

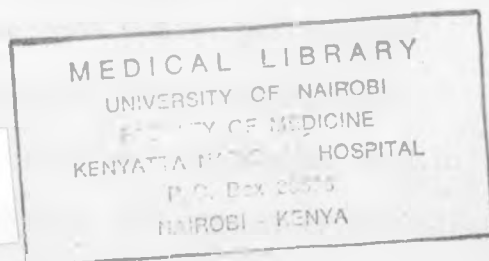
TITLE: A STUDY OF THE ROLE OF FAMILY HISTORY AND TOTAL SERUM  
TESTOSTERONE IN AFRICAN PATIENTS WITH IDIOPATHIC ACNE  
VULGARIS ATTENDING KENYATTA NATIONAL HOSPITAL.

BY

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A THESIS SUBMITTED IN PART FULFILMENT FOR THE  
DEGREE OF MASTER OF MEDICINE, AT THE UNIVERSITY  
OF NAIROBI.

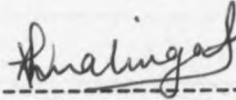
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DECLARARION:

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This thesis is my original work and has not been presented for a degree in any other University.



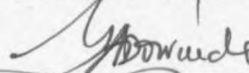
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Abbreviations used in this study

- SBHG - Sex binding hormone globulin  
DHA - Dehydroepiandrosterone  
ACTH - Adrenocorticotrophic hormone

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SUMMARY:

A study of acne vulgaris in black Africans attending Kenyatta National Hospital was done in a case controlled cross-sectional study between October 1987 and March 1988. The study was composed of 32 male and 20 female patients with various grades of acne vulgaris. The patients were matched for age and sex to an identical number of controls who were selected from the surgical out-patients clinic

There was no significant difference in the total serum testosterone levels between the patients and age-sex matched controls as measured by radio-immunoassay. There was no correlation between total serum testosterone levels and the severity of acne. There was no significant difference in the mean total serum testosterone levels when patients with acne alone and those with acne and hirsutism were compared. All the female patients studied had regular menstrual cycles.

Family history was present in 67.3% of the patients with acne and in 40.4% of the age-sex matched controls. This was statistically significant. Severe grades of acne vulgaris were significantly associated with a positive family history.

INTRODUCTION:

Acne is a word derived from the Greek word 'Akme' which means the peak of prime of life. Acne vulgaris is a self limiting chronic inflammatory disorder of the pilosebaceous follicles. It is clinically characterised by a variety of lesions which are polymorphic and dynamic in nature. The lesions consist of comedones, macules, papules, pustules nodules and cysts. The severity of the disease is presumably determined by genetic factors as well as a number of environmental influences. Acne occurs in all races of man but not in other primates. Incidence and severity are very significantly lower in the Japanese than Caucasoids. The condition usually starts in adolescence and resolves by the mid twenties though some patients still have acne worth treating by the age of 25-35 years<sup>(1)</sup>. Acne develops earlier in females than in males. This may reflect the earlier onset of puberty in females<sup>(2)</sup>. At the age of 40 years 1% males and 5% of females have acne vulgaris<sup>(1)</sup>. It is not known why acne resolves or why it is more persistent in females.



The pathogenesis of acne is a complex process that involves the interaction of a number of variables including genetic factors, hormonal factors, sebaceous gland activity, bacterial factors, abnormal cornification and the inflammatory process.

Genetic factors are thought to play a role as suggested by a high degree of concordance between identical twins. Hugo Hetch of Cleveland<sup>(3)</sup> in her study found that if one of the parents had acne, those children who resembled him or her were more likely to get acne. Severe acne has been associated with XYY chromosome pattern<sup>(4)</sup>.

Whatever the relative importance of the various aetiological factors that may be involved in acne, one fact is indisputable, active sebaceous glands are a pre-requisite. Acne patients both male and female secrete on average more sebum than normal subjects<sup>(5)</sup> and the level of secretion correlates well with the severity of the acne<sup>(6,7)</sup>. Acne normally occurs in areas of increased sebaceous gland activity that is the face, chest, back and upper arms. Ductal hyperconification occurs in acne though the reason is not clear. There seems to be a significant correlation between the severity of the acne and the number and size of follicular casts<sup>(8,9)</sup>. It appears that comedogenesis is initiated by this process which is characterised by the production of abnormal horny cells that fail to desquamate properly thus leading to an abnormal build up of these cells in the follicular canal. There is also an increased

proliferation of the underlying basal cells of the follicular epithelium which in turn leads to the production of more cornified cells and eventual increase of these cells in the lumen of the follicle. These two factors lead to the formation of micro-comedo which is the primordial acne lesion and the precursor of all the other acne lesions.

Anaerobic diphtheroids found on human skin that is Propionibacteria particularly Propionibacteria acnes and Propionibacteria Granulosum are thought to play a role in acne. Adolescence and its attendant seborrhoea is associated with a significant increase in Propionibacteria Acnes though there is little or no relationship between the number of bacteria on the skin surface or in the ducts and the severity of acne<sup>(10)</sup>. It is postulated that the environment in the follicle is conducive to elaboration by Propionibacteria Acnes of inflammatory mediators which include proteases, lipase, phosphates, hyaluronate and prostaglandin like substance. In addition the cell wall fraction of Propionibacteria Acnes is a potent chemo-attractant for polymorphonuclear and mononuclear cells<sup>(11)</sup>. There is evidence of activation of classical and alternate complement pathways in early inflamed and non-inflamed lesions. Skin testing with heat killed suspension of Propionibacteria Acnes demonstrated that subjects with severe acne produced a greater inflammatory response at 48 hours than other subjects suggesting that host response may be important.<sup>(12)</sup>

Finally there is the role of hormonal influences which is also the main topic of this study. Among the various factors known to be involved in the pathogenesis of acne, hormonal influences are of considerable importance. Agonadol individuals or castrates do not develop acne<sup>(13)</sup>. It has long been noted that patients with endocrine disorders of the ovary or adrenal may develop acne as one of the manifestations for example congenital adrenal hyperplasia, and androgen producing ovarian tumours.

Androgens have been implicated as the principal class of substances responsible for the development of acne, the target tissue in the skin for this response being androgen sensitive sebaceous glands. Of the androgens produced by gonadol and adrenal organs, testosterone is the most portent in stimulating androgen responsive tissues. In addition weaker androgens such as andro-stene dióne, dehydroe-piandisterone sulphate are also capable of stimulating sebaceous glands<sup>(14,15)</sup>.

Abnormally high levels of sebum secretion could thus result from high overall androgen production or increased availability of free androgen because of deficiency in sex hormone binding globulin. Alternatively the high levels of sebum secretion could involve an amplified target response mediated either through 5 $\alpha$  reduction of testosterone<sup>(16)</sup> or the capacity of the intracellular receptor to bind the hormone. There is also

the possibility that other hormones affect the sebaceous glands either directly or by enhancing their response to androgens for example sebum secretion appears to be low in individuals with isolated growth hormone deficiency<sup>(17)</sup>. In acromegalics the rate of sebum excretion correlates well with the logarithm of the serum growth hormone concentrations<sup>(18)</sup>.

In recent years, numerous reports have appeared in the literature on the measurement of androgens and other hormones in the peripheral blood of both men and women with acne in an effort to define the presence of an abnormality or abnormalities that would allow for rational approach to therapy. There is general agreement that plasma testosterone levels are not abnormally high in males with acne<sup>(14,19,20,21,22)</sup> though Lee Peter<sup>(23)</sup> studied acne and serum androgens during puberty and found that boys with acne had significantly higher mean testosterone levels than age matched controls. The mean testosterone levels tended to be higher with each successive stage of acne.

The findings of Hay and Hodkins<sup>(16)</sup> that the skin of acne patients metabolises testosterone to its presumed active intracellular metabolite dehydrotestosterone more avidly than does skin of normal individuals suggest that this may be an important factor rather than the elaboration of hormones per se.

In females the situation is more variable. Some investigators have shown testosterone levels to be normal (21,22,24,25). Odland et al (24) found that healthy adult women and men with mild acne and severe acne had similar total plasma testosterone concentrations. Others have found the means of total serum testosterone to be significantly above normal (19,27,28,29,30,31). There is more general agreement that mean serum SHBG levels are significantly below normal with free serum testosterone levels consequently above normal (24,29,32). Darley et al (32) found that increased total serum testosterone levels of low serum SHBG levels were present alone or in combination in 60% of women patients with acne starting or persisting after the age of 18 years. He also found no correlation between the androgen levels and the severity, distribution of pattern of acne or the presence of hirsuties or irregular periods.

Forstrom (19) studied women with different types of acne of varying severity and found total serum testosterone levels significantly increased though there was no correlation with type distribution or severity of the acne. Beverly et al (34) studied women of reproductive age and found that women with both acne and hirsutism had higher serum testosterone levels than those with acne alone and that the incidence of irregular menstrual cycles was higher in women complaining of acne. Daphne Lawrence et al (29) obtained similar findings.

High incidence of ovarian dysfunction has been reported in hyperandrogenic women. In addition a high incidence of acne has been reported in women with ovarian dysfunction. In a report by Steinberger et al<sup>(28)</sup> of 139 women who presented with chief complaint of acne and who were resistant to conventional therapy, 90% were found to have increased total plasma testosterone above the normal mean, 17.2% had amenorrhoea and 60% had disturbed ovulatory cycles. Poshi<sup>(35)</sup> obtained menstrual histories in 400 consecutive acne patients seen in his practice over 18 months. Regular cycles for the 400 patients studied were defined as  $28 \pm 5$  days. Of the 400 patients studied 78.8% had regular cycles, 20% had irregular cycles and 1.2% had amenorrhoea.

Testosterone is the most important androgen hormone. It is derived from cholesterol in a series of steps requiring five enzymes. Production mainly takes place in the gonads but also by peripheral conversion of adreno-cortical precursors DHA, and androstenedione. Leutinizing hormone stimulates the production of testosterone from the gonads while ACTH stimulates its production from the adrenals. Testosterone is transported in the blood bound to albumin and SHBG. Women are known to have higher SHBG levels than men. SHBG is synthesized in the liver, and its synthesis is increased by oestrogens and decreased by androgens. The concentration of the free testosterone in the blood circulation is determined by an equilibrium involving plasma SHBG. free testosterone complex. Low plasma SHBG leads to increased free testosterone.

Testosterone is metabolised mainly in the liver where conversion to 17 oxosteroids occurs which are then conjugated and excreted in urine. In the peripheral tissues and target organ, testosterone is converted to dihydrotestosterone and oestradiol by 5 $\alpha$  reductase. Testosterone regulates gonadotrophins and maintains spermatogenesis, takes part in the formation of the male phenotype during sexual differentiation and also induction of sexual maturation and function following puberty.

In women of fertile age the peripheral testosterone levels are approximately one tenth that of normal males. An age related decrease in testosterone levels occurs in males.

Testosterone levels are low before puberty up to eight ten years after which they rise in both sexes. The increase is higher in boys than girls, reaching adult levels by seventeen years. In adults, diurnal variation in the magnitude of episodic secretion of leutinizing hormone and testosterone is minor with peak levels in the morning, only about 10-15% higher than during the rest of the day (40).

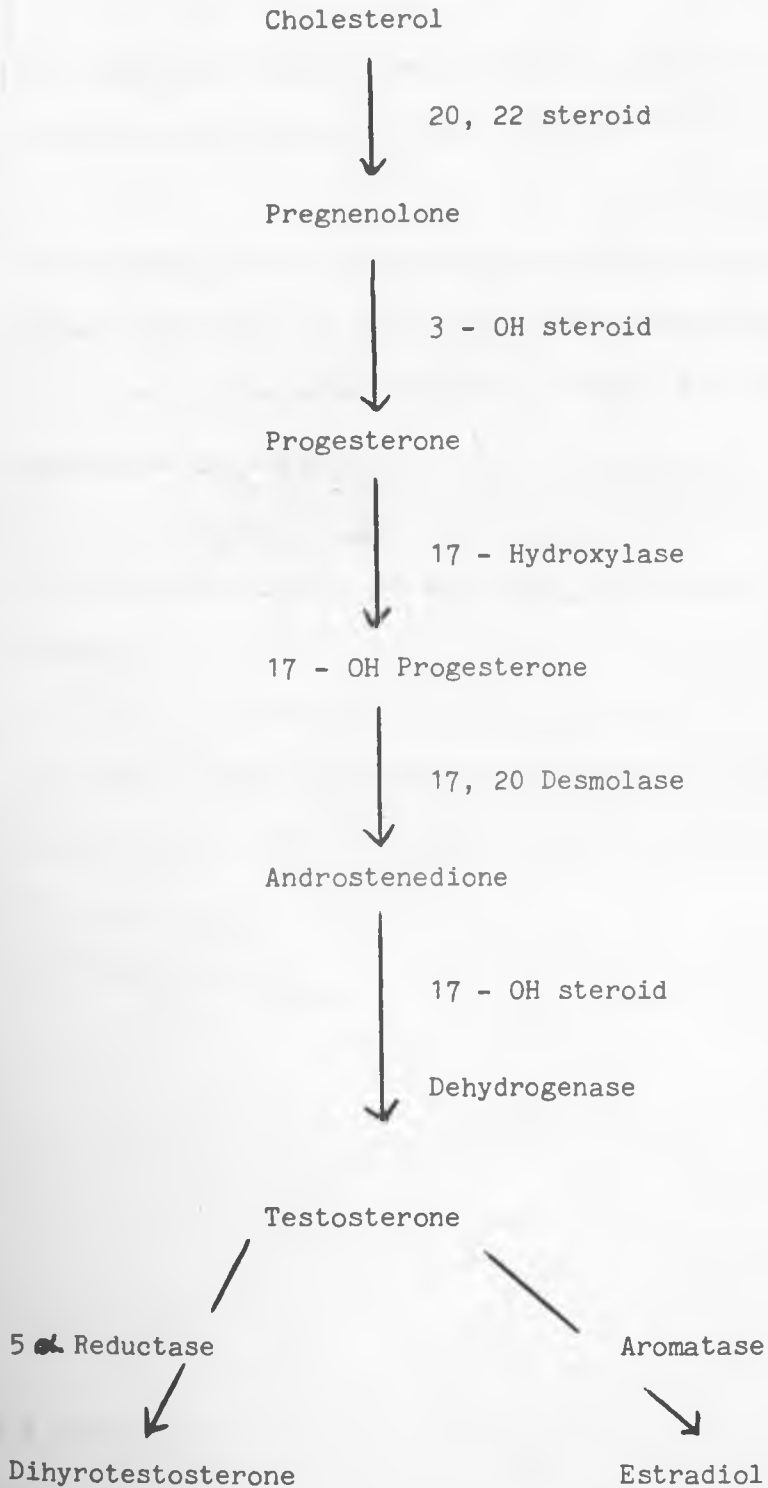
Values vary slightly by day and different times of the year but this is not significant in routine assessment.

In males diurnal variation of testosterone is uncertain. During menstrual cycle in females testosterone levels are maximum at mid cycle. However these cyclic variations are too small to be significant in clinical practice. In women, marked diurnal variation is present due to the important adrenal part of testosterone production. Maximum levels occurs in the morning.

Levels of testosterone can be affected by exogenous compounds, particularly compounds that directly or indirectly affect the gonads or adrenal glands. These include ACTH, gonadotropins, clomiphene citrate, tamoxifen, gluco-corticoids, metyrapone sex hormones and danazol. Substances that affect levels of SHBG - also affect peripheral levels of testosterone such as clomiphene, tamoxifen spironolactone, sex hormones, and danazol. In males it is also known that long lasting alcohol abuse, stress and hard physical exercise, may decrease peripheral levels of testosterone (26).



The pathway of androgen formation in the testes and the conversion of androgens to other active hormones in peripheral tissues, is as shown below in a schematic diagram



AIMS AND OBJECTIVES:

1. To establish the significance of family history in patients with acne vulgaris as compared to the controls.
2. To establish whether family history relates to the severity and duration of acne vulgaris.
3. To determine total serum testosterone in both male and female patients with idiopathic acne vulgaris starting at or persisting after 18 years of age (i.e. after the adolescent period).
4. To correlate severity of acne with total serum testosterone levels.
5. To compare total serum testosterone levels in female patients with acne alone, and those with acne and hirsutism.

METHODOLOGY AND MATERIALS:

The study was carried out between October 1987 and March 1988 at Kenyatta National Hospital. Kenyatta National Hospital is both a national referral and teaching hospital situated in Nairobi the capital city of Kenya.

The study was carried out with permission from the Ethical Committee for Kenyatta National Hospital. Consent from each patient was taken before they could be enrolled into the study.

Fifty two patients both male and female and fifty two controls were studied. Patients were subjects eighteen or more years of age presenting to the dermatology specialist clinic with idiopathic acne vulgaris. All patients were seen by the author as they arrived consecutively until the numbers were adequate. A detailed medical and dermatological history especially to rule out those on drugs that may affect testosterone levels or cause hirsutism, and those with obvious secondary causes for their acne. Patients who had been on the contraceptive pill for the last six months were excluded. The patients personal clinical data was obtained with the help of Appendix 1.

Menstrual irregularity was taken to be any one of the following:-

- Menstrual cycles of less than 21 or more than 36 days.
- Menstrual flow of less than 3 or more than 7 days.
- Scanty or heavy menstrual flow.

A routine medical examination was done on each patient followed by a dermatological examination and grading of their acne as per Appendix 2. For the purpose of this study, grades 1 and 2 acne were regarded as mild whereas grades 3 and 4 as severe acne. A different dermatologist was consulted at each instance to ascertain the diagnosis in acne vulgaris in patients who were being seen for the first time. The other patients who were on follow up had during their first visit been diagnosed to have acne by a dermatologist but if there was any doubt they were re-evaluated. Hirsutism was defined as growth in part or in total of the male sexual pattern.

The control subjects were obtained from surgical outpatients clinic and were matched with the patients for age and sex. They had no evidence of acne, hirsutism or irregular periods by history or physical examination and were not on any compounds that may affect testosterone levels. A family history of acne in their first degree relatives was also taken from them.

Five millilitres of blood was taken from the cubital fossa from each subject between 9-10 am and put in a plain bottle. In the female patients, the period in the menstrual cycle at which blood was taken was not taken into account. The blood was taken to the Department of Chemical Pathology on the same day for storage at  $-20^{\circ}\text{C}$  until hormonal analysis could be done.

The determination of testosterone in serum was done using 1st testosterone radio-immunoassay kit. Code ER-350 as per Appendix 3.

Statistical analysis was done either using Chi squared test or the student T test, where applicable. The probability values of less than 0.05 being counted as significant.

RESULTS:

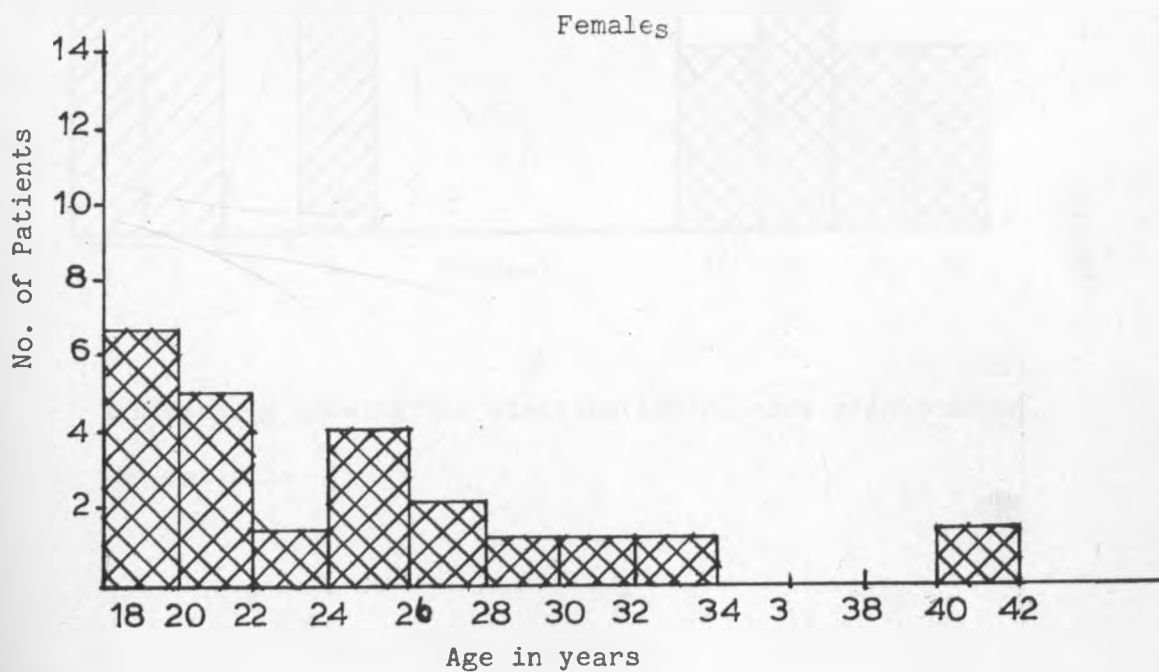
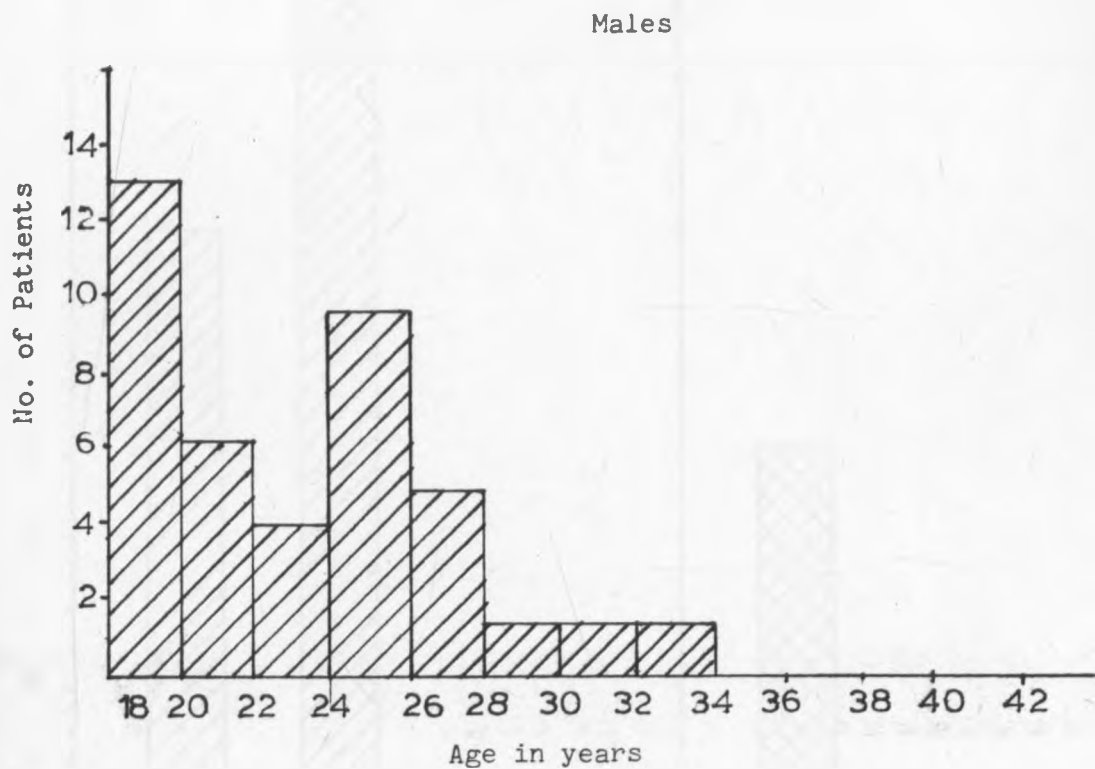
52 patients with acne were studied. All the patients were matched for age and sex and a total of 52 controls were studied. Of these patients 32 were males and 20 were females. Their ages ranged from 18-41 years, peak age being from 18-20 years. Of the females two patients were beyond 30 years their ages being 33 and 41 years respectively. There was only one male patient whose age was 31 years.

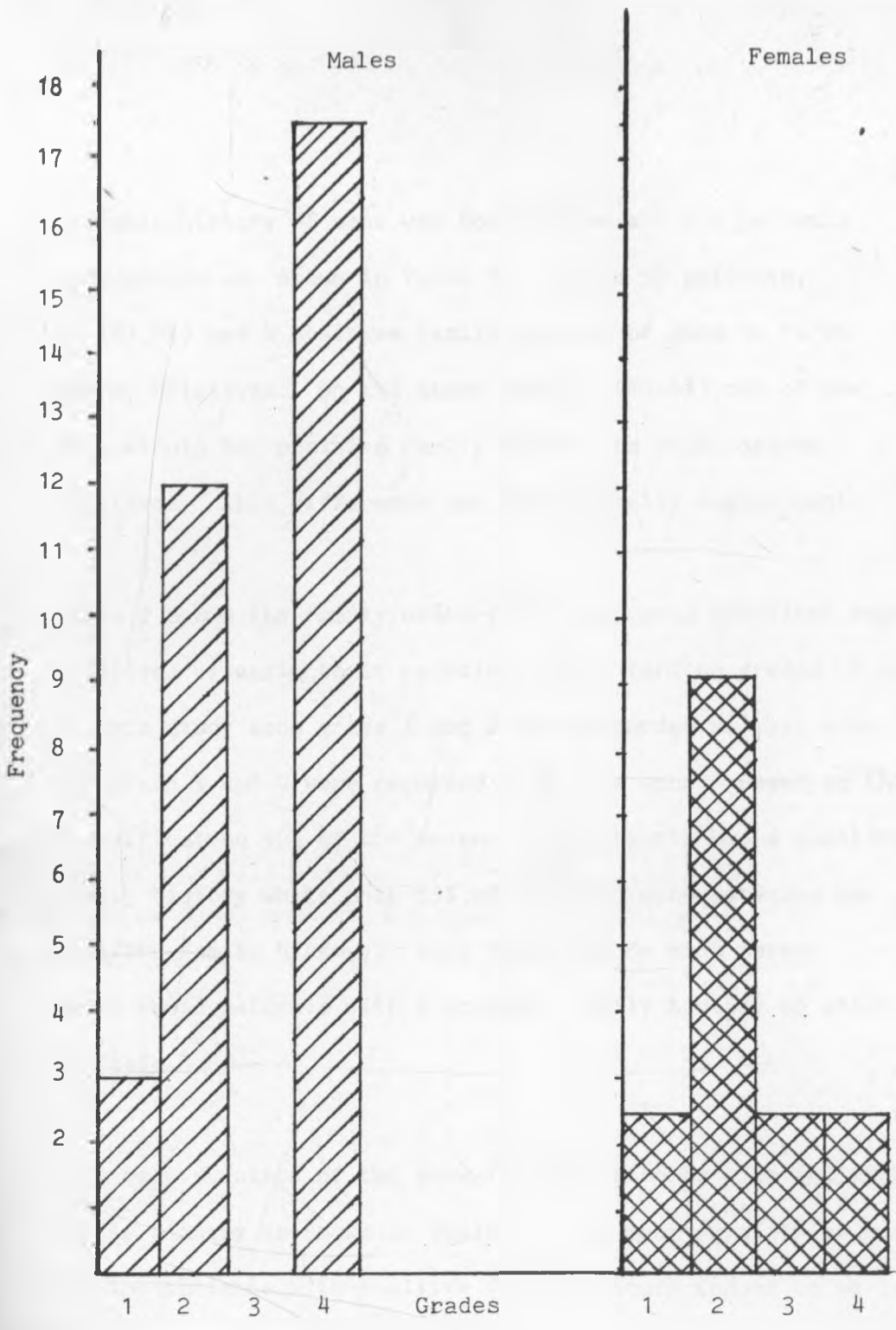
Figure I is a histogram showing the age distribution of both the male and female patients with acne. The occurrence of acne diminishes with age and becomes particularly rare after the mid twenties.

The patients seen had all grades of acne apart from grade 3 acne which seemed to be lacking among the males. The male patients had more severe acne than the female patients. For instance 17 out of the 32 male patients (53%) had a grade 4 acne as compared to 3 out of 20 female patients (20%). There was no relationship between the age and severity of acne.

Figure 2 is a histogram showing the distribution of acne grades among the patients.

Figure 1: A histogram showing the age distribution of both male and female patients with acne vulgaris





A histogram showing the distribution of acne grades among the patients



A family history of acne was sought from all the patients and controls as shown in Table 1. Of the 52 patients, 35 (67.5%) had a positive family history of acne in first degree relatives. On the other hand 21 (40.4%) out of the 52 controls had positive family history in first degree relatives. This difference was statistically significant.

Table 2 shows the family history of acne among the first degree relatives of patients in relation to the various grades of acne. In this study acne grade I and 2 were regarded as mild acne and grade 3 and 4 were regarded as severe acne. Based on this classification 90% of the severe acne patients had a positive family history while only 53% of the mild acne patients had a positive family history. Acne tended to be more severe among those patients with a positive family history as shown in Table 3.

The mean duration of the acne for the patients with and without family history is shown in Table 4. The mean duration of acne for the patients with positive family history tended to be longer.

Table 1: Table showing family history of acne taken from patients and controls

	Positive Family History	Negative Family History	Total
Patients	35	17	52
Controls	21	31	52
Total	56	48	104

When patients with positive family history were compared to controls with positive family history  $\chi^2=6.54$  P value at  $<0.05$  (significant)

Table 2: Showing family history of acne taken from patients with the different grades of acne

Grade of Acne	No. Family History	Positive Family History
1	1	5
2	14	12
3	0	3
4	2	15

Table 3: Table showing relationship between severity of acne and family history

	No. Family History	Positive Family History	
Mild Acne (Grade 1 and 2)	15	17	32
Severe Acne (Grade 3 and 4)	2	18	20
	17	35	52

When patients with mild acne and with a positive family history were compared to patients with severe acne and positive family history  $\chi^2=6.02$  with P value at  $<0.05$  statistically significant.

Table 4: The mean duration of acne in patients with and without family history (in years)

	Family History	No family history
<b>Females</b>		
with mild acne	5.3	4.3
with severe acne	7.2	2.0
<b>Males</b>		
with mild acne	4.7	4.8
with severe acne	6.9	4.7

Key: Mild acne - Grade 1 and 2

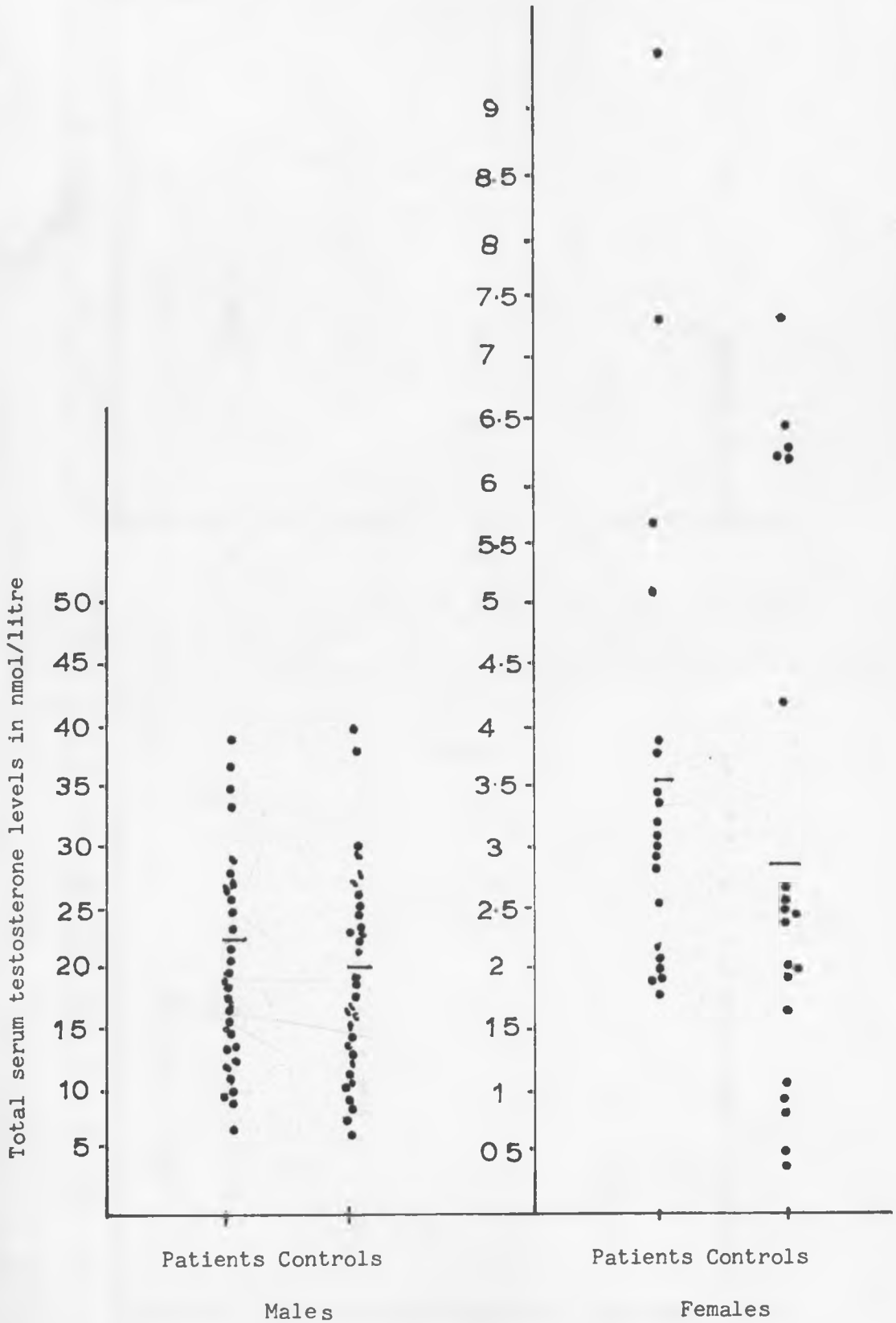
Severe acne - Grade 3 and 4

The levels of total serum testosterone for both the patients and the controls were determined using radio-immunoassay. Figure 3 shows the total serum testosterone levels in both male and female patients and their controls. The mean total serum testosterone for the male patients was  $19.11 \pm 6.84$  nmol/l and that for the male controls is  $18.33 \pm 8.8$  nmol/l. Statistically there was no significant difference between them ( $P > 0.05$ ). The actual levels of the total serum testosterone in the male patients ranged from 6.68-39.3 nmol/litre and were comparable to those of the control group which were 5.15-39.73 nmol/litre. For the female patients total serum testosterone levels ranged from 1.73-9.41 nmol/litre while their controls ranged from 0.31-7.46 nmol/litre. The mean total testosterone levels for the female patients were  $3.66 \pm 3.07$  nmol/litre with the mean for the controls being  $2.89 \pm 2.14$  nmol/litre. Again as for the male patients there was no significant difference between the patients and their controls.

The total serum testosterone levels among the different grades of acne, were observed not to follow any trend as shown in Figure 4a. The mean total serum testosterone levels of the various grades of acne were not significantly different and are compared in Figure 4b.

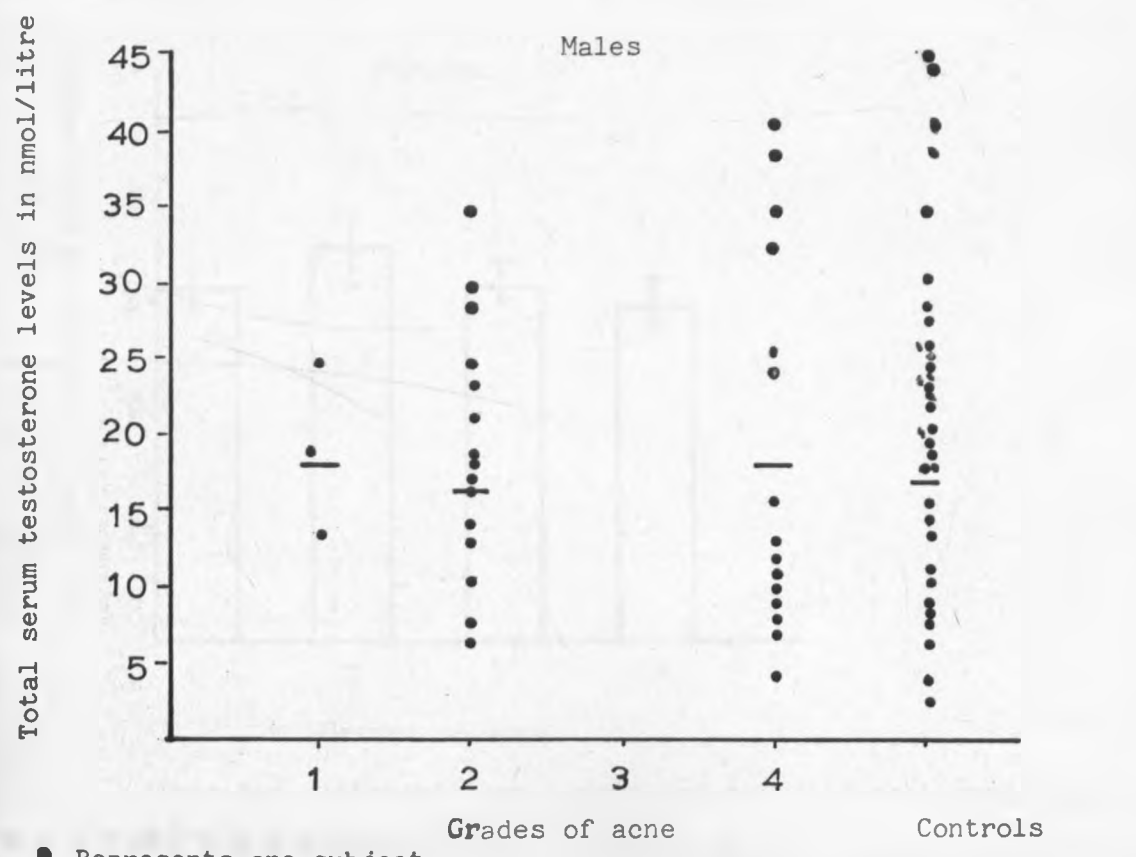
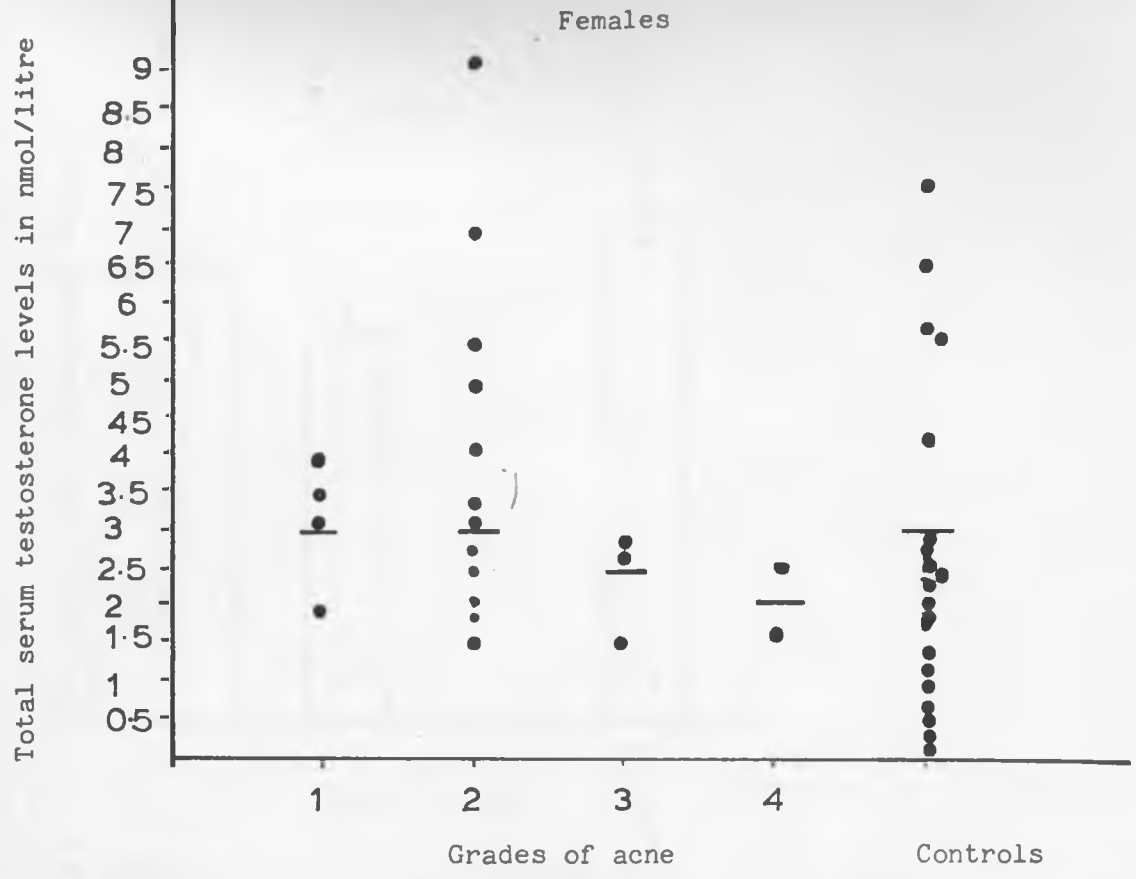
Four female patients with acne vulgaris were found to have hirsutism. There was no significant difference between their mean levels of total serum testosterone and those with acne alone. All the female patients had regular menstrual cycles with a mean cycle duration of 26.6 days and mean duration of each menstrual flow of 3.73 days.

Figure 3 Total serum testosterone levels in male and female patients and their controls



Represents one subject

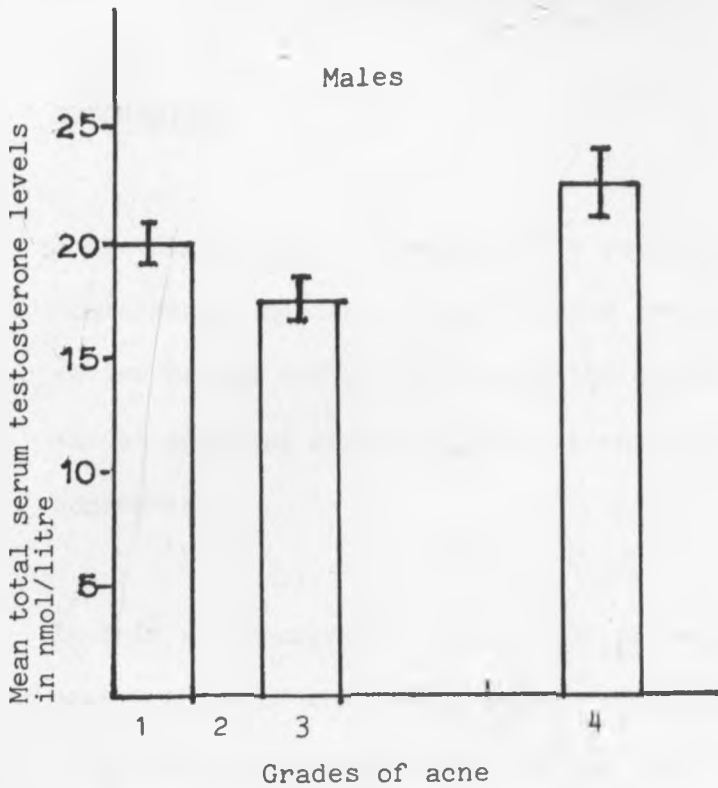
Figure 4a: Showing the total serum testosterone levels plotted against the different grades of acne



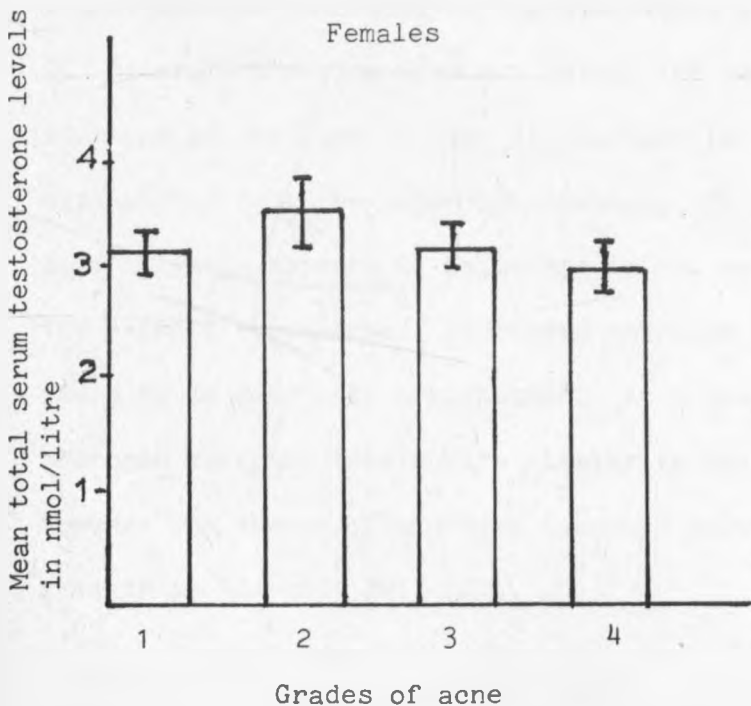
● Represents one subject



Figure 4b: Showing the distribution of the mean serum testosterone levels of the different grades of acne vulgaris  $\pm$  SD



$P > 0.05$  (not significant)



When the different mean levels for the different grades are compared  $P$  value  $> 0.05$  which statistically shows no significant difference.

DISCUSSION:

Acne vulgaris is a disease of the young usually starting at adolescence and rarely going beyond the third decade. Literature review brings out quite clearly the controversies that exist as far as androgen status of the patients with acne vulgaris is concerned.

In this study only 4 out of the 52 patients were beyond thirty years, three of them being women. Literature review<sup>(1)</sup> suggests that acne is more persistent in the female patients though the reason for this is not known.

53% of the male patients and 20% of the female patients had grade 4 acne disease according to the classification given in Appendix 2. Literature review does not reveal the reason for this observed increase in severity of acne in the male patients. A possible explanation could be based on genetics. It is a known fact that acne vulgaris appears to be severe in the genotype XYY implicating the extra Y chromosome. Increased androgen receptor sensitivity could be an alternate explanation. In a study done by Schmidt<sup>(39)</sup> androgen receptor levels were similar in male and female patients, however the number of androgen receptor positive patients was greater in the male patients.

Family history appears to be significant in the patients with acne vulgaris as compared to the **controls** and this observation supports the familial incidence of acne vulgaris<sup>(3)</sup>, though there was no significant difference in the duration of acne.

When the four grades of acne were compared to each other in relation to family history there was no significance. However when mild acne (grade 1 and 2) was compared to severe acne (grade 3 and 4) there was significant family history in severe acne  $P < 0.05$ . 90% of the patients with severe acne had positive family history while 53% of the patients with mild acne had positive family history. This suggests that family history is more significant in severe types of acne vulgaris. In literature reviewed there is no study that has considered this aspect.

Several clinical findings support the old assumption that acne vulgaris has an endocrinological basis. The onset of the disease occurs at puberty and it can be provoked by androgen administration. In most cases it is connected with seborrhoea and the sebaceous gland activity is primarily the result of androgenic stimulation. Thus it has often been suggested that acne may be associated with excessive androgen production.

In this study the total serum testosterone levels in the patients both male and females were all within the normal range, and were comparable to those of the controls. There have been no studies done to determine the normal levels of total testosterone in our population and the normals used were those for the <sup>125</sup>Iodine radio-immunoassay kit as in Appendix 3. In the male patients with acne vulgaris there is general agreement that plasma testosterone levels are within normal<sup>(14,19,20,21,22)</sup>. Unlike this study, Lee's study included adolescents in whom the total serum testosterone pattern may be different.

In female patients some investigations<sup>(24,25,26)</sup> have shown plasma testosterone levels to be normal as in this study, others have found the means of the total serum testosterone to be significantly above normal<sup>(19,27,28,29,30,31)</sup>. Variation in the binding capacity and the percentage of total testosterone bound to serum proteins makes the measurement of free testosterone a more reliable reflection of biologic activity<sup>(36,37)</sup>. When free testosterone levels were done in some studies<sup>(21,22,32,38)</sup>, controversy still persisted and studies<sup>(32,38)</sup> found elevations of free testosterone in female patients. Sultan et al<sup>(21)</sup> found levels to be within the normal limits. Free testosterone levels

in male patients were also done in two studies<sup>(21,22)</sup> and there were no striking differences demonstrated between patients and controls. However there has been more general agreement that in female patients the mean serum SHBG levels are significantly below normal, with free serum testosterone consequently above normal<sup>(24,29,32)</sup>. In this study it was not possible to do either free serum testosterone or serum SHBG due to unavailability of hormonal kits.

In this study there was no relationship between the grades of acne and the total serum testosterone levels, or the mean testosterone levels for grade of acne vulgaris. In a study done by Schiavone et al<sup>(38)</sup>, they did not find any correlation between the free testosterone levels and the severity of acne vulgaris in the 11 out of 24 female patients he found with elevated free testosterone levels, Darley<sup>(31)</sup>, Forstrom<sup>(19)</sup> had similar findings.

Four patients in this study were found to have acne and hirsutism. Their mean serum total testosterone levels were not significantly different from those of the patients with acne alone though the number studied is too small and probably inconclusive. Darley<sup>(31)</sup> also found no significant differences though Daphne<sup>(29)</sup> and Beverly<sup>(34)</sup> found the mean serum total testosterone levels of the patients with acne and hirsutism to be higher than those with acne vulgaris alone. However a recent study

by Schmidt<sup>(39)</sup> on the androgen receptor on the skin of patients with hirsutism and acne found higher levels of androgen receptors in hirsutism than acne. Furthermore there was no correlation between total serum testosterone and the levels of androgen receptors in both conditions. Increased androgen sensitivity at cellular level may explain the occurrence of acne or hirsutism in patients with normal androgen levels.

One setback in doing this study is that no international grading system for acne exists, so that comparison of the studies as far as grading is concerned is not uniformly possible. Individuals have used their own classifications. For example Allen and Smith<sup>(41)</sup> used lesion counts, that is counting the number of comedones papules etc. and attributing a certain count to a grade. Cook et al<sup>(42)</sup> devised a grading system in which overall severity of acne is evaluated on a zero-eight scale anchored to photographic standards. In this study the acne was graded according to the predominant lesion.

CONCLUSIONS:

From the results of this study the following conclusions become evident:-

- That total serum testosterone levels are within normal ranges for the patients with acne attending Kenyatta National Hospital.
- That patients with acne vulgaris may not require further assessment of their serum testosterone status even when hirsutism is present.
- That a patient with acne vulgaris particularly severe acne most likely has a positive family history.

RECOMMENDATIONS:

1. Family history should be taken seriously and should be sought in all patients with acne vulgaris.
2. A single analyte like total serum testosterone is not enough for the evaluation of a patient with acne and multiple analytes including free testosterone, SBHG and skin metabolism of testosterone at cellular level may need to be done.
3. An internationally accepted classification for the grading of acne would go a long way in unifying the different results obtained in many of the studies quoted in this thesis.
4. Futher to this study, a study of patients with persistent acne even after the usual conventional therapy needs to be done. It may be possible that testosterone or other androgen abnormalities may be more prevalent in this group.



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Appendix 1

1. Name of the patient -----  
Unit Number -----
2. Sex: Male ----- Female -----
3. Age (in years) -----
4. Age of onset of Acne -----
5. Duration of Acne -----
6. History of Acne in 1st degree relatives Yes -----  
No. -----
7. Menstrual History: - Cycle -----  
- Duration -----  
- Quantity -----  
Regular ----- Irregular -----
8. Parity -----
9. Hirsutism: Yes ----- No -----

Appendix 1 Cont'd.

10.	Grade of Acne	Sites Involved
	1	-----
	2	-----
	3	-----
	4	-----
11.	Total serum testosterone levels -----	

Appendix 2Acne Grading Scales

1. Comedonal acne mainly
2. Comedonal and papular acne but papular acne predominating.
3. Mainly pustular acne but there may be a few papules and comedones.
4. Nodular cystic Acne mainly but a few of the other lesions that is pustules, papules, and comedones may be co-existing.

Appendix 3 $^{125}\text{I}$  - Testosterone Radioimmuno-assay Kit

The use of radioimmuno-assay provides a simple and inexpensive way of determining levels of steroid hormones in biological liquids. The present test is designed for the determination of testosterone in serum extracts. To that end a  $^{125}\text{I}$ -testosterone tracer is used. Separation of free from antibody bound tracer is achieved by dextran-coated charcoal.

Preparation of Samples

Serum samples should be stored at  $-20^{\circ}\text{C}$ . Prior to the assay, testosterone is extracted from the sample with diethyl ether. Extraction must be performed in glass tubes with glass, teflon or polypropylen stopper. Only the purest quality of ether should be used.

Assay Procedure

1. Pippetting of 'total counts' non specific binding, standards and unknowns is performed as indicated. Duplicate determinations should be done to check for contamination of glass ware and ether an empty glass tube should be extracted with ether as described for serum samples:-

$B/B_0$  for this blank should exceed 95%.

2. Tubes are shaken briefly (vortex) and incubated for 3hr. at room temperature.
3. To each tube (except "total counts") 1ml of chilled (2-8°C) charcoal is added. During pipetting the suspension of charcoal should be stirred. Then the tubes are shaken and incubated at 4°C for 10-20 minutes.
4. After incubation the tubes are centrifuged at 2000xg for 10 minutes. The supernatant is decanted into another tube and counted for 1 minute in a gamma counter (shortly before the expiry date of the kit count for two minutes).

#### Calculation of Results

##### 1. Standard Curve

The concentration of the standard (in pg/tube or f mol/tube) is plotted versus cpm on semi-logarithmic graph paper. Alternatively the quotient of the counts in the standards over the counts in the zero standard ( $B/B_0$ ) can be plotted versus the concentration. The normalised standard curve can also be drawn on logit-log paper.  $B_0$ /total counts usually is 30-40%.

Unknown

The concentrations (in pg/tube or fmol/tube) of the unknown are read from the standard curve. If the extraction has been performed as suggested the concentration in serum (ng/ml or nmol/l) can be calculated.

## Normal values

## Females

0.1-1.0ng/ml = 0.35-3.5 nmol/l

## Males

3-9ng/ml = 10.4-31.2nmol/l

## Children

0.1-0.2ng/ml = 0.35-0.7nmol/l



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