

## Cytodiagnosis of Retinoblastoma by Fine Needle Aspiration Technique : A case report with emphasis on FNA, a premiere diagnostic tool

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*RB is a retinal neoplasm of neurosensory cells. It is the most common intra-ocular neoplasm of children. Traditionally, the diagnosis of RB in a child is based on clinical information, ultrasonography, CT scanning, and Magnetic Resonance Imaging (MRI). Although, the current X-rays technology provides a diagnostic aid, it is very expensive and not cellular basis that is essential for a definite pathological diagnosis. We report a case of RB accurately diagnosed by FNA cytology with histologic confirmation on the specimen of the right eye enucleated a month later. Pre-operative cytodiagnosis can be helpful to the ophthalmologist in planning the extent of surgery, preventing unnecessary enucleation of the eye, and obviating the need for an open biopsy in cases of RB that would be candidates for chemotherapy, thermotherapy, cryotherapy, and plaque radiotherapy. In conclusion, FNA cytology is a useful diagnostic tool for RB and will play a major role in the diagnosis and management of RB, especially when the current trend of RB therapy is going toward focal treatment with eye preservation.*

**Key words:** *Retinoblastoma, Intra-ocular tumor, Malignancy of eye, Tumor of childhood, FNA of eye, Cytology.*

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เยาวเรศ วงศ์ศิระวิลาส, เสาวณีย์ เย็นฤดี. เซลล์วินิจฉัยโรค RB ด้วยเทคนิคการเจาะดูด รายงานผู้ป่วย 1 ราย และเน้นการศึกษาเซลล์วิทยาเจาะดูด. จุฬาลงกรณ์เวชสาร 2544 เม.ย; 45(4): 337-45

เนื้องอกของเซลล์ประสาท RB เป็นเนื้องอกของลูกตาที่พบได้บ่อยที่สุดในเด็ก โดยทั่ว ๆ ไปแล้ว การวินิจฉัยโรค RB ในเด็กต้องอาศัยข้อมูลจากการตรวจทางคลินิกอัลตราโซโนกราฟี CT SCAN และ MRI ถึงแม้ว่าเอกซเรย์เทคโนโลยีที่ทันสมัยในปัจจุบันจะช่วยในการวินิจฉัยโรคนี้ก็ตาม แต่ทว่ามีราคาแพงมากและไม่ได้อยู่บนพื้นฐานการตรวจทางเซลล์ ซึ่งเป็นสิ่งจำเป็นสำหรับการวินิจฉัยโรคทางพยาธิวิทยา ผู้เขียนได้รายงานผู้ป่วยเด็กด้วยโรค RB 1 รายที่ได้รับการวินิจฉัยอย่างถูกต้อง โดยวิธีการเซลล์วิทยาเจาะดูดและได้รับการพิสูจน์ความถูกต้องแม่นยำจากการตรวจชิ้นเนื้อของลูกตา ที่ได้ผ่าตัดออกใน 1 เดือนให้หลัง การวินิจฉัยทางเซลล์วิทยาจะช่วยให้จักษุแพทย์วางแผนการรักษาและกำหนดขอบเขตการทำผ่าตัดได้ล่วงหน้า ช่วยป้องกันการผ่าตัดเอาลูกตาออกโดยไม่จำเป็นและทำให้ไม่ต้องตัดชิ้นเนื้อออกมาตรวจในกรณีที่คนไข้ RB อยู่ในภาวะที่จะให้การรักษาได้ด้วย chemotherapy, thermotherapy, cryotherapy and plaque radiotherapy โดยสรุปแล้ว เซลล์วิทยาเจาะดูดเป็นวิธีการวินิจฉัยที่เป็นประโยชน์และจะมีบทบาทอย่างมาก ในการวินิจฉัยและการดูแลรักษาผู้ป่วยด้วยโรค RB โดยเฉพาะอย่างยิ่ง การรักษา RB ในปัจจุบันมุ่งมั่นอยู่ที่การรักษาเฉพาะส่วนที่เป็นโรคของลูกตาเพื่อการเก็บรักษาดวงตาไว้

Retinoblastoma (RB) is the most common intra-ocular neoplasm of children. <sup>(1-3)</sup> Most cases of retinoblastoma are sporadic but approximately 30 %-40 % of them are inherited. <sup>(4,5)</sup> The inherited cases are often bilateral and may be associated with a similar tumor in the pineal gland. <sup>(6,7)</sup> (Bilateral retinoblastoma plus pineal tumor equals trilateral retinoblastoma). The average age at initial diagnosis is 13 months with 89 % diagnosed before 3 years of age. About 8.5 % of patients are older than 5 years at the time of diagnosis. <sup>(8)</sup> Traditionally the diagnosis of RB in a child is based on the sign of leukokoria, family history, ultrasonography, CT scan, and magnetic resonance imaging (MRI). FNA can be used as a tool to obtain intra-ocular material for cytologic examination.

### Historical background

Cytology was born since the first invention of the microscope by Antoni van Leeuwenhoek (1632-1723), a self-trained Dutch scientist, almost 400 years ago. At the same time, the biological use of the word 'cell' was initiated by his British contemporary, Robert Hooke. <sup>(9)</sup> Medical history indicated that morphologic diagnosis of disease was based on microscopic examination of cell preparation long before the introduction of tissue fixatives, cutting instruments, and stains during the 1870's. Needles and syringes have been used to aspirate collections of fluid for microscopic study since the introduction of these instruments into the medical field. The earliest documents of this diagnostic tool go back to the early nineteenth century. Papers describing its applications in the early twentieth century include the use of aspirates of lymph nodes for the identification of trypanosomes in the diagnosis of sleeping sickness;

aspirates of solid tumor of skin, head and neck for the diagnosis of cancer. The most well known paper about aspiration cytology was the diagnosis of tumors by aspiration that was published by Dr. Fred W. Stewart in 1933. <sup>(10)</sup> Needle aspiration of tumors for cytologic examination was repeatedly described in American and European publications. Unfortunately, it gained little interest in the United States. For more than a century, it was practically replaced by an examination of tissue prepared by tissue processing, embedding, cutting, and staining. The new era of FNA cytology began in the early 1970's when cellular examination for detection of cancer was widely accepted and the FNA technique was recognized as a tool for obtaining specimens for cytologic study.

As in all scientific pursuits, cytology has depended upon technical progress in order to advance rapidly. In the past thirty years, FNA cytology has achieved a broader spectrum its applications in the field of diagnostic pathology. Its continuous growth is attributed chiefly to the advancement in medical technology, i.e., CT scan, MRI, ultrasonography, and endoscopy, etc. Technological advances not only allow an improved visualization of tumors but also an easier access to hardly approachable lesions or organs. Furthermore, it has been well documented that FNA is both useful and harmless. Once again, FNA has become a premiere diagnostic tool in modern medicine. It is currently widespread throughout the world.

There have been only a few reports on cytodiagnosis of RB by fine needle aspiration. We report a case of RB with emphasis on the usefulness of FNA cytology in diagnosing RB, an intra-ocular tumor that hardly approachable by any other means.

### Case report

This 19 month-old male infant was admitted to Queen Sirikit's Children Hospital with the history of being accidentally poked at his right eye with a scissors approximately 5 months prior to this admission. The exact mode of injury was uncertain. He had no other symptoms and his past history and family history were unremarkable. Other physical examination and routine laboratory tests were unremarkable. The diagnosis of small pupil and after cataract was made. He underwent an operation for lysis synergia with iridectomy of the right eye.

An eye examination during operation revealed a white mass with neovascular formation in the posterior chamber (vitreous) of the right eye. Fine Needle Aspiration (FNA) of the right intra-ocular site was performed by the attending ophthalmologist using a 27-gauge needle and a 2.5 cc. disposable plastic

syringe. Four direct smear slides were prepared from a small amount of intra-ocular fluid and immediately immersed in 95 % ethyl alcohol fixative. The slides were sent to the Cytopathology Laboratory where Papanicolaou stain was done. Cytologic examination led to the diagnosis of retinoblastoma. Subsequently, the same diagnosis was confirmed histologically after enucleation of the right eye a month later.

### Cytologic findings

Papanicolaou-stained slides were examined microscopically. The smears were hypercellular and composed of a rather monomorphic population of small round cells that appeared as individual cells, in loose clusters, and in Flexner Winterstiener rosettes,<sup>(11)</sup> - a feature of photoreceptor differentiation.<sup>(11, 12)</sup> (Figures 1, 2) Tumor cells contained prominent and homogeneous nuclei and almost invisible cytoplasm.

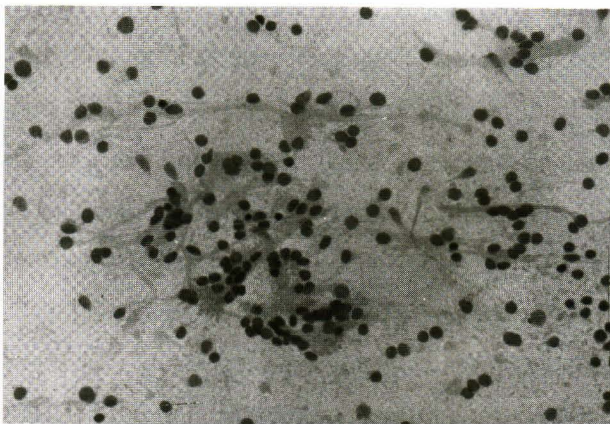


Figure 1. Papanicolaou stain of FNA material demonstrates a highly cellular smear composed of uniform small round cells that appear singly, in loose clusters, and in Flexner Winterstiener rosettes. (X100)

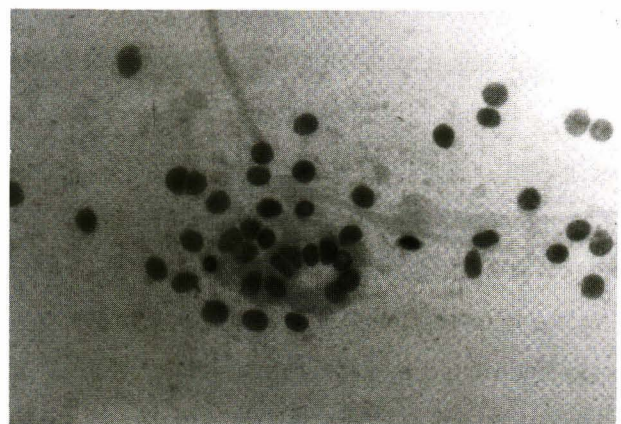


Figure 2. A higher magnification of Pap smear demonstrates uniform small round cells in clusters and in Flexner Winterstiener rosettes. Tumor cells contain enlarged, round to oval, hyperchromatic nuclei with almost invisible cytoplasm. (X400)

Many naked nuclei were also seen. In a Flexner Wintersteiner rosette, tumor cells arrange around a clear central lumen that contains a hyaluronidase - resistant acid mucopolysaccharide. The diagnosis of small round cell tumor, most compatible with retinoblastoma was made. The differential diagnosis includes Medulloblastoma and Neuroblastoma that show no rosettes and are extremely rare in the eye.

### Gross findings

Received fixed in formalin solution was a right eyeball with no optic nerve, measuring 2.2 cm. in diameter. Cut sections of the specimen revealed that the posterior chamber of the eye contained a well-circumscribed, pure white, soft mass, measuring 1.2x 1.1 x 0.8 cm. and clear fluid.

### Histologic findings

Histologic sections were prepared from formalin-fixed, paraffin-embedded specimen of the right eye. Hematoxylin and Eosin stain and immunohistochemical studies were performed. H&E sections revealed a solid mass arising in the retina in the posterior chamber. The mass was composed of rather uniform small round cells arranged around vascular channels, in Flexner Winterstiener rosettes, and in solid nests. These malignant cells contained enlarged, round to oval, hyperchromatic, and homogeneous nuclei and almost invisible cytoplasm. Frequent mitotic figures, prominent vascular proliferation, tumor cell necrosis, and inflammatory infiltrates were observed.

Immunohistochemical stains revealed non-reactivity of tumor cells to glial-fibrillary acidic protein

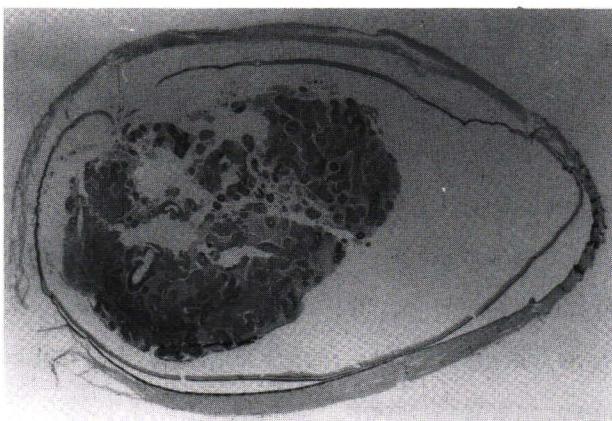


Figure 3. H&E section of the enucleated right eye, viewed under lower magnification shows a mass of RB in the posterior chamber of the eye.

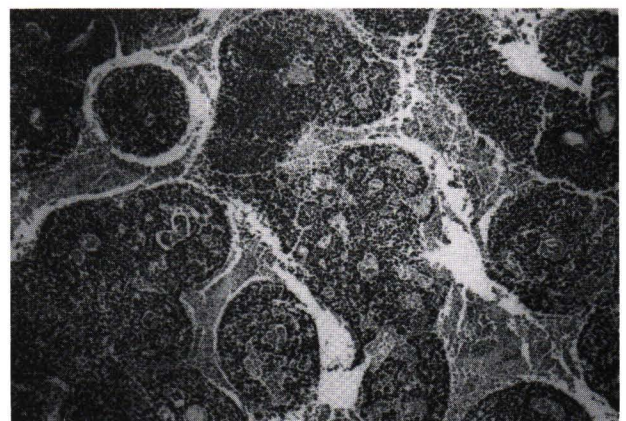


Figure 4. Increased magnification of the right eye mass shows numerous viable tumor cells around blood vessels, foci of necrotic tumor cells, and Flexner Wintersteiner rosettes. (X40)

(GFAP) and S-100 antibodies but strong reactivity to neuron specific enolase (NSE) and synaptophysin antibodies. The stains confirmed that the tumor cells were the neuroreceptor cells.<sup>(12)</sup>

## Discussion

RB is a retinal neoplasm of neurosensory cells akin to neuroblasts.<sup>(12)</sup> Traditionally, the diagnosis of RB in a child is based on the sign of leukokoria, family history, ultrasonography, CT scan and magnetic resonance imaging (MRI). Children with early lesions may be completely asymptomatic or may present with visual difficulties or strabismus.<sup>(8)</sup> Moderate lesions may present as leukokoria that leads to further diagnostic work up. Diagnosis is often times delayed. Furthermore, there are other conditions that may present as leukokoria, i.e., Norrie's disease, congenital non-attachment of retina, orbital cellulitis, Coat's disease, and other intra-ocular tumors, etc. These conditions may simulate RB on clinical examination, ultrasonography, or CT scan. Unfortunately, as high as 16 % of eyes removed with clinical diagnosis of RB contain a simulating lesion.<sup>(13)</sup>

Char et al<sup>(2)</sup> proudly reported diagnostic accuracy of non-invasive diagnostic technique to be over 98 % of RB found in an Ocular Oncology Unit, University of California, San Francisco, USA. At the same time, these authors addressed the difficulty in distinguishing RB from other simulating lesions in smaller lesions or other unusual cases. Actually, diagnostic accuracy of non-invasive techniques varies from center to center.

In Thailand, high technology X-rays equipment is very expensive and available in only a few medical centers. High cost equipment precludes a routine use

of it. The diagnosis of RB in a child is usually based on seeing a white mass in the eye and occasionally with a complimentary examination under ultrasonography. There is no data available as to the diagnostic accuracy of RB based on the sign of leukokoria plus ultrasound.

Although, the current X - rays technology provides a diagnostic aid, it is very expensive and not cellular basis that is essential for a definite pathological diagnosis. Furthermore, the change in the management of children with RB mandates an accurate diagnosis.

The management of RB has gradually changed in the past few decades.<sup>(14-20)</sup> The goal is early detection, early treatment, and preservation of the eye. Enucleation is still performed for a large RB that almost fills the eye, especially when there is a concern for tumor invasion into the optic nerve and choroid. External beam therapy is reserved for treating less advanced RB, especially when there is diffuse vitreous or subretinal seeding.<sup>(14,15,17)</sup> Cryotherapy and photocoagulation provide excellent control of selected small tumors. The current method of treatment of children with RB includes chemoreduction combined with cryotherapy, thermotherapy, and plaque radiotherapy.<sup>(14-20)</sup> Recent advances provide an effective tumor control, allow eye preservation, and leave the child with a reasonably tiny scar, thus preserving better vision.

FNA is a useful technique to obtain intra-ocular material for cytodiagnosis. The information gained is important for clinical management of the patients. Pre-operative cytodiagnosis can assist the ophthalmologist in planning the extent of surgery. Aspiration diagnosis not only prevents unnecessary enucleation of the eye

but also avoids the need for an open biopsy in cases of RB that would be candidates for chemoreduction, thermotherapy, cryotherapy, and plaque radiotherapy.

Orbital FNA requires a competent ophthalmologist who has adequate skill to perform aspiration and capable of handling any untoward effect that may arise. Needle tract seeding of malignant cells and excessive bleeding are the major concern of the ophthalmologist. In fact, the concern over complication resulted in almost abandonment of eye FNA procedures in the 1950's. Aspiration may require an ultrasound or CT scan for localization of an eye tumor. In the studies of Glasgow et al,<sup>(21)</sup> retrobulbar hemorrhage occurred in 6 % and eye perforation was very infrequent which was less than 1%. Bleeding and needle tract seeding of malignant cells can be avoided with the use of 25 gauge-needle or a smaller-bore needle.<sup>(22,23)</sup>

In the past few decades, FNA has been widely accepted for cancer detection of many organs, but FNA cytodiagnosis of intra-ocular tumors and RB has been underutilized. Only few case reports can be found on cytodiagnosis of RB by FNA. Therefore, diagnostic accuracy for FNA cytology of RB cannot be determined. However, a review of the literature regarding cytodiagnosis of lesions of the eyes and adnexa revealed encouraging results. The largest series of 292 cases is belonged to Zajdela et al<sup>(24)</sup> who proudly reported diagnostic accuracy of 87 % with 1.8 % false negative rate, 1.4 % false positive rate, and 6.3 % insufficient specimens. The second largest series of 83 cases of Glasgow et al<sup>(21)</sup> revealed diagnostic accuracy of 70 % with no false negative/false positive rate and 23 % insufficient specimens. The overall result of 13 studies was acceptable with

75.2% diagnostic accuracy and 9.5 % insufficient rate.<sup>(25)</sup> Actually, the more skill and experience of the performers, the better diagnostic accuracy. So far, there has been no data available as to diagnostic accuracy comparison between each individual test for the diagnosis of RB.

In conclusion, FNA cytology is a useful diagnostic tool for RB and will play a major role in the diagnosis and management of RB.

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