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HEMATOLOGICAL AND HISTOPATHOLOGICAL STUDY OF CUTANEOUS LEISHMANIASIS (WET TYPE) IN IRAQ

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ABSTRACT: **Background**: Cutaneous leishmaniasis (CL) is an endemic disease in Iraq and it is become epidemic in Nassiriyah city during the period of 2015-2016. The current study was aimed to explain the blood group and histopathological changes associated with CL or Baghdad boil.

Methods: Blood and several biopsies from skin were obtained by dermatologist under sterilizing condition and at Al-Hussein teaching hospital in Nassiriyah city, south of Iraq, where the patients have entered for examination and treatment, then after, presented to tissue processing by known technique.
Results: The results explain the following changing for two biopsies including: first biopsy (A): granuloma like associated with several layers of vaculated cells filled with parasite around it (a1)**,** dermal areas of chronic granulomatous inflammation mainly formed by vaculated macrophages filled with parasites (a2)**,** epidermis with keratinization and vaculated prickle cells undered it vaculated macrophages with parasites (a3)**,** small granuloma and area of granulamatous inflammatory reaction mostly formed by vaculated macrophages with parasites (a4), few lymphocytes present between the macrophages (a5)**,** nest like of vaculated macrophages associated with dermal infiltration of lymphocytes (a6), vaculated macrophages filled with parasites forming small granuloma (a7)**,** as well as granuloma with vaculated macrophages and multinucleated giant cells with parasites (a8)**.** Second biopsy (B): marked epidermal hyperplasia with hyperkeratosis (b1), small granuloma in the dermis consist of macrophages and fibroblast at the periphery (b2), marked hyperplasia of epidermis , hyperkeratosis and scab formation (b3), marked hyperplasia of epidermis , hyperkeratosis and scab formation , keratin nest in the dermis (b4), two small granulomas of macrophages some with vaculation in the dermis (b5), sever dermal hyperplasia and foliculitis also hyperkeratosis and scab formation (b6), congestion and chronic inflammatory cells with fibrosis in the dermis (b7), and small granuloma in the dermis consisting of macrophages some of with vaculation (b8).
Conclusion: All blood group was target by sand fly and it is not differentiated by the parasite. The number of parasite or amastigote stage was decreased with the increasing of granulation.

KEYWORDS: Cutaneous leishmaniasis , Baghdad boil , Skin , pathology, Iraq

I. INTRODUCTION

Leishmania tropica parasite infects the human body causing leishmaniasis which is highly common parasite return to haemoflagellata and cause some healthy problems in Iraq and other Middle East area [1-3] reporting or estimating 12 million of cases, there are 1.5 million cases of infection or leishmaniasis yearly and 90% of which reported in Iran, Iraq, Syria, Saudi Arabia and Brazil (4). In the urban area *L. tropica* is common while *L. major* is common in rural region (3). Leishmaniasis in Iraq present as an anthroponotic caused by *L. tropica* or it is present as a zoonotic caused by *L. major*, both of the anthroponotic and zoonotic are reported In Iraq (5).

The prevalence of leishmaniasis in Iraq is high and ranged between 2.3-45.5\100000(5) since the infection remains unstable from the epidemiological view. Human when gets the infection with cutaneous leishmaniasis is characterized by presence of single or multiple sore with or in different size on his skin, sores can be changed with a time in size, morphology or its appearance. Clinically sores or lesion can be painful or not (6).

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The current study was designed to explain the histopathological effect for cutaneous leishmaniasis on skin structure and the effect of this changes on parasite viability, further, the relationship between the infection and blood group of patients where also target by this study.

II. MATERIALS AND METHODS

Ethical statement

All skin biopsies were obtained by dermatologist under sterilized condition from patients whom attending to the hospital for detection the disease.

Patients

Patients suffering from presence of the sores or boils in different size, number, and location from body, were target by this study to explain the histopathological changes coming from its (Fig 1).

Blood samples

Blood were collected from 3400 of cases with Baghdad boil by capillary tube to determine the blood group.

Collected biopsy from skin

Two biopsy were collected from two different patients with cutaneous leishmaniasis by the dermatologist and directly placed in 10% of formalin to prepare it for tissue processing. The two biopsy were mention as first and second biopsy in results below.

Tissue processing

Tissues biopsy were sectioned based on Humanson (1972), where the skin tissues were taken in sterilized condition and bring to 10% formalin for discovering the histopathological changing produced form disease, then after the samples were dehydrated by ethanol alcohol with series of its dilution. xylol were used then to remove alcohol from tissue and embedded in paraffin wax to make blocked with 3-5 mm thickness for getting sections by microtome system. glass slides were used finally to carry the tissue section and deparaffinised with xylol, rehydrated by alcohol. hematoxyline and eosin were used for slide staining at end and examined at microscope at different power.

Statistical analysis

Data analysis was carried out using SPSS 17.0 statistical package (SPSS Inc., Chicago, IL, USA). Chi-square test was used for data analysis. $P<0.05$ was considered statistically significant

III. RESULTS

Hematological study

The relationship between the infection with cutaneous leishmaniasis and blood groups of patients were checked and illustrated in the Table (1). The results are revealed that patients with B group were highly infected with cutaneous leishmaniasis compared with other when reported 32.35% followed by patients with A blood group while the infection is low among patients with O blood group where that 0-2.94% and explaining a significant difference were present when study the relationship between the infection and blood groups of patients.

Pathological study

The following histopathological changing in the first biopsy (Figs 2 to 9) as well as second biopsy (Figs 10, and 11) has associated with Baghdad boil, the clinical infection with CL caused by the *L. tropica*.

The results explain the following changing for two biopsies including: first biopsy: granuloma like associated with several layers of vaculated cells filled with parasite around it (Fig. 2), dermal areas of chronic granulomatous inflammation mainly formed by vaculated macrophages filled with parasites (Fig.3), epidermis with keratinization and vaculated prickle cells undered it vaculated macrophages with parasites (Fig.4), small granuloma and area of granulamatous inflammatory reaction mostly formed by vaculated macrophages with parasites (Fig5), few lymphocytes present between the macrophages (Fig.6), nest like of vaculated macrophages associated with dermal infiltration of lymphocytes (Fig.7), vaculated macrophages filled with parasites forming small granuloma (Fig.8), as well as granuloma with vaculated macrophages and multinucleated giant cells with parasites (Fig.9). Second biopsy: marked epidermal hyperplasia with hyperkeratosis (Fig.10a), small granuloma in the dermis consist of macrophages and fibroblast at the periphery (Fig.10b), marked hyperplasia of epidermis , hyperkeratosis and scab formation (Fig.10c), marked hyperplasia of epidermis , hyperkeratosis and scab formation , keratin nest in the dermis (Fig.10d), two small granulomas

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of macrophages some with vaculation in the dermis (Fig.11a), sever dermal hyperplasia and foliculitis also hyperkeratosis and scab formation (Fig.11b), congestion and chronic inflammatory cells with fibrosis in the dermis (Fig.11c), and small granuloma in the dermis consisting of macrophages some of with vaculation (Fig.11d).

IV. DISCUSSION

The body of human is covered by the skin and it is contacted with the out environment. The importance of skin is by their immunological role as well as its activity in protection against the infection.

Pathologically, when the skin harbor the microorganism like *L. tropica* causing of cutaneous leishmaniasis lead to appearance a different clinical patterns, like an erythemic lesion with amastigote or LD bodies which is sometimes diagnosed as malignant remaining untreated for years. This clinical pictures of disease are depending on the stage and type of disease like the appearance of ulcers at the site where healing occur and depends on the immunity of the host since it may persist for months or years (1, 2).

Greenblatt *et al*. (1981) hypothesized that the leishmanial parasite might utilize asystem of camouflage of mimicry of host blood group antigens to invade host defense mechanisms in human (15). Our results showed that the blood group was not a risk factor in the occurrence of (CL). The findings failed to support the hypothesis of Greenblatt *et al*. (1981). So, we conclude that ABO-Rh blood groups are not associated with the occurrence of CL in Syrian patients. The conclusion of our study is similar to that of (16) which failed to support the hypothesis of camouflage, using blood group antigens.

Cutaneous leishmaniasis has a different picture where these different based on the stage of disease, clinical type of disease and the immune system of host has role also in it. The progression of the lesion evolution was from a papule and nodule in to soft boggy, crusted plaque (12). The histological spectrum in CL has wide range with a great variation in morphology. The appearances may range from predominance of leishmanial granuloma with macrophages showing epithelioid. Granuloma formation results from macrophages activation into epithelioid cells, considering clinicopathological relationship (11). In about 80 % of patients, epithelioid cell granulomas with giant cells and a rim of lymphocytes are present. Amastigotes stage is a diagnostic so it is essential to present during searching or examining (13,14).

Histopathological changing of skin during CL explain the granulomatous reaction at site of infection together with presence of amastigote stage or LD bodies in the lesion based on the current study. Proliferation of amastigotes in cells of the mononuclear phagocytic system is the characteristic features of this disease pathology which is responsible for granulomatous inflammatory response. The evolution of these granuloma included three steps, in which the young mononuclear phagocytes was infiltrate at first followed by maturation and aggregation of these cells as an unorganized granuloma and finally an epithelioid or organized granuloma was complete from the maturation of these cells (7,8,9.10).

CL is characterized by lesion filled with LD bodies or amastigotes stages which have oval to rounded shape contain nucleus with anterior kinetoplast as seen or included in its morphology where that amastigotes are proliferated in macrophages in large number and move with the help of macrophages (5,6,8).

V. CONCLUSION

All blood group was target by sand fly and it is not differentiated by the parasite. The number of parasite or amastigote stage was decreased with the increasing of granulation.

Conflict of interest

The authors declare that no conflict of interest in this study.

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Table 1. Relationship between the infection with Baghdad boil and blood groups

No.	Blood groups	Number of patient (%)
	A^+	**630 (18.52)**
	A^-	**220 (6.47)**
	B^+	**1100 (32.35)***
	B^-	**440 (12.94)**
	AB^+	**430 (12.64)**
	AB^-	**250 (7.35)**
	O^+	**308 (2.94)**
	O^-	**22 (0.64)**

*Significant differences P< 0.01

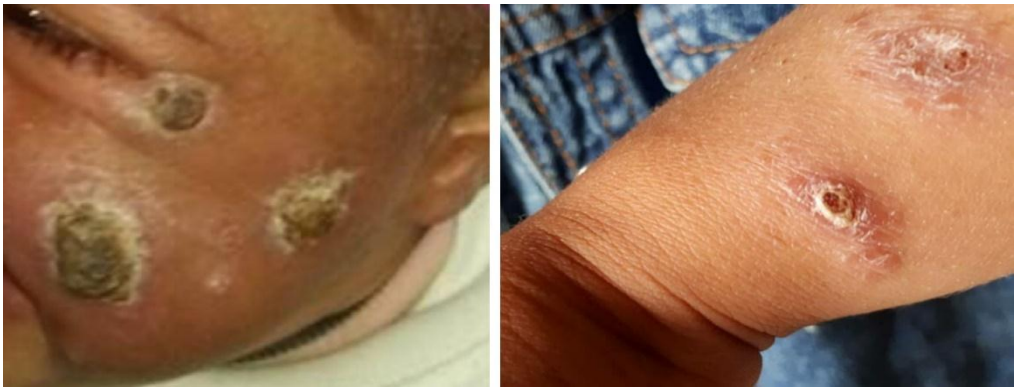

Fig.1. Patients with cutaneous leishmanisis in Iraq

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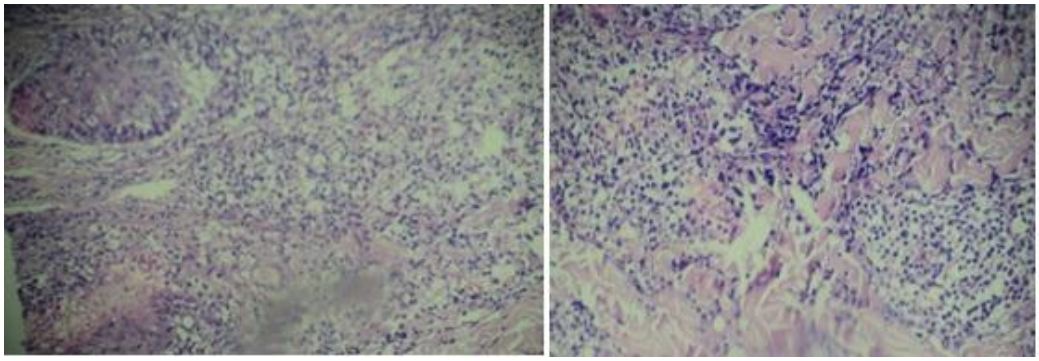

Fig.2. Granuloma like associated with several layers of vaculated cells filled with parasite around it (10X).

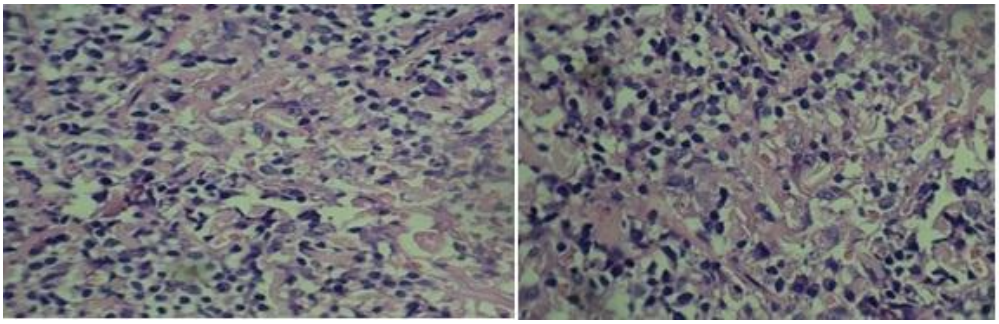

Fig.3. Dermal areas of chronic granulomatous inflammation mainly formed by vaculated macrophages filled with parasites (40X).

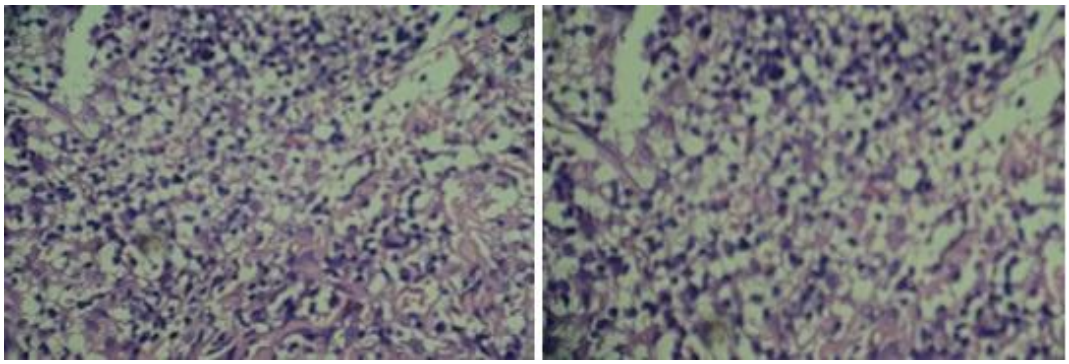

Fig.4. In adjacent another area formed by vaculated macrophages filled with parasites (40X)

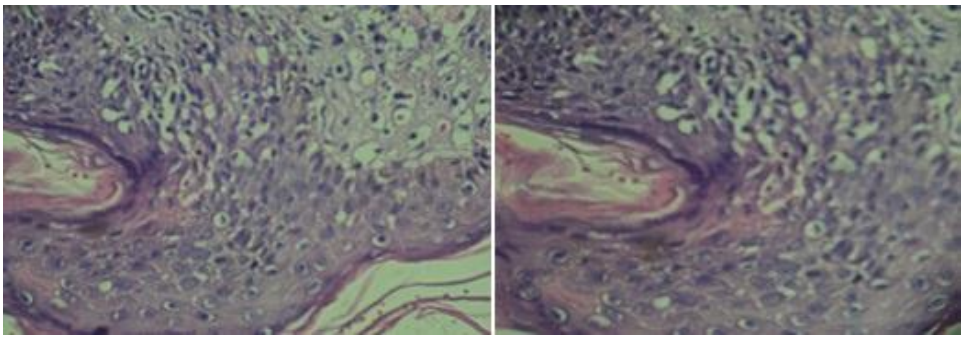

Fig.5. Epidermis with keratinization and vaculated prickle cells undered it vaculated macrophages with parasites (40X).

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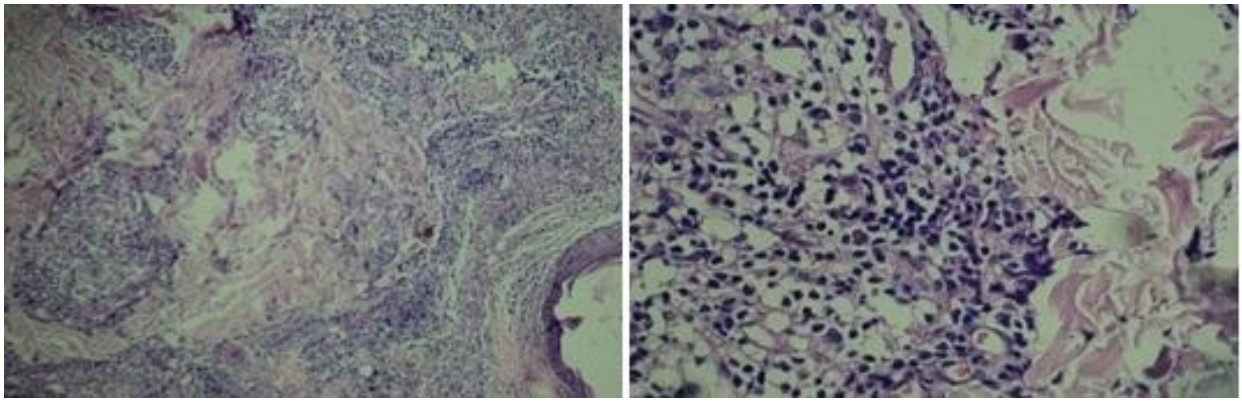

Fig.6. Small granuloma and area of granulamatous inflammatory reaction mostly formed by vaculated macrophages with parasites , few lymphocytes present between the macrophages (10X , 40X)

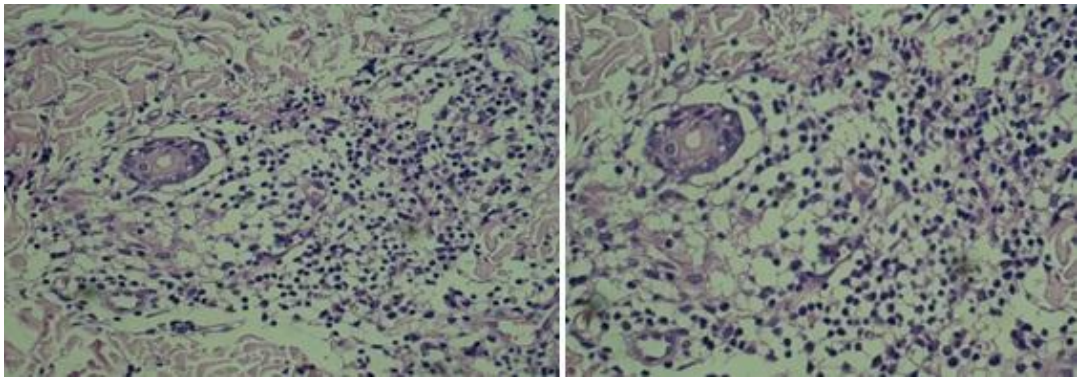

Fig.7. Nest like of vaculated macrophages associated with dermal infiltration of lymphocytes (40x).

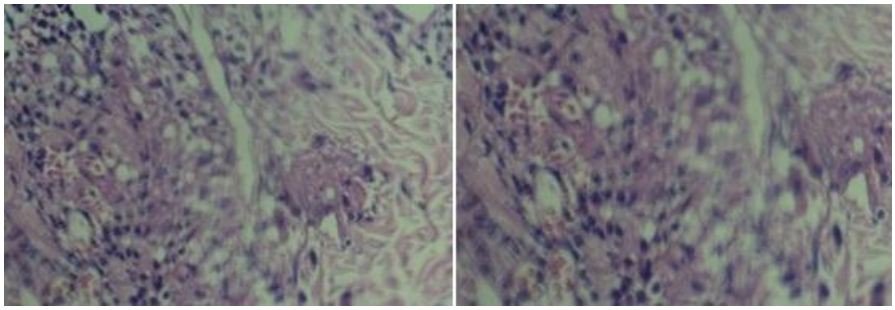

Fig.8. Vaculated macrophages filled with parasites forming small granuloma (40X).

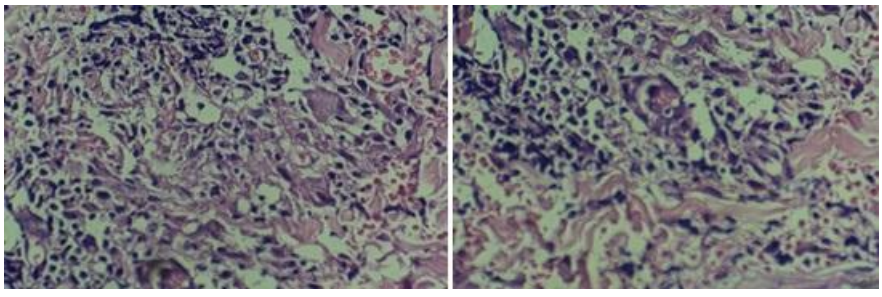

Fig.9. Granuloma with vaculated macrophages and multinucleated giant cells with parasites (40X) **.**

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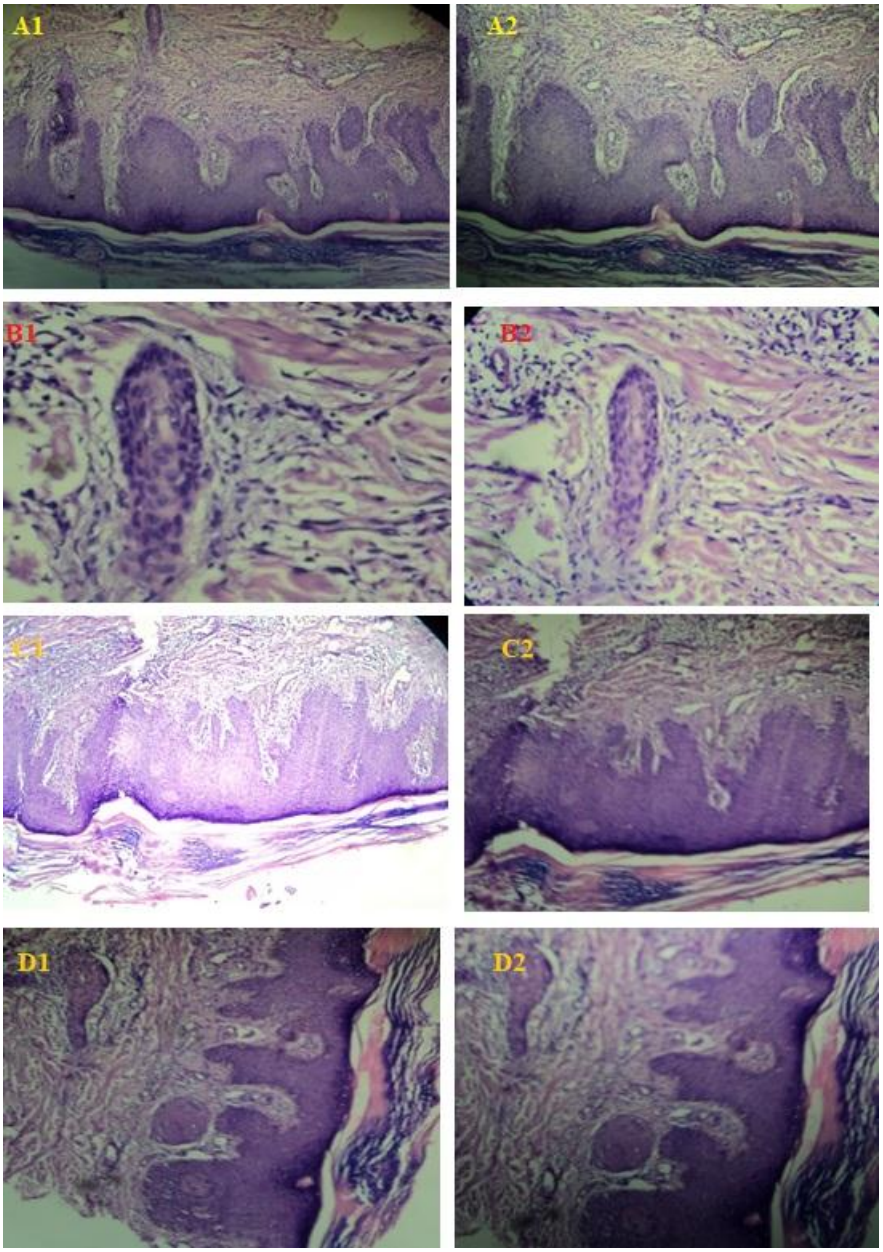

Fig 10. A: Marked epidermal hyperplasia with hyperkeratosis (10X); **B:** Small granuloma in the dermis consist of macrophages and fibroblast at the periphery (40X); **C:** Marked hyperplasia of epidermis , hyperkeratosis and scab formation (10X); D; Marked hyperplasia of epidermis , hyperkeratosis and scab formation , keratin nest in the dermis (10X).

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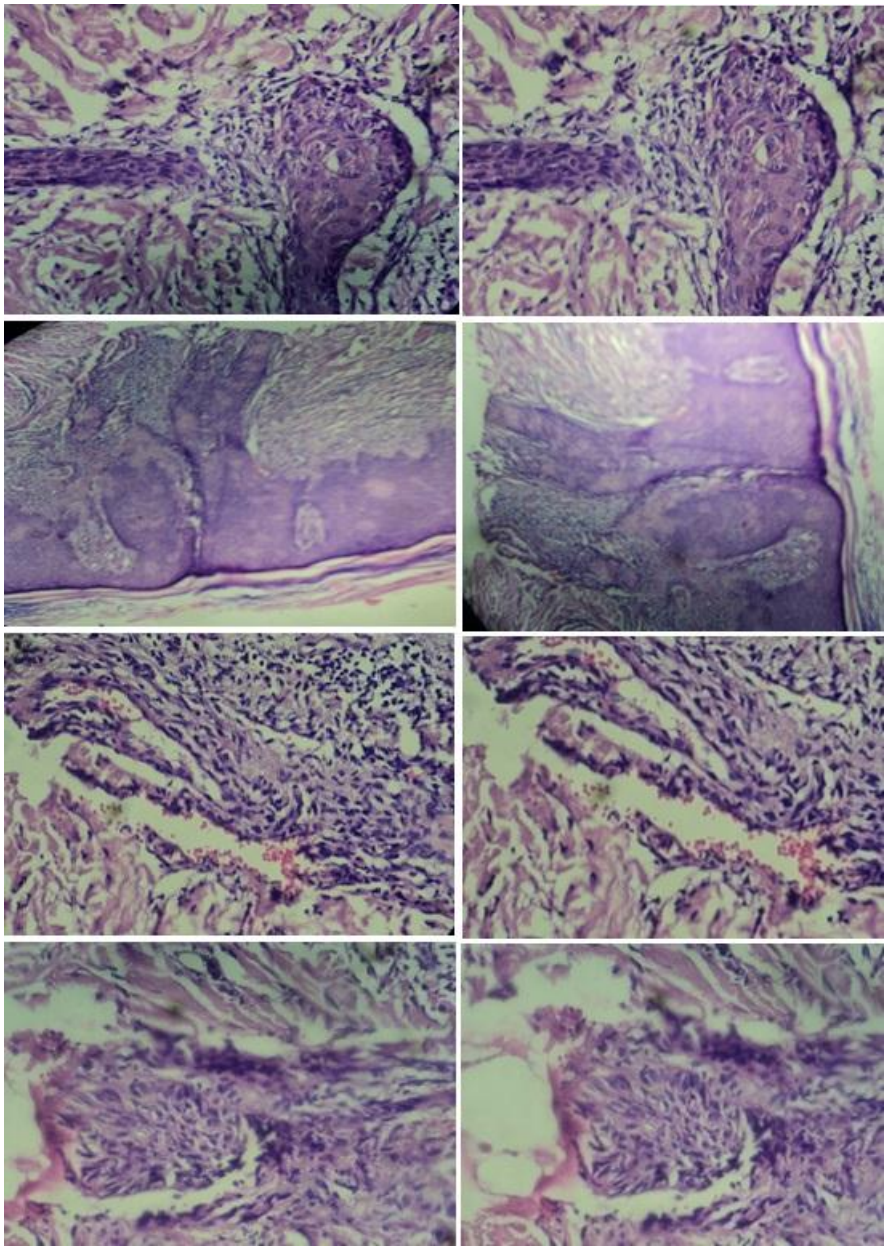

Fig 11. **A:**Two small granulomas of macrophages some with vaculation in the dermis (40X); B: Sever dermal hyperplasia and foliculitis also hyperkeratosis and scab formation (10X)**; C:** Congestion and chronic inflammatory cells with fibrosis in the dermis (40X); D: Small granuloma in the dermis consisting of macrophages some of with vaculation (40X)