Volume 20 Number 1 June 2015

FOCUS

PAPANICOLAOU SOCIETY OF CYTOPATHOLOGY

Companion Society of the United States and Canadian Academy of Pathology

Dedicated to Clinical Practice, Clinical Education and Clinical Research

From the Editor's Desk

Dear colleagues,

The time flies and yet another issue of Focus is here!

As always, we have interesting articles of practical significance to our daily practice with cytopathology angle to it.

On lighter note Dr. Giorgadze has contributed interesting images for you to enjoy!

The details about various benefits of joining PSC membership are highlighted on the last page. Please recommend to your colleague to join PSC membership by sending the membership form downloaded from http://www.papsociety.org/docs/09/pscapp2009.pdf.

Please send the articles or other contributions (eg. interesting images in cytology, book reviews, case reports, reviews etc) to me or any of the Focus editorial board members. Currently, we are targeting the contributions for the December 2015 issue. There is no hard deadline for submitting the contributions, but earlier submissions received at vshidham@med.wayne.edu prior to November 7, 2015 are appreciated.

I wish you a happy reading!

Sincerely,

Vinod B. Shidham, MD, FRCPath, FIAC

President's Message

Tarik Elsheikh, MD

It is my great pleasure and honor to serve as the new president of the Papanicolaou Society of Cytopathology (PSC) for the upcoming two years (March 2015-2017). Since its inception, this incredible organization has been dedicated, through its members, to bridging the gap between cytopathology and surgical pathology via national and international educational efforts in cytopathology and small biopsy histology, and to the development of practical evidence-based practice guidelines.

These incredible efforts and accomplishments would not have been possible without the efforts of past and present PSC executive boards, committees, and members. I would like to use this opportunity to also give special thanks to Zubair Baloch, who has performed with distinction in his past two-year tenure as president. In addition, I would like to welcome

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In This Issue

Membership Application

(Please download, print and complete)
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Focus is published by the Papanicolaou Society of Cytopathology

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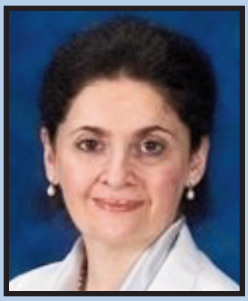
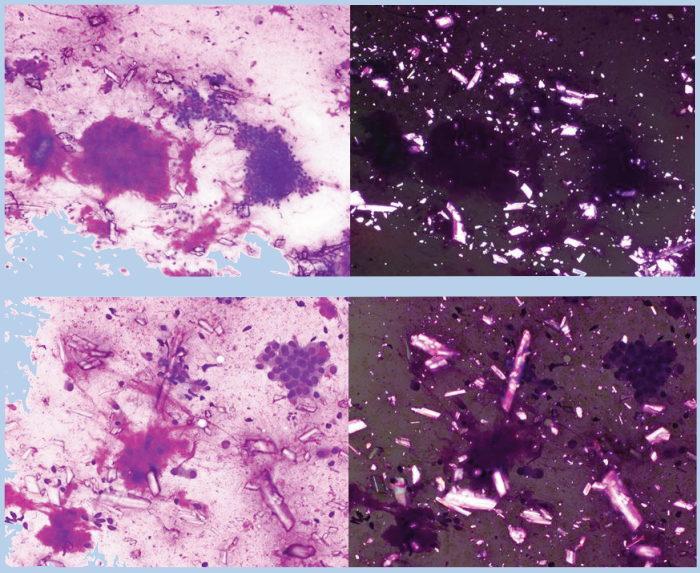
Cholesterol Crystals in Pleomorphic Adenoma of Palate

Alan Marcus, MD and Tamar Giorgadze, MD, PhD, MIAC



Crystalloids and crystals have been described associated both with neoplastic and nonneoplastic salivary gland lesions. [1-5]These include tyrosine, nontyrosine (amylase), and collagenous crystalloids, as well as calcium oxalate and intraluminal crystals. Tyrosine crystalloids are floret-shaped non-birefringent, while amylase crystalloids are non-birefringent and geometric, and vary in shape and size. Both stain orange with Papanicolaou stain and pink with H&E. While tyrosine crystalloids are synthesized by myoepithelial cells, amylase crystalloids are product of acinar cells. Tyrosine crystalloids can be associated with benign salivary gland lesions and tumors as wells as malignant salivary gland neoplasms (pleomorphic adenoma, polymorphous low grade adenocarcinoma, adenoid cystic carcinoma). [1-4]Although generally considered to be associated with sialoadenitis and in cystic changes, including sialocysts lined with oncocytic cells, in rare cases amylase crystalloids may be also seen in Warthin's tumors and pleomorphic adenomas. [4-5]Review of the literature shows that 1-21% of pleomorphic adenomas both of major and minor salivary glands can have associated tyrosine crystals. [1-5]Campbell at al. in their largest series of 294 cases of minor salivary gland tumors, 130 of which were pleomorphic adenoma, found associated crystalloid inclusions (tyrosine and collagenous crystalloids) in 5% of pleomorphic adenomas. [3]Depicted in this image are polarizable cholesterol crystals most likely associated with a long-standing cystic change in this pleomorphic adenoma of a minor salivary gland. To our knowledge, cholesterol crystal incidence in salivary gland tumors has not been reported so far.



References

1. Koss' Diagnostic Cytology And Its Histopathologic Bases 2 vol. Koss LG (Editor) and Melamed MR (Editor). Lippincott Williams & Wilkins; 2005.
2. Paker I, Anlar M, Genel N and Apler M. Amylase Crystalloids on Fine-Needle Aspiration of the Salivary Gland. Turkish Journal of Pathology. Vol. 26, No.2, 2010.
3. Campbell WG, Priest RE, Weathers DR. Characterization of Two Types of Crystalliods in Pleomorphic Adenomas of Minor Salviary Glands. AJP. February 1985; 194-202.
4. Nasuti JF, Gupta PK, Fleisher SR, LiVolsi VA. Nontyrosine Crystalloids in Salviary Gland Lesions: Report of Seven Cases With Fine-Needle Aspiration Cytology and Follow-Up Surgical Pathology. Diagnostic Cytopathology. 2000;Vol22, No3; 167-171.
5. Pantanowitz LP, Goulart RA, Cao QJ. Salivary Gland Crystalloids. Diagnostic Cytopathology. 2006; Vol34, No11; 749-750.

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From the President's Desk

aboard three newly elected members to our executive board, David Chhieng as president-elect, and Andrea Abati and Andrew Field as members at large. The complete list of members of executive board and committees' rosters for 2015-2017 are available on the PSC website.

As always, PSC had another strong showing at the 2015 USCAP annual meeting in Boston. On the evening of March 21st, the PSC companion scientific session took place and was well attended, and received excellent evaluations. The scientific session was titled "Small Biopsy Specimens of Head and Neck with Emphasis on Cell Cytology and the Role of Special Studies", which was moderated by Mat Zarka (past chair of scientific committee). Bill Faquin gave the first presentation entitled "Salivary Gland FNA: New Markers and New Opportunities for Improved Diagnosis". This was followed by Raja R. Seethala who presented "Lumps and Bumps of the Oral Cavity and Oropharynx", and Lester Thompson who presented "SRBCT: Sinonasal Region Biopsies: Cytology of Tumors". The session concluded with a presentation by Margaret Brandwein-Gensler entitled "Challenging Squamoid Biopsies". The contents of the presentations emphasized a practical approach to diagnosing difficult and challenging cases in head and neck small biopsies and cytologic samples, utilizing a standardized approach and practical application of ancillary studies including immunohistochemistry and molecular studies.

At the PSC evening scientific session, several prestigious awards were also presented to distinguished pathologists for their continuing and everlasting contributions to the field of cytology. Britt-Marie Ljung received the life time achievement award, Celeste Powers received the L.C. Tao Educator of the Year award, and David Kaminsky received the Yolanda Oertel Interventional Cytopathologist Award. PSC Research Awards were granted to two pathologists in training. This year the Research Committee reviewed a total of 81 abstracts submitted by first authors in training in the category of Cytopathology. First Place was awarded to Georgios Deftereos, et al. from the University of Washington for their work entitled "Methylation Markers of Pancreatic Carcinoma and Their Usefulness in Pancreatic FNA Cytology". Second place was awarded to Christopher Vytlacil, et al. from Allegheny General Hospital for their work entitled "The Diagnostic Implications of GNAS Point Mutation in Pancreaticobiliary Neoplasia in FNA and Brush Cytology Specimens". Congratulation to all awardees for well deserved recognitions.

Earlier in the afternoon of March 21, 2015, the International Relations Committee presented its annual session at USCAP, moderated by Eric Suba. The session included three presentations by Rosemary Tambouret, Ronald Balassanian, and Eric Suba on "Cervical Screening Programs in Developing Countries", "Developing a Breast FNA Biopsy Service in Peru", and "U.S.-Funded Measurements of Cervical Cancer Death Rates in India", respectively. This was followed by a roundtable discussion.

The PSC book series publications continue to be a success. Three books were published this past year, including "Lymph Node and Spleen Cytohistology" by Field and Geddie, "Head and Neck Cytohistology" by Baloch et al, and "Cytohistology of Focal Liver Lesions" by Wee at al. Two additional books are due to be published in July 2015, including "Cytohistology of the Serous Membranes" by Michael et al and "Pancreatic Cytohistology" by Centeno et al.

The PSC closes the year 2015 strong with more active participation planned on the national and international frontiers, including PSC sponsored sessions and presentations in September in Milan at the European Congress of Cytology, in October at the ASCP annual meeting in Long Beach, and in November at the ASC annual meeting in Chicago.

Our society remains strong and healthy and, with your help and active participation, will continue to grow and have a global influence on the field of cytology and surgical pathology for years to come.

Tarik Elsheikh, MD
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President of the Papanicolaou Society of Cytopathology (PSC)
March 2015-2017

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Courtesy: CytoJournal 2015;12:10
Bhalla A, Meijas-Badillo L, Jencks A, Shidham VB
Thyroid gland and adjacent lesions; Cytomorphological clues!

CytoJournal

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CytoJournal Quiz Case

Thyroid gland and adjacent lesions: Cytomorphological clues!

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Published: 21 May 2015
CytoJournal 2015, 12:10
This article is available from: http://www.cytojournal.com/content/12/2/10

Received: 10 April 15
Accepted: 11 April 15

This article may be cited as:
Bhalla A, Meijas-Badillo L, Jencks A, Shidham VB. Thyroid gland and adjacent lesions: Cytomorphological clues!. CytoJournal 2015;12:10.

A 65-year-old female presented for evaluation of hypertension. Physical examination revealed a painless lump in the neck. Ultrasound examination of the thyroid showed a posteriorly located, hypervascular, single hypoechoic, solid lesion, measuring 1.4 cm, near the upper pole of the left thyroid gland. Figure 1a-d shows the cytomorphological features of the fine needle aspirate in Diff-Quik stained smears. Papanicolaou (Pap) stained smears were suboptimal with scant cellularity.

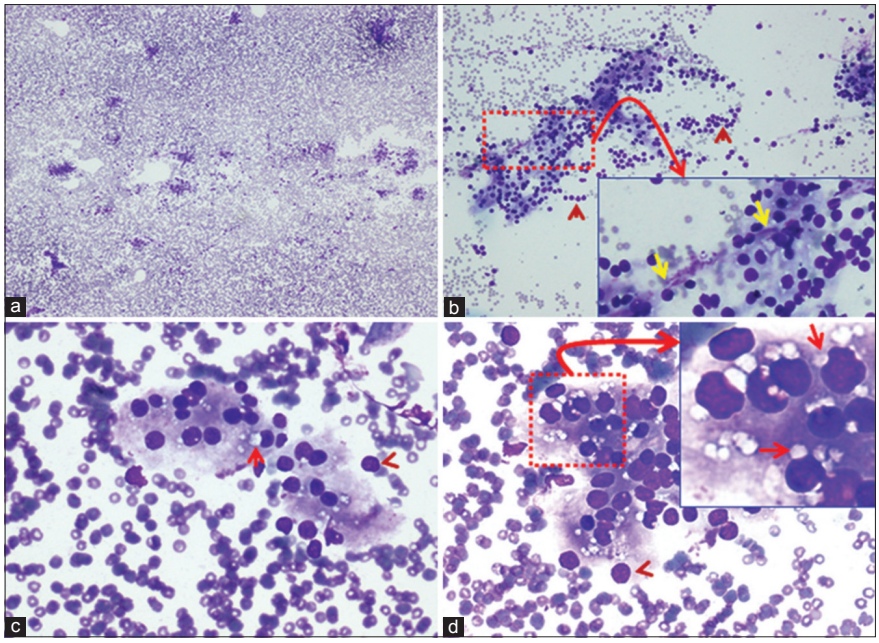

Figure 1: Fine-needle aspiration of neck lesion (Diff-Quik stained preparation). (a) Mildly cellular smear without colloid (×4). (b) Showing small groups of cells forming syncitia, admixed with a few bare nuclei (arrowheads). Inset of "b" (×40 Zoomed) shows occasional fragment of capillary stroma with loosely attached cells (yellow arrows) (×10). (c and d) The cells showed solitary paranuclear intracytoplasmic vacuoles (arrows) without paravacuolar granules. A few bare nuclei are seen at the periphery of the groups (arrowheads). Inset of "d" (×40 Zoomed) highlights the paranuclear intracytoplasmic vacuoles indenting the nuclei (arrows) in many intact cells (×40)

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DOI: 10.4103/1742-6413.157497

WHAT IS YOUR INTERPRETATION?

A Follicular lesion with Hurthle cell change
B Hyperplastic thyroid nodule
C Parathyroid gland tissue
D Thyroiditis with Hurthle cell change.

See next pages for answer and additional Quiz questions.

ANSWER

The correct cytopathologic interpretation is:

- Parathyroid gland tissue

Fine-needle aspiration (FNA) biopsy is a simple and minimally invasive method for evaluating neck mass lesions. The identification of parathyroid gland tissue and its lesions may be challenging. The differential diagnoses include thyroid nodules, lymphoid tissue, adipose tissue, thymus, metastatic tumors, and paraganglioma. The algorithmic approach shown in Figure 2 would facilitate the cytomorphological interpretation.[1-4]

Both thyroid and parathyroid gland lesions may show similar architecture consisting of tissue fragments or loose rounded clusters of epithelial cells. Parathyroid gland tissue may sometimes show follicle formation [Figure 1a-d] with colloid-like material in the background.

Hyperplastic nodular goiter shows groups of follicular cells in honey-comb pattern with evenly spaced nuclei and colloid in the background. Follicular lesions show microfollicles and three-dimensional microfollicle complexes with relative lack of colloid. The nuclei are round to oval, relatively larger, that is, 7–9 μm in diameter, with smooth nuclear membrane, uniformly distributed granular to compact chromatin, and inconspicuous nucleoli.[3-6]

Parathyroid lesions may also show oncocytic changes and may be clinically silent. The definitive distinction from thyroid follicular cells with oncocytic changes may be challenging, and ancillary support such as immunohistochemistry may be needed.[7,8]

Oncocytic change may be associated with hyperplastic nodular goiter, thyroiditis, and follicular lesions. The Hurthle cells are larger than follicular epithelial cells; possess well-defined cell borders and abundant finely granular cytoplasm. The nuclei are enlarged, eccentrically to centrally located, round to oval, with fine to coarsely granular chromatin, and prominent nucleoli.

Features favoring parathyroid over thyroid tissue:

- Regimented pattern of palisading nuclei along branching network of delicate capillaries [Inset of Figure 1b].
- Parathyroid cells are slightly smaller in size, measuring 6–7 μm in diameter. They possess central nuclei and abundant pale cytoplasm without paravacuolar lipochrome granules (seen as gray to blue granules in Diff-Quick stained and as brown granules in Pap stained smears in thyroid follicular cells)[3] [Figure 1b-d].
- Stippled nuclear chromatin. Better seen in Pap stained smears.[3]
- Intracytoplasmic vacuoles indenting the nucleus (seen distinctly in Diff-Quick stained smears) are present in parathyroid gland cells. Intra and intercellular lipid is depleted or absent in parathyroid hyperplasia and adenoma.[2,3] In such cases, other cytomorphological features and ancillary tests may be needed to confirm the nature of cells
- The tendency for bare nuclei [Figure 1a and b].
- Granular lacy background, owing to the ruptured cytoplasm.
- Parathyroid cells are immunoreactive for parathormone with nonimmunoreactivity for thyroid transcription factor-1 (TTF-1) and thyroglobulin (thyroid follicular cells are immunoreactive for TTF-1 and thyroglobulin).[1-4]
- Surgical pathology sections (e.g. - frozen section) may show parathyroid follicles with colloid-like contents. Intraoperative cytopathology of imprint smears of tiny tissue fragments usually submitted for frozen sectioning in these situations is a dependable adjunct. Most of the cytomorphological features are better seen in Diff-Quik stained smears. However, Pap stained smears are also important for studying nuclear features including chromatin.[3]

Follow-up of present case

The serum chemistry showed elevated parathormone and serum calcium levels.

Parathyroid scan showed a parathyroid adenoma.

ADDITIONAL QUIZ QUESTIONS

Q1. Which of the following cytomorphological features is highly reproducible for parathyroid gland tissue in Diff-Quik stained preparations?

a. Intracytoplasmic lipid vacuoles, indenting the nucleus
b. Bare nuclei
c. Para vacuolar granules
d. Oncocytic cytoplasm

Q2. Which of the following cytomorphological features favor parathyroid neoplasms over thyroid follicular neoplasms?

a. Regimented pattern of palisading nuclei along branching network of delicate capillaries
b. Bare nuclei
c. Cytoplasmic vacuoles, indenting the nucleus
d. All of the above

Q3. Which of the following features does not favor parathyroid lesions?

a. Intracytoplasmic lipid vacuoles
b. Immunoreactivity for calcitonin
c. Oncocytic cytoplasm
d. Metachromatic neurosecretory granules

See next pages for
answers to the additional Quiz questions
with brief review of the topic.

Answers to additional quiz questions

1.a; 2.d; 3.b

1. (a): [Figure 1c and d] the fat vacuoles appear as discrete, round to oval intracytoplasmic spaces with a sharp outline, and a tendency to indent a portion of nucleus, touching it, subtly. They are a hallmark of parathyroid gland cells and are most numerous in normal parathyroid glands.[3]
   Both intercellular and intracellular lipid vacuoles decrease in parathyroid hyperplasia and adenoma. The intracytoplasmic fat vacuoles may also be seen in the setting of lipoadenoma and parathyroid hamartoma. Imprint smears preserve the cytoplasm of individual fragile cells better than scrape smears (and hypothetically conventional smears of FNA aspirates), permitting better visualization of intracytoplasmic fat vacuoles[3,5,6] [Figure 1c-d]. Some studies do not describe the vacuoles, but they are observed in their published Diff-Quik images of FNA of parathyroid lesions [Figure 2 pg. 409].[7] Rarely nonspecific vacuoles may be present in other neck lesions. However, they may not be solitary, paranuclear, and do not indent the nucleus.
   Bare nuclei, devoid of cytoplasm may be dispersed singly [arrow heads in Figure 1b-d]. They may also be observed in aspirates from thyroid lesions, lymphoid neoplasms, and metastatic small cell carcinoma.
2. (d): [Figure 1b - Inset] regimented pattern of palisading nuclei along branching network of delicate capillaries is typically seen in parathyroid lesions and differentiates it from thyroid nodules.[1-4] However, similar features may also be seen in carotid body tumors (paraganglioma), metastatic tumors and other neuroendocrine lesions.
3. (b): Chief cells of the parathyroid gland show cytoplasmic lipid, better observed in Romanowski stained preparations such as Diff-Quik stained

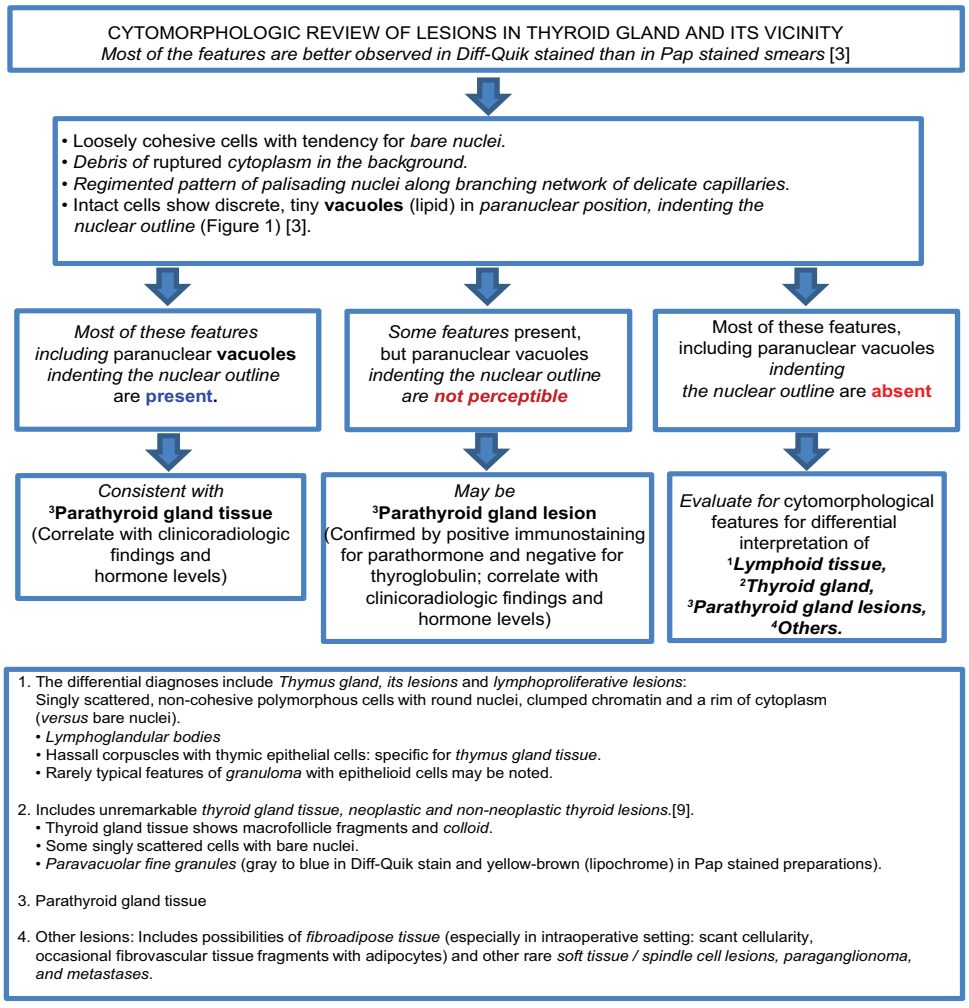

Figure 2: Algorithm to guide broader cytomorphological interpretation of cytology preparations of the lesions in the vicinity of or in thyroid gland (with reference to parathyroid gland tissue/lesions)

smears [Figures 1d - Inset]. Hyperplastic and neoplastic glands tend to have less cytoplasmic lipid and smaller droplets than normal or atrophic parathyroid cells. Glycogen may be present in clear cells and stains with periodic acid-Schiff.

Neurosecretory granules may be seen as metachromatic inclusions in the cytoplasm, stained with Romanowsky stains.[2]

Immunoreactivity for calcitonin is a specific feature of parafollicular cells (C cells) and medullary carcinoma thyroid.

BRIEF REVIEW OF THE TOPIC

Awareness of cytomorphological features of parathyroid and other anatomical structures in the vicinity, inclusive of lymph nodes, thyroid, and branchial cleft remnants is important to make a definitive diagnosis.

Cytomorphological features suggesting parathyroid gland lesion may be encountered in the following clinical scenarios:

- Suspected parathyroid lesion.
- Intraoperative consultation: Frozen section evaluation of tissues in the vicinity of thyroid gland including thyroidectomies and parathyroid gland surgeries.
- Evaluation of hyperparathyroidism.
- Incidental finding on FNA, as in intrathyroidal parathyroid or ectopic parathyroid.
- FNA of hypoechoic thyroid nodules.
- FNA of parathyroid cyst.
- FNA of ectopic parathyroid while evaluation of neck nodule.
- FNA of thyroid bed, status post thyroidectomy.

Clinical associations

- Hypercalcemia
- Nephrolithiasis
- Bone lesions (brown tumors)

Radiological correlation[1,7]

- Normal parathyroid glands are not visualized by ultrasonography.
- Parathyroid adenomas are usually hypervascular, hypoechoic, ovoid or lobulated lesions, associated with extrathyroidal feeding artery and one or more vascular pedicles.
- They may show cysts and calcifications.

Fine-needle aspiration biopsy

Fine-needle aspiration biopsy of parathyroid lesions is a challenging procedure, with variable yield of diagnostic material. The inadequacy rates, range from 8.3% to 28.1%. The rate of contamination with thyroid follicular epithelial cells varies from 8.3% to 31.5%.[7]

FOCUSED DIFFERENTIAL DIAGNOSIS OF PARATHYROID GLAND LESIONS

Normal parathyroid glands

- Architectural patterns: Solid sheets, branching anastomosing cords and acinar structures with rich vascularity.
- Cellularity: Admixture of parenchyma and adipose tissue.
- Cell types: Chief cells, oncocytic/oxyphilic cells, and water clear cells.
- Cell size: Slightly smaller than follicular epithelial cells from thyroid.
- Cytoplasm: Moderate amount of pale granular cytoplasm with small intracytoplasmic lipid vacuoles with a tendency to indent the nucleus [Figure 1c and d]
- Oxyphil cells are slightly larger and have abundant oncocytic cytoplasm. The nuclei are round, central to eccentric, with dense chromatin, and prominent dark nucleoli.
- Water clear cells are rarely seen in normal parathyroid glands. They have faintly eosinophilic to clear cytoplasm with abundant glycogen deposits and sharply defined cell membranes.[1,2,9]
- Immunohistochemistry: Cytoplasmic immunoreactivity for keratin, chromogranin A, and parathormone. Lack of immunoreactivity for vimentin, glial fibrillary acidic protein, neurofilament, and chromogranin B.[10]

Parathyroid cysts

- Derived from embryologic remnants, coalescence of microcysts or degeneration of an adenoma.
- Contents of parathyroid cyst: Clear, watery; occasionally golden brown. The fluid is acellular or hypocellular.
- Cytoarchitecture: Tissue fragments, honeycomb sheets or microfollicles.
- Cells: Small, cuboidal, with round nuclei and granular to compact chromatin.
- Background: Proteinaceous debris.
- Differential diagnosis: Cystic degeneration of nodular goiter, branchial cleft cyst, thymic cyst, and thyroglossal duct cyst.

Parathyroid adenoma and hyperplasia

- Cellularity: Moderate cellularity.
- Architecture/cellular distribution: Two- or three-dimensional clusters, papillary fragments, complex branching, follicular pattern, dispersed single cell pattern. A branching network of capillaries and neoplastic cells arranged alongside capillaries, in a regimented pattern, is characteristic.

- Cell size and shape: Monomorphous round to oval cells that exhibit stippled nuclear chromatin and high nucleo-cytoplasmic ratio. Endocrine atypia may be pronounced. Spindle-shaped cells may be seen.
- Background: Inspissated colloid like material may be present, either mixed with the cells or distributed separately within the vicinity of cells. Macrophages, fat globules, delicate branching, vascularized stromal tissue fragments may be present.
- Bare nuclei: Seen commonly in abundance.
- Nuclear morphology: Round to oval, uniform, smooth membrane, coarsely granular/stippled chromatin, with micronucleoli. Intranuclear inclusions, nuclear pleomorphism/endocrine atypia, nuclear moulding, single vacuoles may be present. Mitosis and karyorrhexis should typically be absent in adenoma.
- Distinction of different parathyroid lesions (hyperplasia *versus* adenoma) may not be possible on cytomorphologic evaluation alone.

Parathyromatosis

- Multiple small nodules, containing bland cells are disseminated in the soft tissues of the neck.
- They may be associated with Multiple Endocrine Neoplasia 1 (MEN 1) syndrome.

Parathyroid carcinoma

- The lesions are extremely cellular
- Cells: Small, medium, or large. Either monomorphic or pleomorphic in appearance. Cells with clear cytoplasm and tumor giant cells may be present. The cells are dyshesive with anaplasia, macronucleoli, typical and atypical mitoses, and necrosis.
- High Ki-67 index, Cyclin D1 expression, and lower p27 indices indicate parathyroid carcinoma. Galectin-3 is expressed in parathyroid carcinomas and not in adenoma.[2,9]

COMPETING INTERESTS STATEMENT BY ALL AUTHORS

The authors declare that they have no competing interests.

AUTHORSHIP STATEMENT BY ALL AUTHORS

All authors of this article declare that we qualify for authorship as defined by ICMJE http://www.icmje.org/#author.

Each author has participated sufficiently in the work and takes public responsibility for appropriate portions of the content of this article.

ETHICS STATEMENT BY ALL AUTHORS

As this is a quiz case without identifiers, our institution does not require approval from Institutional Review Board (IRB) (or its equivalent).

LIST OF ABBREVIATIONS

FNA = Fine-needle aspiration
TTF-1 = Thyroid Transcription Factor-1

REFERENCES

1. Kini S, editor. Thyroid and parathyroid. In: Color Atlas of Differential Diagnosis in Exfoliative and Aspiration Cytopathology. 2nd ed. Philadelphia, PA: Wolters Kluwer/Lippincott Williams and Wilkins; 2011. p. 401-541.
2. DeMay RM, editor. Head and neck. In: The Art and Science of Cytopathology. 2nd ed. Chicago, IL: ASCP Press; 2012. p. 752-73.
3. Shidham VB, Asma Z, Rao RN, Chavan A, Machhi J, Almagro U, *et al.* Intraoperative cytology increases the diagnostic accuracy of frozen sections for the confirmation of various tissues in the parathyroid region. Am J Clin Pathol 2002;118:895-902.
4. Chan JK. Tumors of the thyroid and parathyroid gland. In: Fletcher CD, editor. Diagnostic Histopathology of Tumors. 4th ed. Philadelphia, PA: Elsevier; 2013. p. 1177-250.
5. Sasano H, Geelhoed GW, Silverberg SG. Intraoperative cytologic evaluation of lipid in the diagnosis of parathyroid adenoma. Am J Surg Pathol 1988;12:282-6.
6. Dimashkieh H, Krishnamurthy S. Ultrasound guided fine needle aspiration biopsy of parathyroid gland and lesions. Cytojournal 2006;3:6.
7. Agarwal AM, Bentz JS, Hungerford R, Abraham D. Parathyroid fine-needle aspiration cytology in the evaluation of parathyroid adenoma: Cytologic findings from 53 patients. Diagn Cytopathol 2009;37:407-10.
8. Giorgadze T, Stratton B, Baloch ZW, Livolsi VA. Oncocytic parathyroid adenoma: Problem in cytological diagnosis. Diagn Cytopathol 2004;31:276-80.
9. Rosai J. Parathyroid glands. In: Rosai and Ackerman's Surgical Pathology. 10th ed. Philadelphia, PA: Elsevier; 2011. p. 565-83.
10. Faquin WC, Michael CW, Renshaw AA, Vielh P. Follicular neoplasm, hurthle cell type/suspicious for a follicular neoplasm, hurthle cell type. In: Ali SZ, Cibas ES, editors. The Bethesda System for Reporting Thyroid Cytopathology. Definitions, Criteria, and Explanatory Notes. New York: Springer; 2010. p. 59-73. Based on NCI Thyroid Fine Needle Aspiration State of the Science Conference (Oct 22-23, 2007), Springer.

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To ensure the integrity and highest quality of CytoJournal publications, all Quiz cases are reviewed by Quiz case section team prior to be accepted for publication.

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Research Article

Cytological variations and typical diagnostic features of endocervical adenocarcinoma *in situ*: A retrospective study of 74 cases

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Published: 29 Apr 2015
CytoJournal 2015, 12:8
This article is available from: http://www.cytojournal.com/content/12/1/8

Received: 14 January 15
Accepted: 31 March 15

This article may be cited as:
Umezawa T, Umemori M, Horiguchi A, Nomura K, Takahashi H, Yamada K, Ochiai K, Okamoto A, Ikegami M, Sawabe M. Cytological variations and typical diagnostic features of endocervical adenocarcinoma in situ: A retrospective study of 74 cases. CytoJournal 2015;12:8.

Abstract

Background: The sensitivity of Papanicolaou smears for detecting endocervical adenocarcinoma *in situ* (AIS) is very low. A comprehensive cytological analysis of endocervical AIS is necessary to increase diagnostic accuracy.

Methods: The subjects were 74 patients with pathologically-diagnosed AIS. A total of 140 Papanicolaou smears were reviewed to calculate the sensitivity of the Papanicolaou smears for detecting AIS and the incidence of sampling/screening/diagnostic errors. The cytological review was performed by 6 cytotechnologists, and the final cytological diagnosis was obtained at the consensus meeting. We classified the cases into three differentiation types; typical type (well-differentiated AIS), polymorphic type (poorly differentiated AIS), and mixed typical and polymorphic type. Three cytological subtypes (endocervical, endometrioid and intestinal subtypes) of AIS were also analyzed.

Results: The sensitivity of the original Papanicolaou smears for the detection of AIS was 44.6%, while that for the detection of AIS and adenocarcinoma was 63.5%. The diagnostic accuracy of AIS increased to 78.5% in the final diagnosis. The common characteristic features were microbiopsies/hyperchromatic crowded groups (HCG) (82.0%) and mitotic figures (72.2%). The appearance of single cells (2.8%) was rare, and all the cervical cytology smears showed no evidence of necrotic tumor diathesis. The most common AIS was the typical type (41 cases, 67.2%) among all cytologically-diagnosed AIS or adenocarcinoma cases (61 cases). Although mixed typical and polymorphic AIS existed in 17 cases (27.9%), pure polymorphic AIS was very rare (3 cases, 4.9%). The endocervical subtype was the most predominant subtype (67.2%), followed by a few

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Quick Response Code:	Website: www.cytojournal.com
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CytoJournal 2015, 12:8 http://www.cytojournal.com/content/12/1/8

mixed subtypes. The important diagnostic keys for AIS cytology are as follows: (1) The appearance of microbiopsies/HCG (single-cell pattern is rare), (2) mitotic figures in the microbiopsies/HCG, (3) a lack of necrotic tumor diathesis in cases with polymorphic AIS, and (4) recognition of typical cytological subtypes.

Conclusions: The relatively low diagnostic accuracy AIS was caused by the underestimation of microbiopsies/HCG and the overestimation of polymorphic components. The typical cytological features of AIS are the presence of microbiopsies/HCG with mitotic figures in the absence of necrotic tumor diathesis in specimens containing endocervical samples. The recognition of infrequent AIS subtypes (endometrioid and intestinal subtypes) is also important.

Key words: Adenocarcinoma *in situ*, cytobrush, hyperchromatic crowded cell groups, microbiopsies, papanicolaou test, uterine cervix

INTRODUCTION

Cervical cytological screening is an important procedure for the detection of adenocarcinoma *in situ* (AIS) and cervical intraepithelial neoplasia (CIN) 2/3, both of which are precursors of invasive adenocarcinoma or squamous cell carcinoma (SCC) of the uterine cervix. The detection of these lesions leads to early treatment, and eventually a surgical cure with uterine preservation, enabling patients to become pregnant in the future.[1,2] Cervical conization or a simple hysterectomy without pelvic lymphadenectomy is the standard surgery and results in a favorable prognosis for patients with AIS.[1,2] Östör reported no recurrences during an 8-year follow-up of 53 AIS patients who were treated with cervical conization.[2]

As AIS arises in an inner cervical glandular area and is usually not associated with abnormal genital bleeding, detection by colposcopy or clinical manifestation is quite difficult, and a diagnosis usually relies on cervical cytological screening.[2] Endocervical AIS is classified as an independent category in the 2001 Bethesda system (TBS2001).[3,4] The diagnostic accuracy of AIS is significantly lower than that of the high-grade squamous intraepithelial lesion (HSIL) or SCC because of a low sensitivity resulting from sampling and screening errors.[5-14] Schoolland *et al*. reported a low sensitivity of cervical smear detection for AIS of approximately 50%, based on two large-scale studies of AIS.[9] The retrospective rescreening of 31 negative smears of AIS showed a high rate (55%) of abnormal findings.[7] Very small numbers of AIS cells and blood contamination in the specimens can contribute to false-negative results.[7-11,14] The AIS cells are often misdiagnosed as normal endocervical cells, HSIL, or SCC.[5,8,10] Since many cytological features overlap between endocervical AIS and well-differentiated invasive cervical adenocarcinoma, the Bethesda 2001 Workshop recommended a special caution against the diagnosis of AIS.[3]

Krumins *et al*. first reported the cytologic features of uterine cervical AIS in 1977,[13] and Ayer *et al*. then reported its cytological variations in 1987.[5] They classified AIS into two subtypes according to cytological and nuclear features: Well-differentiated AIS with typical cytological patterns, and poorly differentiated AIS with marked cytological atypia.[5] Since carcinoma *in situ* (intraepithelial neoplasm) is usually not classified according to the degree of differentiation, we designated well-differentiated AIS as typical AIS and poorly differentiated AIS as polymorphic AIS in this article. The AIS can also be also classified to four subtypes: Endocervical, intestinal, endometrioid, and a rare Paneth cell subtype.[5]

We retrospectively analyzed 74 AIS cases to clarify the incidence of sampling/screening/diagnostic errors, factors related to cytological variations, and the diagnostic accuracy of uterine cervical AIS, and finally found that the recognition of cytological variation was crucial for accurate diagnosis of cervical AIS.

METHODS

Subjects

We retrospectively recruited 74 cases of cervical conization or hysterectomy materials with stage 0 cervical AIS that were diagnosed during a 20-year period from 1993 to 2012 at the Department of Pathology, Jikei University Hospital, Tokyo, Japan. The mean age (range) of the 74 subjects was 41.2 years (21–69 years). The surgical treatments were as follows: Cervical conization alone in 47 cases, simple hysterectomy in 22 cases, and conization and subsequent hysterectomy in five cases. Cases with microinvasive or invasive adenocarcinoma were excluded from the present study. Endocervical smears were usually taken using a cytobrush by gynecologists before surgery. A total of 140 Papanicolaou smears were reviewed. All the smears were prepared using the conventional method: The smears were fixed in 95% ethyl alcohol and were stained with the standard Papanicolaou stain. No liquid-based preparations were used.

Cytological review

All the cervical papanicolaou smears were first blindly rescreened individually by six cytotechnologists including

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the authors without knowing the final histological diagnosis. If there was discordance among the cytological diagnosis, the consensus meeting was held to reach the final diagnosis. Initial and review papanicolaou smears were diagnosed according to the TBS2001 system.[3,4] We analyzed the diagnostic accuracy of AIS and the factors influencing the sampling/screening/diagnostic errors. Sampling errors were defined as cases with smears that were initially and finally reported as negative for intraepithelial lesion or malignancy (NILM). Screening errors were defined as cases with possible or definite high-grade epithelial abnormalities initially reported as NILM. Diagnostic errors were defined as AIS cases initially reported as atypical glandular cells (AGC; underdiagnosis), low-grade squamous intraepithelial lesions (LSIL/HSIL; underdiagnosis), adenocarcinoma (overdiagnosis) or SCC (overdiagnosis).

The tissue fragments of abnormal cells were called microbiopsy or hyperchromatic crowded groups (HCG).[15,16] HCG are three-dimensional clusters of crowded cells with hyperchromatic nuclei and a high nuclear/cytoplasmic ratio. We analyzed the presence or absence of detailed cytological features, such as feathering, mitosis, polymorphism, endocervical samples, and necrotic diathesis. We designated well-differentiated AIS as typical AIS and poorly differentiated AIS as polymorphic AIS.[5] When features of both typical and polymorphic were observed, the term mixed typical and polymorphic AIS was applied. Thus, three distinct differentiation patterns of AIS were applied to our series of samples: Typical AIS, mixed typical and polymorphic AIS, and polymorphic AIS. We also classified the AIS into three subtypes: Endocervical, intestinal and endometrioid subtypes. No Paneth cell subtypes were included among the samples.

RESULTS

Sampling/screening/diagnostic errors of the cytological diagnosis

Normal endocervical samples were included in all the Papanicolaou smears; thus, the specimens were suitable for the cytological diagnosis of the endocervix. The overall flow chart of the cytological diagnosis before and after the review is shown in Figure 1. Glandular abnormality was absent in two cases (one each case of HSIL and NILM). The review showed AGC in one case of NILM. Thus, sampling and screening errors were only present in one case each (1.4%). The final cytological diagnosis of glandular abnormality included AIS in 58 cases (78.4%), AGC in 11 cases (14.1%), and adenocarcinoma in 3 cases (4.1%). A total of 25 cases were initially misdiagnosed as other cytological diagnoses than AIS. These misdiagnosed cases are shown in red boxes in Figure 1. The diagnostic error rate was 33.8% and the total error rate was 36.5%.

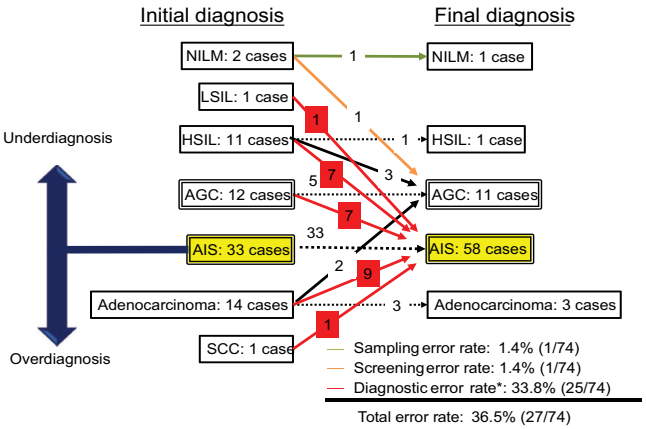

Figure 1: Flow chart of 74 pathologically-confirmed adenocarcinoma *in situ* cases before and after a cytological review. The left column shows the distribution of the initial cytological diagnoses, and the right column shows that of the final cytological diagnoses after the review. A few sampling and screening errors occurred, but diagnostic errors predominated. AGC: Atypical glandular cells, AIS: Adenocarcinoma *in situ*, HSIL: High-grade squamous intraepithelial lesion, LSIL: Low-grade squamous intraepithelial lesion, NILM: Negative for intraepithelial lesion or malignancy, SCC: Squamous cell carcinoma

Cytological details of glandular abnormalities

The cytological features of 72 cases with glandular abnormality are shown in Table 1. The common characteristic features were microbiopsies/HCG (82.0%) and mitotic figures (72.2%). The appearance of single cells (2.8%) was rare, and all the cervical cytology smears lacked necrotic tumor diathesis.

Among 58 cases with AIS, 41 cases showed a typical AIS pattern (67.2%). The remaining 17 cases (32.8%) of AIS showed the apparent polymorphism in some cancer cells, then diagnosed as mixed typical and polymorphic AIS. Typical histological and cytological figures of polymorphic AIS are shown in Figures 2 and 3. Three cases of purely polymorphic AIS were cytologically diagnosed as (invasive) adenocarcinoma. The distribution of the AIS subtypes is shown in Table 2 and typical cytological figures of AIS subtypes are shown in Figure 4. The endocervical subtype was the most predominant subtype, followed by a few mixed subtypes. The pure endometrioid subtype was present only in one case.

Among 11 cases with a final cytological diagnosis of AGC, cytological features favoring a diagnosis of AIS were infrequent; microbiopsies/HCG and frequent mitosis (3 cases each, 27.3%). Abnormal clusters were often obscured by large amounts of blood (one case) or mucus (three cases) and severe inflammation (two cases). Numerous HSIL cells coexisted with AGCs in two AGC favor neoplastic (FN) + HSIL cases, masking glandular abnormalities.

Cytological features of adenocarcinoma *in situ* with diagnostic errors

The cytological features of 25 AIS cases with diagnostic errors are shown in Table 3. The polymorphism was frequently marked among nine cases with an initial diagnosis of (invasive) adenocarcinoma (5/9 cases). In eight cases with the initial diagnosis of SIL (one case of LSIL and seven cases of HSIL), HSIL frequently coexisted (6/8 cases).

DISCUSSION

Characteristic cytological pictures of adenocarcinoma *in situ*

The present study showed that the characteristic cytological features of AIS included microbiopsies/HCG, mitotic figures of the cancer cells, and a lack of necrotic tumor diathesis in polymorphic AIS.

Table 1: Retrospective cytological features of 72 cases* with endocervical glandular abnormalities

Cytologic characteristics	*Cytological diagnosis*			*Total (%)*
	*AGC***	*AIS*	*Adenocarcinoma (purely polymorphic AIS)*	
Number of patients	11	58	3	72
Microbiopsies/HCG	3	53	3	59 (82.0)
Single cells	0	0	2	2 (2.8)
Feathering	0	34	2	36 (50)
Mitosis	3	46	3	52 (72.2)
Polymorphism	0	17	3	20 (27.8)
Necrotic tumor diathesis	0	0	0	0 (0)

*No glandular abnormalities were detected in 2 cases (one each case of NILM and HSIL), **11 cases of AGC included 4 cases of AGC-NOS and 7 cases of AGC-FN. AGC: Atypical glandular cells, AIS: Adenocarcinoma *in situ*, HCG: Hyperchromatic crowded groups, NILM: Negative for intraepithelial lesion or malignancy, HSIL: High-grade squamous intraepithelial lesion, FN: Favor neoplastic, NOS: Not otherwise specified

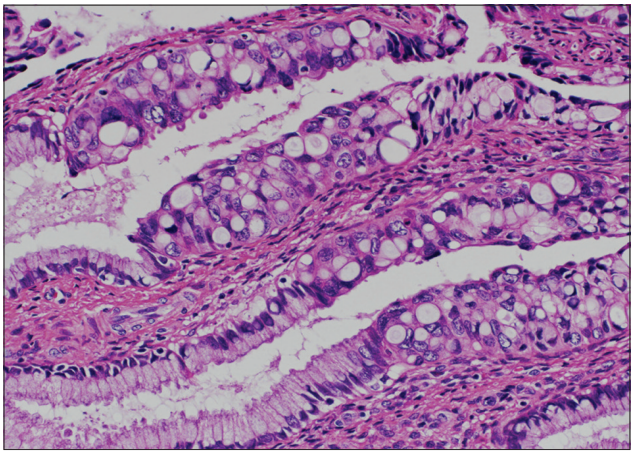

Figure 2: Histological picture of endocervical polymorphic adenocarcinoma *in situ*. Note large polymorphic nuclei with conspicuous nucleoli. No stromal invasion is present (H and E stain, ×400)

Microbiopsies / hyperchromatic crowded cell groups

The appearance of microbiopsy/HCG was a key cytological feature for the AIS diagnosis and was present in 82.0% (59 cases) of the subjects in our study. The cancer tissue seems to be fragmented during sampling with a cytobrush and appears as dense clusters of darkly-stained cells.[15,16] Benign microbiopsy/HCG are also observed in cases of squamous atrophy or metaplasia, or in samples originating from normal endocervical and endometrial cells. Malignant microbiopsy/HCG appear in various cases with HSIL, SCC, AIS, invasive adenocarcinoma, or metastatic carcinoma. False-positive or false-negative results have been noted in these cases.[15,16] The present study showed that microbiopsy/HCG of an AIS origin were often misdiagnosed as HSIL, indicating a need for the careful assessment of microbiopsies/HCG.[5,10,12] Thiryayi *et al*. reported a similar result regarding misdiagnosis, stating that the differentiation of microbiopsies/HCG with a glandular nature from those with a squamous nature was often difficult.[17] The microbiopsies/HCG of an AIS origin had a high proportion of columnar cells at the peripheries of the clusters, while the microbiopsies/HCG of HSIL origin had flattened cells at the periphery, the loss of cell polarity within the clusters and the presence of isolated HSIL cells in the background of the specimens.[4] The presence of mitotic figures within the clusters is also an important feature for the diagnosis of AIS, since the epithelial cells of normal cervical glands lack mitotic figures.[4,11,12,18,19] The mitotic figures within the clusters were actually present in 72.2% of the AIS cases in our study. The microbiopsies/HCG with mitotic figures in the specimens containing endocervical samples are typical cytological features of AIS.

Polymorphic adenocarcinoma in situ and necrotic tumor diathesis

Ayer *et al*. reported a high ratio (50%) of the misdiagnosis of AIS as (invasive) adenocarcinoma.[5] Subsequent studies concluded that a differential diagnosis between AIS and adenocarcinoma was very difficult.[8,10] Adenocarcinoma usually shows marked cytological atypia, with the appearance of single cells and necrotic diathesis.[12,16] The present study revealed the frequent presence of polymorphic subtype and lack of necrotic tumor diathesis among the cases with AIS. The abnormal glandular cells with polymorphic components lacking necrotic tumor diathesis should be diagnosed as polymorphic AIS rather than (invasive) adenocarcinoma.[5,10,11] Single abnormal glandular cells were present in only two cases in the present study.

Subtypes of adenocarcinoma in situ

Our study showed that the endocervical subtype was the most prevalent and cases with intestinal or endometrioid subtypes coexisted with the endocervical subtype. Therefore, the cytological variation due to infrequent subtypes (endometrioid and intestinal subtypes) needs to be recognized for the accurate diagnosis of AIS.

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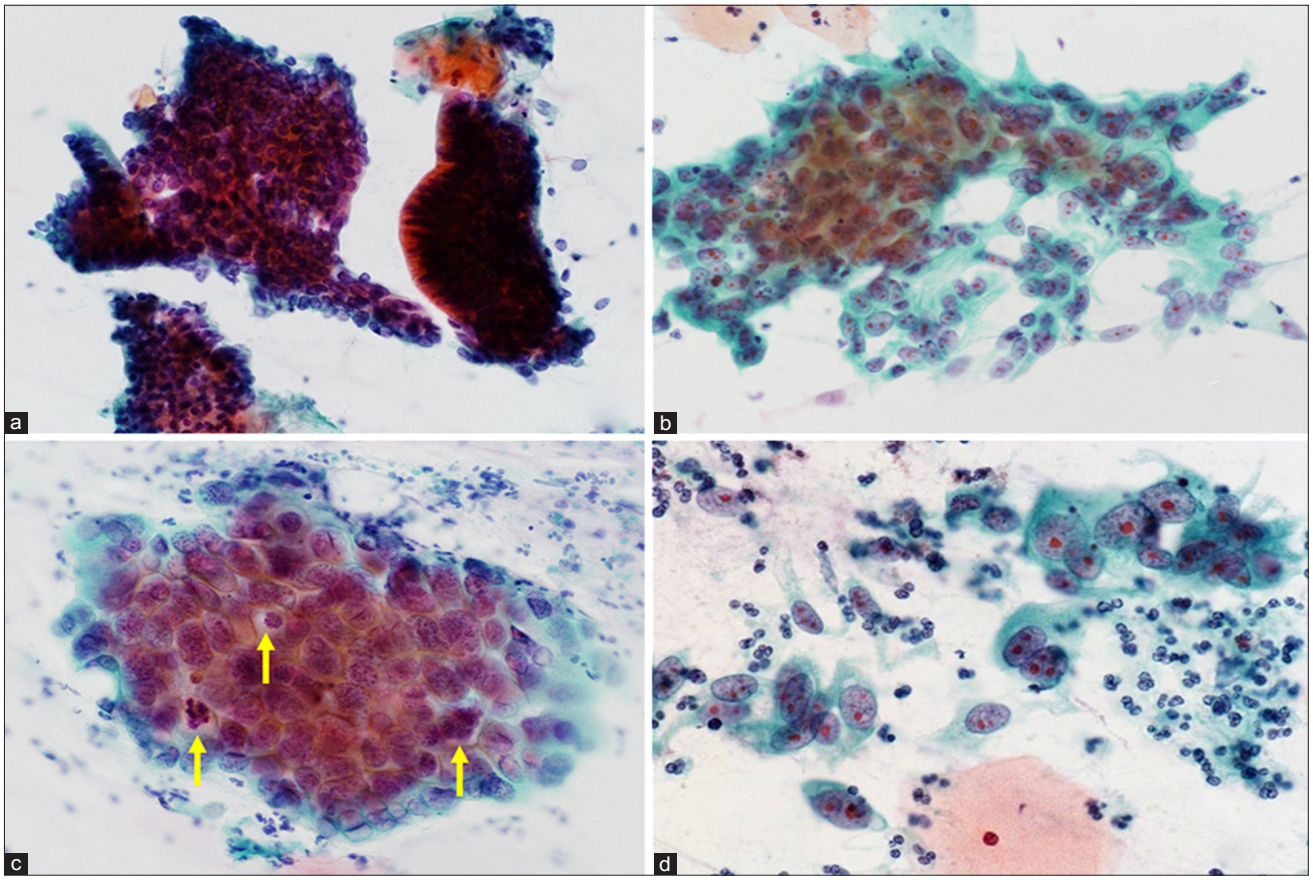

Figure 3: Cytological pictures of endocervical polymorphic adenocarcinoma *in situ* (AIS). (a) The tissue fragments of typical endocervical AIS. Dense clusters of darkly stained AIS cells in microbiopsies/hyperchromatic crowded groups (HCG) are present. The AIS cells at the margin of the clusters show no feathering (Papanicolaou stain, ×400). (b) Polymorphic AIS. The AIS cells have large polymorphic nuclei with prominent nucleoli (Papanicolaou stain, ×400). (c) Polymorphic AIS. The AIS cells often exhibit mitotic activity (arrows) (Papanicolaou stain, ×600). (d) Single or loosely arranged AIS cells in polymorphic AIS. Microbiopsies/HCG or three-dimensional clusters are scarce in this case (Papanicolaou stain, ×600)

Table 2: Subtypes and differentiation patterns of 61 cases with endocervical AIS (*n*=58) or adenocarcinoma (*n*=3)

Subtypes	*Typical AIS*	*Mixed typical and polymorphic AIS*	*Polymorphic AIS**	*Number of patients*	*Percentage*
EC type	26	12	3	41	67.2
EC + IT type	9	3	0	12	19.7
EC + EM type	5	1	0	6	9.8
EC + IT + EM type	0	1	0	1	1.6
IT type	0	0	0	0	0
EM type	1	0	0	1	1.6
Total	41	17	3	61	100

*3 cases of purely polymorphic AIS were cytologically diagnosed as (invasive) adenocarcinoma. AIS: Adenocarcinoma *in situ,* EC: Endocervical subtype, EM: Endometrioid subtype, IT: Intestinal subtype

Causes of misdiagnosis

When abnormal glandular cells in a sample are scarce, the diagnosis of AIS is often difficult and may result in a diagnosis of NILM, rather than AGC.[5,10,11] These cases should be diagnosed as AGC-FN to prevent AIS from being overlooked.[4] The Update on ASCCP Consensus Guidelines recommends an excisional procedure for AIS if the initial cytology is AGC-FN or AIS and no invasion is identified.[20] Only seven cases in this study exhibited AGC-FN. Faraker and Boxer indicated that the false-negative factors in cervical cancer screening were

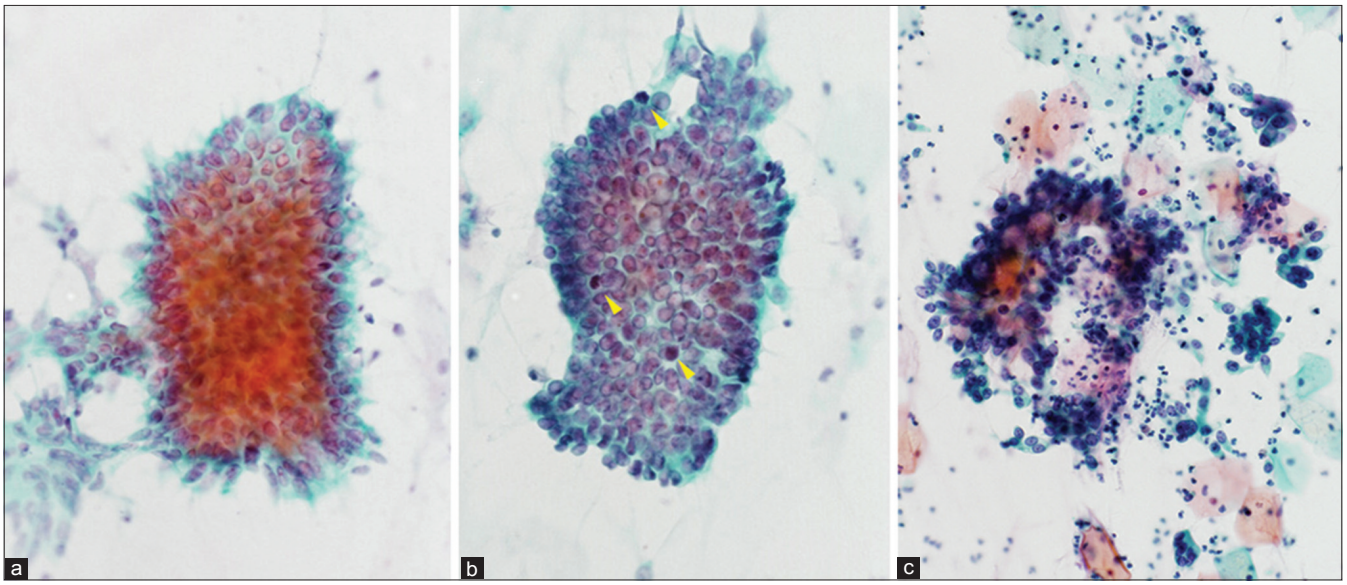

Figure 4: Typical cytological features of adenocarcinoma *in situ*. (a) Endocervical subtype. Pseudostratified strip of cells demonstrating crowding, nuclear enlargement and peripheral feathering (Papanicolaou stain, ×600). (b) Endometrioid subtype. Pseudostratified cluster with nuclear crowding, irregular internuclear distances, and scattered mitotic figures (yellow arrow heads) (Papanicolaou stain, ×600). (c) Intestinal subtype. Note large mucin droplets in the clusters (Goblet cells) (Papanicolaou stain, ×400)

Table 3: Cytological features of 25 misdiagnosed cases* with final cytological diagnosis of AIS

Initial diagnosis (number of cases)	*Final diagnosis (number of cases)*	*Cytological features*			
		Polymorphism	*HCG*	*Mitosis*	*Feathering*
Underdiagnosis (7)					
AGC	AIS	2/7	6/7	4/7	1/7
Overdiagnosis (10)					
Adenocarcinoma	AIS	5/9	7/9	7/9	3/9
SCC	AIS	0/1	1/1	1/1	0/1
Other diagnostic errors (8)					
LSIL	AIS + HSIL	0/1	1/1	0/1	0/1
HSIL	AIS + HSIL (5) and AIS (2)	3/7	6/7	6/7	5/7
Total (%)		10/25 (40)	21/25 (84)	18/25 (72)	9/25 (36)

*These 25 cases were shown in red boxes in Figure 1. AGC: Atypical glandular cells; AIS: Adenocarcinoma *in situ*, HCG: Hyperchromatic crowded groups, HSIL: High-grade squamous intraepithelial lesion, LSIL: Low-grade squamous intraepithelial lesion, SCC: Squamous cell carcinoma

(1) scant abnormal cells and (2) abundant dyskaryotic cells presenting as microbiopsies/HCG.[21]

Limitation of the study

The retrospective nature of this study limits the implication of the results. As we have introduced the liquid-based cytology (LBC) system in our lab shortly after this study, we could not reproduce our results in a prospective way. This study adopted the conventional Papanicolaou method rather than LBC. The LBC system has already been widely introduced in US and other western countries, but the conventional method is still widely used in developing countries, because they could not introduce the LBC system due to the financial limitation. The introduction of LBC system has been delayed in Japan as well (<10%). As the cytological details are slightly different between the conventional method and LBC, we safely conclude our results are at least applicable to the cytology using the conventional Papanicolaou method.

CONCLUSIONS

The present study showed that the main causes of diagnostic errors are the underdiagnosis of microbiopsies/HCG, the overdiagnosis of polymorphic components, and recognition of subtypes of AIS.

Recent studies reported that $p16^{INK4a}$ and Ki-67 immunocytochemistry was useful for predicting CIN2/3 and AIS/adenocarcinoma.[22] However, sampling and diagnostic skills should be first improved to maximize the diagnostic competency for AIS. Cytotechnologists and pathologists are required to be familiar with the typical cytological features and cytological variations of AIS, especially polymorphic AIS and AIS subtypes (endometrioid and intestinal types), to increase the diagnostic accuracy.

ACKNOWLEDGEMENTS

We are greatly thankful to all the staff members of the Department of Pathology, Jikei University Hospital for the preparation of slides. We also thank to Ms. Mio Nakagawa for English editing. The present study did not receive any grant support.

COMPETING INTERESTS

All the authors and their families have no personal conflicts of interest to disclose.

AUTHORSHIP STATEMENT BY ALL AUTHORS

TU perceived the design of the study, acquisition of data, and drafting the article. MU and AH carried out the acquisition of cytological data. KN, HT, and MI participated in the acquisition of pathological data. KY, KO, and AO carried out the acquisition of clinical data. MS drafted and revised the article for important intellectual content. All authors read and approved the final manuscript.

ETHICS STATEMENT BY ALL AUTHORS

This study was retrospectively designed using the usual Pap smears obtained from the gynecological examinations; therefore we didn't obtain any specific oral or written informed consents from the patients. This study was approved by the ethical committee of the Jikei University School of Medicine (24-102 [6868]).

LIST OF ABBREVIATIONS

AGC = Atypical Glandular Cells
AIS = Adenocarcinoma in situ
CIN = Cervical Intraepithelial Neoplasia
FN = Favor Neoplastic
HCG = Hyperchromatic Crowded Groups
HSIL = High-Grade Squamous Intraepithelial Lesion
LBC = Liquid-Based Cytology
LSIL = Low-Grade Squamous Intraepithelial Lesions
NILM = Intraepithelial lesion or Malignancy
SCC = Squamous Cell Carcinoma

REFERENCES

1. Kim ML, Hahn HS, Lim KT, Lee KH, Kim HS, Hong SR, *et al.* The safety of conization in the management of adenocarcinoma *in situ* of the uterine cervix. J Gynecol Oncol 2011;22:25-31.
2. Ostör AG, Duncan A, Quinn M, Rome R. Adenocarcinoma *in situ* of the uterine cervix: An experience with 100 cases. Gynecol Oncol 2000;79:207-10.
3. Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, *et al.* The 2001 Bethesda system: Terminology for reporting results of cervical cytology. JAMA 2002;287:2114-9.
4. Solomon D, Nayar R. The Bethesda System for Reporting Cervical Cytology: Endocervical/Transformation Zone Component, and Explanatory Notes. 2nd ed. New York: Springer; 2003. p. 12-5.
5. Ayer B, Pacey F, Greenberg M, Bousfield L. The cytologic diagnosis of adenocarcinoma *in situ* of the cervix uteri and related lesions. I. Adenocarcinoma *in situ*. Acta Cytol 1987;31:397-411.
6. Renshaw AA, Mody DR, Lozano RL, Volk EE, Walsh MK, Davey DD, *et al.* Detection of adenocarcinoma *in situ* of the cervix in Papanicolaou tests: Comparison of diagnostic accuracy with other high-grade lesions. Arch Pathol Lab Med 2004;128:153-7.
7. Lee KR, Minter LJ, Granter SR. Papanicolaou smear sensitivity for adenocarcinoma *in situ* of the cervix. A study of 34 cases. Am J Clin Pathol 1997;107:30-5.
8. Krane JF, Granter SR, Trask CE, Hogan CL, Lee KR. Papanicolaou smear sensitivity for the detection of adenocarcinoma of the cervix. A study of 49 cases. Cancer Cytopathol 2001;93:8-15.
9. Schoolland M, Segal A, Allpress S, Miranda A, Frost FA, Sterrett GF. Adenocarcinoma *in situ* of the cervix. Sensitivity of detection by cervical smear. Cancer Cytopathol 2002;96:330-7.
10. Ruba S, Schoolland M, Allpress S, Sterrett G. Adenocarcinoma *in situ* of the uterine cervix. Screening and diagnostic errors in Papanicolaou smears. Cancer Cytopathol 2004;102:280-7.
11. Lee KR, Manna EA, Jones MA. Comparative cytologic features of adenocarcinoma *in situ* of the uterine cervix. Acta Cytol 1991;35:117-26.
12. Van Aspert-van Erp AJ, Smedts FM, Vooijs GP. Severe cervical glandular cell lesions with coexisting squamous cell lesions. Cancer Cytopathol 2004;102:218-27.
13. Krumins I, Young Q, Pacey F, Bousfield L, Mulhearn L. The cytologic diagnosis of adenocarcinoma *in situ* of the cervix uteri. Acta Cytol 1977;21:320-9.
14. Jaworski RC. Endocervical glandular dysplasia, adenocarcinoma *in situ*, and early invasive (microinvasive) adenocarcinoma of the uterine cervix. Semin Diagn Pathol 1990;7:190-204.
15. Croll E, Rana DN, Walton LJ. Hyperchromatic crowded cell groups in gynaecological liquid-based cytology samples. Br J Biomed Sci 2010;67:154-63.
16. Demay RM. Hyperchromatic crowded groups: Pitfalls in pap smear diagnosis. Am J Clin Pathol 2000;114 Suppl: S36-43.
17. Thiryayi SA, Marshall J, Rana DN. An audit of liquid-based cervical cytology screening samples (ThinPrep and SurePath) reported as glandular neoplasia. Cytopathology 2010;21:223-8.
18. Biscotti CV, Gero MA, Toddy SM, Fischler DF, Easley KA. Endocervical adenocarcinoma *in situ*: An analysis of cellular features. Diagn Cytopathol 1997;17:326-32.
19. Belsley NA, Tambouret RH, Misdraji J, Muzikansky A, Russell DK, Wilbur DC. Cytologic features of endocervical glandular lesions: Comparison of SurePath, ThinPrep, and conventional smear specimen preparations. Diagn Cytopathol 2008;36:232-7.
20. Apgar BS, Kittendorf AL, Bettcher CM, Wong J, Kaufman AJ. Update on ASCCP consensus guidelines for abnormal cervical screening tests and cervical histology. Am Fam Physician 2009;80:147-55.
21. Faraker CA, Boxer ME. Rapid review (partial rescreening) of cervical cytology. Four years experience and quality assurance implications. J Clin Pathol 1996;49:587-91.
22. Singh M, Mockler D, Akalin A, Burke S, Shroyer A, Shroyer KR. Immunocytochemical colocalization of P16(INK4a) and Ki-67 predicts CIN2/3 and AIS/adenocarcinoma. Cancer Cytopathol 2012;120:26-34.

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