# Androgenic Status of Lepromatous Leprosy Patients With Gynecomastia<sup>1</sup>

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Gynecomastia is one of the known complications of leprosy. In India, its incidence in leprosy has been reported to be 11.8% (<sup>5</sup>). As seen in varied clinical conditions, gynecomastia is thought to be the result of gonadal hormonal imbalance which may result either from a diminished production of androgens or from a reduced detoxication of estrogens, or both. The exact nature of this imbalance is, however, not known. Liver being the main site of inactivation of estrogens, its damage is expected to facilitate a build-up of excessive amounts of estrogens in the body. Since a sufficiently sensitive method for direct estimation of estrogens in the males was not available, certain liver function tests that would give an indirect, albeit gross, evidence of derangement of liver were performed in this study. In leprosy, Grabstald and Swan (3), Rollier and Rebonl (14), and Job (7) estimated urinary 17-ketosteroid as an index of androgenic status, but this does not differentiate between androgenic and some of the nonandrogenic steroids. The present study was undertaken to assess the androgenic status of patients with lepromatous leprosy with and without gynecomastia by employing the competitive protein binding assay method. The histopathologic studies of the liver and the testes were carried out in order to correlate the plasma androgenic levels with the morphology of the liver and the testes.

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. ..... scale) (<sup>13</sup>), 12 of which had gynecomastia, were selected for study from the leprosy hospital, Shahadra (Delhi) and from the dermatology outpatient department of the All India Institute of Medical Sciences, New Delhi. Twelve age-matched healthy males acted as controls. The breast enlargement was classified into three grades using Hall's (<sup>4</sup>) classification.

**Radioactive steroid.** 1,2-<sup>3</sup>H-testosterone (S.A. 137  $\mu$ Ci/ $\mu$ g) from New England Nuclear Corp., Mass., U.S.A. was purified on Bush Paper Chromatography system of benzene: petroleum ether; methanol; water in a ratio of 1:2:4:1 (<sup>2</sup>). Radiochemical purity of the 1,2-<sup>3</sup>H-testosterone was checked further by a thin layer chromatography system. Radioinert testosterone was procured from Sigma Chemical Company. Florisil (60-100) laboratory reagent grade from B.D.H. Poole, England, was washed several times with distilled water and finings (fine particles) were removed. This was dried overnight at 110°C and stored in a tightly closed bottle.

Testosterone binding globulin. Plasma containing testosterone binding globulin (TBG) was obtained from women in the third trimester of pregnancy and stored for up to three months at  $-15^{\circ}$ C to  $-20^{\circ}$ C in 0.5 ml aliquots. For the analysis of plasma androgens blood samples were drawn from normal healthy subjects aged between 20-39 years and patients with lepromatous leprosy with or without gynecomastia. Extraction of plasma androgens. Plasma (0.5 ml) samples to be assayed for testosterone were alkalinized with 0.1 ml of 1N NaOH. Steroids were extracted first with 10 ml and subsequently twice with 5 ml of methylene chloride. The combined extract was washed twice with 5 ml of distilled water and then dried under vacuum. The dried extract was then quantitatively transferred to a conical centrifuge tube and dried under nitrogen.

# **MATERIALS AND METHODS**

Subjects. Twenty-four patients with lepromatous leprosy (LL or BL Ridley & Jopling

Competitive protein binding assay. This was carried out by Murphy's (12) technic.

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Plasma containing testosterone binding globulin was diluted 1:100 with demineralized water. Approximately 500,000 cpm (0.7  $\mu$ Ci) of 1,2-<sup>3</sup>H-testosterone was added to the testosterone binding solution. Each ml of TBG solution contained 5,000 cpm (counts per minute) testosterone. Testosterone standards from 0 to 10 ng with increments of 1 ng were prepared and dried in tubes. The standard and the samples were dissolved in one ml of radioactive TBG plasma solution, mixed in a Vortex mixer, incubated at room temperature for 15 minutes, and transferred to 37°C for another 5 minutes. These samples were then kept at 4°C. After 15 minutes, 40 mg of activated florisil was added in Murphy's spoon (holds approximately 40 mgm of florisil) and the tubes were shaken for two minutes. The tubes were allowed to stand for another ten minutes to allow florisil to settle at the bottom of the tube. A half milliliter of supernatant was pipetted into a counting vial and 15 ml of diotol scintillation fluid (containing 250 ml of dioxan, 250 ml of toluene, 150 ml methanol, 3.25 gm PPO,<sup>3</sup> 0.065 gm dimethyl POPOP,<sup>4</sup> and 52 gm naphthalene) was added. Samples were also processed like the standard testosterone. A standard curve was constructed (Fig. 1). The quantity of testosterone in the unknown samples was read from the standard curve. The results were expressed as nanograms of testosterone per 100 ml of plasma. Total serum proteins were estimated by the method of Lowry et al (10). Estimations of SGOT and SGPT



FIG. 1. Standard curve used in competitive protein-binding radioassay of testosterone.

leprosy group. The biopsy specimens were fixed in Bouin's fixative and processed for histopathologic examination. Five micron thick paraffin embedded sections were cut and stained with hematoxylin-eosin and Ziehl-Neelsen stains. Biopsies were not made on the healthy controls.

## RESULTS

The ages of patients with gynecomastia ranged from 18-60 years with a mean of 33.9 years, those of patients without gynecomastia were 17-40 years with a mean of 29.1 years, and those of the normal healthy controls were 20-39 years with a mean of 27.7 years. The duration of gynecomastia, as assessed from the history, varied from less than six months to more than three years. Of the 12 cases, 8 had bilateral (Fig. 2) and 4 unilateral enlargement of the breast. Five patients complained of pain in the breast. There was no history of secretion from the nipple nor could fluid be expressed manually. Secondary sex characters. In the gynecomastia study group five patients showed loss of pubic hair, and seven showed a female hair pattern (Fig. 3). Nine patients showed scanty axillary and chest hair. In the leprosy patients without gynecomastia, - hair distribution and configuration were normal in three, while in nine cases the hair in the pubic and axillary regions were sparse.

were made by King's micromethod (<sup>8</sup>).

Liver biopsies. Liver biopsy specimens were obtained by percutaneous puncture method under local anesthesia, using a No. 13 Menghini's needle, usually through the eighth intercostal space. The tissue obtained was fixed and processed for histopathologic examinations. Five micron thick paraffin embedded sections were separately stained with hematoxylin-eosin and Ziehl-Neelsen stains. Biopsies were not made on the healthy controls.

Testicular biopsies. Wedge testicular biopsy was performed from one testis under local anesthesia, from each of ten patients of the study group and seven of the control

<sup>&</sup>lt;sup>3</sup>PPO stands for 2,5 diphenyloxazole.

<sup>&</sup>lt;sup>4</sup> POPOP stands for 1,4-bis-2-(4-methyl-5 phenyl-oxazolyl)-benzene.



FIG. 2. Lepromatous leprosy patient with bilaterally well developed grade III gynecomastia.



Measure of sexual function and fertility. Libido in the study group, as elicited from their clinical histories, was diminished and seminal emission was not easily accomplished. In the gynecomastia group seven patients were married but only one had had a single issue which had been born years before the onset of overt clinical disease. The other six patients had been married for more than five years but without their wives having conceived. In the control lepromatous leprosy group, eight patients had been married; of these, six had more than one issue. Of the other two, who did not have any issue, one had been married for only a year.

Male sex organs were fully developed in all the cases. Both testes in seven cases from the gynecomastia group and in two from the control lepromatous group were atrophic. Two cases in the gynecomastia group had one testis much smaller than the other.

Plasma testosterone levels. The plasma testosterone levels of the normal, healthy control group varied between 420 and 1090 ng/100 ml with a mean value of 748.4 ng/ 100 ml. The plasma testosterone levels in the control leprosy group ranged between 400-980 ng/100 ml plasma with a mean of 686.7 ng. In patients with lepromatous leprosy having gynecomastia the values varied between 180-520  $ng/100\ ml$  with a mean of 356.9 ng/100 ml. The differences in the values of plasma testosterone between normal healthy controls and lepromatous leprosy patients with gynecomastia was highly significant (p < 0.01). The differences in the plasma testosterone values in lepromatous leprosy patients without gynecomastia and those of the normal healthy control group were not statistically significant (p > 0.05).

FIG. 3. Lepromatous leprosy patient with gynecomastia showing female pattern of pubic hair.

TABLE	1.	Plasma	testosterone	levels	in	different	groups.
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	Group	No. of cases	Mean value in mg%	Statistical significance
I.	Normal healthy controls	12	748.4 + 215.8	
II.	Lepromatous leprosy without gynecomastia	12	686.7 ± 186.3	$> 0.05^{a}$
III.	Lepromatous leprosy with gynecomastia	12	356.9 ± 124.8	$< 0.01^{b} < 0.01^{c}$

<sup>a</sup> Groups I & II. <sup>b</sup> Groups I & III. <sup>c</sup> Groups II & III p < 0.01. Histopathology of liver and liver function tests. The livers of the patients with leprosy either with or without gynecomastia showed lepromas around the portal tract as well as in other areas (Fig. 4). The hepatic parenchymal cells were quite normal and the architecture was well preserved. Lipofuchsin pigment was present in large quantities. In a few cases acid-fast bacilli could be demonstrated.

The mean serum protein values in the gynecomastia group were 7.48 gm% compared to 6.93 gm% in the control group. The serum albumin levels in the gynecomastia group were lower than in the control groups. The SGOT and SGPT levels were significantly higher than in the control groups (SGOT > 20 units and SGPT > 15 units regarded as abnormal).



Histopathology of testes. Marked testicular atrophy was observed in four of ten cases in the gynecomastia group, and in two of seven in the leprosy control group. Figure 5 exemplifies the testicular atrophy which was so severe that the testicular tissue was replaced by scar tissue. Leydig cell hyperplasia with varying degree of edema was present in one case in each of the leprosy groups. Sixty percent of cases in the gynecomastia group and 28% of the control leprosy group showed lepromatous infiltration in the testes. This, however, had no correlation with the degree of atrophy of the seminiferous tubules.

# DISCUSSION

The etiology of gynecomastia is obscure. A common theme underlying various conditions resulting in gynecomastia seems to be an imbalance of sex hormones, the exact nature of which is unknown. The physical features observed in these patients and the duration of gynecomastia had no correlation with the duration of the disease or the size of the breasts. In four patients enlargement of the breast had begun during the course of an erythema nodosum leprosum reaction and had shown some diminution after the reaction had subsided. A complete remission, unlike those reported by Hall (4) and Job (7), was however never seen, though our follow-up was limited to about two years. The loss of libido and the associated primary and secondary sterility in the study group may be explained on the basis of structural damage to the gonads. The circulating levels of testosterone were low in these patients. The low levels of testosterone due to leprosy may be the cause of loss of libido and consequent sterility. Ejaculation was not possible on manual manipulation in any of the patients. The functional activity of the accessory sex organs depends on the plasma testosterone (1). The low amounts of testosterone may not be sufficient to maintain the secretory activity of the prostate and seminal vesicles. In the gynecomastia group, six of the ten testicular biopsy specimens and in the control group two out of seven such showed lepromatous infiltration. The difference in the fertility rate of these patients may be due to the effect of lepromatous infiltration on the testicles. Such histopathologic obser-

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FIG. 4. Photomicrograph of liver tissue of a lepromatous leprosy patient with gynecomastia.



FIG. 5. Photomicrograph of testicular tissue of a lepromatous leprosy patient with gynecomastia.

vations have been reported (3,7) but were not related to sterility.

The assessment of the androgenic status of the subjects by the competitive protein binding assay method is more sensitive than the estimation of urinary 17-ketosteroid (<sup>3, 7, 14</sup>). Urinary 17-ketosteroid is, at most, a poor indicator of androgenic status since nonandrogenic steroids secreted by the adrenal cortex may also contribute to 17ketosteroids in the urine (9). The present study showed that while the testosterone levels were diminished in most lepromatous leprosy patients, they were significantly so in patients with gynecomastia. Martin and his associates (11) came to similar conclusions using the method of Hudson and Coghlan (6) for the estimation of plasma androgens.

The parenchymal functions of liver were estimated by assessing the serum transaminases. It was found that the gynecomastia group had raised SGOT and SGPT levels as compared to the other lepromatous and healthy controls. They also showed low serum albumin levels. It is difficult to derive a definite conclusion, but the results of the liver function tests did suggest that the liver was more deranged in leprosy patients with gynecomastia than those who did not have gynecomastia. This may imply that, due to a greater derangement of liver functions, the liver is unable to inactivate estrogens. In individuals with lowered androgenic levels the decreased levels may initiate or augment the pre-existing gynecomastia by tilting the balance further in favor of estrogens. On the basis of morphology, the livers did not seem to have been grossly affected and patients from either lepromatous group showed lepromatous infiltration, and some of them also showed the presence of acidfast bacilli. From a study of the liver biopsies it is hardly possible to assess liver functional status. The interesting feature in the liver biopsies was that patients who showed testicular degeneration did not show marked liver changes.

ly diminished in the gynecomastia group as compared to levels in either of the control groups. The patients without gynecomastia showed values not significantly lower than those of normal males. Histopathologic examination of the leprous testes showed a variable admixture of inflammatory, degenerative and fibrotic changes which were more severe and more frequent in the patients with gynecomastia than in those without it.

Parenchymatous liver function tests showed greater derangement in the gynecomastia group as compared to the controls Histopathology of the liver showed lepromatous infiltration in patients with and without gynecomastia though the changes in liver were minimal in patients who showed total testicular degeneration in the biopsies.

### RESUMEN

Se estudió el estado androgénico de 24 pacientes del sexo masculino con lepra lepromatosa (doce pacientes con ginecomastia) y se hizo la comparación con el estado androgénico de 12 personas sanas pertenecientes al mismo sexo. Comparados con los controles sanos y con los pacientes sin ginecomastia, los pacientes con ginecomastia mostraron una disminución importante en sus niveles de testosterona plasmática. Comparando los niveles de testosterona de los pacientes sin ginecomastia con los de los controles sanos no se observo ninguna diferencia significativa. El examen histopatológico de los testículos de los pacientes con lepra revelo una serie variable de cambios inflamatorios, degenerativos y fibróticos los cuales fueron más severos y más frecuentes en los pacientes con ginecomastia. Los pacientes con ginecomastia, también mostraron las mayores alteraciones en sus pruebas functionales hepáticas. En las biopsias, la histopatología del hígado de los pacientes con y sin ginecomastia revelo la presencia de infiltración lepromatosa aún cuando los cambios hepáticos fueron mínimos en aquellos pacientes cuyas biopsias mostraron una total degeneración testicular.

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# SUMMARY

The androgenic status of 24 lepromatous leprosy male patients, 12 with and 12 without gynecomastia, was studied in comparison with that of 12 normal healthy males. Plasma testosterone levels were significant-

# RÉSUMÉ

On a étudié 12 malades lépromateux de sexe masculin atteints de gynécomastie, en vue d'évaluer leur état androgénique. Un groupe assorti pour l'âge de 12 malades lépromateux sans gynécomastie, et de 12 adultes normaux de sexe masculin, ont été utilisés comme témoins. Les niveaux plasmatiques de testostérone étaient

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significativement diminués dans le groupe d'étude par comparaison au niveau observé dans les deux autres groups. Les malades sans gynécomastie ont présenté des valeurs qui n'étaient pas significativement plus basses que celles des individus normaux de sexe masculin. L'examen histopathologique des testicules a montré un mélange variable de modifications inflammatoires, dégénératives et fibreuses; ces modifications étaient plus graves et plus fréquentes chez des malades avec gynécomastie que chez ceux qui ne présentaient pas cette complication.

Les épreuves de la fonction du parenchyme hépatique onto révélé des perturbations plus importantes dans le group d'étude que dans le groupe témoin. L'étude histologique du foie a révélé une infiltration lepromateux chez les malades avec gynécomastie comme chez les malades sans gynécomastie, encore que les modifications décelées dans le foie étaient minimes chez les malades qui présentaient une dégénérescence testiculaire complète.

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