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SHORT COMMUNICATION

Aberrant craniopharyngeal structure in the pituitary of a male Wistar rat

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ABSTRACT

An aberrant craniopharyngeal structure (ACS) in pituitary is a congenital developmental abnormality. The ACS was reported during histopathological examination of safety assessment study. It was entirely located within pars nervosa of pituitary as tubular-acinar pattern made by cuboidal epithelial cells of tubulo-acinar pattern with/without lumen, ciliated, PAS positive luminal cells with secretions and were not pre-neoplastic in terms of microscopic features. The other parameters of neurobehavioral battery, clinical pathology including thyroid profile were normal and the presence of ACS was asymptomatic. The incidence of ACS was 0.002% (N=598) in male wistar rat (aged 10-21 weeks) screened for histopathology from several 28 day and 90-day studies at the facility between 2016-2021. The present case confirmed the earlier reports viz., rare incidence, male predominance, microscopic features which included possible similarity to salivary gland, nasal epithelium and an innocuous presence in the animal.

Keywords: Aberrant craniopharyngeal structure, PAS positive, Rathke's pouch, stomatodeum, Wistar rat

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An aberrant craniopharyngeal structure (ACS) in pituitary is a congenital developmental abnormality. Defects in movement of posterior wall of Rathke's pouch towards ventral wall of the forebrain vesicle during rodent gestation days 12-13[1] and persistence of oro-pharyngeal epithelium of craniopharyngeal duct (i.e. Rathke's pouch) could cause spontaneous pituitary findings in rat such as persistence Rathke's pouch/cleft, craniopharyngioma or leave a remnant called ACS in pituitary gland[2]. Among them, ACS is a rare incidence lesion that had been reported in Wistar, Sprague Dawley (SD)[2] and Fisher rats[3]. ACS was demonstrated to be not of pituitary tissue origin[2] by its lack of pituitary hormone secretion, followed by identification of parotid gland structures, thereby confirming similarity of ACS to salivary gland's tissue of origin, Stomatodeum[3]. With this background status of ACS in rats like rare incidence, identified as an embryological remnant and a tissue origin other than pituitary, we tried to report a case finding of ACS from a male Wistar rat during a histopathology review from a 90-day toxicity study, with emphasis on its incidence based on historical occurrence of pituitary lesions in our facility. The animal's other related parameters from the 90-day study, its microscopic similarities as reported[2,3,4] earlier using hematoxylin and eosin and Periodic acid (PAS)-Alcian blue special stain was considered for its assessment.

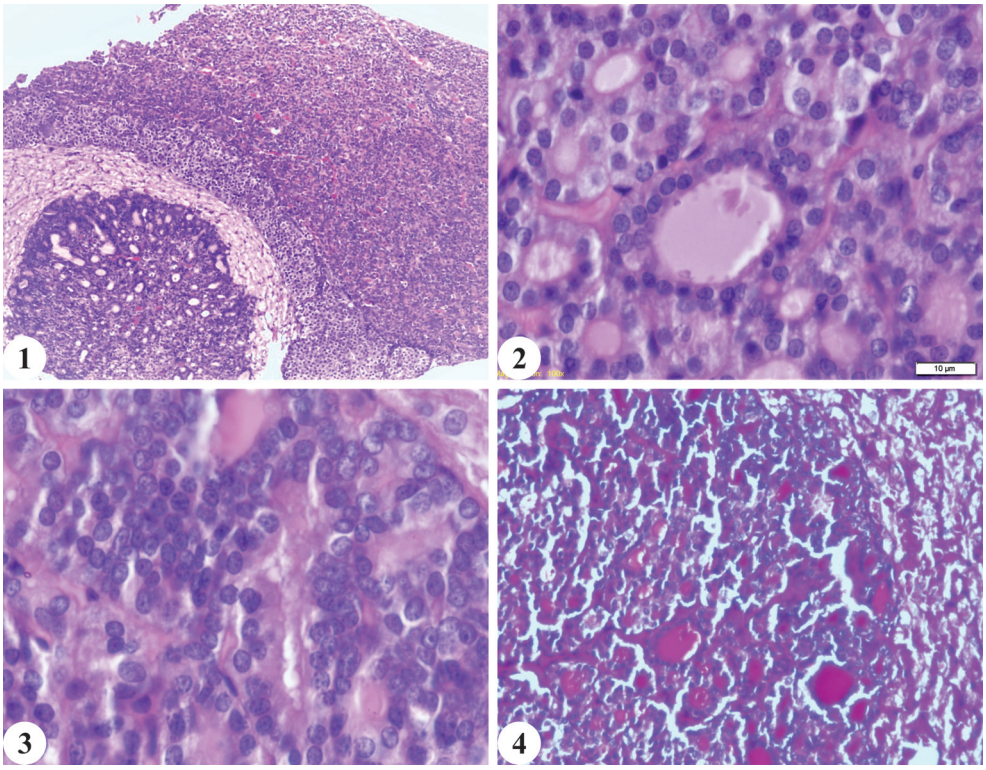
During the 90-day toxicity safety assessment study[5] conducted as per compliance with Organisation for Economic Co-operation and Development (OECD) principles of Good Laboratory Practice (GLP) at Vimta Labs Limited Hyderabad, India, the animals were provided with a standard diet and water *ad libitum* in a controlled environment (temperature 22°C-25°C, relative humidity 50%-70%), and a twelve-hour light-dark cycle. Animal was observed for clinical signs throughout the study period and neurobehavioral examination was conducted during the study period. During terminal sacrifice, the approximate age of male HanTac: WH wistar was 21-week old. Animal was sacrificed with carbon-di-oxide asphyxiation after retro-orbital blood collection under isoflurane anesthesia for analysis of hematology (ADVIA, 2120, Siemens, Germany), coagulation (Diagnostica Start-4, Stago, France) and clinical chemistry parameters including thyroid hormones (Vitros 4600, Johnson & Johnson). During the necropsy, the animal was thoroughly gross examined, and its pituitary was collected *in-situ* with sphenoid bone in 10% neutral buffered formalin for preservation, which was weighed after 24 hour post fixation and processed routinely for histological examination, embedded with its dorso-caudal aspect downwards in paraffin, sectioned at 3-5 micron thickness and stained with haematoxylin and eosin (H&E). On identification of the lesion during histopathological evaluation, sections were recut for PAS-Alcian blue stain (with Hematoxylin counterstain)[6]. All

procedure from the study like clinical examination, clinical pathology procedures, histology processing and evaluation were performed following standard operating procedures of organization and recommended guidelines[5].

No abnormal clinical signs were observed in the animal during daily clinical observation or neurobehavioral examination conducted at the end of the study. At necropsy, gross examination of pituitary was normal including the pituitary organ weight. The routine clinical pathology parameters in a 90-day rodent safety assessment study (including thyroid hormone profile) remained within reference values of age matched Charles River Wistar (Hannover) wistar data of >17-week age animals[7] and 19-21 week Wistar rat's facility historical reference values. Microscopically, the aberrant structure was confined as solid demarcated structure within pars nervosa, without compression of surrounding tissue and occupied entire pars nervosa (Fig. 1). The epithelial cells were plump with eosinophilic cytoplasm, hyperchromatic nuclei and coarse chromatin arranged in tubular and acinar pattern with or without eosinophilic secretion filled lumens. Ciliation was observed in the lumen (Fig. 2) formed by the cells. When luminal formation was absent, cells were found as cords and aggregations (Fig. 3). No mitotic figures were observed. The luminal secretions and cellular content were found to be PAS positive and Alcian blue negative (Fig. 4).

In rodents, aberrant craniopharyngeal structures (ACS) of pituitary gland is a non-proliferative lesion involving acinar, tubular or fusiform cellular structures appearing in neurohypophysis or between pars intermedia and pars nervosa region of pituitary[2]. The earlier reported incidences of the structures were 0.17% (7/4200) in Fisher (F334/DuCrj) male rats, 0.15% (7/4450) in Fisher F334/DuCrj) female rats, 1.25% (5/400) to 1.45% (3/400) in male SD rats, 1.10% (2/183) in female SD rats, 0.45% (3/646) to 1.25% (2/517) in male Wistar rats and 0.40% (2/517) to 1.65% (1/60) in female Wistar rats.

In our facility, based on five years (2016-2021) of historical control incidences of histopathological lesions in pituitary from Wistar rats, aged between 10-21 weeks from 28 and 90-days repeated dose toxicity studies, pituitary cyst was the common finding (1.3% in male and 0.5% in female Wistar rats; N=598/sex). ACS of rodent pituitary in the male Wistar was the first incidence at our facility with a very low frequency in males (0.002%; N=598). None was observed in females of Wistar rats. A male predominance was earlier reported in SD (mean age 106 weeks)[3] and Wistar rat (mean age 41 weeks) studies[3], but was absent in report of Fisher rats (age 26-



Aberrant craniopharyngeal structure. **Fig. 1.** Circumscribed solid demarcated growth within pars nervosa (H&E 40X); **Fig. 2.** Tubulo acinar pattern of growth formed plump cells with eosinophilic cytoplasm, hyperchromatic nuclei and coarse chromatin with ciliated lumen formations containing eosinophilic secretions (H&E 1000X); **Fig. 3.** Cords of epithelial cells without lumen (H&E 1000X); **Fig. 4.** Periodic Schiff positive luminal secretions and cells shown within pars nervosa (PAS-Alcian blue/H&E 200X).

104 weeks)[3]. Although, there was no enough incidence with us to report a male predominance, possibly the only incidence in Wistar rats of both sexes appeared an inclination of ACS to occur in male Wistar rat. In those earlier reported studies[3,4], the animals were of higher age which could possibly be the cause for occurrence of gross findings not observed in our case report, where the pituitary weight remained normal. The reported gross findings were single nodule, large cyst (Fisher rat studies[4]), and enlargement of pituitary (Wistar/SD rat[3]) which corresponded to microscopic findings such as epithelial nests, Rathke's cleft cyst in Fisher rat and adenoma of adenohypophysis in Wistar rat colony respectively. Except the nodule that corresponded to ACS and appeared histologically as epithelial nests, the other gross findings were not associated with ACS.

The studies in SD, Wistar[3] and Fisher rats[4] and recent compilation of proliferative lesion in rodent[1] described the presence of ACS in pars nervosa or at the junction of pars intermedia/nervosa with solid, branched/mesh like growth of cystic structures with acinar/tubular or fusiform pattern and the cells comprising cuboidal (round basal nuclei/abundant eosinophilic cytoplasm), squamous cells (pale nuclei, medium sized cells) and fusiform (scanty cytoplasm and spindle shaped) type. They were arranged as single or double layer or stratified around the lumen of tubules. When cystic structures were involved in ACS, they occurred with lining of ciliated epithelial cells. If they were made of squamous epithelium, had shown keratinisation and lumen lined with cuboidal epithelium, had proteinaceous/colloid secretion which were PAS positive and also resembled salivary gland (serous/mucous)[4]. Fusiform type cells although resembled glial cells were Glial Fibrillary Acid Protein (GFAP) negative indicating its origin not from pars nervosa. In our case study, we found a tubular acinar pattern growth with cuboidal cells. The presence of squamous cells although specifically unconfirmed, no keratinisation occurred. No stratification or double layer of epithelium was observed and there was ciliary like projections in the cells bordering lumen (Fig. 2). As earlier reported[4] ciliation attributed a similarity to nasal epithelium and could probably be a congenital development aberration of the nasal respiratory epithelium which shares similar tissue of origin, Stomatodeum. We also observed tubules with lumen containing eosinophilic secretions which gave PAS positivity including cells lining the lumen (Fig. 4). This was endorsed by earlier work[3] which reported mimicking of ACS with salivary gland in Wistar/SD rats and in succession was confirmed in Fisher rats[4] based on PAS-Alcian method with PAS positivity and mucin negative result. We either could not see any mucin positive staining in ACS structure of the case study. During diagnosis of ACS in rats, one among the differentials include craniopharyngioma a rare epithelial tumour of sellar region in human beings, domestic and laboratory animals[8] which should be differentiated by its compression, marked hyper/para keratosis[2] features which were absent in our case. With no mitotic figure or compressive growth, ACS in our study was not pre-neoplastic. Based on the other parameters of neurobehavioral examination, clinical pathology including thyroid hormone profile and daily signs the finding was considered innocuous. To conclude, the finding of ACS as important differential in the diagnosis of proliferative lesion of pituitary during a rodent chronic study is emphasised and had been further confirmed for its rare incidence, male predominance, microscopic features and PAS positivity through our case report.

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