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Nuclear diameter in parathyroid disease

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SUMMARY The nuclear diameter of chief cells was measured in 17 cases of parathyroid adenomas, four cases of secondary hyperplasia, five cases of primary hyperplasia and six cases of tertiary hyperparathyroidism. All the cases with secondary hyperplasia and tertiary hyperparathyroidism were associated with chronic renal failure. The nuclear diameter in both the adenomatous and hyperplastic areas of tertiary hyperparathyroidism were measured. The adenomatous areas of tertiary hyperparathyroidism contained nuclei of a larger diameter than those in the hyperplastic foci of the same gland. The nuclear diameter in adenomatous foci of tertiary hyperparathyroidism was similar to that in adenomas from primary hyperparathyroidism. These findings lend support to the concept of formation of autonomous adenomas against a background of reactive parathyroid hyperplasia in cases of tertiary hyperparathyroidism. Using statistical methods there were differences between the nuclear diameter in cases of primary adenomata, and cases of primary and secondary hyperplasia. Primary parathyroid hyperplasia stood out as a distinct group. The significance of these findings is discussed.

The condition "tertiary hyperparathyroidism" is usually defined as the development of an autonomous adenoma or adenomas in overstimulated hyperplastic parathyroid glands. This occurs most commonly in chronic renal failure but is also seen in malabsorption syndromes.

The term was first introduced by St Goar[1] in 1963 and subsequently accepted by others.[2–5] However, some workers[6–8] have doubted the existence of this entity and believe that the clinical and biochemical manifestations of tertiary hyperparathyroidism are entirely due to a large mass of hyperplastic parathyroid tissue.

In order to investigate whether tertiary hyperparathyroidism is a distinct disease entity, the nuclear diameters of the chief cells in the adenomatous foci of the glands were compared with those of the hyperplastic areas of the same glands. Cases of secondary hyperplasia, primary chief cell hyperplasia and adenomas of primary hyperparathyroidism were also studied for comparison. We determined the nuclear diameters to see if this would enable the practising pathologist to distinguish adenomata from cases of hyperplasia.

Material and methods

Seventeen adenomas producing primary hyperparathyroidism, four cases of secondary hyperplasia, five of primary chief cell hyperplasia and six cases of tertiary hyperparathyroidism were studied. The diameters of chief cell nuclei of two normal parathyroids, which were accidentally removed during thyroidectomy, were also measured. Most of the cases were collected over a period of nine years (1973 to 1981) at the University Hospital of South Manchester (Withington Hospital). Three cases of primary hyperplasia were obtained from other hospitals in order that a significant number of cases could be studied. All the patients with secondary hyperplasia and tertiary hyperparathyroidism had been on haemodialysis for chronic renal failure. The clinical data and pathological findings of these cases have been previously described.[5]

In cases of primary hyperparathyroidism the diagnosis of adenoma and primary hyperplasia were made according to the criteria of Castleman.[9] Adenomas in our series involved only one parathyroid gland and the other glands were found to be normal or atrophic. Histologically they showed a proliferation which consisted predominantly of chief cells. These were arranged in trabeculae, acini or in diffuse sheets. Most cases showed nuclear pleomorphism. A fibrous capsule with a thin, compressed rim of normal parathyroid tissue outside the capsule was found in some cases. In cases of primary hyperplasia more than one gland was involved. All four glands were involved in three cases but in the other

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two, only two glands were enlarged and the other parathyroid glands were not identified at surgery. Histologically, all the cases showed chief cell hyperplasia with some oxyphil and water-clear (Wasserhelle) cells. The nuclei were uniform and no compressed normal parathyroid tissue was found at the periphery of the gland.

After tissue had been taken for frozen section and electron microscopy the glands were fixed in Carson's fluid. Paraffin-embedded, 5 μm thick sections stained with haematoxylin and eosin were examined. The nuclear diameters of the chief cells were measured with a horizontal eye-piece micrometer graticule (Graticules Ltd). The graticule was calibrated by means of a stage micrometer. All sections were examined using a × 100 oil-immersion objective and × 10 eyepiece. As parathyroid nuclei are perfectly round only one measurement of the diameter was made. Only round, non-distorted chief cell nuclei were measured in different areas of each gland. The nuclei at the periphery of the gland, in areas of haemorrhage or artefact were excluded.

Initially 500 nuclei from each gland were measured from the first two adenomas from cases of primary hyperparathyroidism. The mean nuclear diameters and their standard deviations were calculated after successive groups of 50 nuclei had been measured. Both the mean and the standard deviation fluctuated when only small numbers of nuclei were studied but they varied little after 250 nuclei or more had been measured (Fig. 1). It was therefore decided that 250 nuclei should be measured from each gland. In cases of primary adenomas, primary and secondary hyperplasia, 250 nuclei were measured from each gland. In cases of tertiary hyperparathyroidism 250 nuclei were measured from an adenomatous area and a further 250 nuclei were sampled from a hyperplastic focus in the same gland.

Results

MEAN NUCLEAR DIAMETER (FIG. 2)

The mean nuclear diameters of individual glands were calculated and then the means of the mean nuclear diameters of each group (group mean) were determined for comparison. The lowest group mean was found in secondary hyperplasia (5·7 μm) and the highest in the adenomatous foci of tertiary hyperparathyroidism (6·3 μm). All the other groups showed a group mean of 6·0 μm. The group mean of the two normal glands were much smaller: 5·2 and 5·6 μm respectively. These data have not been shown in the graph as they are too few for inclusion in a formal statistical analysis. Some variation of nuclear diameter has been noted in every group except for cases of primary hyperplasia where the group mean of the 12 glands showed little variation. The mean of one primary adenoma was found to be 7·6 μm and as can be seen in Fig. 2 it was higher than all adenomata studied.

STANDARD DEVIATION (FIG. 3)

The standard deviation (SD) is a measure of variation and showed the distribution pattern of nuclear diameters. The average of the standard deviations of nuclear diameters in each group was determined. Both cases of secondary hyperplasia and hyperplastic areas of tertiary hyperparathyroidism showed a similar grouping with a SD of 0·5. In contrast the SD of the adenomatous foci of tertiary hyperparathyroidism was larger (0·56). The primary adenomas had a SD of 0·61. The lowest SD (0·43) was found in primary hyperplasias.

KURTOSIS[10] (FIG. 4)

Kurtosis is a measure of "peakedness" and is used to distinguish between a flat distribution pattern and a

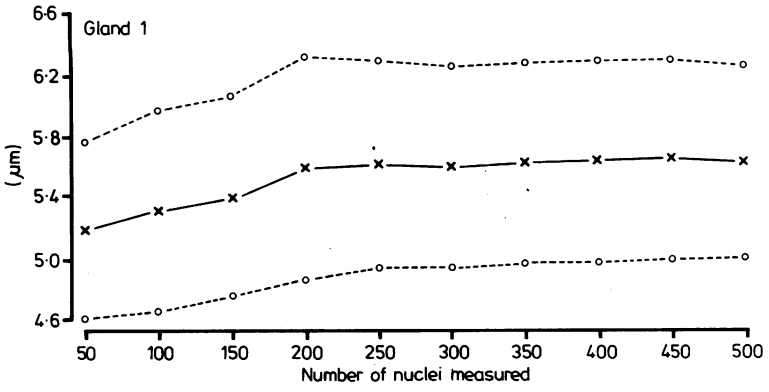

Fig. 1 *Graph showing mean nuclear diameters (× — × — ×) ±SD (O - - - - O) in μm for increasing numbers of nuclei measured for a single gland. The nuclear diameter shown on the abscissa becomes constant after measuring 250 nuclei.*

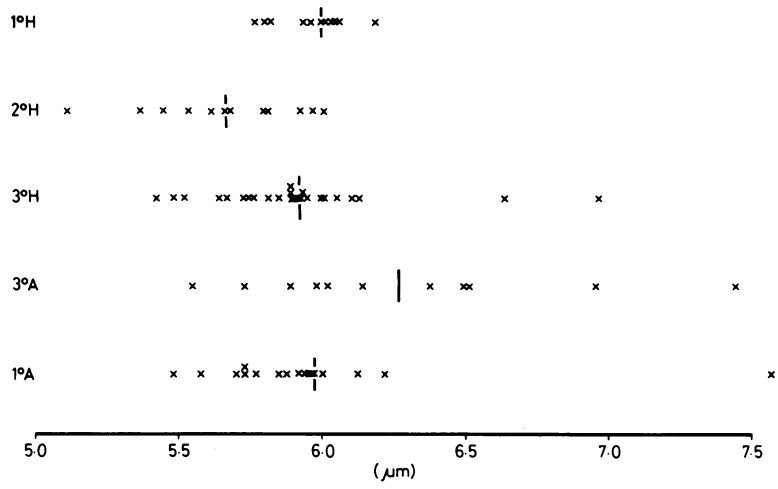

Fig. 2 *Showing the mean nuclear diameter of individual glands marked by crosses and the group mean shown by bars. (1°H = primary hyperplasia, 2°H = secondary hyperplasia, 1°A = primary adenoma, 3°A = adenomatous area of tertiary hyperparathyroidism, 3°H = hyperplastic area of tertiary hyperparathyroidism). More than one gland was studied in some of the cases.*

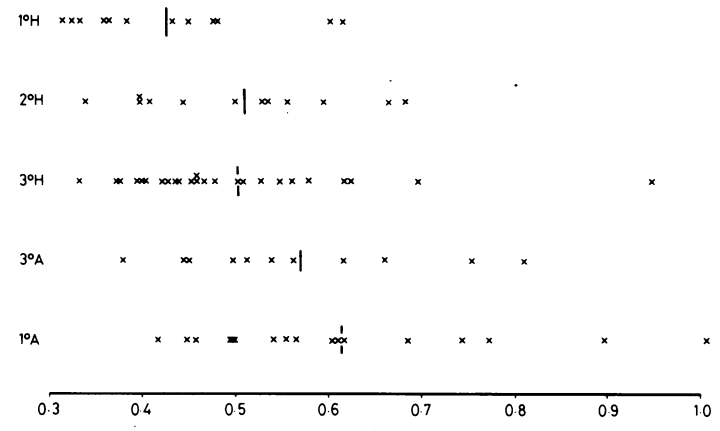

Fig. 3 *Standard deviation in cases of parathyroid disease. Individual glands marked by crosses and the average of standard deviations by bars.*

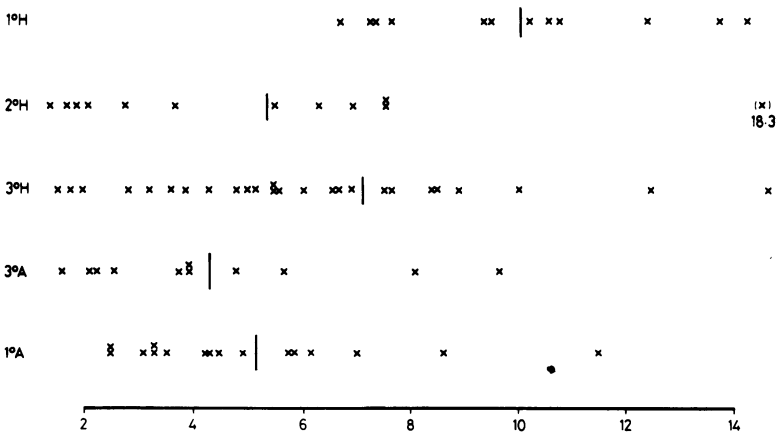

Fig. 4 *Kurtosis in cases of parathyroid disease. Individual values are shown by crosses and group average kurtosis by bars.*

sharply peaked curve. The values obtained tended to group closely together. They also showed a similarity between cases of secondary hyperplasia and hyperplastic areas of tertiary hyperparathyroidism lying in one group and primary adenoma and adenomatous foci of tertiary hyperparathyroidism lying in another group. The cases of primary hyperplasia were once again a distinct group.

Discussion

In the present study the nuclear diameters of parathyroid chief cells were measured initially to see if tertiary hyperparathyroidism was a distinct disease entity or part of secondary parathyroid hyperplasia. The nuclear diameter of chief cells were compared in adenomatous and hyperplastic foci of tertiary hyperparathyroidism. These values were then set alongside cases of adenomata from primary hyperplasia and cases of chief cell hyperplasia due to primary and secondary hyperparathyroidism. Using mean nuclear diameter only as a parameter there was no statistical difference between the groups. However when the standard deviation and kurtosis were determined a distinct grouping of the adenomata in cases of primary and tertiary hyperparathyroidism emerged. Similarly cases of secondary hyperparathyroidism and foci of hyperplasia in tertiary hyperparathyroidism were grouped together. This has led us to believe, apart from the microscopic findings, that there is distinct adenoma formation in cases of tertiary hyperparathyroidism. The average of the SD of the hyperplastic states was identical (0·5) suggesting the same disease process was occurring in these glands.

As has been mentioned above, there has been much controversy about tertiary hyperparathyroidism. Some authors[1–5] believe it to be a distinct disease entity while others[6–8] take the view that it is part of the spectrum of secondary parathyroid hyperplasia. We have argued previously[5] that on purely histological grounds tertiary hyperparathyroidism is a separate entity. Usually there is a capsule around the chief cells and compression of the cells outside. This differs from secondary hyperplasia where the cells tend to lie in a lobular pattern and the connective tissue between them is rather loose and oedematous. The presence of a compressed rim of parathyroid cells has not been accepted by all authors as a good criterion for the diagnosis of adenomata.[7 11] We would agree with this in cases of primary parathyroid adenomata since it is often difficult to find a capsule, let alone compressed parathyroid cells outside it. However, in tertiary hyperparathyroidism there is usually no difficulty in finding compressed parathyroid cells and the fibrous tissue surrounding the adenoma is usually much denser than in secondary hyperplasia. Electron microscopy is of little help in distinguishing adenomatous foci from adenomata.[5]

Adenomata could well develop on a background of hyperplasia since a case has been described with parathyroid carcinoma developing on a background of hyperplasia and adenoma formation[3] as well as coexistent hyperplasia and adenomata in other cases. No specific clinical or radiological features separate secondary parathyroid hyperplasia from tertiary hyperparathyroidism, though many cases of the latter condition have hypercalcaemia. However, histology is the only sure way of diagnosing tertiary hyperparathyroidism. Some workers[12] believe that the immunoreactive parathormone (iPTH) concentration is higher in tertiary hyperparathyroidism. This is not so in our experience: we have seen a case with a serum iPTH of > 20 ng/ml,[5] similar to that in cases of tertiary hyperparathyroidism. However, the histology showed secondary hyperplasia.

A study similar to the present one examined the nuclear diameters in chief cell adenomata from cases with primary hyperparathyroidism.[13] These authors measured nuclear diameter in 55 parathyroid chief cell adenomata. They studied "normal" glands in 34 of these patients, these second glands being removed during exploration of the neck during parathyroid surgery. In 27 out of the 34 cases the mean nuclear diameter was significantly greater in the adenomata than in control cases. However, the selection of controls is open to criticism since in cases of primary hyperparathyroidism due to an adenoma, the non-adenomatous glands may be suppressed and this may lead to a decrease in nuclear diameter. Normal control parathyroid glands are difficult to obtain. The use of necropsy material may not be comparing like with like in that the nuclei of necropsy glands may show autolytic change. Lloyd and his colleagues[13] examined five normal parathyroid glands at necropsy and found the nuclear diameter to be similar to the operation control group. However, there was much more variation in nuclear diameter in the operation normals, suggesting varying degrees of suppression. We were able to obtain two parathyroid glands that had been removed at the time of thyroid surgery and found that their mean nuclear diameter was less than glands with adenomata. The mean nuclear diameter in our cases with adenomata due to primary hyperparathyroidism was 6·0 μm whereas normal controls were 5·4 μm. Unfortunately only two normal glands were examined and it is difficult to make useful comparisons on such a small number of cases. The mean nuclear diameter in adenomas in Lloyd's series[13] was similar to ours at 5·831 ± SEM 0·115 μm. As in our cases there was a considerable scatter of chief cell nuclear diameter in primary

parathyroid adenomata. One of our cases of primary adenomata was of particular interest. A 37-year-old man had a pancreatic pseudocyst and hypercalcaemia. A parathyroid adenoma weighing 22·8 g was found at surgery. The patient subsequently died from his abdominal problems and at necropsy no other parathyroid adenomata were found. The chief cell nuclei in this case measured up to 12 μm in diameter. Giant nuclei (≥12 μm) are a feature of adenomata of primary hyperparathyroidism and may be useful in diagnosis.[9] Lloyd and his colleagues[13] showed that the group mean of adenomas in cases with overt bone disease was greater than in patients with renal stones or just with hypercalcaemia and no other stigmata of hyperparathyroidism. It is important to note that these authors did not examine cases of tertiary hyperparathyroidism.

Bengtsson *et al*[14] approached the problem of the separation of parathyroid adenomata from hyperplasia in a different way. These authors did not study cases of tertiary hyperparathyroidism. They found that in normal and hyperplastic glands diploid cells predominated and tetraploid cells were less than 1%. Diploid cells also predominated in the adenomas but the number of tetraploid cells increased and reached 50% in some cases. Cells of a ploidy higher than four were often seen in adenomata but never in hyperplastic or normal glands. They measured nuclear diameter in three cases with primary adenomas and showed that when the ploidy was low so was the nuclear diameter, but in cases with a ploidy of eight the nuclear diameter was higher. Unfortunately these authors did not look at the bone status of their patients as did Lloyd *et al.*[13] There was no correlation between the frequency of tetraploid cells and the weight of the adenomata, the serum calcium or the type and duration of symptoms. Bengtsson *et al*[14] felt that adenomata tended to have more than 2% tetraploid nuclei whereas the majority of hyperplastic glands had diploid nuclei. Thus these authors could, in most cases, distinguish cases of hyperplasia from adenoma.

Fialkow *et al*[15] studied four parathyroid adenomas for the X-chromosome linked enzyme, glucose-6-phosphate dehydrogenase (G6PD). Normal tissues from a subject heterozygous for a B and A gene at the G6PD locus display both B and A isoenzymes. However, since a single cell synthesises one isoenzyme a tumour with clonal origin will manifest either type A or type B G6PD. They found both B and A isoenzyme in each adenoma in proportions similar to normal tissues, indicating that adenomas have a multicellular origin. These authors use this finding as an argument for a similar biological behaviour between hyperplastic and adenomatous states. However, G6PD may be an unsatisfactory discriminator between adenomas and hyperplasias,[16] since contamination may occur with endothelial cells, fibroblasts and oxyphil cells.

Our results do not tend to favour a multicellular origin of both adenomas and cases of primary hyperplasia since the mean nuclear diameters of cases with primary hyperplasia showed little variation and tended to form a compact group. Other authors[4 9 10 17] have accepted the concept of primary chief cell hyperplasia. It has been thought in the past that primary hyperplasia is the first event and then an adenoma emerges in one gland, subsequently suppressing the others. It is impossible to prove this because of the different times patients present with their disease.

Finally we set out to see if nuclear diameter would enable us to distinguish adenomas, especially those in tertiary hyperparathyroidism, from hyperplasias. Nuclear diameters will do this but unfortunately 250 nuclei have to be measured and statistical analysis carried out. Thus the method cannot be recommended for a normal practising histopathologist. Nevertheless we feel the exercise has been useful in establishing that tertiary hyperparathyroidism does exist.

We wish to thank Dr Lumb for alerting us to one of the cases used in this study, Drs Vasudev (Blackpool) and Taylor (Liverpool) for kindly donating cases of primary parathyroid hyperplasia.

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