

# Impression Cytology of the Chalazion Contents

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## Abstract

To confirm tissue diagnosis of chalazion contents by impression cytology. This prospective randomized cross-section study included 60 eyes of 60 patients with localised eyelid swelling and diagnosed clinically as chalazion after complete ophthalmology examination with excluded the patients that diagnosed as recurrent or infected chalazion. All patients are managed by cruciate incision operation and all the contents are examined by impression cytology through membrane filter paper and rinsed in a fixation solution then stained by haematoxylin and PAS stains. Two broad patterns of granulomatous inflammation with overlapping features have been identified. 48 cases out of 60 cases (80.0%) were recognized as mixed cell granuloma (MCG), while 12 cases (20.0%) were recognized as suppurating granuloma (SG). Statistics showed P value of (0.044) for plasma cells (73.3%), P value of (0.045) for lymphocytes (90%), Neutrophils (83.3%) (P=0.195), and Macrophage (76.6%) (P=0.777) distributed in SG and MCG patterns. Impression cytology is a rapid, safe, and reliable means of documenting a tissue diagnosis of chalazion and excluding malignancy, allowing treatment to be initiated with confidence.

**Keywords** Cytology; Chalazion; Filter paper; Inflammatory cells; Granulomas



## 1. Introduction

Chalazion, a focal chronic inflammatory lesion of the eye lid, is a common cause of lid swelling. Chalazia result from an obstruction in the duct of the Meibomian gland<sup>[1]</sup>. Secreted lipids accumulate and erupt into the collagen of the tarsal plate, provoking a mixed inflammatory reaction and the result is a chronic lipogranulomatous inflammation in the tarsal plate<sup>[2]</sup>. The obstruction of sebaceous gland can be the result of inflammation or infection 'acne rosacea or seborrheic dermatitis' or of neoplastic lesions 'sebaceous gland carcinoma'<sup>[3]</sup> or Merkel cell tumour 'of the lid margin'<sup>[4,5]</sup>.

Impression cytology refers to the application of a cellulose acetate filter to the ocular surface to remove the superficial layers of the ocular surface epithelium, generally removing 2-3 layers<sup>[6]</sup>. Modifications in techniques like use of Biopore membranes or by transferring impressions from cellulose acetate filter to a gelatin coated glass slide; have extended its use in transmission electron microscopy, polymerase chain reaction (PCR), flowcytometry and immunohistochemistry. Its application includes diagnosing a wide range of ocular surface disorders<sup>[7]</sup>.

In this study, impression cytology applied to the chalazia contents as a tissue diagnosis for confirmation and exclusion to malignancy.

## 2. Materials and Methods

### 2.1 Patients

The review board of the Department of Ophthalmology Ain Shams University approved this prospective randomized clinical cross-section study. It included 60 eyes of 60 patients with localized eyelid swelling who presented to outpatient clinic of Ain Shams university hospital, Cairo from January 2014 to January 2016, full

history and ophthalmological examination were done and diagnosed as chalazion and excluded the patients with recurrent or infected chalazion.

### 2.2 Operation and specimen preparation

All patients signed an informed consent and agreed to use the photographs for scientific purposes. The eyelids were scrubbed with Povidone-Iodine 5% soaked dressing for sterilization, then the surrounding eyelid tissue was injected with the anaesthetic Mepecaine L 1.8ml carpule (Mepivacaine HCL 2% + Levonordefrin 1:20000). The eyelid was everted then a chalazion clamp was positioned over the lesion. This helps stabilize the eyelid and assists in haemostasis.

A surgical #11 straight blade was used to make a cruciate incision in the involved Meibomian gland through the conjunctival surface. A 5x5 mm pre-cut cellulose acetate strip (sterilized Membrane filter paper Millipore, 0.45µm pore size) was placed on the surface of the chalazion contents using gentle pressure, carefully peeled off then rinsed in a fixation solution (glacial acetic acid, formaldehyde, and ethyl alcohol in a 1:1:20 volume ratio). A curette was then used to scrape out the chronic granulomatous debris. The chalazion clamp was removed and the eyelid was repositioned. Pressure was applied until all the bleeding has stopped after instillation of antibiotic eye ointment.

### 2.3 Staining protocol

The filter paper with the specimen was fixed for approximately 10 minutes in a fixation solution containing glacial acetic acid, formaldehyde, and ethyl alcohol in a 1:20 volume ratio. The specimens next were rehydrated in 70% ethyl alcohol and then placed successively in periodic acid Schiff reagent, haematoxylin, and tap water substitute for 2 minutes each, they were rinsed twice in two different jars of tap water in between each step. The sections were next dehydrated in two changes of 95% ethyl alcohol, and then stained with orange G for 2 minutes.

Sections were next rinsed with 95% ethyl alcohol for 3 minutes, and stained with eosin Y for 2 minutes,

again rinsed in 95% ethyl alcohol for 5-10 minutes, before dehydration in absolute alcohol for 5 minutes. Xylene was used to make the filter paper transparent. Before mounting, the filter paper was placed with the specimen facing up. The completed slides were examined by light microscopy.

### 3. Statistical analysis

Data were collected, and statistical analysis was performed using IBM SPSS Statistics (version 21, SPSS Inc., Chicago, IL, USA). Significance level was  $P < 0.05$ . minimum, maximum, mean, standard deviation and percentiles for quantitative variables. The chi-square test were performed for qualitative variables.

### 4. Results

The study included 60 patients with chalazia. The mean age was  $26.33 \pm 10.755$  years (Mean  $\pm$ SD) there were 36 males (60.0%) and 24 females (40.0%).

On examination of impression cytology of chalazia contents by light microscope, two broad patterns of granulomatous inflammation with overlapping features were identified; 48 cases out of 60 cases (80.0%) were recognized as mixed cell granuloma (MCG), while 12 cases (20.0%) were recognized as suppurating granuloma (SG). In the first pattern (MCG), the granulomas were epithelioid (Figure 1). There were foreign-body giant cells and granulation tissue in the form of fibroblasts and endothelial cell proliferation. The accompanying inflammatory cells were neutrophils, lymphocytes, plasma cells and macrophages. The giant cells had foamy pink cytoplasm with nuclear debris. These were designated as mixed-cell granuloma (MCG). In the second pattern (SG), the granulomas were epithelioid or histiocytic (Figure 2). There were numerous neutrophils in a proteinaceous background at the spots where the neutrophils aggregated to form microabscesses (Figure 3). Smears showing these features were designated as suppurative granuloma

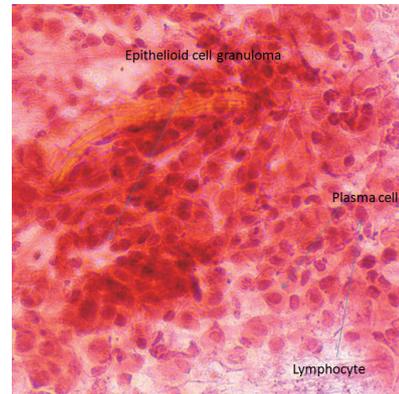


Figure 1. Mixed cell granuloma showing epithelioid cell granuloma, plasma cells and lymphocytes.

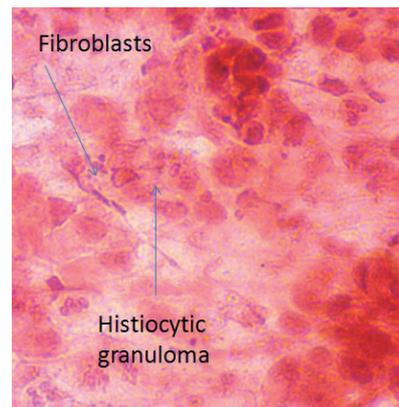


Figure 2. Histiocytic granuloma and fibroblasts

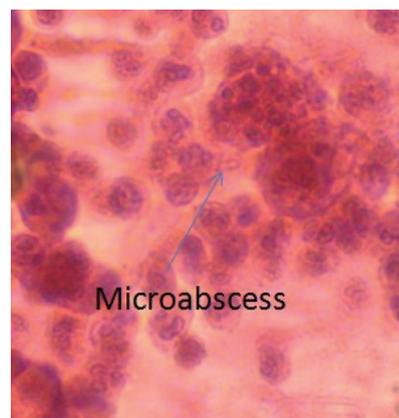


Figure 3. Aggregate of neutrophils resembling microabscess

(SG). What differentiate the SG from MCG are the relative paucity of lymphocytes, plasma cells, isolated macrophages, foreign body giant cells, and granulation tissue with free fat spaces.

The distribution of the inflammatory cells and the percentages of the inflammatory cells; neutrophils (Table 1), macrophages (Table 2), plasma cells (Table 3) and lymphocytes (Table 4) in the two patterns of granulomatous inflammation has been shown. The relative proportions of inflammatory cells were

quantified on a scale of three (few 1+, many 2+ and numerous 3+). Statistical analysis showed a P value of (0.044) for plasma cells and P value of (0.045) for lymphocytes distribution in SG and MCG patterns. On

comparing the cells presence in the two patterns we have noticed the paucity of plasma cells and lymphocytes in suppurative granuloma (Tables3 &4).minimal number of gram positive cocci<sup>[2-5]</sup> are found in 3 cases.

		SG N=12	MCG N=38	Total N=50	P value
Neutrophils	1+	6 (50.0%)	18 (47.4%)	24 (48.0%)	0.195
	2+	0 (0.0%)	12 (31.6%)	12 (24.0%)	
	3+	6 (50.0%)	8 (21.1%)	14 (28.0%)	

**Table 1: Percentage of neutrophils in the patterns of granulomatous inflammation.**  
MCG; Mixed-cell granuloma, SG; suppurating granuloma, N; Number.

		SG N=6	MCG N=40	Total N=46	P value
Macrophages	1+	4 (66.7%)	22 (55.0%)	26 (56.5%)	0.777
	2+	2 (33.3%)	12 (30.0%)	14 (30.4%)	
	3+	0 (0.0%)	6 (15.0%)	6 (13.0%)	

**Table 2 : Percentage of macrophages in the patterns of granulomatous inflammation.**  
MCG; Mixed-cell granuloma, SG; suppurating granuloma, N; Number.

		SG N=6	MCG N=38	Total N=44	P value
Plasma cells	Paucity	2 (33.3%)	0 (0.0%)	2 (4.5%)	0.044
	1+	4 (66.7%)	18 (47.4%)	22 (50.0%)	
	2+	0 (0.0%)	16 (42.1%)	16 (36.4%)	
	3+	0 (0.0%)	4 (10.5%)	4 (9.1%)	

**Table 3: Percentage of plasma cells in the patterns of granulomatous inflammation.**  
MCG; Mixed-cell granuloma, SG; suppurating granuloma, N; Number.

		SG N=4	MCG N=46	Total N=50	P value
Lymphocytes	Paucity	2 (50.0%)	0 (0.0%)	2 (4%)	0.045
	1+	2 (50.0%)	24 (52.2%)	26 (52%)	
	2+	0 (0.0%)	12 (26.1%)	12 (24%)	
	3+	0 (0.0%)	10 (21.7%)	10 (20%)	

**Table 4: Percentage of lymphocytes in the patterns of granulomatous inflammation**  
MCG; Mixed-cell granuloma, SG; suppurating granuloma, N; Number.

## 5. Discussion

Impression cytology has been widely used for the diagnosis of a large variety of inflammatory conditions of the eyelid. In this study chalazia contents had been subjected to impression cytology. Common to most impressions was the presence of granulomas comprising of cohesive aggregates of epithelioid cells

or histiocytes.To the best of our knowledge, this study is the first to subject the contents of the chalazia to impression cytology.

The two patterns of inflammation seen on cytological smears reflect the evolution and clinical spectrum of the life of the lesion, appearing to extend from the early suppurating granuloma, progressively evolving into a chronic mixed granulomatous inflammation, and

finally healing with the formation of granulation tissue and fibrosis.

Since the evolution of the lesion represents a continuous spectrum, the classification of the impressions into these two patterns is somewhat arbitrary and depends on the relative proportions of the various cell types seen in the impressions. Although there was no difficulty in classifying our material into these two categories, it is possible that the distinction may be problematic at times. Evolution of disease is a dynamic process; attempts to classify morphologic patterns represent fixed points within this process and must be understood in this context<sup>[8]</sup>.

Our study agreed with the one performed by Dhaliwalet al<sup>[8]</sup>, in the presence of the two patterns. It was a cross-sectional study in which 16 cases of chalazia were subjected to fine needle aspiration biopsy and cytology (FNAC), but a difference was noticed in the percentage of existence of every pattern as MCG and SG represent in our study 80%, 20% respectively while in Dhaliwal 56.25%, 43.75%. This difference may be due to low number of cases and mean age 39.12±11.42 in Dhaliwal. In the early literature, reviewed by Dvorak-Theobald<sup>[9]</sup>, the chalazion was thought to have always an infectious origin, and this is not in agreement with our study in which we found minimal signs of infection. This also agrees with the literature reviewed by Schall<sup>[10]</sup> who had earlier observed that infection was not always involved. He thought that an obstruction of the Meibomian gland first occurred. Then, perhaps by rupture of the blocked gland duct, the contents leaked into the surrounding tissue, causing an inflammatory response.

Impression cytology combined with histochemistry and immunocytochemistry stainings should be mandatory in the case of patients with atypical symptoms, recurrence, older age, and lesions that are increasing in size, the excised chalazion should be sent for histopathological examination to exclude malignancy.

## 6. Conclusion

Impression cytology is a rapid, safe, and reliable means of documenting a tissue diagnosis of chalazion

and excluding malignancy, allowing treatment to be initiated with confidence.

## Acknowledgement

None.

## Competing financial interests

The authors declare no competing financial interests.

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