RESULTS

I-Patient's data:

This study included 29 patients categorized into three groups. Each patient was subjected to full history taking and proper examination. Investigation and staging were done for MF patients. The raw data of the patients are presented in tables (1,2,3).

Group I: (Hypopgigmented MF): (fig. 8)

This group included nine patients with hypopigmented mycosis fungoides (proved by histological examination). Patient's ages ranged from 11- 52y (mean 22.6± 12.8). five of our patients were females (55%) and four were males (45%). As regards the skin type, three patients were of skin type III (33%), five of skin type IV (55%) and one of skin type V (11%).

All of our patients had hypopigmented mycosis fungoides stage Ia or Ib with variable extent of lesions that ranged from 10%-90% of body surface area. No precipitating factors were found among the patients group. Disease duration varied from four months to 105 months. Two patients (22%) had variable associated diseases; one with hypertension and one with rheumatic fever. Positive family history was encountered only in one patient (11%).

Group II (Vitiligo) : (fig. 9)

This group included ten patients with vitiligo. Patient's ages ranged from 9 - 56y (mean 27.4 ± 16.1) . Seven patients were females (70%) and three were males (30%). As regards the skin type, one patient was of skin type III (10%), eight of skin type IV (80%) and one patient was of skin type V (10%).

All the patients had vitiligo vulgaris with variable extent of lesions that ranged from 5%-60% of body surface area. Three patients (30%) had positive precipitating factors in the form of psychic stress. Disease duration varied from 1.5 month to 120 months. Three patient (30%) had variable associated diseases; two with diabetes mellitus, and one with rheumatic heart disease. Positive family history was encountered in two patients (20%).

Group III (hypopigmented T.V): (fig.10)

This group included ten patients with hypopigmented tinea versicolour. Patient's ages ranged from 15.5 – 52y (mean 26.9 ±11.9). five patients were females (50%) and five were males (50%). As regards the skin types, two patients were of skin type III (20%), six of skin type IV (60%) and two of skin type V (20%). The patients had hypopigmented tinea versicolour with variable extent of lesions that ranged from 5%-20%. Disease duration varied from one week to one month. No associated diseases were found among the patients group, a part from juvenile diabetes in one patient (10%).

Controls data:

Ten healthy volunteers served as control. Their ages ranged from 21 -58y (mean 22.2 ± 10.9). They were 7 females (70%) and 3 males (30%). As regards the skin type, 3 of them were of skin type III (30%), 5 of skin type IV (50%) and 2 of skin type V (20%).

II- Results of histopathological examination using H & E staining:-

In group I (hypopigmented MF), there was diffuse lymphocytic infiltrate within the papillary dermis with prominent atypical lymphocytes within the epidermis (epidermotropism), showing aggregations in some areas (pautrier microabsceses), focal parakeratosis without spongiosis. In addition, hypopigmented lesions may show hydropic degeneration of basal cell layer, partial loss of pigment, preservation of some melanocytes and wiry fibrosis of the papillary dermal collagen (fig. 11,12)

In group II (vitiligo), the most prominent feature is the alteration of melanocytes at the dermoepidermal junction, lesions of vitiligo are totally devoid of melanocytes, lesions show superficial perivascular and occasionally lichenoid mononuclear cell infiltrate (fig. 13,14)

In group III (hypopigmented tinea versicolour), there was slight hyperkeratosis, spongiosis in the epidermis, perivascular lymphocytic infiltrate, lower epidermis showing decrease in melanin within melanocytes and keratinocytes (hypopigmentation in basal cell layer) (fig. 15,16)

III- Results of immunohistochemical staining of tumour necrosis factor-α: (Table 4)

A) There was a highly significant increase ($P<0.001$) in the TNF-α expression, density and staining intensity in all hypopigmented groups as compared with the normal controls. (Table 4)

Group I : (Hypopigmented MF):

This group included nine patients with hypopigmented mycosis fungoides (proved by histological examination using H & E and by immunohistochemical staining of CD3 for T- lymphocytes (fig. 17). TNF- α was expressed in keratinocytes, in mosaic distribution (whole the epidermis in areas, basal and suprabasal in other areas)

Staining density of TNF-α was diffuse and of strong intensity in seven patients (77.8%), diffuse density and moderate intensity of staining was observed in one patient (11.1%), one patient was negative for staining, most propably due to technical error or delayed fixation (fig. 20,21,22).

Group II: (Vitiligo):

This group included ten patients with vitiligo. TNF- α was expressed in keratinocytes in uniform distribution, mainly basal and suprabasal . Staining density of TNF-α was diffuse and of strong intensity in eight patients (80.0%), diffuse density and moderate staining intensity was observed in two patients (20.0%) (fig. 23,24).

Group III: (Hypopigmented T.V):

This group included ten patients with hypopigmented T.V. TNF- α was expressed in keratinocytes, mainly basal and suprabasal. Staining density of TNF-α was diffuse and of strong intensity in two patients (20.0%), diffuse density and moderate staining intensity was observed in eight patients (80.0%) (fig. 25,26).

Group IV: normal Control :

Ten healthy volunteers served as control. TNF-α expression was on basal keratinocytes showing focal density and weak staining intensity in almost all specimens (100%) (fig.18,19).

B) Correlation of TNF-α expression with age in various groups:

There was no significant correlation (P>0.05) between TNF-α expression as regard the age in the different groups (Table 5).

C) Correlation of TNF-α expression and duration of the lesion in the three hypopigmented groups of patients:

TNF-α expression was not found to be significantly affected by the duration of the lesions in the three hypopigmented groups (P>0.05). (Tables 6,7,8).

D) Correlation of TNF-α expression and skin type in various groups:

TNF-α expression was not found to be significantly affected by the skin type in various groups (P> 0.05) (Tables 9,10,11).

Analysis of the results

(I) Table 4, shows comparison between TNF-α expression in the lesional skin of different groups of patients and controls.

TNF- α expression, density and intensity	Group I hypopigmented MF (n= 9)		Group II vitiligo (n= 10)		Group III hypopigmented TV (n=10)		Group IV control (n= 10)		Total	
	No	%	No	%	No	%	No	%	No	%
Negative	1	11.1	0	0.00	0	0.00	0	0.00	1	2.6
Focal density, weak intensity, basal expression	0	0.00	0	0.00	0	0.00	10	100.0	10	25.6
Diffuse density, moderate intensity, basal and suprabasal expression	0	0.00	2	20.0	8	80.0	0	0.00	10	25.6
Diffuse density, strong intensity, basal and suprabasal expression	0	0.00	8	80.0	2	20.0	0	0.00	10	25.6
Diffuse density, moderate intensity, mosaic in distribution	1	11.1	0	0.00	0	0.00	0	0.00	1	2.6
Diffuse density, strong intensity, mosaic in distribution	7	77.8	0	0.00	0	0.00	0	0.00	7	17.9
Total	9	100.0	10	100.0	10	100.0	10	100.0	39	100.0

Chisquare = 92.04 P value = < 0.001

There was a highly significant increase ($p<0.001$) in the TNF-α expression, density and staining in all hypopigmented groups as compared with the normal control.

(II) Table 5, shows correlation of TNF-α expression with age in various groups.

TNF- α expression, density and intensity	Group I hypopigmented MF (n= 9)		Group II vitiligo (n= 10)		Group III hypopigmented TV (n=10)		Group IV control (n= 10)	
	No	$\bar{X} \pm SD$	No	$\bar{X} \pm SD$	No	$\bar{X} \pm SD$	No	$\bar{X} \pm SD$
Negative	1	14.0	0	0.00	0	0.00	0	0.00
Focal density, weak intensity, basal expression	0	0.00	0	0.00	0	0.00	10	22.2±10.9
Diffuse density, moderate intensity, basal and suprabasal expression	0	0.00	2	11±12.8	8	25.1±8.5	0	0.00
Diffuse density, strong intensity, basal and suprabasal expression	0	0.00	8	31.5±15.3	2	34±25.5	0	0.00
Diffuse density, moderate intensity, mosaic in distribution	1	20±0	0	0.00	0	0.00	0	0.00
Diffuse density, strong intensity, mosaic in distribution	7	22.9±14.4	0	0.00	0	0.00	0	0.00
Total	9	22.6±12.8	10	27.4±16.1	10	26.9±11.9	10	22.2±10.9
Test of significance	F=1.17 P>0.05		T=1.94 P>0.05		T=0.49 P>0.05			

There was no significant correlation between TNF-α expression as regards age in the different groups (p>0.05).

(III) Correlation of TNF-α expression and duration of the lesion in the three hypopigmented groups of patients (table 6,7,8).

Table (6) Group- I (hypopigmented MF).

Duration of the lesion / TNF-α expression	$\bar{X} \pm SD$
1- Negative staining (n=1)	48±0
2- Diffuse density, moderate intensity, mosaic in distribution (n=1)	24±0
3- Diffuse density, strong intensity, mosaic in distribution (n=7)	38±40.1

F = 1.95 P> 0.05

Table (7) Group II (vitiligo)

Duration of the lesion / TNF-α expression	$\bar{X} \pm SD$
1- Diffuse density, moderate intensity, basal and suprabasal, uniform in distribution (n=2)	3±1.4
3- Diffuse density, strong intensity, basal and suprabasal, uniform in distribution (n=8)	2.9±1.5

T = 0.09 P> 0.05

Table (8) Group III (hypopigmented TV)

Duration of the lesion / TNF-α expression	$\bar{X} \pm SD$
1- Diffuse density, moderate intensity, basal and suprabasal, uniform in distribution (n=8)	0.6±0.3
3- Diffuse density, strong intensity, basal and suprabasal, uniform in distribution (n=2)	0.6±0.3

TNF-α expression was not found to be significantly affected by the duration of the lesions in the three hypopigmented groups (P>0.05).

(IV) Correlation of TNF-α expression and skin types in various groups (table 9,10,11).

Table (9) Group I (hypopigmented MF)

skin type / TNF-α Density& intensity	3		4		5		Total	
	No	%	No	%	No	%	No	%
Diffuse density, moderate intensity, mosaic in distribution	0	0.0	1	20.0	0	0.0	1	12.5
Diffuse density, strong intensity, mosaic in distribution	2	100.0	4	80.0	1	100.0	7	87.5
Total	2	100.0	5	100.0	1	100.0	8	100.0

$\chi^2 = 0.9$ $P > 0.05$

Table (10) Group II (vitiligo).

skin type / TNF-α Density& intensity	3		4		5		Total	
	No	%	No	%	No	%	No	%
Diffuse density, moderate intensity, uniform in distribution	0	0.0	2	25.0	0	0.0	2	20.0
Diffuse density, strong intensity, uniform in distribution	1	100.0	6	75.0	1	100.0	8	80.0
Total	1	100.0	8	100.0	1	100.0	10	100.0

$\chi^2 = 0.63$ $P > 0.05$

Table (11) Group III. (hypopigmented TV).

skin type / TNF-α Density& intensity	3		4		5		Total	
	No	%	No	%	No	%	No	%
Diffuse density, moderate intensity, uniform in distribution	2	100.0	5	83.3	1	50.0	8	80.0
Diffuse density, strong intensity, uniform in distribution	0	0.0	1	16.7	1	50.0	2	20.0
Total	2	100.0	6	100.0	2	100.0	10	100.0

$\chi^2 = 1.67$ $P > 0.05$

TNF-α expression was not found to be significantly affected by the skin type in various groups (P> 0.05)

Fig. (8): Clinical cases of hypopigmented MF showing hypopigmented patches distributed on the trunk, shoulders, proximal extrimities. Slightly scaly in the young case.

Fig. (9): Clinical cases of vitiligo showing milky white macules and patches distributed bilaterally and symmetrically mainly on the extensor surfaces of extremities.

Fig. (10): Clinical cases of hypopigmented TV showing hypopigmented macules scattered on the trunk, sides of the neck, arms, sometimes may coalease to form a larger patch, with slightly scaly surface.

Routine histopathological examination using hematoxylin and eosin:

Fig.(11): A case of hypopigmented mycosis fungoides showing the characteristic features including atypical lymphocytes among the epidermis (epidomotropism) within a spongiotic hallo ,hydropic degeneration of basal cells, partial loss of pigment with preservation of some melanocytes , increased density of dermal lymphocytic infilterate (H&E X20).

Fig. (12): A case of hypopigmented MF showing atypical lymphocytes within the epidermis (epidermotropism), moderate perivascular lymphocytic infiltrate and wiry fibrosis of dermal collagen.(H&E X 10)

Fig. (13): A case of vitiligo showing complete loss of pigmentation , total absence of melanocytes and sparseness of lymphocytes in the dermal papillae (H&E X 20).

Fig. (14): A case of vitiligo showing complete absence of melanocytes with lichenoid mononuclear cellular infilterate (H&E X 10).

Fig. (15): A case of hypopigmented tinea versicolomr showing dense lymphocytic infiltrate, spongiosis in epidermis, lower epidermis showing decrease in melanin within melanocytes and keratinocytes (hypopigmentation in basal cell layer) (H&E X 20).

Fig. (16): A case of hypopigmented tinea versicolour showing dermal lymphocytic infilterate, spongiosis in the epidermis, hypopigmentation in basal cell layer (H&E X 20).

- Immuno histochemical staining using anti- CD3 for T-lymphocytes.

Fig. (17): A case of hypopigmented MF showing immunohistochemical staining using anti – CD3 antibodies to confirm the diagnosis, infilterating lymphocytes are strongly stained as well as lymphocytes in the epidermis . There is diffuse non-spesific stains in the epidermis which is insignificant (IH X 10).

- Immunohistochemical staining of TNF-α using Avidin- Biotin complex

Fig. (18): Immunohistochemical staining of TNF-α in normal control groups showing TNF-α expression on basal keratinocytes, intracytoplasmic brown staining of focal density, weak intensity (IH X 20).

Fig. (19): Immunohistochemical staining of TNF-α in normal control groups showing expression of TNF -α on basal keratinocytes , intracytoplasmic brown staining of focal density, weak intensity (IH X 20).

Fig. (20): A case of hypopigmented MF with immunohistochemical expression of TNF-α on keratinocytes (intracytoplasmic) , showing diffuse density, of mosaic distribution , strong staining intensity (IH X 10).

Fig. (21): A case of hypopigmented MF with immunohistochemical expression of TNF-α on keratinocytes (intracytoplasnic) , showing the mosaic distribution with diffuse density, strong staining intensity (IH X 40).

Fig. (22): Case no. 3 of hypopigmented MF. Showing negative staining of TNF-α (IH x 20).

Fig. (23): A case of vitiligo with immunohistochemical expression of TNF-α on keratinocytes (basal and suprabasal) , showing diffuse density, moderate to strong staining intensity (IH X 20).

Fig. (24): A case of vitiligo with immunohistochemical expression of TNF-α on keratinocytes (basal and suprabasal) , showing diffuse density, moderate to strong staining intensity (IH X 20).

Fig. (25): A case of hypopigmented T.V with immunohistochemical expression of TNF-α on keratinocytes (basal and suprabasal) , showing diffuse density and moderate to strong staining intensity (IH X 20).

Fig.(26): A case of hypopigmented T.V with immunohistochemical expression of TNF-α on keratinocytes (basal and suprabasal) , showing diffuse density and moderate to strong staining intensity (IH X 20).