First evidence of brain surgery in Bronze Age Harappa

We report here the first unequivocal case of ancient brain surgical practice, known as trepanation, observed ~ 4300 years ago in a Bronze Age Harappan skull. A decade ago, a Neolithic skull from Burzahom[1–3] in the Kashmir Valley was reported with multiple trepanations as the first case from the Indian subcontinent. The trepanation, also called trephination or trephining, had been the oldest craniotomic surgical procedure practised by mankind since the Stone Age by way of drilling or cutting through the skull vault of a living or recently deceased person. It was first noticed in Peru[4,5] and later in Europe as well[6,7] around 5000 years ago, and thought to have spread to Asia ~ 4000 BP in the Bronze Age Jericho of Palestine[8,9]. Most scholars[7] noticed striking similarities in trepanation techniques across the continents, and therefore consider it as an important evidence for prehistoric movements of people and for transfer of surgical skills from one society to another.

Presence of trepanation in the Indus Civilization was suspected about four decades ago on a child's skull from Lothal[10], and on Harappan and Kalibangan skulls[11], but not ascertained, except those on the Burzahom skull[1–3]. The present study confirms and reports the occurrence of trepanation in one Harappan male skull (H-796/B; Figure 1) kept in the Palaeoanthropology Repository of the Anthropological Survey of India, Kolkata. The trepanated Harappan skull has come from Cemetery H, which contained crude red ware but not typical Harappan ceramics. The lower Stratum II (H2) of Cemetery H formed about two dozens of the extended burials with the heads facing eastward and the knees flexed, whereas the upper Stratum I (H1) contained pot/jar/urn burials with skulls and a few long bones along with red ware.

The cause of trepanation in the Harappan skull could be understood in the light of similar cases elsewhere. Scholars argued for different motives for trepanations in different regions and societies of the world, but a majority consider most of these as definitely surgical operations of therapeutic use either for repairing a fracture of the skull resulting from blows of sticks or stones, or to remove splinters and clotted blood, or alleviate persistent headaches. In South America and the Mediterranean region alone more than half of the Iron Age trepanations were practised on traumatic crania by the similar techniques of boring and cutting[12]. In addition, there were trepanated surgical cases intended to get relief from certain intracranial vascular catastrophes, otitis media, mastoid inflammation, vertigo, neuralgia, coma, delirium, meningitis, convulsions, epilepsy, intracranial tumours, mental diseases, and syphilitic lesions in Peru[13]. Interestingly, trepanations are still practised by the traditional medicine-men in Bolivia[14] for head injuries, and in Melanesia[15] in certain cases of headache, epilepsy or insanity, and also to enhance longevity. There were also cases where posthumous trepanations were performed to obtain roundels of human skull bone for making necklace as charms or amulets to ward off the evil spirits or as a talisman to counter the demons[5,16–18]. Some scholars thought that the Burzahom multiple trepanations done for roundels[18], but this proved an error of diagnosis of the trepanation[1] since in parietal osteoporosis, the normal bone shelves into a thinned area which is extremely fragile and breaks down postmortem, for one reason or another, leaving an opening with a more or less bevelled circumference and apparently healed.

The Harappan case is clearly of a cranial trauma visible on the parietals as a horizontal linear depression with cracked margins, probably resulting from a severe blow using a strong wooden stick. The trephined hole is just on the right superior temporal line at the terminus of the traumatic line. A clear rim of 3 mm width at the internal border of the hole is the evidence of osteogenesis or healing, indicating that the victim survived for a considerable time after the operation. Thus, trepanation was practised as a common means of surgery during the Bronze Age in the Indian subcontinent, which could have been a precursor to the later Ayurvedic surgical practices followed in ancient India as well.

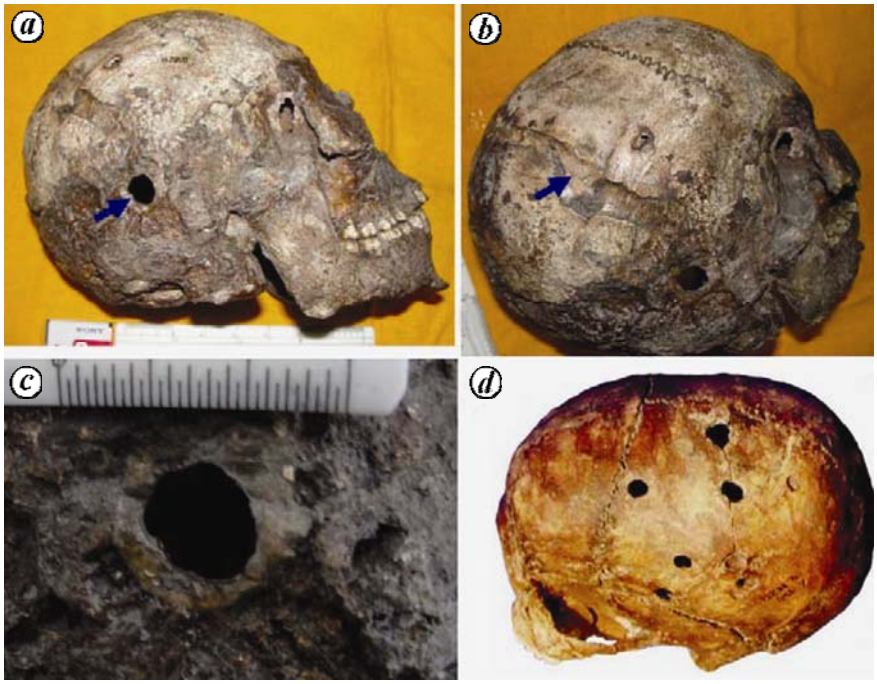

Figure 1. The trepanated Harappan male skull H-796/B in the Palaeoanthropology Repository of the Anthropological Survey of India, Kolkata in three views: ***a***, the left lateral view showing the trepanated hole; ***b***, the postero-lateral view showing the horizontal linear traumatic fracture on the occipital bone; ***c***, an enlarged view of the trepanated site showing the rim of callous formed due to healing, and ***d***, the trepanated Burzahom female skull showing signs of multiple trepanations (after Sankhyan and Weber[1]).

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A modified method to isolate genomic DNA from plants without liquid nitrogen

With the development of various molecular markers based on PCR, like RAPDs, SSRs, STRs, AFLP and PCR–RFLP, molecular biology has greatly enhanced the speed and efficiency of crop improvement and breeding programmes, rDNA technology and genomic DNA library construction. A pre-requisite for applying these methods is the ability to isolate high-quality genomic DNA of adequate quantity. A good extraction procedure is considered to be one which results in DNA of reasonable purity without the use of harmful chemicals. DNA extraction has been reported from various plant species using the cetyltrimethyl ammonium bromide (CTAB) procedure[1] and its modifications[2,3]. Most extraction methods employ expensive and hazardous procedures of grinding plant tissue in liquid nitrogen (N_2) to break down the cell wall of plants[4,5] or freeze-drying (lyophilization)[6]. Procurement and storage of liquid nitrogen may be difficult for many laboratories and handling of the same is also cumbersome. Thus any method not using liquid nitrogen is helpful. The omission of a grinding step in liquid nitrogen has been applied by many workers mostly using soft tissues such as flower petals[7] or young leaves[8,9]. In the present study a quick, simple and cheap procedure to isolate DNA from plants has been developed, which involves alternate cold (–80°C) and heat shock (60°C) treatments in order to break down the cell wall without using liquid N_2, which is suitable for various molecular biology applications. The extracted DNA was successfully subjected to PCR amplification of the ITS (internal transcribed spacer) region of rRNA gene, restriction digestion of the amplified product, microsatellite fingerprinting and RAPD successfully.

Samples of young, tender, leaves of ten plants species (*Desmodium giganicum*, *Aegle marmelos*, *Solanum xanthocarpum*, *Solanum indicum*, *Tribulus terresteris*, *Oroxylum indicum*, *Boerhavia diffusa*, *Trianthema portulacastrum*, *Trianthema monogyna* and *Datura innoxia*) were collected from the Botanical Garden of Panjab University, Chandigarh and various other nurseries. The plant tissue was washed well with water and sterilized by wiping it with 70% alcohol. The fresh weight of the plant tissue was taken and then it was chopped into fine pieces and subjected to DNA isolation. Simultaneously leaves of the same species were dried at 60°C and also processed for extraction of DNA.

DNA was extracted by the following steps:

- Pre-chill the mortar–pestle at –80°C for 15 min prior to the start of the experiment. Alternatively, it can be pre-chilled at –20°C for 1 h.
- Transfer the finely chopped plant tissue (300 mg) and dried tissue separately to chilled mortar–pestle and keep it at –80°C for 20 min or at –20 for 1 h.
- Grind the plant tissue into fine powder, transfer it to 1.5 ml microfuge tube and incubate it at 60°C for 5 min.
- Keep it again at –80°C for 15 min or at –20 for 1 h.
- Finally, thaw the powdered tissue by pouring equal volume (300 µl) of hot (65°C) 2× CTAB buffer (100 mM Tris [pH 8], 20 mM EDTA [pH 8], 1.4 M NaCl, 2% CTAB w/v, 2%PVP 40,000) and 1/10th volume of β-mercaptoethanol, and mix well.
- Add one volume (600 µl) of chloroform/isoamyl alcohol (24 : 1) and mix thoroughly to form an emulsion.
- Centrifuge in a microfuge for 15 min at 12,000 rpm.