

Study of Some Biochemical and Hematological Parameters among Cleaning Workers Infected with Microbial Skin Infections

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Abstract:

This study have been carried out on (100) samples (hair, skin, nail and lesions) from cleaners of hospitals, schools and roads suspected of skin infections, 60 male, 40 female and 40 samples 20 male and 20 female from healthy individuals as control. Patients were divided into 3 age groups: G1 (15-25) years, G2 (26-35) years and G3 (36-45) years, in addition to control group (25-35) years.

Samples were taken from (June-September, 2010) at some regions in Baghdad City. Diagnosis by a potassium hydroxide (KOH) microscopy and culture on Saboraud's dextrose agar (SDA) have showed that most common dermatophyte infections were for tinea pedis with prevalence rate of (53%) followed by tinea unguium of (24.6%), tinea versicolor of (12.1%), tinea corporis of (5.3%), tinea capitis of (4.7%). Bacterial infections have been diagnosed by Gram stain film then cultured on blood agar and Api system.

Staphylococcus aureus was responsible for most infections that appeared. Significant differences ($P < 0.05$) have been appeared between some of the study groups comparing with normal values and control group in the results of hematological tests [hemoglobin level, erythrocyte sedimentation rate (E.S.R.)]. Biochemical analyses were performed on serum samples (blood sugar, blood urea, serum creatinine, total bilirubin, serum cholesterol, serum triglycerides).

الخلاصة:

أجريت الدراسة على (100) عينة من الشعر والجلد والأظافر والقرح القيحية، أخذت من عمال النظافة في المستشفيات والمدارس والطرق ممن لديهم أعراض بإصابات جلدية: 60 ذكر و 40 أنثى، فضلا عن 40 عينة سيطرة جمعت من 40 شخص سليم: 20 من الإناث و20 من الذكور في بعض مناطق بغداد للفترة من (حزيران-أيلول، 2010). قسمت العينات إلى ثلاثة فئات عمرية، المجموعة الأولى (15-25) سنة والمجموعة الثانية (26-35) سنة، أما المجموعة الثالثة فتراوحت أعمارهم بين (36-45) سنة بالإضافة إلى مجموعة السيطرة (25-35) سنة. شخّصت الإصابة الفطرية مجهريا بإستعمال هيدروكسيد البوتاسيوم (KOH) ومن ثم زرعت العينات على وسط أكار دكستروز السابروييد (SDA)، بينما شخّصت الإصابة البكتيرية بعمل مسحات مجهرية وكذلك زرع العينات على وسط أكار الدم والتحرري عن إنتاج إنزيم (Coagulase) وإجراء مجموعة إختبارات (Api) الخاصة ببكتريا العنقوديات. بينت النتائج أن أغلب الإصابات الفطرية كانت لـ (tinea pedis) وبمعدل (53%) ومن ثم (tinea unguium) بمعدل (24.6%) وجاءت بعدها الإصابة بـ (tinea versicolor) بمعدل (12.1%) بعدها (tinea corporis) (5.3%) ومن ثم (tinea capitis) بمعدل (4.7%)، أما الإصابات البكتيرية فكانت أغلبها بسبب بكتريا *Staphylococcus aureus* وظهرت فروقات معنوية ($P < 0.05$) بين نتائج مجاميع الدراسة مقارنة بالمعدلات الطبيعية ونتائج مجموعة السيطرة في إختبارات الدم (مستوى الهيموغلوبين ومعدل ترسيب الكريات الحمر). كما تم التحري عن بعض التغيرات الكيميائية التي قد تحدث عند المصابين مثل مستوى السكر واليوريا والكرياتينين والكوليسترول والدهون والبيليروبين في الدم.

Introduction:

The skin covers and protects the body, is the body's first line of nonspecific defense against pathogens. As a physical barrier, it is almost impossible for pathogens to penetrate the intact skin. Microbes can enter through skin breaks

that are not readily apparent and the larval forms of a few parasites can penetrate intact skin. The skin is an inhospitable place for most microorganisms because the secretions of the skin are acidic and most of the skin contains little moisture. Some parts of the body such as the armpit and the

area between the legs have enough moisture, though to support relatively large bacterial populations. Drier regions, such as the scale, support rather small numbers of microorganism^[1].

Microbial skin infections are communicable diseases acquired from infected animals or from fomites. In people with severe immune problems, these infections can become more serious and invasive, they may range from mild and superficial, almost subclinical, to a few areas of scaling to a highly inflammatory reaction with extensive areas of scaring and lesions^[1, 2]. Two genera of bacteria, *Staphylococcus* and *Streptococcus*, are frequent causes of skin related diseases. Superficial Staphylococcal and Streptococcal infections of the skin are very common. The bacteria frequently come into contact with the skin and have adapted fairly well to the physiological conditions there. Both genera also produce invasive enzymes and damaging toxins.

Dermatophytes, a group of keratinophilic fungi thriving on the keratin substrate, are the etiological agents responsible of causing cutaneous infections. Infections pertaining to mankind particularly those affecting the keratinized tissues are of serious concerns worldwide and are increasing on a global scale. Dermatomyces are infections of the skin, hair and nail caused as a result of colonization of the keratinized layers of the body. This colonization is brought about by the organisms belonging to the three genera: Trichophyton, Microsporum and Epidermophyton^[3].

The infections caused by dermatophytes are commonly referred to as tinea or ring-worm infections due to the characteristics ringed lesions. Based on the site of infection, the tinea infections are divided into: tinea capitis (scalp), tinea corporis (non-hairy, glabrous region of the body), tinea pedis (Athletes foot, foot), tinea unguium (Onchomycosis, nail), tinea versicolor (back, chest and neck)^[4, 5].

Nutritional requirements, growth in special media, *in vitro* perforation, mating studies are procedures used to identify atypical isolates. Serological approaches have revealed difficulties for fungi not bacteria. Many kinds of molecular biologic techniques have been made available for clinical diagnosis. Recently, almost all of these techniques involve the polymerase chain reaction (PCR)^[2, 6].

Objectives: The aim of this study is to isolate and diagnose the most common pathogenic dermatophytes and bacteria that infect cleaners and study the effect of these infections on the values of certain hematological factors and some biochemical tests.

Materials and Methods:

Materials:

1- Skin Materials:

A total number of 100 (60 males and 40 females) clinical samples collected from cleaners have been chosen from large specimen, who have symptoms of dermatophytic infection, those patients work in hospitals, schools and roads, as well as 40 samples (20 male and 20 female) have been used as control, from (June-September 2010).

Specimen Collection:

Nail Samples:

Should include clippings from any discoloured, dystrophic or brittle part of the nail and scraped material from underneath the nail preferably from its edge.

Scales from Skin Lesions:

Using a blunt scalpel, the skin lesion is scraped outwards from the edges of the lesions, where most viable fungus is likely to be present. Specimens of scalp should include hair stabs, the contents of plugged follicles and skin scales. Infected hairs are plucked from the scalp using forceps.

Skin Samples for Bacterial infection:

1- Clean the infected site with sterile saline, wipe gently with alcohol and allow it to air-dry.

- 2 - Aspirate a fluid sample from fresh intact vesicles with a 25- gauge needle and transfer the specimen to the transport medium by ejecting it from the syringe.
- 3 - If the fluid cannot be aspirated, open the vesicles and use cotton to swab the base of the lesion to collect infected cells, place the swab directly into transport medium and culture on blood agar.
- 4 - To make smears for Gram stain, scrape the lesion by glass slide and spread scraped material in a thin layer on slide.

2-Venous blood samples were collected using EDTA tubes for hematological investigations: Erythrocyte Sedimentation Rate, hemoglobin level, platelets and white blood cells count.

3- Serum samples have been used to evaluate some biochemical tests: blood sugar, blood urea, total bilirubin, serum creatinine, cholesterol and triglycerides.

Methods:

Diagnosis of Dermatophytosis:

The differential diagnosis of dermatophytes infection includes the site of infection, type of the clinical symptoms were present by the fungus, for example texture and color of the inflammatory lesions followed by microscopic examination of skin scrapings and hair clippings (in case of tinea capitis) and by fungal cultures as follow ^[7].

1-Direct microscopy:

A potassium hydroxide KOH (10-20%) mount of the keratinized specimen show filamentous branching hyphae arthrospore can be seen either inside or outside the hair (endothrix or ectothrix infection) depending on the causative agent (species) ^[7].

2-Culture of dermatophytes Species:

It is necessary for species identification. The specimen is inoculated into Sabouraud's dextrose agar containing chloramphenicol and cycloheximide to inhibit bacterial growth and incubated aerobically at (25-30) °C up to three weeks. If *Malassezia spp.* specimen of the culture was stained using gram technique and observed under light microscope with a 100 fold magnification.

If dermatophytes were identified by colony morphology, two subcultures were prepared using Phenol Red Agar and Potato Dextrose Agar. Specimens of cultures positive for dermatophytes were stained with cotton blue and observed under a 40 fold magnification searching for micro conidia and macro conidia ^[7].

Diagnosis of Bacterial Species:

Swabs from lesions, pus, exudates were collected. Gram positive films were made to observe bacteria especially typical forms of G⁺ve cocci then cultured on blood agar followed by coagulase test, Api Staph. and mast Staph. ^[8].

Biochemical analysis:

Serum Samples were analyzed for general biochemical tests and measured with -Automated Biochemical Analyzer-type Optima 600. Normal values ranges of each test are included at table 5, ^[7, 8].

Statistical Analysis:

The statistical computer software package MINITAB was used to analyze the data. All data was expressed as mean ± Stander deviation (SD). Repeated measurement analysis of variance ANOVA was used to study changes between the study groups and the control. A probability of less than 0.05 was accepted significant.

Results:

Table -1: Frequency distribution of age and gender among study groups

Sex	Age Group: Number (Percentage)			Total
	G1 (15-25)year	G2 (26-35)year	G3 (36-45)year	
Male	14(14%)	27 (27%)	19 (19%)	60 (60%)
Female	5 (5%)	9 (9%)	26 (26%)	40 (40%)
Total	19 (19%)	36 (36%)	45 (45%)	100 (100%)

A total of 100 skin samples (60 male and 40 female) were distributed on three age groups: group I (15-25) year, group II (26-35) year and group III (36-45) year. In addition to control group (20 male and 20 female) with ages ranged between (25-35) years, as shown at table-1.



Figure -1: Direct microscopy of Pityriasis versicolor: Few spores and short septate yphae penetrating epidermal cells.

Table -2: Prevalence Rates of Superficial Fungal Infections According to Age and Sex

Diagnosis sex	G1 (15-25)y		G2 (26-35)y		G3 (36-45)y		Total	
	M 14	F 5	M 27	F 9	M 19	F 26	M 60	F 40
Tinea versicolor	2	1	5	2	5	3	12	6
Total	3 (16.6%)		7 (38.8%)		8 (44.4%)		18 (12.1%)	
Tinea capitis	3	0	2	1	0	1	5	2
Total	3 (42.9%)		3 (42.9%)		1 (14.3%)		7 (4.7%)	
Tinea corporis	1	0	3	1	2	1	6	2
Total	1 (12.5%)		4 (50%)		3 (37.5%)		8 (5.3%)	
Tinea pedis	11	5	23	7	14	19	48	31
Total	16 (20.2%)		30 (38%)		33 (41.7%)		79 (53%)	
Tinea unguium onchomycosis	1	4	3	9	2	18	6	31
Total	5 (13.5%)		12 (32.4%)		20 (54.1%)		37 (24.6%)	

Table-2 demonstrates the prevalence rate of superficial infections among the three groups. Diagnosis of dermatophytes has been made mainly by (KOH) preparation and culture on SDA and other culture media. Direct microscopy, although false negative up to 50% of cases, is a highly efficient screening technique,

scraping and hairs should be mixed with 10-15% KOH, as shown in figure-1.

The results of bacterial identification were listed in table-3 showed that the agent which cause boils, carbuncles and sycosis barbae, was *Staph. aureus* mainly is the causal organism.

Table -3: Bacterial Skin Infections

Infection	Site		Causal Microorganism
Boil	Hair Follicle		<i>Staphylococcus aureus</i>
	Male	Female	
	3	7	
Carbuncle	Multiple Hair Follicle		<i>Staph. aureus</i>
	Male	Female	
	2	5	
Sycosis barbae	Shaving Area		<i>Staph. aureus</i>
	Male	Female	
	8	0	
Acne vulgaris	Face and Back		<i>Propionibacterium acnes</i>
	Male	Female	
	5	13	

Table - 4: Results of Hematological Analysis of all Study Groups

Hematological Test Normal Value (N.V)	G1 N(14)	G2 N(27)	G3 N(19)	Control N(20)	G1 N(5)	G2 N(9)	G3 N(26)	Control N(20)
	Male				Female			
Hemoglobin gm/dl N.V (12-16) gm/dl (F) N.V(12-17) gm/dl (M)	13.6 ± 1.1	14.6 ± 0.6	14 ± 0.7	14.3 ± 1.1	11.6 * ± 0.6	12.3 ± 0.6	12 ± 0.7	12.6 ± 0.7
White Blood Cells Count (WBCs) N.V(4-11)x 1000cell	8600 ± 400	8000 ± 600	7200 ± 400	8200 ± 600	7200 ± 600	7800 ± 400	6400 ± 600	7200 ± 200
Platelets Count N.V(150-450)x 1000	310 ± 20	320 ± 10	280 ± 20	300 ± 10	240 ± 20	280 ± 10	210 ± 10	230 ± 20
E.S.R N.V (0-20) mm/hr	15 ± 3	20 ± 5	28 * ± 5	16 ± 5	10 ± 3	18 ± 5	22 * ± 3	12 ± 5

*Significant probability ($P<0.05$). Data are mean \pm S.D.

Table-4 show the results of hematological tests: hemoglobin level, white blood cells count, platelets count, erythrocyte sedimentation rate of the three age groups (males and females) and control group (20 healthy males and 20 healthy females), their ages were ranged

between (25-35) year, data in the table as mean \pm S.D. Patients who have chronic diseases: *Diabetes melitis* and blood hypertension, have been excluded.

Table-5 shows the biochemical analysis of the study groups in comparison with control group and normal values.

Table - 5: Biochemical Results Analysis of all the Study Groups

Biochemical Test Normal Value Range	G1 N(14)	G2 N(27)	G3 N(19)	Control N(20)	G1 N(5)	G2 N(9)	G3 N(26)	Contr ol N(20)
	Males				Females			
Blood Sugar N.V (3.5-5.5) mol/L	3.2-4	3.5-4.1	3.3- 4.3	4-5	3.5-4	3.4-4.3	3.1-3.8	3.8- 4.3
Blood Urea N.V (15-50)mg/dl	22-36	18-38	25-40	16-37	18-25	20-31	22-38	19-36
Serum Creatinine N.V (0.2-0.9)mg/dl	0.2-0.3	0.2-0.3	0.2- 0.4	0.2-0.3	0.2-0.3	0.2-0.3	0.2-3.5	0.2- 0.3
Total Bilirubin N.V (0.1-1) mg/dl	0.2-0.3	0.2-0.4	0.1- 0.4	0.1-0.3	0.1-0.3	0.2-0.3	0.2-0.3	0.2- 0.4
Serum Cholestrol N.V (150-250) mg/dl	180-220	200-260	*215- 285	180-230	170-200	185-215	190-270	180- 210
Serum Triglycerides N.V (50-85) mg/dl	60-75	65-80	65-85	70-82	65-80	65-90	70-98	65-80

*Significant probability (P < 0.05). Data are mean \pm S.D.

Discussion:

As shown in table 1 the prevalence rate of dermatophytosis in the first group was 14% for males and 5% for females, while in the second group was 27% for males and 9% for females, whereas in the third group prevalence rate was 19% for males and 26% for females.

In this study, microscopy using 10-20% KOH was followed by cultures on modified Sabouraud's dextrose agar (SDA) with chloramphenicol 0.05mg/ml and cycloheximide 0.5mg/ml. Sabouraud's peptone glucose agar is the most common medium used for dermatophytes isolation^[9]. Fungal species were identified by morphological characters like: spore forms, hyphae, mycelia, cultural characteristics, pigment production and rates of growth.

According to the diagnostic data at table 2, it has been noticed that the most common infection was for tinea pedis 53% followed by tinea unguium 24.6%, and then tinea versicolor 12.1%, that agrees with the studies of^[10,11] who has performed epidemiological survey in Japan and revealed that dermatophytosis was the most prevalent cutaneous fungal infection

89.1% followed by candidiasis 8.4% and the malassezia infections 2.4%.

Among dermatophytes, tinea pedis was the most frequent then in decreasing order, tinea unguium, tinea corporis, tinea cruris, tinea manuum and tinea capitis, including kerion. Among all dermatophyte infections, *Trichophyton rubrum* was the most frequent isolated.

Tinea Pedis, this disease also known as "athlete's foot", is a common diagnosis in white urban populations. Clinically, the first is the interdigital type; thickening of web spaces of the feet, it may extend to toes and the soles, pruritus and foul odour be presented. Organisms most often isolated from infected tissue are *T. rubrum*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum*^[12,13]. have been reported that *T. rubrum* was the most common isolated from the glabrous skin, groins and feet. Percentage of tinea pedis was (53%) table 2.

Tinea pedis, *Malassezia furfur*, the fungus that causes tinea versicolor, is normally present in small numbers on the skin and scalp, overgrowth leads to infection^[3].^[3] and^[14] have determined the risk factors of tinea versicolor more

common in men, the adolescents and young adults, people who suffer from profuse sweating or high production of sebum, people with an immunodeficiency following diabetes, Cushing syndrome, people on medications such as corticosteroids and genetic predisposition and moreover it is more common in warm and humid climates (tropical and subtropical areas).

Superficial dermatomycosis were the most common diagnosis with a total prevalence rate of 33.9% in Morogoro region in Tanzania. The prevalence rates of tinea versicolor, tinea capitis, corporis and pedis were 26.2%, 5.5%, 2.6% and 3.2% respectively ^[15]. That is in contrast with our results especially for tinea versicolor may be because of difference in specimen sources. Samples of ^[15] had been taken from school children, while in our study samples were from three age groups of cleaners (males and females), as well as difference in weather between two countries.

Mathur, 2008^[14] has been referred that tinea versicolor is a common condition. It is estimated that 2 to 8% of the population of the United States have it. This skin disease affects adolescents and young adults especially in warm and humid climates. 1.9% of factory workers were examined in central Turkey presented with tinea versicolor lesions ^[16]. However, this infection has been found to be associated with malnutrition ^[17].

Dermatophytes may also invade nails; this infection is called tinea unguium. *T. rubrum* and *T. mentagrophyte* are the most common dermatophytes causing this infection ^[18]. Nails are thickened, discolored and brittle, that is in agreement with the results of our study. These two species were the most common causes of tinea unguium, which have high percentage 24.6% of dermatomycosis, table 2. ^[13]. Have been also reported that these two species as well as *T. violaceum* the most common organisms isolated from 166 nail samples.

Several species of *Trichophyton* and *Microsporum* have been isolated from scalp ringworm lesions (tinea capitis). Disease manifestations range from small, scaling patches to involvement of the entire scalp with extensive hair loss. Percentage of tinea capitis reached to 4.7% of total dermatomycosis in this study, table-2.

Organisms have been caused tinea corporis (ringworm), were *Epidermophyte floccosum* and several species of *Trichophyton* and *Microsporum*. Lesions appear as advancing annular rings with scaly centers. Lesions most often occur on non-hairy areas of the trunk. Percentage of tinea corporis was 5.3% of total infections in our study, table 2, this considered low percentage when compared with the study of Mathur (2008) in Mumbai, which have been reported that most prevalent clinical type that cause dermatomycosis is tinea cruris 60% followed by tinea corporis 33%.

Bacterial skin Infection is usually endogenous due to a strain of *Staph. aureus* which carried in the nose and on the skin. Lesion tends to recur appearing in crops at the same site often over weeks or months. Generally, it is more common in males than females ^[8].

Sycosis barbe, seen only in males is a chronic septic pustular rash on the shaving area spread by the minor inflected on skin and hair follicles.

Acne vulgaris: was common and disfiguring skin disease of adolescence that sometimes persists into adult life. While *Propionibacterium acnes* and *P. granulosum* can regularly be isolated from inflamed black heads.

Bacteria are cofactors and may induce inflammatory reaction in the skin by the production of lipase, which liberates irritant fatty acids from lipid in the sebum within sebaceous glands ^[9].

When we examined some of hematological parameters significant differences have been found between the three patients' group when compared with

control group and normal values according to the statistical analysis were used. Hemoglobin levels was normal for male groups in comparison with control group, while levels for female groups were decreased significantly ($P < 0.05$) in group 1 (11.6) mg/dl, that may be contribute to many factors like: malnutrition, period cycle and tiredness because of nature of their work and exposure to different diseases because they contact with parasites (nematodes and protozoan), bacteria, viruses...etc, in addition to contact with dust and fungal spores.

WBCs count remained unchanged significantly even was decreased in number in some of study group like group 3 comparing with control group or with normal values. E.S.R was elevated significantly ($P < 0.05$) in the 3rd patients' group of males (28) mm/hr and females 22mm/hr in comparison with control group, the reason could be attributed to several infections, especially bacterial infections that may coincide fungal infection.

The possible route of entry for the dermatophytes into the host body is the injured skin, scars and burns. Infection is caused by arthrospores or conidia. The fungal pathogens induce both immediate hypersensitivity as well as cell mediated or delayed type hypersensitivity, mediated or delayed type of hypersensitivity. Acquired resistance to the infection may also results from dermatophytic infection. The fungal growth is restricted by the inflammatory reactions produced as a result of infection with dermatophytes [1,5].

The results of biochemical analysis table 5 have been shown that biochemical tests were within normal limits with significant elevation ($P < 0.05$) have been noticed in serum cholesterol of the 3rd group of males (215-285) mg/dl compared with control group (180-230) mg/dl.

Little increases have recorded in serum triglycerides levels of the 2nd group of females (65-90) mg/dl but it is not

significant when compared with control group (65-80) mg/dl.

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