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Karyomorphology of skin diseases in human population

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Abstract
The aim of the present study is to determine the incidence of chromosomal abnormalities in skin diseases. Patients were analyzed for chromosomal aberrations using peripheral lymphocyte culture. Cytogenetic analysis was carried out in 27 people affected by Vitiligo, Psoriasis, Parthenia. Lymphocytes culture was done and subjected to colcemid, hypotonic treatment, fixation and air drying. GTG banded chromosomes were identified and counted for abnormalities. One way ANOVA was used to analyze the collected data. Tukey-Kramer Multiple Comparisons Test was used to determine differences between the means. Comparing the groups on the basis of type of chromosomal abnormalities, the differences between the means was found to be non significant (P>0.05) in all the comparisons. However, it was found to be significant for acrocentric association in Vitiligo subjects as compared to the controls. The means for premature centromeric divisions (Parthenia Vs Vitiligo) were also found to be significantly different.

Keywords: chromosomal aberrations, vitiligo, psoriasis, parthenia, cytogenetic analysis.

INTRODUCTION

Skin is a vital organ for thermoregulation and excretion of non soluble fat and the dirt from the body in form of sweat. Skin diseases are predominant in Asian subcontinents. For dermatologist, sometimes to identify skin diseases are a big question and for a proper diagnosis and prognosis. Microbial, biochemical and other sporadic causes are well known but genetic involvement for skin diseases are still unveiled. The present study is an attempt to find out the associated chromosomal aberrations, if any in vitiligo, psoriasis and parthenia.

Vitiligo is one of the commonest skin disorders affecting the human race. It is a hypo pigmentation disorder where the loss of functioning melanocytes causes the appearance of white patches on the skin. It affects 1% of the world population (1). The etiology of Vitiligo is still unknown, but there are some factors e.g. genetic factors, oxidative stress, autoimmunity, neurologic factors, toxic metabolites and lack of melanocyte growth factors might contribute for occurrence of the disease in susceptible people (2).

Psoriasis is a chronic immune-mediated disease that appears on the skin. It occurs when the immune system sends out faulty signals that speed up the growth cycle of skin cells. The cause of psoriasis is not fully understood, but it is believed to have a genetic component and local psoriatic changes can be triggered by an injury to the skin known as the Koebner phenomenon (3). Various environmental factors have been suggested as aggravating to psoriasis, including stress, withdrawal of systemic corticosteroid, as well as other environmental factors, but few have shown statistical significance.

Parthenium (Parthenia) is an allergy, caused by direct and indirect contact with Parthenium hysterophorus L. of the Compositae family. Affected people suffer from severe contact dermatitis which becomes chronic after longer exposition.

The present investigation attempted to unveil the overall chromosomal aberration that might be responsible for the cause and progression of skin diseases, especially vitiligo, psoriasis and parthenia.

MATERIAL AND METHODS

The study was conducted at Jawaharlal Nehru Cancer Hospital and Research Center, Bhopal. Cytogenetic analysis was carried out in 27 people who did not undergo any treatment. The study was ethically approved by the Institutional Human Ethical Committee of Jawaharlal Nehru Cancer Hospital & Research Centre, Bhopal. 1 ml of peripheral blood sample was drawn with a sterile syringe containing 30 units of Heparin (1000 IU/ml). The blood is allowed to settle by gravity sedimentation. Culture media was thawed and temperature was adjusted to 37ºC. All aseptic operations were carried out in laminar air flow. 0.5 ml of blood plasma lymphocyte was inoculated into a culture bottle having 8 ml of RPMI media and 300 µL of PHA. The cultures were incubated in a CO_2 incubator for 72 hours, at 37ºC and 5% CO_2. At the 70th hour, 50 µL of colchicine was added and kept at 37ºC in CO_2 incubator for 45 minutes. After 45 minutes, the samples were centrifuged at 2000 rpm for 10 minutes. Supernatent was discarded carefully, so as not to disturb the pellet. 0.57% KCl was used as the hypotonic agent. KCl was added while cyclomixing. Again sample was kept at 37ºC in CO_2 incubator for 45 minutes incubation and again centrifuged for 10 minutes at 2000 rpm. The supernatant was discarded. Cyclomixing of pellet was done and Carnoy's fixative (glacial acetic acid: methanol) was added to it and kept overnight at 4ºC. Washing was done repeatedly until a clear white pellet was obtained. Slides were prepared by air drop method. 3-4 days old slide were immersed in

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2% trypsin solution for a time according to age of slides. Older the slides, more time should be taken. After this, slides were immersed in normal saline to remove the action of trypsin. They were then washed in distill water and then stained in Giemsa at 6.8 pH for 10-15 minutes. Stained and scattered metaphase plates were identified and counted for abnormalities under 100X under Trioccular microscope and images were captured. All chromosomal aberrations are shown in Fig.1, 2, 3 and 4.

DATA ANALYSIS

All the subjects were screened for numerical and structural variations of their chromosomes. In each case, total 20 metaphases were analyzed. Mean frequency and standard error of mean were calculated for normal and aberrant metaphases in the study group and control group (Table-1). Data were analyzed by using one way analysis of variance (ANOVA). Tukey-Kramer Multiple Comparisons Test was used as post test to determine statistically significant mean differences. All the analysis was carried out using GraphPad InStat version 3.

RESULTS

Vitiligo Vs Controls

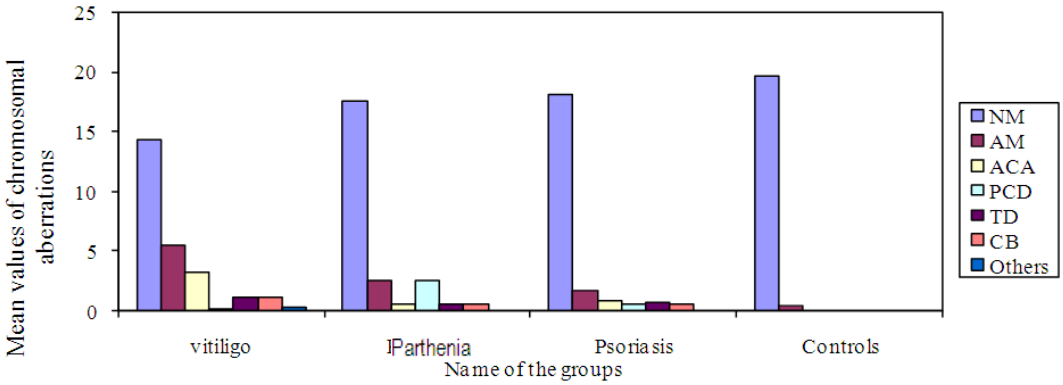
All the chromosomal aberrations including acrocentric associations (ACA), premature centromeric divisions (PCD), terminal deletions (TD) and chromatid breaks (CB) were found to be increased in vitiligo patients as compared to the controls (Graph-1). The difference between the means of normal metaphase in vitiligo group and controls was found to be significant statically ($P < 0.01$). The difference between the means for PCD, TD, CB and others was found to be statically non significant ($P > 0.05$). However, it was found to be significant in (ACA-Vitiligo Vs ACA- Control).

Parthenia Vs Controls

Chromosomal aberrations in parthenia group were found to be increased as compared to the control groups (Graph- 1). However, the difference between the means of normal metaphase in parthenia group and controls was found to be non significant ($P> 0.05$). The difference between the means for ACA, TD and CB was found to be statistically non significant ($P> 0.05$) but the means for PCD (shown in Fig.2) were found to be statistically different ($P < 0.05$).

Psoriasis Vs Controls

Chromosomal aberrations were found to be increased in psoriasis patients as compared to controls (Graph- 1). The P values for ACA, TD, CB and PCD revealed non-significant differences between the study group and controls. The difference between the mean of normal metaphases in psoriatic group and controls was found to be statistically non significant.



Graph 1. Comparison of mean value of chromosomal aberrations in study groups and controls

Table 1 -Showing mean frequency and standard error of mean of normal and aberrant metaphases in the study group and controls.

Mean ± Std error of mean							
Group	NM	AM	ACA	PCD	TD	CB/ICB	Others
Vitiligo	14±0.96	5.83±0.91	3.58±0.90	0.16±0.16	1.08±0.48	1.16±0.57	0.25±0.25
Psoriasis	18.16±0.98	1.66±0.95	0.66±0.42	0.33±0.33	0.33±0.33	0.33±0.33	0.33±0.33
Parthenia	17.5±2.50	2.5±2.5	0	2.5±2.5	0	0	0
Control	19.6±0.24	0.4±0.24	0	0.4±0.24	0	0	0

NM- Normal metaphases, AM- Abnormal metaphase, ACA- Acrocentric association, PCD- Premature centromeric division, TD- Terminal deletion, CB- Chromosomal Break.

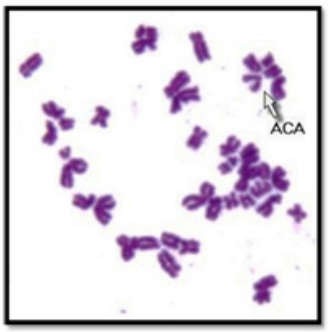


Fig 1. Metaphase spread with ACA

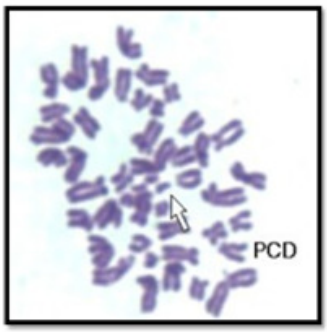


Fig 2. Metaphase spread with PCD

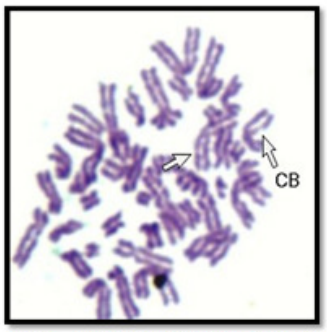


Fig 3. Metaphase spread with CB

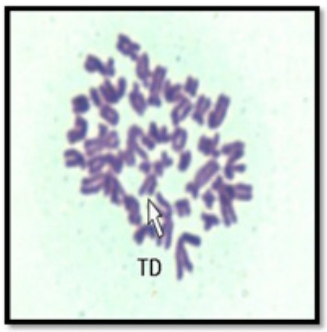


Fig 4. Metaphase spread with TD

DISCUSSION AND CONCLUSION

Chromosomal abnormalities have been held responsible for numerous genetic disorders, which include mainly congenital anomalies and cancer. Apart from these, researchers have revealed their involvement in many other diseases. Literature suggests that skin diseases most importantly vitiligo is regulated by some genetic factors. Majumdar et al. (4) assumed that vitiligo might be determined by multiple recessive loci. Zhang et al. (5) concluded that complex genetics of vitiligo involved multiple susceptible loci, genetic heterogeneity and incomplete penetrance with gene- gene and gene-environment interaction. Shajil et al. (6) from his studies, carried out in different populations, proposed that there may be candidate gene regulating oxidative stress that might contribute to the pathogenesis of vitiligo. They further stated that mammalian skin pigments possibly regulated by more than hundred genes which along with genes regulating oxidative stress and immune responses qualify as a potential candidate genes for vitiligo. Genome wide association studies by Birlea SA et al. (7) suggested significant association of vitiligo with SNPs (single nucleotide polymorphism) in 30 kb LD block on chromosome 6q27.

Cytogenetic analysis of 477 unrelated psoriatic patients was carried out by Enerback et al. (8) that pointed out an increased frequency of chromosomal aberrations found in chromosome region 11q.

Tjio and Levan (9) first reported the satellites on human chromosomes. All D and G group chromosomes i.e. chromosome numbers 13, 14, 15, 21 and 22 have satellites. The satellite chromosomes tend to associate with their satellites directed towards each other and this phenomenon is known as satellite association (SA) or acrocentric association (Fig.1). These associations were first of all observed in the human metaphase chromosomes by Ferguson-Smith and Handmaker (10). The acrocentric chromosomes associate together due to the sticky nucleolar material.

The increase in various chromosomal abnormalities in the diseased group as compared to the controls suggests the possible involvement of these anomalies in the genesis of the aforementioned skin diseases. However, identification of particular chromosome involved is needed. For this special kind of banding procedure like G and C banding and molecular cytogenetic techniques like FISH, SKY and CGH need to be adopted. Extensive molecular cytogenetic studies are required to come to a final conclusion**.**

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