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Study by TEM to the Effects Occurred in Leukocytes in Patients Suffering from Hydatid Cysts

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ABSTRACT

The research aimed to determinate the effects of hydatid cyst disease on leukocytes.

By using Transmission electron microscope (TEM).
Samples of blood were collected from different patient of different ages and sexes.

The result showed that degranulation was observed in the cytoplasm of neutrophil, eosinophil and basophil.

High phagocytosis activity were observed in neutrophil, eosinophil and monocyte, furthermore the result illustrated the presence of cytotoxic lymphocyte.

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1- INTRODUCTION

Hydatid cyst disease is one of the widespread parasitic diseases that affect humans and livestock [1, 2]. It has several names, such as Echinococciasis, Hydatid disease, hydatidosis, hydatosis unicystic, or Unilocular hydatid disease [3], and it is caused by the larval stage of the tapeworm Echinococcus granulosus [4,5]. The definitive hosts of the adult stage are dogs, wolves, and foxes [14, 6). The adult stage lives in the small intestines of infected animals [7].

Studies in Iraq have confirmed that dogs are the definitive hosts of the adult stage [8). Additionally, sheep, cattle, goats, horses, and humans are intermediate hosts for the parasite [9, 10]. In humans, infection occurs through close association with dogs, especially in children, or by consuming improperly washed vegetables and food contaminated with the feces of infected dogs [11]. The developing embryos progress from eggs to a watery cyst that grows about 5-10 centimeters per year in the first year, and then continues to grow slowly in the target organ for several years [12].

The embryos (oncospheres) face resistance from white blood cells during their passage through the bloodstream, which may lead to their destruction [13].

If they settle in the host's body, the body's resistance and white blood cells persist, as the growing hydatid cyst is surrounded by fibrous tissues containing epithelial cells, acidophilic cells, and giant cells [14].

Moreover, hydatid cyst infection has a negative impact on red blood cells, leading to certain cases of anemia (15), as well as a negative effect on all white blood cells.

2- MATERIALS AND METHODS

In this study, blood samples were examined using a transmission electron microscope (TEM) from thirty diagnosed patients with Echinococcus granulosus infection, along with a control group of healthy individuals of different ages, both male and female, at a hospital Refech al herery in Baghdad city. A volume of 1 cm3 of saline solution was drawn, and the volume was completed to 5 cm3 with a syringe.

Blood was drawn directly from the median cubital vein before the procedure. The blood was preserved in test tubes containing anticoagulant EDTA-K+ and stored in a plastic container.

The samples method. The preparation of the blood samples for (TEM) was carried out as described in [16].

3- RESULTS

1- Study of Neutrophils:

Neutrophils in the blood samples of healthy individuals were characterized by the presence of pseudopodia. The nucleus appeared segmented, resembling multiple separate nuclei. The rough endoplasmic reticulum was observed. The cytoplasm contained primary circular granules and a larger number of secondary granules, which appeared as rod-shaped with varying densities [Figure 1].

When examining the neutrophils in the blood samples of patients infected with *Echinococcus granulosus*, it was evident that some granules disappeared, while others moved towards the plasma membrane edge. Some granules were observed leaving the cell.

These neutrophils exhibited numerous pseudopodia and unfolded cytoplasmic folds in their plasma membrane, indicating phagocytic activity. Furthermore, toxic cytotoxic neutrophils were observed, characterized by having more than three lobes in the nucleus and highly effective engulfment. They also exhibited increased electron density in the cytoplasm and the disappearance of secretory granules.

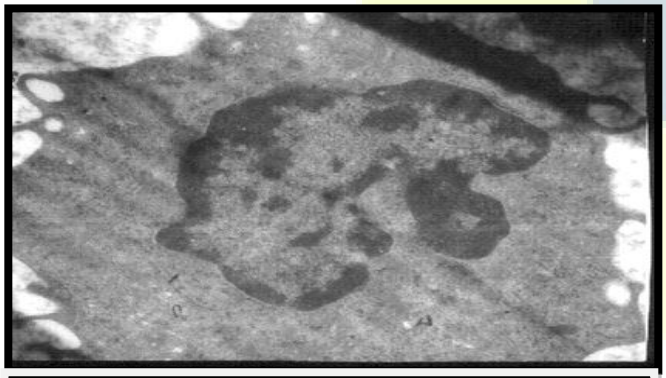

Figure (2): A neutrophil cell magnified under a TEM (uranyl acetate and lead citrate (× 48,000)) in patients' blood samples, showing secretory granule structures migrating toward the plasma membrane.

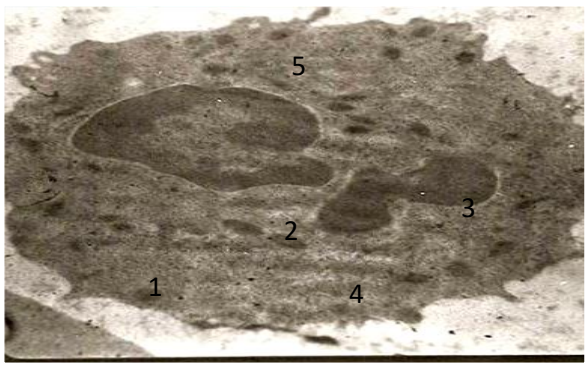

Figure (1): A normal neutrophil cell magnified under a TEM (uranyl acetate and lead citrate) (×48000) showing the following structures: 1- Rough plasmic reticulum 2- Primary secretory granules 3- Secondary secretory granules 4- Lysosome 5- Nucleus

2- Study of Eosinophil's:

Eosinophils in the blood samples of healthy individuals were characterized by the presence of a nucleus with two separate lobes. The cytoplasm contained oval-shaped secretory granules in various stages of maturation, with some exhibiting different electron densities. The presence of prominent Golgi bodies within the cytoplasm was observed, along with the presence of mitochondria. The rough endoplasmic reticulum appeared more distinct compared to neutrophils [Figure 3].

When examining eosinophil's in the blood samples of patients, it was evident that they were almost devoid of secretory granules, except for a very small number. The presence of empty secretory vesicles within the cytoplasm was prominent. This eosinophil's exhibited more pronounced phagocytic activity compared to their counterparts in the healthy blood samples, as evidenced by an increase in the number of pseudopodia [Figure 4].

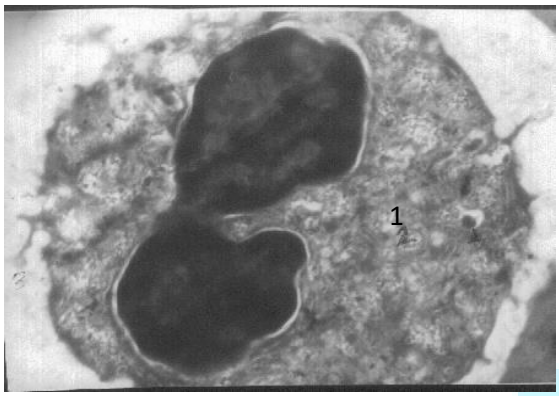

Figure (4) An eosinophil in patients' blood samples, magnified under TEM (uranyl acetate and lead citrate (48,000 x), showing the following structures: 1- Secretory granules 2- Vesicle -3 Exocrine granule outside the body

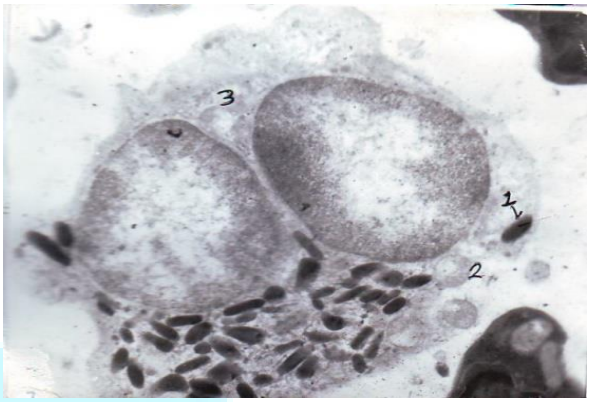

Figure (3) A normal eosinophil cell magnified under TEM (uranyl acetate and lead citrate (48000 x) showing the following structures: 1- Secretory granules 2- Golgi body 3- Plasma reticulum

3: The study of basophil cell:

The presence of basophile cells was observed when examining a sample from patients infected with *Echinococcus granulosus*.

They were diagnosed using (TEM), which revealed the disappearance of most secretory granules. Some of them were found close to the plasma membrane, along with an increased electron density of the cytoplasm. Clear phagocytic activity was observed through the observation of non-fused cytoplasmic folds extending from the plasma membrane, as well as the presence of phagocytic vacuoles near the plasma membrane [Figure 5].

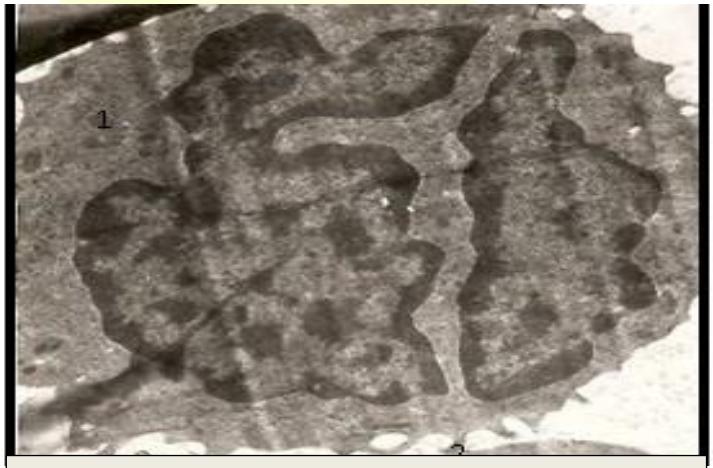

Figure (5) A kidney basophil cell in a patient's blood sample, magnified under a TEM, uranyl citrate and lead citrate (48000 x) in which the disappearance of most secretory granules.

4- Study of Monocytes

Upon detailed examination of mononuclear cells using (TEM) in healthy blood samples, the nucleus was observed to have a horse's hoof-like shape, and the cytoplasm appeared to have moderate electron density. Particulate matter was clearly seen scattered within the cytoplasm, at various stages of maturation. The mitochondria were observed to be dispersed within the cytoplasm, and there were cytoplasmic extensions from the plasma membrane as well as incomplete cytoplasmic folds. The phenomenon of cellular drinking (pinocytosis) was evident [Figure 6].

When examining the cells in samples from patients, the cells were characterized by significant phagocytic activity, indicated by the abundance of pseudopodia. In one of the cytoplasmic extensions, the presence of a secretory granule, possibly representing one of the types of secretory vesicles, was observed. The cytoplasm was filled with ribosomes and rough endoplasmic reticulum. Additionally, small structures with low electron density were observed, which may represent early stages of primary particle formation. The cytoplasm appeared to have higher electron density [Figure 7].

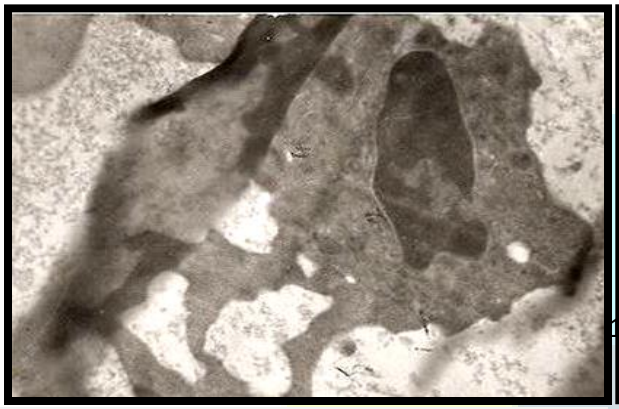

Figure (7) A mononuclear cell in patients' blood samples magnified under TEMe (uranyl acetate and lead citrate) (×48000) showing the following structures: 1- Rough plasmatic reticulum 2- Free ribosomes 3- Pseudopodia 4- Cell drinking 5- Secretory granule released outside the cell body.

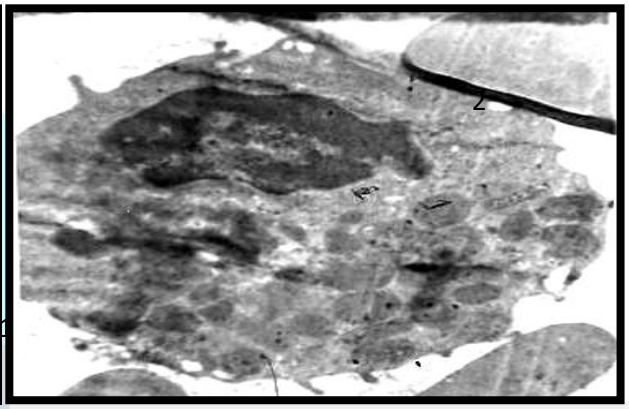

Figure (6) A mononuclear cell in healthy blood samples, magnified under a TEM (uranyl acetate and lead citrate (48,000 x), showing the following structures: 1- Secretory granules 2- Vesicle

5- Study of Lymphocytes

When studying lymphocytes in healthy blood samples, their nuclei appeared densely chromatin-rich. Ribosomes were observed to be dispersed in the cytoplasm, along with a few mitochondria and a small number of secretory granules. When examining the cells in blood samples from individuals infected with *Echinococcus granulosus*, the nuclei appeared eccentrically located. The cytoplasm appeared to be devoid of organelles and exhibited high electron density.

The cells were observed in the process of transitioning into plasma cells [Figure 8].

Other lymphocytes undergoing transformation were also observed, with clear nuclear elongation. Additionally, a small number of secretory granules were scattered, and the cytoplasm contained some strands of rough endoplasmic reticulum [Figure 9].

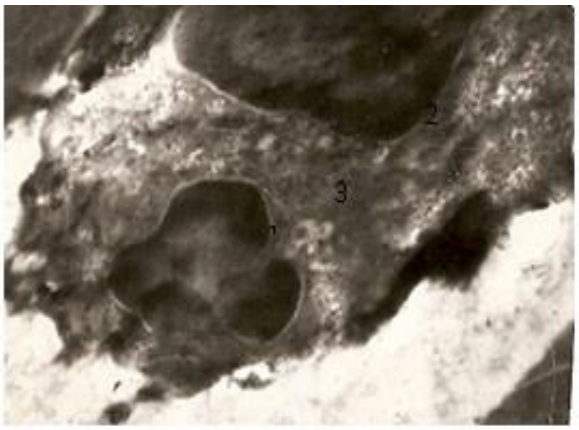

Figure 9: A lymphocyte in patient samples, undergoing degeneration, magnified under a TEM, uranyl acetate, magnified at 48,000 x, sodium citrate, showing: 1- Golgi bodies, 2- Free ribosomes, 3- Plasmodium reticulum

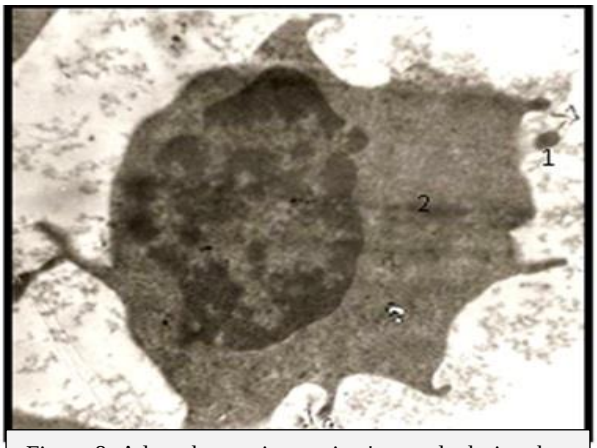

Figure 8: A lymphocyte in a patient's sample during the transformation into a plasma cell, magnified under a TEM at 48,000x, treated with uranyl acetate and lead citrate, showing phagocytic activity and secretion of secretory granules and rough plasmatic reticulum.

4- DISCUSSION

The results of the TEM examination of peripheral blood leukocytes in patient samples revealed changes in the morphology of these cells, such as nuclear deformation, which reflects the speed of movement and activity of these cells [17].

The occurrence of injury and the growth of the parasitic cyst, which led to increased migration of these cells to the site of injury and their encapsulation by the cyst due to chemotactic factors [18], may have resulted in increased phagocytic cell activity due to increased secretion of chemotactic factors from the cyst, including the leakage of calcium and magnesium ions and changes in glucose concentration, which stimulate phagocytic cell activity [19].

The presence of incomplete cytoplasmic folds, along with the presence of fine structures within these folds that could be bacteria, fungi, or cell secretions, can be considered as factors contributing to the development of inflammation associated with cystic injury [18].

Further studies, such as histological or immune histochemical studies, are needed to identify the nature of these materials observed in the folds of these cells.

The cells appeared to be degranulation, as they contained a small number of secretory granules. They appeared to be moving towards the phagocytic cup to release their contents of basic phosphatases and vagostatin.

The scarcity of secondary granules secreted by these systems supports this observation [19].

The increased phagocytic activity, characterized by the presence of rough endoplasmic reticulum and ribosomes involved in protein synthesis used in the body's defense mechanism against foreign bodies, indicates the body's clearance of debris from dead cells resulting from the body's resistance to foreign invaders [20].

The phenomenon of cellular drinking is evident, adding further evidence of increased bacteria or parasites, which may be due to concomitant inflammation with the cystic injury.

In this study, the presence of toxic mononuclear cells was observed, characterized by the absence of secretory granules that could have been released as a means of defense by the body to eliminate bacteria by secreting basic phosphatases and vagostatin, which are secreted by secondary granules [18].
The examination of acidophilic leukocytes in the blood samples of patients infected with Echinococcus parasites showed the disappearance of most secretory granules, except for a few scattered near the plasma membrane surface compared to the control group.

This may be due to the appropriate chemotactic factor that led to the movement of secretory granules from the cell to its plasma membrane through physical barriers in the plasma membrane components, as the plasma membrane of acidophilic cells contains receptors sensitive to immunoglobulin (IgE), and upon interaction, the granules are removed, releasing histamine and peroxidase [27].

The migration of the remaining secretory granules towards the plasma membrane confirms the occurrence of the immune reaction involving IgE.

Furthermore, TEM examination of acidophilic leukocytes revealed the presence of pseudopodia around the plasma membrane, indicating increased phagocytic activity of these cells due to cystic injury [24], and the observed inflammation may be a consequence of cystic injury [20], which stimulated all phagocytic leukocytes, including acidophilic cells, to eliminate bacteria, parasites, fungi, and debris from dead cells.

The results of the TEM examination of the monocytes blood cells in the samples of patients infected with echinococcus parasites, when compared to the healthy group, revealed significant variations that reflect their high effectiveness [21].

Numerous pseudopodia were observed around the plasma membrane, along with cytoplasmic extensions, as well as the spread of phagocytic vesicles in the cytoplasm, indicating increased phagocytic activity of the cell for the purpose of engulfing specific antibodies, possibly due to the spread of antigens as a result of the infection [22, 23] and an increase in the rate of bacterial killing [25]. Furthermore, the clear phenomenon of cellular drinking indicates significant bacterial activity, possibly due to acute inflammation resulting from acute infection.

Examination of blood samples from patients infected with eosinophilic granulocytes revealed the presence of lymphocytes undergoing transition to plasma cells, which is an indicator of accompanying acute inflammation resulting from hydatid cyst disease [26, 27].

It is worth noting that these cells were observed for the first time in this study in peripheral blood, as plasma cells are normally present in connective tissues, but their appearance in peripheral blood may be a result of the immune response to the infection with the protozoan, leading to the transformation of B lymphocytes into plasma cells for the production of antibodies as a defensive mechanism for the body.

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دراسة بإستعمال المجهر الألكتروني النافذ (TEM) للتأثيرات التي تحدث في كريات الدم البيض عند المرضى المصابين بالأكياس المائية

الخـلاصــة

إستهدف البحث تحديد تاثيرات الأصابة بمرض الأكياس المائية (الأكياس العدارية) في التركيب المستدق لخلايا الدم البيض عن طريق الفحص بالمجهر الإلكتروني النافذ. وشمل البحث سحب عينات من دم المرضى المصابين وبأعمار وأجناس مختلفة.

أظهرت نتائج البحث إختفاء التحبب من سايتوبلازم خلايا الدم البيض العدلة والحمضات والقعدات. كما لوحظ زيادة النشاط البلعمي للخلايا العدلة والحمضات ووحيدات النواة, فضلا عن ملاحظة وجود بعض الخلايا اللمفاوية المتنكسة.