Introduction

INTRODUCTION

Napkin dermatitis stands as one of the most common problems that physicians meet during their practice espicially below two years age group. It is commonly seen in children whose napkins are left wet for a long time without frequent changing and cleaning of the diaper area particulary in hot weather.

Napkin dermatitis is an inflammatory disorder induced by prolonged contact with urine;faeces or both, or occasionly by irritant chemicals contained in the napkin itself (Beare and Rook, 1977).

Aim Of The Work

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The present work is planned to study the Bacteriological and Mycological causes of napkin dermatitis in outpatient pediatric and Dermatology Clinics at Benha University Hospital.

NAPKIN DERMATITIS

Definition :

Napkin dermatitis is a term used to encompass a wide range of inflammatory processes that occur in the area covered by the diaper. The term "Napkin dermatitis is thus simply a description of the location of a rash, Numerous factors can be involved in the pathogenesis of dermatitis in the diaper area. These factors including the role of the diaper, friction and contact irritation, urine and ammonia, faeces, infection, and of underlying dermatologic conditions (Leyden JJ 1986).

Aetiology :

It was previously blunted that napkin dermatitis caused by syphilis. At the turn of that century various ofrts were being made to explain diaper rash on the basis of nutrition, Alkalinity of faeces, infection and irritation both chemical and mechanical causes.

In 1915 Zahorsky called attention to the almost constant association of the odour of ammonia in the diaper area with cruption in it. This association was not clarified however untill Cook at (1921) isolated ammoniagenes and demonstrated the role of the bactrium in the production of ammonia in urine. The clarification of the problem of ammonical dermatitis was important, but lead unfortunately, to over emphasis of this mechanism of diaper rash production to the exclusion of all other factors. In fact until recently most text books of pediatrics mentioned only ammonical dermatitis under the heading " Diaper Rash " (Jacobs, 1978).

Leyden _et_ _al_. (1978) mentioned that amonia is not the primary cause of napkin dermatitis, but it may play a possible role for further irritation in an already existed condition where he noticed that the experimental application of highly ammonical urine on intact skin of infant and adult failed to provoke dermatitis. Erythema could be induced only when ammonical urine was applied occlusively to scarified skin.

In 1961 Burgon _et al_ stated that diaper rash is not specific disorder but rather areaction of the skin in a localised area. They pointed out that a variety of morphological changes are produced by multiple causative factors. They divided these factors into predisposing and activating factors.

Predisposing factors influences on the skin may alter its response to irritation. These may be genetically inhirited traits, as in the seborrheic diathesis and the reactive skin orthey may be due to an altered response of the cutaneous system due to systemic disease.

The reactive skin :

It is recognized that some children inherit a kind of reactive skin from their parents which may be more easily irritated by contact with sensitizing preparation or more liable to irritation than the normal skin of others.

Seborrheic diathesis :

The seborrheic diathesis is an inborn physiologic trait which seems to be genetically transmitted. It has several distinct characteristics, including the distribution of the cruption, frequent association with an oily skin during adolesence and adult life, an vulnerability to secondery bacterial and yest infection, in infancy, the influence of this sort of inheritence is manifested in the diaper area.

Systemic diseases :

A few systemic diseases are frequently associated with a persistant dermatitis reaction in the diaper area, Those diseases most frequently mistaken for persistent ammonical dermatitis these diseases are letterer Siwe disease, acrodermatitis chronica enteropathica and Syphilis. (Burgoon et al 1961).

Activating factors :

There are a number of activating factors which may initiate the dermatitis in the predisposed infant as maceration, sweat retension, contactant factors, goneigenic factors, infection and trauma (Burgoon *et al*.,1961).

1. **Maceration :**

The damp environment produced by continous contact with the moist diaper results in water logging of the skin Damage produced by moisture is probably one of the most important triggering factors in the predisposed skin (Burgoon *et al*.; 1961).

The macerating effect is increased by applying an impervious diaper cover or two or three diapers at bed time to prevent soaking of the bed.

Fresh urine containing bacteria do not cause a dermatitis wheareas urine allowed to stand for 18 hours and "putriefy" does produce a dermatitis, This suggests that wetness alone may not account for the genesis of the dermatitis, but that water plus overgrowth of bacteria are required (Rapp, 1955).

Wetness of the diaper area skin alone can not account for the generation of a dermatitis, but acts as a cofactor perhaps by altering the epidermal barrier (Weston et al., 1980).

2. Sweat Retension :

Hyper keratotic plugging of the poral orrifices of the sweat ducts and sweat retension are the natural sequences following maceration and water logging of the stratum corneum. In infant without predisposing factors the resultant irritation is minimal, but in infant with predisposing factors these changes may mark the beginning of "diaper dermatitis. (Burgoon et al., 1961).

3. Contactant factors :

Contactants may be divided into allergic and primary irritant types. The allergic contact type of dermatitis is the result of epidermal sensitization and may be produced after an innitial contact and an incubation period followed by recontact. In contrast, a primary irritant type of reaction can be produced regularily in all patients at the time of first exposure. (Burgoon *et al*., 1961).

Allergic contactants play a relatively minor role in infant due to immunologic immaturity of the cutenous system, sensitization reactions are most frequenty produced by the flurochromes used in detergent soap preparations, rubber and plastic diaper covers (Burgoon et al; 1961 and Beare and Rook, 1979).

The Primary Irritants :

a) Faeces. regularly produce a primary irritant effect on the skin, this is well evident in the infant who has a frequent loose stools and a dermatitic reaction in the areas where contact with faeces occurs, and unless faeces are removed rapidly dermatitis proceeds to eczematization and erosions within a short time from four to six hours.

The issue of alkalinity playing a role in diaper dermatitis remains unresolved, although the skin pH usually does not differ in those infants with and without dermatitis (Rapp. 1955).

Another occasional source of chemical irritation is the acid stool produced by some childrens depending on a diet high in protein, other than milk and which can produce the so called " acid scald" (Brown and Wilson 1964).

b) Urine : As previously mentioned the freshly voided sterile urine will not produce any visible reaction on normal intact skin, But "Rapp" (1915) found that old bacterially contaminated urine produced dermatitis, where he found that there were at least two types of skin irritants produced in urine by the action of putrefactive and proteolytic enzymes from the bacteria "Ammoniagenes" of the faeces and the skin which flourish in wet warm diaper; when diaper changes are infrequent and not be adequatly sterielized.

c) Ammonia :

In 1921, Cooke was the first who implicated ammonia in the genesis of diaper dermatitis. He related that a strong ammonia odour with Napkin dermamtitis. He found a Gram positive, non motile bacteria that grow from urine of infants with diaper dermatitis but not from those without. This bacteria liberated ammonia from urea in culture and was called "bacillus ammoniagenes" .

Cooke's theory of ammonical diaper dermatitis was accepted for a long time, but it has recently been challenged by studies which demonstrated that concentration of ammonia obtained from 26 infants with diaper dermatitis did not differ from levels obtained from 82 infants without diaper dermatitis (Leyeden et al., 1977).

They also reported that even when ammonia was liberated from infant's urine with urease, the increased urine ammonia levels did not differ in the two groups. Also, after application of ammonia (1.6 %) to the skin of ten infants for 24 hours, it did not produce erythema or dermatitis .

Further, bacillus ammoniagenes was not recovered from all infant's urine who had diaper dermatitis or diaper area candidiasis by using standard microbiologic techniques (Leyeden an Kligman 1978).

These findings produced considerable doubt on the role of ammonia in diaper dermatitis. In addition the clinical observations that not all infants with napkin dermatitis have a strong ammonia odour, suggests that ammonia is not involved in the genesis of diaper dermatitis (Weston et al., 1980).

Although, it may play a secondery role in aggrevating already damaged skin (Leyden et al., 1977).

3. Goneigenic factors :

Goneigenic causation is a term used to describe the association of emotional problems in the mother with production or persistence of dermatitis in the diaper area; for example, the mother may neglect the infant because she is too involved with her own problems or she may produce additional irritation by being over assiduous in cleaning of the affected area, the goneigenic factors should be evaluated in the presence of refractory dermatitis. (Burgoon et al., 1961).

4. <u>Infection</u> :

In the order of the relative frequency with which may be seen, yeast, bacterial or viral infection may be localised to the diaper area,this localization to this area is conditioned by the damage to the skin produced by constant moisture.

a) <u>Candida</u> :

Diaper dermatitis present for greater than 72 hours is likely to yield Candida albicans when cultured (Montes et al., 1971).

Dixen et al., (1969) suggested that Candida albicans in most cases of diaper dermatitis, present as a secondery invader, though, they could not exclude the possibility that primary Candida infection sometimes occur.

Most authoritis now agree that Candida albicans is a frequent invader of the diaper area, Montes et al (1971). isolated it from 77.1 % of infants with diaper dermatitis. It is controversial whether, Candida albicans initiates diaper dermatitis or merely aggravates a dermatitis already caused by friction and maceration (Munz et al., 1982).

b) Bacteria :

Bacteria may be involved in diaper dermatitis, the studies of Rapp (1955) showed that "putrefied urine" produced a dermatitis when applied to the skin of 3 month old infants.

Leyden and Kligman (1978) re-examined bacteria obtained from the skin of infants with and without various types of napkin dermatitis and could defect no significant differences in the type or quantity of microbial flora recovered. They did, however, find that 50 % of infants with napkin dermatitis had staphylococcus aureus recovered while healthy infants did not. The role of colonization with Staplylococcus aureus was not clear from their study.

Bacterial over growth on the skin of infants with diaper dermatitis may represent a secondery event. There is no firm proof, however, that bacteria account for the dermetitis (Westen et al.; 1980).

c) Viral infection :

Viral infection in the diaper area are uncommon, but may be produced by Vaccinia or Herpes simplex viruses, vaccinia infection may follow vaccination in the presence of irritation of the skin in the diaper area. Herpes infection of the skin is of primary type and may be associated with a severe systemic reaction and regional lymphadenopathy, infection is usually transmitted from an infected member of the family that is in close contact with the infant.

d) Trauma :

Tight application of the diaper and mechanical irritation result in the localization of the dermatitis to the convex portion of the buttocks and other areas of pressure from the diaper, this is accentuated with wetting the diaper with ammonical urine where ammoniacal urine where ammonia acts as a primary irritant. (Burgoon et al, 1961) and, Hurwitz 1981).

Clinical Features :

The morphologic changes range from a parchement like erythema resembling a scaled or severe sunburn, through areas of papules, vesicles, and small superficial erosions, to ulcerated nodules which may measure a centimeter or more Koblenzer (1973).

This eruption is characteristically found at sites of most intimate contact with the diaper, such as convexities of the buttocks, lower abdomen, proxymal thighs, the mons pubis and the external genitalia Koblenzer, (1973); Jacobs, (1978) and Hurwitz, 1981). In some cases, the sides of the thighs, the calves and the heel may also be involved,Beare and Rook, (1979). The skin in the intertriginous area of the genito-crural fold and between the buttocks is clear (Burgoon et al., 1961).

In chronic forms fine scaling combined with glazed erythema are characteristic (Beare and Rook, 1979).

The term Jacquet's dermatitis is often used to describe a sever papulo-erosive eruption with umblicated or crater, like appearence. In male infants, ulceration and crusting of the glans penis and urinary meatus may create difficulty or discomfert on micturation (Hurwitz 1981).

CANDIDA

Yeasts are unicellular fungal organisms that reproduce by budding which are members of the closs Blastomycetes. Many of them constitute a resident population regularly and universally part of the normal flora of the skin surfaces, buccal mucosa, the intestinal tract and vaginal mucosa (Rippon, 1982).

The genus Candida belongs to the family Cryptococcaceae, it accomodates a heterogenous collection of 81 species of asporogenous yeasts (imperfect yeasts) that characteristically reproduce by budding only, without formation of ascospores (Al-Doory, 1980).

Candida albicans grow rapidlyon Sabroud's glucose agar at 27°C producing cream-coloured and pasty colonies after 3 days. Within one month the colonies become glistening, some what waxy, soft and smooth. older cultures may become tough,

Fig. 1 Candida albican colonies

displaying a wrinkled or folded surfaces. pseudo-hyphae bearing bull-like clusters of blastospores measuring up to 4 um in diameter may develop while, large, rounded to pear-shaped chlamydospores (8-12 um) that are characterised by thick walls may be formed terminally on the pseudohyphae. Some strains of candida albicans are known to produce true mycelium.

On serum or eggalbumine at 37°C for two hours sprout mycelium formed. Essenticly all strains of Candida albicans are positive in this test, and no other commonly encountered species demonstrated this reaction.

Also C. albicans ferment, glucose, galactose, sucrose and maltose and assimilate, glucose, golactose, sucrose and maltose (Rippon 1982).

Candida albicans is scarcely found in the skin of healthy young adults other than the specialised areas such as the axillae, groions and toewebs. The inability of Candida to establish itself on intact, dry skin may be due to lack of suitable nutrients, lack of suitable environmental conditions, particulary humidity and the presence of inhibitory substances, such as lactic acid and fatty acids (Marples, 1966). On the other hand, C. albicans is more common on skin in seniles over 65 years of age and in infants (Somerville, 1969), However in infants, C. albicans seldom lives saprophytically on the skin, and it should be regarded as a pathogen whenever it is isolated from a cutenous site (Taschdjian and Kozinn, 1957).

C. albicans, the normal flora, has a pathogenic role under certain conditions where the normal host characteristics are altered. Whenever the host/parasite balance is upset, for example in extreme youth, after prolonged treatment with antibiotics and in seriouslly ill, the yeast shoots noticeable morphological changes and mycelial forms predominate (Noble and Somerville, 1974). Also any damage to skin or environmental change leads to rapid colonization, for this reason, Candida is not infrequently isolated from a variety of dermatologic conditions (Rippon, 1982).

On the other hand, C. albicans appears to be the only member of genus candida that are regulary able to evoke fatal disease in man and animals C. stellatoidea and C. tropicalis, though less pathogenic than C. albicans, are significantly more virulent than other candida species (Mourad and Friedman, 1961). Nucleic acid-base composition studies indicate that C. albicans, C. tropicalis, C. clausenii and C. stellatoidea are related (Stendcup and Bak, 1968). However, by D.N.A homolgy studies, C. albicans has close relationship to C. clausenii and candida stellatoidea but not C. tropicalis (Back and Stendcup 1969). On the other hand, C. albicans is antigenitically divided into :

Serotype A) which it shares with C. tropicalis and

Serotype B) which is shared by C. stellatoidea (Sweet and Kufman, 1970).

Organism	Assimilation: Dextrose	Maltose	Sucrose	lactose	Galactose	Trehalose	Raffinose	Cellobiose	Melibiose	inositol	-xylose	Fermentation: Dextrose	Maltose	Sucrose	Lactose	Morphology on cornmeal tween 80 agar
C.albicans	+	+	+	-	+	+	-	-	-	-	+	AlG	AlG		-	Hyphone and pseudolyphae formed with clusters of blastospores (4-5 um) at internodes, Terminal chlamydospores (8-12 um) formed in most species.
C.stellatoidea	+	+	-	-	+	+	-	-	-	-	+			-	-	Extensive pseudomycelium with irregular or spterical clusters of blastospores (4um) at internods or septa-chlanydospores are rare
C.Tropicalis	+	+	+	-	+	+	-		-	-	+				-	Abundent pseudomycelium with branching blastospares (4-7 by 5.11 um) occur singly or in clusters any where along mycelium. True hyphae also formed.
C.pseudo-tropicats	+	-	+	+	+	-	+	+	-	-	+	-				Abundant pseudomycelium of elongate cells that fall apart and lie parallel like legs in a stream. Blastospores are infrdquent.
C.paropsilosis	+	+	+	-	+	+	-	-	-	-	+					Fine and coarse pseudomycelium (giant forms) Blastospores (3-7 um) single or in short chains at septa a terminal ends.
C. gullermondii	+	+	+	-	+	+	+	+	+	+						pseudomycelium is sometimes rare and usually tin, and short with small cells-small clusters of blastospores at internodes.
C. Kruesi	+	-	-	-	-	-	-	-	-	-						A branched pseudomycelium is formed by elongate cells Elongated cells at internodes have-crossed match books appearance Blastospores (3-10 um) occur along mycelium.
C. rugosa	+	-	-	-	-	-	-	-	+	-	+					Pseudomycelium well developed with abundant blastospores (3-12 um) at internodes.

A. acid reaction
G. gas production

strain variation.

source Haley and Standerd (1973).

NAPKIN CANDIDIASIS

* Defenition :

Candidiasis is a primary or secondery infection involving a member of the genus Candida. Essentially, however, the disease is an infection caused by Candida albicans (Rippon, 1982).

Candida albicans is perhaps the best example of an opportunistic organism, which can changes its habit from commensalism to parasitism when conditions warrant such a change. When candida is living as a commensal, it is in balance with its host, the balance is delicate and is upset by changes in the host (Winner, 1969).

Candida exhibit both yeast and filamentous (mycelium) growth on the skin, Lever and Shaumburg-Lever (1983). In parasitic life the organism often undergoes an initial multiplication in yeast phase, and transfered to the mycelial phase when it starts to invade the host tissues Winner, (1969). The yeast form is principly responsible for the invasion and the filamentous form for the pathologic response (Taschdjian et al., 1960). Also the filamentous phase help the organism to escape macrophage ingestion and it is necessary for invasion of the tissue,(Montes and Wilborn (1968).

The organism is always found outside the living portion of the skin in the cracks and fissuers of the outermost desqumating stratum corneum utilising soluble nutrients which permeate the interstices of this tissue (Maiabach and Kligman, 1962).

The investigators had attributed the ability of C. albicans to produce cutaneous disease to two different mechanism. There were those who mentioned that infection through invasion and subsequent digestion of keratin by the fungus, is the basic process Kapica and Blank, (1957). Such a view had been supported by Mortes and Wilborn in (1968) by electornmicroscopic observations.

Expermintal models of C. albicans infection demonstrated that this organism itself when applied to the skin surface, had the ability to invade through the epidermal barrier, perhaps by the role of keratinases liberated by the C. albicans (Repora et al., 1973).

Thus Candida albicans need only to overgrow on the skin surface to invade the skin without requiring any additional factors to further compromise the epidermal barrier Weston. (1980).

On the other hand, there were others who believed that cutaneous candidiasis is a primary irritant type of contact dermatitis (Maibach and Kligman, (1962) and Montes et al., (1971). In the latter mechanism as shown by Maibach and Kligman (1962) occlusion was a necessary factor.

In 1982 Rippon reported that C. albicans is an endogenous species, the new born is inoculated from the vagina during birth. Infants harbour C. albicans organism in the lower intestine and from this focus that infected faces present the primary source for monitial diaper eruptions, Kozinn et al., (1961).

On the skin of normal infant, C. albicans may be recovered, but was found in only 1 % to 3 % in several studies (Brookes et al., 1971; Dixon et at., 1972 and Leiden and Kligman, 1978), and it was found in 12 % of cases in a study done by Montes et al., (1971).

Recently Repora and Leyden (1981), recovered C. albicans from only five out of 145 sites in forty infants free from napkin rash.

On the infant's skin with diaper dermatitis the presence of C. albicans had been recognised in a number of studies. Bound (1956) isolated C. albicans from over 50 % of 75 infants with diaper dermaites. Fergusson et al. (1966) obtained positive culture for C. albicans from almost

50 % of 29 cases of diaper dermatitis. Also Dixon et al. (1969) found an over all prevalence of 41 % in 117 patient.

Candida albicans was recoverd from 80 % of those diagnosed as "candidiasis" and this result lead to conclusion that C. albicans is an important etiological factor in the more severe forms of napkin dermatitis (Leyden and Kligman, 1978).

The role of alimentry C. albicans in persistence or recurrent diaper dermatitis had been will studied (Dixon et al., 1969). Although commonly recoverd from the rectum, British authoritis had convincingly demonstrated that aliminly C. albicans did not play a role in diaper candidiasis (Dixen et al, 1969 and Dixen et al, 1972).

In a recent study done by Rebora and Leyden (1981) C. albicans was recoverd from pustules of all thirty infants with typical candidiasis and from the rectum in 93 %. This result strongly suggests that the gastrointestial tract serves as a reservior and that infection results from spread into the napkin area.

Cutaneous candidiasis is a common sequela to systemic antibiotic therapy, the most important effect of antibacterial antibiotics is the elimination and alteration of the bacterial flora that holds the population of candida in check Rippon (1982). However, Maibach and Kligman (1962) and Dixon et al. (1969) found that neither prior treatment with oral antibiotics, nor treatment of the diaper skin area with topical glucocerticoids increased the recovery rate of C. albicans.

Clinical picture :

The typical Candidal diaper rash presents as a more or less wide spread erythema on the buttocks lower abdomen and inner aspects of the thighs often with fissuring in the intertriginus area (Kozinn et al., 1961). It is characterized by a vivid beefy red colour. The eruption is characterised by a raised edges, sharp marginization. with white scales at the border of the lesions,and pin point pastulo-vesicular satellate lesions marks the region of active spread of the infection (Harwitz, 1981).

In 1981, Hurwitz mentiond that although cutaneous candidiasis frequently occurs in association with oral thrush, commonly the mouth is by passed and the infection is frequently confined exclusively to the diaper area. But Rippon (1982) reported that Extension of the lesions to include the buttock and inner thighs is common, as well as the genitalia , lower abdomen, and entire diaper area are some times involved. These is minimal pruritis and in healthy children the condition resolves quickly following therapy.

and irregular ones (Noble and Somerville, 1974)

Aerobic corynebacteria are commonly found in large numbers on the skin, especially in adult life. Together with staphylococci and micrococci, they are dominant residents on many skin sites (Marples, 1965). However, others are derived from the environment and are termed transient or nomadic (Somerville-Millar and Noble, 1974).

In fact, number and incidence of aerobic corynebacteria on the skin appear to be controlled by the presence of sebum. Being capable of breaking down the lipids of sebum producing free fatty acids (Smitth and Willett , 1968), they may exert some control on the composition of the rest of the skin microflora or be controlled by it (Smith 1969).

At present, most medically oriented studies of the aerobic cutaenous coryneforms classified them into strains that produce a coral -red fluoroscence (prophyrin) on special media or whose growth is stimulatd by the presence of oleate (lipophiles) supplied as Tween 80, a those which passess esterases capable of breaking down Tween 80 (lipophylic) Pitcher and Jackman, 1981).

Lipophylic coryneforms vary in their skin site distribution. Mc Ginley et al. (1975) detected them on 50 % Scalps sampled. According to somerville (1973) and Aly and Maibach (1976), lip phylic coryneforms are the most common types in the axilla, groin and toe webs.

Lipolytic coryneforms showed no preferred site of growth, though, they are most common in the groin and least common in the nostrils (Noble and Somerville, 1974).

Regarding the anaerobic corynebacteria, these spieces should be assigned to the genus probionibacterium on the basis of its serological cross-reactions with members of this genus and its ability to produce propionic acid from lactate (Moore and Cato, 1963). There are two types of them; P. acre and P. granulosum .

Anaerobic corynebacteria are normal inhabitants of the skin especially of the hair follicles. They are present in large number in the areas of high sebum secretions such as the face, chest and back. They become more numerous at puberty and decrease with age and sebaceous activity is reduced at eldery age (Marples, 1965).

Organisms of corynebacteria are also found in pathological conditions, ranging from extremly mildform where an over growth of the organisms produce simple visible concentrations on hair to serious system infections. Diphteroids are involved in the aetiology of at least three specific skin infections, trichomycosis axillaris, erythrasma and acne, and also probably play some part in pitted keratolysis. They may also act as opportunist pathogens, invading a deblitated host causing systemic illness and even death (Moble and Somerville, 1974).

Coryneforms may also be a possible cause of diaper dermatitis. Brevibacterium ammoniageneus, a potent producer of ammonia from urea, was implicated by Cooke (1921); However, Leyden and Kligman (1978) found no difference between ureolytic coryneforms from diapers in the affected and normal childrens. of 18 strains of large-colony coryneforms, five were found to be potent producers of ammonia, but their colonial morphology did not resemble that of B. ammoniageneus coryneforms like this organisms were only rarely isolated (Pitcher and Jackman, 1981).

2) Micrococcaceae :

The genera staphylococus, Micrococcus and sarcina, constitute the family Micrococcaceae, represting Gram + ve , catalase positive cocci (Baird, Parker, 1963). Staphylococci and micrococci represent the second major group of bacteria inhabiting human skin, (Noble and somerville, 1974 and kloos, 1981).

Staphyllococci and micrococci can be identified from each other by several routine laboratory tests. Staphyllococcus has the ability to ferment glucose under anaerobic conditions, while the micrococcus lack this facility (Baird Parker, 1963). Other tests proposed by Schleifer and Kloos (1975) are the ability of staphyllococus to produce acid, aerobically, from glycerol in presence of 0.4 ug erythromycin per ml and their susceptibility to lysostaphin at a concent. of 200 ug/ml. Curry and Borovian (1976) had shown that a nitrofuran containing medium (FTO agar) permited the growth of micrococci but not staphylococci. Another fundamental difference based on the DNA base composition, is that micrococci have a high percentage of guanine and in contrast to the low percentage in staplylococci (Noble and somerville, 1974).

Extensive systemic and ecological studies had identified at ten different staplylococcal species living on human skin. These species include , staph aureus, staph epidermidis, . staph. hominis, staph haemolyticus, staph capitis, staphwaneri , staph sabroptiticus, staph. cohinii, staph xylosus and staph simulans (Carr and Kloos, 1977 and Durham and Kloos, 1978).

Staph. aureus is considered the most potentially pathogenic species, it is co-agulas -positive. On the other hand the Co-agulas negative species found living on human skin may be organised into three species groups (Kloos et al., 1976) , the main of which is the staph epidermidis species group. This latter is composed of the species staph. epidermidis, staph hominis, staph warneri, and staph. capitis (Schleifer et al., 1979). Until recently, a scheme was a survivor of the earlier nomenclature divided strains on the basis of their colony colour, hence staphylococcus pyogenes aureus, staph pyogens albus, citreus, roseus, etc. (Noble and Somerville, 1974).

Staphylcocci are round or some what oval cells , arranged in grape-like clusters. This is more evident on solid than in liquid media. All staph. are non motile, non flagellated, and non sporing and most of them are uncapsulated. They are uniformly Goram-positive in young cultures. Nearly all staph. grow abundantly in unenriched media. On nutrient agar they form smooth, circular, opaque, pigmented colonies, 1-2 mm in diameter after overnight incubation of 37°C (Parker, 1984).

Staph epidermidis colonies are usually grey or grey-white with prolonged incubation the centre of colonies becomes dark (Kloos et al., 1974). On the other hand, colonies of staph aureus are usually golden yellow, but may be pale-yellow or fawn. Those of staph intermedius and the co-agulose negative staph are usually white, but less often may be pale yellow (Parker, 1984).

Staphylococci are facultative anaerobes but, their growth is improved by oxygen. They attack sugars fermentatively, though this activity may be very weak.

Anaerobically they form mainly lactic acid and a little CO_2 is formed (Parker, 1984). Staph aureus usually reduces nitrates, demonstrats weak to moderate phosphatase activity, and produces acid, aerobically form D. mannitol (Kloos, 1981).

The nose, axillae, perineum and toewebs are the only common resident carrier sites of staph. aureus. The general skin surface in often regarded as yielding staph aureus in about 5 % of the normal population (Mable and Somerville, 1974). While Staph. epidermidis is uniformlly found on the normal skin in 100 % of the population (Weinberg and Swartz, 1971). On the other hand, micrococci are usually found in much smaller population on human skin than staplylococci, though they may account for a large propertion of the aerobic bacteria in cutenous habitates supporting relatively small numbers of aerobic bacteria on certain individuals (Kloos and Mussel white, 1975).

Micrococci are currently classified on the basis of a combination of key phenotypic characters (Kocur et al., 1972), genetic transformation (Kloos et al., 1974) and to some extent, DNA - relation (ogarwara-Fujita and Saraguchi, 1976). Eight different micrococcal species had been isolated from human skin, including M-luteus, M. varians, M. lylae, M. nishinomiyaensis, M. Kristinae, M. roseus, M. sedentarius, and M. agilis, in order of prevalence (Kloos, 1981). Temporal studies had suggested that certain strains of M. luteus, M. varians, M. Kristinae and M. sedentarius probably maintain a resident status, whereas others appear to be temporary residents or transients (Kloos and Musselwhite, 1975). Some of the micrococci are a little larger than staph., and unlike staph., some form tetrads or cubical packets. Some of the pink micrococci are motile. Colonies are opaque and round, with smoothor rough surface. Some strains form bright yellow and others pink colonies; mony, however, have only a slight lemon-yellow tinge that is not intensified by growth on milk agar. Some yellow micrococci produce violet pigment on potato but not on nutrient agar (Parker 1984), M. luteus is the major species of human skin. Its colonics are usually convex and uniformly shades cream white to yellow.

It does not produce acid aerobically from glucose , fructose, galactose, mannose, rhamnose, xylose on sorbital, produce aceton or grow on Simmons citrate agar, Most strains are oxidase-positive and fail to reduce nitrates (Kloos, 1981).

Regarding, Sarcina, this name which was at one time used to describe aerobic cocci that formed packets of eight cells, is now reserved for strict anaerobs that are similarly arranged. Sarcina grows on simple media and can grow at very low pH (Parker, 1984).

Sarcina had domed yellow-pigmented colonies, though white forms also occurs. It is Gram positive, co-agulose negative and catala e positive (Noble and Somerville, 1974). It derives its energy from the fermentation of Sugars (Parker, 1984). Sarcina, is a soil organism. so, it is regularly present in the faeces of vegetarians (Parker, 1984). It is seldom carried by infants and frequently by children and less frequently by adults and elderly. It is apparently non pathogenic (Noble and Somerville, 1974).

3. <u>The Streptococci</u> :

It is frequently stated the streptococci do not form part of the normal skin flora. Although this is true in most cicrumstances of the beta-haemolytic streptococci other groups may be found as residents in certain sites or in certain groups of individuals (Noble and Somerville, 1974). Streptococci are more or less spherical in shape and are arranged in chains. All the streptococci are non. motile, except for some of the enterococci.

Capsulation is not a regular character of streptococci but some form a capsule of hyaluronic acid in the early phase of growth. They are frankly Gram-positive. Various types of extracellular filament have been described in streptococci (Parker, 1984).

They may require rich media, such as blood agar, for adequate growth; even so, small colonies are generally produced (Noble and Somerville, 1974). All the streptococci are aerobic and facultatively anaerobic, some strains need 5 % of CO_2. All of them ferment glucose and maltose and most of them ferment lactose and sucrose, and all of streptococci give a negative catalse reaction (Parker, 1984). The initial subdivision of the streptococci is based on the type of haemolysis produced on sheep blood agar into : Alphahaemolytic streptococci (St. viridans) which produce partial lysis with greenish discolouration Beta-haemolytic streptococci (Streptococus pyogens) which produce complete lysis or clearing of the red cells in the area surrounding the colony. Non. haemolytic streptococci (Streptococcus faecalis) which does not produce haemolysis (Noble and Somerville, 1974).

Lancefield's grouping scheme, divided the beta haemolytic cocci into many groups based on the polysaccharide antigen. Group A is the most common human pathogen, Groups B,C and G may be isolated from human infections and the enterococci, group D, are also pathogenic in some situations

(Colman 1968). Beta haemolytic streptococci are rare found on normal skin though they may be carried in the throat of about 100 percent of the normal population (Kligman, 1965).

Marples (1965) found transient skin carriage in only 0.5 - 1 % of individuals. Streptococcus viridans have their principle niche in the mouth and from it , they spread over the face and hands; and in infants, over much of the body. It was found that non-haemolytic streptococci are more common in infants than in older age groups (Noble and somorville 1974).

4. " THE GRAM-NEGATIVE BACILLI " :

Gram negative bacilli are comparatively rare on normal healthy skin. The exception are the Mima/Herella group which may be part of the normal flora of the axillae, groin and toe-webs (Taplin et al., 1963), and proteus species, which may inhabit the nasal mucosa of about 5 per cent and the toe spaces of between 5 and 10 percent of the population (Noble and semorville, 1974).

On glabrous skin, faecal califormis disappear within two hours presumably as a result of desication. Coliform organisms found on the skin can therefore be assumed to be purely transient (Noble and somerville, 1974).

Somerville (1969) found children and adults carried Gram negative bacilli more often than did infants or the aged. Stratford and et al., (1968) demonstrated an increase in the Gram negative organism on the skin of patients with pyrexia. Sweating, giving an increase in surface moisture, bed baths and antibiotic treatment contribute to this increase. On occasion, Gram negative rods may produce or superinfect many types of lesions. As antibiotic are most effective against the gram positive bacteria, infections with gram-negative rods have become more important in general hospital practice (Barber, 1961).

Infections of the skin involving Gram-negative organisms fall conventantly into four groups : ulcers, paronychia, toe-web infections and misellaneous infections. Noble and somerville (1974) isolated Gram-negative rods from 9.0 percent of ecrema lesions, 5 per cent of psoriatic plaques, 49 percent of ulcers and 9 percent of lesions in other skin diseases.

Gram-negative rods are also frequently isolated in napkin rash, but the significance is difficult to determine (Montes et al., 1971).

In clinical microbiology it is a common practice to divide the Gram-negative bacilli isolated from the skin into a limited number of groups : pseudomonas, protous klebsiella, Mima/ Herella and "coliform-like organisms (Noble and somer ville, 1974). The last being a heterogenous group comprising about 40 species including the genera Escherichia, citrobacter, and Enterobacter (Show fermentation of lactose) and the non lactose fermenting Edwardsiella and Serratia

(Free man, 1973). According to Jawetz et al.(1982) family of Enterobacteriaceae consists of Gram. negative, non-sporing bacilli. They grow will on ordinary media and on Mcconkey's media. They may be motile or non motile, aerobes and facultative anaerobes active carbohydrate fermenters, with production of acid only or acid and gas. Many of the Enterobacter are fimbriate. Some species are normally capsulated and form mucoid colonies when first isolated. However the ability to form capsules may be lost on prolonged subculture on antificial media (Cross and Holmes 1984).

Escherichia coli, is a motile organism. On McConkey's media it produce rose pink-colonies, on nutrient agar plates, the colonies are greyish-white semi-transparent, smooth and low convex, and on blood agar, some strains produce. B haemolysis. It is indole and methyl red positive and vogs-proskauer reaction negative (Cruickshank et al., 1973). E. coli usually produce gas from fermentable carbohydrates. Mannitol is fermented and most strains acidify lactose promptly. It does not grow on simmon's citrate medium. Most strains fail to hydrolyse urea or to produce H_2S detectable in triple sugar iron agar. Phenyl alanin is not deaminated and gluconate is not oxidised (Gross and Holmes, 1984). E. coli inhabits the intestine of man and animals causes suppurative and diarrheal diseases. Also, it causes urinary tract infection and sepsis of operation wounds and abscess in a variety of organs and neo-natal meningitisand septicaemia (Gross and Holmes, 1984).

Citrobacter occurs infrequently in the normal stool. They are closely resemble E. coli in many biochemical reaches, but differ in that they can utilise citrate as a sole source of carbon. The two main species in this group are citrobacter Freundii which is indole-negative and citrobacter Hoseri which is indole-positive (Orskov and Orskovi, 1981).

Members of the genus Klebsiella tend to be some what shorter and thicker than the other enterabacteria. The cells are either in pairs end-to-end or arranged singly. They are non-motile and usually capsulated. Most strains are fimbriated. They ferment mannitol, salicin, adonitol, inositol and sorbital, and often also lactose and sucrose. Most strains form gas from sugars; gas production from starch is an important diagnostic feature. characteristically they give a negative methyl red and a positive voges-proskauer reaction. Nearly all grow on Simmon's citrate medium (Gross and Holmes, 1984). Typical klebsiella organisms produce large, non pigmented, moist colonies which include klebsiella pneumoniae (Firedlanders bacillus), klebsiella rhinoscheromatis (organisms associated with rhinoscleroma) and klebsiella ozaenae (Jawetz et al., 1982).

The genus Enterobacter is motile, less often and less heavily capsulated than klebscilla. They ferment mannitol and form gas from some sugars including cellobiase but not starch. Generally methyl-red negative voges. Proskaner positive, and grows on simmon's citrate medium with non pigmented or yellow pigmented growth (Gross and Holmes, 1984). They occur in the intestinal tract of men and animals and in soil. Occasionally cause septic infection (Oroskv & Orskovi 1981).

The genus proteus is classified into four species, proteus valgaris, proteus mirabilis, proteus morganii and proteus retgeri. On agar plates proteus produces swarming, on Mc Conkey's medium it gives pale yellow growth. It is non lactose fermenter and it is urease positive (Finegold and Martin, 1982).

S rratia orgnisms are small, motile, Gram-negative rods. Ferment mannitol, sucrose and salicin with the production of acid and sometimes of small bubble of gas. They generally give a negative indole, negative methyl red and a positive voges - proskauer reaction. They grow on simmon's citrate medium. Some strains produce a red non-diffusible pigment. Typically found in soil and water, but strains occur in the animal body. May cause septic infection in man (Kross and Holmes, 1984).

Mima/Herellae group, are Gram-negative, non motile rods which resemble Neisseria morphologically but differ biochemically. Two species were described, Mima polymorpha and Herellae vaginicola (Hoble and Somer ville, 1974). Somerville (1969) found these organisms in 41 per cent of adult females, and in only 6 percent of geriatric patients while, no infants were colonized.

TREATMENT

Prophylactic treatment :

Diaper dermatits syndrome is a completely preventable reaction especially if the various aetiologic factors are taken into casideration.

Removal of impervious diaper covering and wearing of one diaper are the 1st line of attack, since maceration and secondary sweat retension are the first consequences of continued moisture on the skin. The skin may be protected in such instance by application of lassar's past with each diaper change.

Mothers must be warned against using two or three diapers at night to avoid the moisture underneath, for this has the same macerating tendency as does an impervious diaper covers.

Through cleansing of the skin after diaper changing is essential to remove bacteria and fecal contamination.

Active treatment :

Study was carried out in general practice to assess the effectivness, acceptability and tolerability of a 1 % clotrimozole plus 1 % hydrocortisone cream in the treatment of 112 infant with napkin dermatitis. The cream was applied twice daily to the affected area for 7 days and treatment was extended to 14 days for those who failed to respond adequately, Assesments made of the severity of symptoms showed that there was significant improvement in erythema, irritation and pustulation in all but a few patients and an over all impression of clinical response made at the last patient visit indicated that 92 % of the patients were considered to have been cured or markedly improved. Accoptability and tolerability were good. (Hayden GF 1985).

Material & Methods

MATERIAL AND METHODS

Case material :

one hundered infants of ages varying from 15 days to 20 months, with various napkin-dermatitis were the subject of this study. They were collected from the outpatient Pediatric and dermatological clinics of Benha University Hospital.

They were categorised clinically into two groups:

1) Napkin Moniliasis.

This group consisted of 34 cases 29 of them were suffering from malnutrition and 5 cases were suffering from gastroenteritis.

2) Napkin Dermatitis.

This group consisted of 41 patient 38 of them were suffering from gastroenteritis and 3 cases were suffering from malnutrition.

In addition 25 normal infants without rash in Diaper area were studied as a control group.

For whole cases studied the following were done.

1) Careful history.

2) Thorough clinical examination.

3) Routine investigations.

4) Mycological and Bacteriological investigations

I) History and Sheet :

Include a) Name, age, sex, adress and complaint

b) History of the present disease including onset, course and duration of the disease.

c) family history of similar conditions.

d) History of previous topical and systemic therapy.

The history include also the details of the care of the infant's skin, the frequency of diaper changing (specially at night), the dietary and general health data as the type of feeding and presence or absence of diarrhea or vomiting.

II. Clinical examination :

Beside dermatological exam. for the present illness, general medical examination for every case was routeinly done to know the state of nutrition, gastrointestinal or urinary trouble, allergic manifestation or any associated disease.

III. Bacleriological and Mycological Investigations:

A sterile swab was taken from each case. The samples were transmitted to the laboratory as soon as possible and each sample were cultured on nutrient agar, blood agar and Sabouraud's glucose agar (with chloramphenical 40 mg per litre) plates. These plates were incubated aerobically at 37°C for 24 hours. Then the growing organisms were identified according to their colonial and cell morphology and biochemical Reactions.

(1) <u>For Micrococcaceae</u> :

These were identified by their colonial character on agar, and on blood agar. Also co-agulase test, soluble and bound - was done. Soluble co-agulase can be demonstrated by tube method in which a drop of fresh staphylococcus culture is added to ½ ml of $\frac{1}{10}$ dilution of citrated human or rabbit plasma. Co-agulation will take place within 1 - 6 hours at 37°C.

* Bound co-agulase can be demonstrated by adding a loopful of undiluted plasma to a saline suspension of the organism on a slide. clumping will occurs within 15 seconds.

Co-agulase of the plasma was observed to differentiate Co-agulase positive and co-agulase negative organisms.

(2) <u>For streptococci</u> :

Again these were identified by their colony character , the changes they produce in sheep blood agar and microscopical appearance.

3) For corynebacterium group :

Diphteriod were spotted by their characteristic growth which is usually poor on ordinary media 24 hours colony about 1 mm in diameter, greyish white or creamy colour, and on blood agar not produce haemolysis. And by their morphology being Gram + ve, bacilli , non motile and arranged in pairs Parallel or at angles.

4) For Gram negative bacilli :

These were identified by their colony characters. The species were differentiated by biochemical reactions using entero tube II (F. Hoffmann-LaRoche Co limited company, Diagnostica , Basle, switzerland) which is a commorcial preparation used for identification of G-ve bacilli; aerobic and anaerobic, the Enterotube II contains the following biochemical reactions :

1) Sugars fermentation glucose,

, lactose, arabinose, sorbitol,

2) H_2S production.

3) Indol production test.

4) V.P. reaction.

5) urease test.

6) Citrate test.

The Enterotube was used as follow :

1) Both caps were removed, the tip of inoculating needle is under the white cap. without flaming the needle picked a well isolated colony directly onto the tip of the needle, without puncturing the agar.

2) Enterotube II was inoculated by first twisting the needle, then withdrowing it through all the compartments using a turning motion. Then the needle was reinserted without sterilizing, into Enterotube II until the notch on the needle was aligned with the opening of the tube. The tip of the needle should be

Fig. 2 Entero. tube II
for identification of Gr.-ve bacilli

seen in the citrate compartment.

3) The needle was broken at the notch by bending the portion of the needle remaining in the tube maintained anaerobic conditions necessary for the fermentation of glucose, decarboxylation of lysine and ornithine and the detection of gas production.

4) With the broken off part of the needle, holes through the foil covering the air inlets of the last eight compartments (adonitol , lactose, arabinose , sorbitol, v.p., dulcitol/ PA, urea and citrate) were punched, in order to support aerobic growth in these compartments. Then both caps were replaced.

5) Enterotube II was incubated at 37°C, standing it upright as possible in the test tube support with its glucose compartment pointing upwards, or laying it on its flat surface, incubation period was 24 hours.

* Germ tube formation test :

- The yeast is inoculated into 0.5 ml of pooled human sera and incubated for 2 hours at 37°C.

- For convenience, the inoculation can be done with a Pasteur pipet and the pipet left in the tube through the incubation period and then used to transfer the drop of the suspension to a slide.

- A germ tube is a true lateral hyphae extension of the yeast cell with no c nstriction present at the base.

* Chlamydospores formation :

Cornmeal Tween agar, a formulated agar medium stimulates the production of chlanydospores, and blastospore formation in yeasts.

Further identification of candida species were carried out by using API Auxanogram (API system S.A La Balm-Les corottes 38390 Mortaleu vrrcieu as follow :

Fig 3 Germ tube.

Fig. 4 Chlamydospores.

1) Preparation of the inoculum :

a) an ampule of API medium was liquified in a boiling bath, then the ampoule was placed in a water bath at + 42°C + 44°C and the temperature was stabilized for about 10 minutes.

b) The top of the ampoule was snaped off by applying thumb pressure to the flattend end of the plastic cap.

c) A suspension was made in 1 ml of distilled sterile water with fraction of colony from the isolatation medium. From this suspension 2 drops were introduced into the ampoule of API medium, the medium was then homogenised by using a sterile pipette.

2) Preparation of the gallery :

a) In an incubation tray (top and bottom). 5 ml of water was distributed into the small wells in the bottom of the tray.

Fig. 5 API 20c gallary for identification of
Candida Sp.

b) Then a gallary was removed from its packing and placed in the tray. Then the entire gollary was inoculated with the pipette, with avoiding making any convex or concave meniscus.

c) Then closed tray was placed in an incubator at 30°C.

3) <u>Reading</u> :

a) the reactions were read after 24, 48 and possibly 72 hours of incubation.

b) The 10 cupule acted as a negative control for assimilation reactions. Cupules showing a turbidity heavier than that in the control were considered positive.

c) The results were recorded on the appropriate sheet.

4) <u>Identification of organisms</u> :

This was mode from the results obtained with the help of the index. The tests were grouped in threes, and each positive reaction recorded was given a determined numerical value. By adding up the values in each group a seven - figure number was obtained which corresponds to a numerical profile.

Media

1) Sabouraud's dextroseagar which contains :

Dextrose	40 gm.
Peptone	10 gm.
agar	25 gm.
Distilled water.	1 litre.

The pH of the medium is adjusted to 5.6 then sterilized by autoclaving at 120°C for 15 minutes.

2) Sabouroud's dextrose agar with antibiotics
. chloramphenical is added to inhibit the bacterial growth.

3) Corn meel agar (Oxoid LTD, Basing stoke, Hampshire, England).

Formula :

Corn meal extract (from 50 g. whole maize)	2.0 gm
Agar No. 3 (Oxoid L 13)	15.0 gm

Suspend 17 gm of the dehyderated medium in 1 litre of distilled water. Bring to boiling to dissolve completely. Sterilization by autoclaving at 121°C for 15 minutes.

4) MacConkey's agar (Cruickshank, 1982)

* Formulas :

- Pepton 20 gm
- Sodium tourecholate commercial 5 gm
- Water 1 L
- Agar 20 gm
- Neutral red solution. 2 per cent in 50 percent ethanol 3.5 ml.
- Lactose, 10 percent aqueous solun. 100 ml.

Dissolve the p__con and taurochelate (bile solt) in the water by heating. Add the agar and dissolve it in the steamer or autoclave. Adjust the pH to 7.5, Add the lactose and the neutral which should be well shaken before use, and mix. Heat in the autoclave with free steam 100°c) for one hour, then at 115°C for 15 minutes. pour plates.

5. Nutrient agar.

6. and blood agar.

were used to study associated bacteria.

Enterotube II for identification of Gm-ve bacilli

(F. Hoffmann-la Roche-Sco. limited company,

Diagnostrica, Basle, Switzerland).

API 20c system for identification and isolation of Candida

and yeast species.

(API system S.A La Balme-les-Corottes 38390 France.

Montaleu-Vercieu).