



Histology, histochemistry and scanning electron microscopy of the pineal gland of the pig

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ABSTRACT

The present study was conducted on 10 adult pigs to reveal structure of pineal gland by light and scanning electron microscopy. The pineal gland was surrounded by a layer of dense capsule consisting of reticular and collagen fibres. At places connective tissue trabeculae from the capsule penetrated the parenchyma dividing it into lobes and lobules. The parenchyma of the gland was constituted mainly by pinealocytes whereas, 4 types of glial cells categorized on the basis of nuclear morphology, were present mainly towards the glial zones. The light and dark pinealocytes were arranged in small clusters, irregular groups, rosettes, cords and follicular patterns. At places, melanin containing cells were interspersed in between pinealocytes and glial cells. Corpora arenacea was observed only at few places throughout the pineal parenchyma. The mast cells were absent. The carbohydrates in the form of mucopolysaccharides were uniformly distributed in the cytoplasm of pinealocytes and glial cells whereas, a moderate reaction was observed in processes of pinealocytes and glial cells. The proteins in very less concentration were observed towards capsule of gland however, a moderate reaction was seen in blood capillaries containing blood cells. The lipids distribution in the gland was almost negligible. The scanning electron microscopy of dorsal surface of pineal gland presented fibrous strands oriented in different directions forming an irregular meshwork. However, cut surface of the gland depicted pinealocytes, glial cells, glial fibres and cellular debris. The surface of round to oval shaped pinealocytes was studded with microvilli like structure whereas round to oval, glial cells presented smooth surface and possessed large sized processes which frequently intermixed with those of other glial cells.

Key words: Glial cells, Pig, Pineal gland, Pinealocytes

The morphology and functions of the mammalian pineal gland appeared to be due to circadian rhythms synchronized by changes in environmental lightening conditions. Metabolically it synthesizes a collection of biologically dynamic substances like serotonin (5-hydroxytryptamine), histamine, nor-epinephrine, melatonin and other indoles. The pineal gland of mammals seems to be a sole source for synthesis of melatonin due to presence of enzyme hydroxyindole-o-methyl transferase. Studies on rodents suggested that the pineal gland may influence the actions of recreational drugs, such as cocaine (Uz *et al.* 2003) and its hormone melatonin can protect against neurodegeneration (Manev *et al.* 1996). Wide ranging functional entities and paucity of literature were instrumental to plan the present histological study on the pineal gland of local inhabitant pigs.

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MATERIALS AND METHODS

The brain tissues containing pineal gland were collected from 10 adult pigs immediately after slaughter by exposing the cranium. The tissues fixed in 10% neutral buffered formalin for 48–72 h were processed for paraffin technique of light microscopy to make sections of 6 μ . Thick sections of 15 μ were also cut for the purpose of a few special stains. The sections were stained with routine Harris' haematoxylin and eosin stain, Gomori's method for reticulum, Weigert's method for elastic fibres, Bielschowsky's method for axis cylinder and dendrites, Holmes method for nerve cells and fibres, Sevier-Munger method for neural tissue, Kluver-Barrera method for myelin and nerve cells, Lapham's method for myelinated glial fibres, Kossa's method for calcium, McManus' PAS method for glycogen, PAS Alcian blue method for mucosubstances (pH 2.5), diastase digestion method (Luna 1968), Crossman's trichrome stain for collagen fibres (Crossman 1937), Quick toluidine blue stain for mast

cells (Humason 1979), mercury bromphenol blue method for protein (Pearse 1968).

For lipid histochemistry, the fresh pineal tissues were immediately frozen at -20°C in cryostat to cut 10–12 μ sections which were taken on glass slides and stained by oil-red-o in propylene glycol and Sudan black B methods for fats (Luna 1968). For scanning electron microscopy (SEM), the fresh tissues of pineal gland were washed with chilled 0.1 M phosphate buffer (pH 7.4). The fixation was carried out in chilled 2% glutaraldehyde solution prepared in 0.1 M phosphate buffer (pH 7.4) at 4°C for 6–8 h. The tissues were again washed thrice with the buffer, dehydrated, critical points dried and sputter coated with gold before viewing under scanning electron microscope at EM Laboratory, AIIMS, New Delhi.

RESULTS AND DISCUSSION

The pineal gland of pig was surrounded by a layer of capsule having loose irregular connective tissue consisting of collagen and reticular fibres as observed in ox (Jain and Koranne 1976), goat (Sharma *et al.* 1980, Kumar *et al.* 1995), sheep (Saggar *et al.* 2001) and horse (Kumar *et al.* 2007). In addition, smooth muscle fibres observed in camel (Sudhakar *et al.* 1987) could not be observed in pig during present study.

The capsule of pineal gland towards its outer surface was lined by a simple cuboidal epithelium and telochoroidea of III ventricle (Fig. 1). At places the connective tissue trabeculae from the capsule penetrated the parenchyma and divided the gland into lobes and lobules as observed in goat (Kumar *et al.* 1995, Pathak *et al.* 2009), sheep (Saggar *et al.* 2001) and camel (Sudhakar *et al.* 1987). However, a small cortex at periphery and a bulky medulla towards centre of the gland was reported in ox (Jain and Koranne 1976). In contrast, the peripheral portion of the gland had been reported denser as compared to central portion in goat (Sharma *et al.* 1980).

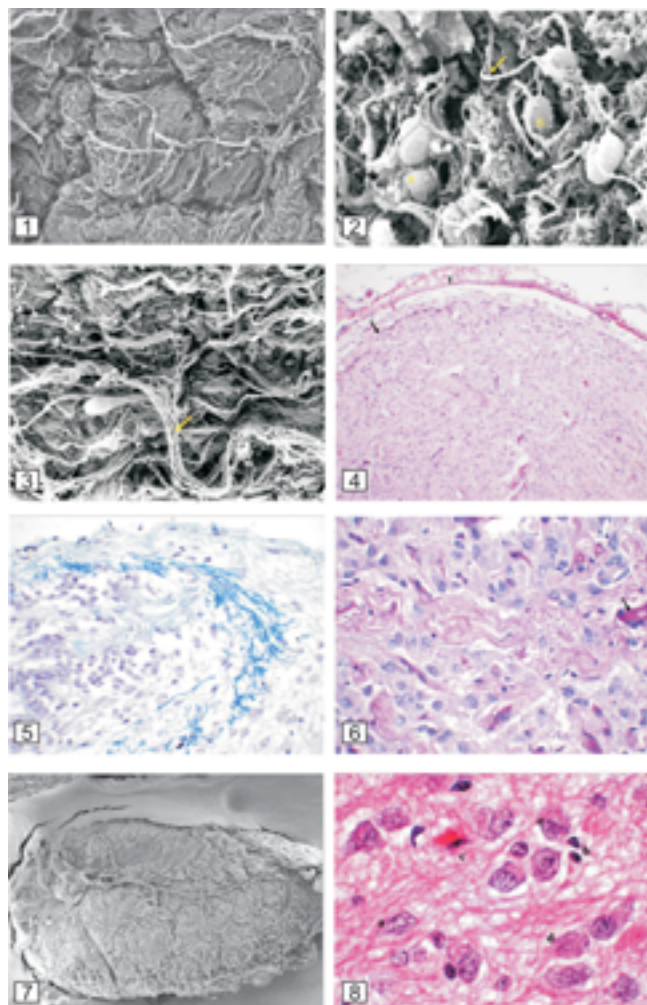
The parenchyma of the gland was constituted by pinealocytes, glial cells, fine blood capillaries and the nerve bundles. The pinealocytes constituting the majority of cell population were distributed throughout the gland having an arrangement in the form of irregular groups, small clusters, rosettes or follicles. Similar observations were made in goat (Kumar *et al.* 1995, Pathak *et al.* 2009), sheep (Saggar *et al.* 2001) and horse (Kumar *et al.* 2007). The chief cells representing the pinealocytes were distributed diffusely in a narrow zone at the periphery whereas; these cells were closely packed to form a syncytium like arrangement towards the centre in buffalo calf (Lalitha and Seshadri 1986). The pinealocytes in the form of typical follicle had been reported in camel (Dellmann and Fahmy 1966 a).

The pinealocytes of pig were categorised into two types on the basis of distribution of chromatin material and staining affinity of cytoplasm (Fig. 2). The round to oval shaped lighter pinealocytes had fine dusting of euchromatin material

irregularly throughout the nucleus whereas, small sized dark pinealocytes possessed fine aggregation of deeply basophilic heterochromatin material masking the details of nucleoli (Fig. 2). The pinealocytes were reported rounded in goat (Sharma *et al.* 1980, Pathak *et al.* 2009), round or oval in buffalo calf (Lalitha and Seshadri 1986) and polyhedral in camel (Sudhakar *et al.* 1987). One or more generally eccentric nucleoli were clearly visible in the lighter pinealocytes. The pinealocytes possessed fine processes which intermingled with those of glial cells or other pinealocytes. However, nodular club shaped endings of the processes of chief cells were observed in buffalo calves (Lalitha and Seshadri 1986).

The glial cells interspersed in between groups of pinealocytes could not be distinguished in the form of astrocytes, oligodendroglia and microglial cells even with the special stains. However, the glial cells were categorized into 4 types based on nuclear morphology (Figs 2, 3) as reported in goat (Kumar *et al.* 1995, Pathak *et al.* 2009), sheep (Saggar *et al.* 2001) and horse (Kumar *et al.* 2007). Type-I glial cells had large round to oval, less basophilic nuclei with fine chromatin material condensed in smaller clumps irregularly throughout the nucleus. The glial processes presented more eosinophilic and granular feature as compared to the cytoplasm of the cell. Type-II glial cells (Fig. 3) were significantly smaller in size with round to oval nuclei having strongly basophilic chromatin material which tended to accumulate in larger clumps particularly towards the outer nuclear membrane. These cells were having a sparse distribution along with type-I glial cells. Type-III glial cells were elongated, ovoid in shape mainly associated with fine blood capillaries. The number of these cells showed a positive correlation with the vascularity of the gland parenchyma (Fig. 3). The type-IV cells possessed nuclei which were largest amongst all the cell types in the pineal gland of pig as reported in goat (Kumar *et al.* 1995) and sheep (Saggar *et al.* 2001). One or more nucleoli were placed centrally or peripherally in various cells types except type-II where appearance of nucleoli was masked by deeply basophilic chromatin material. Lalitha and Seshadri (1986) in buffalo calves distinguished three types of neuroglial cells. The first type of neuroglial cells was larger with two to three processes, second without processes and third being smaller with numerous processes. However, astrocytes, oligodendroglia and microglial cells had been demonstrated in buffalo (Dellmann and Fahmy 1966 b), goat (Sharma *et al.* 1980) and camel (Dellmann and Fahmy 1966 a, Sudhakar *et al.* 1987).

Nerve fibres revealed by various special stains were distributed mainly towards periphery of the gland, in the interlobular spaces (Fig. 4) and also surrounding the blood capillaries. An increased distribution of nerve bundles at the junction of pineal gland with pars-supracommissuralis portion of the subcommissural organ was also seen as reported in sheep (Saggar *et al.* 2001). Nerve cells and mast



Figs 1–8. Photomicrograph of pineal gland showing: **1.** telorchoroidea (T) and capsule (arrow) towards outer surface along with its parenchyma. H & E $\times 100$; **2.** light (L) and dark (D) pinealocytes and different types of glial cells (G). H & E. $\times 1000$; **3.** showing type-I (a), type-II (b), type-III (c) and type-IV (d) glial cells. H & E. $\times 1000$; **4.** bundles of nerve fibres (blue coloured) placed mainly towards the periphery of the gland and in the interlobular space. Kluver-Barrera method. $\times 100$; **5.** Scanning electron-micrograph of pineal gland showing irregular dorsal surface of the pineal gland with connective tissue fibres and blood vessels forming the capsule. $\times 31$; **6.** dorsal surface at higher magnification depicting network of fibrous strands oriented in different directions. Note connective tissue fibres appeared to be penetrating deep into the parenchyma of the gland; $\times 337$. **7.** cut surface having rough surfaced pinealocytes (P), smooth surfaced glial cells (G) and their processes (arrow) running in different directions. $\times 2870$; **8.** cut surface presenting large number of glial fibres (arrow) forming meshwork like structure corresponding to glial zones. $\times 282$.

cells could not be localized during present study. At places melanin containing cells were interspersed in between pinealocytes and glial cells especially towards the periphery of the gland parenchyma as reported in goat (Saigal *et al.*

1976), buffalo (Lalitha and Seshadri 1986) and horse (Kumar *et al.* 2007). Presence of calcium deposits in the form of fine corpora arenacea were seen irregularly at few places throughout the pineal parenchyma. Similarly, the concretions of brain sand made-up of calcareous material with concentric lamellar structure had been reported in adult buffalo (Dellmann and Fahmy 1966 b, Lalitha and Seshadri 1986), ox (Jain and Koranne 1976) and goat (Pathak *et al.* 2009). The corpora arenacea was reported to be absent in goat (Sharma *et al.* 1980, Kumar *et al.* 1995), pig (Jain and Koranne 1976) and sheep (Saggar *et al.* 2001).

The carbohydrates revealed by various histochemical techniques presented PAS positive activity throughout the pineal parenchyma in pig. A mild and strong PAS activity was seen towards capsule and lumen of blood capillaries, respectively. The cytoplasm and processes of pinealocytes and the glial cells had PAS positive fine granular feature. The PAS reactivity was almost similar after diastase treatment indicating presence of other mucopolysaccharides along with glycogen (Fig. 5). The pinealocytes of Gaddi goat also showed strong bounded amino groups when stained with NH-Schiff method indicating presence of hydroxyindole-o-methyl transferase (HIOMT) enzyme responsible for conversion of serotonin to melatonin (Pathak *et al.* 2009). The authors also observed a distinct PAS positive activity in corpora arenacea in contrast to the mild reaction observed in pinealocytes. The proteins demonstrated by mercury bromphenol blue were observed only at the periphery of parenchyma. The remaining portion of pineal gland was lacking any protein activity except a moderate reaction which was observed towards the lumen of blood capillaries where RBCs were present as reported in sheep (Saggar *et al.* 2001). A very negligible amount of lipids was observed as demonstrated by oil-red-o and Sudan black B methods.

SEM of dorsal surface of pineal gland of pig presented the fibres of different shape and dimensions representing connective tissue of the capsule (Figs 5, 6). These fibrous strands were oriented in different directions and formed an irregular meshwork entrapping the cellular debris as reported in goat, sheep and buffalo calves (Kumar *et al.* 1996 a, Saggar *et al.* 2002, Kumar *et al.* 2006). These fibres appeared to be penetrating deep into the parenchyma of the gland (Fig. 6). The cut surface of pig pineal gland presented pinealocytes, glial cells, glial fibres and cellular debris. The surface of round to oval shaped pinealocytes was studded with microvilli like structure (Fig. 7) as reported in buffalo calves (Kumar *et al.* 2006). The pinealocytes had characteristic depression which presented cauliflower like appearance in goat (Kumar *et al.* 1996 a) and buffalo calves (Kumar *et al.* 2006). The round to oval, smaller glial cells presented smooth surface (Fig. 7) and possessed large sized processes which frequently intermixed with those of other glial cells. At places, a few areas having predominance of glial fibres (Fig. 8) were observed as reported in goat (Kumar *et al.* 1996b).

These areas corresponded to the glial zones observed in the light microscopy during present study.

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