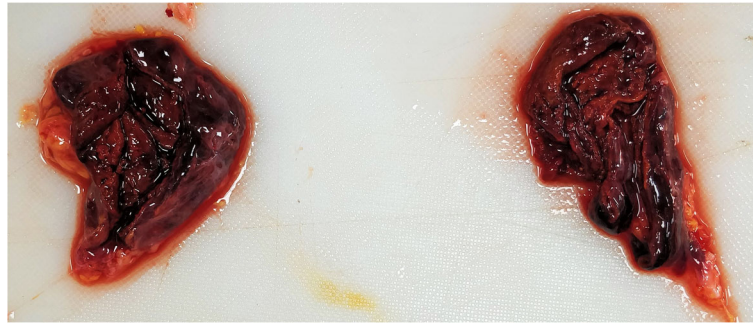


Fig. 3 Gross view of bilateral adrenal glands showing hemorrhage

interstitium (Fig. 5). Hemorrhagic infarction was the predominant feature reported in the histopathology of pituitary in 20 (52.6%) cases followed by congestion in nine (23.7%) cases (Fig. 6). Histology of the pancreas and thyroid largely showed normal architecture with one case that showed hemorrhage in the pancreas in which the species of snake was not identified by the victims and the bystanders. Lymphoid infiltrates were commonly noted in the thyroid gland (10 cases) which may be due to the inflammatory state (Table 3).

Association between the epidemiological factors and gross features of study samples

Table 4 shows that the epidemiological factors did not alter the gross features of the outer surface of the kidney, pancreas, thyroid, and adrenals. However, there was an association between the snake species and gross feature of the heart and between time delay to receive anti-snake venom (ASV) and gross feature of kidney cut surface and pituitary.

Association between the epidemiological factors and histological features of study samples

Table 5 shows that the association between snake species and histopathological features of study samples was insignificant. First aid prior to hospitalization had effects on the histopathology of renal tubules and adrenals, but association was insignificant for other study samples. Time delay to receive ASV did not alter the histopathology of any study sample. Survival duration had effect on the histopathology of pancreas, and association was not significant for other samples.

Discussion

Gross findings of study samples

On cut section of the kidneys, pale cortex and congested medulla (distinct cortico-medullary demarcation) and corticomedullary hemorrhage were noted in 65.8% of cases, and congestion in 34.2% of cases. These results are in concordance with a study by Yogesh et al., where corticomedullary hemorrhage was present in 45.3% of

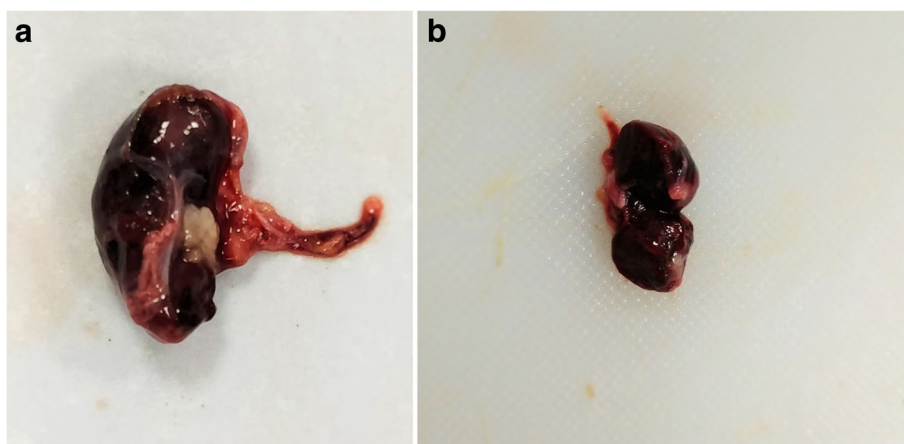


Fig. 4 a, b Gross view and cut section of pituitary show hemorrhage

Table 2 Gross findings in endocrine glands

S.No	Findings	Pituitary (%)	Thyroid (%)	Pancreas (%)	Adrenals (%)
1.	Intact and congested	09 (23.7)	33 (86.8)	34 (89.5)	26 (68.4)
2.	Hemorrhagic surface/cut-section	29 (76.3)	05 (13.2)	04 (10.5)	12 (31.6)

cases. (Yogesh and Satish 2014). A review on community-acquired acute kidney injury in Asia showed that petechial hemorrhage was seen on the surface of the kidneys in cases of snake bite on autopsy (Jha and Chugh 2008). The mechanism underlying the corticomedullary distinction suggests the cortical ischemia and shunting mechanism in the kidneys due to the toxin (Lauler and Schreiner 1958). Comparable to our study reports like Raghavendra et al. showed sub-endocardial hemorrhage in a case of snake bite (Babu et al. 2013). Surface epicardial petechial hemorrhage and sub-endocardial hemorrhage were noted in a study by Welton et al. (Welton et al. 2017). The cause for hemorrhage in the heart was proposed as snake venom metalloproteinase (SVMPs) which impedes platelet aggregation and potentiates bleeding (Fatima and Fatah 2014). Another study on hemotoxic effects of snake venom stated that SVMPs also directed inactivation of factor X, prothrombin, and fibrinolytic effects (Slagboom et al. 2017). The different type of snake produces certain gross characteristic findings in the heart which was significant. On the findings of the endocrine glands, fatty necrosis of the pancreas along with parenchymal hemorrhage was demonstrated by Milani et al. in Bothrops snake (Milani Junior et al. 1997). Pituitary hemorrhage after a bite of Russell's viper causing hypopituitarism was demonstrated in a case report (Antonypillai et al. 2011). Pituitary and adrenal hemorrhage

was noted on autopsy in a case report by Tun et al. (Tun et al. 1987).

Histopathological findings of study samples

Acute tubular necrosis was present predominantly in 21 (55.3%) cases. Two cases in our study demonstrated RBC casts which were due to the hemoglobinuria which in turn was due to direct toxicity of the snake venom on the renal tubules, and hyaline casts were noted in seven (18.4%) cases. These were usually during the end-stage renal function at the time of death. Mild to moderate interstitial inflammation was present in 10.5% of cases. Renal biopsy in a study by Naqvi et al. showed acute tubular necrosis in 44% of cases (Naqvi 2016). Two cases of renal biopsy showed acute tubular necrosis, pigment casts, interstitial inflammation, and acute cortical necrosis (Chugh 1989). A case report by Ramachandran et al. presented both necrotic glomeruli, tubules, interstitial edema, and cellular infiltration (Ramachandran and Perera 1974). The renal lesion arising from snake bite differs from those due to any other cause of acute renal failure. The features specific to the snake bite-induced acute renal failure were severe tubular and vascular lesions, increased apoptosis of distal tubules, and inflammatory infiltrates in the interstitium. The histologic changes varied with duration of illness. Renal changes in snake bite can be

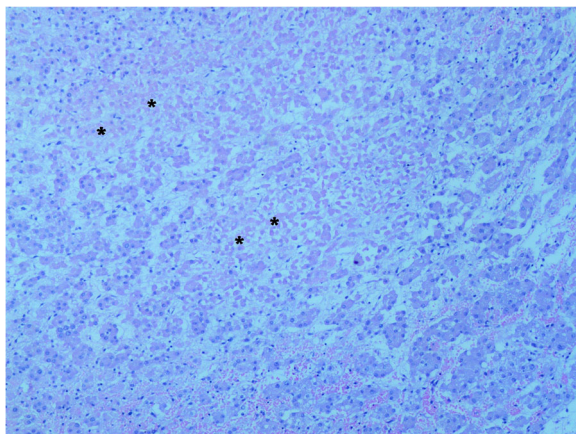


Fig. 5 Section from adrenal gland asterisk area shows coagulative necrosis/infarction, H&E stain, $\times 200$

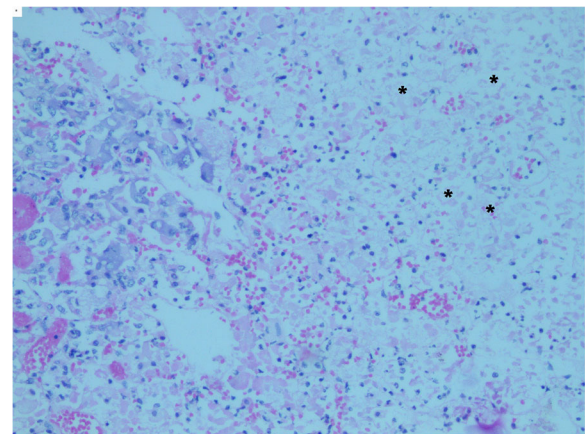


Fig. 6 Section from pituitary asterisk area shows coagulative necrosis/infarction, H&E stain, $\times 200$

Table 3 Histopathology of endocrine glands

S.No	Histopathological profile	Pituitary (%)	Thyroid (%)	Pancreas (%)	Adrenals (%)
1.	Normal	07 (18.4)	23 (60.5)	30 (78.9)	18 (47.4)
2.	Congestion of interstitium	09 (23.7)	05 (13.2)	-	12 (31.6)
3.	Hemorrhagic infarction	20 (52.6)	-	01 (2.6)	07 (18.4)
4.	Autolysis	02 (5.3)	-	05 (13.2)	01 (2.6)
5.	Inflammatory cell infiltration	-	10 (26.3)	02 (5.3)	-

ascribed to the nephrotoxicity of venom, hypersensitivity to the venom, immune complex disease, bleeding, hemolysis, myoglobinuria, and sepsis (Chugh 1989).

The heart was unremarkable in 31 (81.6%) cases. Congestion in the epicardium and myocardium was present in two cases. Only one case showed coagulative necrosis of the ventricles. Three cases presented with narrowed coronary arteries; all of which were due to atherosclerosis. Chen et al. demonstrated small focal necrosis, inflammatory infiltrate, and edema in myocardium due to the pit viper toxicity (Chen and Huang 2013). Coagulative necrosis was reported in a case which is due to the proteolytic, coagulant, and hemorrhagic effects of the venom (Benvenuti et al. 2007).

Among the endocrine glands, pituitary frequently (52.6%) showed hemorrhagic infarction, and 23.7% of cases of pituitary showed congestion of interstitium. In 12 (31.6%) cases, adrenals demonstrated congestion, whereas seven (18.4%) cases showed hemorrhagic infarction. Inflammation of the pancreas was present in two (5.3%) cases. Lymphocytic infiltration of thyroid was present in 10 (26.3%) cases and congestion in five (13.2%) cases. Rajagopala et al. showed adrenal and pituitary necrosis in the autopsy of a Russell's viper bite (Rajagopala et al. 2015). Similar findings such as pituitary necrosis on autopsy were reported by Murthy et al. (Murthy et al. 2002). Pituitary necrosis due to snake venom is attributed to two mechanisms. In the first phase, stimulus in the pituitary and enlargement occur

due to direct effects of the venom, capillary leak syndrome, and hypotension. In the second phase, more bleeding may cause relative ischemia to the (swollen) pituitary stalk causing ischemic necrosis. This is expected to be exacerbated by microvascular thrombosis due to DIC. Phospholipase A2 present in most of the snake venom as "direct lytic factor" is implicated in the inflammatory changes in the pancreas and thyroid (Kihara et al. 2005; Uma and Gowda 2000).

Conclusion

Snake bite remains a significant cause of accidental deaths in this modern era, and its occurrence usually underestimated because of less reporting. It is considered an occupational hazard among the high-risk population especially those working in agricultural fields, plantations, and other outdoor activities. It is also termed as a disease of poverty endemic to the geographical distribution of farming and its related activities. Snake envenomation leading to damage in the kidney, heart, and other endocrine glands is more likely if there is an untoward delayed referral to an appropriate tertiary center. Early referral to a specialized care center can reduce mortality and morbidity, but social and cultural and financial limitations are the major deterrents. This study highlights the histological changes in the organs that of primary target by the snake venom and may help and guide the pathologist and physician in their early intervention.

Table 4 Association between the epidemiological factors (snake species, first aid prior to hospitalization, time delay to receive ASV, and survival duration) and gross features of study samples (kidney, heart, and endocrine glands)

Gross features	Snake species (<i>p</i> value)	First aid prior to hospitalization (<i>p</i> value)	Time delay to receive ASV (<i>p</i> value)	Survival duration (<i>p</i> value)
Kidney outer surface	0.358	1.000	0.762	0.158
Kidney cut surface	0.134	0.186	0.011*	1.000
Heart	0.025*	0.691	0.815	0.216
Pituitary	0.082	0.113	0.031*	0.122
Pancreas	0.626	0.321	0.366	0.607
Thyroid	0.395	1.000	0.490	0.127
Adrenals	0.240	0.513	0.624	0.284

*indicates *p* value less than 0.05

Table 5 Association between the epidemiological factors (snake species, first aid prior to hospitalization, time delay to receive ASV, and survival duration) and histological features of study samples (kidney, heart, and endocrine glands)

Histology features	Snake species (<i>p</i> value)	First aid prior to hospitalization (<i>p</i> value)	Time delay to receive ASV (<i>p</i> value)	Survival duration (<i>p</i> value)
Tubules of the kidney	1.000	0.025*	0.109	0.608
Heart	0.800	0.923	0.930	0.368
Pituitary	0.426	0.718	0.469	0.063
Pancreas	0.396	0.915	0.428	0.048*
Thyroid	1.000	0.301	0.518	0.945
Adrenals	0.940	0.043*	0.686	0.150

*indicates *p* value less than 0.05

Abbreviations

WHO: World Health Organization; AKI: Acute kidney injury; ASV: Anti-snake venom; SVMP: Snake venom metalloproteinase; DIC: Disseminated intravascular coagulation

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None

Authors' contributions

KS and KKS conceived the idea of writing this paper. KS, KKS, APP, and JSR were involved in the design of the work and analysis and interpretation of the data. KS wrote the initial draft of the manuscript. KS, KKS, APP, and JSR have read and approved the final version of the manuscript. All authors have equally participated in this work. All authors read and approved the final manuscript.

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Availability of data and materials

The dataset used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Institutional Ethics Committee of Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER) vide reference no. JIP/IEC/2016/1028, Puducherry. Written informed consent was obtained from the legally acceptable representative of the deceased for collecting the sample and its scientific use.

Consent for publication

Not required by the ethics committee.

Competing interests

The authors declare no competing interests.

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