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Original & Clinical Research

Eczema in Early Childhood is Strongly Associated with the Development of Asthma and Rhinitis in A Prospective Cohort

Dr. Praveen Kumar Bhopalka[1], Dr. Priam Pankaj[2]

ABSTRACT

Background : This study aimed to estimate the association between eczema in early childhood and the onset of asthma and rhinitis later in life in children.

Methods : A total of 3,124 children aged 1–2years were included. The association between eczema in early childhood and the incidence of asthma and rhinitis later in life was estimated by univariable and multivariable logistic regression modelling.

Results : The prevalence of eczema in children aged 1–2years was 17.6% at baseline. Children with eczema had a 3-fold increased odds of developing asthma (adjusted odds ratio [aOR], 3.07; 95% confidence interval (CI) 1.79–5.27), and a nearly 3-fold increased odds of developing rhinitis (aOR, 2.63; 1.85–3.73) at follow-up compared with children without eczema, adjusted for age, sex, parental allergic disease, parental smoking, length of breastfeeding, site of living, polyvinylchloride flooring material, and concomitant allergic disease. When eczema was divided into subgroups, moderate to severe eczema (aOR, 3.56; 1.62–7.83 and aOR, 3.87; 2.37–6.33, respectively), early onset of eczema (aOR, 3.44; 1.94–6.09 and aOR, 4.05; 2.82–5.81; respectively), and persistence of eczema (aOR, 5.16; 2.62–10.18 and aOR, 4.00; 2.53–6.22, respectively) further increased the odds of developing asthma and rhinitis. Further independent risk factors increasing the odds of developing asthma were a parental history of allergic disease (aOR, 1.83; 1.29–2.60) and a period of breast feeding shorter than 6 months (aOR, 1.57; 1.03–2.39). The incidence of rhinitis was increased for parental history of allergic disease (aOR, 2.00; 1.59–2.51) and polyvinylchloride flooring (aOR, 1.60; 1.02–2.51).

Conclusion : Eczema in infancy is associated with development of asthma and rhinitis during the following 4-year period, and eczema is one of the strongest risk factors. Early identification is valuable for prediction of the atopic march.

INTRODUCTION

The prevalence of eczema has increased to levels of public health relevance in the Western world, especially in children. As the most frequent inflammatory condition in childhood, eczema affects physiological and psychological wellbeing of affected children and results in substantial costs. It has been suggested that early life eczema is a risk factor for the development of asthma later in life. However, evidence for the progression to asthma comes mainly from cross-sectional studies. There are only a few prospective cohort studies that have investigated the association between early life eczema and later onset of asthma and rhinitis. Some of the existing longitudinal studies found no association between eczema and later onset of asthma, and other prospective studies found an eczema/asthma relationship much weaker than expected. This overall weak association might be partly explained by a different effect across eczema subgroups on allergic airway diseases. The severity of eczema has been found to be more closely associated with the risk of developing asthma than the timing of onset, or duration of eczema symptoms. We examined whether eczema in early childhood predicts the later onset of asthma and rhinitis in children and determined the importance of severity, time of onset and persistency of the childhood eczema by analysing data from a large prospective population-based cohort with a follow-up period of 4-years.

MATERIAL AND METHODS

Data collection

Four thousand and twenty children initially aged 1–2-years had answered a baseline questionnaire. The cohort of the current study consisted of 3,124 children who responded both to the baseline and a follow-up questionnaire (response rate: 77.7%).

Inclusion criteria of the baseline survey were all children living in V‰rmland aged 1–5years whose parents gave consent to participate and who answered a postal questionnaire. The study was approved by the regional ethical committee in KMCH, Katihar.

Definition of variables

In addition, questions on doctor-diagnosed asthma and doctor-diagnosed rhinitis were included. The additional questions were: "Has your child been diagnosed with asthma by a physician?" and "Has your child been diagnosed with rhinitis by a physician?" The main outcomes were "4-year cumulative incidence of asthma" and "5-year cumulative incidence of rhinitis". The "4-year cumulative incidence of asthma" was defined as no report of physician-diagnosed asthma and no wheezing at baseline, but physician-

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diagnosed asthma was reported. The main explanatory variable was eczema. Eczema in early childhood was defined by affirmative responses to the question "Has your child had an itchy rash at any time in the last 12-months?" in the baseline.

Secondary analysis was performed for subgroups of eczema: persistent eczema, severe eczema and early onset of eczema, and their association with later development of allergic airway diseases.

Statistical analysis

The association between eczema in early childhood and the development of asthma and rhinitis was estimated by using univariable logistic regression and expressed as odds ratios (ORs) with confidence intervals (CIs). Statistical significance was assessed using the likelihood ratio test (LRT; $p<0.05$). Characteristics of participants and non-participants were compared in drop-out analysis using the chi-square test ($p<0.05$).

RESULTS

The study population consisted of 3,124 children, aged 1–2 years from 2,526 families due to siblinghood. The study population showed equal distribution regarding age and sex. Most children were breastfed (97.4%), and living with two adults (93.8%) in a single-family house (82.4%), with non-smoking parents (77.8%). A high proportion of children had at least one parent with a history of allergic disease (49.0%). In the 1–2-year-old children, the prevalence of baseline eczema was 17.6% (n=551; 95% CI, 16.3–19.0%). The cumulative 4-year incidence of asthma was 3.1% (2.5–3.9) and rhinitis was 5.6% (4.8–6.5).

DISCUSSION

Our study found that eczema in early childhood was strongly associated with the development of asthma and rhinitis during the following 4-year period. Eczema was one of the strongest independent risk factors. Interestingly, when eczema was divided into subgroups, children with early onset of eczema, moderate to severe eczema, and persistence of eczema had the highest odds of developing asthma and rhinitis.

This population-based prospective study confirms that early eczema affects later development of asthma and rhinitis.

Although some previous prospective studies were not able to show an association between early childhood eczema and later development of asthma and rhinitis, our findings are robust and in line with the study by Arshad et al. In addition, similar results regarding severity have been found in both Gustavsson's and Ricci's eczema cohorts, which reported that eczematous children with high severity scores were at increased risk of developing asthma. To the best of our knowledge, our study is the first prospective cohort to show that early onset of eczema or persistent eczema increases the odds of later onset of asthma in both boys and girls, which is in contrast to a previous study that only showed a relationship in boys. Definitions of asthma and rhinitis are important for the interpretation of results. Our sensitivity analysis confirmed that the association between eczema and asthma/rhinitis remained when symptom-based criteria for asthma and rhinitis were used.

Possible explanations for the relationship between eczema and asthma and rhinitis

Evidence from several experimental studies has suggested that impaired epithelial function results in increased sensitization and IgE production. In humans, the theory of epicutaneous sensitization is supported by the observation that exposing atopic children to topical emollients containing peanut protein leads to an increased risk of airway peanut sensitization. Genetic factors, such as the common loss of function mutations within the filaggrin gene, are a risk factor for incident eczema and account for skin barrier dysfunction. Recently, it has been shown that filaggrin mutations affect asthma, which supports the hypothesis that impaired skin function acts as a gateway for allergens, increasing the risk of atopic airways diseases.

Advantages and limitations

A major advantage of our study was its prospective design, which made results less subject to recall bias and allowed assessment of temporal relationships. Our study had a large sample size compared with earlier studies. Our results should be less prone to selection and ascertainment bias because of its population-based design. We also had a high response rate and limited loss to follow-up. There were no differences between the analysed sample and drop-outs in health-related variables; therefore results might have been biased towards 1. A higher prevalence of socioeconomic risk factors in children leaving the study, while assuming that low socioeconomic status is a risk factor for the incidence of asthma, might have biased the results towards 1 as well. Therefore, our conclusions that eczema is associated with later onset of asthma and rhinitis would not change if all children had participated. The advantages of the study design and performance allow generalization of results.

Reporting of eczema by questionnaire might have advantages compared with assessment by physicians, because eczema can be intermittent. The term *"itchy flexural rash in the last 12months"* has been shown to correlate well with diagnosis by a physician in a validation study performed in the UK on children aged 3–11years. Sensitivity in this previous study was 84% and specificity was 93%.

Currently, there is no clear definition of persistence of eczema, which is consistent as reported by Williams. Illi and M^hrenschlager considered eczema as persistent when signs of the disease were present at different points of time. In our study, persistence of eczema was defined as having had eczema at least three times. Therefore, the risk of including children with "short-term rashes only" might be low, but we cannot exclude the possibility that some children classified as having persistent eczema had longer symptom-free intervals. It would have been advantageous to assess the prevalence of eczema more often during the study period.

CONCLUSIONS

Eczema in infancy independently increases the odds of developing asthma and rhinitis during the following 4-year period. This association is present for children with eczema at baseline; however when they are divided into subgroups (severe eczema, early onset of eczema and persistence of eczema) the odds of the incidence of asthma and rhinitis further increases. Identifying risk groups is important for healthcare planning.

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CME Notice

249th CME Programme—Monthly Clinical Meeting

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traumatic injury, anorectal malformation (especially in grade 3) and some cases of obstruction of distal large intestine (benign or malignant).

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ORIGINAL & CLINICAL RESEARCH

Association of Toll-Interacting Protein Gene Polymorphisms with Atopic Dermatitis

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ABSTRACT

Background : Atopic dermatitis (AD) is a common inflammatory skin disorder, affecting up to 15% of children in industrialized countries. Toll-interacting protein (TOLLIP) is an inhibitory adaptor protein within the toll-like receptor (TLR) pathway, a part of the innate immune system that recognizes structurally conserved molecular patterns of microbial pathogens, leading to an inflammatory immune response.

Methods : In order to detect a possible role of TOLLIP variation in the pathogenesis of AD, we screened the entire coding sequence of the *TOLLIP* gene by SSCP in 50 AD patients. We identified an amino acid exchange in exon 6 (Ala222Ser) and a synonymous variation in exon 4 (Pro139Pro). Subsequently, these two variations and four additional non-coding polymorphisms (-526 C/G, two polymorphisms in intron 1 and one in the 3'UTR) were genotyped in 317 AD patients and 224 healthy controls.

Results : The -526G allele showed borderline association with AD in our cohort ($p = 0.012$; significance level after correction for multiple testing 0.0102). Haplotype analysis did not yield additional information. Evaluation of mRNA expression by quantitative real-time polymerase chain reaction in six probands with the CC and six with the GG genotype at the -526 C/G locus did not reveal significant differences between genotypes.

Conclusion : Variation in the *TOLLIP* gene may play a role in the pathogenesis of AD. Yet, replication studies in other cohorts and populations are warranted to confirm these association results.

INTRODUCTION

Atopic dermatitis (AD) is an inflammatory skin disease characterized by pruritus and chronic or relapsing eczematous lesions that commonly presents during early infancy and affects up to 16% of children. AD has a multifactorial background, with genetic predisposition and environmental factors contributing to disease susceptibility. In industrialized countries AD prevalence has increased during the past decades, and it has been postulated in the so-called 'hygiene hypothesis' that the lack of contact to microbial products in early infancy might at least in part be responsible for this increase. There is evidence from prospective studies to support an inverse relationship between AD and exposure to endotoxin, a cell membrane component of gram negative bacteria, early day-care attendance and animal exposure.

Recognition of microbial products such as endotoxin is mediated by the innate immune system. Toll-like receptors (TLRs) are a family of evolutionarily conserved receptors that recognize pathogen-associated molecular patterns (PAMPs), leading to an inflammatory response by induction of interleukins and other pro-inflammatory proteins. Polymorphisms in *TLR* genes have been implicated in various diseases including AD. Yet, the effect of genetic variation in TLR downstream signalling pathways has not been sufficiently studied yet. Toll-interacting protein (TOLLIP) is an adaptor protein that acts as an inhibitory factor in the TLR-signalling cascade. It functions downstream of MyD88 and TIR domain containing adaptor protein (TIRAP) through inhibition of Interleukin-1

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receptor associated kinase 1 (IRAK1) and controls the magnitude of inflammatory cytokine production in response to endotoxin. The *TOLLIP*gene is located on chromosome 11p15 and comprises 6 exons encoding a 274 amino acid transcript. The 11p15 region has so far not been reported as a linkage region for AD in the four published genome screens. Yet, association studies are generally supposed to have a greater power to detect common alleles with modest effects on disease susceptibility than linkage studies. Furthermore, dysregulated inhibition in the TLR-signalling cascade may cause a pathologically increased or reduced inflammatory response, and variations in the *ST2* gene, encoding another inhibitory protein in the TLR pathway, were recently found to be associated with AD. Therefore, we considered the *TOLLIP* gene an interesting candidate gene for AD.

We screened the entire coding region of the *TOLLIP* gene by single strand conformation polymorphism (SSCP) analysis in 50 AD patients in order to identify coding variation that might play a role for AD pathogenesis. Subsequently, the identified polymorphisms were genotyped in 317 AD patients and 224 healthy controls to evaluate a possible association with AD. In order to provide a more complete and valuable assessment of variation in the *TOLLIP* gene, we additionally typed four non coding polymorphisms (located in the promoter and intronic regions as well as in the 3'UTR) that were chosen from the HapMap database.

MATERIAL AND METHODS

Subjects

317 unrelated patients with atopic dermatitis were admitted at Department of Dermatology, Katihar Medical College & Hospital, Katihar, Bihar including 193 children and 124 adults. The AD diagnosis was based on the presence of clinical features, including pruritus, eczema with age-dependent differences in location, xerosis and chronic or relapsing dermatitis. In addition, all investigated AD patients had a positive family history for atopic diseases. 224 control samples from adults without known allergies, asthma or AD were collected in the same private practice as the AD patients. We specifically chose to use non-allergic adults as controls because for diseases as frequent as AD, the risk remains very high for asymptomatic children to develop an allergic disease during childhood or even adulthood. The control subjects underwent clinical examination in order to exclude symptoms of AD, asthma or allergic rhinitis, had no self-reported allergies or allergic symptoms and no first degree relatives with known allergic diseases. Informed consent was obtained from all subjects.

Preparation of DNA and RNA

DNA was extracted from EDTA anti-coagulated peripheral blood by using a standard salting-out method. Total RNA samples were extracted from EBV-transformed lymphoblastoid cell lines using TriFast reagent (Peqlab) according to the manufacturer's instructions up to the point of phase separation. Afterwards, the aqueous phase was transferred to KMCH laboratory and processed according to the manufacturer's protocol. OD_{260} and OD_{280}were determined and the RNA was stored at -70 C until use.

Single strand conformation polymorphism analysis

The entire coding region of the *TOLLIP* gene was screened for polymorphisms by polymerase chain reaction (PCR) with consecutive single strand conformation polymorphism (SSCP) analysis. PCR reactions were performed in a total volume of 10 µl, containing 50 ng DNA, 200 mmol of each dNTP, 0.4 U Taq polymerase, GC-Buffer, and 0.1 µl [^{32}P] ±-CTP (10 mCi/ml). Thermal cycling was conducted in a thermal cycler. After a denaturation step at 95 C for 5 minutes followed by two initial cycles at 6 C and 3 C above the annealing temperature, 28 cycles with 95 C (30 sec), annealing temperature (30 sec) and 72 C (30 sec) were run. Further details for primers and conditions are given in table 1. For SSCP analysis, 3 µl of the PCR product was mixed with 7 µl of SSCP loading buffer (95% formamide, 20 mM EDTA, 0.05% xylene cyanol and 0.05% bromophenol blue), denatured for 5 minutes at 95 C and thereafter directly cooled on ice. Then 2.5 µl were applied on 6% polyacrylamide gels (38% acrylamide, 2% bisacrylamide) containing 1 TBE buffer (890 mM Tris-borate, 20 mM EDTA, pH 8.3) and 10% glycerine in a SQ3 apparatus at 55 W for about 210 minutes. The room temperature was constantly held at 4 C during SSCP analyses.

DNA sequencing

DNA samples showing mobility shifts on SSCP gels were directly sequenced on an automated capillary DNA sequencer, using the BigDye cycle sequencing kit as described in the manufacturer's instructions.

Genotyping

All six polymorphisms in the *TOLLIP* gene were genotyped by restriction enzyme digestion. Patient and control samples were amplified using the same primers and conditions as described above, except for the inclusion of radioactivity. After digestion with the respective enzymes for at least three hours, the fragments were separated on 2% agarose gels in 1 TBE buffer (45 min, 200 V) and visualized by staining with ethidium bromide.

Measurement of mRNA levels by quantitative real-time PCR

We carried out real-time PCR on an I-Cycler. The quantity of PCR products was determined after each round of amplification using the fluorescent dye SYBR Green I (Qiagen), which binds double-stranded DNA. The primer sequences (F: 5'-AGTACGGAGGCGCAGTGG-3'; R: 5'-AGGCGCAGTCGGCAGTAG-3') represent parts of two adjacent exons in order to prevent amplification of potential traces of contaminating genomic DNA. PCR reactions were run in triplicates (95 C for 5 min, 56 C for 30 sec, 95 C for 1 min, 55 C for 30 sec). Fluorescence was recorded at the end of each extension step, and a melting curve was obtained at the end of each run. Quantification of the house-keeping gene glyceraldehyde-3-phosphate dehydrogenase-(GAPDH-) mRNA (primers F: 5'-TGTGTCCGTCGTGGATCTGA-3'; R: 5'-CCTGCTTCACCACCTTCTTGA-3'; product size 76 bp) was used as a control for data normalization. After the PCR, products were analyzed on agarose gels in order to verify band sizes and purity. Expression was assessed by evaluating threshold cycle (C_T) values. The C_T values were calculated by the system software (iCycler), and the

relative amount of expressed RNA was calculated using Livak 's method.

RESULTS

Screening of the coding region of the *TOLLIP* gene revealed two exonic polymorphisms: a synonymous variation in exon 4 (Pro139Pro) and an amino acid substitution in exon 6 (Ala222Ser). Four additional SNPs in non-coding regions (-526 C/G, Intron1a, Intron1b, 3'UTR) were chosen from the HapMap database. Evaluation of these polymorphisms in 317 AD patients and 224 controls showed a modest association of the -526 C/G promoter SNP with AD. The G allele of the -526C/G promoter polymorphism was significantly more frequent in AD patients than in healthy controls (12.7% *vs.* 7.7%, uncorrected p-value = 0.012). This polymorphism was in strong linkage disequilibrium (LD) with the Intron1a SNP, and the Pro139Pro variation was in moderate LD with the 3'UTR SNP (r^2 = 0.64 and 0.20, resp.). Therefore, we felt that Bonferroni correction for independent tests would be overly conservative and applied Li & Ji's multiplicity correction for SNPs that are in LD with each other and, thus, dependent. This approach yielded a significance level of 0.0102 to keep an overall level of 0.05 in both the cases and the control group. Our finding (p = 0.012), therefore, showed borderline significance, being slightly higher than the significance level. Additionally, the Intron1a G allele was more frequent in AD patients (8.8% *vs.* 5.6%) while the 222Ser allele showed an increased frequency in healthy controls (5.4% *vs.* 2.7%). Yet, significance was not evident after correction for multiple testing for both polymorphisms. For the other three variations, no difference in allele or genotype frequencies was found between AD patients and controls. We repeated our analyses within the subgroup of patients with elevated IgE levels and saw the same trend within this subgroup, with the -526G, the Intron1a G allele and the 222Ala allele being more prevalent in cases than in controls (data not shown). Yet, because of the reduction in sample size, the results did not reach statistical significance. All investigated polymorphisms were in Hardy-Weinberg equilibrium in cases and controls. We performed power analyses with the Genetic Power Calculator. Given a multiplicative model with a genotypic relative risk of 2 for heterozygotes and 4 for homozygotes and a D' of 0.9, we would have 96% power to detect a potential effect. Choosing more conservative parameters, with a genotypic relative risk of 1.5/2.25 and D' of 0.8, would yield only 48% power. Haplotype analysis did not yield significant results. We did not find evidence for a pair wise interaction between SNPs within the *TOLLIP* gene.

DISCUSSION

The innate immune response initiated by TLRs is an important mechanism in defense against pathogenic microorganisms, and variations in *TLR* genes have been implicated in the pathogenesis of allergic as well as autoimmune diseases. Several studies have pointed out that the innate immune system plays an important role in AD pathogenesis. For example, epidemiological studies showed an inverse relationship between AD and exposure to endotoxin (lipopolysaccharide, LPS), early day-care attendance and animal exposure, suggesting that lack of contact to microbial products might increase risk for AD. Further, TLRs were found to be up-regulated in circulating monocytes from AD patients. A polymorphism in the *TLR2* gene was associated with a severe AD phenotype. Additionally, variations in the *CD14* gene, encoding part of the cell membrane receptor for LPS, as well as in the *CARD15* gene, encoding an intracellular LPS receptor, have shown associations with AD. Thus, genes in the downstream pathway of TLR signalling also constitute reasonable candidate genes for this frequent skin disease. We demonstrate here first evidence for an association of AD with variation in the *TOLLIP* gene, encoding a protein with an inhibitory function in the TLR signalling pathway.

Screening of the coding region of the *TOLLIP* gene by SSCP identified two coding variations: Pro139Pro and Ala222Ser. The rate of detection of nucleotide variants by SSCP varies between 80% and close to 100% under optimized conditions. Thus, it is possible that rare variations might have been missed with this approach. Yet, for multifactorial diseases like AD, common instead of rare variations have been suggested to play a role for pathogenesis. Therefore, we chose to additionally type four frequent SNPs in non-coding regions from the HapMap database. We found a modest association of a promoter polymorphism (-526 C/G) in the *TOLLIP* gene with AD. Since Bonferroni correction has been controversial for genetic association studies because it might be overly conservative, we chose to use a method for multiplicity correction when SNPs in linkage disequilibrium (LD) are tested that was introduced by Li & Ji. Our observed p-value of 0.012 for the -526 C/G promoter SNP was borderline significant with respect to Li & Ji's method (0.0102). We therefore believe that it might represent a true association. Haplotype analysis did not yield additional information.

Interestingly, functional SNPs in the distal promoter of the *ST2* gene were recently found to be associated with AD in the Japanese population. Both TOLLIP and ST2 exert inhibitory functions in the TLR cascade, and for the *TOLLIP*gene we also saw the most significant although borderline association with the promoter SNP. Therefore, we decided to further explore the role of the -526 C/G variation for AD. Investigation of potential binding sites for transcription factors at the -526 C/G locus using a transcription factor prediction program predicted differential binding of two transcription factors (AP2- and E2F) for the two different alleles at this locus. Interestingly, E2F is an important factor in the control of skin proliferation, and the AP2 family appears to regulate the expression of genes required for the development of ectodermal tissues, including skin. In order to detect a possible direct influence of the *TOLLIP* -526C/G polymorphism on mRNA expression, we performed quantitative real-time PCR for measurement of mRNA amounts in lymphoid cell cultures from six probands with the C/C and six with the G/G genotype at this locus. Yet, we were unable to find differences in mRNA expression with this method. There are several explanations for this finding. First, a variation that is more distal to the promoter and in LD with the -526 C/G polymorphism or some promoter haplotypes might be the true disease-associated variation. In fact, the recently

identified functional SNPs in the *ST2* promoter are also located very distal from the transcription start (-26999 and -27639, resp. Second, measuring mRNA expression *via* quantitative real-time PCR may not represent the most sensitive method to detect moderate differences in promoter function. Third, expression studies in keratinocytes instead of blood B-lymphocytes would be more informative for a chronic skin disease such AD. Finally, since our association results are somewhat borderline, there may indeed be no functional significance of the analyzed promoter SNP. Yet, functional studies including electro-mobility shift assays (EMSA) are needed to further evaluate the functional relevance of the -526 C/G promoter polymorphism in the *TOLLIP* gene.

We cannot exclude the possibility that the association result we found for the *TOLLIP* promoter polymorphism may be caused by another SNP in *TOLLIP* or a neighbouring gene that is in LD with this variation. For example, the intron 1a SNP that is in LD with the -526C/T polymorphism and the Ala222Ser variation in exon 6 also showed marginal evidence for an association with AD. Further, we are aware of the fact that genetic association studies bear the risk of false-positive results caused by hidden population substructures. We consider the risk that our results are caused by hidden population substructures to be marginal, at most. Yet, population stratification could in principle contribute to the borderline significant results we observed in our association study. Finally, the relatively small size of our sample bears the risk of small power, potentially causing false-negative association results. Yet, power analyses indicated that considering a multiplicative model with a genotypic relative risk of 2 for heterozygotes and 4 for homozygotes and a D′ of 0.9, we would have a 96% power to detect a potential effect of a chosen marker. Choosing more conservative parameters, with a genotypic relative risk of 1.5/2.25 and D′ of 0.8, would yield only 48% power. On the other hand, the cohort we present here has been thoroughly recruited by a single physician, so that it constitutes a highly controlled sample with exclusion of many potential confounders. In any case, the borderline evidence for association of *TOLLIP* variation with AD that we present in this study clearly needs to be replicated in additional studies and populations in order to rule out false positive results.

CONCLUSION

We present first evidence for an association of *TOLLIP* variation with atopic dermatitis. Our results, although borderline, support the concept that genetic variation in the TLR system may play an important role in the pathogenesis of AD. In combination with recently published association findings for the *ST2* gene, we suggest that dysregulation of inhibition in the TLR pathway might be of special importance. Yet, the exact mechanism by which variation in negative regulators of TLR signalling may influence AD pathogenesis still needs to be explored. Targeting TLRs has already been discussed as a novel therapeutic option for allergic diseases. Thus, better understanding of the molecular pathogenesis of AD could eventually lead to new options in prevention and treatment of this frequent skin disease.

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E 1 : Prolongation of pregnacy with tocolytic therapy

›n	Nifedipine (50)	Ritodrine (50)
	5	15
:8hrs	45	35
7days	35	30
37wks	25	13

BLE 2 : Outcome of treatement

	Nifedipine	Retodrine
	45	35
	5	15

.E 3 : Side effects accociated with tocolytic therapy

ects	Nifedipine	Retodrine
tation	4	21
iache	10	—
iing	2	—
hlessness	—	5
onary oedema	—	2
6. Fetal tachycardia	—	20
7. Nausea vometing	—	5

TABLE 4 : Neonatal outcome

Parameters	Nifedipine	Ritodrine
Mean gestation age of birth	35 wks	34wks
NICU admission	27	32
RDS	7	8
Perinatal death	5	7

CONCLUSION

Nifedipine as a tocolytic was more successful in delaying the delivery for the next 48hrs. Mean prolongation of gestation was also higher. It is cheap , effect9ive, with fewer and less serious side effects and is more compliant to patient than ritodrine.

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RACT

kground : Vitiligo is the most nt pigmentary disorder which occurs ide, with an incidence rate between ercent. It is anticipated that the ry of biological pathways of vitiligo nesis will provide novel therapeutic rophylactic targets for future hes to the treatment and prevention go. The purposes of this study were ng the efficacy of supplemental zinc treatment of vitiligo.

hods : This randomized clinical trial nducted for a period of one year. ve patients among 86 participants gible to entrance to the study. The in two equal randomized groups ical corticosteroid and combination zinc sulfate-topical corticosteroid.

lts : The mean of responses in the eroid group and the zinc sulfate-teroid combination group were and 24.7%, respectively.

clusion : Although, the response to eroid plus zinc sulfate was more ticosteroid, there was no statistically nt difference between them. It appeared that more robust long-term randomized controlled trials on more patients, maybe with higher doses of zinc sulfate, are needed to fully establish the efficacy of oral zinc in management of vitiligo.

Comparison of Therapeutic Efficacy of Topical Corticosteroid and Oral Zinc Sulfate-Topical Corticosteroid Combination in the Treatment of Vitiligo Patients

Dr. Praveen Kumar Bhopalka[1], Dr. Pritam Pankaj[2]

INTRODUCTION

Vitiligo has been known for thousands of years because of its visually phenotype. It is characterized by acquired, idiopathic, progressive, circumscribed hypomelanosis of the skin and hair, with total absence of melanocytes microscopically.

Vitiligo is the most prevalent pigmentary disorder, occurs worldwide, with an incidence rate between 0.1-2%, irrespective of age, race, ethnic origin, or skin color. Both sexes are equally afflicted. In some studies, a female preponderance has been reported, but the discrepancy has been attributed to a presumed increase in

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reporting of cosmetic concerns by female patients. Vitiligo commonly begins in childhood or young adulthood, with peak onset of 10 to 30 years, but it can develop at any age.

It is generally agreed that there is an absence of functional melanocytes in vitiligo skin and that this loss of histochemically recognizable melanocytes is the result of destruction. The etiopathogenesis of vitiligo is complex, and includes genetic factors, autoimmune process, infectious factors, and psychological factors (stress and personality characteristics of patients).

Zinc is one of the important trace elements related to health and disease. Zinc in combination with other micronutrients such as copper, cobalt, nickel, iron, manganese, and calcium plays an important role in the process of melanogenesis. With searching the computerized bibliographic database Pub Med, we found no study of zinc efficacy in treatment of vitiligo, which motivated us to carry out this study.

MATERIAL AND METHODS

This clinical trial was conducted for a period of one year from March of 2015 to February of 2016. Eighty six vitiligo patients from 102 patients who attended the Department of Dermatology, Katihar Medical College & Hospital, Katihar, Bihar participated in the study. The KMCH, Katihar, Ethical Committee permission was obtained before performing the study. The informed consent was prepared including the definition of vitiligo, traditional therapeutic approaches and their efficacy and safety, the process of trial and the probable complication of zinc as a therapeutic new approach. According to this consent, the patients could deny the study whenever the drug complication was intolerable for them.

At first, a questionnaire was completed for each patient, which included the data of demographic status, duration of vitiligo, medical and drug history, familial status for vitiligo and pregnancy status in females. Then, for all participants, laboratory tests were recommended which comprised complete blood count and differentiation of white blood cells, fasting blood sugar, serum calcium, phosphorus and zinc levels, liver function (AST, ALT, Alk Ph and Bil), renal function (BUN, Creatinin), and thyroid function tests (T3, T4, TSH and T3RUP), urinalysis and stool examination.

In the second step, among the patients, the eligible ones who had inclusion criteria were selected; The eligible patients for continuing the second step were randomized in two treatment groups. The first group took topical corticosteroid as 0.05% clobetasol propionate cream in isopropyl alcohol 65 preparation (in equal proportion) for the body and 0.1% triamcinolone acetonide cream for the face and flexures, two times daily. For the second group, topical corticosteroid (compatible with the first group) admixed with oral zinc sulfate (220-mg capsule) in dose of 2 capsules per day in teenager and adults and 10 mg/kg of capsule or syrup for children, were prescribed. For the second group, serum zinc level was repeatedly measured 1 and 3 months after commencing the treatment.

All patients were assessed 1, 3 and 4 months after beginning the treatment. For comparing, we considered the largest patch as the target lesion. This target patch was selected in the way that lesions in exposed area and distal parts of limbs were not included as target lesions; so we omitted the probable bias in evaluation of response regarding to probable more rapid response in exposed areas or slower response in the hairless areas of extremities. The surface of the target lesion was measured by two physicians with a crossed sheet and a photograph was prepared for the next comparing. At the next stages, we determined the response rate regarding to the size of the target lesion.

Eventually, using the software of SPSS (Version 15), results were analyzed. P value < 0.05 was considered to be statistically significant.

RESULTS

A total of 86 patients with vitiligo were studied. Among these patients, 39 (45.3%) were female and 47 (54.7%) were male. Totally, 39 (45.3%) of the patients had abnormal laboratory tests, who were excluded from continuing study. The serum zinc were increased in 4 (4.7%) patients, and decreased in 9 (10.5%) patients.

According to the results with considering the inclusion criteria, out of the 86 patients, 35 were eligible for continuing the study. Then, the patients were divided in two groups, randomly; randomization in the two groups of therapy and control was performed by computerized number tables. The first group receiving topical corticosteroid included 16 (45.7%) subjects, and the second group receiving topical corticosteroid plus oral zinc sulfate was consistent of 19 (54.3%) subjects.

Considering the two treatment groups based on the sex frequency, using Pearson Chi-Square test with P-value of 0.45, showed no statistically significant difference.

The minimum, maximum and mean of age in the first group were 13.0, 57.0 and 32.2 (± 12.58), respectively, and for the second group were 11.0, 59.0 and 30.5 (± 12.11), respectively. Comparing the two treatment groups, in the view of age, with T-test and P-value of > 0.05 showed no statistically significant difference.

In the aspect of vitiligo involvement, using T-test and P-value of 0.8, no significant difference was seen between the two groups. The mean of involvement was 11.0% (± 6.6%) of body surface in the first group, whereas was 10.6% (± 8.1%) of the body surface in the second group.

In the first group, one patient (6.3%), and in the second group also one patient (5.3%) showed decreased serum zinc level. To compare the two groups in the view of serum zinc level, there was no statistical significance according to Fisher's exact test and P-value of 1.00.

From the first group, one patient (6.3%) was excluded from the study because of discontinuing the drug. In second group, 3 patients (15.8%), because of refuting reference, and one case (5.3%), because of rising of serum zinc level, were excluded from the study. So, in both of the two groups, 15 patients continued the study to the end of forth month. In the first group, out of 15 patients, one (6.3%) showed no response during 4 months of the study, considering with Fisher's exact test and P-value of 1.00, had no statistically significance.

Both of the two groups showed no response during the first month of the therapy. The mean of responses in the third

and forth months, in the first group were 19.3% (± 9.3%) and 21.43% (± 11.6%), respectively and for the second group, were 20.8% (± 8.7%) and 24.7% (± 11.0%), respectively (Table 2). Although, the response in the second group were more than the first group, T-test revealed no statistically significant differences between the two groups, in the third and forth months with P-values equal to 0.6 and 0.4, respectively. To conclude, topical corticosteroid plus oral zinc sulfate had no preference on topical corticosteroid only.

In the view of the complication of zinc sulfate, only 2 (13.3%) patients of the second group complained of a little tolerable gastric burning.

DISCUSSION

Vitiligo is an acquired depigmenting disorder due to loss of melanocytes and the resultant absence of pigment production affecting skin and mucosal surfaces, with a prevalence of about 1-4%.

Although neither life threatening, nor symptomatic (except that depigmented patches burn easily when exposed to the sun) the effect of vitiligo can be cosmetically and psychologically devastating, resulting in low self-esteem, poor body image, and difficulties in sexual relationships. It is a frustrating condition to treat, spontaneous repigmentation occurs in more than 15% to 25% of cases. Sun protection of the vitiliginous areas with sunblocks is important, which help prevent sunburn and thus may lessen photodamage as well as the chance that a Koebner phenomenon will occur. Sunscreens also decrease tanning of the uninvolved skin and therefore lessen the contrast with vitiliginous lesions. Cosmetic improvement can be achieved by camouflage products and self-tanning dyes.

Because the disease is still not understood, there is a plethora of different treatments including topical corticosteroids, calcineurin inhibitors, vitamin-D derivatives, phototherapy (ultraviolet [UV] A, narrowband UVB), photochemotherapy (psoralen plus UVA [PUVA], psoralen with sunlight [PUVAsol]), surgical techniques, excimer laser, topical prostaglandin E (PGE2), and combinations of topical therapies and light treatment. Complementary therapies have also been used, the most interesting being ginkgo biloba, and levamisole which have been reported to have immune-modulating properties. Pseudocatalase cream with Dead Sea climatotherapy are also compatible with repigmentation. Topical fluorouracil, topical melagenina I and II, minoxidil, oral L-phenylalanine, homeopathy, ayurvedic medicine, climtologic, and balneologic therapies are as altemative therapy for vitiligo.

Zinc is one of the important trace elements related to health and disease. Essentiality of zinc is related mainly to its function as the metal moiety of important enzymes. The most important of these processes are cellular respiration, cellular utilization of oxygen, DNA and RNA reproduction, maintenance of cell membrane integrity, and sequestration of free radicals.

Zinc in combination with other micronutrients such as copper, cobalt, nickel, iron, manganese, and calcium plays an important role in the process of melanogenesis. They catalyze the rearrangement of dopachrome to form 5,6-dihydroxy indole-2 carboxylic acid (DICA), and enhancement of eumelanin polymer formation from monomers. This process is at the final stage of eumelanin formation in melanogenesis.

The most frequent adverse effects of zinc salts given orally are gastrointestinal and include abdominal pain, dyspepsia, nausea, vomiting, diarrhea, gastric irritation, and gastritis.

There are few controlled trials assessing efficacy of natural health products (e.g. vitamins, minerals, herbal medicines and other supplements) for vitiligo, but those that have been published generally show weakly positive outcomes with few adverse reactions. On the other hand, with searching the computerized bibliographic database Pub Med, we found no study of zinc efficacy in treatment of vitiligo. It appeared that our study is the first one to investigate zinc efficacy in the treatment of vitiligo.

Analysis of the zinc level in the study of Shameer *et al* revealed a reduced level in 21.6% of the patients. Only one patient showed elevated level of zinc. In this study, the serum zinc level in the control group was within the normal range. This differences between two groups was statistically significant ($P < 0.0002$). In another study, Arora *et al* showed that serum zinc was lower in vitiligo patients than control group, but this difference was not statistically important. In our study, the serum zinc level were normal in 73 (84.9%), increased in 4 (4.7%), and decreased in 9 (10.5%) of the patients. Unfortunately, we had no control group for comparing the serum zinc level. In spite of these, our study compared with Shameer's one, revealed lower frequency of reduced serum zinc level and higher frequency of increased serum zinc level.

This study showed that the response to the oral zinc sulfate-topical corticosteroid combination was more than the topical corticosteroid alone, but T-test revealed no statistically significant difference between them.

CONCLUSION

We conclude that topical corticosteroid plus oral zinc sulfate had no preference on topical corticosteroid only. Considering the more effect of corticosteroid plus zinc sulfate compared with corticosteroid alone, it appears that more robust long-term randomized controlled trials with more patients, maybe with higher doses of zinc sulfate, are needed to fully establish the efficacy of oral zinc in management of vitiligo.

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Trends in Lipid Profiles in Patients with Psoriasis

Dr. Praveen Kumar Bhopalka[1], Dr. Pritam Pankaj[2]

ABSTRACT

Background : Psoriasis is associated with an atherogenic lipid profile but longitudinal changes in lipids around disease onset are unknown. The purpose of our study is to examine the effect of psoriasis onset on serum lipid profiles.

Methods : We compared changes in lipid profiles in a population based incident cohort of 689 patients with psoriasis and 717 non-psoriasis subjects. All lipid measures performed 4 years before and after psoriasis incidence/index date were abstracted. Random-effects models adjusting for age, sex and calendar year were used to examine trends in lipid profiles.

Results : There were significant declines in total cholesterol (TC) and low-density lipoprotein (LDL) levels during the 4 years before and after psoriasis incidence/index date in both the psoriasis and the non-psoriasis cohorts, with a greater decrease noted in the TC levels (p=0.022) and LDL (p=0.054) in the non-psoriasis cohort. High-density lipoprotein (HDL) levels increased significantly both before and after psoriasis incidence date in the psoriasis cohort. Triglyceride (TG) levels were significantly higher (p<0.001), and HDL levels significantly lower (p=0.013) in patients with psoriasis compared to non-psoriasis subjects. There were no differences in prescriptions for lipid lowering drugs between the two cohorts.

Conclusions : Patients with psoriasis had a significant decrease in TC and LDL levels during the 4 years before psoriasis incidence. Higher mean TG and lower mean HDL levels were noted in the 4 years before psoriasis incidence. These changes are unlikely to be caused by lipid lowering treatment alone and require further exploration.

KEYWORDS : *Psoriasis Lipids Epidemiology.*

INTRODUCTION

Psoriasis is becoming understood as a systemic and inflammatory disease with increased associated comorbidities, including risk for cardiovascular (CV) disease. Among the comorbidities which predispose patients with psoriasis to increased risk of CV disease are psoriatic arthritis and sleep disorders, as well as other traditional risk factors for CV disease, including atherogenic lipid profiles.

Little is known about the impact of psoriasis on lipids in patients with new onset psoriasis. A number of studies have demonstrated a pro-atherogenic lipid profile in psoriasis, but not all. There is now growing evidence that psoriasis is associated with enhanced atherosclerosis and unfavorable lipid profiles, but some of the conflicting results examining this relationship may be at least in part influenced by the effects of inflammation related to psoriasis, as well as its treatment.

To address the relationship between psoriasis and the profile of potentially atherogenic lipids, we performed a longitudinal study of changes in lipid profile during the period surrounding psoriasis incidence in a population-based cohort of patients with psoriasis and a comparison cohort of non-psoriasis subjects.

AIM OF THE STUDY

The aim of this study was to determine the effect of psoriasis onset on serum lipid profiles by comparing lipid profiles in patients with psoriasis and non-psoriasis subjects during the 4 years before and 4 years after psoriasis incidence/index date.

MATERIAL AND METHODS

This retrospective longitudinal cohort study was conducted in Department of Dermatology, Katihar Medical College & Hospital, Katihar, Bihar.

In case of a doubtful diagnosis, the medical record was reviewed by the dermatologist co-investigator. Incidence date was defined as the physician diagnosis date. Subjects with prevalent psoriasis, subjects with missing medical records, and those who denied research authorization were excluded.

For each patient with psoriasis, a non-psoriasis subject of similar age, sex, calendar year and length of medical history prior to index date was randomly selected from the same population. Each non-psoriasis subject was assigned an index date corresponding to the psoriasis incidence date of the corresponding patient with psoriasis.

All lipid measures performed for clinical indications from 4 years prior to psoriasis incidence/index date to last follow-up were abstracted. These included total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides [TG]). In accordance with Adult Treatment Panel III (ATPIII) guidelines, abnormal lipid levels were defined as TC 240 mg/dL, LDL 160 mg/dL, TG 200 mg/dL or HDL <40 mg/dL. Data on prescription of lipid-lowering medications (i.e., statins and other lipid-lowering drugs) and body mass index were also collected. Obesity was defined as body mass index 30 kg/m2. The study protocol was approved by the Institutional Review Boards from KMCH, Katihar, Bihar.

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Statistical methods

Descriptive statistics (means, percentages, etc.) were used to summarize the lipid measures. Demographics of patients with psoriasis and non-psoriasis subjects, as well as subjects with and without lipid measures, were compared using Chi-square tests and t-tests. Random effects models adjusting for age, sex, and calendar year of psoriasis incidence/index date were used to analyze the trends in lipid profiles during the time period from 5 years before to 5 years after psoriasis incidence/index date. These models accounted for multiple measurements per subject using random effects to fit individual intercepts and slopes for each subject. Generalized additive models with smoothing splines were used to illustrate the trends in lipid levels over time.

RESULTS

A total of 963 Olmsted County, MN residents aged 35 years were diagnosed with psoriasis. Of these, 689 patients had at least one lipid measure during the time period from 5 years before to 4 years after psoriasis incidence date and thus were included in the study (Table 1). The total number of lipid measurements in patients with psoriasis was 3,561 (median 3 measurements per patient). Patients with psoriasis without lipid measures during the time period of interest (n=274) were younger (mean age 51.9 years, p<0.001) and were more likely to be male (59.5% vs. 46.2%) than patients with psoriasis in whom lipid measures were obtained. Within the same time period, lipid measurements were also available for 717 non-psoriasis subjects with a total of 3,678 lipid measurements (median 3 measurements per subject). The spread of measurements were similar for both groups (mean 9.38 years, standard deviation (SD) 1.36 years for psoriasis and mean 9.32 years, SD 1.45 years for non-psoriasis (p=0.68). Non-psoriasis subjects had similar age and sex characteristics compared to patients with psoriasis with lipid measures. In addition, the prevalence of lipid-lowering drug use was similar among psoriasis and non-psoriasis subjects (37% vs. 39%, respectively; p=0.33). Data on systemic medication used was available in 551 of the 689 psoriasis subjects. Of these, 49 (8.9% of the 551) used systemic medication during the study period.

Demographic characteristics and length of follow-up were similar in both psoriasis and non-psoriasis subjects. The median follow-up was 4 years in each cohort as the follow-up was truncated at 4 years for these analyses. A full 4 years of follow-up after psoriasis incidence/index date was available for 533 (77%) of patients with psoriasis and 563 (79%) of non-psoriasis subjects. In addition, 628 (91%) of psoriasis and 650 (91%) of non-psoriasis subjects had 4 years of available information prior to psoriasis incidence/index date. The mean time prior to psoriasis incidence/index date was 4.7 years in both groups with a median of 4 years.

Table 2 shows the mean difference in lipid levels between the psoriasis and non-psoriasis cohorts adjusted for age, and calendar year. Overall, TG levels were significantly higher in psoriasis compared to non-psoriasis subjects (on average 16.8 mg/dL; p<0.001) and HDL levels were significantly lower in patients with psoriasis compared to non-psoriasis subjects ("1.9 mg/dL; p=0.013). Subgroup analyses were performed for males and females, and non-obese and obese subjects, as well as statin users. Differences in TG levels between psoriasis and non-psoriasis subjects were more pronounced among males, obese subjects and statin users, and less pronounced among females, non-obese patients and those who did not use statins. No difference in mean lipid levels of TC or LDL between psoriasis and non-psoriasis subjects were noted overall or in any subgroups of patients.

The trends in lipids during the 4 years before and 4 years after psoriasis incidence/index date in the psoriasis and non-psoriasis cohorts. There were significant declines in TC and LDL levels during the 4 years before and the 4 years after psoriasis incidence/index date in both the psoriasis and the non-psoriasis cohort. HDL levels increased significantly both before and after psoriasis incidence date in the psoriasis cohort. There was no significant change in HDL during the 4 years before index date in the non-psoriasis cohort. Subgroup analyses were performed for statin users. Statin users experienced significant declines in TC and LDL in both the psoriasis and non-psoriasis cohorts, as expected. However, there were no differences in the trends in lipids between the psoriasis and non-psoriasis groups among the subgroup of patients taking statins.

DISCUSSION

Both psoriasis and dyslipidemia are risk factors for cardiovascular disease in patients with psoriasis. To examine this relationship, we performed this retrospective study of lipid profiles during the period surrounding psoriasis incidence in a population-based cohort of patients with psoriasis and a comparison cohort of non-psoriasis subjects. There were significant declines in TC and LDL levels during the 5 years before and the 4 years after psoriasis incidence/index date in both the psoriasis and the non-psoriasis cohorts with a greater decrease noted in the TC levels and LDL in the non-psoriasis cohort. HDL levels increased significantly both before and after psoriasis incidence in the psoriasis cohort, but significant increases were seen only in the 4 years after index date for the non-psoriasis cohort. TG levels showed an increase in the 4 year period before psoriasis incidence in the psoriasis cohort and a decline prior to index date in the non-psoriasis cohort, but they did not achieve statistical significance. Lipid trends during the 4 years after incidence/index date were largely similar in both cohorts, with a decrease in TC, LDL and TG and an increase in HDL, with no significant differences between the trends in both groups.

There is conflicting information about how lipid profiles might be affected by psoriasis. An atherogenic lipid profile namely, higher cholesterol, LDL and TG levels were noted in psoriasis patients in some studies, with no significant difference between patients and controls noted in others. It is also unknown whether the observed lipid changes are primary or secondary to the chronic inflammatory process or its treatment.

We sought to examine the potential relationship of psoriasis and dyslipidemia by examining lipid profiles in patients around the time of psoriasis onset. As far as we can ascertain, this is the first population-based study to describe longitudinal lipid trends in psoriasis and non-psoriasis populations both before and after psoriasis

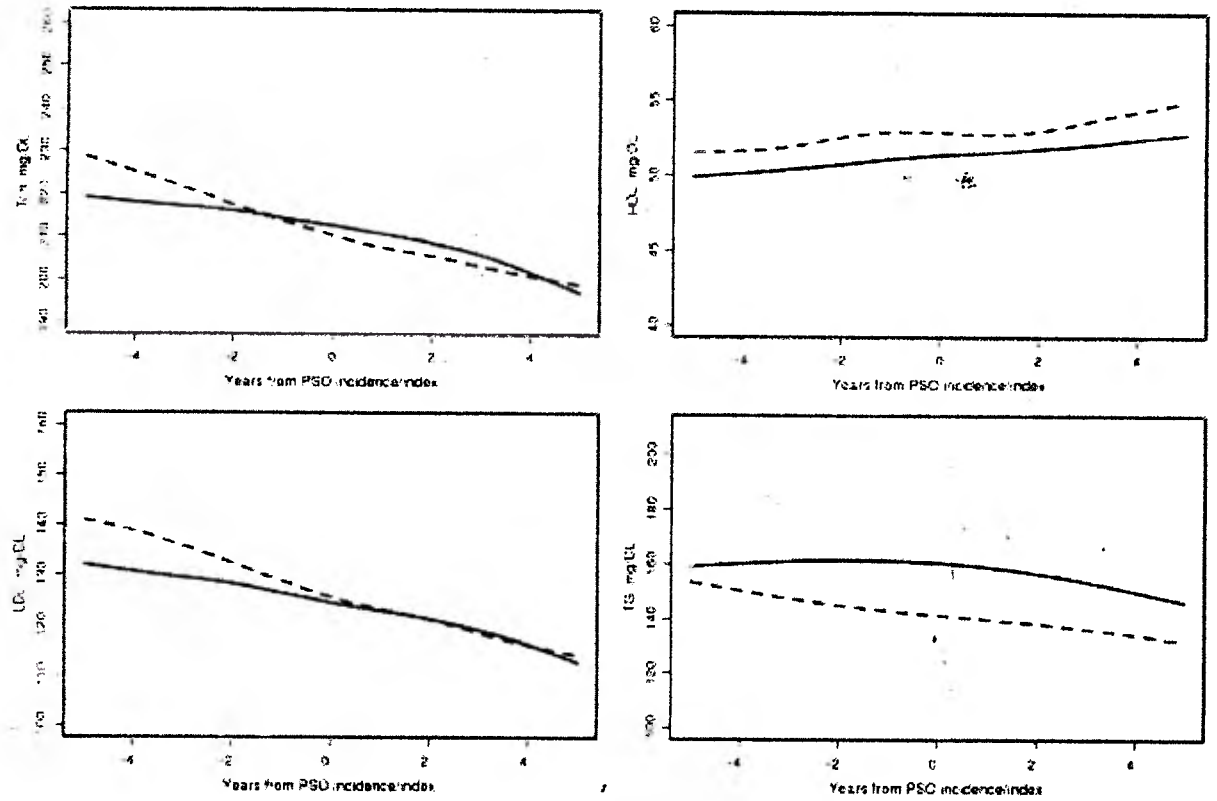

Figure : Trends in lipid levels in psoriasis patients (solid lines) and non-psoriasis subjects (dashed lines) during the time period from 4 years before to 4 years after psoriasis incidence/index date adjusting for age, sex and calendar year of psoriasis diagnosis.

incidence/index date. The data from our study show that although there is indeed a downward trend in TC and LDL in both populations in the five years before psoriasis incidence/index date, the decline in TC and LDL levels was smaller in the psoriasis patients than in the non-psoriasis subjects. This atherogenic trend in the psoriasis subjects is further demonstrated by significantly increased TG levels and significantly lower HDL levels in psoriasis subjects compared to non-psoriasis subjects over the duration of the study.

There is an emerging consensus as to the role of the chronic inflammatory state in diseases like systemic lupus erythematosus and rheumatoid arthritis and the accompanying proinflammatory milieu in promoting development and progression of dyslipidemia and atherosclerosis. It is likely that psoriasis, a chronic immune mediated inflammatory skin disease, may predispose individuals to dyslipidemia. This association is demonstrably stronger for severe psoriasis and psoriatic arthritis. Psoriasis has also been shown to be an independent risk factor for cardiovascular mortality. In addition, there appears to be a significant association between psoriasis and traditional risk factors for atherosclerosis and heart disease in the general population such as diabetes mellitus type II, coronary artery disease, peripheral vascular disease and hypertensive heart disease.

In our study, patients with psoriasis tended to have a smaller decline in the TC and LDL levels compared to non-psoriasis subjects in the 5 years before psoriasis incidence/index date, a finding which is concordant with some previous studies. Direct comparisons with these findings cannot be made due to the differences in study design, time periods and populations involved. The downward trend in TC and LDL prior to psoriasis incidence is not without precedent and has also been demonstrated in other chronic inflammatory conditions such as rheumatoid arthritis. The apparent paradox of downward trending total cholesterol levels and increased cardiovascular mortality risk in psoriasis may be explained by the altered cytokine milieu and inflammation. This is also consistent with the lowering of the plasma cholesterol concentrations seen in chronic inflammatory conditions.

The lowering of TC and LDL in the 5 years prior to psoriasis incidence was unlikely to have been caused by treatment for psoriasis. The broad downward trend in lipids in both the patients with psoriasis and the comparator subjects who did not have psoriasis could be partly explained by the increasing usage of lipid-lowering drugs in the general population during time period covered by the study. A similar explanation might be suggested for the changes in HDL. However, the changes probably cannot be attributed solely to lipid lowering medications, as there was no difference in the number of subjects on lipid lowering medications in either cohort. An effect of onset of psoriasis contributing to the less marked decline in TC and LDL, with significantly higher TG and lower HDL,

compared to non-psoriasis subjects cannot be excluded.

Strengths of this study include the longitudinal population based study design using the data from the Rochester Epidemiology Project with comprehensive data collection of the target population. We analyzed lipid trends before and after the incidence of psoriasis to ascertain longitudinal trends in these subjects. It is possible that patients with psoriasis had greater provider contact leading to greater number of lipid measurements and interventions. However, no differences in the number of lipid measures per person in patients with and without psoriasis were noted, and multiple measurements per person were accounted for during statistical analysis.

CONCLUSIONS

Patients with psoriasis had a significant decrease in TC and LDL levels during the 5 years before psoriasis incidence but the magnitude of the reduction was smaller than that seen in non-psoriasis subjects. Although there was no difference in the trends of TG and HDL levels between the two cohorts, mean TG levels were significantly higher in psoriasis subjects compared with non-psoriasis subjects. HDL levels were significantly lower in patients with psoriasis compared with non-psoriasis subjects. Lipid trends were otherwise similar in psoriasis and non-psoriasis cohorts during the 4 years after psoriasis incidence/index date. These changes are unlikely to be caused by lipid lowering treatment alone. The cause and implications of the apparent changes in lipid profile before psoriasis incidence require further exploration.

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Treatment of Vitiligo with Topical Pimecrolimus

Dr. Pritam Pankaj[1]

ABSTRACT

Background : As the pathogenesis of vitiligo is still obscure, its treatment is a great challenge and is often unsatisfactory. Topical calcineurin inhibitors are claimed to be a suitable option with good safety profile.

Aims of the work : To evaluate the immunomodulatory effect of Pimecrolimus cream in vitiligo patients. Repigmentation in vitiligo is due to immunomodulatory effects of Pimecrolimus.

Methods : Thirty patients with focal vitiligo without any previous history of spontaneous repigmentation were included (12 females and 18 males). The depigmented lesions extended from 3% to 15% of body surface area. Although focal/segmental vitiligo is defined globally as <20% involvement of Body surface area (BSA). Pimecrolimus cream 1% was topically administered to the lesions twice daily for 12 weeks.

Results : Out of 30 patients, none had excellent response, 9 had good response, 11 had moderate response, 8 had poor response and 2 showed to no response. No significant relation was detected between clinical response and the age of patients, family history, stress, or skin type. Better response was noticed on the face and trunk while the peripheral sites showed poor or no response. Side effects were minimal and tolerable.

Conclusion : Pimecrolimus cream through its immunomodulatory effect has shown to be effective in patients with vitiligo without causing the adverse effects associated with other common treatments for this pigmentary disorder.

INTRODUCTION

Vitiligo is a common dermatological disorder characterized by milky-white depigmented macules devoid of identifiable melanocytes. Its incidence varies from 1-2% worldwide and has been shown to be as high as 3-4% in India. There is a familial incidence of 30%. The disorder results in substantial cosmetic disfigurement. In some cultures, patients with vitiligo are regarded as social outcasts and are emotionally and physically affected.

The underlying mechanism is still unclear. Several hypotheses have been put forward including genetic predisposition, neural involvement and self destruction of melanocytes (autotoxicity theory). There is however a growing evidence for an autoimmune mechanism involving humoral as well as cellular immunity. Vitiligo is far more common in patients suffering from autoimmune disease such as thyroiditis, pernicious anemia and diabetes.

Patients with vitiligo produce special melanocytes antibodies. The direct relation of these antibodies titres and the extent of vitiligo favors a direct pathogenic role of these antibodies to the disease. Studies have addressed the role of peripheral blood and lesional cytokine expression in patients with vitiligo.

Beside other factors such as oxidative stress, nitric oxide and monoaminergic system are claimed to be involved in the pathogenesis. A composite hypothesis has been suggested.

As the pathogenesis is still obscure, the treatment of vitiligo has generally been stressful, unsatisfactory and often disappointing and spontaneous regression of this disease is unusual. It remains a challenge for the dermatologist although numerous modalities have been proposed and are currently available.

Conventional therapies include topical steroids, phototherapy and photochemotherapy (NBUVB and PUVA respectively). Helium neon laser treatment may be a an option for patients with segmental vitiligo.

Topical calcineurin inhibitors, tacrolimus and pimecrolimus are novel topical immunomodulatory drugs that are originally used for the treatment of atopic dermatitis. When compared with conventional topical corticosteroid therapy, they have more selective mode of action, with the less adverse events related to the long term use of corticosteroids and are not associated with significant systemic absorption.

Preferred sites for the use of topical calcineurin inhibitors are areas such as the face, neck, flexures and genital areas, which are more susceptible to topical corticosteroid. The efficacy of topical calcineurin inhibitors has been demonstrated for vitiligo.

Calcineurin inhibitors act on T cells and mast cells inhibiting T cell activation and release of inflammatory mediators and preventing degranulation of mast cells.

AIMS OF THE WORK

The aim of this study is to evaluate the therapeutic effect of Pimecrolimus as an immuno-modulatory topical treatment for vitiligo.

MATERIAL AND METHODS

The study involved randomly selected 30 patients with focal vatiligo affecting no more than 15% of body surface area depending on the hand palm rule (1%).

Exclusion Criteria : Cases with dermatomal

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Table

	Good	Moderate	Poor	No response
Face (No. 7)	3 (42.8%)	3 (42.8%)	1 (14.3%)	0 (0%)
Trunk (No. 9)	3 (33.3%)	5 (55.5%)	1 (11.1%)	0 (0%)
Limbs (No. 8)	3 (37.5%)	2 (25.0%)	3 (37.5%)	0 (0%)
Peripheral Parts (No. 6)	0 (0%)	1 (16.6%)	3 (50.0%)	2 (33.3%)
Overall (No. 30)	9	11	8	2

vitiligo, pregnancy, lactation, women with childbearing potential not using adequate contraceptive method, immunosuppression or concomitant use of immunosuppressive medications, patients who had used any other topical or systemic treatment for vitiligo within the past 2 months, and known hypersensitivity to the used treatment.

METHODS :

All patients were subjected to :

1. Complete history taking including personal history, present history, family history and history with special relevance to the vitiligo condition. History of related skin or systemic disease as alopecia areata, diabetes mellitus or thyroid disease, and response to previous therapy.

2. Clinical Examination :

a. General physical examination.

b. Complete dermatological examination including site, shape, number and distribution of lesions and the extent of involvement using the "hand palm rule" (size of physician's hand palm equals 1% of the total body area). The boundaries of vitiligo patches were defined using woods light.

c. An informed written consent was obtained before treatment.

d. Pimecrolimus cream 1% was topically administered to the lesions twice daily for 3 months.

3. Clinical Assessment :

a. Clinical assessment consisted of clinical response determination monthly.

b. Monitoring for repigmentation: perifollicular pigmentation was assessed as an initial response to therapy.

c. The clinical response to therapy was visually scored as the percentage of repigmentation of the depigmented lesions and rated as follows :

i. Excellent response : If >75% repigmentation of the depigmented lesions at end of therapy.

ii. Good response : If between 51-75% repigmentation of the depigmented lesions at end of therapy.

iii. Moderate response : If between 26-50% repigmentation of the depigmented lesions at end of therapy.

iv. Poor response : If <25% repigmentation of the depigmented lesions at end of therapy.

v. No response : If 0% repigmentation of the depigmented lesions at end of therapy.

vi. Evaluation of side effects each visit.

vii. Follow up was done for 3 months after completion of treatment to assess the stability of lesional repigmentation.

RESULTS

This study was conducted on 30 patients with focal vitiligo. They included 12 females and 18 males. The age ranged from 11 to 45 years. The duration of disease ranged from 2 to 30 months. Five patients (16.7%) reported positive family history. The depigmented lesions extended from 3% to 15% of body surface area.

A significance clinical response to treatment was noticed. Evaluation of the clinical response showed that no patients (0%) had excellent response, 9 patients (30%) had good response, 11 patients (36.66%) had moderate response, 8 patients (26.66%) had poor response and 2 patients (6.66%) showed no response.

No significant correlation was found between the clinical response and the variables, skin type, age of patients, family history or history of stress.

Although it was noticed that good results were more on the trunk and face than the peripheral parts of the body, no significant relation was present (Table).

A significant negative correlation was detected between duration of disease and clinical response. Short duration of disease was associated with better results.

Follow up evaluation for 3 months after cessation of treatment showed no recurrence of responding lesions or appearance of new lesions.

Side effects were minimal and transient. They were reported in 5 patients in the form of mild burning sensation or irritation. They were relieved with short courses of antihistamines and bland emollients.

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Study of Skin Disorder During Pregnancy and Its Association with Fetal Outcome

Dr. Pritam Pankaj[1]

ABSTRACT

BACKGROUND : In our society maternal and child health is major concern. During pregnancy there are many metabolic, physiological and endocrine changes of the body which may produce various physiological and pathological alteration results in various skin problems. Almost every women during her period of gestation may present with skin problem either physiological and pathological.

AIMS : To determine the demographic profile various physiological skin changes different type of skin diseases and their effect on maternal health.

METHODS : Study was conducted to any pregnant women who have any dermatoses during pregnancy, irrespective of their period of gestation. Patient attended Obstetrics and Gynaecological department of Katihar Medical College and Hospital, have been selected for study, 270 cases were evaluated. Patients were mainly from urban poor background and study was conducted from March 2014 to February 2015. Patients were subjects to complete blood count, urine routine examination, sugar (fasting / postprandial), Liver function test, thyroid function tests, VDRL, HbSAg, USG and microscopic examination of fungus by KOH smear. HIV screening was also done.

RESULTS : Most common physiological changes notes was pigmentation (89.6%), itching (24%) followed by vaginal discharge (18.1%). Regarding specific dermatoses of pregnancy pruritic urticarial papules and plaque of pregnancy was commonest (63.6%) followed by pruritus gravidarum (22.7%).

CONCLUSION : In a community the type of disease depends on genetic constitution, racial characteristic, nutritional status, hygiene, customes, occupations. Of our study sample there are very high incidence of treatable infection and infestation which reflect the lack of sanitation, congested living condition and hot and humid climate. All these factors brew a cauldron and microbes which need to be eliminated and improve the overall condition of the people.

INTRODUCTION

Maternal and child health is always the prime concern of any society and as well as its country. Skin disorders during pregnancy are very common. Almost every woman during her period of gestation may presents with skin problem either physiological or pathological. During pregnancy many metabolic and endocrine changes though physiological in nature, may produce various pathological alterations, ranging from trivial cutaneous changes to changes that are pathological, recurrent and specific to pregnancy. The patients may be worried about the impact of these changes on their fetus. With conception a new endocrine system is established in the women's body and the endocrine changes begin to manifest soon after the fertilized ovum gets implanted in the endometrium. Placental hormones as well as hormones like human chorionic gonadotrophin, estrogen, progesterone, follicle stimulating hormones are responsible for various physiological changes observed during pregnancy including breast enlargement.

A new feto materno placental endocrine unit creates various dermatological manifestation which are physiological like pigmentation manifested as hyperpigmentation of nipple areola and genital areas. Melasma is also very common in Indian women. Hirsutism often associated with pregnancy due to increase number of anagen hair and nail changes are not so prominent but few women become conscious when there is sudden hair loss and nails become brittle.

Endocrine changes are mainly due to increase in sebum secretion during pregnancy which manifest as Milliaria. Apocrine gland activity apparently reduced as Fox-Fordyces disease is reported to improve during pregnancy. Vascular changes during pregnancy are mainly due to increased level of estrogens. They are mainly palmar erythema, varicose veins in legs and vulval varicosities. They disappear gradually after delivery.

Few skin disease may changes their course in the period of pregnancy. Diminished cell mediated immunity makes gravid women more prone to infectious diseases for the first time and also some pre-existing infections becomes more severe. Candidal vaginitis, trichomonas vaginitis, bacterial vaginosis are the real threat to neonatal health. Herpes virus infection during pregnancy particularly herpes genitalis need close supervision from the part of obstetrician. Pityrosporum folliculitis, genital wart are common during pregnancy. Leprosy need energetic management during pregnancy. Immunological disorder shows improvement during the period of pregnancy.

Pruritus during pregnancy is the common complain along with vaginal discharge. In Indian women pruritus may be due to infections like scabies, pediculosis, infestations, atopic dermatitis, neurodermatitis and most importantly iron deficiency anemia, which is very common in Indian women may manifest as severe pruritus. Dermatoses specific to

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pregnancy need complete evaluation for the diagnosis. Pruritus gravidarum, herpes gestationis, pruritic urticarial papule and plaque of pregnancy, pruritic folliculitis of pregnancy, impetigo herpetiformis, popular dermatitis of pregnancy, linear IGM dermatoses of pregnancy, autoimmune progesterone dermatitis of pregnancy – all come under the group of pregnancy specific dermatoses.

Every dermatological condition whether it is physiological or pathological make an impact on women's mind and gets her disturbed psychologically. So correct diagnosis appropriate counseling and proper treatment is necessary for a healthy baby and healthy nation.

AIMS AND OBJECTIVES

1. To study the demographic profile of the subjects.
2. To study various physiological skin changes during pregnancy.
3. To study various skin diseases during pregnancy.
4. To study the effect of skin diseases on maternal and fetal health.

MATERIAL AND METHODS

The present study of skin disorder during pregnancy and its association with fetal outcome was undertaken at the Department of Obstetrics and Gynaecology and Department of Dermatology, Katihar Medical College & Hospital, Katihar, Bihar.

Study was conducted from March 2014 to February 2015, for a total duration of one year. This period was divided into 3 parts representing the three seasons, namely summer extending from April to June, Monsoon from July to September and winter from October to February. This enabled as to try and study the seasonal variations in the pattern of dermatoses.

Any pregnant women who have any dermatoses during pregnancy are included in our study, irrespective of their period of gestation.

The patient were not decidedly but were chosen at random in an attempt to eliminate observers bias in the sample.

A detailed history was taken and following were given special importance. Age, residential background and status of hygiene.

Present and past obstetric history, menstrual, personal and social history along with family history and taken in details.

A thorough general survey was done followed by a meticulous examination of the cutaneous system.

The lesions were described in detail with reference to their morphology, site of distribution and any distinctive character.

The mucous membranes, hair and nails were also examined in each case.

Investigations such as routine blood examination, microscopic examination with KOH solution and skin biopsy were done as and when necessary.

RESULTS AND ANALYSIS

Skin disorder during pregnancy is quite common in pregnancy. In this study 20% women were in the age group <20 years. Most of the patients were from the lower socioeconomic strata of our society. Most common physiological changes noted was pigmentation among them 89.6% women presented with linea nigra. Commonest symptom was itching noted in 24% cases along with 18.1% women presented with vaginal discharge. Infections and infestations cases were maximum 131(48.5%) cases were documented in our study. 15 cases if pregnancy specific dermatoses were documented in our study of them 10 cases were PUPPP and 3 cases were of pruritus gravidarum. Pruritus in iron deficiency were noted in significant number 21.7% women with iron deficiency presented with pruritus. LBW babies (<2.5 kg) were born to 66.9% of study population. 10.74% babies suffered from birth asphyxia (low Apgar score at 1 and 5 min) and required admission in nursery. No neonatal mortality was recorded in our study. In our study no adverse fetal outcome associated with maternal skin disorder was found.

CONCLUSION

The type and amount of disease in any community has been said to reflect the genetic constitutions of its members, most conspicuously evident as their racial characteristics, nutritional status and hygienic standards, customs, occupations. It is affected directly and indirectly by the climate and is influenced by the quality and quantity of medical care available.

Considered in this light, the very high incidence of treatable infections and infestations dominated pattern of our study sample only reflects the lack of sanitations, poverty and congested living environ of our draining population. To add to this is the hot and humid climate in this part of the world. All these factors brew a cauldron of microbes which need to eliminated.

The cell for the creation of a healthy environment and good nutritional status for the community was the declaration of Alma Ata Convention and till date it is the target of Millennium Development Goal. However till date variety of factors still hindering its proper implantation.

Our study though hospital based and hence with an inherent bias, still seeks to improve the overall condition of the people who depend on the services of the state, by giving a broad overview of the pattern of dermatoses afflicting these people and hence enabling those with a mission, the necessary tools to lighter their task.

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Date 29/4/16.

To,
DR. Pritam Pankaj,
Associate Professor, Dept. of Dermatology,
KMCH, Katihar, Bihar.

SUB: "Metabolic changes in Psoriatic skin under topical corticosteroid treatment."

Respected Sir/Madam,

We would like to inform you that the above mentioned article has been accepted suitable for publication in IMJ (NIC/BID/ICMR/JR/ 233 Dt. 14.12.12, INDEX MEDICUS by NIC, NEW DELHI, GOVT. OF INDIA).
Please bear with us as we have space constraint to publish the said article.
We will try to accommodate as soon as possible when space provided.
Thanking You.
Yours Sincerely,

Prof. (DR.) Niladri Sarkar
Editor in Chief
INDIAN MEDICAL JOURNAL

METABOLIC CHANGES IN PSORIATIC SKIN UNDER TOPICAL CORTICOSTEROID TREATMENT

ABSTRACT

Background

MR spectroscopy of intact biopsies can provide a metabolic snapshot of the investigated tissue. The aim of the present study was to explore the metabolic pattern of uninvolved skin, psoriatic skin and corticosteroid treated psoriatic skin.

Methods

The three types of skin biopsy samples were excised from patients with psoriasis (N = 10). Lesions were evaluated clinically, and tissue biopsies were excised and analyzed by one-dimensional ^{1}H MR spectroscopy. Relative levels were calculated for nine tissue metabolites. Subsequently, relative amounts of epidermis, dermis and subcutaneous tissue were scored by histopathological evaluation of HES stained sections.

Results

Seven out of 10 patients experienced at least 40% reduction in clinical score after corticosteroid treatment. Tissue biopsies from psoriatic skin contained lower levels of the metabolites *myo*-inositol and glucose, and higher levels of choline and taurine compared to uninvolved skin. In corticosteroid treated psoriatic skin, tissue levels of glucose, *myo*-inositol, GPC and glycine were increased, whereas choline was reduced, in patients with good therapeutic effect. These tissue levels are becoming more similar to metabolite levels in uninvolved skin.

Conclusion

This MR method demonstrates that metabolism in psoriatic skin becomes similar to that of uninvolved skin after effective corticosteroid treatment. MR profiling of skin lesions reflect metabolic alterations related to pathogenesis and treatment effects.

Keywords:

Tissue; Metabolites; Corticosteroids; Psoriasis treatment; MR spectroscopy

INTRODUCTION

Psoriasis is a common immune-mediated disease that affects the skin and joints. The cause of the disease remains unknown. Many patients have a genetic predisposition. The disease affects around 2–3% of the population worldwide. Clinically, psoriatic plaques are characterized by sharply demarcated erythematous lesions with thick silvery scales, often distributed in a symmetrical pattern. Histopathologically there is hyperproliferation of epidermal cells and an inflammatory cell infiltrate. There is increasing awareness that psoriasis is a multisystem affection with substantial comorbidity, particularly of cardiovascular diseases and metabolic syndrome. The course is that of a chronic, relapsing disease which requires long term treatment. Various topical and systemic treatment options exist for psoriatic lesions. Topical corticosteroids remain the cornerstone, either used as monotherapy or in combination with other treatment modalities. These agents exert anti-inflammatory and immunosuppressive effects by stimulation or inhibition of the genes involved in

inflammatory pathways, including inhibition of cytokine production and reduction of such mediators of inflammation as prostaglandins and leucotrienes, inhibition of T-cell proliferation and T-cell dependent immunity, and suppression of fibroblast and endothelial cell functions. Corticosteroids also have anti-proliferative effects, by delaying the onset of DNA synthesis and decreasing the mitotic rate.

Molecular studies of outbreak and healing of psoriatic lesions can provide insight in the underlying biological processes. Genome wide association scans (GWAS) have identified genetic susceptibility factors, and molecular analysis have revealed associations of psoriasis with specific molecular pathways. Detailed molecular characterization of autoimmune diseases can provide information about mechanisms involved in disease progression and action of drugs, and also provide biomarkers to predict and monitor disease course. Cellular enzymatic processes involve small molecular metabolites as substrates, intermediates and end products, and such metabolites are crucial in energy turnover and membrane synthesis. Metabolic studies have been applied in numerous biomedical settings, and for instance metabolic characterization of cancerous tissue is expected to contribute to a more detailed tumor portrait by defining specific fingerprints reflecting diagnostic status or predicting therapeutic response. Magnetic resonance spectroscopy (MRS) analysis of intact tissue specimens can provide a detailed description of the biochemical composition of the tissue, using so-called high resolution (HR) magic angle spinning (MAS) MRS. This technology requires a minimum of preparation of samples, and detailed biochemical information can be obtained from small specimens (typically 20 mg). Multiple cellular metabolites can be measured simultaneously, and the sample is kept intact for subsequent analysis by other techniques.

The purposes of the present study were to characterize the metabolic patterns of intact uninvolved and affected skin in psoriasis patients and to monitor the biochemical changes in psoriatic skin accompanying corticosteroid treatment. Ten patients were included, three biopsy samples being excised from each: uninvolved skin, psoriatic skin, and corticosteroid treated psoriatic skin, respectively. All biopsy samples were investigated by MAS MRS, and the resulting spectra were further analyzed by peak area calculations to obtain relative measures of tissue metabolite contents.

MATERIAL AND METHODS

Subjects

Ten patients with stable light to moderate plaque psoriasis volunteered to participate in the study. Eight of the patients were men and two were women (not pregnant or nursing) with a median age of 52 (range 28–75) years. None of the patients used systemic treatment for psoriasis. Three patients were on systemic medication for non-dermatological reasons: irbesartan (hypertension) and terbinafine (tinea unguium), aspirin and pravastatin (hypercholesterolemia) and amlodipine (hypertension).

Study design

Two symmetrical psoriatic lesions were chosen for each patient. The psoriatic lesions were localized at elbows (n = 3), knees (n = 3), upper back (n = 1), hips (n = 1), flanks (n = 1) and buttocks (n = 1). After at least two weeks of treatment with only emollient (Locobase®, Yamanouchi), one plaque was assigned for continued treatment with emollient. The other chosen psoriatic lesion was treated once daily (evening time) with the very potent corticosteroid clobetasol propionate ointment 0,05% (Dermovate®,

GlaxoSmtihKline). In addition, the emollient was used both on the lesion treated with corticosteroid and the control lesion according to needs. Both psoriatic lesions were evaluated clinically before the start of the treatment and after four weeks of treatment. The severity of scaling, erythema and infiltration of the lesions was scored on a scale from 0–4 for each parameter (0 absent and 4 severe). After four weeks, three punch biopsies (4 mm) were taken after local anaesthesia with lidocaine with epinephrine: from uninvolved skin, from psoriatic skin and from corticosteroid treated psoriatic skin in the same body area.

Sample treatment

The punch biopsy samples were put in a cryo-tube and frozen in liquid nitrogen (-195.8°C) within one minute after tissue resection, and further stored in liquid nitrogen until MR analysis. Samples weighed 21.9 mg on average (range from 11.6 to 35.7 mg).

MR spectroscopy

The MR experiments were performed as previously described. Briefly, samples were thawed on an ice-block to provide a cold environment, and transferred to a 4 mm MAS rotor (total sample volume 50 µL) containing 40 µL phosphate buffered saline with TSP (1 mM). The rotor was thereafter placed in a Bruker AVANCE DRX600 spectrometer equipped with a $^{1}H/^{13}C$ MAS probe with gradients (Bruker BioSpin GmbH, Germany). During signal acquisition, which started within 42 minutes in average after sample thawing (maximum 1 hour and 35 minutes), the samples were spun at 5 kHz and kept at 4°C. One-dimensional ^{1}H spectra were recorded, using a spin-echo sequence which suppresses broad peaks and the water signal. The resulting spectra are highly resolved, with relatively enhanced signals from small metabolites. Spectral assignments were performed based on metabolite appearances in previously recorded MR spectra of intact human tissue and in MR spectra of extracts from skin tissue. Totally 17 metabolites were assigned.

Analysis of MR spectra

The spectral region 4.7 to 3.0 ppm was used for peak area calculations, which was performed using the curve fitting program PeakFit version 4 (SeaSolve Inc, USA (MA)), by combined Lorenzian and Gaussian functions (Voigt area) for curve area estimation. Areas were calculated for the nine peaks arising from glucose, lactate, *myo*-inositol, glycine, taurine, glycerophosphocholine (GPC), phosphocholine (PCho), choline and creatine. The program uses a least squares function to optimize the fit to the real spectrum, and the correlation factor which describes the goodness of fit was better than 0.95 for all area calculations. To obtain a semi-quantitative measure for each metabolite, its peak area was normalized to the total peak area of all nine metabolites for every spectrum. Kruskal-Wallis multiple sample analysis was applied for paired comparisons of metabolite content in the three types of skin samples. Differences in tissue metabolites between corticosteroid treated and untreated psoriatic skin were calculated for all patients. Metabolic changes ascribed to corticosteroid treatment were compared between the group with poor ($N = 3$) and good ($N = 7$) clinical effect using Mann–Whitney significance test. Statistical analyses were performed using SPSS (SPSS 16, SPSS Inc.).

Histopathology

Tissue samples were stored in liquid nitrogen for 20 months after MAS MRS analysis. For histological evaluation, samples were thawed and immersed in 4% buffered

formaldehyde fixative solution for 24 hours, followed by embedment in paraffin. From each block, one 5 µm tissue section was cut and stained with haematoxilin, erythrosine and saffron (HES). The microscopic sections were photographed with a digital camera, and the relative amounts of epidermis, dermis and subcutaneous tissue were determined by point counting. Briefly, the micrographs were overlaid with a randomly positioned point grid, and the number of points falling on each of the three tissue components was counted, considering the stratum corneum as part of the epidermis. The relative number of points falling on one particular component was taken as an estimate of the section area occupied by the respective tissue element. The area fraction thus obtained is an unbiased estimate of the corresponding volume fraction in the tissue sample, provided the section is chosen randomly.

RESULTS

Patients

All patients experienced a reduced degree of psoriatic affection after four weeks of treatment with corticosteroid ointment. Seven of the patients experienced at least 40% reduction of the clinical score of the skin, of which four patients had almost complete normalization of the skin (score grade 1 for erythema and infiltration, no scaling). Three patients had 40% or less reduction of clinical skin scoring, and were considered to have poor effect of the corticosteroid treatment. Concerning the untreated psoriatic lesions, five of the patients showed no change over four weeks, three experienced less scaling after application of emollient whereas in two patients a worsening was noted.

Histology

All samples could be evaluated with respect to tissue composition after MR analysis and long-term storage in liquid nitrogen. Epidermis was thicker in psoriasis lesions, and comprised a significantly larger fraction of the biopsies both in untreated (12%) and corticosteroid treated (9%) skin than in the uninvolved skin (3%) ($p < 0.05$, ANOVA). All patients but one had lower epidermal fraction in corticosteroid treated than in untreated psoriatic skin, with about 40% reduction in epithelial thickness. The one patient with an increased thickness of epidermis after corticosteroid treatment was also clinically scored as showing poor response to treatment.

Metabolites

The MR spectra of the skin samples showed signals from numerous small molecular weight metabolites and lipids. The nine metabolites were detectable in all spectra, and were identified as cell building blocks (amino acids and choline compounds), osmolytes (taurine) and metabolites involved in energy consumption (glucose and lactate). Peak areas of the nine selected metabolites could be calculated for all samples. In addition, the anesthetic lidocaine contributed significantly to most spectra, giving rise to a total of seven peaks. Statistical analysis showed that the tissue content of glucose, *myo*-inositol, taurine, GPC and choline were different in the three types of sample ($p < 0.05$, Kruskal-Walis). The levels of *myo*-inositol and glucose were highest in uninvolved skin and lowest in psoriatic skin, whereas those of taurine and choline were highest in psoriatic skin and lowest in uninvolved skin. The levels of GPC were highest in corticosteroid treated skin and lowest in psoriatic skin. We observed no differences in the tissue levels of creatine, glycine, lactate or phosphocholine between the three types of sample.

Seven of the patients showed a good clinical effect of topical corticosteroid treatment (at least 40% reduction of clinical score), whereas three patients had poor effect (less than 40% reduction of clinical score). We found metabolic changes that were different in these two patient groups for five of the metabolites ($p < 0.05$, Mann–Whitney). In skin samples from patients with good treatment results, glucose, *myo*-inositol, GPC and glycine increased with treatment, whereas choline decreased.

CONCLUSION

This study demonstrated detectable differences in tissue metabolites between uninvolved skin, psoriatic skin and corticosteroid treated psoriatic skin. We also found that metabolic differences induced by corticosteroid treatment were related to the actual therapeutic effect. The application of MR spectroscopy in dermatological research provides information about tissue metabolites, thus presenting a novel approach for studies of pathogenesis and treatment effects in the skin.

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Date 29/4/16

To,
DR. Pritam Pankaj,
Associate Professor, Dept. of Dermatology,
KMCH, Katihar, Bihar.

SUB: "Topical treatment with fresh human milk versus emollient on atopic eczema spots in young children."

Respected Sir/Madam,

We would like to inform you that the above mentioned article has been accepted suitable for publication in IMJ (NIC/BID/ICMR/JR/ 233 Dt. 14.12.12, INDEX MEDICUS by NIC, NEW DELHI, GOVT. OF INDIA).
Please bear with us as we have space constraint to publish the said article.
We will try to accommodate as soon as possible when space provided.
Thanking You.
Yours Sincerely,

Prof. (DR.) Niladri Sarkar
Editor in Chief
INDIAN MEDICAL JOURNAL

TOPICAL TREATMENT WITH FRESH HUMAN MILK VERSUS EMOLLIENT ON ATOPIC ECZEMA SPOTS IN YOUNG CHILDREN: A SMALL, RANDOMIZED, SPLIT BODY, CONTROLLED, BLINDED PILOT STUDY

ABSTRACT

Background

Public health nurses report on effects of fresh human milk as treatment for conjunctivitis, rhinitis and atopic eczema (AE), the latter being highly prevalent in early childhood. Emollients and topical corticosteroids are first line treatment of AE. As many caregivers have steroid phobia, alternative treatment options for mild AE are of interest. The aim of this small pilot study was to assess the potential effects and risks of applying fresh human milk locally on eczema spots in children with AE.

Methods

This was a split body, controlled, randomized and physician blinded pilot study, of children with AE with two similar contralateral eczema spots having a mother breastfeeding the child or a sibling. Fresh expressed milk and emollient was applied on the intervention spot and emollient alone on the control area, three times a day for four weeks. The severity and area of the eczema spots was evaluated weekly, and samples from milk and the spots were analysed weekly with respect to bacterial colonisation.

Results

Of nine patients included, six completed the study. Mean age at inclusion was 18.5 months. The spots examined were localized on the arms, legs or cheeks. The spots were similar in severity, but differed in area. In one patient the eczema ceased after inclusion. In four patients both control and intervention areas increased during the intervention. The relative change in eczema area compared to baseline showed less increase in the intervention spots in two patients, whereas the opposite was observed in three. In four children *Staphylococcus aureus* was found in their eczema once or more. In three of the 28 human milk samples, *Staphylococcus aureus*, *alfa haemolytic streptococci* or *coagulase negative staphylococci* were detected. *Staphylococcus aureus* was found once both in human milk and in the eczema spots, no clinical signs of infection were however observed. No secondary infection due to milk application was detected.

Conclusion

In this small pilot study, no effect was found on eczema spots treated with topical application of fresh human milk.

Keywords:

Atopic eczema; Children; Human milk; Emollient; Topical treatment; Pilot study

INTRODUCTION

Atopic eczema (AE) is a common, chronic, pruritic, relapsing skin disease which affects up to 20% of children in the Nordic European countries. AE is strongly associated with other atopic disorders, such as allergic rhinitis and asthma. The pathogenesis is interplay between barrier dysfunction, genetic, immunological, environmental factors and colonization by *Staphylococcus aureus* (*S. aureus*).

The treatment algorithm in AE is based on treating the barrier defect, the inflammation, the infection and the pruritus [4]. First line treatment is treating the barrier defect with optimal skin care by the use of emollients and baths. If a clinical infection is present in the eczema, local antiseptics may be utilized; for severe cases systemic antibiotics are needed. The inflammation is treated with topical steroid creams. Topical steroids have been shown to be a well-tolerated treatment, but in spite of this many caregivers have steroid phobia, mainly because of the potential side effects. A treatment option in chronic eczema is topical calcineurin inhibitors; these are however not to be used in children under two years of age. Alternatives without side effects for young children are therefore of interest.

Human milk may represent a source with potential treatment properties. Knowledge of the immunological qualities of mammalian milk can be traced back to 1892, when Paul Ehrlich demonstrated that newborn mice were protected against the toxic effects of phytotoxins if they were fed milk from an immunized mouse. Today, numerous studies have contributed to our present knowledge of the short- and long-term effects of human milk in the breastfed child. Mammalian milk is species specific. Human milk contains specialized immune components, including factors with anti-microbial and anti-inflammatory properties, which theoretically could be responsible for an effect on eczema spots when applied topically.

In Norway, public health nurses report several cases where parents have had positive experiences with topic applications of expressed human milk in eyes of children with conjunctivitis and on eczema spots in children with AE. We have not been able to find any studies investigating such treatment in children with AE. However, local use of expressed human milk has been studied for diaper dermatitis, rhinitis and conjunctivitis .

The aim of this small pilot study was to assess the potential positive and/or negative effects of topical use of expressed, fresh human milk on eczema spots in young children with AE by evaluating the eczema areas. A secondary aim was to evaluate any bacterial transmission from human milk to the eczema spots, causing infection in the child. Finally the mothers' compliance to the treatment was of interest.

MATERIAL AND METHODS

Trial design

This was a split body controlled, randomized and physician blinded study of expressed human milk and emollients on contralateral eczema spots in children, the trial was conducted at Department of Dermatology, Katihar Medical College & Hospital, Katihar Bihar.

Inclusion criteria were children with AE according to Hanifin and Rajkas criteria with a mother breastfeeding the child or a sibling. The eczema spots in the treatment and control areas were to be similar in features and extent as well as being localized on contralateral parts of the body. Children were excluded if the severity of the eczema spots indicated need for treatment with antibiotics and/or steroids.

Recruitment

Study patients were recruited through advertisement posters from three different well baby clinics in Katihar, Bihar, in the period 2014–2015. Mothers interested in the study contacted the study team. The consultations mainly took place at the hospital; a few were carried out in the child's home. The mothers were able to contact the examining physician if they experienced any problems with the treatment.

Follow-up

After inclusion, the children were examined once weekly for four consecutive weeks. The overall severity of AE was evaluated by the use of SCORAD, which defines mild disease as score <25, moderate disease between 25–50 and severe disease as scores >50 [12]. The severity of the study and control areas were evaluated by scoring the erythema, lichenification, excoriation and pruritus on a scale from 0 to 3, where 0 is none and 3 is severe. Study and control areas were measured using Visitrak™ (Smith & Nephew), a portable device used to measure the area of wounds. A transparent folio is placed over the area and the borders are outlined, whereafter a computer determines the area measured in cm^2. In the present study this device was used to follow the development of the extent of the eczema spots.

Outcome

The primary outcome was to register proportional change in the area of the eczema spot from baseline, as measured by Visitrak™. The secondary outcome was to assess transmission of bacteria from mother's milk to eczema spots in the child. The mother's compliance was also evaluated.

Statistical methods

Descriptive statistics were performed. The areas of the intervention and control sites for each child were not identical; therefore differences were calculated as percentages: Changes in the areas of the control and intervention sites each week were calculated as change in proportion of area related to baseline area.

RESULTS

Study population

Nine children, four male, were recruited for the study through advertisement posters from three different well baby clinics in Katihar, Bihar in the period 2014–2015. Three of these nine children were lost to follow-up consultations; one experienced remission from AE, the second suffered from severe AE and was hospitalized, the third never met for follow up. Two children were treated with mothers' milk produced for a younger sibling. The mean age of the children was 18.5 months (min, max; 4, 32). At inclusion mean SCORAD was 35 (min, max; 22, 45) and at the end of the study mean SCORAD was 34 (min, max; 18, 52). The spots examined were localized on the arms or legs in five of the children and on the cheeks in one. The spots were similar in severity, however the extent differed some.

Changes in measured area of eczema

The weekly change in the control and intervention eczema area related to baseline eczema area is illustrated. At the end of the study, child number one and seven displayed less area involvement in the area treated with human milk compared to the emollient treated area. In child number two, five and nine the emollient treated area showed at study end less involvement than the area treated with human milk. The eczema spots in child number eight disappeared after inclusion.

Most of the children showed an improvement of their general eczema, except for child five, who showed a slight increase. Child seven differs from the other children: this child experienced a worsening of the total eczema, having mild atopic eczema at inclusion, and severe atopic eczema at week four.

Changes in presence of bacterial species

Four of the children had positive *S. aureus* cultures in their eczema once or more (Table 1). However, only in four of twelve occasions this coincided with clinical signs of infection. Gram-negative rods were found in child number one at one visit. *S. aureus, alfa haemolytic streptococcior coagulase-negative staphylococci* were detected in three of the 28 human milk samples. Only on one occasion the same bacteria (*S. aureus*) were detected in both the eczema lesions and the human milk (child number five), and signs of clinical infection were present. The intervention areas differed some from the control areas, as *S. aureus* was found in intervention area but not in the control area on four occasions in three different children.

Compliance

The mothers experienced the application of human milk as an uncomplicated treatment option.

DISCUSSION

In this small, split body controlled randomized pilot study of human milk and emollient applied topically on eczema spots in six children, no effect was found on eczema spots treated with the topical application of fresh human milk. In two of five children with persistent eczema lesions during the study, there was less involvement of the human milk treated area compared to the emollient area at study end compared to baseline. However, the opposite was found in three children.

There are few studies looking at the effect of human milk on eczema. One study of children with diaper dermatitis examined the effect of applying human milk after each breastfeeding or hydrocortisone 1% ointment twice a day, detecting after one week an effect of human milk comparable to that of hydrocortisone. The application frequency was higher than in our study, but we still believe that an application rate of three times a day would be enough to show an effect of human milk, after four weeks of treatment.

There are many theoretical indications of how human milk can be effective on eczema lesions. One aspect of atopic disease is the type 2 helper T cell (Th2) dysbalance with production of interleukin- 4 (IL-4), IL-5, and IL-13 in the acute phase. In the chronic phase there is a Th1/Th0 dominance with production of interferon-γ, IL-2, IL-5 and granulocyte-macrophage colony-stimulating factor. Of interest, therefore, are findings from an animal model, where the effect of human colostrum used locally on an acute inflammatory process was shown to be as potent as oral indomethacin and superior to oral dexamethasone at suppressing polymorphonuclear leukocyte influx. In humans, the milk contains, among a wide variety of biologically active hormones, glucocorticoids. These are transferred from plasma to the milk in levels fairly highly correlated (in the .6-.7 range)

Milk samples from healthy donors have revealed bacteria in 10–23% of the samples. In the present study, bacteria were found in three of 28 samples; 11%. In one child only (child number five), *S. aureus* was found once in both the eczema spot and in the milk. Clinically, however, the eczema was not infected, and this child had *S. aureus* on both eczema lesions at every visit. This suggests that no iatrogenic infections due to application of fresh mothers milk occurred. Surprisingly, *S. aureus* was found in the intervention area but not in the control area on four occasions in three different children. One speculation might be that the preservative (eg. phenoxyethanol) in the emollient had some antimicrobial effect when applied alone.

Human milk contains several different substances that act against bacteria, virus and fungi, such as Secretory IgA and Secretory IgM, lactoferrin, lysozyme, oligosaccharides, Toll-like receptors and fatty acids [8]. In a study evaluating the inhibition in vitro of human colostrum against bacterial cultures from eye swabs of neonates with neonatal conjunctivitis, the inhibitory activity was ≥ 50% against *S. aureus* and coliform bacteria, demonstrating an antimicrobial effect also in vitro. A consistent inhibitory effect of human milk was found against *Neisseria gonorrhea* in children with conjunctivitis. A significant but less pronounced effect was also found against *Moraxella catarrhalis* This strengthens the evidence for an antimicrobial effect of human milk also when used topically. When comparing different methods for preventing omphalitis in newborns, an application frequency of topical human milk twice a day demonstrated a shorter separation time of the umbilical stump compared to the use of antiseptics. In the study of Ibhanesebhor, the mean duration of inhibition of human milk against *S. aureus* was three hours. Considering the huge amount of different biologically active components and cells in human milk, there might be a variety of candidates for explaining the positive effects described above.
The children participating in the present study were recruited through advertisement posters in well baby clinics, and responding mothers were presumably highly motivated and inclined to trust alternative/new treatment methods. We cannot rule out that the mothers co-treated the intervention sites with for instance steroid cream, but the instructions with respect to treatment of the intervention and control site were clear, and the results do not indicate performance bias.

CONCLUSION

The results of this small randomized, controlled pilot study of six children with AE does not support an effect of topical applied human milk. Treatment with fresh expressed human milk seems safe and easy for mothers to carry out.

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Original & Clinical Research

Recent Concept

Prevalence, Incidence and Predictive Factors for Hand Eczema in Young Adults – A Follow-Up Study

Dr. Seeba Hussain[1], Dr. Praveen Kumar Bhopalka[2]

ABSTRACT

Background : Hand eczema is common in the general population and affects women twice as often as men. It is also the most frequent occupational skin disease. The economic consequences are considerable for society and for the affected individuals.

Methods : To investigate the prevalence and incidence of hand eczema and to evaluate risk factors for development of hand eczema in young adults. Subjects and methods; This is a prospective follow-up study of 2,403 young adults, 16 – 19 years old in 2002 and aged 29 – 32 years, 13 years later, in 2015. They completed a postal questionnaire that included questions regarding one-year prevalence of hand eczema, childhood eczema, asthma, rhino-conjunctivitis and factors considered to affect hand eczema such as hand-washing, washing and cleaning, cooking, taking care of small children and usage of moisturisers. These factors were evaluated with the multinominal logistic regression analysis.

Results : The one-year prevalence of hand eczema was 15.8% (females 20.3% and males 10.0%, p<0.001). The incidence was 11.6 cases per 1000 person-years (females 14.3 and males 5.2, p<0.001). Childhood eczema was the most important risk factor for hand eczema. The odds ratios were 13.17 when having hand eczema 1995 and 2008 compared to 5.17 in 2008 (p<0.001). A high frequency of hand washing was important in predicting hand eczema only when having 1-year prevalence 2008, OR 1.02 (p=0.038).

Conclusions : After 13 years an increased 1-year prevalence of hand eczema was found. The significant risk factors for hand eczema changed over time from endogenous to exogenous factors.

INTRODUCTION

Hand eczema is common in the general population. In a recent review of studies in the general population from mostly Asian countries, the 1-year prevalence rates ranged from 6.5% to 17.5%. Hand eczema is 1.5 – 2 times more common in females compared with males. Indian estimates of 1-year prevalence of hand eczema in different age-groups have varied from 6.5% to 11.8%. Among Indian 20–29 year olds, the 1-year prevalence of hand eczema was reported to range from 7.5% to 10.8%. Furthermore, hand eczema is the most common occupational skin disease.

Several exogenous risk factors for hand eczema have been reported: occupational exposure, use of detergents and wet work at home. The identification and evaluation of risk factors for the development and persistence of hand eczema are important especially among young adults. During this period of life, type of occupation, household work and childcare are factors that are important to study because they might be related to the development of hand eczema. Taken together, these circumstances justify follow up studies in early adulthood.

AIM OF THE STUDY

The aim of the present study was to investigate the prevalence and cumulative incidence of hand eczema and to evaluate factors that can influence the development and recurrence of hand eczema in young adults.

MATERIAL AND METHODS

Study group

This is the 13 year prospective follow-up study of a cohort of pupils in upper secondary school, 16–19 years old at the baseline assessment, and consequently they were 29–32 years old at follow-up. In 2002, 2,572 pupils in the four secondary schools in Katihar completed a self-administrated questionnaire regarding hand eczema, the response rate was 98.6%. Katihar is a town in Bihar with approximately 70,000 inhabitants.

Topics surveyed by the questionnaire were: hand eczema, childhood eczema, asthma and rhino-conjunctivitis, household size and family structure, occupation and everyday activities, hand washing and skin care.

Data analysis and statistics

One-year prevalence of hand eczema was estimated from reported hand eczema at present or having had hand eczema some time during the last 12 months.

The cumulative incidence was calculated on the individuals reporting having 1-year prevalence or ever having had hand eczema 2015 minus those who had 1-year prevalence or ever had had hand eczema in 2002. The cumulative incidence is presented as the percentage of

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new cases of hand eczema in the cohort. Incidence rate is presented as new cases per 1000 person-years, i. e. the cumulative incidence/13 years 1000.

Four groups were constructed with the intention to analyse risk factors and the development of hand eczema over time.

Potential exogenous risk factors for developing hand eczema such as household size, time required for household work, frequency of hand washing, skin protective habits, working hours outside home and leisure activities were investigated by dividing the cohort into two groups.

Regarding occupation, the respondents were asked not only to tell their profession, but also to give information about work tasks.

RESULTS

Out of the 2,403 participants from the original cohort who received a questionnaire in the mail, 1,516 responded to the questionnaire, which was a response rate of 63%; 56% of the respondents were females. Significantly more females than males answered the questionnaire, 69.4% of the reachable original female cohort and 56.4% of the males ($p<0.001$). However, in 2015 there were no significant differences between the respondents and non-respondents in reporting 1-year prevalence of hand eczema in 1995 ($p=0.677$). No significant differences were found within the genders in reported hand eczema in 1995 (females, $p=0.490$; males, $p=0.297$).

In the first dispatch, 899 (37%) responded, the first postcard reminder yielded 158 (10%) responses. On the second reminder 437 (32%) responded. With the final postcard reminder, 22 (2%) responded, which left 887 non-respondents.

One-year prevalence of hand eczema

The 1-year prevalence of hand eczema in 2015 was 15.8%, females reported hand eczema twice as often as males, 20.3% versus 10.0%, ($p<0.001$). The estimated true 1-year prevalence for this cohort was: $(0.158+(0.99 - 1)) / (0.73+(0.99 - 1))=20.6\%$, 26.8% for females and 12.5% for males. The 1516 participants were allocated to any of the four groups. One hundred and sixty respondents (10.6%) reported that they had had hand eczema at some time, but not in 2002 nor in 2015, 29 individuals, 1.9%, did not answer the question.

Incidence of hand eczema

In 2002 in total 13.3% (202/1516) reported they had or had had hand eczema, 139 females, (16.2%) and 63 males (9.6%), $p<0.001$. In 2008 an additional 198 individuals reported themselves having or having had hand eczema. Thus the cumulative incidence over the 13 years was 15.1% (198/1314), for the females 18.6% and for the males 10.7%, $p<0.001$. The incidence rate was estimated as 11.6 cases per 1000 person-years, 14.3 for females and 5.2 for males ($p<0.001$).

Hand eczema versus childhood eczema, asthma, rhino-conjunctivitis and gender

Childhood eczema was reported by 400/1516 (26.4%) of the participants. The proportions of having had childhood eczema, asthma and rhino-conjunctivitis in the four groups in total and by gender for 2015. The proportions of the individuals reporting only childhood eczema; i.e. not in combination with asthma and/or rhino-conjunctivitis (146/1516, 9.6%).

Factors predicting hand eczema

The analysis of endogenous and exogenous factors was performed with multinominal logistic regression.

DISCUSSION

In this study comprising 1,516 young adults, the 1-year prevalence of hand eczema was more than 15%. One third of these individuals also had 1-year prevalence at the baseline 2002. The 1-year prevalence, and not the point prevalence, was used in all calculations because it better reflects the persistency, the relapsing course and the seasonal variations of the disease. The increase in the one-year prevalence between the two occasions is in accordance with previous large Swedish cross-sectional studies with respect to the age groups.

The estimated incidence of hand eczema in our study was 11.6 cases per 1000 person-years, 14.3 among females and 5.2 among males. Our figures are in the upper amplitude compared to an earlier population based study from Sweden, which showed between 11.4 and 3.7 cases/1000 person-years among 20–29 year-old females and males, respectively. One explanation could be that our study is prospective, and underreporting is to be expected in retrospective questionnaire studies. Based on 7 European hand eczema studies performed among 16–77 years-olds, the median incidence rate of hand eczema was 9.6 cases/1000 person-years (range 4.6 11.4) among women and 4.0 cases/1000 person-years (range 1.4–7.4) among men, which is also slightly lower than our current findings, probably due to age-differences. To the best of our knowledge there are no comparable studies of the cumulative incidence in this age group. The cumulative incidence of hand eczema in our study across 13 years was 15.1% (18.6% for females and 10.7% for males). This can be considered to be a high proportion. When using a questionnaire for estimating the true occurrence of a disease it is important to know the sensitivity and specificity of the question used. The question on 1-year prevalence of hand eczema underestimates the occurrence. However, regarding childhood eczema the occurrence has been found to be overestimated especially if the true prevalence is low. Based on prevalence as well as incidence, the occurrence of hand eczema is approximately twice as common among females compared to men, which is similar to other population-based studies.

CONCLUSIONS

This study demonstrated that incidence of hand eczema in early adulthood tends to be associated with factors in everyday life such as frequent hand-washing. Regarding childhood eczema, the odds ratio for having hand eczema was twice as high in the compared to the another group, indicating a high vulnerability in this group. Furthermore, early onset of hand eczema seemed to be related to endogenous risk factors such as a history of childhood eczema. The higher frequency of hand eczema among women depended on exogenous factors.

The Treatment of Melasma by Silymarin Cream

Dr. Seeba Hussain[1], Dr. Praveen Kumar Bhopalka[2]

ABSTRACT

Background : Melasma is an acquired increased pigmentation of the skin characterized by symmetrical and confluent grey-brown patches usually on the areas of the face exposed to the sun. Silymarin strongly prevents photocarcinogenesis, and significantly prevented melanin production. The objectives of this study were the assessment of safety and efficacy of topical Silymain (SM) cream in a double-blind placebo controlled study for treatment of melasma patients.

Methods : Experimentally on 24 Albino rabbits were randomly divided into 4 equal groups. [A] No treatment, [B] received placebo, [C] treated with SM cream (0.1), & [D] treated by SM (0.2), were applied topically before UV sun light exposure for 30 days, assessed clinically & tissue pathology. Clinically on 96 adults diagnosed with melasma randomized to three equal groups to receive one of the tested drugs applied twice daily for 4 weeks, evaluated by the response; lesion size, melasma area and severity index score, Physician global assessment, and subjective assessment.

Results : The Clinical and histopathology observations were reduced significantly in SM groups. Clinically; all patients showed significant excellent pigment improvement & lesion size reduction with SM treatments from the 1st week. All patients were fully satisfied 100%. No side effects were observed.

Conclusions : Silymarin showed tremendous improvement of melasma in a dose-dependent manner, and was effective in prevention of skin damage caused by U.V. sunlight. It is a safe new candidate effective treatment for melasma.

INTRODUCTION

Melasma is a common acquired pigmentary disorder that occurs usually in women (more than 90% of cases) of all racial and ethnic groups. Melasma presents as brown to grey macules and patches, with serrated, irregular, and geographic borders. The pigmented patches are usually sharply demarcated and symmetrical. Melasma has a predilection for sun-exposed areas. The etiology is not entirely elucidated; however, the ultraviolet sunlight exposure appears to be the most significant factor. In those patients with epidermal type melasma, there are several treatments available. Topical agents include phenols, e.g., hydroquinone; retinoids, e.g., tretinoin; azelaic acid; kojic acid; and glycolic acid.

Silymarin, derived from the milk thistle plant *Silybum marianum* (L.) Gaertn] is a natural polyphenolic flavonoid. Its main component silybin (silibinin), is considered to be the most biologically active with potent antioxidant properties. Cutaneous photoprotection mechanisms triggered by silymarin and silybin are numerous and mainly demonstrate mainly their ability to reduce and suppress harmful effects of solar UV radiation, such as UV-induced oxidative stress, inflammation, immune responses and DNA damage as well as induction of apoptosis. Silymarin significantly prevented melanin production in a dose-dependent manner with an IC50 value (concentration producing 50% maximal inhibition) of 28.2 ºg/ml, without effects on cell viability. Even in high doses, silymarin does not show any toxic effects and, in fact has no harmful effects on the embryo.

MATERAL AND METHODS

Experimental study

Twenty-four healthy rabbits Albino rabbits (weight: 1500±500 g each), were individually housed in suspended cages, for 1 week before the experiment. They were kept in the same environmental and nutritional conditions (temperature 25±2 C, relative humidity 40%-60%, and 12 hours in light and 12 hours in darkness cycles) in the animal house of the college of medicine. At the beginning of the study, animals were randomly divided into 4 equal groups (n=6); group [A] did not receive any treatment, [B] received placebo, [C] treated with SM (0.1 mg/ml.kg^{-1}) cream, and [D] treated by SM cream (0.2 mg/ml.kg^{-1}). 3 cm^2 of Albino rabbits' back were shaved. Then after 48 hours, the tested drugs were applied topically by cotton pad stick on the shaved area of all groups daily, 30 minutes before each UV sun light exposure. The rabbits were exposed to UV sunlight (11+ extreme) for 3 hours during each day of June (temperature 43±2 C) for 30 days. All animals were painlessly killed by chloroform and samples were taken from the shaved area for tissue pathology study, the samples were put in formalin buffer 10%.

Histopathological examination

All samples were cut into small blocks. These blocks of tissues were then routinely processed. These paraffin blocks were sectioned into 5 ºm thick and stained with hematoxylin ... eosin. The stained tissues were examined for histopathological alterations. The histopathology departments' staffs were blinded to the tested drugs.

Clinical study

This is a double blind, randomized clinical study on patients with melasma

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attending the outpatient clinic of the Dermatology department of Katihar Medical College & Hospital, Katihar, Bihar. The inclusion criteria were adults with melasma without any topical, systemic, laser, and surgical treatment on face during the previous months. While the exclusion criteria were pregnant and nursing women, patients with history of hypersensitivity to some of the components of the formulas of the study, and coexistence of associate diseases and other pigmentation diseases, and concomitant use of other skin care products or systemic treatments. A history was taken from each patient, regarding age, gender, occupation, time of onset, history of pregnancy, contraceptive pills, and sun exposure. Patients were randomized in a double-blind manner to receive one treatment of the tested drugs; Group I (G I) SM (7 mg/ml) cream, Group II (G II) SM (14 mg/ml) cream, or Group III (G III) placebo, applied topically to the affected areas, twice daily for 4 weeks, also advised to avoid sun exposure and to use topical sunscreen with sun protection factor (SPF) of 15+during the entire period of treatment and thereafter. The patients were seen regularly every week for one month for assessment; the response to treatment was rated by the size of lesions. Skin pigment evaluation by melasma area and severity index (MASI), physician global assessment (PGA); assessment of overall treatment of disease activity, used a scale from 0 to 10, by an independent observer blinded to the treated groups, and record the presence of any side effect. The Subjective assessment depending on recording improvement in patient satisfaction measures during the time course, and graded as follows: Grade 0 =not satisfied, Grade 1 =moderately or partially satisfied, Grade 2 =greatly but not fully satisfied, Grade 3 =fully or completely satisfied.

Statistical analysis

In order to analyze the data, Chi-square test, Student *t* test and *X* 2 were used, *P* value of less than 0.05 was considered significant, data showed as mean ± SD (SPSS 18)

RESULTS

Experimental study results

Some clinical features such as skin scaling, skin irregularity, erythema, skin hyperpigmentation, and edema were evaluated. The clinical observations are as follows:

Group A (G A), without treatments, dermal scaling, skin irregularity, erythema & hyperpigmentation, and edema were observed at the highest level (100%).

Group B (G B), received placebo, dermal scaling, skin irregularity, erythema & hyperpigmentation, and edema were observed at the level (90%).

Group C (G C), treated with SM (0.1) cream, no clinical features were observed.

Group D (G D), treated with SM (0.2) cream, no clinical features were observed.

The significance between groups was; G A *vs.* B, C, & D groups, p=0.0001, G B *vs.* C, & D groups, p=0.0001, and G C *vs.* D; p>0.05.

Histopathology results

Epidermal hyperpigmentation, epidermal hyperkeratosis, lymphocyte infiltration into epidermis, squamous cell proliferation, edema and dermal thickness increase, infiltration of lymphocytes, plasma cells, and eosinophils into dermis were noticed in all cases of G A (100%), in G B were (58%, 50.2%, 4%, 45%, 60.2%, 60.2%) respectively. While in G C, & G D all the microscopic observations were not seen. The level of significance was G A vs B, C, & D groups, p=0.0002, G B vs C, & D groups, p=0.0001, and G C vs D, p>0.05.

Clinical study results

Ninety-six melasma patients were enrolled in this study, female (F) 80 (83.3%) and male (M) 16 (16.66%), the ratio of sex distribution (F: M) within the groups were (G I (26:6), G II (27:5), and G III (27:5). The patients' age ranged from 28 to 55 years (median, 41 years). The duration of melasma varied from 2 to 6 years (median 4 years). Family history of melasma was found in 46 (47.916%) patients. The most frequent precipitating factors were the sun exposure (90%), and pregnancy (10%)

The response to treatment was evaluated as per the size of the lesion at the end of each week till 4 weeks. G I & G II showed significant reduction in size of the lesion at the end of 1st week compared to G III, complete clearing of the lesion was shown in G II after 3 weeks of treatment, while G I in the fourth week.

DISCUSSION

Skin exposure to solar UV radiation induces a number of skin disorders, including erythema, edema, sunburn cell formation, hyperplasia, immune suppression, DNA damage, photoaging, melanogenesis and skin cancers. It is well documented that UV irradiation, both its UVB (290–320 nm) and UVA (320–400 nm) component, induces the generation of reactive oxygen species (ROS), which create the oxidative stress in skin cells and play an important role in the initiation, promotion and progression of skin aging and carcinogenesis. Thus, the use of antioxidants, namely naturally occurring herbal compounds, is receiving considerable interest to protect skin from adverse biological effects of solar UV radiation. In the SKH-1 hairless mice silymarin inhibited UVB induced skin edema, formation of sunburn and apoptotic cells, prevented UVB-induced infiltration of inflammatory leukocytes, and significantly reduced the activity of myeloperoxidase, a marker of tissue infiltration.

Cellular antioxidant status plays a crucial role in modulating the effects of unrepaired DNA lesions and cellular sensitivity to the DNA damaging effects of solar UV radiation. Benefits have been observed from both systemic and topical administration. Significant inhibition of UVB-induced sunburn, apoptotic cell formation, and edema has been associated with topical application of silymarin. The present study agrees with the previous studies that the experimental study showed no clinical or histopathological features in Silymarin treated groups (both concentrations) compared to placebo and none treated

groups after exposure to UV sunlight. Also, it may be one of the mechanisms of action of Silymarin in the treatment of pigmented lesion.

The body possesses endogenous defense mechanisms, such as antioxidative enzymes (superoxide dismutase, catalase, glutathione peroxidase) and nonenzymatic antioxidative molecules (vitamin E, vitamin C, glutathione, ubiquinone), protecting it from free radicals by reducing and neutralizing them[15]. Some can be inhibited by ultraviolet (UV) light. It has been reported that UVA decreases intracellular glutathione status and subsequently increases UVA sensitivity of keratinocytes. The DNA-damaging effect of UVA can be reduced by improving the regulation of intracellular antioxidant status by suitable antioxidants.

Silymarin shows strong free radical-scavenging activity that is severalfold greater than that of vitamin E. It inhibits lipid peroxidation and provides significant protection against UVB-induced depletion of catalase activity. Therefore, silymarin can effectively terminate the harmful biochemical reactions by scavenging free radicals and ROS, and by strengthening the cellular antioxidant status[. This may be the other mechanism of action of Silymarin in the treatment of melasma.

Silymarin inhibited l-DOPA oxidation activity of tyrosinase, the rate-limiting melanogenic enzyme, in cell based-systems, but it did not directly affect cell-free tyrosinase activity. Furthermore, Western blot analysis indicated that silymarin decreased the expression of tyrosinase protein. This explains the exact main mechanism of action of silymarin in the treatment of melasma.

CONCLUSION

This study showed a significant reduction of pigment, melasma lesion in a short period of time. Silymarin has the efficacy to treat melasma in a dose dependent manner. It is safe. No side effect was observed. All patients were fully and completely satisfied from the first week of treatment with Silymarin.

The author suggests studying other pharmaceutical preparations of Silymarin like gel or paint and other dosing intervals in the treatment of melasma.

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Original Article

Comparative evaluation of hydroquinone, tretinoin and mometasone versus glycolic acid versus trichloroacetic acid peel in melasma

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Abstract

ObjectiveComparative evaluation of 2% hydroquinone, 0.025% tretinoin and 1% mometasone versus glycolic acid and trichloroacetic acid peel alone in melasma.

MethodsSeventy five patients were included in the study. Total duration of study was one and a half years. Melasma area severity index (MASI) score of more than 10 were included in the study. The patients were divided into three groups. Group A consisted of 25 patients and were given 2% Hydroquinone, mometasone 1% and 0.025% tretinoin. Group B consisted of 25 patients and underwent glycolic acid peel. Group C consisted of 25 patients and underwent trichloroacetic acidpeel.

Results Group A had better response, followed by group B and group C, respectively.

ConclusionCombination of hydroquinone, mometasone and tretinoin is a good option to treat melasma only when used judiciously along with sunscreen, whereas glycolic acid peels are a safer option with fewer side effects.

Keywords
Melasma, hydroquinone, tretinoin, mometasone, glycolic acid, trichloroacetic acid.

Introduction

Melasma is one of the most common pigmentary disorders seen in dermatology outpatient department. Melasma is known to have a multifactorial etiology. Contributing factors include increased UV exposure, pregnancy, cosmetics, genetic factors, endocrine factors, and hormonal therapy.[1-3] It is a definite cause of cosmetic concern in females.Melasma is a dysfunction of pigmentary system, resulting in an irregular brown or grayish-brown facial hypermelanosis. Different topical and peeling agents have been tried for the treatment of this recurrent condition with varied success. Glycolic acid and trichloroacetic acid (TCA) have been used alone and also in combination to treat melasma.[4,5]

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Methods

Seventy-five patients were included in the study. Total duration of study was one and a half year. The study was conducted in a tertiary care centre in Bihar in the Department of Dermatology, Venereology and Leprosy outpatient section after approval of the ethical committee of the institute.Patients were diagnosed clinically and Wood's lamp examination was done to differentiate epidermal, dermal and mixed pattern of melasma.Patients with melasma area severity index (MASI) of more than 10 were included in the study. The patients were divided in to three groups. Group A consisted of 25 patients who were treated with topical 2% hydroquinone, mometasone 1% and 0.025% tretinoin. Group B consisted of 25 patients and underwent glycolic acid peel. Group C consisted of 25 patients and underwent TCA peel. Patients with previous history of herpes, keloids, hypertrophic scars, pregnancy, on isotretinoin, oral contraceptive pills, non-compliant patients, hypersensitivity to any of the treatment regimen were excluded from the study. Patients' consent was taken prior to the study. Sunscreen with SPF >30 wasadvised during daytime. During every visit, clinical improvement was graded as shown in **Table 1**.

Group A were given 2% hydroquinone, mometasone 1% and 0.025% tretinoin to be applied at night followed by judicious use of sunscreen in the morning. Group B underwent glycolic acid peel with concentration of 20-35% at 2 weekly interval with time being increased on an incremental basis starting with thirty seconds, one minute, one and half minutes, twominutes in different concentrations. All Patients

Table 1 Grades of clinical improvement.

Grade	*Clinical improvement*
1	Slight improvement barely noticeable (<25%)
2	Moderate improvement, noticeable (25-50%)
3	Obvious improvement (50-75%)
4	Very marked improvement (>75%)

were advised a sunscreen with SPF>30 in the morning.Group C underwent TCA peel withconcentration of 10-20% at 2-weekly interval with time being increased on an incremental basis for a total of six sittings.

Results

Majority of the cases were females and they comprised 80% of the total cases with housewives as the majority constituting 49.5% of the cases. Clinical pattern of the malar type was seen in 66.5% of the cases. Duration of melasma was less than 5 years in 67% of the patients. Positive family history was elicited amongst 35% of cases. History of use of cosmetics was present in 73.5% of them. Most common aggravating factor was sunlight exposure, seen in 17.5% of cases. On Wood's lamp examination accentuation of the lesion was seen in 26% of cases.

Table 2 shows the treatment response in three groups. Grade 4 response i.e. >75% improvement was seen in 10 (40%) patients of group A, 3 (12%) of group B and 2 (8%) patients of group C. Similarly, grade 3 improvement i.e. 50%-75% was seen in 6(24%), 9 (36%) and 8 (32%) patients in group A, group B and group C, respectively. The respective grade 2 improvement i.e. 25-50% in three groups A, B and C was 20%, 28% and 32%. Slight improvement i.e. grade 1, <25% was seen in 16%, 28% and 28% patients in three groups, respectively.

Table 2 Grades of improvement in three groups, group A (hydroquinone + mometasone + tretinoin), group B (glycolic acid peel), and group C (trichloroacetic acid peel).

Grades of improvement	*Group A*	*Group B*	*Group C*
Grade 4 (very marked improvement, >75%)	10 (40%)	3 (12%)	2 (8%)
Grade 3 (obvious improvement, 50-75%)	6 (24%)	9 (36%)	8 (32%)
Grade 2 (moderate improvement, noticeable, 25-50%)	5 (20%)	7 (28%)	8 (32%)
Grade 1 (slight improvement barely noticeable, <25%)	4 (16%)	7 (28%)	7 (28%)

Overall, group A had better response, followed by group B and group C, respectively.

Discussion

Melasma is one of the commonest pigmentary disorders seen in dermatology outpatient. It isThe exact pathogenesis is unknown though the major triggering factors are sunlight and female hormones. Difficult to treat melasma also has a dermal component.Three clinical patterns are generally seen: malar, centrofacial and mandibular type. The principles of therapy include protection from UV light, inhibition of melanocyteactivity and melanin synthesis and the disruption and removal of melanin granules.[6] An effective treatment for epidermal hypermelanosis is a combination of HQ, a topical steroid, and tretinoin. The combination strongly inhibits the production of melanin without the destruction of melanocytes.

Kligman and Willis[7]proposed a preparation containing HQ 5%, tretinoin 0.1%, and dexamethasone 21-acetate 0.1%, to be applied daily for 5 to 7 weeks, and it was found to be effective in the treatment of melasma. In addition, they discovered that omitting any one component resulted in a loss of effectiveness. Lowering the concentrations of the components decreased the frequency of irritancy, but alsodecreased the potency of the mixture.[7] Javaheri *et al.*[8]performed a study with 25 womenwith melasma. The degree of improvement was measured based on changes in MASI scores. The response of each patient was graded as no response (no decrease in MASI score), mild (<25%), moderate(25% to<50%), good (50% to<75%), and very good ([75%). A total of 23 patients completed the study.

In the 70% of patients, reduction of pigmentationwas apparent after the first peel. At the end of thethird peel, 4 patients demonstrated a good response,11 had a moderate response, and 6 showed a mildresponse. Two patients did not show improvement.Overall, improvement in melasma (reduction inMASI) was observed in 91% of patients (P<0.01).Patients with epidermal type melasma demonstrateda better response to treatment than those with mixedtype melasma (P<0.05). At treatment end, onepatient experienced a mild degree of treatmentinducedhyperpigmentation, but during follow-upno other patient developed anysymptoms.[8]In a randomized, investigator-blind, split-faceprospective trial,[9,10]Hispanic women with melasmawere treated with 4 GA peels (either 20% or30%) plus 4% HQ on one side of the face and 4% HQcream alone on the other. Of the 18 patients whocompleted the study there was no significant differencein the degree of lightening, or difference in theMASI scores from baseline to study end, between thetwo groups. The physician global evaluation showedthat 8 patients had more improvement on the peeledside versus 7 patients with more improvement on thenonpeeled side. Most patients felt tingling and some developed mild erythema. From this study it seemsthat, although GA peels may improve melasma, they are no more effective than HQ alone. However, it should be noted that these investigators recommended that more studies be done comparing the efficacyof HQ with GA peels.[9]

Chun *et al.*[11]explored the use of focal TCA, a derivative of acetic acid, peeling on dark-skinned individuals with various pigmented lesions, including melasma. There was focal application of TCA at concentrations of 10% to 50% to 20 patients with melasma. In all, 11 patients (55%) experienced goodclinical response. There were no significant complications, such as persistent erythema,hyperpigmentation, herpes simplex flare up, scarring,or keloids. Mild erythema and transient PIHoccurred only in rare cases. The study concluded that focal TCA peels are a safe and effective method of treating benign pigmented lesions.[11]

Conclusion

Combination of hydroquinone, mometasone and tretinoin is a good option to treat melasma only when used judiciously along with sunscreen, whereas glycolic acid peels are a safer option with fewer side effects.

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Section Dermatology | Original Article

Comparison of Repigmentation of Stable Vitiligo via Punch Grafting, Thin-thiersch's grafting and Suction Blister Grafting

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ABSTRACT

Background: *Vitiligo is defined as an acquired melanocytopenia of obscure aetiology and is characterized by circumscribed hypomelanosis and depigmentation of skin and hair which is often progressive. The aim of present study is to compare between 3 surgical modalities i.e. miniature punch grafting, thin split-skin thickness grafting and suction blister grafting and to experiment a novel method according to the site, size and location of the lesion and to study the extent of re-pigmentation after doing these procedures and comparison of the results in patients of stable vitiligo. We also aim to assess the complications and disadvantages of different surgical techniques.* ***Methods****: The study was conducted on total 60 vitiligo patients, who were divided into 3 groups of 20 each, in the age group of 15-60 years, attending Dermatology Department of Katihar Medical College in the span of 2 years****Results****. Results are comparable overall but vary considerably according to site of lesions. Punch grafting is very good for mobile areas like elbow, ankle and other joints; Thin – thiersch's skin grafting gives better results for flat areas like trunk, thigh, arms and face while Suction blister grafting gives satisfactory results for lips vitiligo and also over small, oval lesions over flat sites.* ***Conclusions****: Thus, it can be finally concluded from this study that the surgical modality for treating a case of vitiligo cannot be generalized. Every patient should be evaluated individually according to anatomical site involved, size and shape of lesion, time required to achieve pigmentation, infrastructure available and patient's preferences.*

Key words*: vitiligo, punch graphting, suction blister graphting, repigmentation.*

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INTRODUCTION

Vitiligo is an acquired cutaneous hypomelanosis with a 0.5–2% incidence worldwide, without predilection for sex or ethnicity. The clinical presentation is characterized by well-circumscribed white macules. Generalized vitiligo is characterized by acquired depigmentation due to melanocyte loss, in a pattern that is non-focal and generally bilateral across the midline, though not necessarily symmetric.[1] Vitiligo is characterized by a disappearance of epidermal and/or follicular melanocytes. It is likely that melanocytes are destroyed by an as-yet unknown process.[2] It is believed that vitiligo is of polygenic trait and that a convergence theory, combining elements of all the different theories, autoimmune, neural , auto-cytotoxic and growth factor

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defect is the most accurate aetiology. Familial studies have shown the increased prevalence of vitiligo in close relatives of affected individuals. In a large series performed in India, this increase was about 4.5-fold in close biological relatives.[3] The pattern of relationship between relative risk and degree of kinship indicates involvement of genetic factors, although it is not consistent with single-locus Mendelian transmission. The major genetic component in vitiligo pathogenesis and also the role of environmental factors were recently emphasized. Sporadic generalized vitiligo is associated with autoimmune thyroid disease, pernicious anemia, Addison's disease, systemic lupus erythematosus.[4] Familial generalized vitiligo is also characterized by a broad repertoire of associated autoimmune diseases, such as thyroiditis, rheumatoid arthritis, psoriasis, adult-onset-dependent diabetes mellitus, pernicious anemia, and Addison's disease.[5]

The natural course of disease is usually unpredictable, but is often progressive. After years of stabilization, a sudden exacerbation may occur. A more rapid progressive from of vitiligo may lead to a complete de-pigmentation within 6-12 months after onset of disease. Vitiligo can be treated by a number of medical and surgical interventions. The different surgical interventions are needling, tattooing, miniature punch grafting, split thickness skin grafting and cultured or non-cultured melanocytes transplantation.

AIM OF STUDY

The aim of present study is to compare between the 3 modalities i.e. miniature punch grafting, thin split-skin thickness grafting and suction blister grafting and to experiment a novel method according to the site, size and location of the lesion and to study the extent of re-pigmentation after doing these procedures and comparison of the results in patients of stable vitiligo. It was also aimed at assessing the complications and disadvantages of different surgical modalities and to provide treatment to patients of vitiligo that were resistant to medical therapies and to cut short the length and duration and the total cost treatment.

Surgical procedures aim to replace the melanocytes with ones from a normally pigmented autologous donor site. Several melanocyte transplantation techniques can be performed under local anaesthesia in an outpatient facility [6]. Punch grafting (tissue graft) is the easiest and least expensive method, but it is not suitable for large lesions and seldom produces even repigmentation. Epidermal blister grafting gives excellent cosmetic results, but it is time-consuming, and large areas cannot be treated. Ultrathin epidermal sheet grafting can treat larger areas (up to 200 cm2) but requires skill and experience. The highest incidence of adverse events occurs with punch grafting (scar formation at the donor site, cobblestoning of the acceptor area) followed by ultrathin epidermal grafting (transient or permanent hypopigmentation, hypertrophic scars on the donor site, milia formation on the recipient site) and suction blister epidermal grafting (transitory hyperpigmentation on donor site, imperfect colour matching on the recipient site).[7]

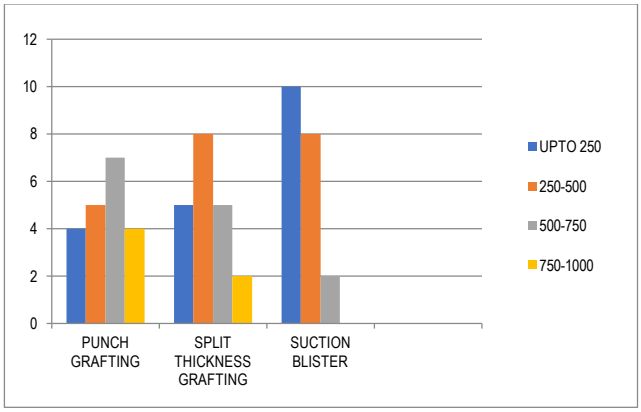
METHODS

The study was conducted on stable vitiligo patients of both sexes in the age group of above 15 years, attending Dermatology, Venereology and Leprosy Department of Katihar Medical College & Hospital in the span of 2 years. Patients with active disease or those having > 10% BSA involvement were excluded from the study. Miniature punch grafting, Thin – thiersch's skin grafting and Suction blister grafting was done on stable, localised and resistant cases of vitiligo in 60 patients with altogether 75 sites in a span of 2 years. After a through pre-operative check-up and charting the area to be grafted, they were divided in 3 groups with 20 patients in each group and grafted with the three different methods.

RESULTS

Most of the patients were in age-group 15-30 years (78%) . The sex ratio was almost same with slight female preponderance with 34 females and 26 males. Age of onset of disease ranged from 9-58 years. In most of the patients onset of disease was in between 11-20 years. In 20% it was between 21-30 years. Lesser patients were in the age group of 41-50 years (11%) and 51-60 years (9%). 58% had disease of 1-2 years duration, 12 patients(27%) had disease of duration of 3-5 years while only 15% patients were having disease of longer duration. Cases included in the study were stable since last 1-6 years. 62% patients have stable disease since last 1-2 years whereas only 7% patients have stable disease of more than 6 years. Majority of patients (75%) were having focal vitiligo while lesser patients had segmental vitiligo (15%) and only 6 patients had lesions at multiple sites.

In this study, smaller lesions were subjected to suction blister and split-skin thickness grafting while larger lesions were dealt with punch grafting but the overall total VASI in all three groups was equivalent.

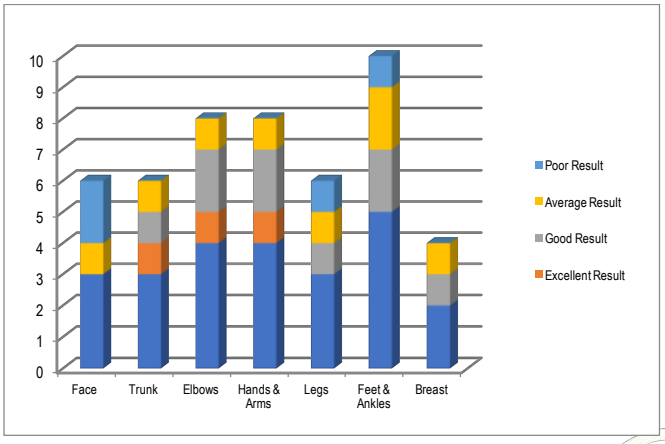


Pre-Treatment VASI

A total 75 sites in 60 patients were subjected to surgery. The distribution of lesions in all the groups was comparable except the lip lesions which were more subjected to suction blister technique. Rest all the body parts were unequivocally dealt with the 3 different modalities. Out of 60 patients only 12% showed superficial scarring and 13% had infection at the donor site, while majority (73%) had no complications.

The complications seen had similar incidence in all 3 groups. Out of 60 patients, about 28% of patients developed complication at the recipient sites. Cobblestoning was a unique complication seen in 5 out of 24 (20%) patients of punch grafting, colour mismatch was also more common in punch grating appearing as polka-dots. Erythema was the most common complication seen in 12% of total patients.

In this study, majority of patients showed good and average result, about 67% . Excellent results were seen in lesions over trunk, elbow and hand . 17% Of patients showed poor results and half of them were face lesions.



Post Treatment VASI Group 'A' Punch grafting

The following pictures show the result obtained in a patient with vitiligo patch in the neck region who underwent punch grafting. Figure 1 shows the pre-treatment photograph of the patch in the neck region. Figure 2 and figure 3 shows the graft donor site from where multiple punch grafts were taken and the graft site where the grafts were placed respectively. Figure 4 shows grafts in situ. Figure 5 and 6 shows post treatment response after 6 weeks and 12 weeks respectively.

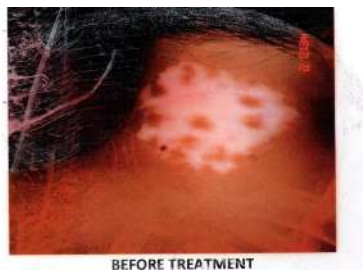


Fig 1

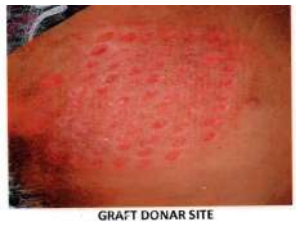


Fig 2

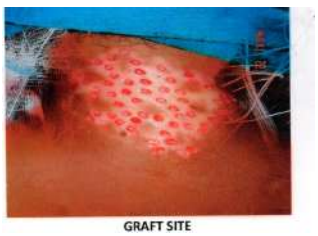


Fig 3

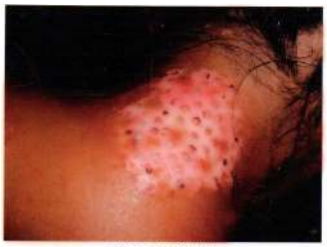


Fig 4

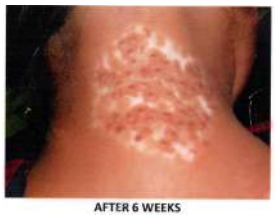


Fig 5

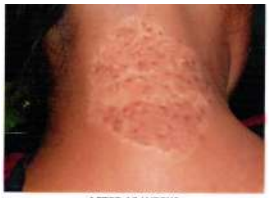
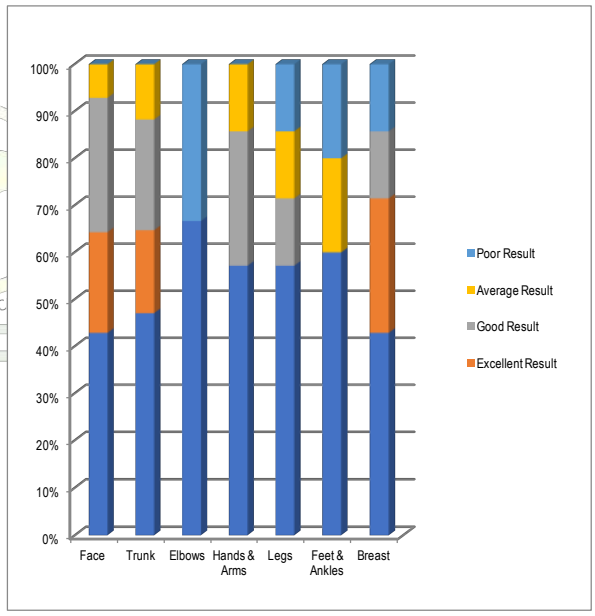


Fig 6

Post Treatment Vasi Group 'B'– Thin – Thiesch's Grafting

In This Study, 27% Of Cases Showed Excellent Pigmentation and a Total Of 40% cases showing good or better results. Only 13% Had Poor Results; these cases had lesions over joints.



Post Treatment Vasi Group 'B'– Thin – Thiesch's Grafting

The following pictures show the result obtained in a patient with vitiligo patch over the waist region who underwent thin-thiesch's grafting. Figure 7 shows the pre-treatment picture of the patient. Figure 8 shows the graft being taken from the donor site. Figure 9 shows dermabrasion being done done at the donor site and Figure 10 shows graft in situ. Figure 11 shows results obtained after 12 weeks.

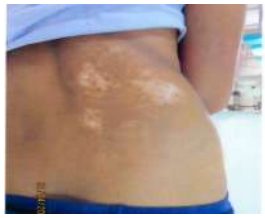


Fig 7

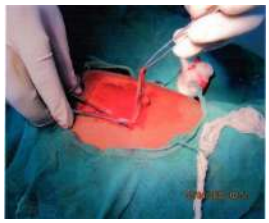


Fig 8

Fig 9

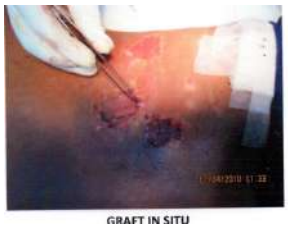


Fig 10

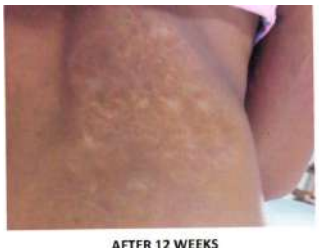
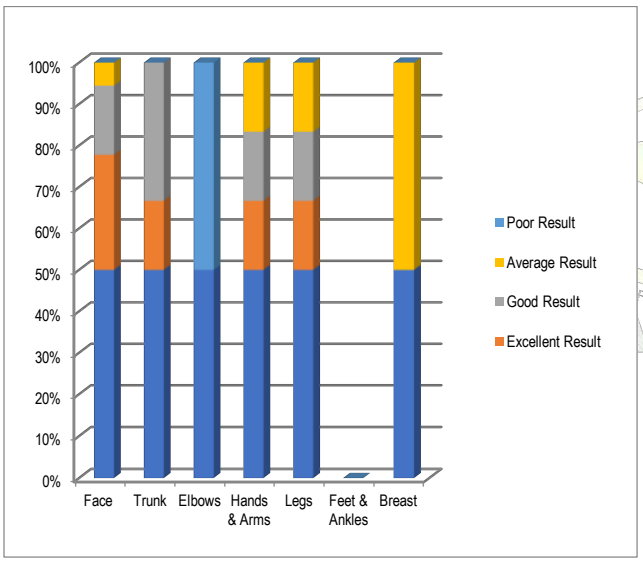


Fig 11

POST Treatment VASI- GROUP "C" SUCTION BLISTER

In this study, about 40% of patients showed excellent to good results, the lesions were mostly over face and lips. Only 1 patient (5%) with lesion over elbow showed poor result.



POST Treatment VASI- GROUP "C" SUCTION BLISTER

The following pictures show how suction blisters were raised for a patient who underwent suction blister grafting.

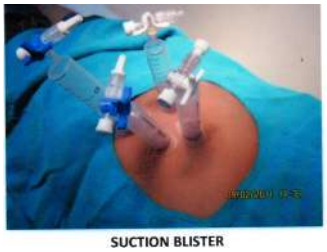


Fig 12

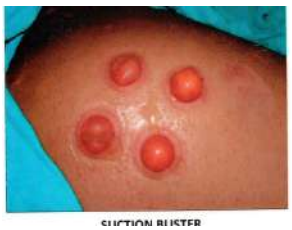
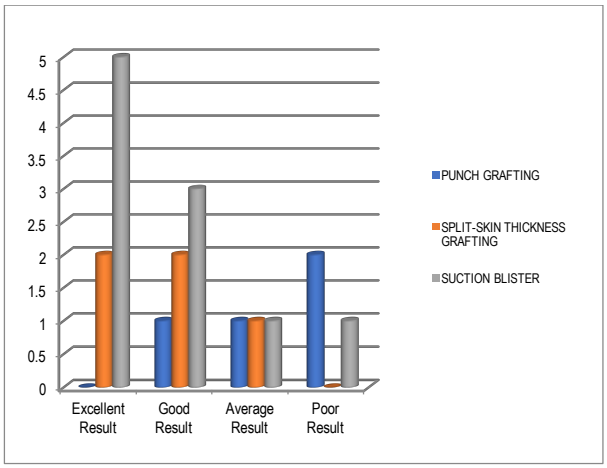


Fig 13

This is study showed that almost equivalent results are seen in all 3 groups in terms of total pigmentation, but they have differences only in terms of sites of lesions. In this study, the difference in result of surgery over face and lip lesions was showed. While 57% lesions in group C showed excellent results, in split-skin thickness grafting about 50% of cases showed excellent pigmentation while group A patients mainly showed average to poor results over lip vitiligo.



DISCUSSION

In the present study, 60 patients with 75 vitiliginous sites were selected and divided into three groups. The first group contained 20 patients with 30 disease sites over which miniature punch grafting was done. In the second group, 20 patients with 25 vitiligo sites on different parts of body were subjected to thin-split-skin thickness grafting. The last group contained 20 patients with 20 disease sites the maximum of which were lip lesions, and subjected to suction blister grafting technique.

This study showed various complications obtained at donor sites in different grafting techniques. In miniature punch grafting, superficial scarring was the most common complication encountered in 12% of the cases followed by infection in 8%. In the Thin – thiersch's grafting patients, superficial scarring and infection was seen in equal number of patients (15% each) while no complication was seen in rest 70% of patients. In the third group of suction blister grafting, even lesser degree of complications were seen at donor sites, infection in 2 out of 15 sites and superficial scarring in 1 case.

This study showed the complications seen at the recipient sites in different groups. Erythema was most common and was found almost equally in all treatment groups Cobblestoning was unique complication seen only in patients undergone miniature punch grafting in 21% of the sites. Colour mismatch of the graft with surrounding area was seen almost equally in all 3 groups.

Study showed the results obtained after 6 months of punch grafting. Excellent results were obtained in 3 out of 24 sites, 1 each over trunk, hands & arms and legs. Good pigmentation i.e achievement of 75% IN PRE-TREATMENT VASI was obtained in a fair number of patients, about 34% . Good results were seen at almost every site except for face and lips. 1 case lip vitiligo showed average result while 2 showed poor re-pigmentation on mobile areas like elbows and ankles, the results were also not good.

The study showed the re-pigmentation obtained in 15 patients with 20 sites undergone thin split-skin thickness grafting. Excellent results were obtained in 25% of cases, 2 each on face and trunk and 1 case over breast. About 40% sites showed good degree of re-pigmentation over almost every site except for joints like elbows and ankles. Poor results were seen at these sites.

Study showed the result of suction blister done over 20 sites. Excellent re-pigmentation at the end of 6 months was seen in one – third of patients but the majority of them were on vitiligo of lips. Over trunk and extremities the results were good (33%) and average (27%) while on joints, it was poor.

The study showed the overall pigmentation results in the 3 treatment groups . Punch grafting patients mainly showed average to good pigmentation over trunk and joints but poor pigmentation over lips. Suction blister and thin split skin thickness grafting patients had excellent results over lips and other flat areas while they showed poor re-pigmentation over elbows and ankles.

CONCLUSION

Punch grafting is very good for mobile areas like elbow, ankle and other joints, but gives poor results for angle of mouth, lips and fingers. Thin – thiersch's skin grafting gives better results for flat areas like trunk, thigh, arms and also face including lips, but is technically difficult to obtain thinner graft and the thinner the graft, the better the result. Suction blister grafting gives very satisfactory results for lips vitiligo and also over small, oval lesions over flat sites, but gives poor results for mobile areas and is unsuitable for larger lesions.

Thus, it can be finally concluded from this study that the surgical modality for treating a case of vitiligo cannot be generalized. Every patient should be evaluated individually according to anatomical site involved, size and shape of lesion, time required to achieve pigmentation, infrastructure available and patient's preferences. Patient should be counselled for all possible therapeutic options and should be given liberty to choose from the available modalities.

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Section Skin & VD

Acne Vulgaris and its Effect on Quality of Life: A Cross-Sectional Study

Original Article

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ABSTRACT

Background: *Acne vulgaris affects about 85% of adolescents, often extending into adulthood. Though considered to be merely a cosmetic problem, it is associated with considerable psychological impairment, such patients are prone to low self-esteem, low self-confidence and social dysfunction which may lead to anxiety, depression, obsessive compulsiveness and sometimes suicidal ideation.*

AIM: *This study was aimed to assess the impact of acne and its sequelae on the quality of life.*

Methods: *The current cross sectional study was conducted in Patients diagnosed as acne vulgaris attending OPD of HIMSR in department of dermatology for a period of 6 months. Patients aged 15 years and above were included in our study. A detailed history was taken after obtaining consent from all the participants of study. Dermatology life quality index (DLQI) was administered on patients to determine the impact of acne vulgaris on their quality of life (QOL).*

Results: *This study included 200 patients with females being more in number 130 (65%) as compared to male patients which were 70 in number (35%). The mean age was 20.49 with majority of patients being in 15-20 years of age group. Mean DLQI score was 8.22, statistically influenced by the age of the patient, duration and grade of acne, acne scar, and post acne hyperpigmentation.*

Conclusions: *This study showed significant impairment of quality of life in acne patients. Counseling along with early treatment of acne vulgaris both are important as to reduce disease-related psychosocial sequelae and enhancing the efficacy of treatment.*

Keywords: *Quality of life, Acne vulgaris, DLQ*

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INTRODUCTION

Acne vulgar is a chronic inflammatory disease of the pilosebaceous unit affecting at least 85 percent of adolescents and young adults.[1] More than 85% of adolescents suffer from acne and in 50% cases, it extends into adulthood.[2]

All body areas where pilosebaceous glands are present are prone to acne but the face, back and chest are more prone to it. It results due to androgen induced sebum production, altered keratinisation, inflammation and bacterial colonisation by *Propionibacterium* acne.[3,4]

Acne is considered to be merely a cosmetic problem; however it is compared to diseases such as asthma, epilepsy, diabetes, or arthritis which impose considerable psychological impairment These patients suffer from low self-esteem, low self-confidence and social dysfunction which may lead to anxiety, depression, and obsessive compulsiveness and sometimes even patient's have suicidal tendency.[5] These patients are more prone to embarrassment, social withdrawal, depression, anxiety, and anger.[6]

Assessment of impact of acne on health-related quality of life (QoL) is needed to fully characterize the overall disease burden and effectiveness of treatment.[7] QoL is defined by

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WHO as the "individual's perception of their position in the context of culture and value systems in which they live and in relation to their goals, expectations, standards, and concerns".[8] The QoL questionnaires helps us understand how acne affects the patient on a day-to-day basis and can also measure treatment success. DLQI is one such questionnaire. It is widely used in research and clinical practice to assess changes in health-related QoL, as it is a sensitive measure.[9,10] The objective of this study was to assess the impact of acne,and its sequelae on the QoL in our study patients.

METHODS

This study was cross sectional study conducted in Patients diagnosed as acne vulgaris attending OPD of HIMSR, New Delhi, in department of dermatology for a period of 6months.

Study tools:

Case record form to record clinical data.

Acne severity assessed by grading system on clinical grounds according to Indian classification

DLQI questionnaire to be filled by all included patients.

Inclusion criteria:

Patients diagnosed as acne vulgaris aged 15 years and above attending OPD dermatology.

Exclusion criteria:

Patients below 15years of age.

Patients with history of mental disorder or any somatic disease.

Those who have used topical & systemic drugs known to predispose them to acne

Patients who did not give consent

200 patients attending the Dermatology OPD with diagnosis of acne vulgaris were taken for the study. Patients aged 15 and above were included in our study. The study was approved by the IEC.

The procedure of the study started with identifying the participants for the study. Following this identification, before the collection of the data, informed consent was taken from all the participants and they were assured about the confidentiality by telling them about the purpose of the study. Patients were asked to fill up the DLQI questionnaire without assistance.

A detailed history about presenting complaints, duration of acne, and history of any topical / systemic drugs known to cause acne was taken. Proper cutaneous examination was done on all patients by the single dermatologist and the following features were noted: (I) Type of skin (dry/normal/oily), (ii) site of lesion (face, chest, or back), (iii) grade of acne, (iv) post acne hyper pigmentation (present/absent), and (v) acne scars.

Acne was graded into 4 types (Grade I, II, III, IV) according to Indian classification based on number, type and severity of lesion. DLQI is a general questionnaire for evaluation of quality of life in dermatology patients and consists of 10 questions about disease symptoms, feelings, daily activities, type of clothing, social or physical activities, exercise, job or education, interpersonal relationships, marriage relationships and treatment.

Statistical analysis:

IBM SPSS Statistics software version 20 was used for analyzing data. Value of $P < 0.05$ was considered significant and $P < 0.01$ as highly significant.

RESULTS

This study included 200 patients with females being more in number 130 (65%) as compared to male patients which were 70 in number (35%). The mean age was 20.49 and maximum patients were between 15-20 years of age group.

Comparison of gender with site of acne, grade of acne and acne scar (Table 1, Table1a, Table 1b)

Table 1: SITE: The face was the most common site involved (67%) followed by face and back together (13%), face & chest involved 10.5% & 3.5 % involved each chest & back together and face, chest & back together.

Table 1: Sites involved

Site	Female (N)	Males	Males & Females(Combined)	%Age
Face	100	34	134	67%
Back	2	3	5	2.5%
Face & Back	11	16	27	13%
Face & Chest	11	10	21	10.5%
Chest & Back	3	4	7	3.5%
Face Chest & Back	3	3	7	3.5%

ACNE GRADING: In our study Grade I I was the most common type 45 % of acne, followed by grade I I I (32.5%), Grade I (15%) and grade I V was seen in only 7.5%.

Table 1a.

Grade	Females	Males	Males & Females (Combined)	%Age
Grade I	20	10	30	15%
Gradei I	70	20	90	45%
Gradei I I	30	35	65	32.5%
Gradei V	10	5	15	7.5%

POST ACNE SCARS: The majority of patients 32.5 % in our study didn't have post acne scars, 28.5 % patients had severe scars followed mild scars in 20% of patients and 19% of patients had moderate scarring.

Table 1b:

Scars	Females	Males	Males &Females(Combined)	%Age
Mild	35	5	40	20%
Moderate	29	9	38	19%
Severe	31	25	57	28.5%
Absent	35	31	65	32.5

Table 2: Duration of Acne

Duration	Females	Males	Males & Females(Combined)	%Age
0 To 6 Months	42	43	85	42.5%
7 To 12 Months	30	30	60	30%
13 To 24 Months	15	12	27	13.5%
25 To 36 Months	5	10	15	7..5%
≥36 Months	6	7	13	6.5%

Around 57.5% patients had oily skin, 21.5% had dry skin and 21% had normal skin.

Table 3: Type of Skin

Skin Type	Females	Males	Males & Females (Combined)	%Age
DRY	30	13	43	21.5
OILY	80	35	115	57.5%
NORMAL	20	22	42	21%

Post hyperpigmentation was absent in 56.5% whilst 43.5% of patients had post acne hyperpigmentation.

Table 4: Post Ace Hyperpigmentation

Post acne hyperpigmentation	Females	Males	Males & Females(combined)	%Age
Present	67	20	87	43.5%
Absent	63	50	113	56.5%

Dermatology Life Quality Index scores:
DLQI scores ranged from 1 to 20 with mean DLQI score of (8.22 ± 4.45) which showed an impairment of 25%.
Mean DLQI scores as per Age, acne grading, gender, duration, scar & hyperpigmentation.
Mean DLQI was highest in age group 25 years & above. However, there was no significant difference between the age groups of 21 to 25 years and 15 to 20 years.

Table 5: Age

AGE (YEARS)	DLQI
15-20	8
21-25	8.04
>25	11.04

Mean DLQI was highest in grade IV followed by Grade III , Grade II and was seen lowest in grade I

Table 6: Grading

GRADES	DLQI
Grade I	4.01
Grade I I	8
Grade I I I	11.01
Grade I V	18.09

Table 7: DLQI was on high in male patients.

Gender	DLQI
MALE	7.95
FEMALE	6.84

Table 8: Patients with acne of duration of 13 to 24 months and 25 to 36 months had higher DLQI followed by acne of duration more than 36 months.

DURATION(MONTHS)	DLQI
0-6	6.08
7-12	7
13-24	9.50
25-36	9.60
≥36	8.31

Table 9: Patients with hyperpigmentation had higher DLQI.

HYPERPIGMENTATION	DLQI
PRESENT	9
ABSENT	3.59

Table 10: Patients with severe acne scarring had highest DLQI.

ACNE SCAR	DLQI
MILD	5.95
MODERATE	7
SEVERE	10.02
ABSENT	6.02

Interpretation of Dermatology Life Quality Index scores:
90% of the patients had elevated DLQI, mild effect being the most common seen in 39.5% of patients. None of the patients had DLQI score greater than 20.

DLQI Interpretation	Number Of Patients	%AGE
No Effect (0-1)	20	10%
Mild Effect (2-5)	79	39.5%
Moderate Effect (6-10)	68	34%
Very Large Effect (11-20)	33	16.5%
Extreme Large Effect (21-30)	0	0

Distribution of grade of acne based on DLQI interpretation:
SIX out of 18 cases with grade III acne and all grade IV acne had a very large effect on patient's life.

Grade	No Effect (0-1)	Mild Effect (2-5)	Moderate Effect (6-10)	Very Large Effect (11-20)	Extreme Large Effect (21-30)	Total
I	7	14	7	2	0	30
I I	11	50	56	33	0	150
I I I	0	6	6	6	0	18
I V	0	0	0	2	0	2
TOTAL	18	70	69	43	0	200

Distribution of acne scar based on DLQI interpretation:
20 out of 51 cases with severe acne scars had DLQI score in range of 11–20 interpreted as very large effect on quality of life.

Acne Scar	No Effect (0-1)	Mild Effect (2-5)	Moderate Effect (6-10)	Very Large Effect (11-20)	Extreme Large Effect (21-30)	Total
MILD	6	18	16	6	0	46
MODERATE	3	22	18	12	0	55
SEVERE	4	14	13	20	0	51
ABSENT	6	10	23	9	0	48
TOTAL	19	64	70	47	0	200

Factors affecting Dermatology Life Quality Index score
Statistically significant association was noted between DLQI scores and variables such as the age of the patient, duration and grade of acne, acne scar, and postacne hyperpigmentation (Table 1).

DISCUSSION

Acne vulgaris is a chronic inflammatory disease of pilosebaceous unit.[11] 85% of adolescents and young adults affected.[12] Multiple factors such as increased sebum production, follicular hyperkeratinization, proliferation of *Propionibacterium acnes* within the follicle, alteration of the quality of sebum lipids, regulation of cutaneous steroidogenesis, androgen activity, interaction with neuropeptides, and exhibition of pro- and anti-inflammatory properties contribute to its pathogenesis.[13] Inflammation plays a key role and may be the primary process in the pathogenesis, led by the perifollicular T-helper cells through IL-1. It also upregulates sebum production through leukotriene B4.[14]

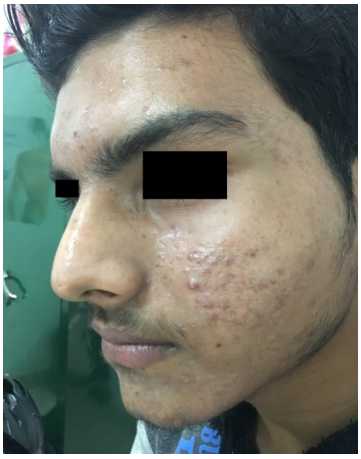

Fig 1: papules with scars.

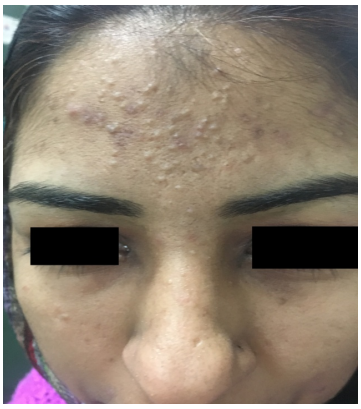

Figure 2: Comedones

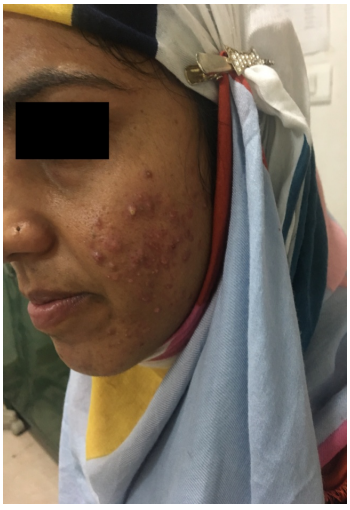

Figure 3: pustules & nodules.

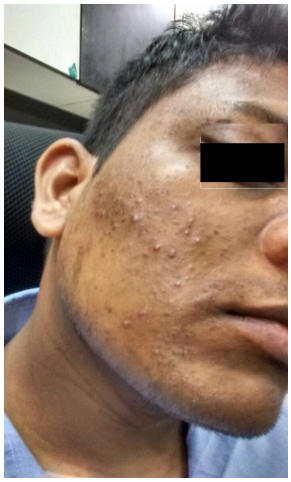

Figure 4: papules with hyperpigmentation

Acne vulgaris is graded using a simple grading system as follows [15]:

- Grade 1 - comedones, occasional papules
- Grade 2 - papules, comedones, few pustules
- Grade 3 - predominant pustules, nodules, abscesses
- Grade 4 - mainly cysts, abscesses, widespread scarring.

Acne and its sequelae has a significant impact on emotions, daily activities, social activities, study/work, and interpersonal relationships.[16]

Finlay and Khan, in 1994 was the first to introduce the DLQI questionnaire.[9] DLQI is a validated questionnaire which grades QoL by assessing the following domains: (a) physical symptoms and feelings (questions 1 and 2), (b) daily activities (questions 3 and 4), (c) leisure (questions 5 and 6), (d) work/school (questions 7), (e) personal relationships (questions 8 and 9), and (f) treatment (question 10). Each question is scored as “very much” (score 3), “a lot” (score 2), “a little” (score 1), and “not at all” (score 0), keeping in mind the problems faced the previous week due to the disease. Final DLQI score is the sum of all scores (range 0–30). High scores indicate poor QoL.

DLQI score interpretation is done as follows:

- 0–1 no effect on patient's life
- 2–5 small effect on patient's life
- 6–10 moderate effect on patient's life
- 11–20 very large effect on patient's life
- 21–30 extremely large effect on patient's life.

This was a hospital-based study which included 200 patients and visited to dermatology OPD. This study was of 6 months duration. Hazarika and Archana did a similar study and included 100 patients over a period of 3 months.[16] Durai and Nair[17] included 140 cases over 5 months whilst Kulthanan et al[18] included 110 cases in a period of 1 year.

Acne lesions start around 15 years of age.[19] The acne lesions can even persist into the thirties and forties1. In this study the patients of the age of 15 years and above were taken. The mean age of the study population was 20.49, study done by Tasoula et al reported mean age of 15.77 amongst the study group aged between 11-19 years.[20]

Mean DLQI scores in this study increased with increasing age, 11.05 in (>25) year old compared to 8.00 among 15–20 year olds. With advancing age the severity of acne worsens and affects the QoL as has been reported in few studies.[17,21,22] The mot possible reason for this could be that in late adolescents and early adult life, peer and romantic relationships form an important component and thus appearance has significant affect comparatively, in early adolescence, family is still the key and appearance does not matter much.[20]

In our study 57.5 %had oily skin. Kulanthan *et al* [18] found two-thirds of acne patients to have oily skin.

In this study grade grade II acne was seen in 45% patients while Durai and Nair[17] reported comedones to be most common (95%).

A significant correlation between DLQI scores and grade of acne ($P < 0.001$) was observed in this study and similar results were seen in studies done in Greece, Iraq, Turkey, and France.[20,21] Few past studies have shown no such association.[23,24]

67.5% patients had acne scars while acne scars were reported in 40.2%18[25] and 25%[26] was reported by some

authors. Hayashi *et al* [27] observed acne scars in 90.8% and opined that acne scars had a negative impact on patient's QoL.

A common complication of acne vulgaris is Post-inflammatory hyperpigmentation particularly in pigmented skin.[28] Postacne pigmentation was seen in 43.5% which was slightly lower when compared to earlier studies.[29,30]

The mean DLQI score of (8.22) in this study showed an impairment of 25%. Mean DLQI in different studies ranged from 1.7 to 8.95.[2, 32, 20,17]. Interestingly, it was found that few patients with grade II acne and some cases with mild scar had elevated DLQI scores which implied that even mild acne and scars can pose a cosmetic problem to some patients, affecting their quality of life.

CONCLUSION

Overall present study showed that quality of life is significantly impaired in patients of Acne vulgaris. Worsening of QoL was observed with advancement in age, increase in severity of acne and acne scars, and the presence of postacne hyperpigmentation. There was no gender difference in the QoL scores. Few patients with low-grade of acne and with minimal scarring also presented higher DLQI scores, implying that even mild acne can lead to psychosocial morbidity. Our study suggests counseling along with early treatment of acne vulgaris as to reduce disease-related psychosocial sequelae and enhancing the efficacy of treatment.

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