PREVALENCE OF HYPERPROLACTINEMIA AMONG WOMEN ATTENDING INFERTILITY CLINICS IN CALABAR, SOUTH-SOUTH NIGERIA

A

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DECLARATION

I hereby declare that this work is original. This work has not been presented to any other college for a fellowship nor has it been submitted for publication.

DR. UBONG BASSEY AKPAN

18th June, 2012
DEDICATION

This research work is dedicated to Almighty God, and all the women who took part in this study.
CERTIFICATION
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ABSTRACT

Background: Infertility is a worldwide problem affecting about 10% of couples. Hypothalamic-pituitary dysfunction due to Hyperprolactinemia is one of the leading causes of anovulatory infertility. However, there has been paucity of data on this subject in Sub-saharan Africa, hence, the need for this work.

Objective: The purpose of this study was to determine the contribution of Hyperprolactinemia to female infertility in Calabar and also assess any association with bio-social factors and hypothyroidism.

Methods: In this comparative cross sectional study, prolactin levels of 152 women in infertility clinics in Calabar were assessed. One hundred and thirty-three fertile subjects with similar age and socio-economic status were recruited from the family planning clinics and enrolled as controls. Prolactin measurement was performed using immunoassay auto-analyzer. Data were collected and analyzed using SPSS version 16.
**Results:** The mean age of the women in infertile group was 30.7±1.38 (SD) and the mean age in the control was 29.3±1.39 (p-value > 0.05). The mean duration of infertility was 3.89 years ± 3.433 (SD). Fifty seven patients among the infertile group had high level of prolactin giving the prevalence of 37.5%. The prevalence in the fertile group was 4.5%. The incidence of hypothyroidism was rare. The correlation of hypothyroidism with Hyperprolactinemia was 1:29. Oligomenorrhoea was the commonest menstrual irregularity encountered in the infertile women with Hyperprolactinemia and it occurred in 35.6% of these women.

**Conclusion:** The prevalence of Hyperprolactinemia is significantly high in infertile women. Hence, assessment of serum prolactin levels should be included in the initial work up of all infertile women especially those presenting with menstrual disturbances.

**Key words:** Infertility, Hyperprolactinemia, galactorrhoea, oligomenorrhoea.
CHAPTER ONE

1.1 INTRODUCTION

Infertility is a worldwide problem occurring in about one in ten couples and leading to increasing number of them seeking specialist fertility care.\(^1,^2\) In the general population, conception occurs in 84% of women within 12 months and 92% within 24 months of regular unprotected sexual intercourse.\(^3\) Infertility is said to occur when there is inability of couple to conceive after one year of regular intercourse without contraception.\(^4\)

Infertility is primary if a pregnancy has never occurred, and secondary if there has been a preceding pregnancy, irrespective of the outcome of the pregnancy.\(^2,^4,^5\) According to standard protocols, infertility evaluation usually identifies different causes, including female infertility (35%), male infertility (30%), combination of both (20%) and finally unexplained or idiopathic infertility (15%).\(^4\)

Most of those presenting with childlessness have reduced fertility, rather than absolute sterility, and many may conceive spontaneously.\(^2\) Subfertility affects one in seven couples in the
United Kingdom and in the United States, infertility occurs in about 28 million couples or 14% of couples.\textsuperscript{1,6} Compared to other parts of the world, several countries in sub-Saharan Africa have high prevalence rates of infertility. In some parts of Nigeria, community based studies have reported rates of infertility as high as 20% to 45%.\textsuperscript{5} It accounts for nearly 40% of all gynaecological consultations in Maiduguri, North-east Nigeria.\textsuperscript{7} Infertility is the commonest problem in gynaecological clinics in Calabar.

Fertility depends on complex psychological, anatomical, endocrinological and immunological factors.\textsuperscript{1,2,9} The male needs normal sperms produced and deposited in the reproductive tract of the female. The female needs a functionally intact hypothalamic-pituitary-ovarian axis to regulate and provide normal follicular development, ovulation and priming of the endometrium for implantation of the zygote, which has to pass through a normal fallopian tube following fertilization. Failure of any of these complex processes leads to infertility.

Hyperprolactinemia is a leading cause of anovulatory infertility in Africa\textsuperscript{5}. Elevated serum prolactin level is associated with failure of
ovulation. Women with hyperprolactinemia typically present with a history of oligomenorrhea, amenorrhea, or infertility, which generally results from prolactin suppression of gonadotrophin releasing hormone (GnRH). The physical findings most commonly encountered in patients with hyperprolactinemia are galactorrhoea and, occasionally, visual field defects. Galactorrhoea is due to the direct physiologic effect of the prolactin on breast epithelial cells. Typically, the diagnosis is made via the aid of laboratory studies. Normal fasting values are generally less than 25ng/ml irrespective of the differences in the laboratory used.

The causes of hyperprolactinemia fall into 3 main categories: physiological, pharmacological and pathological. Pathologic hyperprolactinemia is due to hypothalomo-pituitary lesions and secondary causes such as hypothyroidism, renal or adrenal insufficiency.

Pharmacological causes of hyperprolactinemia are dopamine receptor antagonists example phenothiazone, butyrophenons,
thioxanthines, riperidone, metoclopramide, sulphide and pimozide and dopamine – depleting agents such as methyldopa and reserpine.\textsuperscript{9,10,13,14} Other pharmacological agents that have been known to be associated with hyperprolactinemia include tricyclic antidepressant, monamine antihypertensives, estrogens, anti-androgens, opiates, H-2 blockers (cimetidine) and cocaine.\textsuperscript{9,10,13} The main physiologic function of prolactin is in breast development and lactation.\textsuperscript{9} Secretion is pulsatile and is increased during pregnancy, reaching a peak at the time of parturition.\textsuperscript{9,10} After delivery, the plasma concentration falls to non-pregnant levels within 2 weeks except the woman is lactating.\textsuperscript{9}

Traditionally, measurements of prolactin and thyroid stimulating hormone (TSH) have been considered important components of the evaluation of women presenting with infertility.\textsuperscript{16} Many obstetricians and gynaecologists check serum levels of prolactin and thyroid stimulating hormone (TSH) in every female patient undergoing infertility evaluation regardless of their menstrual rhythm.\textsuperscript{16}
Primary hypothyroidism is associated with increased production of thyrotropin releasing hormone (TRH), which is known to stimulate a pituitary TSH and prolactin release. Thyroid dysfunction is implicated in a broad spectrum of reproductive disorders, ranging from abnormal sexual development to menstrual irregularities and infertility.\textsuperscript{4} It is important to be aware of the limitations of some of the commonly used tests and balance the risks and the cost versus potentials benefits.

This study seeks to determine the prevalence of hyperprolactinemia among women attending infertility clinics in Calabar. This will help in the assessment of the contribution of hyperprolactinemia to infertility in Calabar.
1.2 RELEVANCE OF THE STUDY TO GYNAECOLOGICAL PRACTICE

Infertility is one of the commonest problems in gynaecological clinics in Calabar. The manner of clinical presentation varies from one place to another, depending on the socio-economic and cultural environment from which the patients come. Anovulation due to hyperprolactinemia is a leading cause of infertility in Africa and beyond.

The economic burden of infertility is also enormous as most infertile couples spend a significant proportion of their resources on medical treatment. The National Health Insurance Scheme is yet to extend its coverage to infertility treatment. The causes are so wide and the treatment options are varied. Hence, the investigation tools used in diagnosing the definitive cause of the problem has become so large and sophisticated, but most patients in developing countries have little or no knowledge of these tools nor can they afford their cost.
Many obstetricians and gynaecologists check serum levels of prolactin and thyroid stimulating hormone in every woman undergoing infertility evaluation regardless of their menstrual rhythm.\textsuperscript{16} Also it is not uncommon to see some women with infertility taking dopaminergic agonist such as bromocriptine on their own or prescribed by practitioners especially if there is galactorrhoea, without checking the serum levels of prolactin.

This study therefore seeks to determine the prevalence of hyperprolactinemia among women being evaluated for infertility in Calabar.
CHAPTER TWO

LITERATURE REVIEW

2.1 THE BURDEN OF INFERTILITY

The problem of infertility of pathological origin is so high in sub-Saharan Africa that it has become a public health problem.\textsuperscript{18,19} The prevalent rate varies from one region to the other. In a study by Ugwuja \textit{et al}\textsuperscript{18}, prevalent rate of 30\% was reported in a rural Nigerian community, of which 9.2\% and 21.1\% represent primary and secondary infertility respectively.

An “infertility belt” has been described in Africa that stretches from West Africa, through Central to East Africa.\textsuperscript{5} Several countries with high rates of infertility that lie within this belt include Nigeria, Cameroon, Gabon, Democratic Republic of Congo, Burundi, Uganda and Kenya. In Gabon, it is estimated that more than 33\% of women are childless at the end of their reproductive lives.\textsuperscript{5}

The high prevalence of infertility in Africa has profound implication for women’s reproductive health. As a result of the
high premium placed on childbearing in many African societies, infertility is a social destabilizing condition for couples. In Africa, infertility carries a stigma, especially for women, and it is a cause of marital disharmony, social ostracization and physical violence against women.5

The prevention and treatment of infertility is an important unmet reproductive health need in Africa. Interviews with women in several countries across Africa have revealed that infertility is the most frightening reproductive health problem for women.5 The fear of infertility has been identified as the most important reason that women often do not use contraceptives, believing that these could lead to infertility.20

2.2 THE PREVALENCE OF HYPERPROLACTINEMIA

Hyperprolactinemia occurs in less than 1% of the general population.4,10 The occurrence of clinically apparent Hyperprolactinemia depends on the study population. The prevalence has been reported to range from 0.4% in an unselected healthy adult population in Japan to 5% among clients
at a family planning clinic in the same country. These studies may not reflect the true incidence among infertile couples in certain areas since certain demographic factors and fertility profile are influenced by socio-cultural and environmental factors such as alcohol consumption, cigarette smoking, and occupational stress.

Some other studies have shown that the rate is even higher among patients with specific symptoms that may be attributable to Hyperprolactinemia: it is estimated at 9% among women with amenorrhea, 25% among women with galactorrhoea and as high as 70% among women with amenorrhoea and galactorrhoea. The prevalence is about 5% among men who present with impotence or infertility.

Hyperprolactinemia was found in 21% of women with secondary infertility by Idrisa et al in a Northern Nigeria University Teaching Hospital. However, this study was limited to a government tertiary hospital and did not consider infertile women attending private clinics. Also, in retrospective studies, poor or
inadequate information recording in case notes may cause limitation.

Approximately 30% of women presenting with galactorrhoea, amenorrhoea and Hyperprolactinemia may have prolactin secreting tumors.\textsuperscript{10} Mortality is unlikely, however, in cases where the condition is due to a large prolactin secreting tumor, local mass effect can lead to significant morbidity.\textsuperscript{10,23,24} The condition often causes systematic complaints that often resolve when the prolactin level returns to normal or once the tumour shrinks.\textsuperscript{10,25,26} It is not a routine practice in most centers in the tropics to investigate for pituitary tumours in hyperprolactinemic women. It is important to determine the proportion of women in our centers with very high levels of prolactin, 100mg/dl and above, who have macroadenomas.

2.3 THE REPRODUCTIVE FUNCTIONS OF PROLACTIN

Prolactin is a pituitary-derived hormone that plays a pivotal role in a variety of reproductive functions. It is an essential factor for normal production of breast milk following childbirth. Prolactin
stimulates breast epithelial cell proliferation and induce milk production.\textsuperscript{10,13} Estrogen stimulates the proliferation of pituitary lactotroph cells, resulting in an increased quantity of these cells in premenopausal women, especially during pregnancy.\textsuperscript{27,28}

Furthermore, prolactin negatively modulates the secretion of pituitary hormones responsible for gonadal function, including luteinizing hormone (LH) and follicle-stimulating hormone (FSH).\textsuperscript{21}

The possible mechanism of action of prolactin had been studied by many authors. It is still not well understood. It is generally believed that dopamine has the dominant influence over prolactin secretion. Secretion of prolactin is under tonic inhibitory control by dopamine which acts via D2-type receptors located on the lactotrophes.\textsuperscript{10,13,22}

Prolactin production can be stimulated by hypothalamic peptides, thyrotropin releasing hormone (TRH) and vasoactive intestinal peptide (VIP).\textsuperscript{10,13} Thus primary hypothyroidism, a high TRH state, can cause hyperprolactinemia. VIP increases prolactin in response to suckling, probably because of its action on receptors that increase adenosine 3′5′ – cyclic phosphate (CAMP).\textsuperscript{10,13}
The association between ovulatory dysfunction and hyperprolactinemia is well documented. Its primary mechanism appears to be the inhibition of pulsatile secretion of Gonadotropin releasing hormone (GnRH) that results in a hypoestrogenic state. Elevated prolactin levels may cause the direct inhibition of steroidogenesis at the level of the ovary.

### 2.4 HYPERPROLACTINEMIA AND INFERTILITY

In a study by Serri et al. mild prolactin excess of 25-50ng/ml was associated with short luteal phase, decreased libido and infertility. Moderate prolactin excess of 51-75ng/ml was associated with oligomenorrhoea and marked prolactin excess >100ng/ml was associated with hypogonadism, galactorrhoea and amenorrhoea. Therefore, women with oligomenorrhoea, amenorrhoea, galactorrhoea or infertility must have serum prolactin levels measured. Hyperprolactinemia can cause delayed puberty by inducing the suppression of hypothalamic-pituitary gonadal axis or resistance of the ovary to gonadotropin action which results in amenorrhoea and lack of ovulation. It is
imperative to conduct a prospective study to assess the impact of Hyperprolactinemia on infertile women in our center.

2.5 OTHER CLINICAL CONDITIONS ASSOCIATED WITH HYPERPROLACTINEMIA

Numerous clinical conditions may cause elevated prolactin levels, among them are pituitary tumors and empty sella syndromes. Transient elevation had been documented following protein ingestion, stress, Herpes Zoster, sexual intercourse and hypoglycaemia. Although adenomas tend to be associated with high level of prolactin, they may be associated with any degree of hyperprolactinemia so Rein et al had suggested that evaluation of the hypothalamus and pituitary with Magnetic Resonance Imaging (MRI) should be considered for all patients with persistent Hyperprolactinemia.

Amenorrhea and/or galactorrhoea may precede the eventual full clinical expression of a tumour that secretes adrenocorticotropic hormone (ACTH) or growth hormones (GH). Cysts, tuberculosis, sarcoidosis and fat deposits have been reported as
causes of pituitary compression leading to hypogonadotrophic amenorrhoea.\textsuperscript{10} Lymphocytic hypophysitis is a rare autoimmune infiltration of the pituitary that can mimic a pituitary tumor, often occurring during pregnancy or the first 6 months postpartum.\textsuperscript{25,26} In the initial phase of hypophysitis, hyperprolactinemia is common, followed by hypopituitarism.\textsuperscript{25} Nearby lesions, such as internal carotid artery aneurism, destruction of the aqueduct of Sylvius, can also cause amenorrhoea.\textsuperscript{10,25}

Elevated gonadotropins in the presence of pituitary microadenoma in a woman with amenorrhoea may not be a consequence of secretion by the tumour, another explanation must be pursued.\textsuperscript{1} Galactorrhoea is frequently, but not always seen in patients with pituitary tumours.\textsuperscript{1,19} Because amenorrhea and galactorrhoea may precede neurologic symptoms by years, a central nervous lesion must be considered in any patient with the syndrome of amenorrhoea, galactorrhoea, and hyperprolactinemia. Although galactorrhoea remains the clinical hallmark of these disorders, increased prolactin levels may be present without recognizable clinical symptoms.\textsuperscript{6,10,24}
It is suggested that if a galactorrheic patient has a normal prolactin level, a prolactin-secreting tumour is unlikely unless it is associated with amenorrhoea, then, a non functioning or poorly functioning tumour should be excluded.\textsuperscript{10,25,26} Although as earlier documented by many studies, the likelihood of finding a pituitary adenoma may not correlate well with the level of hyperprolactinemia.\textsuperscript{26}

In the ovary, prolactin directly inhibits basal as well as gonadotrophin-mediated oestradiol and progesterone production and ovulation.\textsuperscript{5,16} It does this by inhibiting LH induced production of plasminogen activator in pre-ovulatory follicles which reduces the availability of plasmin.\textsuperscript{19} Plasmin is necessary for the digestion of the follicle wall that results in the release of the ovum.\textsuperscript{19} Interestingly, postmenopausal women with elevated levels of prolactin do not experience vasomotor symptoms (hot flushes) until prolactin levels are restored to normal.\textsuperscript{10,21}

Postictal patients also develop hyperprolactinemia within 1-2 hours after a seizure and this condition usually produce a prolactin level of less than 50ng/ml.\textsuperscript{2,10} And many other studies
had linked hyperprolactinemia to the results of disease of other organs such as the liver, kidneys, and thyroid.\textsuperscript{31} It it also important to determine what percentage of our women with Hyperprolactinemia have other systemic diseases.

The data regarding the role of prolactin in human cancer have been conflicting. Some studies have suggested that higher circulating levels of prolactin are associated with an increased risk of radiographically dense breast tissue.\textsuperscript{32} Others have noted that postsurgical hyperprolactinemias are associated with a significant lower recurrence rate and longer disease-free and overall survival in node-negative breast cancer patients.\textsuperscript{33} In addition, in patients with chronically elevated prolactin levels such as those with prolactinomas, no increase in neoplasia in general or breast cancer in particular has been noted.\textsuperscript{34} Therefore there is no sufficient evidence establishing the relationship between excess prolactin and the risk of neoplasia to form the basis for recommending inhibition of prolactin for postmenopausal women.\textsuperscript{35} So, in this study, breast, ovarian and endometrial malignancies are not included as risk factors.
2.6 THE CHEMISTRY OF PROLACTIN

Human prolactin circulates in at least 3 discrete forms in the serum; monomeric (free) prolactin, which accounts for 85% of the prolactin; a big-prolactin species which account for 10-15%, and a very big prolactin or macroprolactin, which is an antigen-antibody complex of monomeric prolactin and IgG.\(^{36}\) As macroprolactin remains confined in the vasculature, it does not have any biologic activity and patients with macroprolactinemia do not require treatment with dopaminergic agonists.\(^{36}\) The prevalence of macroprolactinemia in patients with hyperprolactinemia has been reported to be between 18 to 45% in various studies.\(^{36,37}\)

2.7 THE ASSOCIATION BETWEEN PROLACTIN, THYROID STIMULATING HORMONE (TSH) AND INFERTILITY

Data on the relationship between thyroid disorders and infertility remain scarce and the association with a particular cause of infertility has not been thoroughly analyzed.\(^{38}\) Hypothyroidism, have all come under a lot of discussion recently. The prevalence
of sub-clinical thyroid disorders in infertility patients and indications for treatment have been discussed, but no consensus has been obtained.\textsuperscript{39} In a recent study conducted among 140 infertile women, the prevalence of sub-clinical hypothyroidism was 6.5\% and 15\%, and the prevalence of Hyperprolactinemia was 43\% and 21\% in primary and secondary infertility respectively.\textsuperscript{40}

In a prospective study in the U.S.A., Serum, TSH and prolactin (PRL) were checked at the time of the couple’s initial consultation for infertility. There were 2.48\% of patients with abnormal levels of TSH, and 1.77\% with elevated levels of PRL. From this result, it was concluded that the practice of routinely ordering serum levels of TSH and PRL in infertility with normal periods is questionable.\textsuperscript{40} It is necessary in our centers to assess the correlation of Hyperprolactinemia with hypothyroidism among infertile women.
2.8 TREATMENT OF HYPERPROLACTINEMIA

Objectives of Treatment

The main objectives of treatment of Hyperprolactinemia are: restoration and maintenance of normal gonadal function, restoration of normal fertility and prevention of osteoporosis.²¹ If a pituitary tumour is present, the aim is to correct visual or neurological abnormalities, reduce or remove tumour mass, preserve normal pituitary function and prevent pituitary or hypothalamic disease.²¹

Medical Therapeutic Options for Management of Hyperprolactinemia

Dopamine agonists are currently the first therapeutic option.¹⁰,¹⁴¹,⁴² Dopamine agonists have proven efficacy in reducing prolactin levels, restoring ovulation in premenopausal women and restoring gonadal function in men.⁴³

Prolactin levels remain above normal in about 20% of cases of macroprolactinemia despite dopamine agonist therapy.⁴³ Bromocriptine restore ovulation in 70-80% of women.² Long-term
treatment with bromocriptine results in pregnancy rates of 35% - 70% per woman.\textsuperscript{2} Cabergoline and Quinogolid are newer dopamine agonists which have recently been licensed for treatment of Hyperprolactinemia.\textsuperscript{2} Fewer side effects and longer half-lives allow a once daily dose for quinogolid and a twice weekly dose for cabergoline.

Data from randomized controlled trials have shown cabergoline to be more effective than bromocriptine in restoring normoprolactinemia and ovulation. Cabergoline has greater affinity and selectivity for pituitary dopamine D\textsubscript{2} receptors and longer duration of action.\textsuperscript{43,44,45}

**Surgical Treatment of Hyperprolactinemia**

Treatment of Hyperprolactinemia is mainly medical. In rare cases, surgery may be considered in women with pituitary tumours who despite normalization of prolactin levels do not show adequate shrinkage.\textsuperscript{2,10} Alternatively, it may be considered in patients with large macroadenoma who are intolerant or resistant to drug treatment.\textsuperscript{2,10} Despite the invasive nature of surgery, prolactin
values return to normal in 50% of microadenomas and 10-15% of macroadenomas.²

2.9 COMPLICATIONS OF TREATMENT

Some of the common side effects associated with the use of bromocriptine include hypotensive reaction, palpitation, epistaxis, epigastric pain, syncope, asthenia, headache, drowsiness and hot flushes.²⁴ Longer term side effects include Raynaud’s phenomenon, constipation and psychiatric manifestations.⁵,⁴⁷ Recent cross sectional studies have investigated valvular heart disease in patients taking cabergoline for Hyperprolactinemia.⁴⁸-⁵⁴ These studies do not support an association between treatment of prolactinomas with cabergoline and clinically significant valvular heart disease. However, mild and moderate, and mitral valve tenting have been described.⁴⁹,⁵⁰,⁵¹,⁵²,⁵³,⁵⁴ However, these studies are limited by their cross sectional design and relatively small number of patients. The need for studies with larger sample sizes is imperative.
The recent MHRA guidelines advise that cabergoline should not be used in pregnancy and must be stopped in women one month before trying to conceive\textsuperscript{55}. However, stopping treatment before planning a pregnancy may cause recurrence of Hyperprolactinemia and prevent ovulation\textsuperscript{55}. Taking cabergoline during pregnancy has not been associated with miscarriage or foetal malformation.\textsuperscript{55}

Infertility associated with Hyperprolactinemia is reversible with treatment.\textsuperscript{5} Lowering of prolactin levels to normal or near normal is often necessary to restore ovulation\textsuperscript{4,5}. 
CHAPTER THREE

OBJECTIVES

3.1 GENERAL OBJECTIVE
To assess the contribution of Hyperprolactinemia to female infertility in Calabar.

3.2 SPECIFIC OBJECTIVES
(1) To determine the prevalence of Hyperprolactinemia among women attending infertility clinics in Calabar.
(2) To establish the demographic characteristics of women with Hyperprolactinemia who are attending infertility clinics in Calabar.
(3) To assess for any association between Hyperprolactinemia and hypothyroidism among the women in the study population.
(4) To compare the prevalence of Hyperprolactinemia in infertile and fertile women in Calabar.
CHAPTER FOUR
METHODOLOGY

4.1 STUDY DESIGN, POPULATION AND AREA

This is a sample type prospective study aimed at determining the prevalence of Hyperprolactinemia among infertile women attending gynaecological clinics in Calabar. The research also assessed the correlation of Hyperprolactinemia with hypothyroidism among these women. The research was conducted in the University of Calabar Teaching Hospital and two private clinics. The private clinics were chosen along with UCTH to enable us cover different classes of women in Calabar.

The University of Calabar Teaching Hospital is the only tertiary health facility in Calabar metropolis, the capital of Cross River State which is located in south-south zone of Nigeria. There are six private specialist gynaecological clinics in Calabar. The two specialist clinics chosen – Mevon Clinic & Victoria Itam Specialist clinic provide services to all patients who present themselves for treatment but with particular interest in obstetrics and
gynaecological patients. They also have large patients turn-out compared to the rest.

4.2 DETERMINATION OF SAMPLE SIZE (LEEDY, 1992)

\[ N = \frac{Z_\alpha^2 pq}{d^2} \]

Where \( Z_\alpha = \alpha \) value at 95% confidence interval (≈ 1.96 t-test)

\( p = \) Probability of presence of the condition

\( q = \) Probability of absence of the condition

\( d = \) Precision at 5%

Substituting: \( P \) here ≈ 10% convenience probability of presence (i.e. 0.10)

\( q = 1-P = (1-0.10) = 0.90 \)

\( d = 5\% = 0.05 \)

\[ N = \frac{Z_\alpha^2 pq}{d^2} \]

\[ = \frac{1.96^2 \times 0.10 \times 0.90}{(0.05)^2} \]

\[ \approx 138 \]

The sample size of 150 was chosen so as to cater for attrition.
4.3 RECRUITMENT OF SUBJECTS

Women with infertility were enrolled during the initial consultation at the clinics. The research and its purpose and expected benefit to the patient and the community was explained and consent was obtained from the willing participants. To facilitate easy completion of Questionnaire, semi structured and closed response questions were used. A detailed history was obtained and physical examination was carried out depending on the complaint. The weight and height were measured and body mass index (BMI) calculated. Then blood sample was obtained using standard venupuncture technique. In order to determine the prevalence of Hyperprolactinemia in the fertile population matched controls were developed by recruiting 133 fertile women with similar age and socio-economic status attending family planning clinics who were not on hormonal contraception.

Using the records in the family planning clinics that were used, clients who had been in the clinic in the past two years were contacted and the research and its purpose explained to them. A good number of them who reside in Calabar happily responded.
Their transport fares to the clinics were refunded and the result of
the hormonal test given to them. They were also told their blood
pressure and body mass index. Also, a good number of nurses
working in the maternity complex who heard about the research
happily volunteered as controls. A total of 133 fertile women were
recruited as control within the six weeks study period.

4.4 THE EXCLUSION CRITERIA FOR THE INFERTILE
GROUP
(1) Patients who are not infertile based on the definition.
(2) Patients who refuse to give consent.
(3) Women with known psychiatric problems.
(4) Women who are presently on dopaminergic agonists or
drugs known to influence the serum concentration of
prolactin.
(5) Pregnant or lactating mothers.

4.5 EXCLUSION CRITERIA FOR THE CONTROLS
(1) Women who refused to give consent
(2) Women on hormonal contraception
(3) Pregnant or lactating mothers.
4.6 SAMPLE COLLECTION

Five milliliters of venous blood samples was drawn from subjects at least one hour after their arrival at the clinic to ensure that the value is not influenced by momentary rise in prolactin level due to the stress of journeying to the hospital. Samples were transferred to the laboratory within an hour of collection. Each sample was properly labeled using a number tag.

4.7 SAMPLE ANALYSIS (PROLACTIN IMMUNOASSAY)

Methodology

Serum prolactin was determined by a commercially available quantitative enzyme linked immunosorbent assay for human prolactin (Diagnostic automation Inc. 23961 Craftsman Rd Suite E/F, Calabarsas Califonia 91302 USA). The analysis was supervised by a chemical pathologist.

Measurement principles

A monoclonal anti-prolactin antibody was immobilized on microtitre-wells. Another mouse monoclonal anti-prolactin
antibody was conjugated with the enzyme horseradish peroxidase (HRP) to form an antibody-enzyme conjugate.

The prolactin in the sample bound simultaneously with both antiprolactin antibodies to form a sandwich of prolactin between solid phase and enzyme-linked antibodies. After 60 minutes incubation at room temperature the microwells were washed with water to remove unbound labeled antibodies. A TMB solution was added and the wells incubated for 20 minutes, to develop a blue colour. The colour development was halted by addition of a stop solution which changed the colour to yellow.

The enzymatic reaction was proportional to the amount of prolactin in the sample. Absorbance was measured using a microwell reader, which plotted a standard curve and read out the amount of prolactin in concentration units.

**Reagents**

1. Serum samples
2. Enzyme conjugate reagent
3. Wash buffer
4. TMB substrate
5. Stop solution
6. Prolactin Standards (lyophilized)
7. Prolactin precision Controls

**Materials**

1. Microwell plates (each comprising of 96 wells)
2. Test tube/cuvettes
3. Cardboard Sealers
4. Calibrated Micropipettes
5. Vortex Mixer
6. Absorbent Paper
7. Deionized Water
8. Automatic Microplate washer
9. Water Bath
10. Microtitre well reader

**Procedures**

All reagents were brought to temperature before use. Frozen serum was allowed to thaw at room temperature before analysis. Lyophilized standards were reconstituted by addition of 0.5ml of deionized water to 50ul of prolactin standards, serum samples
and controls was pipette. 100ul of enzyme conjugate was pipette into the microwells. The microwell plate was then covered and incubated for 60 mins at room temperature after thorough mixing for 10 seconds. Thereafter the microwells were washed for 5 times using the washing buffer. 100ul of TMB substrate solution was added into each well at timed intervals.

The microwell plate was then covered and incubated for 20 minutes at room temperature in the dark. The whole reaction was stopped by the addition of 100ul of stop solution into each well at timed intervals. The plate was shaken gently to mix the solutions. Absorbance was measured at 450nm using a microwell reader within 30mins of adding the stop solution.

**Calculation**

The microwell reader plotted the standard curve and reads out the prolactin concentrations expressed in ng/mL from the curve. The normal upper limit used in the UCTH Chemical Pathology Laboratory is 20.15ng/ml. serum TSH levels was checked for
women with prolactin levels above the upper limit. Women with serum prolactin levels > 100ng/ml were advised for skull x-ray (coned down view), Computed Tomographic (CT) scan or Magnetic Resonance Imaging (MRI) to rule out prolactin secreting tumours.

4.8 ANALYTICAL PRECISION CONTROL

The prolactin assays were carried out in batches of 20 samples each.

Analytical quality control was ensured by assaying precision control sera during the batch analyses of subjects’ samples. Intra-assay and between-assay precision studies were carried out using appropriate control sera from the Prolactin Kit.

The intra-assay precisions for levels 1, 2 and 3 control sera at the prolactin concentrations of 10.5ng/ml, 28.5ng/ml and 77.5ng/ml are 3.3%, 3.0% and 2.5% respectively.

The between-assay precisions for levels 1, 2 and 3 control sera at the prolactin concentrations of 11.5ng/ml, 27.8ng/ml and 78.5ng/ml are 1.7%, 1.8% and 3.0% respectively.
4.9 DATA ANALYSIS
Data obtained was analyzed with statistical package for social sciences (SPSS) version 16 Inc. Chicago, Illinois – USA. Test of statistical significance in outcome shall be computed using the student t-test and the Chi-square test (X2 test). A p-value of <0.05 was considered statistically significant.

4.10 ETHICAL CONSIDERATION
The participation of subjects in this research study was voluntary; the principle of patient confidentiality was strictly adhered to. Each participant was duly counseled and a prepared consent form was signed (See Appendix 1 and 2).

Formal approval was obtained from the Research Ethical Committee of the University of Calabar Teaching Hospital, Calabar. Approvals were also obtained from the two Private Specialist Clinics where the study was conducted.

4.11 LIMITATIONS OF THE STUDY
1) This study relied in part on information obtained with a precoded questionnaire and history obtained from the
patients. Some important facts might not have been volunteered.

2) This study also relied on results obtained from laboratory procedures; errors may arise from the human element or from equipment used.

3) The cost of the reagents.
CHAPTER FIVE

RESULTS

One hundred and fifty two (152) women attending infertility clinics in Calabar were enrolled in the study group and one hundred and thirty three (133) fertile women were recruited from family planning clinics as controls. The mean age in years of women in the infertile group was 30.7 \(\pm 1.38\) (SD) and that of the control was 29.3 \(\pm 1.39\) (SD). Table I and II show the age distribution in the two groups.

**Table I: Age distribution of study group (infertile women)**

\[ N = 152 \]

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(&lt;20)</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>21- 25</td>
<td>18</td>
<td>11.8</td>
</tr>
<tr>
<td>26 – 30</td>
<td>58</td>
<td>38.2</td>
</tr>
<tr>
<td>31 – 35</td>
<td>42</td>
<td>27.6</td>
</tr>
<tr>
<td>(\geq36)</td>
<td>33</td>
<td>21.7</td>
</tr>
<tr>
<td>Total</td>
<td>152</td>
<td>100</td>
</tr>
</tbody>
</table>
Table II: Age distribution of participants in the control group (fertile women) N = 133

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>˂ 20</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>21- 25</td>
<td>43</td>
<td>32.3</td>
</tr>
<tr>
<td>26 – 30</td>
<td>28</td>
<td>21.1</td>
</tr>
<tr>
<td>31 – 35</td>
<td>11</td>
<td>8.3</td>
</tr>
<tr>
<td>˃ 36</td>
<td>41</td>
<td>30.8</td>
</tr>
</tbody>
</table>

About 93% of the women in the infertile group were married, four women were single but have been co-habiting with their prospective husbands for more than one year desiring to conceive, five of the women were officially engaged to their partners and were unable to conceive while 2 women had been separated from their husbands due to infertility. Among the 133 fertile women 97.7% were married, two were single and one was deceased. Table III and IV show the marital status in the two groups.
Table III: Marital status of study group (n = 152)

<table>
<thead>
<tr>
<th>Marital status</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married</td>
<td>141</td>
<td>92.8</td>
</tr>
<tr>
<td>Single</td>
<td>4</td>
<td>2.6</td>
</tr>
<tr>
<td>Engaged</td>
<td>5</td>
<td>3.3</td>
</tr>
<tr>
<td>Separated</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td>Total</td>
<td>152</td>
<td>100</td>
</tr>
</tbody>
</table>

Table IV: Marital status of control group (n = 133)

<table>
<thead>
<tr>
<th>Marital status</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married</td>
<td>130</td>
<td>97.7</td>
</tr>
<tr>
<td>Single</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>Separated</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Divorced</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Deceased</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>Total</td>
<td>133</td>
<td>100</td>
</tr>
</tbody>
</table>
The social classes of the respondents in both groups were determined using the classification by Olusanya and Okpere\textsuperscript{59} based on the highest educational attainment of the woman and the occupation of her spouse as demonstrated as shown below:

**Table Va: Protocol for social classification (Olusanya, Okpere & Ezimokhai)**

<table>
<thead>
<tr>
<th></th>
<th>A. Husband</th>
<th>B. Wife</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scores</td>
<td>Occupation</td>
<td>Score</td>
</tr>
<tr>
<td>1.</td>
<td>Professionals</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>Middle Level</td>
<td>1</td>
</tr>
<tr>
<td>3.</td>
<td>Unskilled</td>
<td>2</td>
</tr>
</tbody>
</table>

The sum of scores A and B will be the social class of the woman.

The table $V_b$ below shows that majority of women in both groups were in social class 2 and 3.
Table Vb: Social class of respondents

<table>
<thead>
<tr>
<th>Social class</th>
<th>Study group n = 152</th>
<th>Control group n= 133</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency (n)</td>
<td>Percentage (%)</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>9.9</td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>32.2</td>
</tr>
<tr>
<td>3</td>
<td>59</td>
<td>38.8</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>15.1</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Table VI shows that more than half of the women (65.3%) in the infertile group were nulliparous.

Table VI: Parity distribution of the women

<table>
<thead>
<tr>
<th>Parity</th>
<th>Study group n = 152</th>
<th>Control group n= 133</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency (n)</td>
<td>Percentage (%)</td>
</tr>
<tr>
<td>Nulliparity</td>
<td>99</td>
<td>65.3</td>
</tr>
<tr>
<td>Para 1</td>
<td>23</td>
<td>15.1</td>
</tr>
<tr>
<td>Para 2</td>
<td>15</td>
<td>9.7</td>
</tr>
<tr>
<td>Para 3</td>
<td>8</td>
<td>5.3</td>
</tr>
<tr>
<td>Para 4 and above</td>
<td>7</td>
<td>4.6</td>
</tr>
<tr>
<td>Total</td>
<td>152</td>
<td>100</td>
</tr>
</tbody>
</table>
The average duration of marriage in the control group was 7.65 years. More than 20% of the women in the infertile group have been married for more than 10 years. Table VII compares the duration of marriage in the study and the control group. One hundred and forty-one (92.8%) of the women in the infertile group were married.

**Table VII: Duration of marriage of respondents**

<table>
<thead>
<tr>
<th>Duration of marriage/relationship (years)</th>
<th>Study group n = 152</th>
<th>Control group n= 133</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency (n)</td>
<td>Percentage (%)</td>
</tr>
<tr>
<td>≤ 5</td>
<td>89</td>
<td>58.6</td>
</tr>
<tr>
<td>6- 9</td>
<td>31</td>
<td>20.4</td>
</tr>
<tr>
<td>≥ 10</td>
<td>32</td>
<td>21.1</td>
</tr>
<tr>
<td>Total</td>
<td>152</td>
<td>100</td>
</tr>
</tbody>
</table>

Secondary infertility was found in 118 women (77.6%) and primary infertility occurred in only 34 women (22.4%) in the study group. Table VIII below gives the summary of the types of infertility found in the study group.
Table VIII: Types of infertility amongst study group n=152

<table>
<thead>
<tr>
<th>Types of infertility</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>34</td>
<td>22.4</td>
</tr>
<tr>
<td>Secondary</td>
<td>118</td>
<td>77.6</td>
</tr>
<tr>
<td>Total</td>
<td>152</td>
<td>100</td>
</tr>
</tbody>
</table>

The mean duration of infertility was 3.89 years ±3.433(SD). The duration of infertility was calculated in years from the last conception/childbirth to the time of presentation for the women with secondary infertility. For those with primary infertility, it was calculated from the time of marriage or desiring pregnancy to the time of presentation at the clinic for evaluation. Table IX shows that 44.7% of the respondents were unable to conceive within 2 years of marriage/relationship while 8.6% were infertile for more than 9 years.
Table IX: Duration of infertility of study group n = 152

<table>
<thead>
<tr>
<th>Duration of infertility (years)</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 2</td>
<td>68</td>
<td>44.7</td>
</tr>
<tr>
<td>3 – 5</td>
<td>54</td>
<td>36.2</td>
</tr>
<tr>
<td>6 – 8</td>
<td>16</td>
<td>10.5</td>
</tr>
<tr>
<td>&gt; 9</td>
<td>13</td>
<td>8.6</td>
</tr>
<tr>
<td>Total</td>
<td>152</td>
<td>100</td>
</tr>
</tbody>
</table>

Mean duration of infertility = 3.89 years ± 3.433 (SD)

The body mass index (BMI) in the infertile group range from 19.3 Kg/m$^2$ to 38.5 Kg/m$^2$ and the mean BMI was 27.19. The mean BMI in the control was 25.84 Kg/m$^2$ (range 18.9 to 39.3). Table X shows that the infertile women were more likely than the controls to be obese (BMI of 30 Kg/m$^2$ and above) although the difference was not statistically significant (p value > 0.05). More than 50% of the respondents in both groups had body mass index between 20 and 30 Kg/m$^2$. 
Table X: Body mass index of both groups

<table>
<thead>
<tr>
<th>Body mass index (BMI)</th>
<th>Study group n = 152</th>
<th>Control group n = 133</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency (n)</td>
<td>Percentage (%)</td>
<td>Frequency (n)</td>
</tr>
<tr>
<td>≤ 19.9</td>
<td>2</td>
<td>1.3</td>
<td>4</td>
</tr>
<tr>
<td>20 - 24.9</td>
<td>46</td>
<td>30.3</td>
<td>54</td>
</tr>
<tr>
<td>25 - 29.9</td>
<td>70</td>
<td>46.1</td>
<td>56</td>
</tr>
<tr>
<td>30 – 34.9</td>
<td>28</td>
<td>18.4</td>
<td>13</td>
</tr>
<tr>
<td>≥ 35</td>
<td>6</td>
<td>3.9</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>152</td>
<td>100</td>
<td>133</td>
</tr>
</tbody>
</table>

Hyperprolactinemia was found in 57 (37.5%) infertile women compared to only 6 (4.5%) in the controls giving a very high prevalence of Hyperprolactinemia among the infertile women, as shown in table XI below:

Table XI: Prevalence of Hyperprolactinemia in both groups

<table>
<thead>
<tr>
<th>Prolactin levels (ng/ml)</th>
<th>Study group n = 152</th>
<th>Control group n = 133</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency (n)</td>
<td>Percentage (%)</td>
</tr>
<tr>
<td>&lt; 20.15</td>
<td>95</td>
<td>62.5</td>
</tr>
<tr>
<td>≥20.15</td>
<td>57</td>
<td>37.5</td>
</tr>
<tr>
<td>Total</td>
<td>152</td>
<td>100</td>
</tr>
</tbody>
</table>

p value = 0.0001
The serum prolactin level ranged from 1.1ng/ml to 325ng/ml among the infertile women, and the median prolactin level was 15.75ng/ml. That of the control ranged from 0.5 to 47.9 ng/ml, giving a mean prolactin level of 7.24ng/ml ±7.77 (SD) and a median of 4.4ng/ml.

Out of the 34 women with primary infertility, 15 (44.1%) of them had Hyperprolactinemia whereas 42 (35.6%) of women with secondary infertility had Hyperprolactinemia, (p = 0.384). See table XII

Table XII: The prevalence of Hyperprolactinemia in primary and secondary infertility

<table>
<thead>
<tr>
<th>Prolactin levels</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of Infertility</strong></td>
<td><strong>Normal level</strong></td>
<td><strong>Excess prolactin level</strong></td>
</tr>
<tr>
<td>Primary</td>
<td>19 (55.9%)</td>
<td>15 (44.1%)</td>
</tr>
<tr>
<td>Secondary</td>
<td>76 (64.4%)</td>
<td>42 (35.6%)</td>
</tr>
</tbody>
</table>

p value = 0.384

In assessing the degree of Hyperprolactinemia based on the serum levels21 39 out of the 57 hyperprolactinemic women had mild prolactin excess, that is, serum level of 20.15 – 50ng/ml,
fifteen of them had moderate prolactin excess and 3 had marked prolactin elevation, that is, 100 ng/ml and above.

The table below shows the comparison between the prolactin levels in primary and secondary infertility.

**Table XIII: Serum prolactin levels n=152**

<table>
<thead>
<tr>
<th>Serum prolactin level (ng/ml)</th>
<th>Primary infertility n=34</th>
<th>Percentage (%)</th>
<th>Secondary infertility n=118</th>
<th>Percentage</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-20.15 (normal)</td>
<td>19</td>
<td>55.9</td>
<td>76</td>
<td>64.4</td>
<td>0.0365</td>
</tr>
<tr>
<td>20.2-50 (mild)</td>
<td>9</td>
<td>26.5</td>
<td>30</td>
<td>25.4</td>
<td>0.9017</td>
</tr>
<tr>
<td>50.1-99.9 (moderate)</td>
<td>5</td>
<td>14.7</td>
<td>10</td>
<td>8.5</td>
<td>0.02823</td>
</tr>
<tr>
<td>100 and above (marked)</td>
<td>1</td>
<td>2.9</td>
<td>2</td>
<td>1.7</td>
<td>0.644</td>
</tr>
</tbody>
</table>
Piechart showing the percentage of the different degree of prolactin excess

Bar chart comparing the prolactin excess in primary and secondary infertility groups
Thirty six (77.2%) out of the 57 infertile women with Hyperprolactinemia had Body Mass Index within normal range (20.0 to 29.9 Kg/m²) and 3 out of the 6 hyperprolactinemic women in the control had normal BMI. The remaining 3 in this group were obese (BMI > 30Kg/m²) as shown Table XIV

Table XIV: BMI and Hyperprolactinemia among the respondents

<table>
<thead>
<tr>
<th>BMI</th>
<th>Control group</th>
<th>Study group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤19.9</td>
<td>-</td>
<td>1 (1.8%)</td>
<td>-</td>
</tr>
<tr>
<td>20-24.9</td>
<td>2 (33.3%)</td>
<td>19 (33.3%)</td>
<td>-</td>
</tr>
<tr>
<td>25-29.9</td>
<td>1 (16.7%)</td>
<td>25 (43.9%)</td>
<td>0.1981</td>
</tr>
<tr>
<td>30-34.9</td>
<td>2 (33.3%)</td>
<td>11 (19.3%)</td>
<td>0.4202</td>
</tr>
<tr>
<td>&gt;35</td>
<td>1 (16.7%)</td>
<td>1 (1.8%)</td>
<td>0.0492</td>
</tr>
</tbody>
</table>
Compared to only 3(2.3%) in the control, galactorrhoea was found in 45(29.6%) of the infertile women. The incidence of galactorrhoea in hyperprolactinemic patients was 57.8%

**Table XV: Incidence of galactorrhoea**

<table>
<thead>
<tr>
<th>Galactorrhoea</th>
<th>Serum Prolactin Levels</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Hyper</td>
</tr>
<tr>
<td>Yes</td>
<td>19 (42.2%)</td>
<td>26 (57.8%)</td>
</tr>
<tr>
<td>No</td>
<td>76</td>
<td>31</td>
</tr>
</tbody>
</table>

As shown below (Table XVI), secondary infertile women with galactorrhoea were more likely than the primary infertile women to have Hyperprolactinemia, although the difference was not statistically significant (p>0.05).

**Table XVI: Comparison of Hyperprolactinemia and galactorrhoea in secondary and primary infertility groups**

<table>
<thead>
<tr>
<th></th>
<th>Primary n=34</th>
<th>Secondary n=118</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperprolactinemia</td>
<td>15 (44.1%)</td>
<td>42 (35.6%)</td>
<td>0.3653</td>
</tr>
<tr>
<td>Galactorrhoea</td>
<td>8 (23.5%)</td>
<td>37 (31.4%)</td>
<td>0.3782</td>
</tr>
</tbody>
</table>
Hyperprolactinemia was more likely to be associated with menstrual irregularities and infertile women were more likely than the women in the control to have menstrual irregularities. Oligomenorrhea was the commonest menstrual disorder among the hyperprolactinemic group. Table XVII shows that only 36.8% of the Hyperprolactinemic patients had regular menstruation.

**Table XVII: Hyperprolactinemia and Menstrual Disorders**

<table>
<thead>
<tr>
<th>Menstrual disorder</th>
<th>Prolactin group (Study group)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal level</td>
<td>Hyperprolactinemia</td>
</tr>
<tr>
<td>Regular</td>
<td>50 (52.6%)</td>
<td>21 (36.8%)</td>
</tr>
<tr>
<td>Oligomenorrhoea</td>
<td>17 (17.9%)</td>
<td>20 (35.1%)</td>
</tr>
<tr>
<td>Amenorrhoea</td>
<td>21 (22.1%)</td>
<td>11 (19.3%)</td>
</tr>
<tr>
<td>Others</td>
<td>7 (7.4%)</td>
<td>5 (8.8%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>95</strong></td>
<td><strong>57</strong></td>
</tr>
</tbody>
</table>

The incidence of menstrual disorders was less in the fertile group (control) when compared to the women in the infertile group. The table below shows that 86.5% of the fertile women had normal menstrual pattern compared to 46.7% in the infertile subjects.
Table XVIII: Incidence of menstrual disorder in both groups

<table>
<thead>
<tr>
<th>Menstrual disorder</th>
<th>Control Group (n = 152)</th>
<th>Study group (n = 133)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>Percentage</td>
<td>Frequency</td>
</tr>
<tr>
<td>Regular menses</td>
<td>118</td>
<td>86.5%</td>
<td>71</td>
</tr>
<tr>
<td>Oligomenorrhoea</td>
<td>11</td>
<td>8.3%</td>
<td>37</td>
</tr>
<tr>
<td>Amenorrhoea</td>
<td>3</td>
<td>2.3%</td>
<td>32</td>
</tr>
<tr>
<td>Menorrhagia</td>
<td>4</td>
<td>3%</td>
<td>12</td>
</tr>
</tbody>
</table>

The possible influence of the various social classes of the respondents and prolactin excess were assessed. Table XIX below shows that the incidence of Hyperprolactinemia was more among women in social class one in the controls while the incidence was highest in the infertile women in social class 2. Infertile women in social class 2 and 3 were more likely than the controls to be hyperprolactinemic and the difference was statistically significant: p=0.0001.
Table XIX: Incidence of Hyperprolactinemia in various social classes of the respondents

<table>
<thead>
<tr>
<th>Social class</th>
<th>Study n=152</th>
<th></th>
<th>Control n=133</th>
<th></th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Freq.</td>
<td>%</td>
<td>PRL n=57</td>
<td>Freq.</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>9.9</td>
<td>4 (26.7%)</td>
<td>16</td>
<td>12.0</td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>32.2</td>
<td>24 (48.9%)</td>
<td>54</td>
<td>40.6</td>
</tr>
<tr>
<td>3</td>
<td>59</td>
<td>38.8</td>
<td>23 (38.9%)</td>
<td>44</td>
<td>33.1</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>15.1</td>
<td>6 (26.1%)</td>
<td>15</td>
<td>11.3</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>5.0</td>
<td>0</td>
<td>4</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Only two women with Hyperprolactinemia in this study had TSH level above normal (0.5 – 4.1miu/L) giving a very poor correlation with Hyperprolactinemia of 1:29. Higher values of normal levels were observed among oligomenorrheic women. The mean TSH level in this group was 2.54±2.45 (SD) compared to the mean serum level of 1.26±0.78 in women with normal menstrual pattern (p =0.3618).
DISCUSSION

Hyperprolactinemia is a common problem encountered in reproductive disorders.\textsuperscript{14,59,60} The understanding that prolactin hypersecretion not only causes galactorrhoea and amenorrhoea but also gonadal dysfunction and infertility led to the wider use of prolactin estimations. The prevalence of Hyperprolactinemia among infertile women in this study was 37.5 percent. This is slightly higher than that reported in other parts of Nigeria.\textsuperscript{1} In a retrospective study of 104 infertile women in a northern Nigerian tertiary hospital, Idrisa et al\textsuperscript{1} reported a prevalence of 31.7%. Among the fertile women recruited in this study as controls, the prevalence was 4.5%. This was similar to prevalence of 5% among family planning clients in a Japanese study\textsuperscript{21} but much lower than 15% prevalence in an unselected population in Asia reported by Goswami et al\textsuperscript{61}. This high prevalence in the infertile women emphasizes the possible impact of Hyperprolactinemia on the reproductive profile of the women in the study group. In a study evaluating 111 infertile women in India, Kumkum A.1et al\textsuperscript{14} reported a higher prevalence of 46%. A much higher prevalence
of 61% was reported by Estekhari N. et al,\textsuperscript{62} among 100 infertile women with abnormal uterine bleeding.

In this study, there were 34 (22.4%) women with primary infertility and 118 (77.6%) women with secondary infertility giving the ratio of primary to secondary infertility of about 1:4 compared to the ratio of 1:3 (31.7% primary and 68.3% secondary) documented by Idrisa et al\textsuperscript{1} in Northern Nigeria. This is in contrast to what is obtained in some studies outside sub-Saharan Africa. For instance, in a study assessing the prevalence of Hyperprolactinemia among infertile women in India by Kumkum A. et al\textsuperscript{14}, 60% of the women had primary infertility and 40% had secondary infertility. This significant difference may not be unrelated to the etiological factors that are peculiar to sub-Saharan African countries like infection from unsafe abortion which may be a major contributing factor to infertility.

In this study, the serum concentration of prolactin among the infertile group ranged from 1.1 to 325ng/ml and the median concentration was 15.75 whereas Mishera et al\textsuperscript{63} and Kumkum et al\textsuperscript{14} reported mean prolactin concentration of 128.28±12.74ng/ml
(SD) and 76.33±55.97 (SD) respectively among infertile women. This wide variation may be due to the sensitivity of the immunoassay kits used in different laboratories and also the differences in the demographic characteristics of the subjects in these studies.

The cut off for normal (20.15ng/ml) used in this study was based on the prolactin kit used at the University of Calabar Teaching Hospital Chemical Pathology Laboratory where the analysis was done. Some studies especially outside the country use 25.0ng/ml as the upper limit of normal\textsuperscript{14}.

Generally, prolactin levels in females show a moderate increase at puberty and remain at this level during the reproductive years.\textsuperscript{1} Hormonal studies of each menstrual cycle reveal a mid-cycle, oestrogen related modest prolactin peak. This transient increase may not have any significant impact on reproduction\textsuperscript{1}. Estimation in these women was done at various points of the cycle and the impact of this on the assays was not assessed.
In this study, nulliparous women were more likely than the parous women to have Hyperprolactinemia and the result was statistically significant: 46.9% vs 11% (p value < 0.05). Also those with primary infertility (44.1%) were more likely to be hyperprolactinemic compared to those with secondary infertility, though the difference was not statistically significant (p = 0.384). Similar findings were obtained by Idrisa et al in Northern Nigeria. Galactorrhoea was present in 45 (29.6%) of the total women in the infertile group compared to 3 (2.3%) in the controls. Secondary infertile women were more likely than the primary infertile subjects to have galactorrhoea (31.4% vs 23.8%) although the difference was not statistically significant (p > 0.05). The incidence of Hyperprolactinemia in women with galactorrhoea was 57.8%. This was less than the incidence of 90% reported by Kumkum et al. Thus the practice of routinely prescribing dopaminergic agonists like Bromocriptine for women with symptomatic galactorrhoea in Calabar without measuring the serum levels of prolactin is not encouraged as 4 out of 10 of such women may not be hyperprolactinemic. It has been stressed that
the absence of galactorrhoea does not exclude Hyperprolactinemia and galactorrhoea can occur with normal serum prolactin levels.

Menstrual disturbances were found in 82 (54%) of the women in the infertile group compared to 18 (13.5%) in the fertile group. Oligomenorrhea/hypomenorrhea was the most common menstrual disorder encountered in the women with infertility and it accounted for 37 (45.1%) of women with menstrual disorders in this study. This was followed by amenorrhea 32 (39.1%) and the remaining disorders like menorrhagia accounted for 14.6%.

In a recent publication by Adele B. et al\textsuperscript{64}, menstrual disorder was detected in 45.2% of the subjects and 23.5% in Hyperprolactinemic patients and 21.8% in normal prolactin groups. Raber et al\textsuperscript{63} reported a lower incidence of menstrual disorder : 26% in the Hyperprolactinemic patients.

The mechanisms by which Hyperprolactinemia leads to menstrual abnormalities, galactorrhoea and anovulation had been described by various authors. It is also pertinent to note that interpretation of impacts of Hyperprolactinemia may not be as straightforward
as it is with other hormones. Interpersonal variations can occur in secretion pattern of prolactin among patients and also the occurrence of different molecular forms, which have been found to exert characteristic physiological effects. This may be the reason for poor correlation between the degree of prolactin excess and the occurrence of symptoms like galactorrhoea and menstrual disorders.

In this study, 15 out of the 152 women in the study group had other medical disorders. Hypertension was the most common and was found in 5.9% of the people. Three of the women (2.0%) had received treatment for pulmonary tuberculosis and 3 women came with ultrasound diagnosis of uterine fibroids. The small numbers of these other systemic medical conditions made it statistically difficult to assess any positive correlation between these medical problems and Hyperprolactinemia.

Contrary to the views by Klufio C. A.\textsuperscript{24} that all cases of Hyperprolactinemia should be tested for hypothyroidism, in this study, hypothyroidism (raised TSH) was very rare. Out of the 57 subjects with Hyperprolactinemia in this study, only 2 women had
serum TSH above normal level giving a ratio of hypothyroidism to Hyperprolactinemia of 1:29. Far less than a positive correlation of 1:4 reported by Kumkum A. et al.\textsuperscript{14} In a cross sectional study by Goswami et al\textsuperscript{61} the crude prevalence of hypothyroidism was slightly higher in the infertile women in comparison with the general population and there was a positive correlation between serum TSH and prolactin levels in the infertile subjects. It was concluded that there was a greater propensity for thyroid disorders in infertile women than the fertile ones. Shalver et al\textsuperscript{63} studied the routine thyroid function tests in infertile women and reported that the low incidence of hypothyroidism in pregnant patients was related to the association between infertility and hypothyroidism. This was also supported by Raber et al\textsuperscript{65}.

Although all the hyperprolactinemic women in the infertile group except two had within normal levels of TSH the levels were relatively higher in those with menstrual disorders compared to those with normal menstrual pattern. The mean TSH in women with Hyperprolactinemia with menstrual disorders was 1.64miu/L+_1.56(SD) which was higher than that in the women
with normal menstrual pattern (1.26±0.78). There is a paucity of information in the literature in our country on this subject. Sharma et al. reported that 20% of primary infertile women with menstrual disorders in India showed abnormal values of thyroid hormones. The wide variations in reported correlation of hypothyroidism with Hyperprolactinemia may be due to differences in environmental factors like diets.

CONCLUSION/RECOMMENDATIONS

(1) The prevalence of Hyperprolactinemia is high among infertile women in Calabar and prolactin assay should be included as a first line hormonal screening in our infertility clinics. It high prevalence among nulliparous infertile subjects in this study showed that anxiety may be a contributing factor.

(2) The routine use of dopaminergic agonist by women with galactorrhoea without measuring the serum levels of prolactin should be discouraged as many as 4 out of 10 of
such women in Calabar may have normal plasma prolactin levels except if there is menstrual disorders.

(3) The prevalence of hypothyroidism in infertile women with Hyperprolactinemia is very rare (1:29) in Calabar. Routine thyroid hormone estimation should no more be included as a first line hormonal work up in our infertility clinics as it may not be cost effective in evaluating these women except patients present with menstrual disorder or symptoms of hypothyroidism or has a history of residing in an endemic area.
REFERENCES


APPENDIX 1

STATEMENT OF CONFIDENTIALITY

PREVALENCE OF HYPERPROLACTINEMIA AMONG WOMEN ATTENDING INFERTILITY CLINICS IN CALABAR

All information obtained from the patients and the results of this research study shall be treated as strictly confidential. No part of this information shall be divulged to anyone except with the patient’s approval.

The language of communication shall be English but where the patient does not understand English Language, she will be communicated to in her local dialect if need be; through the help of an interpreter.

Every patient shall be treated with fairness, equity and a sense of human dignity irrespective of the patient’s age, socio-economic circumstances, ethnic or religious lineage.

Informed consent shall be obtained from all subjects.

......................................................
Dr. Ubong Bassey Akpan
APPENDIX 2

CONSENT TO PARTICIPATE IN A RESEARCH

This is an informed consent to participate in a research which objective is to measure the serum level of prolactin in women with complaints of inability to conceive. This test is one of the first line investigations to determine the cause of anovulation. Participants are not to pay for the test but would benefit from the result which would be sent to their folders. And the benefit to the community is that recommendation would be made on possible cost effective modalities of evaluation and treatment of patients with anovulatory infertility.

You are at liberty to accept or refuse to participate. Refusal to participate will not affect the attention and treatment you will receive from me or your doctor.

Full confidentiality is assured.
5 milliliter of blood would be drawn from each participant and sent to laboratory for analysis.

I,........................................of................................................hereby consent to take part in this research study after explanation by:............................................................
Dated this:...........................day of........................................

Signed:.......................Signature of Researcher:.....................
(Participant) (Researcher)
Full Name:..........................Full Name:.............................
Address:.............................Address:.............................
Date:.................................Date:.................................
APPENDIX 3

QUESTIONNAIRE

PREVALENCE OF HYPERPROLACTINEMIA AMONG WOMEN ATTENDING INFERTILITY CLINICS IN CALABAR

DEMOGRAPHIC DATA

Age (a) 20 and below   (b) 21-25 (c) 26-30 (d) 31 - 35   (e) 36 and above

Occupation/qualification

Occupation of spouse

Parity  (a) Nulliparity  (b) Para1  (c) Para2  (d) Para3
        (e) Para4 and above

Marital Status: (a) Married   (b) Single

Duration of Marriage/relationship:

BACKGROUND INFORMATION

Previous pregnancy  (a) Within 1 year  (b) Within 2 years
            (c) 3 years or more

Previous abortion or miscarriage Or ectopic pregnancy  (a) None
            (b) 1  (c) 2  (d) 3 or more
Current usage of drugs known to affect prolactin secretion example metoclopramide, methyldopa, reserpine, largactil, opiates, cimetidine (Yes/No); specify :.......................................................... 

Menstrual Disturbance: 

(a) Regular menses (b) Oligomenorrhoea (menstrual cycle 35 days or above (c) Amenorrhoea (d) Menorrhagia (e) specify.................................. 

Duration of menstrual disturbance in months:.......... 

Duration of Infertility in years:............................................ 

Any other medical condition? (a) Thyroid disease (b) Epilepsy (c) Hypertension (d) Diabetes mellitus. Specify................................................................. 

Sexual Intercourse in the preceding one week? 

(a) None (b) Once (c) Twice (d) Three or more. Specify........................................... 

Contraception (Yes/No).......Specify type............................. 

Duration used................................................................. 

Galactorrhoea (Yes/No). Duration in months, if yes......... 

............................................................................ 

Previous use of dopaminergic drugs e.g. bromocriptine, cabergoline(Yes/No)..............................Duration of use if yes..............................................................