The Chemical Pathology of Leiomyoma

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DECLARATION

The experimental work described in this thesis was carried out at the Department of Molecular Medicine, School of Medical Sciences, K.N.U.S.T. This work has not been submitted for any other degree.

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ABSTRACT

Uterine leiomyomas or fibroids are a major public health problem among women, occurring more frequently in women of reproductive age and are associated with diverse symptoms. In spite of the frequency with which fibroids occur, their biology is poorly understood. Studies indicate that oestrogen and progesterone play a role in the regulation of tumour growth, and there is increasing evidence that this response may be mediated via a number of growth factors. The literature regarding predisposing risk factors for development of myomas in Ghanaian women is very limited by the paucity of studies available. This is against the background that Ghana is thought to have a high prevalence of fibroids due to its indigenous black population, since uterine fibroids are more prevalent in black women. Almost all the studies done so far have been on women in the U.S.A or some other developed country where the environmental factors that are thought to influence the development of fibroids are very different from what pertains in the under developed world, particularly, Africa. Against this background, there is the need to investigate the risk factors that are believed to influence the development of fibroids in Ghanaian women. Therefore, the chemical pathology of fibroids was studied to bridge the gap in information on fibroids between the developed and the under developed world. The specific objectives of this study were to: 1) establish the demographic characteristics of women with fibroids in Ghana. 2) assess the association between fibroid development and growth, and the gynaecologic history of patients. 3) assess the relationship between the life styles of patients and the risk of developing fibroids among Ghanaian women. 4) determine the haematological profile of Ghanaian women with fibroids in relation to the tumour growth and development. 5) assess the impact of fibroids on the renal and liver functions of Ghanaian women with fibroids. 6) assess the association of oxidative stress with the development and growth of fibroids among Ghanaian women.

A consecutive study of 200 women with fibroids between the ages of 20 to 40 was done in which questionnaires were designed to elicit information on their socioeconomic background and gynaecologic history. Anthropometric features were also taken and their blood samples analyzed for oxidative stress markers, biochemical, and haematological profiles. Women with obvious hormonal imbalances and chronic or malignant diseases were excluded. Control subjects recruited had similar age distribution as the patients and had been examined to exclude fibroids.

Significant difference exist between the patients and the controls in terms of socio-economic characteristics most especially education and income earnings. Abortion, nulliparity, early onset of menarche and history of sexually transmitted diseases were observed to be strongly associated positively with the risk of fibroid development. BMI and waist-to-hip ratios of the patients were significantly higher than those of the controls. The results of the liver and renal function tests of patients were not significantly different from those of the controls and generally showed that both patients and controls had normal liver and renal functions. The patients had higher serum malondialdehyde and lower vitamin C levels compared to the controls. It was observed that most red blood cell indices were higher in the patients compared to the controls. However all other haematological parameters of the patients were not significantly different from those of the controls.

Though generally, this study's findings were similar to what has been observed by previous studies, the observation that higher incidence of abortions, PIDs, and STIs in patients may interplay through mediating hormonal and probably growth factors leading to the development of fibroids is quite novel.

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ABBREVIATIONS

ΔAbs – change in absorbance

μL – micro litre

16 α-OHE1 – 16 α-hydroxyesterone

2 -OHE1 – 2-hydroxyesterone

3, 5 –DHBS – 3, 5-dichloro-2-hydroxybenzene

4 –AAP – 4- aminoantipyrine

AB – age at first birth

AFA – age at first abortion

AIDS – acquired immunodeficiency syndrome

ALB – albumin

ALP – alkaline phosphatase

ALT – alanine aminotransaminase

AP – age at first pregnancy

AST – aspartate aminotransaminase

ATP – adenosine triphosphate

BCG – bromocresol green

bFGF – basic fibroblast growth factor

BMI – body mass index

BUN – blood urea nitrogen

CI – confidence interval

Conc – concentration

CR – coronary risk

Crt – creatinine

DAP – dihydroxyacetone phosphate

DB – direct bilirubin

DMPA – depot medroxyprogesterone acetate

DNA – deoxyribonucleic acid

EDTA – ethylenediaminetetraacetic acid

EGF – epidermal growth factor

ER – estrogen

Fe – Iron

G3P – glycerol-3-phosphate

G6PD – glucose-6-phosphate dehydrogenase

GGT – gamma glutamyl transferase

GLO – globulin

GnRH – gonadotropin releasing hormone

GPO – glycerophosphate oxidase

Hb – hemoglobin

HC – hip circumference

HCT – haematocrit

HDL – high density lipoprotein

HRP – horseradish peroxidase

IB – indirect bilirubin

IGF – insulin-like growth factor

IUD – intrauterine device

JNK – c-Jun N-terminal kinases

K^+ - potassium ion

KATH – Komfo Anokye Teaching Hospital

Kg – kilogram

L – liter

LDH – lactate dehydrogenase

LDL – low density lipoprotein

LMC – length of menstrual cycle

MCH – mean cell hemoglobin

MCHC – mean cell hemoglobin concentration

MCV – mean cell volume

MDA – malondialdehyde

MDH – malate dehydrogenase

ml – milliliter

mmol – millimole

MPA – medroxyprogesterone acetate

mRNA – messenger ribonucleic acid

Na^+ - sodium ion

NAC – N-acetyl cysteine

NAD – nicotinamide adenine dinucleotide

NADH – reduced NAD

NPA – number of previous abortions

OC(s) – oral contraceptive(s)

OCP – oral contraceptive pill

OR – odds ratio

PCV – packed cell volume

PDGF – platelet derived growth factor

Pg – picogram

PID – pelvic inflammatory disease

PLT – platelet

PR – progesterone

RBC – red blood cell

RNA – ribonucleic acid

ROS – reactive oxygen species

SEM – standard error of mean

SGPT – serum glutamic pyruvic transaminase

SIA – sandwich enzyme immunoassay

SMS – School of Medical Science

Std – standard

STIs – sexually transmitted infections

TB – total bilirubin

TBA – thiobarbituric acid

TC – total cholesterol

TCA – trichloroacetic acid

TG – triglyceride

TGF-β – transforming growth factor-β

TI – total iron

TIBC – total iron binding capacity

TMB – tetramethylbenzidine

TP – total protein

TW – tumour weight

U/L –unit per litre

UIBC – unsaturated iron binding capacity

VEGF – vascular endothelial growth factor

VLDL – very low density lipoprotein

VVF – vesico-vaginal fistula

WBC – white blood cell

WC – waist circumference

WHR – waist-to-hip ratio

Chapter 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

1.1.1 Definition

A leiomyoma (plural is 'leiomyomata') is a benign smooth muscle neoplasm that is not premalignant. They can occur in any organ, but the most common forms occur in the uterus, small bowel, and the oesophagus. Leiomyoma is derived from three Greek words: leios - meaning, smooth; muV (pronounced as 'myo') – meaning muscle; and oma – meaning tumour. Uterine fibroids (fibroid is derived from fibra which is Latin for fiber) are leiomyomata of the uterine smooth muscles. Just as other leiomyomata, they are benign. Uterine leiomyomata originate from the myometrium and are classified according to their location. They are monoclonal tumours derived from a single myometrial cell (Townsend *et al.*, 1970). The uterus, small bowel and the esophagus are composed of large amounts of extracellular matrix containing collagen, fibronectin, and proteoglycan. Collagen type I and type III are abundant, but the collagen fibrils are formed abnormally and are in disarray, much like the collagen found in keloid formation (Buttram and Reiter, 1981; Stewart *et al.*, 1994; Walker and Stewart, 2005).

1.2 LITERATURE REVIEW

1.2.1 Incidence of leiomyomata

Myomas are remarkably common. Fine serial sectioning of uteri from 100 consecutive women who underwent hysterectomy found myomas in 77%, including some as small as 2mm (Cramer and Patel, 1990). Myomas were found no less frequently in women who had a hysterectomy for other indications than for uterine myomas, although they were smaller and less numerous. Because most imaging techniques lack resolution <1 cm, they underestimate the true incidence of

this condition, although small myomas may be of no clinical significance. The hysterectomy specimens from premenopausal women with myomas have had an average of 7.6 myomas; postmenopausal women have had on average 4.2 myomas (Cramer and Patel, 1990).

A random sampling of women aged 35 to 49 who were screened by self-report, medical record review, and sonography found that by age 35 the incidence of myomas was 60% among African-American women; the incidence increased to over 80% by age 50. Caucasian women had an incidence of 40% by age 35, and almost 70% by age 50 (Day Baird *et al.*, 2003). Myomas are an enormous healthcare concern; they were the primary indication for surgery in 199,000 hysterectomies and 30,000 myomectomies performed in the United States in 1997 (Farquhar and Steiner, 2002). Inpatient surgery for myomas cost $2.1 billion in the United States in 1997, and the cost of outpatient surgeries, medical and nonmedical costs, and time away from work or family adds significantly to these expenditures (Myers *et al.*, 2002). Knowledge of the prevalence of uterine fibroids in other countries could provide clues to the importance of diet, environmental factors, and ethnicity, but unfortunately, few such studies exist in the literature. One study in Japan (Sato *et al.*, 2000a) reported that "uterine leiomyomas are the most common pelvic tumours" but provided no data of the actual prevalence among their patients. Others (Ezem and Otubu, 1981) have cited a 68% incidence of uterine fibromyomata among their hysterectomy cases in Nigeria. A study from Malaysia (Ravindran and Kumaraguruparan, 1998) listed fibroids as the main indication for hysterectomy in their series (47.6% of cases). Similarly, other investigators have implicated fibroid uterus as the main indication for hysterectomy in northern France (66.7% of cases) (Debodinance, 2001). Although no firm statistical

conclusions can be drawn, these reports suggest that uterine fibroids occur commonly in women in many parts of the world.

1.2.2 Aetiology of leiomyomas

Although the precise causes of myomas are unknown, advances have been made in the understanding of the hormonal factors, genetic factors, growth factors, and molecular biology of these benign tumours (Flake *et al.*, 2003). Factors possibly responsible for the initiation of acquired genetic changes found in myomas include intrinsic abnormalities of the myometrium, congenitally elevated oestrogen receptors in the myometrium, hormonal changes, or a response to ischaemic injury at the time of menses. Once established, these genetic changes are influenced by promoters (hormones) and effectors (growth factors).

1.2.2.1 Myoma Genetics

Myomas are monoclonal, and about 40% are chromosomally abnormal (Hashimoto *et al.*, 1995). Commonly found abnormalities include translocations between chromosomes 12 and 14, deletions of chromosome 7, and trisomy of chromosome 12 (Ligon and Morton, 2000). Cellular, atypical, and large myomas are most likely to show chromosomal abnormalities. The remaining 60% may have undetected mutations. More than 100 genes have been found to be up-regulated or down-regulated in myoma cells, including the sex-steroid associated genes oestrogen receptor a, oestrogen receptor b, progesterone receptor A, progesterone receptor B, growth hormone receptor, prolactin receptor, and extracellular matrix genes, and collagen genes (Lee *et al.*, 2005). Many of these genes appear to regulate cell growth, differentiation, proliferation, and mitogenesis.

1.2.2.2 Uterine Sarcoma Genetics

Genetic differences between myomas and leiomyosarcomas indicate they most likely have distinct origins, and leiomyosarcomas do not result from malignant degeneration of myomas (Walker and Stewart, 2005). Although myomas are proliferative tumours, they remain differentiated and have chromosomal rearrangements similar to other benign lesions. In contrast, leiomyosarcomas are undifferentiated and have complex chromosomal rearrangements and aneuploid karyotypes. Cluster analysis of 146 genes in leiomyosarcomas has shown that the majority is down-regulated, but in myomas or myometrium they are not. Comparative genomic hybridization has not found specific anomalies shared by myomas and leiomyosarcomas (Quade *et al.*, 2004).

1.2.2.3 Hormones

Both oestrogen and progesterone appear to promote the development of myomas. Myomas are rarely observed before puberty, are most prevalent during the reproductive years, and regress after menopause. Factors that increase overall life time exposure to oestrogen, such as obesity and early menarche, increase the incidence. Decreased exposure to oestrogen found with exercise and increased parity is protective (Cook and Walker, 2004). Although blood levels of oestrogen and progesterone are similar in women with and without clinically detectable myomas, levels of oestradiol within myomas are higher than in normal myometrium. De novo production of oestrogen within myoma tissue is suggested by increased levels of aromatase, an enzyme that converts androgens to oestrogen. Low levels of enzymes that convert oestradiol to estrone have been found in myoma cells and may promote accumulation of oestradiol within the cells, leading to up-regulation of oestrogen and progesterone receptors, hyper-responsiveness to oestrogen, and myoma growth. Consistent with this idea, myomas show a higher

proliferative index than normal myometrium throughout the menstrual cycle (Cook and Walker, 2004). Biochemical, clinical, and pharmacologic evidence confirm that progesterone is important in the pathogenesis of myomas. Myomas have increased concentrations of progesterone receptors A and B compared with normal myometrium (Englund *et al.*, 1998; Nisolle *et al.*, 1999). The highest mitotic counts are found during the secretory phase at the peak of progesterone production, and mitotic counts are higher in women treated with medroxyprogesterone acetate (MPA) than in untreated controls (Tiltman, 1985; Kawaguchi *et al.*, 1991). Gonadotropin-releasing hormone (GnRH) agonists decrease the size of myomas, but progestin given concurrently with GnRH prevents a decrease in size. One study found that use of progestin-only injectable contraceptives was inversely associated with risk of having myomas (Wise *et al.*, 2004a). Mifepristone, a progesterone-receptor modulator, decreases myoma size (Murphy *et al.*, 1995).

1.2.2.4 Growth Factors

Growth factors, proteins or polypeptides produced locally by smooth muscle cells and fibroblasts control the proliferation of cells and appear to stimulate myoma growth, primarily by increasing extracellular matrix. Some of the identified myoma-related growth factors are transforming growth factor b (TGF-b), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), and prolactin (Flake *et al.*, 2003). Growth factors affect cells in complex ways, and the response to combinations of growth factors may be different from the response to an individual factor. Many of these growth factors are over expressed in myomas and either increase smooth muscle proliferation (TGFb, bFGF), increase DNA synthesis (EGF, PDGF), stimulate synthesis of

extracellular matrix (TGF-b), promote mitogenesis (TGF-b, EGF, IGF, prolactin), or promote angiogenesis (bFGF, VEGF) (Flake *et al.*, 2003). It is likely that other myoma-related growth factors will be discovered, and it remains to be seen which factors will be important.

1.2.2.5 Risk Factors

The literature regarding predisposing risk factors for development of myomas should be interpreted with caution. Analysis is limited by the paucity of studies available, the study populations (mostly in Caucasian women), and the conflicting results, suggests other unexamined factors may be involved. The high background prevalence of myomas, and possible detection bias as a consequence of increased medical surveillance of symptomatic women, may make interpretation of epidemiologic data difficult. The reliability of self-reported diagnoses may be questioned; the development of myomas may have preceded the exposure to risk factors but may not have been recognized until after presentation to a healthcare provider. Prospective, longitudinal studies are underway to better characterize the factors that influence the development of uterine myomas.

1.2.2.5.1

Age

An increase with age in the prevalence of fibroids during the reproductive years has been demonstrated by several epidemiologic studies (Ross *et al.*, 1986; Velebil *et al.*, 1995; Marshall *et al.*, 1997). Studies that define cases by pathologic diagnosis, thus restricting cases to those having surgery (Ross *et al.*, 1986), have shown a rapid increase in fibroid diagnoses among women in their forties. Whether the risk of new fibroids actually increases rapidly in women during their forties is not known. The observed increase could also result from increased growth of, or increased symptomatology from, already existing fibroids, as well as from a greater

willingness of women in the later reproductive years to have gynaecologic surgery. If the likelihood of fibroid development and growth actually accelerates during the late reproductive years, hormonal factors associated with perimenopause may be important modulators; alternatively, the apparent increase in the late reproductive years may simply represent the cumulative culmination of 20-30 years of stimulation by oestrogen and progesterone.

1.2.2.5.2

Endogenous hormonal factors

Early menarche (<10 years old) has been found to increase (relative risk [RR] 1.24) and late menarche (>16 years) to decrease (RR 0.68) the risk of uterine myomas (Marshall *et al.*, 1998a). Myomas are smaller and less numerous in hysterectomy specimens from postmenopausal women when endogenous oestrogen levels are low; myoma cell size is significantly smaller in postmenopausal women (Cramer and Patel, 1990; Cramer *et al.*, 2000). Family history First-degree relatives of women with myomas have a 2.5 times increased risk of developing myomas (Vikhlyaeva *et al.*, 1995; Schwartz *et al.*, 2000). Women reporting myomas in two first-degree relatives are more than twice as likely to have strong expression of VEGF-a (a myoma-related growth factor) than women who have myomas but no family history (Okolo *et al.*, 2005). Monozygous twins are reported to be hospitalized for treatment of myomas more often than dizygous twins, but these findings may be the result of reporting bias (Treloar *et al.*, 1992).

1.2.2.5.3

Oral Contraceptives

Reports in the literature present inconsistencies with regard to the effect of oral contraceptive (OC) use upon the growth of myomas. An earlier report suggested that OCs may play a role in the development or growth of leiomyomata (John and

Martin, 1971). Some have found no association between the occurrence of fibroids and the use of OCs (Parazzini *et al.*, 1996e; Samadi *et al.*, 1996) while others have reported a reduction in risk of fibroids with OC use (Ratech and Stewart, 1982; Ross *et al.*, 1986). Further, in the study by Ross et al., a consistent decrease in the risk of fibroids was noted with increasing duration of OC use (approximate 17% reduction in risk with each 5 years of use); this apparent protective effect was attributed to reduced exposure to unopposed oestrogen due to the modifying effect of progestogens (Ross *et al.*, 1986). This study was criticized, however, for indication bias (Ratner, 1986), as fibroids had commonly been considered a contraindication to OC use, thus resulting in a selected group for study.

These conflicting findings with regard to the effect of OCs upon the growth of myomas may relate to the differing content of oestrogen and the type of progestogen in each specific OC preparation (Cramer, 1992). In fact, a study (Ross *et al.*, 1986) attempted to address this issue by analyzing the oestrogen and progesterone content of each formulation. Although no conclusions could be drawn regarding the oestrogens present, the authors found that the higher the dose of the progestogen norethisterone acetate, the lower the incidence of fibroids, in preparations containing the same quantity of the oestrogen ethinyloestradiol. In contrast, all preparations containing the progestogen ethynodiol diacetate were associated with an increased incidence of fibroids, regardless of the quantity present or the type or amount of the accompanying oestrogen. The authors offered no explanation for the latter finding and stated that additional studies were needed for confirmation. A significantly elevated risk of fibroids has been reported among women who first used OCs in their early teenage years (13-16 years of age) compared with those who had never used them (Marshall *et al.*, 1998a).

1.2.2.5.4

Racial Differences

There has been a general acceptance in the literature that uterine fibroids are more prevalent in black women than white women. The reference often cited is an early study (Witherspoon and Butler, 1934) that had reported that 89.9% of the fibroid patients seen at Charity Hospital in New Orleans, Louisiana, were African American, whereas the total gynaecologic admissions were only slightly higher among African Americans than whites. Although this disparity has now been substantiated in a few more current studies, the magnitude of the difference has been less than the factor of 3-9 times sometimes cited (Buttram, 1986; Vollenhoven *et al.*, 1990). For instance, in one study (Day Baird *et al.*, 2003), 73% of black women and 48% of white women had uterine fibroids by ultrasound examination. In a study of hysterectomy specimens, (Kjerulff *et al.*, 1996), 89% of the black women and 59% of the white women had leiomyomas, which in black women were often larger, more numerous, and more symptomatic, and had developed at a younger age. In a recent report (Marshall *et al.*, 1997), 95,061 premenopausal nurses with no history of uterine leiomyoma were followed prospectively and had an incidence rate of leiomyoma approximately 2-3 times greater among black women than among white women. Although there was a higher prevalence of risk factors, including a higher mean BMI, among black women in this latter study, these factors could not account for the excessive rate of uterine leiomyomata among premenopausal black women. Although the basis for the higher prevalence among black women is unknown, ethnic differences have been found in circulating oestrogen levels while on control diets, and differences in oestrogen metabolism have been noted. In control groups of healthy, premenopausal women placed on a high-fat, low-fiber diet similar to their usual diet, African-American women had significantly higher serum levels of estrone, oestradiol, and free oestradiol than

Caucasian women. When subsequently placed on a low-fat, high-fiber diet, both groups responded with a significant lowering of their oestrogen levels (Woods *et al.*, 1996). In addition, significantly lower 2-hydroxyestrone (2-OHE1)/16α-hydroxyestrone (16α-OHE1) urinary metabolite ratios have been found in African-American women than in Caucasian women (Taioli *et al.*, 1996), which could also contribute to greater oestrogen exposure, as 2-OHE1 metabolites are devoid of peripheral biologic activity, whereas 16α-OHE1 is oestrogenic. Whether the difference in oestrogen metabolism might be due to genetic or environmental factors is unknown. Fewer data are available regarding the prevalence of uterine fibroids in Hispanics and Asians. In a study of premenopausal nurses in the United States (Marshall *et al.*, 1997), the incidence rates among these two groups, determined by ultrasound or hysterectomy, were similar to those of the white women (rate per 1,000 woman-years = Hispanic 14.5, Asian 10.4, white 12.5, in contrast to black 37.9). In summary, it was concluded that the prevalence of myomas is high among both blacks and whites, and probably also high among Hispanics and Asians, in the United States. The prevalence is relatively higher among African Americans than other ethnic groups based upon ultrasound data, and, more importantly, the clinical prevalence (symptomatic cases) is higher among African Americans because of a higher frequency of multiple lesions and greater size of the fibroids (Marshall *et al.*, 1997). The issue of clinical prevalence versus total prevalence is an important distinction from an aetiologic standpoint, as it indicates that the initiating causes of fibroids may require consideration separate from those factors that could promote their growth to clinically significant proportions. A large study of women screened for the presence of myomas by self-report, medical record review, and sonography found that African-American women had a 2.9 times greater risk of having myomas than Caucasian women, and that this risk was unrelated to other known risk factors. African- American women

also have myomas present at a younger age, and have more numerous, larger, and more symptomatic myomas (Kjerulff *et al.*, 1996; Marshall *et al.*, 1997). It is unclear whether these differences are genetic or due to known differences in circulating oestrogen levels, oestrogen metabolism, diet, or environmental factors. However, a recent study found that the Val/Val genotype of an enzyme essential to oestrogen metabolism, catechol-O-methyltransferase (COMT), is found in 47% of African American women but only 19% of white women. Women with this genotype are more likely to develop myomas, which may explain the higher prevalence of myomas among African-American women (Al-Hendy and Salama, 2006). It is also interesting that myomas and keloids, both more common in African-American women, have similar gene characteristics (Al-Hendy and Salama, 2006).

1.2.2.5.5

Weight

A prospective study found that the risk of myomas increased 21% with each 10 kg increase in body weight and with increasing body mass index (Ross *et al.*, 1986). Similar findings have been reported in women with greater than 30% body fat (Shikora *et al.*, 1991). Obesity increases conversion of adrenal androgens to estrone and decreases sex hormone–binding globulin. The result is an increase in biologically available oestrogen, which may explain an increase in myoma prevalence and/ or growth. Several studies have found an association between obesity and an increased incidence of uterine leiomyomas. In a prospective study from Great Britain (Ross *et al.*, 1986), the risk of fibroids increased approximately 21% for each 10-kg increase in body weight; similar results were obtained when the body mass index (BMI) was analyzed rather than weight. In a case-control study from Thailand (Lumbiganon *et al.*, 1996), a 6% increase in risk was observed for each unit increase in BMI. Similarly, a large prospective study of registered nurses

in the United States (Marshall *et al.*, 1998b) found an increased fibroid risk with increasing adult BMI, as well as an increased risk associated with weight gain since age 18 years. A case-control study from Japan (Sato *et al.*, 1998) likewise reported that women with occult obesity (BMI < 24.0 and percent body fat >= 30%) or women with upper-body fat distribution (> 0.80 waist-to-hip ratio) were at significantly higher risk. In a study from Boston, Massachusetts (Shikora *et al.*, 1991), 51% of the hysterectomy- or myomectomy-confirmed patients with leiomyomata were overweight, and 16% were severely obese; the authors compared their patients with a national study group of women in the United States included in The National Health and Nutrition Survey (Abraham and Johnson, 1980; Van Itallie, 1985; Flegal *et al.*, 1998), quoting comparison figures of 25% overweight and 7.2% severely obese. However, it should be noted that the latter study (Shikora *et al.*, 1991) had no control group of its own, used the percent of desirable body weight as the yardstick rather than BMI, and included fibroid patients from a slightly later time period when the prevalence of obesity was increasing generally in the United States. In contrast to these studies, there are two reports (Parazzini *et al.*, 1988; Samadi *et al.*, 1996) in which no association was found between the incidence of leiomyomas and BMI. Disparate reports of overweight prevalence may relate to definitional criteria, the method of measurement, and choice of comparison groups (Troiano and Flegal, 1999).

This apparent association between obesity and an increased risk of fibroids may be related to hormonal factors associated with obesity, but other pathologic pathways might also be involved. Several relevant hormonal associations with obesity are known. A significant increase occurs in the conversion of circulating adrenal androgens to estrone by excess adipose tissue. The hepatic production of sex hormone-binding globulin is decreased, resulting in more unbound

physiologically active oestrogen. Because almost all circulating oestrogens postmenopausally are derived from metabolism of circulating androgens by peripheral tissues, including fat, these two mechanisms probably have more impact in postmenopausal than premenopausal women (Glass, 1989). In obese premenopausal women, decreased metabolism of oestradiol by the 2-hydroxylation route reduces the conversion of oestradiol to inactive metabolites, which could result in a relatively hypero-estrogenic state (Schneider *et al.*, 1983).

1.2.2.5.6

Diet

The potential role of diet in the genesis of fibroids has received little attention in the literature. In a case-control study in Italy (Chiaffarino *et al.*, 1999a), a moderate association was found between the risk of uterine myomas and the consumption of beef, other red meat, and ham, whereas a high intake of green vegetables seemed to have a protective effect. Unfortunately, no estimate of the total caloric intake was obtained, and no attempt was made to estimate the amount of fat in the diet for cases and controls, although one might assume that a higher intake of beef would be associated with a greater amount of fat in the diet. Despite the limitations of the study, the results are interesting and raise a number of issues. Although fibroids are known to be hormonally responsive tumours, the question as to whether the dietary risks noted above (Chiaffarino *et al.*, 1999a) are secondary to the effects of various food groups upon the bioavailability of oestrogen or progesterone is yet to be answered. Also the protective effect of a high intake of green vegetables related to the fiber, some other undetermined component, such as a vitamin, or a corresponding reduction of fat in the diet is yet to be established. The role, if any, of phytoestrogens is also still being investigated. In a study of premenopausal vegetarian and nonvegetarian women (Goldin *et al.*, 1982; Gorbach

and Goldin, 1987), the vegetarians excreted 3-fold more oestrogen in their feces, had lower urinary oestrogen excretion, and exhibited 15-20% reduced plasma oestrogen levels. This reduction is apparently related to the increased fecal excretion of the oestrogen fraction normally excreted in the bile, resulting in diminished enterohepatic circulation of oestrogens. There are several possible explanations for the greater fecal excretion of oestrogens in vegetarians, including *a*) the greater bulk of undigested and nonabsorbed fiber that may shield the oestrogens from bacterial deconjugation and reabsorption; *b*) some characteristic of the vegetarian diet that decreases the ability of the intestinal flora to deconjugate biliary oestrogen conjugates, a necessary step for their reabsorption; or *c*) an effect related to lower dietary fat levels that might diminish oestrogen absorption. In Goldin's study (Goldin *et al.*, 1982), the vegetarians consumed less total fat and more dietary fiber than did the omnivores. Rose et al. demonstrated that both high-fiber diets (Rose *et al.*, 1991) and low-fat diets (Rose *et al.*, 1987) will reduce serum oestrogen levels, probably by altering the fecal flora and reducing the enterohepatic circulation of oestrogens. Regardless of the relative importance of dietary fat and fiber, such studies have established that modulation of the diet can influence oestrogen metabolism in premenopausal women, which may in turn influence the risk for fibroids. Likewise, a 17% reduction in plasma oestradiol concentration was accomplished in postmenopausal women who participated in a low-fat diet intervention program (Prentice *et al.*, 1990). In recent years plant derivatives, known as phytoestrogens, have gained attention in both the lay and scientific press. Phytoestrogens are diphenolic compounds that become converted into oestrogenic substances in the gastrointestinal tract (Ginsburg and Prelevic, 2000). Although these compounds are present in some 300 plants, the quantities present in most are trivial compared with the concentrations in soy and flax; in most populations the major dietary source of phytoestrogens is thought to be soy

(Tham *et al.*, 1998). These substances generally act as weak oestrogens, but they may also have anti-estrogenic effects, depending upon their concentration, the concentration of endogenous oestrogens, and individual characteristics such as gender and menopausal status (Tham *et al.*, 1998; Ginsburg and Prelevic, 2000) in addition, the effect is probably not identical in different organs (Adlercreutz and Mazur, 1997). In this regard, some investigators have suggested that phytoestrogens may act as "natural" selective oestrogen receptor (ER) modulators (SERMs, such as tamoxifen) (Ginsburg and Prelevic, 2000; Nikov *et al.*, 2000). The observed anti-oestrogenic effects of phytoestrogens may be partially explained by their competition with endogenous oestradiol for ERs. Prediction of the effects of phytoestrogens is uncertain because there are so many variables involved. Despite their weak oestrogenic activity, however, phytoestrogens could conceivably have a significant clinical impact, as their concentrations in the body may exceed those of the endogenous oestrogens (Adlercreutz *et al.*, 1982).

1.2.2.5.7

Exercise

The possibility of a relationship between exercise and the occurrence of fibroids has been addressed by comparing prevalences among a large group of former college athletes and non-athletes (Wyshak *et al.*, 1986). Former non-athletes were found to be 1.4 times more likely than former athletes to develop benign uterine tumours. In addition to differences in the degree of physical activity, however, an athletic lifestyle may have been associated with long-term differences in diet and relative leanness and, in turn, with reduced conversion of androgens to oestrogens in adipose tissue (Frisch *et al.*, 1986; Wyshak *et al.*, 1986).

1.2.2.5.8

Menopause

A reduced risk of fibroids requiring surgery in postmenopausal patients (Ross *et al.*, 1986; Parazzini *et al.*, 1988; Samadi *et al.*, 1996) could be due to tumour shrinkage in the absence of hormonal stimulus following the menopause. Sectioning of uteri at 2-mm intervals revealed a similar incidence of leiomyomas in pre- and postmenopausal patients (74 and 84%, respectively) although the postmenopausal leiomyomas were smaller and fewer (Cramer and Patel, 1990). The estimated risk in postmenopausal patients could be reduced by selection bias because of a tendency toward a more conservative nonsurgical, clinical approach in postmenopausal women (Parazzini *et al.*, 1988).

1.2.2.5.9

Menarche

There is a suggestion of slightly increased risk of fibroids associated with early menarche, although the risk has often not been statistically significant (Parazzini *et al.*, 1988; Cramer *et al.*, 1995a; Samadi *et al.*, 1996). Recently, a significant inverse association between risk of fibroids and age at menarche was reported (Marshall *et al.*, 1998a). some studies (Sato *et al.*, 2000b) found that women with uterine leiomyomas more often exhibited an early normal menstrual cycle pattern, and concluded that early menstrual regularity may enhance leiomyoma growth in early reproductive life. The early onset of menstrual cycles may increase the number of cell divisions that the myometrium undergoes during the reproductive years, resulting in an increased chance of mutation in genes controlling myometrial proliferation (Marshall *et al.*, 1998a).

1.2.2.5.10

Parity

Several studies have shown an inverse relationship between parity and the risk of fibroids (Ross *et al.*, 1986; Lumbiganon *et al.*, 1996; Parazzini *et al.*, 1996b; Samadi *et al.*, 1996). A relative risk of fibroids among parous women of 0.5, compared with nulliparae (Parazzini *et al.*, 1988), and a progressive decline in risk relative to the number of births have been reported (Ross *et al.*, 1986; Lumbiganon *et al.*, 1996; Parazzini, 1996; Parazzini *et al.*, 1996b; Parazzini *et al.*, 1996c; Parazzini *et al.*, 1996f; Marshall *et al.*, 1998a). An explanation that has been sometimes cited in the literature (Parazzini et al. 1996a; Ross et al. 1986) for these findings is that pregnancy reduces the time of exposure to unopposed oestrogens, whereas nulliparity or reduced fertility may be associated with anovulatory cycles characterized by long-term unopposed oestrogens. The alternative possibility exists that uterine fibroids are actually the cause of the infertility, rather than the consequence of it; however, the diminished relative risk of fibroids associated with parity remains essentially the same after exclusion of women with a history of infertility (Marshall et al. 1998a).

1.2.2.5.11

Pregnancy

Increased parity decreases the incidence and number of clinically apparent myomas (Lumbiganon *et al.*, 1996; Parazzini, 1996; Baird and Dunson, 2003). Myomas share some characteristics with normal myometrium during pregnancy, including increased production of extracellular matrix and increased expression of receptors for peptide and steroid hormones. The postpartum myometrium returns to normal weight, blood flow, and cell size via apoptosis and dedifferentiation (Cesen-Cummings *et al.*, 2003). This remodeling process may be responsible for the

involution of myomas. Another theory postulates that the vessels supplying myomas regress during involution of the uterus, depriving myomas of their source of nutrition (Burbank, 2004). Childbearing during the mid-reproductive years (age 25 to 29 years) provides the greatest protection against myoma development. Pregnancies early in the reproductive years, before age 25, may occur before the formation of myomas, and pregnancies after age 30 may occur when myomas are too large to regress (Baird and Dunson, 2003).

1.2.2.5.12

Smoking

Several studies have revealed a reduced risk of fibroids associated with current smoking, but not past smoking (Ross *et al.*, 1986; Wyshak *et al.*, 1986; Parazzini *et al.*, 1988; Lumbiganon *et al.*, 1996; Parazzini *et al.*, 1996b; Samadi *et al.*, 1996). In one study current smokers had a 50% reduced risk of uterine myomas requiring surgery (Parazzini *et al.*, 1996b). In another study, (Ross *et al.*, 1986) the reduction in risk among smokers was dose dependent; women who smoked 10 cigarettes per day had an 18% decreased risk compared with nonsmokers, whereas smokers of 20 cigarettes per day had a risk approximately 33% lower than that of nonsmokers. In contrast to these results, another survey (Marshall *et al.*, 1998b) found no indication of reduced risk in smokers. A number of factors decrease bioavailability of oestrogen at the target tissue; reduced conversion of androgens to estrone secondary to inhibition of aromatase by nicotine, increased 2-hydroxylation of oestradiol, or stimulation of higher sex hormone–binding globulin levels (Barbieri *et al.*, 1986; Michnovicz *et al.*, 1986; Daniel *et al.*, 1992). An epidemiologic study of African-American women did not find an increased risk of myomas among smokers, and postulated that a decrease in oestrogen may be countered by cell proliferation stimulated by components of smoke such as dioxin (Ohtake *et al.*,

2003; Wise *et al.*, 2004b; Wise *et al.*, 2004a). The inverse correlation between smoking and fibroids has been commonly attributed to an anti-oestrogenic effect of cigarette smoking, suggested by other epidemiologic associations of smoking, including a reduced risk of endometrial cancer, earlier natural menopause, and increased osteoporosis. The pathophysiology of this apparent anti-oestrogenic effect is not entirely clear, however, because the levels of estrone and total oestradiol are often similar in postmenopausal smokers and nonsmokers (Baron *et al.*, 1990), and investigation of hormonal levels in premenopausal smokers has yielded inconsistent results (MacMahon *et al.*, 1982b; Longcope and Johnston, 1988; Zumoff *et al.*, 1990; Westhoff *et al.*, 1996). On the other hand, several derangements of steroid metabolism have been identified in smokers. Increased 2-hydroxylation of oestradiol occurs in smokers, resulting in decreased bioavailability at oestrogen target tissues (Michnovicz *et al.*, 1986). Nicotine inhibition of aromatase reduces the conversion of androgens to estrone (Barbieri *et al.*, 1986). Significantly higher serum levels of sex hormone-binding globulin have been found, resulting in less unbound physiologically active oestrogen (Daniel *et al.*, 1992). Increased androstenedione and cortisol levels have been noted in postmenopausal smokers, suggestive of increased adrenal activity; elevated androgens may be significant, as some evidence exists that androgens can inhibit oestrogen-mediated effects in the rat uterus (Hung and Gibbons, 1983; Cassidenti *et al.*, 1992). These studies indicate that the hormonal metabolic effects of smoking are probably multifactorial. In addition, smokers as a group consistently exhibit lower body weights than nonsmokers, possibly because of a lower efficiency of calorie storage and/or an increased metabolic rate (Wack and Rodin, 1982). A lower body weight associated with a reduced risk of fibroids might be expected to be another indirect contributing mechanism through which smoking exerts an effect, but in three studies (Bedogni *et al.*, 1986; Ross *et al.*, 1986; Lumbiganon *et al.*, 1996; Parazzini *et*

al., 1996c), the effect of smoking was not changed by correction for BMI (Schwartz, 2001).

1.2.2.5.13

Tissue injury

Cellular injury or inflammations resulting from an environmental agent, an infection, or hypoxia have been proposed as mechanisms for initiation of myoma formation (Stewart and Nowak, 1998). However, no increased incidence has been found in women who have had sexually transmitted infections, an increased number of sexual partners, a younger age at first intercourse, prior intrauterine device use, or prior talc exposure. Herpes simplex virus I or II, cytomegalovirus, Epstein-Barr virus, or chlamydia have not been found in myomas. Vasoconstriction-induced hypoxia during menstruation has been proposed, but not confirmed, as another possible source of myometrial injury.

1.2.3 Initiators of Tumourigenesis

1.2.3.1 Theories of Initiation

The most important aspect of the aetiology of fibroids (the initiator(s)) remains unknown. Several theories have been advanced. One hypothesis states that increased levels of oestrogen and progesterone result in an increased mitotic rate that may contribute to myoma formation by increasing the likelihood of somatic mutations (Rein, 2000). Another favors an inherent abnormality in the myometrium of those who develop fibroids, based upon the finding of significantly increased levels of ER in the myometrium of fibroid uteri (Richards and Tiltman, 1996). A predisposing genetic factor has been suggested by others on the basis of ethnic and familial predilections (Marshall *et al.*, 1997; Schwartz *et al.*, 2000). Another interesting theory postulates that the pathogenesis of uterine leiomyomas might be similar to a response to injury (Stewart and Nowak, 1998) in

a manner analogous to the development of keloids following surgery. One avenue of potential injury might be ischaemia associated with the release of increased vasoconstrictive substances at the time of menses. Increased secretion of prostaglandins and vasopressin by the endometrium has been noted in patients with dysmenorrhea (Emans and Biro, 1998), which occurs in up to 70% of women by the fifth year after menarche (Silverman *et al.*, 2000). Might the smooth muscle cells of the myometrium react to injury in a manner analogous to vascular smooth muscle cells by undergoing a transformation from a contractile phenotype to a proliferative-synthetic phenotype? Certainly, morphologic similarities exist, as fibroids exhibit both an increased proliferative rate (Dixon *et al.*, 2002) and the synthesis of extracellular fibrous matrix. After vascular injury, basic fibroblast growth factor (bFGF) is critical to smooth muscle proliferation, and this factor is also overexpressed in leiomyomas (Lindner and Reidy, 1991; Mangrulkar *et al.*, 1995). Finally, injury related to menses is worthy of consideration in view of the universality of menstruation and the commonality of fibroids. When we consider the various risk factors, including those that have been attributed in the literature to increased exposure to "unopposed oestrogens," such as early menarche and nulliparity, we observe that such patients also experience more menstrual cycles than their counterparts. Of equal uncertainty in the genesis of fibroids is the role of genetic and/or epigenetic changes. The possibility of hereditary genetic predisposition to fibroids cannot be excluded at this time. On the other hand, evidence has been presented, though limited in scope, that karyotypic changes may occur secondarily (Mashal *et al.*, 1994) during the evolution or aging of some fibroids. Regardless of whether acquired karyotypic changes occur *ab initio* or during clonal evolution of fibroids, we can assume that preceding stimuli, conditions, or injuries must be responsible for the induction of genetic or epigenetic changes, and in this sense acquired genetic changes may be regarded as

secondary. These changes are therefore discussed in this section, not from the standpoint of purported initiators, but as possible potentiators or effectors of currently unrecognized initiating conditions.

1.2.3.2 The Genetic Findings

There have been numerous studies and reviews of the clonality and cytogenetics of uterine leiomyomas (Mark *et al.*, 1988; Nilbert *et al.*, 1990; Pandis *et al.*, 1991; Ozisik *et al.*, 1993b; Ligon and Morton, 2000; Gross and Morton, 2001).

1.2.3.2.1 Heritability.

The question of whether there is evidence of a genetic predisposition to fibroids has been approached from four perspectives: ethnic predisposition, twin studies, familial aggregation, and association with an inherited syndrome. The higher incidence of clinically significant fibroids among African-American women in the United States has been discussed above. Two studies comparing monozygous and dizygous twins may be cited. The first of these reported a 2-fold higher correlation for hysterectomy in monozygotic than dizygotic twins (Treloar *et al.*, 1992). Because leiomyomata represent the most common indication for hysterectomy in the United States, this finding in monozygous twins suggests a genetic liability for fibroids. Because the study did not report the actual incidence of leiomyomata, however, it is recognized that heritable conditions other than fibroids could contribute to the observed correlation in twins (Gross and Morton, 2001). A more recent twin study specifically addressed the risk of fibroids in twins by examining hospital discharge diagnoses from the Finnish Twin Cohort Study and by performing transvaginal ultrasounds in a random sample of these women (Luoto *et al.*, 2000). The casewise concordance for hospitalization due to uterine fibroids was significantly higher in monozygous twins than dizygous twins, providing

support for a genetic contribution. On the other hand, by ultrasound examination the risk ratio for fibroids in a monozygous twin whose sister had been diagnosed with fibroids was only 1.1, the same as for a dizygous twin; however, the authors noted that the low participation rate decreased the power of the study to detect potential differences between the twins. The study concluded that anthropometric and reproductive factors, such as a higher BMI and nulliparity, may play at least as large a role in pathogenesis of fibroids as genetic factors.

Four studies of the familial clustering of fibroids may be cited. The first was a German study, reported in 1938 (Winkler and Hoffmann, 1938), in which fibroids were found to be 4.2 times more common in first-degree relatives of women with fibroids than those without. Similar findings were noted in two studies from Russia in which a higher incidence of fibroids was found in first-degree relatives (Vikhlyaeva *et al.*, 1995) and sisters (Kurbanova *et al.*, 1989) of affected probands than in controls. Last, in a study of 638 fibroid patients and 617 controls in the Puget Sound area of Washington State (Schwartz *et al.*, 2000), fibroid patients again were found more likely than the controls to report a history of fibroids in a mother or sister (33.2% vs. 17.6%). Furthermore, the odds ratio increased to 5.7 in cases of early-onset fibroids, as might be expected for a genetically influenced trait. Unfortunately, these studies may be influenced by reporting and detection bias. A woman having clinical problems that could be attributed to fibroids may be more likely to seek a diagnosis if a close relative has had fibroids. A woman who has been diagnosed may also be more likely to learn about diagnoses among her female relatives.

Finally, a rare inherited disorder known as Reed's Syndrome (Fisher and Helwig, 1963; Reed *et al.*, 1973; Thyresson and Su, 1981), or multiple leiomyomatosis, is characterized by the appearance of multiple leiomyomas in the skin, uterus, or

both. The family histories in these cases suggest an autosomal dominant inheritance with incomplete penetrance. Recent reports of several families in England and Finland with multiple uterine and cutaneous leiomyomata, and a subset of these with papillary renal cell carcinoma, have independently linked this disorder to a predisposition gene in the region of chromosome 1q42.3-q43 (Alam and Ratner, 2001; Kiuru *et al.*, 2001; Launonen *et al.*, 2001). In follow-up studies of this chromosomal region, mutations were detected only in the fumarate hydratase gene (Tomlinson *et al.*, 2002) --a surprising finding, as this enzyme is a component of the essential energy-producing tricarboxylic acid cycle (Rustin *et al.*, 1997). Furthermore, the gene appears to act as a classic tumour suppressor in that loss of the wild-type allele was observed frequently in the leiomyomata and renal cell cancers (Alam and Ratner, 2001; Kiuru *et al.*, 2001; Launonen *et al.*, 2001). Although this hereditary syndrome is itself rare, the association with inactivation of the fumarate hydratase gene is of interest, as it is possible that other mechanisms of transcriptional silencing of this gene such as promoter hypermethylation could be involved in the development of sporadic leiomyomas (Kiuru *et al.*, 2001).

1.2.3.2.2

Clonality.

There is general acceptance in the literature that these tumours are monoclonal. The underlying premise of these studies has been based on the Lyon hypothesis, which assumes that only one X chromosome is active in any female cell, the other X chromosome remaining in an inactive state as a Barr body, and that the X chromosome that is inactivated (methylated) is determined randomly. Thus, genetic loci known to be located on the X chromosome can be studied in these tumours for evidence of homogeneity of expression in those patients identified as heterozygous for a particular gene in their normal, nontumour tissues. The first studies of clonality used the X-linked glucose 6-phosphate dehydrogenase (G6PD)

isozymes. After screening patients for G6PD heterozygosity by analysis of red blood cells, the resected fibroids and myometrium were analyzed for the presence of one or both electrophoretic types of G6PD. In two studies (Linder and Gartler, 1965; Townsend *et al.*, 1970), both G6PD types (A and B) were identified in almost all samples of myometrium, whereas only one G6PD type (A or B) was identified in each of the leiomyomas. Furthermore, both A and B tumours were often identified in the same patient, indicating independent origins of the individual fibroids. These results suggested that the tumours arose from single cells, although selective overgrowth of one cell type from a tumour originally composed of both G6PD types cannot be excluded. The major limitation of these studies is the minor degree of G6PD polymorphism in the population, as most Caucasian females (> 99%) are homozygous type B, and only 30% of African-American females are heterozygous, and therefore only a minority of cases would be informative by studies of this gene. More recently, clonality studies have taken advantage of methylation-sensitive restriction enzymes to discriminate between active and inactive alleles of X-linked genes known to be highly polymorphic (Vogelstein *et al.*, 1985). Tumours arising from single cells should contain only one type of inactive (methylated) allele, which will be amplified exclusively following restriction-enzyme digestion of the active (unmethylated) allele, whereas tumours of multicellular origin should contain some cells with one type of inactive allele and other cells with a second type of inactive allele, resulting in the amplification of both alleles following digestion and polymerase chain reaction. This method has been employed for analysis of both the X-linked androgen receptor gene (Mashal *et al.*, 1994) and the X-linked phosphoglycerokinase gene (Hashimoto *et al.*, 1995). Both studies concluded that the uterine fibroids examined were monoclonal in origin. One report has described chromosome 7 biclonality in four uterine leiomyomas (Ozisik *et al.*, 1993a), with the breakpoint regions in two of these such

that one clone could not possibly have originated from the other clone. Taken in sum, however, the concept of monoclonal origin of most fibroids appears to be a valid one, recognizing that some could be biclonal in origin (Ozisik *et al.*, 1993a) and some are biclonal or oligoclonal because of clonal evolution (Pandis *et al.*, 1990a), and that monoclonality itself could be the result of selective overgrowth of one clone from an originally polyclonal proliferation (Vogelstein *et al.*, 1987; Fey *et al.*, 1992).

1.2.3.2.3

Cytogenetics.

Most of the investigations of leiomyomas seeking chromosomal aberrations have used classic cytogenetic karyotyping, a valuable tool because it is the only method that allows one to survey the entire genetic constitution of a tissue with a single assay. Standard cytogenetic methodology with G-band analysis can identify translocations, deletions, and duplications, but does require the *in vitro* culture of leiomyoma cells to obtain metaphase preparations. An alternative method that has been employed in a few studies (Packenham *et al.*, 1997; Levy *et al.*, 2000) is comparative genomic hybridization, which permits the recognition of cytogenetic changes such as deletions and amplifications without the need for cell cultures of the tumour, although not allowing for detection of balanced rearrangements. Neither standard karyotyping nor comparative genomic hybridization permits the detection of small, submicroscopic chromosomal abnormalities such as point mutations or epigenetic changes such as methylation.

1.2.3.3 Most common cytogenetic changes.

Because the studies of tumour cytogenetics are limited to tissue samples removed at surgery and may be taken from larger fibroids, the possibility exists that they may not be representative of leiomyomas in general. Nonetheless, based upon

such samples, approximately 40-50% of uterine fibroids are reported to have nonrandom chromosomal abnormalities.

1.2.3.3.1

t(12;14)

One of the most common of these is a translocation between chromosomes 12 and 14, specifically t(12;14) (q14-q15;q23-q24), which is present in about 20% of karyotypically abnormal leiomyomata (Ligon and Morton, 2000). This abnormality is of interest for several reasons. First, the region q14-q15 on chromosome 12 is also commonly rearranged in a variety of other mesenchymal solid tumours, including angiomyxomas, haemangiopericytomas, lipomas, and pulmonary chondroid hamartomas, as well as breast fibroadenomas, endometrial polyps, and salivary gland adenomas. In addition, evidence exists that a critical gene located in the chromosome 12q14-q15 region may be *HMGIC* (now designated *HMGA2*), a gene encoding a member of the high-mobility group (HMG) of proteins. These are DNA-binding proteins that can induce conformational changes in DNA, thereby indirectly regulating transcription by influencing the access of other DNA-binding proteins to target genes. The HMGIC protein may play a role as a proliferation factor in growing tissues, particularly those of mesenchymal origin; accordingly, expression of this protein has been detected in leiomyomata with 12q14-15 rearrangements, but not in matched normal myometrium (Gattas *et al.*, 1999). In addition, the region on chromosome 14 involved in this translocation, q23-q24, is of particular interest because of its specificity for fibroids compared with other mesenchymal tumours in which *HMGIC* is rearranged. The ER-ß gene (*ESR2*) is located in this region of chromosome 14 and would seem to be a logical fusion partner with *HMGIC*, as the growth of fibroids is responsive to oestrogen. More recently, *ESR2* has been

mapped to a region approximately 2 Mb centromeric to the t(12;14) breakpoint, suggesting that *ESR2* is not involved with *HMGIC*. However, this finding may not exclude the possibility that *ESR2* might be deregulated by chromosomal translocation in view of its proximity to the breakpoint (Pedeutour *et al.*, 1998). Evidence has also been presented that *RAD51L1* (formerly *RAD51B*), a member of the *RAD51* recombination repair gene family (Shinohara *et al.*, 1992; Albala *et al.*, 1997; Villanueva *et al.*, 1997), is the chromosome 14 target gene and preferential fusion partner of *HMGIC* in uterine leiomyomas with t(12;14) (Ingraham *et al.*, 1999; Schoenmakers *et al.*, 1999; Amant *et al.*, 2001; Takahashi *et al.*, 2001). Although the RAD51L1 protein has not yet been shown to catalyze recombination reactions, *RAD51L1* appears to be an essential gene (Shu *et al.*, 1999; Zhai *et al.*, 1999) expressed in almost all organs and tissues (Rice *et al.*, 1997) and probably plays a role in regulation of cell cycle progression (Havre *et al.*, 1998; Havre *et al.*, 2000). In view of the purported function of *HMGIC* in regulation of cell proliferation (Reeves, 2000) and the probable role of *RAD51L1* in cell cycle regulation, it is reasonable to speculate that the disruption of genomic structure associated with the *RAD51L1/HMGIC* fusion (Ingraham *et al.*, 1999; Schoenmakers *et al.*, 1999; Takahashi *et al.*, 2001) might result in dysregulated cell growth.

1.2.3.3.2

del(7q)

Another frequently encountered karyotypic abnormality in fibroids is a deletion of chromosome 7, del(7)(q22q32), which is present in about 17% of karyotypically abnormal fibroids (Ligon and Morton, 2000). In some series del(7q) has been the most common cytogenetic abnormality in fibroids (Nilbert *et al.*, 1990; Ozisik *et al.*, 1993b). Although interstitial deletions and translocations involving chromosome 7q have also been reported in other benign tumours, such as lipomas and

endometrial polyps, the deletion is more commonly observed in fibroids than in any other solid tumour. Because this region, 7(q22q32), is physically large and gene-rich, pinpointing a specific gene that could be implicated in the genesis of fibroids has proven difficult. Recently, however, the critical area on band 7q22 has been narrowed to a 4-cM (centiMorgan) region by allelotype analysis (van der Heijden *et al.*, 1998). In the latter study loss of heterozygosity in the leiomyomas was rare except in 7q22, where a minimal deletion was observed in 34% of the tumours, leading the authors to speculate that this site probably harbors a novel tumour-suppressor gene involved in the aetiology of this tumour (van der Heijden *et al.*, 1998).

1.2.3.3.3

6p2

A third cytogenetic subgroup consists of aberrations of 6p21, including deletions, inversions, translocations, and insertions. Interest in this region has been related in part to the frequently observed alterations of band 6p21 in other benign mesenchymal tumours, such as lipomas, and to the identification of another high mobility group gene, *HMGIY* (now designated *HMGA1*), in this region. Rearrangements of 6p21 are much less common in fibroids than in these other tumours, however, occurring with a frequency of < 5% (Nilbert *et al.*, 1990).

1.2.3.3.4

Trisomy 12

A variety of other cytogenetic abnormalities have been identified in leiomyomata. The reporting of trisomy 12 in as many as 12% of karyotypically abnormal fibroids (Nilbert *et al.*, 1990; Vanni *et al.*, 1992) raises the question of whether this anomaly might reflect pathogenetic similarities to t(12;14), acting to increase the gene dosage

of HMGIC. Many of the other abnormalities, such as ring chromosomes, occur less frequently and often concomitantly with other chromosomal changes and are therefore thought to represent secondary abnormalities.

1.2.3.4 Correlations with tumour phenotype

No indication of systematic histologic differences between leiomyomas with normal karyotypes and those with chromosomal aberrations were found in one study (Nilbert *et al.*, 1990); however, there is some evidence from other reports (Pandis *et al.*, 1990b; Meloni *et al.*, 1992) that leiomyomas that are either cellular with mitotic activity or atypical histologically are more likely to demonstrate karyotypic abnormalities or to show massive karyotypic aberrations indicative of clonal evolution. In a study of 114 myomas from 92 patients, myomas > 6.5 cm demonstrated a significantly higher proportion of abnormal karyotypes than myomas < 6.5 cm (75% vs. 34%) (Rein *et al.*, 1998). In the same study a relationship between particular karyotypes and fibroid size was identified, with the largest tumours carrying t(12;14) abnormalities and the smaller tumours exhibiting chromosome 7 deletions, suggesting that chromosomal abnormalities associated with individual myomas may enhance myoma growth. A correlation between the location of the fibroid and the likelihood of a cytogenetic abnormality has also been reported (Brosens *et al.*, 1998); submucous myomas presented significantly fewer abnormal karyotypes (12%) than did either the intramural (35%) or the subserosal (29%) tumours, and furthermore, this correlation remained significant regardless of the diameter of the myoma. Despite the large number of cytogenetic studies, many unanswered questions remain. Are the chromosomal aberrations primary to the genesis of these tumours or are they secondary events? In one study chromosomal abnormalities were interpreted as secondary events because they were preceded by monoclonality (Mashal *et al.*, 1994); however, the data are limited and additional

studies are needed for verification. Certain karyotypic abnormalities such as the t(12;14) and the del(7q) occur with sufficient frequency to warrant consideration as differing pathways leading to leiomyoma development, or at least to consider that these sites may contain genes that are important in the proliferation and differentiation of smooth muscle cells. Because at least one-half of fibroid tumours appear to be cytogenetically normal, there may exist an unidentified submicroscopic mutation in this karyotypically normal subgroup or even in the cytogenetically abnormal group as well. Histologic subtypes such as the cellular and atypical leiomyomas may ultimately be correlated with certain karyotypic aberrations that are either distinctive primary events or represent secondary changes of clonal evolution. Finally, regarding heritability, a particular gene or genes may one day be identified as predisposing to the development of leiomyomata, as suggested by the familial clustering studies. If so, it must be a very common gene, widespread in the general population, in view of Cramer and Patel's finding of a 77% incidence of leiomyomas in a thorough examination of 100 consecutive, non-selected hysterectomy specimens (Cramer and Patel, 1990).

1.2.4 Promoters

1.2.4.1 Evidence for the Role of Oestrogen and Progesterone

1.2.4.1.1

Clinical Observations

Oestrogen has been traditionally proposed as the primary promoter of uterine leiomyoma growth. This supposition has been based in part upon the clinical observations that fibroids occur only after menarche, develop during the reproductive years, may enlarge during pregnancy, and frequently regress following menopause. Furthermore, because the risk of fibroids is greater in nulliparous women who might be subject to a higher frequency of anovulatory cycles and obese women with greater aromatization of androgens to estrone in the fat, the concept of unopposed oestrogens as an underlying cause of uterine fibroids has sometimes been proposed in the literature (Ross *et al.*, 1986; Romieu *et al.*, 1989; Cramer, 1992; Parazzini *et al.*, 1996b). Increased growth of myomas among women taking tamoxifen or receiving transdermal or injected oestrogen-replacement therapy further supports the importance of oestrogen. The oestrogen hypothesis has also been supported by clinical trials evaluating the medical treatment of myomas with GnRH agonists, the effective result of which is hypoestrogenism accompanied by regression of the fibroids (Friedman, 1989). As noted by Rein, however, distinguishing the relative importance of oestrogen versus progesterone is difficult, as progesterone levels, in a manner similar to those of oestrogen, are also cyclically elevated during the reproductive years, are significantly elevated during pregnancy, and are suppressed after menopause (Rein *et al.*, 1995).

Furthermore, regression of uterine leiomyomata has been induced by treatment with the antiprogesterone drug RU 486, accompanied by reduction in the progesterone receptor (PR) but not the ER in the tumours, suggesting that the regression was attained through a direct antiprogesterone effect (Murphy *et al.*, 1993). In addition patients treated with leuprolide (a GnRH agonist capable of reducing the size of fibroids) who were concomitantly given medroxyprogesterone acetate demonstrated no significant reduction in myoma or uterine volume (Friedman *et al.*, 1988; Carr *et al.*, 1993). Indeed, clinical and laboratory evidence to date would appear to indicate that oestrogen and progesterone may both be important as promoters of myoma growth (Rein, 2000).

1.2.4.1.2

Pregnancy.

A common clinical perception prevails that myomas increase in size during pregnancy (Buttram, 1986). With the advent of ultrasonographic studies, however, several reports have noted that only a minority of myomas (one-third or less) increase in size during pregnancy, whereas the majority remain stable or decrease in size (Aharoni *et al.*, 1988; Rosati *et al.*, 1992; Strobelt *et al.*, 1994). The larger the myoma, the greater the likelihood of growth (Strobelt *et al.*, 1994). Myoma size can increase as a result of hypertrophy and edema, while shrinkage of the tumour may occur as a result of degenerative changes secondary to ischaemia. A 10% complication rate related to myomas has been reported during pregnancy (Katz *et al.*, 1989). The most common complication was the syndrome of painful myomas, sometimes associated with bleeding, and probably related to haemorrhagic degeneration or infarction. Although the aetiology of the syndrome of painful myomas of pregnancy is unclear, high concentrations of progesterone, as in pregnancy, may play a role, as similar changes of "red degeneration" have been

induced by high-dosage progestin therapy (Goldzieher *et al.*, 1966). Other reported complications of myomas in pregnancy include premature rupture of the membranes, malpresentation, increased caesarean delivery rate, and postpartum endomyometritis (Katz *et al.*, 1989). It has also been suggested that fibroids are a more important feature in pregnancy now than in the past because many women are delaying childbearing to their late thirties, the time of greatest risk for fibroid growth (Vollenhoven *et al.*, 1990).

1.2.4.2 Laboratory Studies

1.2.4.2.1

Oestrogen and progesterone levels.

Patients with uterine leiomyomas have plasma oestradiol and progesterone levels similar to those of women without detectable myomas, as indicated in three studies (Spellacy *et al.*, 1972; Maheux *et al.*, 1986; Dawood and Khan-Dawood, 1994). An older report noted that the urinary oestrogens of approximately one-third of the fibroid patients were elevated with respect to their laboratory normal range, but no control group was available for comparison (Timonen and Vaananen, 1959). Quantitative differences, however, have been demonstrated between leiomyomas and myometrium in the tissue concentrations of ovarian hormones, their receptors, and a key metabolizing enzyme. In one study, the concentration of 17ß-oestradiol was significantly higher in leiomyomas than myometrium, especially in the proliferative phase, whereas no difference in the concentration of progesterone was found (Otubu *et al.*, 1982). The authors speculated that the higher levels of oestradiol in the leiomyomas could be related to lower levels of the enzyme 17ß-hydroxysteroid dehydrogenase, which accelerates the conversion of oestradiol to estrone. Other investigators have also demonstrated higher oestradiol concentrations (Folkerd *et al.*, 1984) and more

frequent expression or overexpression of aromatase activity in leiomyomata than in matched myometrial samples (Folkerd *et al.*, 1984; Yamamoto *et al.*, 1984; Sumitani *et al.*, 2000), leading these authors to entertain the possibility that increased androgen to oestrogen conversion in fibroids may potentiate their growth.

1.2.4.2.2

Oestrogen and progesterone receptors.

The ER and PR literature comprises a rather extensive and sometimes contradictory collection of data that spans several decades of research. Disparate results are probably attributable to the diversity of methodologies employed (including assessment of the cytosol alone versus the combined nuclear and cytosolic fractions), the use of human versus nonhuman tissues, the phase of the menstrual cycle at the time of collection of specimens, and the heterogeneity of myomas in the same patient (Englund *et al.*, 1998). In the absence of experimental unanimity, the generalizations or conclusions that follow are therefore based upon assessment of the weight of the evidence. In the majority of the studies reviewed, the concentrations of both the ERs and PRs were greater in leiomyomata than the myometrium (Tamaya *et al.*, 1975; Pollow *et al.*, 1978a; Wilson *et al.*, 1981; Soules and McCarty, 1982; Sadan *et al.*, 1987; Marugo *et al.*, 1989; Rein *et al.*, 1990; Vollenhoven *et al.*, 1990; Kawaguchi *et al.*, 1991; Andersen, 1996a; Lessl *et al.*, 1997; Viville *et al.*, 1997; Englund *et al.*, 1998; Nisolle *et al.*, 1999; Vij *et al.*, 2006). In addition, Sadan et al. found the ER and PR to be elevated in fibroids during all phases of the menstrual cycle when compared with matched myometria (Sadan *et al.*, 1987). Interestingly, in one study (Marugo *et al.*, 1989) the ER and PR levels were significantly higher in submucous than subserosal leiomyomas, leading the authors to speculate about different aetiologies and types of leiomyomas. The

receptor concentrations were independent of the size of the tumour in one report (Sadan *et al.*, 1987). Another investigation found marked variation in ER and PR levels in different tumours from the same subject (Englund *et al.*, 1998); such heterogeneity may relate to the degree of hyalinization and involution of individual tumours.

1.2.4.2.3

ER-α and ER-ß.

Because a second subtype of the ER, designated ER-ß, was not discovered until 1996 (Kuiper *et al.*, 1996; Mosselman *et al.*, 1996), the significance of ER-ß relative to that of the classic ER, ER-α, has not been fully determined. Nuclear expression of both ER-α and ER-ß throughout the entire myometrium has been demonstrated immunohistochemically (Taylor and Al-Azzawi, 2000). One group (Pedeutour *et al.*, 1998) found ER-ß mRNA in 14 of 15 leiomyomata, with no striking difference in expression from the matched myometrial tissues. Another group (Benassayag *et al.*, 1999) showed expression of both ER-α and ER-ß mRNA in leiomyomata, with the levels of both receptors higher in most of the leiomyomas than in the corresponding non-pregnant myometria. Andersen noted that the highest expression of ER-ß in non-pregnant myometrial and leiomyoma tissues is at the beginning of the menstrual cycle, and the lowest expression is at the early mid-lacteal phase; however, low levels of ER-ß protein were detected in these tissues, in contrast to the more abundant expression in myometrial tissue from pregnant women at term (Andersen, 2000). Despite the lack of consensus regarding the quantitative levels of ER-ß, the possibility of a role for ER-ß in leiomyomata cannot be ruled out at this time, as the ER-ß gene, *ESR2*, has been mapped to 14q22-24 (Enmark *et al.*, 1997), close to the breakpoint site of one of the more common genomic rearrangements of fibroids.

1.2.4.2.4

Progesterone receptor-A and progesterone receptor-B.

Both forms of PR (PR-A and PR-B) are expressed in leiomyomas and myometrium, with the concentration of PR-A higher than that of PR-B in both tissues (Viville *et al.*, 1997). In one study PR-A levels were increased in leiomyomata compared with the matched myometrium (Brandon *et al.*, 1993).

1.2.4.2.5

Interaction between oestrogen, progesterone, and their receptors.

The interaction between the two hormones and their respective receptor levels has been the subject of numerous studies and is of interest with regard to the promotion of fibroid growth. Strong evidence exists that the effect of oestrogen is to increase the levels of both ER and PR in the myometrium, whereas the effect of progesterone is to decrease the level of the ER (Hsueh *et al.*, 1975; Thi *et al.*, 1975; Katzenellenbogen, 1980). These conclusions are consistent with the sequential presentation of these two hormones during the menstrual cycle and the predominant observations that in the myometrium both ER and PR rise during the follicular (proliferative) phase and then fall during the luteal (secretory) phase of the menstrual cycle (Janne *et al.*, 1975; Schmidt-Gollwitzer *et al.*, 1979; Soules and McCarty, 1982; Sadan *et al.*, 1987; Marugo *et al.*, 1989; Kawaguchi *et al.*, 1991; Lessl *et al.*, 1997; Englund *et al.*, 1998; Rein, 2000). Because PR levels also fall during the luteal phase, some feel that progesterone may downregulate its own receptor (Englund *et al.*, 1998); this conclusion was also reached by Thi et al. (1975), who demonstrated a fall in PR in the myometrium of ovariectomized guinea pigs when given progesterone (Thi *et al.*, 1975). However, the alternative explanation that the fall in PR is related to the fall in levels of oestradiol during the luteal phase is difficult to exclude (Schmidt-Gollwitzer *et al.*, 1979; Englund *et al.*, 1998). The

majority of studies have reported the occurrence of similar cyclic rises and falls in ER and PR in uterine fibroids during the menstrual cycle, although there is some controversy regarding the degree, or the existence, of such a fall in ER during the luteal phase. In one study, ER expression occurred throughout the menstrual cycle in leiomyomas (Kawaguchi *et al.*, 1991). Likewise, another investigation showed that elevated levels of the ER in fibroids continue throughout the cycle, suggesting that leiomyomas may have lost a negative regulation that is maintained in the myometrium and limits the myometrial response to oestrogen in the beginning of the menstrual cycle (Andersen and Barbieri, 1995). On the other hand, it is clear that these tumours are subject to hormonal modulation during the cycle, as mitotic activity is reported to be significantly higher during the secretory phase than during the proliferative phase (Kawaguchi *et al.*, 1991; Lamminen *et al.*, 1992; Nisolle *et al.*, 1999). These latter reports are consistent with a study by Tiltman (Tiltman, 1985) that demonstrated a significantly higher mitotic activity in the leiomyomas of patients who received a progestin-only preparation. In lone contrast to these studies is an earlier report that had noted no mitotic activity in the myomas of patients given progestin therapy (Goldzieher *et al.*, 1966). When considered in sum, however, these studies support the concept of a mitogenic effect of progesterone in fibroid tumours. Although these data show that progesterone plays an important role in the growth of leiomyomas, it is also evident that some degree of cell proliferation occurs continuously during the menstrual cycle, as mitotic activity, albeit of a lesser degree, is present during the follicular phase of the cycle as well (Kawaguchi *et al.*, 1989; Lamminen *et al.*, 1992). Although the possibility of progesterone carryover effect from the luteal phase cannot be excluded, this suggests that oestrogen may exert a mitogenic effect as well, and there are some clinical data (Romieu *et al.*, 1989) as well as tissue culture work (Chen *et al.*, 1973; Maruo *et al.*, 2000) to support this supposition. In addition,

we might reason that the mitogenic effect of progesterone is dependent upon prior exposure to oestrogen, as oestrogen priming increases the concentration of PRs in myomas. In summary the evidence available suggests that during the follicular phase, oestrogen upregulates ER and PR, thus setting the stage for the luteal phase progesterone surge associated with a heightened mitogenic effect and subsequent downregulation of ER and PR.

1.2.4.2.6

Metabolism of oestradiol.

The metabolism of oestradiol involves a series of enzymatically catalyzed oxidative transformations, which may occur by several pathways. Because some oestradiol metabolites possess significant oestrogenic activity whereas others are virtually devoid of activity, the levels of the specific metabolizing enzymes and the predominant pathways employed could play important roles in fibroid tumourigenesis. Of interest, therefore, is the demonstration of alterations in two of these enzymes, 17ß-hydroxysteroid dehydrogenase and oestradiol 4-hydroxylase, in uterine leiomyomas.

1.2.4.2.7

17ß-Hydroxysteroid dehydrogenase.

Regardless of the phase of the cycle, the proliferative index of leiomyomas is significantly higher than that of the myometrium (Kawaguchi *et al.*, 1991; Maruo *et al.*, 2000; Dixon *et al.*, 2002). This finding is not surprising in view of the elevated levels of both ERs and PRs in leiomyomas throughout the menstrual cycle. Because oestradiol up-regulates both of these receptors, the increased concentration of oestradiol in these tumours compared with that in the myometrium (Otubu *et al.*, 1982) could be indicative of a pathogenetic link to the development of leiomyomata. The demonstration of reduced activity in leiomyomas of the enzyme

17ß-hydroxysteroid dehydrogenase (Pollow *et al.*, 1978b; Eiletz *et al.*, 1980), the enzyme responsible for the conversion of oestradiol to estrone, would seem to provide a plausible explanation for the accumulation of oestradiol in these tumours (Otubu *et al.*, 1982). Although estrone is weakly oestrogenic, it exhibits a lower binding affinity for ERs than oestradiol, and it diffuses out of the cell more rapidly than oestradiol. In the myometrium, the activity of this enzyme is maximal during the early secretory phase because of upregulation by progesterone (Tseng and Gurpide, 1973), resulting in a diminished oestradiol effect during the second half of the cycle. In leiomyomas, on the other hand, the reduced activity of 17ß-hydroxysteroid dehydrogenase may allow for the accumulation of oestradiol in the cells during the secretory as well as the proliferative phase of the cycle, thus resulting in continual stimulation by oestrogen, with up-regulation of both the ERs and PRs, accompanied by the associated growth-promoting effects. Whether the enzymatic deficiency is a quantitative or qualitative one, and regardless of whether it is a primary or secondary development in the genesis of fibroids, the reduced activity of this enzyme could play a significant role in the pathogenesis of these tumours.

1.2.4.2.8

Oestradiol 4-hydroxylase.

Both oestradiol and estrone may be metabolized by irreversible hydroxylation at several sites, including the C-2 and C-4 positions (forming catechol oestrogens) and the C-6, C-15, and C-16 positions. These various hydroxylated metabolites may have quite different biologic properties. For example, the C-2 metabolites (the predominant form in humans) have limited or no activity, whereas the C-4 and C-16 metabolites possess potent oestrogenicity (Martucci and Fishman, 1993). For this reason, it is of great interest that the mean rate of 4-hydroxylation of oestradiol is 8-

fold higher than that of 2-hydroxylation in myomas, and further, that 4-hydroxylation is substantially elevated in myomas compared with surrounding myometrial tissue (Liehr *et al.*, 1995). Because the dissociation rate of 4-hydroxyoestradiol from the ER complex is also reduced compared with oestradiol (Zhu and Conney, 1998), this catechol metabolite may also function as a long-acting oestrogen, suggesting that overexpressed 4-hydroxylase activity may play a role in the aetiology of uterine fibroids (Liehr *et al.*, 1995).

1.2.5 Effectors:

1.2.5.1 Growth Factors and Their Receptors

The growth-promoting effects of oestrogen and progesterone upon the myometrium and uterine myomas may be mediated through the mitogenic effects of growth factors produced locally by smooth muscle cells and fibroblasts (Mangrulkar *et al.*, 1995; Rein, 2000). Growth factors are polypeptides or proteins that are secreted by a number of cell types, have a wide range of biologic effects, and generally act over short distances either in an autocrine or paracrine manner **(Pusztai *et al.*, 1993)**. They are essential elements in controlling the proliferation rate of cells, and overexpression of either the growth factor or its receptor may contribute to tumourigenesis. Growth factors exert most of their effects on target cells by interaction with specific cell-surface receptors, with subsequent message transmission via signal transduction systems in the cell. Even in the physiologic state, the cellular responses evoked by growth factors are complex and dependent upon a number of variables, including the cell type, the differentiation stage of the cell, other stimuli acting simultaneously upon the cell (e.g., two growth factors together may have a different effect than either one alone), and the tendency for

most growth factor receptors to interact with an entire family of growth factors (Pusztai *et al.*, 1993).

1.2.5.2 Evidence for Regulation of Growth Factors by Oestrogens and Progestins

The evidence is 2-fold. First, several studies have demonstrated increases or decreases in production of particular growth factors in tissue culture cell lines or laboratory animals *in vivo* when given oestrogen or progesterone (Presta, 1988; Charnock-Jones *et al.*, 1993; Cullinan-Bove and Koos, 1993; Takahashi *et al.*, 1994; Hyder *et al.*, 1996; Fujimoto *et al.*, 1997; Rider *et al.*, 1997). Second, there is the indirect evidence that certain growth factors or their receptors are reduced in leiomyoma tissues from patients who are hypoestrogenic because of treatment with GnRH agonists (Lumsden *et al.*, 1988; Rein *et al.*, 1990). Although acknowledging this evidence that growth factors may be regulated by the sex steroids and simply play the role of secondary effectors in fibroid tumourigenesis, the alternative possibility that abnormal expression of a growth factor or its receptor could represent a primary event in the genesis of these tumours cannot be excluded.

1.2.5.3 Growth Factors Identified in Fibroids

Several growth factors and their receptors have now been identified in both myometrium and leiomyomas. Those that have received the most attention in the literature include transforming growth factor (TGF)-ß, bFGF, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF).

1.2.5.3.1

Transforming growth factor-ß.

The TGF-ß superfamily includes more than 30 structurally related polypeptide growth factors (Miyazono, 2000), which are multifunctional cytokines that can act both as inhibitors and stimulators of cell replication (Arici and Sozen, 2000). Within this large family of related factors is the TGF-ß subfamily, which is composed of three major isoforms (Massague, 1998) of particular interest with regard to fibroids, because they are capable not only of promoting mitogenesis but also of upregulating the synthesis of many components of the extracellular matrix, leading to fibrosis (Lyons and Moses, 1990). Both of these features are characteristic of uterine fibroids. Expression of all three types of TGF-ß, as well as TGF-ß receptors I-III, has been detected in human myometrial tissue (Chegini *et al.*, 1994; Tang *et al.*, 1997). One study (Arici and Sozen, 2000) found that the TGF-ß3 mRNA levels in leiomyomas were 3.5-fold higher than in the myometrium, and similarly, Lee and Nowak found TGF-ß3 expression to be elevated in leiomyomas compared with matched myometrium (Lee and Nowak, 2001). In contrast, no significant difference was observed between fibroids and myometrium in TGF-ß1 mRNA abundance (Vollenhoven *et al.*, 1995). Although these data suggest that TGF-ß3 could be important in uterine leiomyoma growth by stimulating cellular proliferation and the production of extracellular matrix, the effects of TGF-ß may be either stimulatory or inhibitory, depending upon multiple factors, including the specific target cell, the concentration of TGF-ß, and the presence of other growth-regulatory molecules. In low concentrations, both TGF-ß1 (Battegay *et al.*, 1990) and TGF-ß3 (Arici and Sozen, 2000) have elicited significant increases in smooth muscle cell proliferation, whereas at higher concentrations this effect has not been observed. Mitogenesis induced in cultures of aortic smooth muscle cells by TGF-ß appears to be mediated indirectly through stimulation of autocrine secretion of

PDGF, whereas higher concentrations of TGF-ß result in downregulation of PDGF receptors (Battegay *et al.*, 1990). An observed striking increase of TGF-ß3 mRNA levels in luteal phase leiomyoma samples compared with those in the follicular phase suggests a pivotal role of progesterone in the regulation of TGF-ß3 expression (Arici and Sozen, 2000). In contrast, no variation was observed in one study in the expression of TGFß mRNAs and proteins in myometrial tissue during the menstrual cycle (Chegini *et al.*, 1994), and other investigators concluded that TGF-ßs had no significant effect on myometrial cell proliferation (Tang *et al.*, 1997). In view of the probable role of this growth factor in fibroid pathophysiology, it is of particular interest that the gene coding for TGF-ß3 is located near the 14q23-24 breakpoints (Andersen, 1998), one of the most common translocation sites identified in cytogenetic studies of fibroids.

1.2.5.3.2

Basic fibroblast growth factor (bFGF).

Basic fibroblast growth factor causes proliferation of smooth muscle cells, including leiomyoma and myometrial cells (Stewart and Nowak, 1996), and also promotes angiogenesis. This factor can also bind to a component of the extracellular matrix (Mangrulkar *et al.*, 1995; Dixon *et al.*, 2000). In one study there was much stronger immunohistochemical staining for bFGF in fibroids than in the myometrium because of the large amount of extracellular matrix in uterine myomata; this finding led the authors to conclude that large quantities of bFGF are stored in the extracellular matrix of these tumours (Mangrulkar *et al.*, 1995). In addition, increased expression of bFGF mRNA was found in the leiomyomas compared with the myometrium. Some immunoreactivity for the FGF type 1 receptor in the extracellular matrix of leiomyomas has been demonstrated,

although the cellular staining for the receptor was greater in the myometrium than in the leiomyomas (Anania *et al.*, 1997).

Thus, apparently both TGF-ß3 and bFGF are overexpressed in leiomyomas compared with matched myometrium, and both factors may contribute to the enhanced growth of leiomyomas. Indeed, Stewart and Nowak feel that these two factors may be central to the pathogenesis of uterine leiomyomas (Stewart and Nowak, 1996).

1.2.5.3.3

Epidermal growth factor (EGF).

Epidermal growth factor is mitogenic for the cells of both myometrium and leiomyomas in tissue cultures (Fayed *et al.*, 1989). Equally important, and possibly a unique feature of this factor, is its apparent upregulation in fibroids by progesterone (Maruo *et al.*, 2000). The concentration of EGF mRNA in leiomyomas is similar to that of the myometrium during the follicular phase but significantly elevated in leiomyomas during the luteal phase, whereas the concentration in the myometrium remains essentially unchanged (Harrison-Woolrych *et al.*, 1994). Because the mitotic activity of leiomyomas is maximal during the luteal phase of the cycle, this finding suggests that the production of EGF may be one mechanism through which progesterone stimulates mitotic activity in fibroids.

The mRNA for the EGF receptor has been detected in both myometrial and leiomyoma cells (Yeh *et al.*, 1991). Although the levels of EGF receptors are not significantly higher in leiomyomas than in the myometrium and do not seem to fluctuate during the menstrual cycle (Hofmann *et al.*, 1984; Chegini *et al.*, 1986; Lumsden *et al.*, 1988), there is a sharp reduction of EGF-receptor levels in the leiomyomas but not in the myometrium of women treated with GnRH agonists

prior to surgery (Lumsden *et al.*, 1988). These data suggest that the EGF receptors in fibroids are more sensitive to regulation by the ovarian sex steroids than those in the myometrium. More importantly, because the reduction of EGF receptor levels correlates with shrinkage of the fibroids as a result of the GnRH-agonist therapy, it suggests that the effects of sex steroids on fibroid growth may be mediated, in part, by EGF (Rein and Nowak, 1992). In this regard, it is of interest that in cultures of leiomyoma cells, oestradiol augmented the expression of the EGF receptor, whereas progesterone increased the expression of EGF, suggesting to the authors that oestradiol and progesterone may act in combination to stimulate proliferation in fibroids through the induction of EGF and its receptor (Maruo *et al.*, 2000).

1.2.5.3.4

Platelet-derived growth factor (PDGF).

Platelet-derived growth factor is a potent mitogen for vascular smooth muscle cells and another of the heparin-binding growth factors along with bFGF and VEGF. Because of the capacity of these factors to bind to heparin, they may become sequestered in the extracellular matrix, which is typically abundant in fibroids and may therefore serve as a reservoir for these growth factors (Nowak *et al.*, 1999). The mRNA for PDGF is expressed in leiomyomas, but the levels are similar to those found in the myometrium (Boehm *et al.*, 1990). On the other hand, significantly more PDGF receptor sites per cell are seen in leiomyomas than in the myometrium, although the PDGF receptor binding affinity in the tumour cells is lower than that of the myometrium (Fayed *et al.*, 1989).

Perhaps the most interesting aspect of PDGF in leiomyomas, however, may not be its growth factor role, acting in isolation, but rather its action in conjunction with other growth factors such as EGF and IGFs. For example, when normal myometrial cells are treated with both PDGF and EGF, there is a synergistic decrease in DNA

synthesis, whereas treatment of leiomyoma cells with both factors results in an additive increase in DNA synthesis (Fayed *et al.*, 1989). Insulin and PDGF exert an additive effect upon DNA synthesis in myometrial and leiomyoma cells **(Fayed *et al.*, 1989)**; previous studies using other cell systems have found that target cells must have prior exposure to a competence growth factor such as PDGF before IGF stimulation will promote movement through the cell cycle (Pledger *et al.*, 1978; Stiles *et al.*, 1979).

1.2.5.3.5

Vascular endothelial growth factor (VEGF).

Five VEGF isoforms have been identified (Neufeld *et al.*, 1999). All but one (VEGF-121) contain heparin-binding regions that can mediate binding to the extracellular matrix (Hyder *et al.*, 2000), which may thus serve as a reservoir for this factor as with the other heparin-binding factors bFGF and PDGF. Although VEGF seems to be a highly specific mitogen for vascular endothelial cells, VEGF mRNA and VEGF protein expression have now been identified in the smooth muscle cells of both myometrium and leiomyomata (Harrison-Woolrych *et al.*, 1995; Dixon *et al.*, 2000), and VEGF receptors have been demonstrated in the smooth muscle cells of the myometrium (Brown *et al.*, 1997). Leiomyomata apparently do not have significantly different levels of VEGF mRNA than the myometrium, do not exhibit differences in VEGF mRNA levels between the proliferative and secretory phases of the cycle, and show similar levels of VEGF mRNA after treatment with a GnRH analog (Harrison-Woolrych *et al.*, 1995). Despite these findings, and evidence that VEGF is not mitogenic to smooth muscle cells (Ferrara *et al.*, 1992), interest remains in the potential role of this factor in fibroid growth, for several reasons. VEGF stimulates angiogenesis, which is essential for actively growing tumours, and VEGF is the most potent agent known for increasing capillary permeability, which

could enhance the growth of fibroids by increasing their nutrient supply. VEGF could also have an indirect effect by inducing the proliferation of endothelial cells, which themselves produce a number of growth factors. In addition, VEGF acts synergistically with fibroblast growth factor (FGF) (Hyder *et al.*, 2000), and it can release the angiogenic factor bFGF from its storage on heparan sulfates of the extracellular matrix (Jonca *et al.*, 1997), with the resulting combination of the two angiogenic mitogens having a synergistic effect on angiogenesis (Goto *et al.*, 1993; Asahara *et al.*, 1995). Further, the resulting availability of bFGF permits the expression of its mitogenic effect upon the smooth muscle cells.

1.2.5.3.6

Insulin-like growth factor (IGF).

The IGFs have received considerable attention in the literature. The family of IGFs consists of two IGFs (IGF-I and IGF-II), two cell membrane receptors (IGF-IR and IGF-IIR), and six IGF binding proteins (Yu and Berkel, 1999). Thus, the actions of the IGFs are mediated through the IGF receptors, primarily IGF-IR, and are regulated by the IGF-binding proteins. The IGFs are produced by most tissues of the body, are abundant in the circulation, and have the potential to act through endocrine, autocrine, and paracrine mechanisms (Cohick and Clemmons, 1993). These factors are structurally related to proinsulin and promote cellular proliferation, differentiation, and cell survival (Strawn *et al.*, 1995; Yu and Berkel, 1999). Evidence exists for dissimilar roles of the two IGFs, in that IGF-II appears to be primarily responsible for the terminal differentiation of skeletal muscle cells and the down-regulation of IGF-I receptor gene expression, whereas IGF-I is responsible for myogenesis (Rosenthal *et al.*, 1994; Strawn *et al.*, 1995). In most situations the IGF binding proteins inhibit the actions of IGFs by blocking their binding to the receptor; in certain circumstances, however, these binding proteins

may be able to enhance the action of IGF-I by binding to it and preventing its degradation, thereby increasing its bioavailability in target tissues (Yu and Berkel, 1999). Several investigators have identified mRNAs for IGF-I and IGF-II and their receptors in both the myometrium and fibroid tumours. IGF-I, but not IGF-II, was mitogenic in leiomyoma cell cultures (Strawn *et al.*, 1995). The levels of IGF-I mRNA were reported higher in leiomyomas than in the myometrium in two studies (Hoppener *et al.*, 1988; Boehm *et al.*, 1990), whereas two other studies concluded that the levels were not significantly different (Gloudemans *et al.*, 1990; Vollenhoven *et al.*, 1993). Increased IGF-I peptide has been detected in some, but not all, leiomyomata compared with myometrium in immunohistochemical studies (Dixon *et al.*, 2000). The variation in relative amounts of IGF-I mRNA reported in these studies may have been due to the heterogeneity that exists among fibroid tumours. In three of these studies (Hoppener *et al.*, 1988; Boehm *et al.*, 1990; Vollenhoven *et al.*, 1993) the mRNA levels of IGF-II were higher in leiomyomas than in the myometrium, whereas one study reported low levels in both tissues (Gloudemans *et al.*, 1990). Giudice *et al.*, (1993) found the IGF-I gene expression to be most abundant in leiomyomata during the late proliferative phase of the cycle, suggesting that oestrogen upregulates this growth factor in leiomyomas; on the other hand, IGF-II gene expression did not vary with the phase of the cycle. Both IGFs can bind to the IGF-I receptor with similar affinity, whereas the IGF-II receptor preferentially binds IGF-II (Van der Ven *et al.*, 1997). The IGF-I receptor mediates most of the biologic actions of both IGF-I and IGF-II (Cohick and Clemmons, 1993), including the mitogenic, metabolic, and cell-survival properties of IGFs through tyrosine kinase signaling activity. The IGF-II/mannose 6-phosphate receptor appears to be a bi-functional receptor serving as both a lysosomal enzyme-targeting system and a suppressor of the action of IGF-II by increasing its degradation (Nissley and Lopaczynski, 1991; Oates *et al.*, 1998). The

levels of IGF-I receptors in leiomyomas have been reported to exceed those of the myometrium in three studies (Tommola *et al.*, 1989; Chandrasekhar *et al.*, 1992; Van der Ven *et al.*, 1997), whereas Chandrasekhar et al. found no difference in the levels of the IGF-II receptors. The levels of neither IGF-I nor IGF-II receptors seem to vary with the stage of the menstrual cycle (Giudice *et al.*, 1993). The conclusion of most of these studies has been that IGF-I may play a mitogenic role in the growth of uterine fibroids because of increased levels of IGF-I receptors and overexpression of the growth factor itself. Lower levels of the IGF binding protein-3 in leiomyomas than in myometrium could also be significant, as this would increase the bioavailability of free bioactive IGF in fibroids (Vollenhoven *et al.*, 1993).

1.2.6 Statement of problem

The literature regarding predisposing risk factors for development of myomas in Ghanaian women is very limited by the paucity of studies available. This is against the background that Ghana, a West African country is thought to have a higher prevalence of fibroids due to it indigenous black populace. There has been a general acceptance in the literature that uterine fibroids are more prevalent in black women than white women (Witherspoon and Butler, 1934). Although this disparity has now been substantiated in a few more current studies, the magnitude of the difference has been less than the factor of 3-9 times sometimes cited (Buttram, 1986; Vollenhoven *et al.*, 1990; Marshall *et al.*, 1997; Day Baird *et al.*, 2003). Some studies have also reported that fibroids developed in black women were often larger, more numerous, and more symptomatic, and developed at a younger age. Although there is a higher prevalence of risk factors, including a higher mean BMI, among black women, these factors could not account for the excessive rate of uterine leiomyomata among premenopausal black women. Although the basis for the higher prevalence among black women is unknown, ethnic differences have been found in circulating oestrogen levels while on control diets, and differences in oestrogen metabolism have been noted (Woods *et al.*, 1996). In addition, significantly lower 2-hydroxyestrone (2-OHE1)/16 α-hydroxyestrone (16 α-OHE1) urinary metabolite ratios have been found in African-American women than in Caucasian women (Taioli *et al.*, 1996), which could also contribute to greater oestrogen exposure, as 2-OHE1 metabolites are devoid of peripheral biologic activity, whereas 16 α-OHE1 is oestrogenic. Whether the difference in oestrogen metabolism might be due to genetic or environmental factors is unknown.

Almost all the studies done so far have been on women in the U.S.A or some other developed country where the environmental factors that are thought to influence the development of fibroids are very different from what pertains in the under

developed world, particularly, Africa. In Ghana, medical records at the Komfo Anokye Teaching Hospital indicate that 5 to 8 women are admitted weekly at the Obstetrics and Gynaecology Department for either hysterectomy or myomectomy due to fibroids. Given the limited availability of diagnostic equipment such as ultrasound facilities and the limited number of clinics that offer gynaecological services in the country, this figure might only represent a small fraction of the actual number of women suffering from fibroid. Women make up more than half the population of Ghana (Census, 2000) and such a medical problem affecting the majority of Ghanaian women should be a matter of concern to all considering the contributions women make to the economy of Ghana.

More and more women in Ghana are accessing higher education and thus are getting jobs that are more sedentary compared to a decade or two ago. This has resulted in more of them gaining higher BMIs and thus attaining those risk factors that are postulated as being the ones that influence the development of fibroids.

Given the fact that most of the studies have been done in the developed world where the environmental risk factors are very different from Ghana where the prevalence is also thought to be higher, there is the need to investigate these risk factors that are believed to influence the development of fibroids in Ghanaian women. Haematological, biochemical, and the demographic characteristics of Ghanaian women suffering from fibroids should be investigated and documented so as to help bridge the gap in information on fibroids between the developed and the under developed world. Such a study will also enable clinicians to manage patients according to the peculiar needs of our women since the difference in environment between the west and Africa could result in difference in patient management and care.

1.2.7 Aim of the Study

The aim of this study is to provide baseline information on the biochemical, haematological, and demographic characteristics of women suffering from fibroids in Ghana.

1.2.7.1 The specific objectives of this study are

- To establish the demographic characteristics of women with fibroids in Ghana
- To assess the association between fibroid development and growth, and the gynaecologic history of the patients
- To assess the relation between the life styles of patients and the risk of development of fibroids among Ghanaian women
- To determine the haematological profile of Ghanaian women with fibroids in relation to the tumour growth and development
- To assess the impact of fibroids on the kidney and liver function of Ghanaian women with fibroids
- To assess the association between serum oestrogen and progesterone levels, and the growth of fibroids in Ghanaian women
- To assess the association of oxidative stress with the development and growth of fibroids among Ghanaian women

Chapter 2

MATERIALS AND METHOD

2.1.1 Subjects

2.1.1.1 Subject selection

A total of two hundred (200) patients diagnosed as having fibroids from clinical history, physical examination, ultrasound examination and also confirmed at surgery to have fibroids were recruited at the obstetrics and gynaecology department of the Komfo Anokye Teaching Hospital for the study. Patients were recruited from premenopausal women aged 20 years to 40 years with benign gynaecological pathology who had been proposed for surgery by gynaecologists at the department of obstetrics and gynaecology at the Komfo Anokye Teaching Hospital. After the informed consent of the subjects had been sought, questionnaires were administered to them and physical examination done by a gynaecologist to determine their suitability or otherwise for the study. Women with obvious hormonal imbalance and chronic or malignant diseases such as diabetes, galactorrhoea, HIV/AIDS, tuberculosis, hepatitis, ovarian tumours and other gynaecological malignancies were excluded. Pregnant women, recently delivered women (delivered less than 6 months) and lactating mothers were also excluded. The questionnaires were also used to determine the socioeconomic background, clinical and gynaecological history (appendix). Ethical clearance was obtained from the committee on human research, publications, and ethics of the Komfo Anokye Teaching Hospital/School of Medical Science, K.N.U.S.T.

2.1.1.2 Control selection

Two hundred (200) control subjects with similar age distribution as the patients were also recruited for the study after obtaining their informed consent. The control subjects were examined to exclude those with fibroids. The exclusion criteria for the control subjects were the same as for the patients. To make sure that

the controls were subjected to the same conditions as the patients, only those who came to the obstetrics and gynaecology theatre for surgery and had all the other qualifications for the study as controls were used. Thus, they mostly were made up of women with vesico-vaginal fistulae (VVF), recto-vaginal fistulae, urethro-vaginal prolapses, old third and fourth degree perineal tears, and patients with hydrosalpinges.

2.1.1.3 Subject and control preparation

Both controls and subjects were given appointments by an obstetrician/gynaecologist for the surgery. They were admitted a day to the surgery at the obstetrics and gynaecology ward of the Komfo Anokye Teaching Hospital.

2.1.2 Blood sample collection

Blood samples were collected at the theatre prior to setting up an intravenous infusion line. The blood samples were collected from the ante-cubital vein before anaesthesia was administered. A rubber tourniquet was applied for less than one minute and the site to be punctured cleaned with 70% methylated spirit. Five (5) ml of blood sample were collected from each subject and 2ml of it was dispensed into a vacutainer® containing two drops of the anticoagulant, ethylenediaminetetraacetic acid (EDTA). This sample was then used for the haematological analysis within three hours of collection. The rest of the blood sample was dispensed into a vacutainer®, allowed to clot and centrifuged at 3000 X g for 10 minutes to obtain the serum which was stored at -20^{0}C until it was later used for the biochemical analysis.

2.1.3 Haematological Assay

Various haematological parameters including white blood cell count (WBC), , haemoglobin concentration (HGB), packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), and platelet concentration (PLT) were determined by an automated blood analyzer CELL-DYN® 1800 (Abbott Laboratories Diagnostics, Abbot Park, Illinois, USA).

2.1.4 The CELL-DYN® 1800

The CELL-DYN® 1800 is an automated, multi-parameter haematology analyzer designed for in vitro diagnostic use in the clinical laboratory. It is menu-driven, controlled by a microprocessor, and comes in an Open System model.

2.1.5 Mode of Operation of the CELL-DYN®1800

2.1.5.1Aspiration

The CELL-DYN® 1800 aspirates approximately 30 µl (30 microliters) of whole blood from an open collection tube that is held under the Sample Aspiration Probe, and transfers the sample to the Pre-Mixing Cup.

2.1.5.2Dilution

A 7.5 milliliter (ml) volume of diluent is added to the Pre-Mixing Cup to obtain a dilution ratio of 1:251. The diluted sample is then divided into two. Hundred (100) µl of the 1:251 sample dilution is aspirated and mixed with an additional 5 ml of diluent in the RBC/PLT mixing chamber and this gives a dilution ratio of 1:12801. A specimen of the 1:12801 dilution is analyzed and this generates results for the red blood cell and platelet parameters. The remainder of the 1:251 sample dilution is then mixed with 1.0 ml of lyse reagent in the WBC Mixing Chamber. The lyse

reagent ruptures the membrane of each red blood cell and this causes the cytoplasm and haemoglobin to be quickly released.

2.1.5.3 Cell Measurement

The CELL-DYN ®1800 uses two independent measurement methods which are Electrical Impedance Method for determining WBC, RBC, and PLT data and the modified methaemoglobin method for determining haemoglobin (Stadie, 1920).

2.1.6 White Blood Cell Count (WBC) Determination

Electrical impedance is used to count the White Blood Cells (WBC) as they pass through the aperture of the von Behrens WBC transducer. As each cell is drawn through the aperture, a change in electrical resistance occurs and this generates an equivalent voltage pulse. The number of pulses sensed during each cycle corresponds to the number of white cells counted. The amplitude of each pulse is essentially proportional to the cell volume.

2.1.6.1 WBC Measurement Process

The 1:251 WBC/HGB dilution is delivered to the WBC mixing chamber where it is bubble-mixed with 1.0 ml of lyse reagent. A metered volume of the lysed sample is drawn through the aperture into the Counting Chamber by vacuum. The WBCs are then counted by impedance.

2.1.7 Haemoglobin concentration (HGB) Determination

A modified methaemoglobin method is used for the colorimetric determination of haemoglobin. A portion of the lysed, diluted sample from the WBC Mixing Chamber is used for HGB estimation. A low-energy Light-Emitting Diode (LED) is used as the light source. The LED shines through the HGB flow cell and a 540 nm narrow-bandwidth filter onto a photo detector. The HGB concentration is directly proportional to the absorbance of the sample.

2.1.8 Platelets (PLTs) Measurement

The 1:12801 RBC/PLT dilution is delivered to the RBC/PLT Mixing Chamber where it is bubble-mixed. A precise volume of the diluted specimen is drawn by vacuum through the aperture into the Counting Chamber. The PLTs are then counted by impedance.

2.1.9 HCT, MCV, MCH and MCHC Determination

The CELL-DYN 1800® determines the mean cell volume (MCV) from the RBC size-distribution data. Haematocrit (HCT) results were calculated from the RBC count and the MCV value as follows:

$$HCT = (RBC \times MCV)/10$$

Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) values were calculated automatically whenever appropriate parameters were measured, for example, red blood cell count (RBC), haematocrit (HCT), and haemoglobin (HGB). Results for the Mean Cell Haemoglobin (MCH) were calculated from the HGB and RBC count as follows:

$$MCH = (Hb/RBC) \times 10$$

Results for the Mean Cell Haemoglobin Concentration (MCHC) were calculated from the HGB and RBC count as follows:

$$MCHC = (Hb/HCT) \times 100$$

2.1.10 Total Iron and Total Iron Binding Capacity (TIBC)

Serum iron assays measure transport iron bound to the protein transferrin. Increase in serum iron levels may indicate increased erythrocyte destruction, decreased erythrocyte formation, increased absorption, or defects in storage

capabilities. Decrease in serum iron levels may indicate iron deficiency or inability to retrieve storage iron. Iron binding capacity is usually increased in iron deficient anemia and decreased in haemochromatosis, malignancies, rheumatic fever, Hodgkin's diseases, and collagen vascular disease (Tietz, 1976).

Most successful iron methodologies remove iron from transferrin, reduce it to the ferrous state, bind it to a chromophore, and quantitate it by measuring the amount of color developed (Henry, 1984). The determination of Iron binding capacity involves adding sufficient iron to saturate transferrin and then determining either the total amount of bound iron or the excess unbound iron. The later procedure is applied to determine Iron Binding Capacity.

The test was done with a kit obtained from Atlas Medical™ (ATLAS Medical, Cambridge, UK) which is designed for the simultaneous determination of iron and iron binding capacity in human serum. Its principle involves the removal of iron from transferrin, reducing it to the ferrous state, binding it to a chromophore, and quantifying it by measuring the amount of color developed The iron in serum is dissociated from its Fe (III) - transferrin complex by the addition of an acidic buffer containing hydroxylamine. This addition reduces the Fe (III) to Fe (II). The chromogenic agent, Ferrozine, forms a highly colored Fe (II) -complex that is measured photometrically at 560 nm.

The unsaturated iron binding capacity (UIBC) is determined by adding Fe (II) ion to serum so that they bind to the unsaturated iron binding sites on transferrin. The excess Fe (II) ions are reacted with Ferrozine to form the color complex, which is measured photometrically. The difference between the amount of Fe (II) added and the amount of Fe (II) measured represents the unsaturated iron binding. The total iron binding capacity (TIBC) is determined by adding the serum iron value to the UIBC value.

For the determination of total iron, 2.5 ml of iron buffer reagent were added to test tubes for samples, controls, and standards whiles 1.0ml of iron free water was added to the test tubes for the blank test tubes and mixed. Then, 0.5ml of samples, controls, standards and iron free water was added to the sample, control, standards and blank test tubes respectively. A spectrophotometer was zeroed with the blank at 560 nm and the absorbances of all the test tubes read (A1 reading). After this, 50 µL iron color reagent were added to all the test tubes and incubated at 37^{0}C for ten minutes. The spectrophotometer was zeroed again with the blank and the absorbances read at 560nm (A2 reading). The total iron was calculated as follows:

$$\frac{(A2 - A1)\ Test}{(A2 - A1)\ standard} \times \text{Concentration of standard} = \text{Total Iron (}\mu g/dL$$

SI Unit Conversion: $\mu g/dl\ x\ 0.179\ =\ mmol/L$

The determination of total iron binding capacity, followed the same procedure as above only that 2.0 ml of unsaturated iron-binding capacity buffer reagent was used instead of the 2.5 ml of iron buffer reagent.

Calculation of total iron binding capacity was done as follows:

$$\left\{\text{Conc. of Std.} - \left[\frac{(A2 - A1)\ \text{Test}}{(A2 - A1)\ \text{Std}}\right]\right\} \times \text{concentration of std} = \text{UIBC } (\mu g/dl)$$

$$TIBC\ (Total\ Iron - Binding\ Capacity): Iron\ Level\ +\ UIBC\ =\ TIBC\ (\mu g/dl)$$

2.2 BIOCHEMICAL ASSAY

Biochemical parameters assayed include Aspartate Aminotransaminase (AST), Alanine Aminotransaminase (ALT), Alkaline Phosphatase (ALP), Gamma Glutamyl Transferase (GGT), Blood Urea Nitrogen (BUN), Creatinine, Sodium (Na^+), Potassium (K^+), Chloride (Cl^-), Total Bilirubin, Direct Bilirubin, Indirect Bilirubin, Total Protein, Albumin, Uric Acid, Total Cholesterol, Low Density Lipoproteins, High Density Lipoproteins, Triglycerides, Progesterone, Oestradiol, Vitamin C and Malondialdehyde.

2.2.1 HORMONAL ASSAYS

Since both oestrogen and progesterone appear to promote the development of leiomyomas in studies done elsewhere (Englund *et al.*, 1998) it was necessary to assess the influence of the two hormones on the growth and development of leiomyomas among Ghanaian women so as to determine any differences with the observations of other studies in the past. To do this, serum circulating levels of oestradiol and progesterone were assayed in subjects with leiomyomas (patients) and those without the condition (controls).

2.2.1.1 PROGESTERONE

Progesterone was assayed using the NoviWell™ progesterone assay kit (HySkill Diagnostics, Bahlingen, Germany) which employs the sandwich enzyme immunoassay (SIA) microtiter method. It is a competitive enzyme immunoassay for the performance of quantitative determination of progesterone in human plasma and serum (Ratcliffe *et al.*, 1988). Assays were carried out as described by the manufacturer. The assay is based on simultaneous binding of hormone to two monoclonal antibodies; one is immobilized on the microplate, the other is soluble and conjugated with horseradish peroxidase (HRP).

The reagents and samples were brought to room temperature before 50 ul standards and samples were dispensed into their respective wells. One well was left for substrate blank. After that, 50 ul of progesterone- HRP conjugate were added to each well with the exception of the one meant for substrate blank. The wells were then covered with a foil and incubated for one hour at 37^0C. When incubation had been completed, the foil was removed, and the content of the wells aspirated and each well washed twice with 300 ul distilled water. Overflows from the reaction wells were avoided. The soak time between each cycle was greater than 5 seconds. At the end of the reaction, the remaining fluid was removed by tapping the strips on tissue paper. Afterwards, 100 ul of tetramethylbenzidine (TMB) substrate solution were dispensed into the wells and incubated for exactly 15 minutes at room temperature in the dark. To stop the reaction, 100 ul stop solution were dispensed into all wells in the same order and at the same rate as for the TMB substrate solution. Any blue colour developed during the incubation turned into yellow. The absorbance of the specimen was measured at 450 nm within 30 minutes after addition of the stop solution. The values of the samples were obtained from a graph constructed using the standards.

2.2.1.2 17β – OESTRADIOL

17β – oestradiol was assayed using the NoviWell™ 17β – Oestradiol assay kit (HySkill Diagnostics, Bahlingen, Germany) which employs the sandwich enzyme immunoassay (SIA) microtiter method. It is a competitive enzyme immunoassay for the performance of quantitative determination of 17β – Oestradiol in human plasma and serum (Ratcliffe *et al.*, 1988). Assays were carried out as described by the manufacturer. The assay is based on simultaneous binding of hormone to two monoclonal antibodies; one is immobilized on the microplate, the other is soluble and conjugated with horseradish peroxidase (HRP).

Having brought the samples and reagents to room temperature, 25 ul of standards and samples were dispensed into their respective wells. Following this, 100 ul oestradiol - HRP conjugate were added to each well. One well was left for substrate blank. The wells were covered with a foil and incubated for 2 hours at 37^0C. When incubation had been complete, the foil was removed, the content of wells aspirated and each well washed twice with 300 ul distilled water. Overflows from the reaction wells were avoided. The soak time between each cycle was greater than 5 seconds. At the end of the reaction, remaining fluid was carefully removed by tapping strips on tissue paper. After this 100 ul of TMB substrate solution were dispensed into wells and incubate for exactly 30 minutes at room temperature in the dark. To stop the reaction, 100ul stop solution was added into all wells in the same order and at the same rate as for the TMB substrate solution. Any blue colour developed during the incubation turned into yellow. The absorbance of the specimen was measured at 450 nm within 30 minutes after addition of the stop solution. The values of the samples were obtained from a graph constructed using the standards.

2.2.2 ASSESSMENT OF LIVER FUNCTION

To assess the effect of the growth and development of leiomyoma on the liver, parameters that were assayed include: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyltranspeptidase (GGT), total bilirubin (T-BIL), direct bilirubin (D-BIL), indirect bilirubin (I-BIL), total-protein (T-PROT), albumin/ globulin ratio. Results of these parameters in the patients were then compared to those of the controls. All reagents were acquired from JAS™ diagnostics, Inc. (JAS Diagnostics, Inc. Miami Florida, USA).

2.2.2.1 ALBUMIN

Since serum albumin levels are helpful as an aid in diagnosing disease states of the liver, they were assayed to assess the effect of the tumour on the liver function of women with the condition. Moderate to large changes in the concentration of albumin have significant effects on the relative amounts of the bound and free concentrations of the ligands it carries. Hypoalbuminemia is very common in many illnesses and results in most instances from impaired synthesis, increased catabolism, reduced absorption of amino acids, altered distribution which may sequester large amounts of albumin in an extravascular compartment, or protein loss by way of urine or faeces (Fischbach, 2000).

Albumin was assayed using the JAS™ diagnostic albumin test kit which is based on a procedure proposed by (Doumas *et al.*, 1971). This is a colorimetric method based on the bromocresol green principle. At a controlled pH, bromocresol green (BCG) forms a coloured complex with albumin. The intensity of colour at 630 nm is directly proportional to albumin content. The instantaneous initial absorbance is obtained as proposed by (Webster, 1977).

$$\boldsymbol{BCG + Albumin \xrightarrow{controlled\ pH} Green\ BCG/Albumin\ Complex}$$

The reagents and samples were brought to room temperature before 2.0 ml of bromocresol were pipetted into test tubes labelled blank, samples and standard. Then 10 ul of samples and standard were added into their respective test tubes and mixed and left to stand for 10 minutes at room temperature. The absorbance of the samples and the standard were read at 630 nm against the reagent blank. The concentrations of the samples were calculated as follows:

$$\left[\frac{Asorbance\ of\ sample}{Absorbance\ of\ standard}\right] \times \text{concentration of std} = \text{albumin conc. of sample}$$

2.2.2.2 Protein

Total protein is useful for investigating and monitoring changes in protein levels caused by various disease states such as chronic inflammatory and lymphoproliferative disorder, malnutrition, immunodeficiency and nephritic syndrome. Hyperproteinemia is particularly useful in diagnosing some liver diseases, multiple myeloma, collagen disorders, and chronic infections and inflammatory states (Fischbach, 2000).

Protein was assayed using the JAS™ diagnostic protein test kit. This is a colorimetric method based on the Biuret reaction. The method is based on the modification of (Gornall *et al.*, 1949). Protein in serum forms a blue coloured complex when reacted with cupric ions in an alkaline solution. The intensity of the violet colour is proportional to the amount of protein present when compared to a solution with known protein concentration.

$$\boldsymbol{Protein + Cu^{2+} \xrightarrow{Alkali} Colored\ Complex}$$

The reagents and samples were brought to room temperature after which 1.0 ml of Biuret reagent were dispensed into test tubes labelled blank, samples and standard. Then 20 ul of samples and standard were added into their respective test tubes and mixed and let to stand for 10 minutes at room temperature. The absorbance of the samples and the standard were read at 540 nm against the reagent blank. The concentrations of the samples were calculated as follows:

$$\left[\frac{Asorbance\ of\ sample}{Absorbance\ of\ standard}\right] \times \text{concentration of std} = \text{protein conc. of sample}$$

2.2.2.3 Alkaline Phosphatase

Alkaline phosphatase is a hydrolytic enzyme found in serum in many distinct forms which originate mainly from bone and liver. Pathological increases are usually associated with hepatobiliary and bone diseases (Fischbach, 2000).

Alkaline phosphatase analysis was done using the JAS™ diagnostic alkaline phosphatase kit. Alkaline phosphatase in serum is determined by measuring the rate of hydrolysis of various phosphate esters under specified conditions. The JAS™ method is based on the kinetic photometric test, according to the IFCC (Shaw *et al.*, 1983). The principle involves the hydrolysis of p-nitrophenyl phosphate by alkaline phosphatase form p-nitrophenol, the rate of formation of which is directly proportional to the levels of alkaline phosphatase. Absorbance is read at 405 nm.

$$\boldsymbol{p-Nitrophenylphosphate + H_2O \xrightarrow{Alk.Phos.} Phosphate + p-Nitrophenol}$$

The reagents, standards and samples were brought to room temperature by placing them on the work bench at room temperature for 30 minutes. One vial of substrate was reconstituted with 20ml of buffer and 1000 ul of the reconstituted reagent pipetted into test tubes labelled; blank and sample respectively. Into the test tube labelled blank, 20 ul of distilled water was added whiles 20 ul of the sample was pipetted into the test tube labelled sample. The tubes were mixed thoroughly and their absorbance read initially at 405 nm wavelength and at 25^{o}C. The absorbances were read again after 1, 2, & 3 minutes and the change in absorbance per minute (ΔAbs) over the course of the reaction calculated. The concentration of alkaline phosphatase in the sample was calculated as follows:

$$ALP\ conc\ =\ \Delta Abs\ x\ 2760\ U/L$$

2.2.2.4 Alanine Amino Transferase (ALT) or Serum Glutamic Pyruvic Transaminase (SGPT)

ALT is widely distributed in tissues with highest concentrations found in the liver and kidney. Elevated levels of ALT are often observed in hepatocellualr diseases such as cirrhosis, hepatitis, or metastatic carcinoma. There can also be elevated levels of ALT with infectious mononucleosis, muscular dystrophy, and dermatomyositis (Fischbach, 2000).

ALT analysis was done using a reagent kit from JAS™ diagnostics. The procedure is based on the method of (Bergmeyer and Horder, 1980). The principle involves ALT catalysing the transfer of the amino group from alanine to 2-oxoglutarate, forming pyruvate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the lactate dehydrogenase (LDH) coupled reaction. Endogenous sample pyruvate is rapidly and completely reduced by LDH during the initial incubation period so that it does not interfere with the assay.

$$L-Alanine + 2-Oxoglutarate \xrightarrow{ALT} Pyruvate + L-Glutamate$$

$$Pyruvate + NADH \xrightarrow{LDH} L-Lactate + NAD^{+}$$

The reagents and samples were brought to room temperature by placing them on the work bench at room temperature for 30 minutes after which 1000 ul working reagent was pipetted into test tubes labelled; blank and sample respectively. Into the test tube labelled blank, 100 ul of distilled water was added and 100 ul of sample was pipetted into the test tube labelled sample and mixed thoroughly and the initial absorbance read at 340 nm wavelength and at 30°C. The absorbance was read again after 1, 2, & 3 minutes and the change in absorbance per minute (ΔAbs)

over the course of the reaction calculated. The concentration of ALT in the sample was calculated as follows:

$$ALT\ conc\ =\ \Delta Abs\ x\ 1746\ U/L$$

2.2.2.5 Aspartate Transaminase (AST) or Serum Glutamyl Oxaloacetic Transaminase (SGOT)

AST is widely distributed with high concentration in the heart, liver, skeletal muscle, kidney and erythrocytes. Disease to any of these tissues such as myocardial infarction, hepatitis, liver necrosis, cirrhosis and muscular dystrophy may result in raised serum levels of AST (Fischbach, 2000).

ALT analysis was done using a reagent kit from JAS™ diagnostics. The principle of test involves AST catalyzing the transfer of the amino group from aspartate to 2-oxoglutarate, forming oxalacetate and glutamate (IFCC, 1986). The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the malate dehydrogenase (MDH) coupled reaction. Lactate dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate which is normally present in serum.

$$\boldsymbol{L-Aspartate\ +\ 2-Oxoglutarate\ \xrightarrow{AST}\ Oxaloacetate\ +\ L-Glutamate}$$

$$\boldsymbol{Oxaloacetate\ +\ NADH\ \xrightarrow{MDH}\ L-Malate\ +\ NAD^{+}}$$

The reagents and samples were brought to room temperature by placing them on the work bench at room temperature for 30 minutes before 1000 ul of the working reagent was pipetted into test tubes labelled; blank and sample respectively. Into the test tube labelled blank, 100 ul of distilled water was added and 100 ul of the

sample was pipetted into the test tube labelled sample and mixed thoroughly and the initial absorbance read at 340 nm wavelength and at 30°C. The absorbance was read again after 1, 2, & 3 minutes and the change in absorbance per minute (ΔAbs) over the course of the reaction calculated. The concentration of AST in the sample is calculated as follows:

$$AST\ conc\ =\ \Delta Abs\ x\ 1746\ U/L$$

2.2.2.6 Gamma Glutamyl Transferase (GGT)

GGT was assayed using a kit from JAS™ (JAS Diagnostics Incorporated, Miami-Florida). The JAS™ method is based on the kinetic photometric test, according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (Shaw *et al.*, 1983). GGT in the sample catalyses the transfer of the glutamyl group from L-gamma-glutamyl-3-carboxy-4-nitroanilide to glycylglycine. The amount of 5-amino-2-nitrobenzoate formed is proportional to GGT activity and may be measured kinetically at 405 nm by the increasing intensity of the yellow color formed.

$$\boldsymbol{L-\gamma-glutamyl-3-carboxy-4-nitroanilide\ +\ Glycylglycine\quad \overrightarrow{Gamma-GT}}$$

$$\boldsymbol{L-\gamma-glutamyl-glycylglycine\ +\ 5-amino-2-nitrobenzoate}$$

The reagents, standards and samples were brought to room temperature by placing them on the work bench at room temperature for 30 minutes before 1000ul of working reagent was pipetted into test tubes labelled; blank and sample respectively. Into the test tube labelled blank, 100 ul of distilled water was added and 100 ul of sample pipetted into the test tube labelled sample and mixed thoroughly and the initial absorbance at 405nm wavelength and at 25°C read against reagent blank. The absorbance was read again after 1, 2, & 3 minutes and

the change in absorbance per minute (ΔAbs) over the course of the reaction calculated. The concentration of alkaline phosphatase in the sample as follows:

$$GGT\ conc\ =\ \Delta Abs\ x\ 2737\ U/L$$

2.2.2.7 Bilirubin

Bilirubin was assayed using a kit from JAS™ (JAS Diagnostics Incorporated, Miami- Florida). The JAS™ direct bilirubin reagent uses an acid diazo method. Conjugated bilirubin reacts with diazotized sulphanilic acid to produce an acid azobilirubin, the absorbance of which is proportional to the concentration of direct bilirubin in the sample and can be measured at 550 nm.

The JAS™ total bilirubin method is based on a modification of the (Pearlman and Lee, 1974) method in which a surfactant is used as a solubilizer. Sodium nitrite is added to sulphanilic acid to form diazotized sulphanilic acid. Bilirubin in the sample reacts with the diazotized sulphanilic acid to produce azobilirubin which absorbs strongly at 550 nm. The absorbance measured at 550 nm is directly proportional to the total Bilirubin concentration in the sample.

The test for total bilirubin and direct bilirubin was performed according to Table 2.1.

Table 2.1 Procedure for determination of serum bilirubin

Pipette into test tubes	Total bilirubin		Direct bilirubin	
	blank	test	blank	test
Reagent R1 (Total bilirubin)	1 ml	1 ml		
Reagent R2 (Direct bilirubin)			1 ml	1 ml
Distilled water	50 ul		50 ul	
Reagent R3 (Nitrite)		50 ul		50 ul
Mix well				
sample	100 ul	100 ul	100 ul	100 ul

The test tubes were then mixed and a stop watch started. For total bilirubin, the test tubes were incubated for more than 5minutes at room temperature whiles for direct bilirubin the test tubes were incubated for exactly 5minutes. The spectrophotometer was zeroed using distilled water. All blanks were read first, and then, the tests were read. The absorbance of the samples was calculated by subtracting the absorbance of the blank from that of the test. The bilirubin concentration of the samples was then calculated as follows:

$$Sample\ bilirubin\ conc.\ (\mu mol/L) = absorbance\ of\ sample \times 195$$

$$Indirect\ bilirubin = total\ bilirubin - direct\ bilirubin$$

2.2.3 Renal Function

To assess the effect of the growth and development of leiomyoma on the kidney function, serum creatinine and blood urea nitrogen (BUN) were assayed. Results of these parameters in the patients were then compared to those of the controls.

2.2.3.1 Creatinine

Creatinine measurements are used in the assessement of renal dysfunction. Elevated creatinine levels are found in renal diseases and insufficiency with decreased glomerular filtration; urinary tract obstruction; reduced renal blood flow including congestive heart failure, shock and dehydration.

Serum creatinine was assayed using a Cromatest™ (Barcelona, Spain) creatinine test kit. This is a kinetic colorimetric method based on a modified Jaffe reaction which is fast, simple and avoids interferences (Fabiny and Ertingshausen, 1971), incorporating a surfactant and other ingredients to minimize protein and carbohydrate interferences. Creatinine reacts with picric acid in alkaline conditions to form a colour complex (yellow-orange) which absorbs at 510 nm. The rate of formation of colour is proportional to the creatinine in the sample.

$$\boldsymbol{Creatinine + Sodium\ Picrate \xrightarrow{Alkali} Creatinine - picrate\ complex}$$

The Working reagent was prepared by mixing one volume of picric acid with one volume of alkaline buffer. The working reagent, samples and standard were pre-incubated to reaction temperature (37^0C). A photometer was set to zero absorbance with distilled water. One millitre of working reagent was aspirated into test tubes labelled samples and standard and 100 ul of standard and samples added into the respective test tubes and mixed gently. A cuvette was inserted into temperature-controlled photometer and stopwatch started. The increase in the absorbance with respect to time of standard and sample at 510 nm after 30 seconds (A_1), and exactly

90 seconds later (A_2) were recorded. The concentrations of the samples were calculated as follows:

$$\frac{(A2 - A1)\ sample}{(A2 - A1)\ standard} \times conc\ of\ standard = conc\ of\ sample\ creatinine$$

2.2.3.2 Blood Urea Nitrogen (BUN)

Determination of urea nitrogen in serum is commonly done as a screening test for renal function, usually in conjunction with the determination of creatinine in serum. Serum urea was assayed using a Cromatest™ (Barcelona, Spain) urea test kit. The present procedure is based on a modification of the method of (Talke and Schubert, 1965). This is a colorimetric method based on the urease/salycilate enzymatic reactions as shown below:

$$\boldsymbol{Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO_2}$$

$$\boldsymbol{NH_3 + \alpha - Ketoglutarate + NADH \xrightarrow{GLDH} L - Glutamate + NAD^+}$$

The reagents and samples were brought to room temperature before 1ml of working reagent was pipetted into test tubes labelled blank, samples and standard. Then 10 ul of samples and standard were added into respective test tubes and mixed well and incubated for 5 minutes at 37^{0}C. The absorbance of the standard and the samples were read at 600 nm against the reagent blank. The urea concentrations of the samples were calculated as follows:

$$\left[\frac{Asorbance\ of\ sample}{Absorbance\ of\ standard}\right] \times \text{concentration of std} = \text{BUN conc. of sample}$$

2.2.4 LIPID PROFILE

The lipids assayed include total cholesterol, triglycerides, high density lipoprotein (HDL), and low density lipoprotein (LDL).

2.2.4.1 Total cholesterol

Total cholesterol was assayed using a kit from Human Diagnostic™ (Wiesbaden, Germany) which employs an enzymatic colorimetric method with lipid clearing factor. The cholesterol is determined after enzymatic hydrolysis and oxidation (Allain *et al.*, 1974). The indicator quinoneimine is formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase (Tamaoku *et al.*, 1982).

$$\textbf{Cholesterol Esters} \xrightarrow{\textbf{Cholesterol Esterase}} \textbf{Cholesterol} + \textbf{Fatty Acids}$$

$$\textbf{Cholesterol} + O_2 \xrightarrow{\textbf{Cholesterol oxidase}} \textbf{Cholest} - 4 - \textbf{en} - 3 - \textbf{one} + H_2O$$

$$2H_2O_2 + \textbf{HBA} + 4\textbf{AAP} \xrightarrow{\textbf{Peroxidase}} \textbf{Quinoneimine}\,(\textbf{red dye}) + 4H_2O$$

The reagents, standards and samples were brought to room temperature and 1 ml of enzyme reagent pipetted into test tubes labelled blank, samples and standard. 10 ul of samples and standard was added into respective test tubes and mixed well and incubated for 5 minutes at 37^{0}C. The absorbance of the standard and the samples were read at 550 nm against the reagent blank. The total cholesterol concentration of the sample was calculated as follows:

$$\left[\frac{Asorbance\ of\ sample}{Absorbance\ of\ standard}\right] \times \text{concentration of std} = \text{TC conc. of sample}$$

2.2.4.2 Triglycerides

Triglycerides were assayed using a kit from Human Diagnostic® (Wiesbaden, Germany) which employs an enzymatic colorimetric method with lipid clearing factor. The present method uses a modified Trinder (Trinder, 1969; Barham and Trinder, 1972b) colour reaction to produce a fast, linear, endpoint reaction (Fossati and Prencipe, 1982; McGowan *et al.*, 1983). Triglycerides in the sample are hydrolyzed by lipase to glycerol and fatty acids. The glycerol is then phosphorylated by ATP to glycerol-3-phosphate (G3P) and ADP in a reaction catalyzed by glycerol kinase. G3P is then converted to dihydroxyacetone phosphate (DAP) and hydrogen peroxide by glycerophosphate oxidase (GPO). The hydrogen peroxide then reacts with 4-aminoantipyrine (4-AAP) and 3, 5-dichloro-2-hydroxybenzen (3,5-DHBS) in a reaction catalyzed by peroxidase to yield a red coloured quinoneimine dye. The intensity of the colour produced is directly proportional to the concentration of triglycerides in the sample.

$$\boldsymbol{Triglycerides + H_2O \xrightarrow{Lipase} Glycerol + Fatty\ Acids}$$

$$\boldsymbol{Glycerol + ATP \xrightarrow{Glycerol\ kinase} G3P + ADP}$$

$$\boldsymbol{G3P + O_2 \xrightarrow{Glycerolphosphate\ oxidase} DAP + H_2O_2}$$

$$\boldsymbol{H_2O_2 + 4AAP + 3,5 - DHBS \xrightarrow{Peroxidase} Quinoneimine\ (red\ dye) + 2H_2O}$$

The reagents, standards and samples were brought to room temperature and 1 ml of monoreagent pipetted into test tubes labelled blank, samples and standard before 10 ul of samples and standard were pipetted into their respective test tubes and mixed well and incubated for 5 minutes at 37^{0}C. The absorbance of the standard and the samples were read at 550 nm against the reagent blank. The triglycerides concentration of the sample was calculated as follows:

$$\left[\frac{Asorbance\ of\ sample}{Absorbance\ of\ standard}\right] \times \text{concentration of std} = \text{Triglyceride conc. of sample}$$

2.2.4.3 HDL and LDL Cholesterols

High density lipoprotein (HDL) Cholesterol was assayed using a kit from Human™ Diagnostic (Wiesbaden, Germany) which employs a precipitant and a standard. The chylomicrons, very low density lipoproteins (VLDL) and low density lipoproteins (LDL) are precipitated by the addition of phosphotungstic acid and magnesium chloride (Warnick *et al.*, 1985; Stein and Myers, 1994). After centrifugation the supernatant fluid contains the HDL fraction, which is assayed for HDL Cholesterol with the HUMAN™ cholesterol liquicolor test kit. The reagents, standards and test samples to room temperature and 1 ml of precipitant pipetted into test tubes labelled samples. Then 500 ul of samples were added into the respective test tubes and mixed well and incubated for 10 minutes at room temperature. The mixture was then Centrifuged for at least for 10 minutes at 4000 g. After the centrifugation the clear supernatant was separated from the precipitant within one hour. One millitre of enzyme reagent (HUMAN™ cholesterol liquicolor reagent) was pipetted into test tubes labelled blank, samples and standard. After that 100 ul of samples and HDL standard were added into respective test tubes and mixed well and incubated for 5 minutes at 37^0C. The absorbance of the standard and the samples were read at 550 nm against the reagent blank. The HDL cholesterol concentration of the sample was calculated as follows:

$$\left[\frac{Asorbance\ of\ sample}{Absorbance\ of\ standard}\right] \times \text{concentration of std} = \text{HDL conc. of sample}$$

LDL Cholesterol is calculated in mmol/L as follows:

$$LDL = TC - \left\{HDL - \left(\frac{TG}{2.2}\right)\right\}$$

2.2.5 Oxidative Stress Markers

2.2.5.1 Ascorbic Acid

Ascorbic acid (vitamin C) was assayed using the micro techniques of clinical chemistry developed by Samuel Natelson in 1961 (Natelson, 1961). Ascorbic acid in plasma is oxidized by Cu^{+2} to form dehydroascorbic acid, which reacts with acidic 2,4- dinitrophenylhydrazine to form a red dishydrazone, which is measured at 520 nm. Ascorbic acid should be analyzed immediately or not latter than 3 hour if the specimen is refrigerated. To start the test, 0.4ml of serum was added rapidly to 1.6ml of 10% trichloroacetic acid and mixed well and allowed to stand at room temperature for five minutes. The mixture was centrifuged at 2000g for five minutes. One millitre of the supernatant was pipetted into the test tube for test. Then 0.4ml of dinitriphenyl hydrazine reagent was pipetted into three test tubes; labelled sample, standard, and blank; and 1ml of sample and standard added to the respective test tubes. One millitre of trichloroacetic acid was added to the test tube labelled blank. The test tubes were stoppered and incubated at 37^0C for three hours. The mixture was then chilled in ice bath after which 1.6ml of cold 65% H_2SO_4 were added and mixed well. The mixture was allowed to stand for 30 minutes at room temperature, and the absorbance of the standard and test read against blank at 520nm in a spectrophotometer. The concentration of ascorbic was calculated as follows:

$$\left[\frac{Asorbance\ of\ sample}{Absorbance\ of\ standard}\right] \times \text{concentration of std} = \text{Vit C conc. of sample}$$

2.2.5.2 Malondialdehyde (MDA) Determination

The method used for this assay was based on that described by Kamal and co (Kamal *et al.*, 1989). MDA levels were determined by the MDA- Thiobarbituric acid (TBA) test which is the colorimetric reaction of MDA and TBA in acid solution. TBA reacted with MDA, a secondary product from lipid peroxidation, which generated an adduct of a red colour, which was measured spectrophotometrically. 0.5 ml of serum was added to 2.5 ml of 20% trichloroacetic acid (TCA) after which 1ml of 0.67% TBA was added to the mixture and incubated at 100^{o}C for 30 minutes. After cooling, the sample was extracted with 4 ml n-butanol and centrifuged at 3000g for 10 min. The absorbances of supernatant were measured at 535 nm and the results were expressed as µmol/l, using the extinction coefficient of 1.56 x 10^{5}l/mmol cm.

2.2.5.3 URIC ACID

Serum uric acid was assayed using a Cromatest™ (Barcelona, Spain) uric acid test kit. This is an enzymatic colorimetric method (Barham and Trinder, 1972a; Tamaoku *et al.*, 1982). The reagents and samples were brought to room temperature. 1ml of monoreagent was pipetted into test tubes labelled blank, samples and standard and 25 ul of samples and standard added into respective test tubes and mixed well and incubated for 5 minutes at 37^{0}C. The absorbance of the standard and the samples were read at 550 nm against the reagent blank. The uric acid concentrations of the samples were calculated as follows:

$$\left[\frac{Asorbance\ of\ sample}{Absorbance\ of\ standard}\right] \times \text{concentration of std} = \text{uric acid conc. of sample}$$

2.3TUMOUR EXTRACTION

The operations were undertaken by obstetrician/gynaecologists of the K. A. T. H. At laprotomy for myomectomy or hysterectomy, the uterine size was estimated in comparison to a gravid uterus. The number of myoma nodules removed were counted and recorded. The locations of the tumours within the uterus were also stated. The total weight of the tumour, that is the weight of all myomas removed at myomectomy or the uterus plus myomas in the case of hysterectomy were taken using a weighing scale.

2.4ANTHROPOMETRIC MEASUREMENTS

The weight of the subject was taken using bathroom weighing scale (Zhongshan Camry Electronics Co. Ltd., Guangdong,China) while barefooted and on light clothing to the nearest Kg. The height of the subject was also measured to nearest centimeter using a Gulick II Tape Measure (model 67020) which was mounted on a wall. The floor of the room where these measurements were done was flat and tiled. The waist circumference of the subject was taken using a measuring tape midway between the inferior angle of the rib cage and the suprailiac crest. The hip circumference of the subject was also taken using a tape measure at the outermost point of the greater trochanters. The body mass index (BMI) was then calculated by dividing the weight of the patient in kilograms by the square of her height in meters. The waist to hip ratio was computed by dividing the waist circumference by the hip circumference.

2.5 STATISTICAL ANALYSIS

The data on haematological, biochemical, anthropometric and oxidative stress markers are presented as mean (± SEM). Data were analyzed by unpaired t-test analysis. The data obtained from questionnaire were analysed by Fisher's exact test. All statistical analysis were done using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.grphpad.com). In all statistical test, a value of $p<0.05$ was considered significant. All graphs were plotted using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.grphpad.com).

Chapter 3

RESULTS

3.1 HAEMATOLOGICAL INVESTIGATION

3.1.1 Haemoglobin

As Table 3.1 shows, analysis of data obtained on haemoglobin using the unpaired t-test revealed a significant difference (t=8.276, *p* <0.0001) between the blood haemoglobin levels of the patients and the control patients. The mean blood haemoglobin level of the patients was 12.83 ± 0.2548 gdl^{-1} and that of the control group was 10.12 ± 0.2045 gdl^{-1}. Using the Pearson's correlation, there was a significant (r^2= 0.8264, *p<0.0001*) between the blood haemoglobin level and tumour weight as shown in Figure 3.1A.

3.1.2 Haematocrit

The analysis of data on haematocrit using the unpaired t-test revealed a significant difference (t=4.334, p<0.0001) between the blood Haematocrit level of the patients and the control subjects. The mean blood Haematocrit level of the patients was 41.46 ± 1.088% and that of the control group was 36.44 ± 0.5012% as shown in Table 3.1. There was a significant correlation (r^2= 0.8105, p= 0.0407) between the blood Haematocrit of the patients and tumour weight using Pearson's analysis as shown in Figure 3.1B.

3.1.3 White blood cell (WBC) count

Unpaired t-test analysis of data revealed an insignificant difference (t=0.4241, p= 0.6721) between the WBC count of the patients and the control group. The mean WBC count of the patients was 5.359 ± 0.2504 x 10^9 L^{-1} and that of the control was 5.209 ± 0.2476 x 10^9 L^{-1} as shown in Table 3.1. The Pearson's correlation analysis showed no significant correlation (r^2= 0.0794, *p*= 0.0823) between the WBC counts of the patients and their tumour weight as shown in Figure 3.1C.

3.1.4 Platelet count

As shown in Table 3.1, unpaired t-test analysis, revealed an insignificant difference (t=1.007, p= 0.3156) between the means of the platelet count of the patients and the control group. The mean platelet count of the patients was 181.9 ± 8.440 x $10^9 L^{-1}$ compared to that of the control group which was 193.6 ± 8.008 x 10^9 L^{-1}. Pearson's correlation analysis showed that there was no significant correlation (r^2= 0.0001, *p*= 0.9230) between the platelet counts of the patients and their tumour weight as shown in Figure 3.2A.

Table 3.1 Haematological indices of controls and patient subjects

Parameters	Controls	Subjects	P value
HB (g dl^{-1})	10.12 ± 0.20	12.83 ± 0.26	0.0001
HCT (%)	36.44 ± 0.50	41.46 ± 1.09	0.0001
WBC (x 10^9 L^{-1})	5.21 ± 0.25	5.36 ± 0.25	0.6721
PLT (x 10^9 L^{-1})	193.60 ± 8.01	181.90 ± 8.44	0.3156
MCV (fL)	80.10 ± 0.79	80.88 ± 0.90	0.5123
MCH (pg)	25.55 ± 0.31	30.72 ± 0.36	*0.0001*
MCHC (g dl^{-1})	24.06 ± 0.39	33.40 ± 0.30	*0.0001*
RBC (x 10^{12} L^{-1})	3.88 ± 0.10	6.018 ± 0.15	0.0001
TI (mmolL^{-1})	16.92 ± 1.02	20.66 ± 0.90	0.0133
TIBC (mmolL^{-1})	66.20 ± 2.10	55.10 ± 2.96	0.0067

HB: Haemoglobin, HCT: Haematocrit, WBC: White Blood Cells, TI: total serum iron, TIBC: total iron binding capacity, PLT: Platelets, MCV: Mean Corpuscular Volume, RBC: Red Blood Cells, MCH: Mean Cell Haemoglobin, MCHC: Mean Cell Haemoglobin Concentration

Data are expressed as mean±SEM. Unpaired t-test analysis of patients compared with controls

3.1.5 Mean Corpuscular Volume (MCV)

The use of unpaired t-test to compare data on patients and control subjects, revealed an insignificant difference (t=0.6567, P= 0.5123) between the MCV of the patients and that of the control group. The mean MCV of the patients was 80.88 ± 0.8974 fL and that of the control group was 80.10 ± 0.7866 fL as shown in Table 3.1. Further analysis of the data using Pearson's correlation method showed an insignificant correlation between the MCV and the weight of tumour (r^2= 0.0013, p= 0.8263) as shown in Figure 3.2B.

3.1.6 Mean Corpuscular Haemoglobin (MCH)

There was a significant difference (t=10.97, *P<0.0001*) between the MCH of the patients and that of the control group using unpaired t-test analysis. The mean MCH value of the patients was 30.72 ± 0.3577 pg and that for the control group was 25.55 ± 0.3090 pg as shown in Table 3.1. Using Pearson's correlation analysis there was a significant correlation between the tumour weight and the MCH obtained (r^2= 0.76231, p= 0.0308) as shown in Figure 3.2C.

3.1.7 Mean Corpuscular Haemoglobin Concentration (MCHC)

A significant difference (t=18.99, *P <0.0001)* between the MCHC of the patients and the control group was observed using the unpaired t-test analysis. The mean MCHC value for the patients was 33.40 ± 0.2989 gdL^{-1} and that for the control group was 24.06 ± 0.3901 gdL^{-1} as shown in Table 3.1. Using Pearson's correlation analysis there was a significant correlation between the tumour weight and the MCHC obtained (r^2= 0.7724, p<0.0001) as shown in Figure 3.3A.

3.1.8 Red Blood Cell (RBC) count

A significant difference (t=12.01, P<0.0001) was observed between the patients and the control group after analysis of the data obtained on RBC count using the unpaired t-test analysis. The mean RBC value for the patients was 6.018 ± 0.1479 x 10^{12} L^{-1} and that of the control group was 3.876 ± 0.0996 x 10^{12} L^{-1} as shown in Table 3.1. When the data was further analyzed using Pearson's correlation method, there was a significant (r^2= 0.9417, P<0.0001) correlation between the weight of the tumour and the RBC count as shown in Figure 3.3B.

3.1.9 Total iron and total iron binding capacity

Significant differences were observed for both TI (t=2.747, p= 0.0133) and TIBC (t= 3.062, p= 0.0067) levels between the controls and the patients when the data was subjected to unpaired t-test analysis. The mean TI levels for patients and controls were 20.66 ± 0.8985 $mmolL^{-1}$ and 16.92 ± 1.023 $mmolL^{-1}$ respectively as shown in Table 3.1. The TIBC levels between the two groups were also significantly different as shown in Table 3.1. The patients had a mean TIBC value of 66.20 ± 2.097 $mmolL^{-1}$ whiles the controls had a mean value of 55.10 ± 2.957 $mmolL^{-1}$.

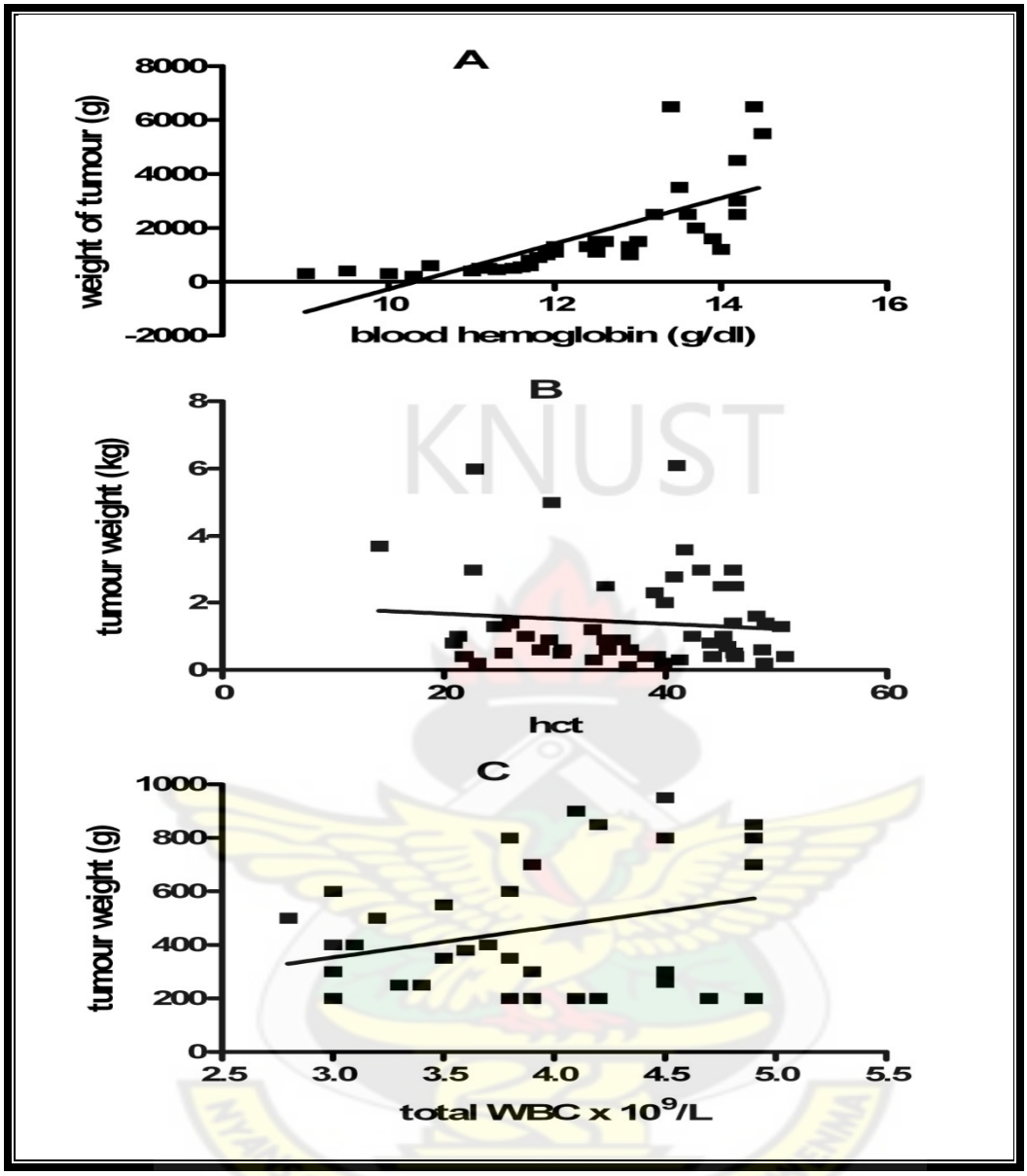

Figure 3.1 The correlation between tumour weight and, A: Patient haemoglobin, *B: Patient haematocrit (HCT) C: Patient total white blood cell count (WBC)*

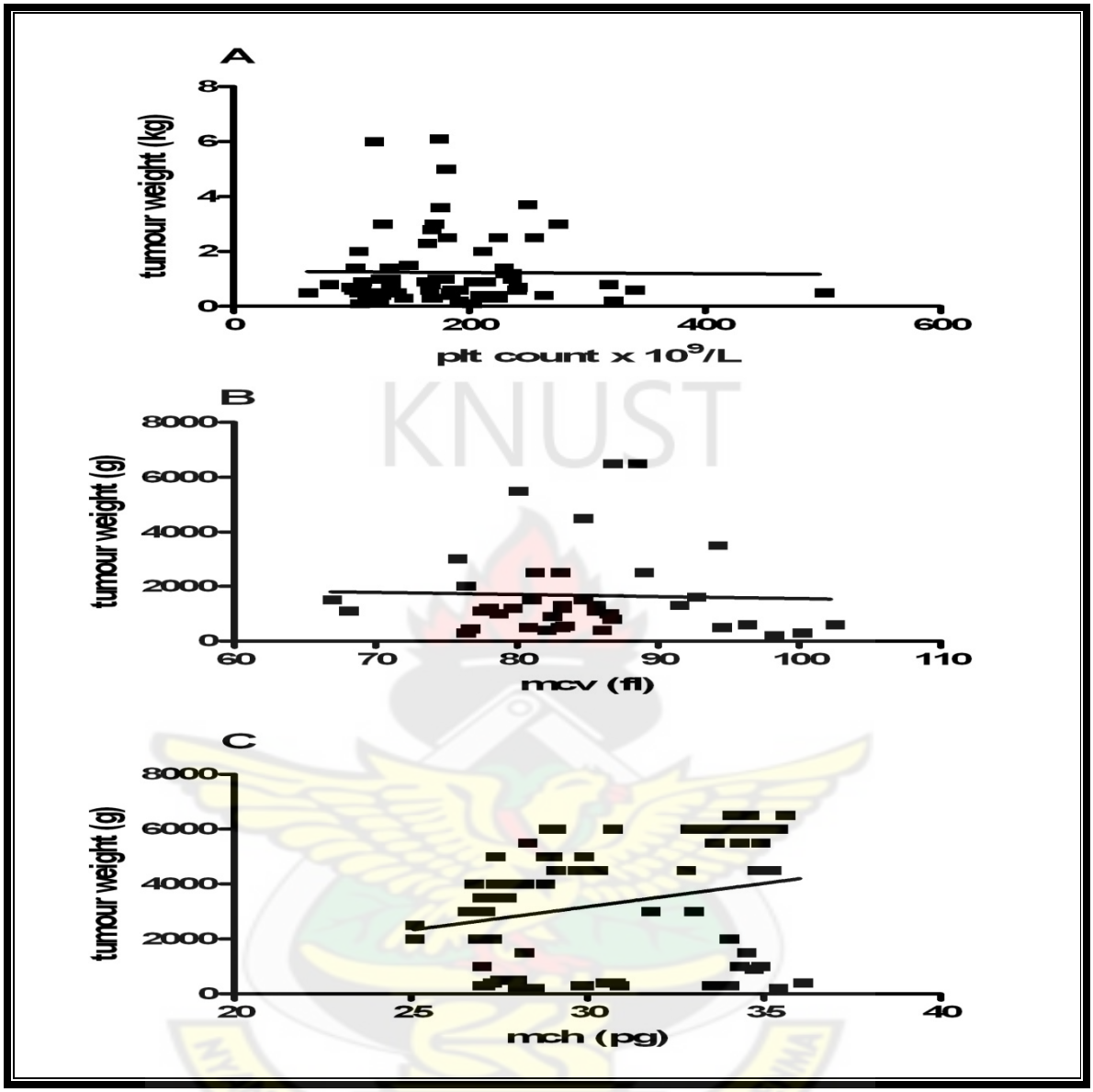

Figure 3.2 Pearson's correlation analysis of tumour weight against, A: Platelet (PLT) count, B: Mean Corpuscular Volume (MCV), C: Mean Corpuscular Haemoglobin (MCH)

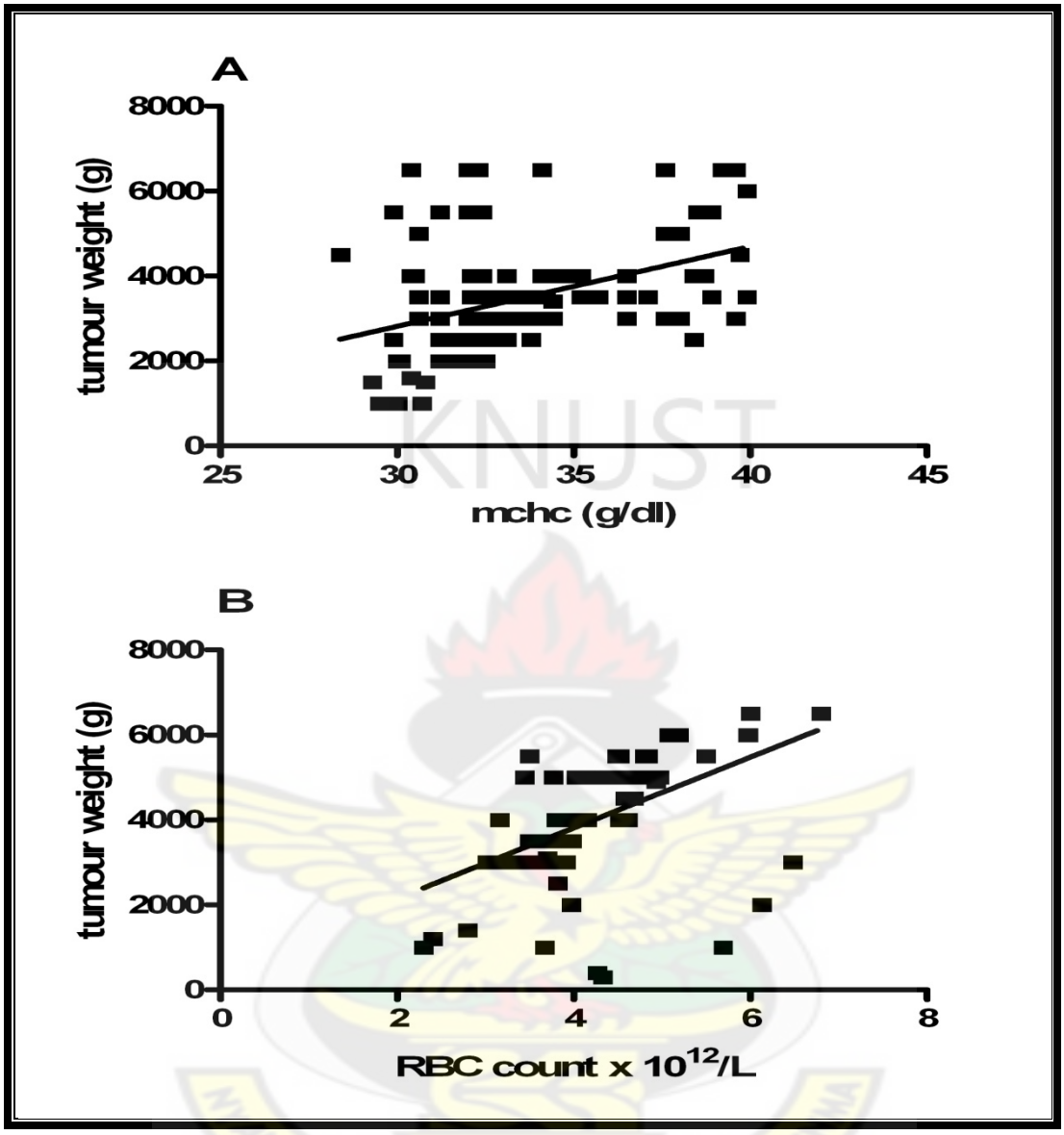

Figure 3.3 Pearson's correlation analysis of tumour weight and, A: the Mean Corpuscular Haemoglobin Concentration (MCHC), B: the Red Blood Cell (RBC) count of patients

3.2 BIOCHEMICAL ASSAYS

3.2.1 LIVER FUNCTION TEST

3.2.1.1 Aspartate Aminotransferase (AST)

Using the unpaired t-test analysis there was a significant difference (t=2.226, p= 0.0277) between the AST of the patients and the control group as shown in Table 3.2. The mean AST value for the patients was 29.78 ± 1.184 UL^{-1} and that for the control group was 25.38 ± 0.8587 UL^{-}. Pearson's correlation analysis however did not show any significant correlation between the serum AST level and the size of the tumour obtained at surgery (r^2= 0.0459, p= 0.0868) as shown in Figure 3.4A.

3.2.1.2 Alanine Aminotransferase (ALT)

The unpaired t-test analysis revealed that there was a significant difference (t=2.369, p= 0.0193) between the ALT of the patients as shown in Table 3.2. The mean ALT value for the patients was 26.81 ± 0.9139 UL^{-1} and that of the control group was 22.91 ± 1.062 UL^{-1}. Pearson's correlation analysis however did not show any significant correlation between the serum ALT level and the size of the tumour obtained at surgery (r^2= 0.0026, p= 0.6571) as shown in Figure 3.4B.

3.2.1.3 Gamma-Glutamyltransferase (GGT)

It was revealed that there was a significant difference (t=2.371, p= 0.0202) between the GGT levels of the patients and the controls after unpaired t-test analysis as shown in Table 3.2. A value of 48.75 ± 2.211 UL^{-1} was the mean GGT value obtained for the patients while 41.08 ± 2.365 UL^{-1} was the mean value for the control group. Pearson's correlation analysis however did not show any significant correlation between the serum GGT level and the size of the tumour obtained at surgery (r^2= 0.0255, p= 0.3248) as shown in Figure 3.4C.

3.2.1.4 Alkaline Phosphatase

Using the unpaired t-test analysis it was shown that there was a significant difference (t=2.119, p= 0.0354) between the serum alkaline phosphatase levels of the patients and the control group. The mean serum alkaline phosphatase value for the patients was 53.69 ± 1.784 UL^{-1} and that of the control group was 48.76 ± 1.492 UL^{-1} as shown in Table 3.2. When the data was further analyzed using Pearson's correlation method, there was no significant (r^2= 0.0088, P= 0.5135) correlation between the weight of the tumour and the serum alkaline phosphatase level as shown in Figure 3.5.

3.2.1.5 Total Bilirubin

There was a significant difference (t=3.363, p= 0.0010) between the total bilirubin levels of the patients and that of the control group using the unpaired t-test analysis as shown in Table 3.2. The mean total bilirubin value for the control group was 12.53 ± 0.9632 $\mu molL^{-1}$ and that of the patients was 17.68 ± 1.093 $\mu molL^{-1}$. Pearson's correlation analysis revealed a significant correlation between the serum total bilirubin level and the size of the tumour obtained at surgery (r^2= 0.6420, p= 0.0314) as shown in Figure 3.6A.

3.2.1.6 Direct Bilirubin

Using the unpaired t-test analysis, there was no significant difference (t=0.0333, p= 0.4073) between the direct bilirubin levels of the patients and those of the control group as shown in Table 3.2. The mean direct bilirubin value for the patients was 3.368 ± 0.4109 $\mu molL^{-1}$ and that of the control group was 2.954 ± 0.3013 $\mu molL^{-1}$. When the data was further analyzed using Pearson's correlation method, there was no significant (r^2= 0.0118, P= 0.3775) correlation between the weight of the tumour and the serum direct bilirubin level as shown in Figure 3.6B.

3.2.1.7 Indirect Bilirubin

Unpaired t-test analysis of the indirect bilirubin data revealed that there was a significant difference (t=2.656, p= 0.0095) between levels of serum indirect bilirubin in patients and the control group as Table 3.2 shows. The mean indirect bilirubin value for the patients was 14.31 ± 0.9727 $\mu molL^{-1}$ and that of the control group was 10.37 ± 1.133 $\mu molL^{-1}$. The weight of the tumour correlated significantly (r^2= 0.6012, p= 0.0324) with the serum indirect bilirubin levels when the data was subjected to Pearson's correlation analysis as depicted in Figure 3.6C.

3.2.1.8 Total Protein

Analysis using the unpaired t-test revealed that there was no significant difference (t=0.5308, p= 0.5965) between the serum total protein of the patients and the control group as shown in Table 3.2. The mean total protein value for the patients was 72.78 ± 1.196 gL^{-1} and that of the control group was 71.43 ± 1.613 gL^{-1}. Using Pearson's correlation method of analysis, there was no significant (r^2= 0.0026, P= 0.7549) correlation between the weight of the tumour and the serum total protein level as shown in Figure 3.7A.

3.2.1.9 Albumin

Serum albumin data obtained was analyzed using the unpaired t-test and it revealed that there was no significant difference (t=1.654, p= 0.1006) between the albumin levels of the patients compared to the control patients. The mean serum albumin level of patients was 39.23 ± 0.8681 gL^{-1} and that of the control group was 36.69 ± 1.208 gL^{-1} as shown in Table3.2. Pearson's correlation method of analysis, did not reveal any significant (r^2= 0.0045, P= 0.6843) correlation between the weight of the tumour and the serum albumin level as shown in Figure 3.7B.

3.2.1.10 Globulin

Serum globulin data obtained was analyzed using the unpaired t-test and it revealed that there was no significant difference (t=1.436, p= 0.1550) between the globulin levels of the patients compared to the control group. The mean serum globulin level of patients was 37.65 ± 1.981 gL^{-1} and that of the control group was 33.33 ± 2.269 gL^{-1} as shown in Table 3.2. Pearson's correlation method of analysis, did not reveal any significant (r^2= 0.0219, P= 0.3614) correlation between the weight of the tumour and the serum globulin concentration as shown in Figure 3.7C.

Table 3.2 Liver function profile for controls and patient subjects

Parameter	Controls	Subjects	p Value
AST (UL^{-1})	25.38 ± 0.86	29.78 ± 1.18	0.0277
ALT (UL^{-1})	22.91 ± 1.06	26.81 ± 0.91	0.0193
GGT (UL^{-1})	41.08 ± 2.37	48.75 ± 2.21	0.0202
AP (UL^{-1})	48.76 ± 1.49	53.69 ± 1.78	0.0354
TB ($\mu molL^{-1}$)	12.53 ± 0.96	17.68 ± 1.09	0.0010
DB ($\mu molL^{-1}$)	2.95 ± 0.30	3.37 ± 0.41	0.4073
IB ($\mu molL^{-1}$)	10.37 ± 1.13	14.31 ± 0.97	0.0095
TP (gL^{-1})	71.43 ± 1.61	72.78 ± 1.20	0.5965
ALB (gL^{-1})	36.69 ± 1.21	39.23 ± 0.87	0.1006
GLO (gL^{-1})	33.33 ± 2.27	37.65 ± 1.98	0.1550

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase,

GGT: Gama-glutymyl transferase, AP: Alkaline phosphatase,

TB: Total bilirubin, DB: Direct bilirubin, IB: Indirect bilirubin

TP: Total protein, ALB: Albumin, GLO: Globulin

Results are expressed as mean±SEM. Unpaired t-test analysis of patients compared with controls

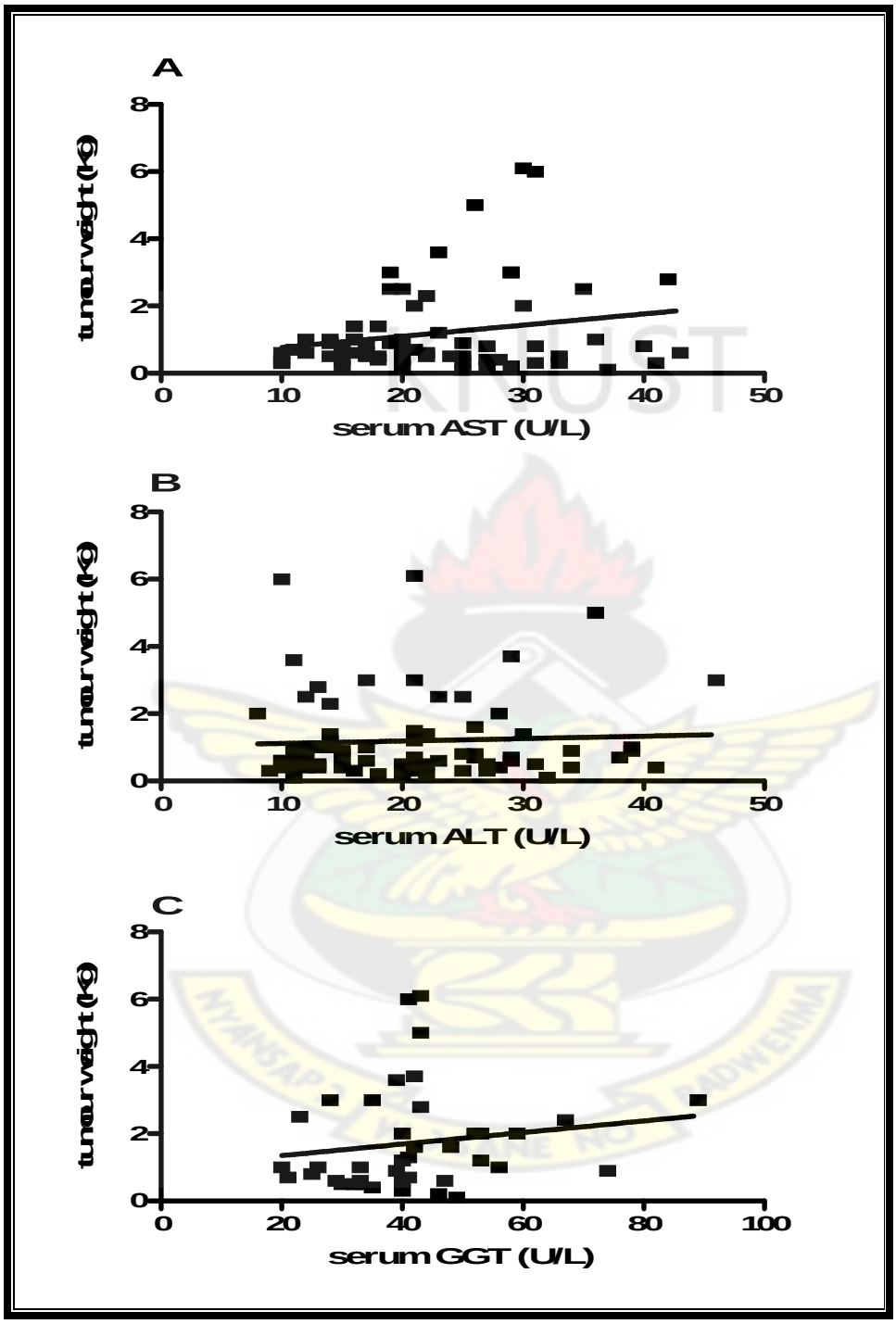
A
tumour weight (kg)
0
2
4
6
8
0
10
20
30
40
50
serum AST (U/L)
B
tumour weight (kg)
0
2
4
6
8
0
10
20
30
40
50
serum ALT (U/L)
C
tumour weight (kg)
0
2
4
6
8
0
20
40
60
80
100
serum GGT (U/L)

Figure 3.4 Correlation analysis of tumour weight against A: Aspartate aminotransferase (AST), B: Alanine aminotransferase (ALT), C: Gama glutymyl transferase (GGT).

All three liver enzymes show no significant correlation with the tumour weight with p values of 0.0868, 0.6571, and 0.3248 for AST, ALT, and GGT respectively.

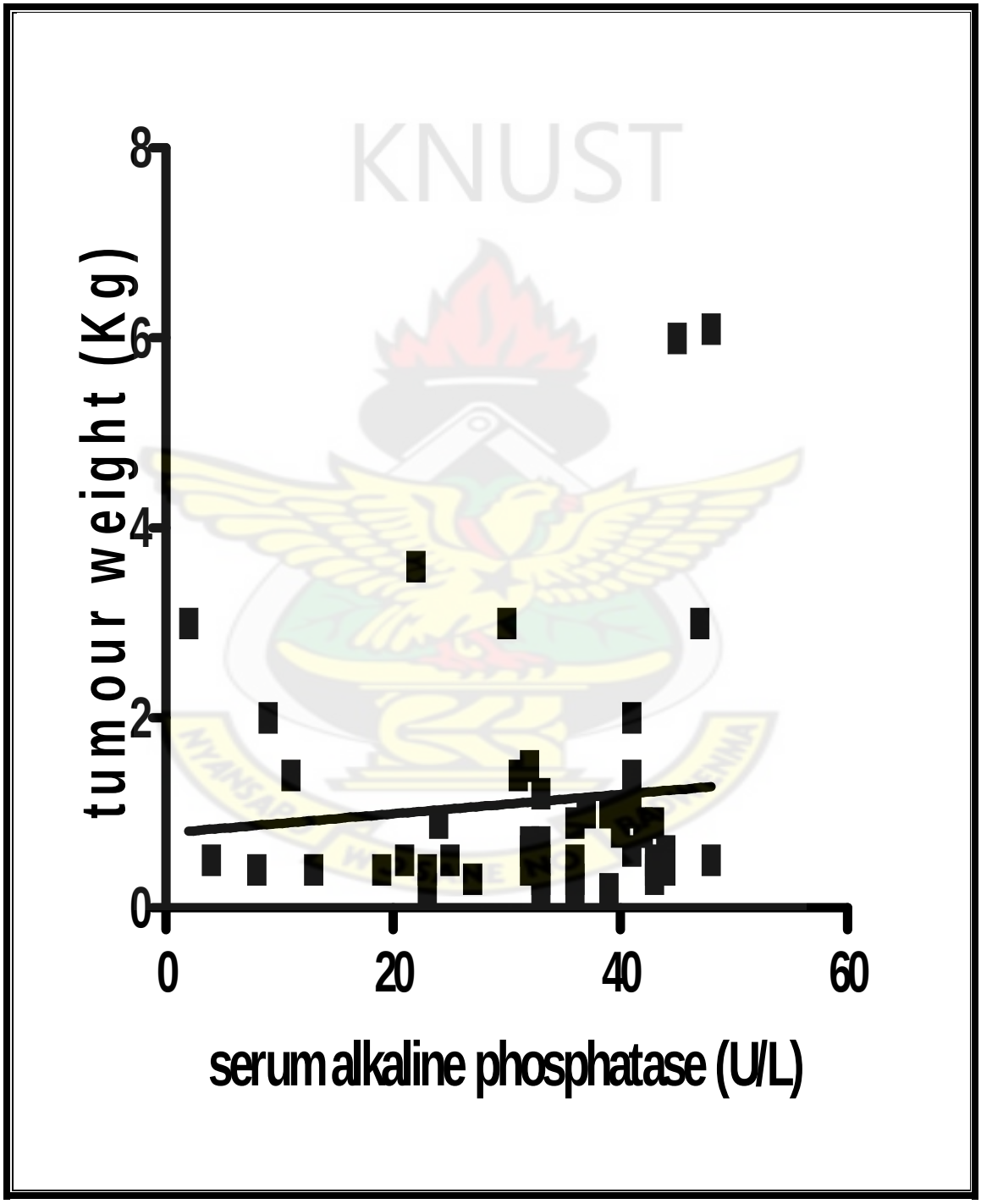

Figure 3.5 The relationship between serum alkaline phosphatase and the weight of fibroid tumour obtained. The correlation analysis shows no significant correlation between the weight of tumour and the serum alkaline phosphatase.

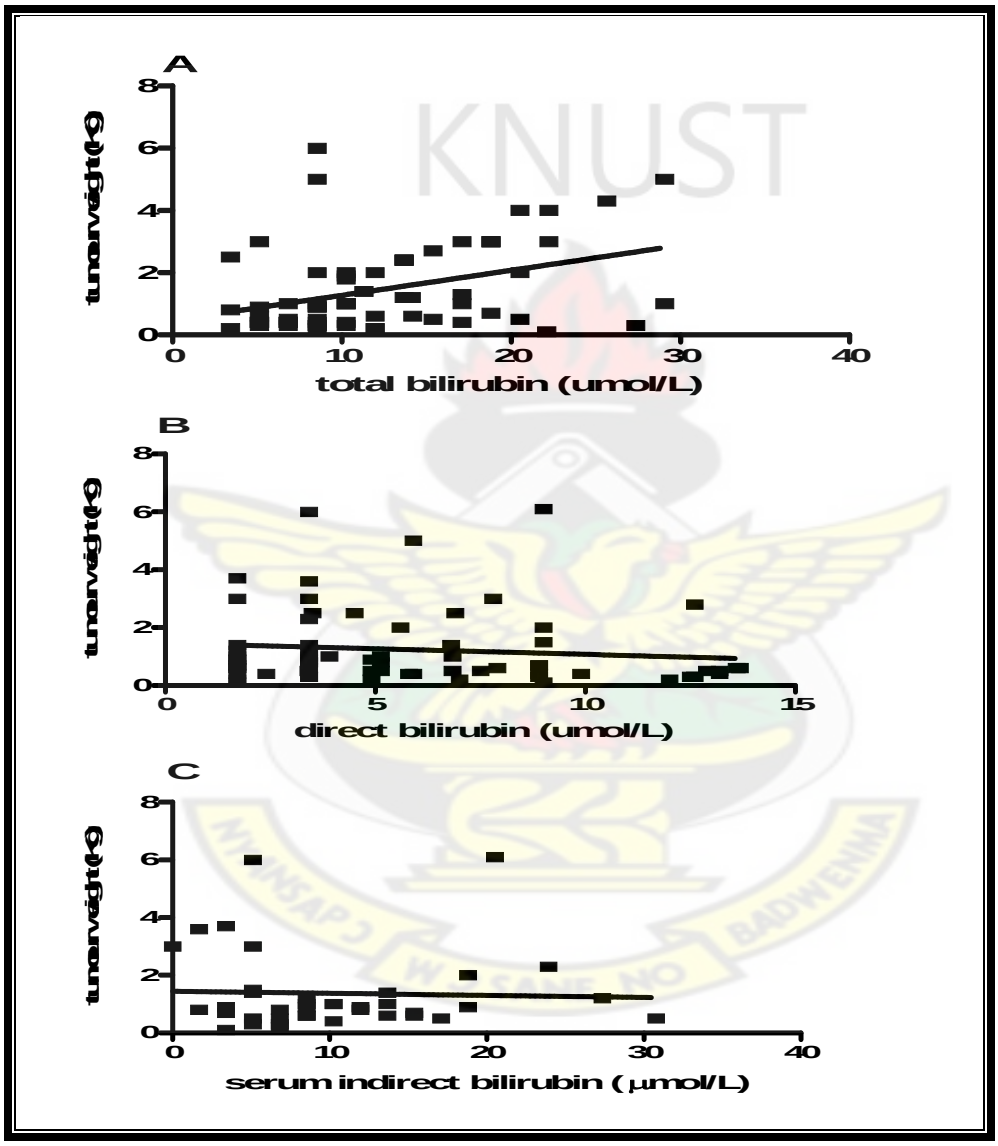

Figure 3.6 Analysis of correlation between tumour weight and; A: serum total bilirubin, B: serum direct bilirubin, C: serum indirect bilirubin; using Pearson's analysis.

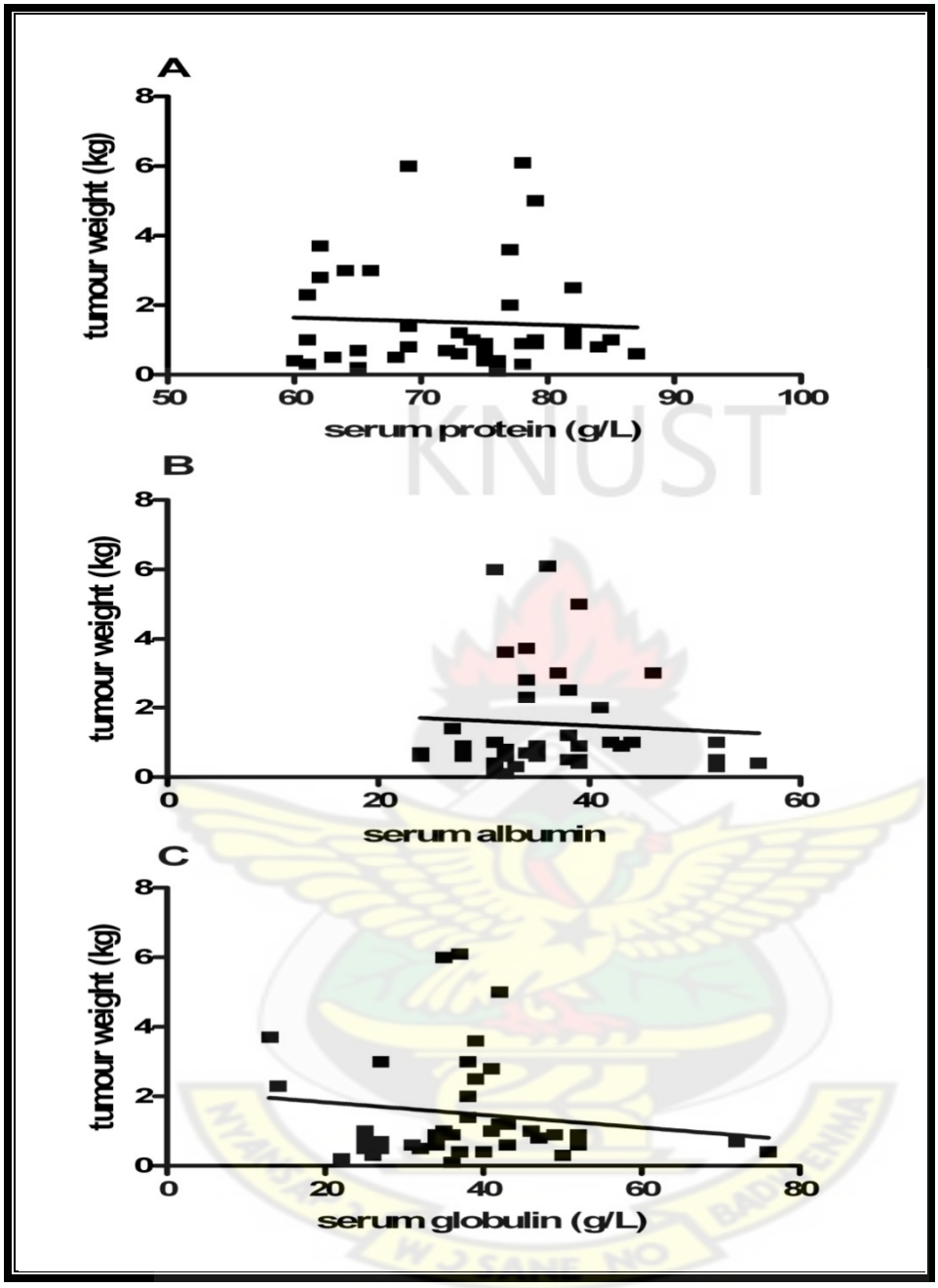

Figure 3.7 Pearson's correlation analysis of A: serum total protein, B: serum albumin, and, C: serum globulin concentrations against the weight of tumour. No significant correlation is observed between the three parameters and the weight of tumour as seen in the p values of 0.7549, 0.6843, and 0.3614 for total protein, albumin and globulin respectively.

3.2.2 RENAL FUNCTION TEST (RFTs)

Table 3.3 Renal function test results for patients and controls subjects

Parameter	Controls	Subjects	p Value
BUN ($mmolL^{-1}$)	3.35 ± 0.11	3.61 ± 0.13	0.1285
Crt ($mmolL^{-1}$)	93.78 ± 0.79	97.98 ± 1.50	0.0143
BUN/Crt	13.05 ± 0.65	14.26 ± 0.16	0.0978
Na^+ ($mmolL^{-1}$)	141.00 ± 0.29	141.90 ± 0.54	0.1417
K^+ ($mmolL^{-1}$)	4.23 ± 0.049	4.25 ± 0.061	0.7881
Cl^- ($mmolL^{-1}$)	105.70 ± 0.56	105.20 ± 0.68	0.5947

BUN: Blood Urea Nitrogen, Crt: Creatinine, Na^+: Sodium ions, K^+: Potassium ions, Cl^-: Chloride ions. Results are expressed as mean±SEM. Unpaired t-test analysis of patients compared with controls

3.2.2.1 Urea (Blood Urea Nitrogen)

The blood urea data was analyzed using the unpaired t-test and it revealed that there was no significant difference between the serum urea levels of the patients and that of the control group (t=1.528, p= 0.1285). The mean serum urea level of the patients was 3.606 ± 0.1264 $mmolL^{-1}$ and that of the control group was 3.354 ± 0.1053 $mmolL^{-1}$ as shown in Table 3.3. When the data was further analyzed using Pearson's correlation method, there was no significant (r^2= 0.0029, P= 0.6497) correlation between the weight of the tumour and the blood urea nitrogen level as shown in Figure 3.8A.

3.2.2.2 Creatinine (Crt)

Unpaired t-test was used to analyze serum creatinine data obtained and it was observed that there was a significant difference between the serum creatinine levels of the patients and the control group (t=2.473, p= 0.0143). The mean serum creatinine level of the patients was 97.98 ± 1.503 $\mu molL^{-1}$ and that of the control was 93.78 ± 0.7882 $\mu molL^{-1}$ as shown in Table 3.3. Using Pearson's correlation method for analysis of the data, there was no significant (r^2= 0.0034, P= 0.6022) correlation between the weight of the tumour and the creatinine concentration as shown in Figure 3.8B.

3.2.2.3 BUN/Crt

Unpaired t-test was used to analyze BUN/Crt data obtained and it was observed that there was no significant difference between the serum BUN/Crt ratios of the patients and the control group (t=1.664, p= 0.0978). The mean serum creatinine level of the patients as can be seen in Table 3.3 was 14.26 ± 0.1599 $\mu molL^{-1}$ and that of the control was 13.05 ± 0.6465 $\mu molL^{-1}$.

3.2.2.4 Sodium

Using the unpaired t-test to analyze the data obtained it was realized that there was no significant difference (t=1.477, p= 0.1417) between the serum sodium levels of the patients and the control group. The mean serum sodium level of the patients was 141.9 ± 0.5419 $mmolL^{-1}$ and that of the control group was 141.0 ± 0.2899 $mmolL^{-1}$ as shown in Table 3.3. Using Pearson's correlation method for analysis of the data, there was no significant (r^2= 0.0172, P= 0.2756) correlation between the weight of the tumour and the serum sodium ion concentration as shown in Figure 3.9B.

3.2.2.5 Potassium

Using the unpaired t-test to analyze the data obtained it was realized that there was no significant difference (t=0.2693, p= 0.7881) between the serum potassium levels of the patients and the control group. The mean serum potassium level of the patients was 4.249 ± 0.06060 mmolL^{-1} and that of the control group was 4.228 ± 0.04947 mmolL^{-1} as shown in Table 3.3. Using Pearson's correlation method for analysis of the data, there was no significant (r^2= 0.0098, P= 0.3699) correlation between the weight of the tumour and the serum potassium ion concentration as shown in Figure 3.9B.

3.2.2.6 Chloride

Using the unpaired t-test to analyze the data obtained it was realized that there was no significant difference (t=0.5335, p= 0.5947) between the serum chloride levels of test patients and the control group. The mean serum chloride level of the patients was 105.2 ± 0.6810 mmolL^{-1} and that of the control was 105.7 ± 0.5579 mmolL^{-1} as shown in Table 3.3. Using Pearson's correlation method for analysis of the data, there was no significant (r^2= 0.0022, P= 0.6719) correlation between the weight of the tumour and the serum chloride ion concentration as shown in Figure 3.9C.

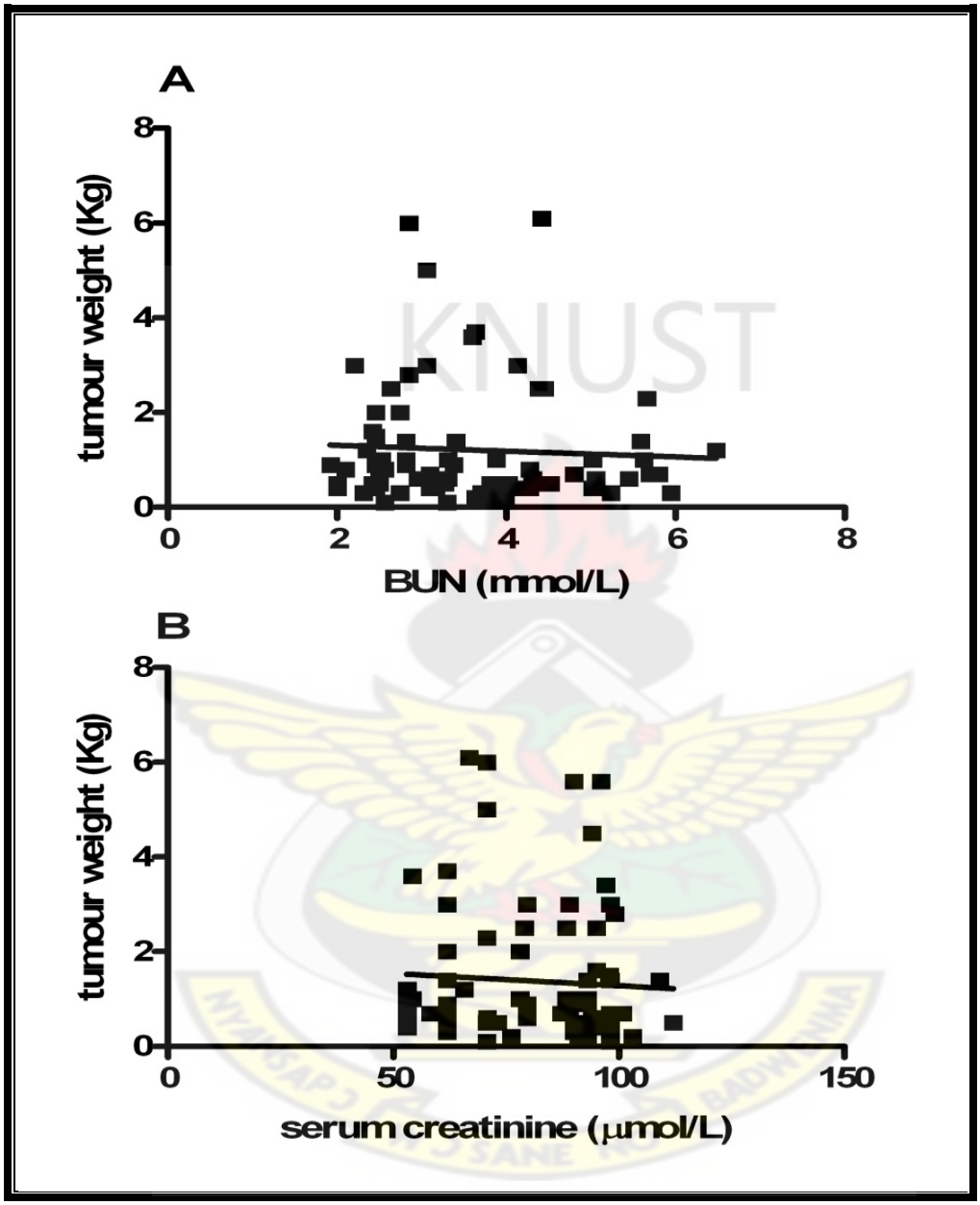

Figure 3.8 Pearson's correlation analysis for data on Blood Urea Nitrogen (BUN) and serum Creatinine concentration versus the weight of tumour obtained from patient subjects. The two graphs show that there is no significant correlation between the serum Blood Urea Nitrogen and serum creatinine on one hand and the tumour weight on the other hand. The p value for the serum BUN is 0.6497 and that of the serum creatinine is 0.6022.

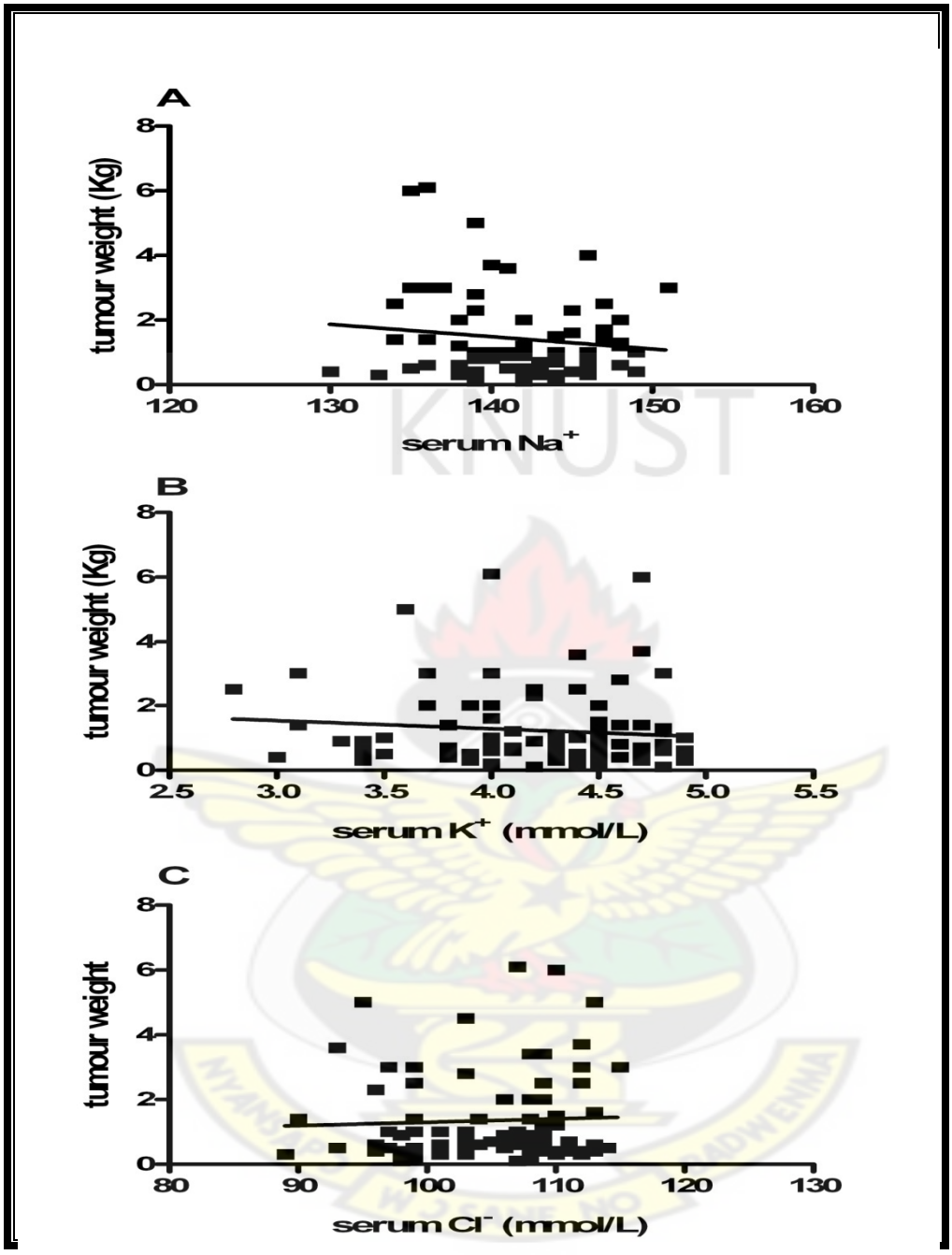

Figure 3.9 Pearson's correlation analysis for tumour weight against serum electrolytes. The three graphs show no significant correlation between the tumour weight and A: serum sodium ions (Na^+), B: serum potassium ions (K^+), C: serum chloride ions with p values of 0.2756, 0.3699, and 0.6719 respectively.

3.2.3 Hormone Assays

3.2.3.1 Progesterone

Data obtained after progesterone assay was analyzed using the unpaired t-test and was revealed that there was a significant difference (t=2.042, p= 0.0448) between the serum progesterone levels of the patients and those of the control group. The mean progesterone level of the patients was 106.5 ± 6.255 $ngml^{-1}$ and that of the control group was 84.63 ± 8.949 $ngml^{-1}$ as shown in Figure 3.10. The Pearson's correlation analysis showed no significant correlation (r^2= 0.4161, p<0.2001) between the serum progesterone levels of the patients and their tumour weight as shown in Figure 3.11. When the subjects were divided into those who were in the secretory phase of the menstrual cycle and those who were in the proliferative phase the patients again recorded higher serum progesterone levels compared to the controls as shown in figure 3.19A and figure 3.20A.

3.2.3.2 Oestradiol

The unpaired t-test analysis of the data obtained from the oestradiol assay showed a significant difference (t=2.525, p= 0.0138) between the serum oestradiol level of the patients and the control group. The mean serum oestradiol level of the patients was 117.4 ± 3.498 $pgml^{-1}$ and the mean serum level of the control group was 103.3 ± 4.348 $pgml^{-1}$ as shown in Figure 3.12. Using the Pearson's correlation analysis it was revealed that there was a significant correlation (r^2= 0.7749, p = 0.0111) between the serum oestradiol concentration of patients and their tumour weight as shown in Figure 3.13. When the subjects were divided into those who were in the secretory phase of the menstrual cycle and those who were in the proliferative phase the patients again recorded higher serum oestradiol levels compared to the controls as shown in figure 3.19B and figure 3.20B.

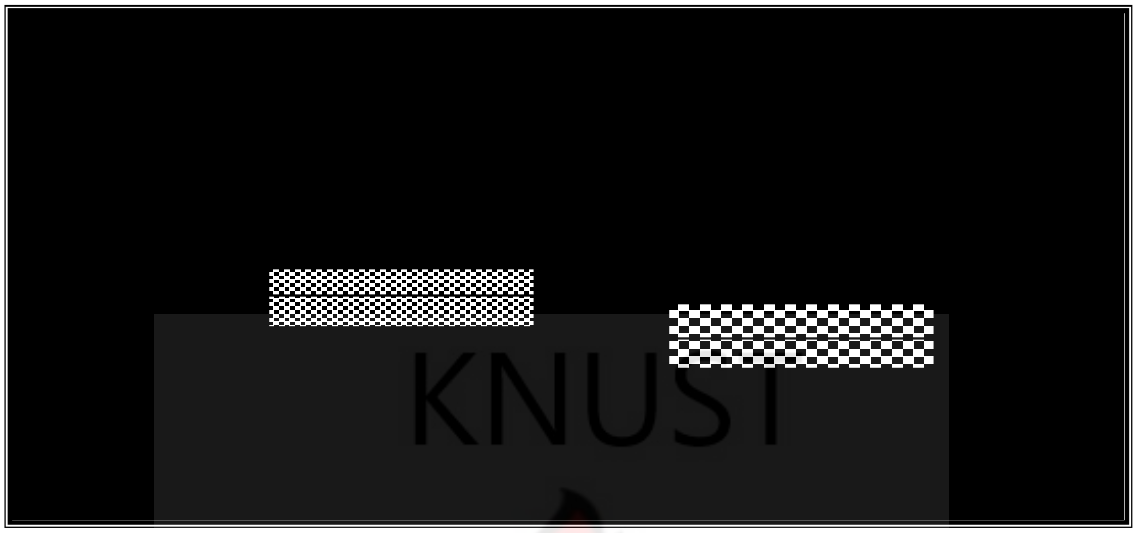

Figure 3.10 T-test comparison of the serum progesterone concentration of patients to controls. Data are presented as mean±SEM. The graph shows a significant difference between the two with a p value of 0.0448.

Figure 3.11 The correlation between tumour weight and serum progesterone concentration as analyzed using Pearson's correlation.

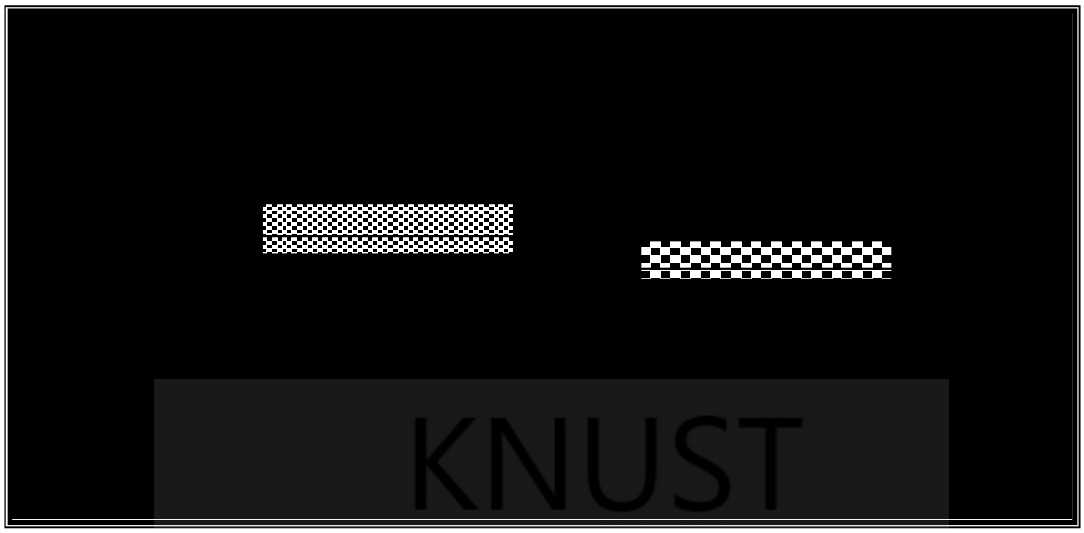

Figure 3.12 T-test analysis of serum oestradiol levels of patients and controls. The results show a significant difference between the serum oestradiol levels of the patients and the controls with a p value of 0.0138. The data are expressed as mean±SEM.

Figure 3.13 Pearson's analysis of data on serum oestradiol showing a significant positive correlation between tumour weight and serum oestradiol concentration.

LIPID PROFILE

Table 3.4 Lipid profile for patients and controls

Parameters	Controls	Subjects	p value
TC ($mmolL^{-1}$)	3.64 ± 0.15	4.35 ± 0.13	0.0015
HDL ($mmolL^{-1}$)	2.73 ± 0.26	3.05 ± 0.20	0.3322
LDL ($mmolL^{-1}$)	1.33 ± 0.19	1.52 ± 0.11	0.3865
TG ($mmolL^{-1}$)	1.42 ± 0.18	2.05 ± 0.18	0.0187
CR	4.51 ± 0.29	4.13 ± 0.26	0.3441

TC: Total Cholesterol, HDL: High Density Lipoproteins, LDL: Low Density Lipoproteins TG: Triglycerides, CR: Coronary Risk. The table presents the results of unpaired t-test analysis of data on serum lipids of patients and controls. The data are expressed as mean±SEM

3.2.3.3 Serum Total Cholesterol

From the unpaired t-test analysis of the data obtained, there was a significant difference (t=3.248, p= 0.0015) between the serum total cholesterol levels of the patients and the control group. The mean serum total cholesterol level of the patients was 4.350 ± 0.1268 $mmolL^{-1}$ and that of the control was 3.642 ± 0.1526 $mmolL^{-1}$ as shown in Table 3.4. There was a significant correlation (r^2 = 0.1016; p= 0.0045) between the serum total cholesterol values of patients and their tumour weight as shown in Figure 3.14A.

3.2.3.4 Serum High Density Lipoprotein (HDL) Cholesterol

Serum HDL data obtained was analyzed using the unpaired t-test and it revealed that there was no significant difference (t=0.9823, p= 0.3322) between the HDL levels of the patients compared to the control patients. The mean serum HDL level of patients was 3.045 ± 0.2019 $mmolL^{-1}$ and that of the control group was 2.725 ± 0.2557 $mmolL^{-1}$ as shown in Table 3.4. Pearson's correlation method of analysis, did not reveal any significant (r^2= 0.0619, P= 0.2901) correlation between the weight of the tumour and the serum HDL level as shown in Figure 3.14B.

3.2.3.5 Serum Low Density Lipoprotein (LDL) Cholesterol

No significant difference (t=0.8761, P= 0.3865) was observed between the patients and the control group after analysis of the data obtained on serum LDL concentration using the unpaired t-test analysis. The mean LDL value for the patients was 1.520 ± 0.1055 $mmolL^{-1}$ and that of the control group was 1.330 ± 0.1895 $mmolL^{-1}$ as shown in Table 3.4. When the data was further analyzed using Pearson's correlation method, there was no significant (r^2= 0.0034, P= 0.8070) correlation between the weight of the tumour and the LDL level as shown in Figure 3.14C.

3.2.3.6 Serum Triglycerides

From the unpaired t-test analysis of the data obtained, there was a significant difference (t=2.457, p= 0.0187) between the serum triglyceride levels of the patients and the control group. The mean serum triglyceride level of the patients was 2.050 ± 0.1837 mmolL^{-1} and that of the control was 1.415 ± 0.1817 mmolL^{-1} as shown in Table 3.4. There was no significant correlation (r^2 = 0.0047; p= 0.7745) between the serum triglyceride values of patients and their tumour weight as shown in Figure 3.15A.

3.2.3.7 Coronary Risk

No significant difference (t=0.9580, P= 0.3441) was observed between the patients and the control group after analysis of the data obtained on coronary risk using the unpaired t-test analysis. The mean coronary risk value for the patients was 4.130 ± 0.2591 and that of the control group was 4.505 ± 0.2935 as shown in Table 3.4. When the data was further analyzed using Pearson's correlation method, there was no significant (r^2= 0.0202, P= 0.5502) correlation between the weight of the tumour and coronary risk as shown in Figure 3.14B.

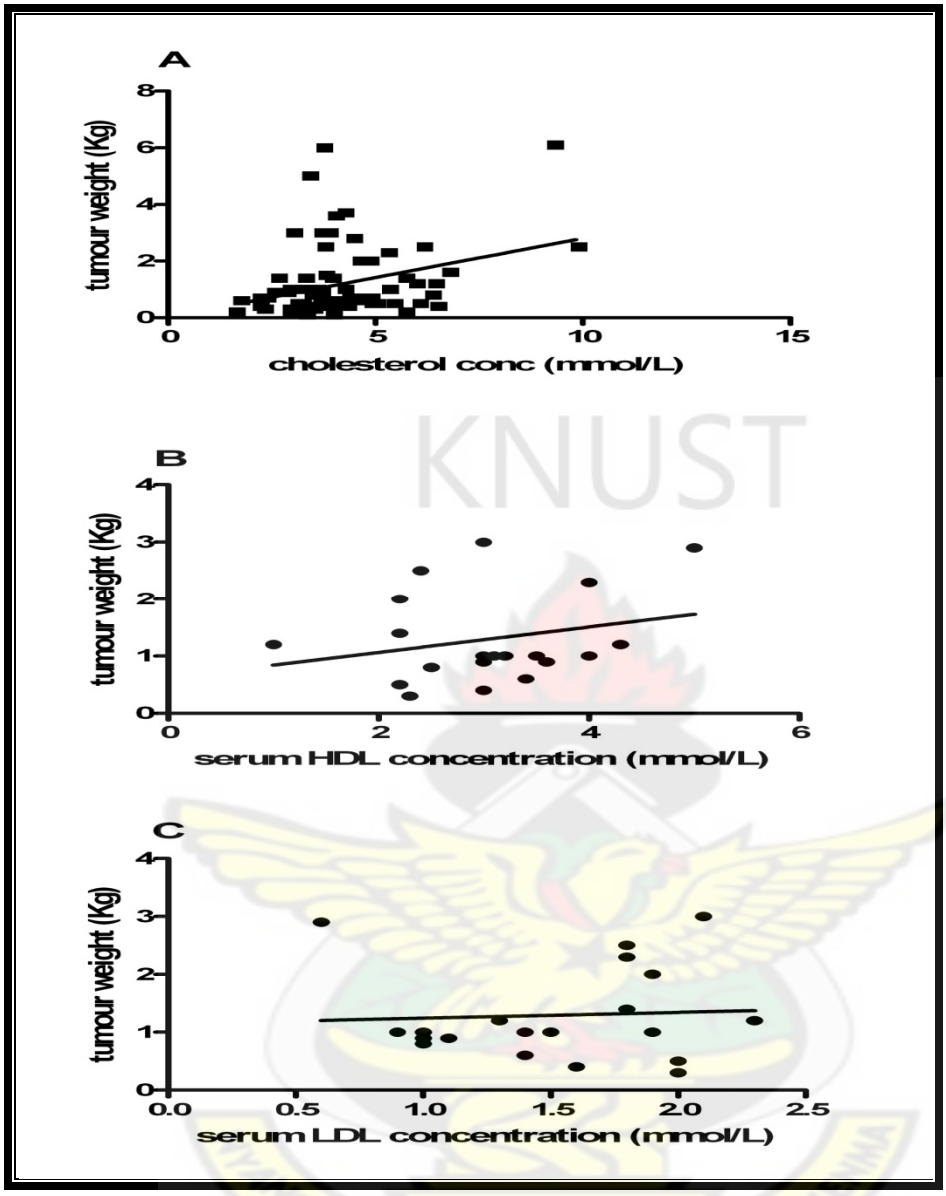

Figure 3.14 The correlation between the tumour weight and; A: serum total cholesterol concentration, B: serum HDL concentration, C: serum LDL concentration; using Pearson's analysis. The results show a significant correlation between the tumour weight and the serum total cholesterol concentration with a p value of 0.0045. Serum HDL and LDL however do not have any significant correlation with the tumour weight as seen in p values of 0.2901 and 0.8070 for HDL and LDL respectively.

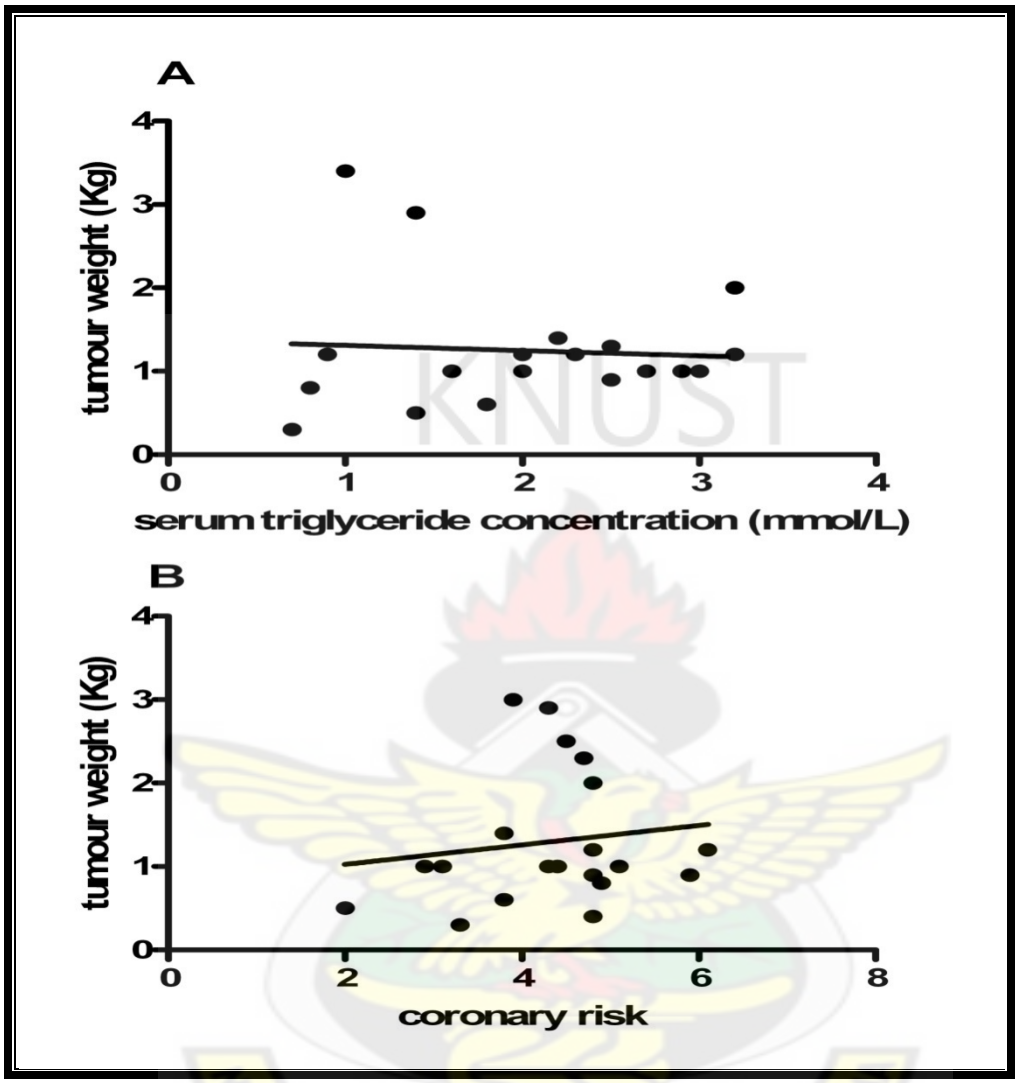

Figure 3.15 Pearson's correlation of tumour weight with A: serum triglycerides concentration, B: Coronary risk of patients.

There is no significant correlation between the tumour weight and the serum triglyceride concentration of patients a p value of 0.7745. There also exist no significant correlation between the tumour weight and the coronary risk of patients as seen in the p value of 0.5502.

3.2.4 Oxidative stress markers

3.2.4.1 ***Malondialdehyde (MDA)***

Comparison of serum malondialdehyde concentrations in patients and control subjects using unpaired t-test analysis yielded a significant difference between the two groups (t=2.489, p=0.0158) with mean values of 1.486 ± 0.1230 $mmolL^{-1}$ and 1.928 ± 0.1277 $mmolL^{-1}$ for controls and patients respectively (Figure 3.16a). MDA levels also had a significant correlation with the serum levels of vitamin C, total cholesterol, LDL, and triglycerides but not with the HDL concentration and the weight of tumour developed (Table 3.5).

3.2.4.2 Vitamin C (Ascorbic Acid)

There was a significant difference between the patients' serum vitamin C levels and those of the controls using t-test analysis (t=3.201, p=0.0021). The patients had a mean serum vitamin C concentration of 34.38 ± 1.432 $\mu molL^{-1}$ compared to a mean serum concentration of 40.33 ± 1.205 $\mu molL^{-1}$ for the controls (Figure 3.16b). Serum vitamin C levels also correlated significantly with the serum levels of MDA, total cholesterol, LDL, and triglycerides as seen in Table 3.5.

Table 3.5 Pearson's correlation coefficients between oxidative stress markers and lipid profiles for controls (lower left-hand side) and patients (upper right-hand side)

	MDA	VC	TC	HDL	LDL	TG	TW
MDA		-0.90***	0.91***	0.23	0.90***	0.95***	0.22
VC	-0.94***		-0.95***	-0.28	-0.93***	-0.95***	-0.28
TC	-0.67***	-0.71***		0.18	0.87***	0.96***	0.46*
HDL	-0.29	0.24	0.16		0.40*	0.43*	0.23
LDL	0.94***	-0.99***	0.70***	0.65**		0.90***	0.52*
TG	0.94***	-1.00	0.71***	0.46*	0.99***		0.55*
TW							

**correlation is significant at the level of 0.05 (two-tailed), **correlation is significant at the 0.01 level (two-tailed), ***correlation is significant at the 0.001 level (two-tailed). MDA: Malondialdehyde, VC: Vitamin C, TC: Total Cholesterol, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, TG: Triglycerides, TW: Tumour Weight*

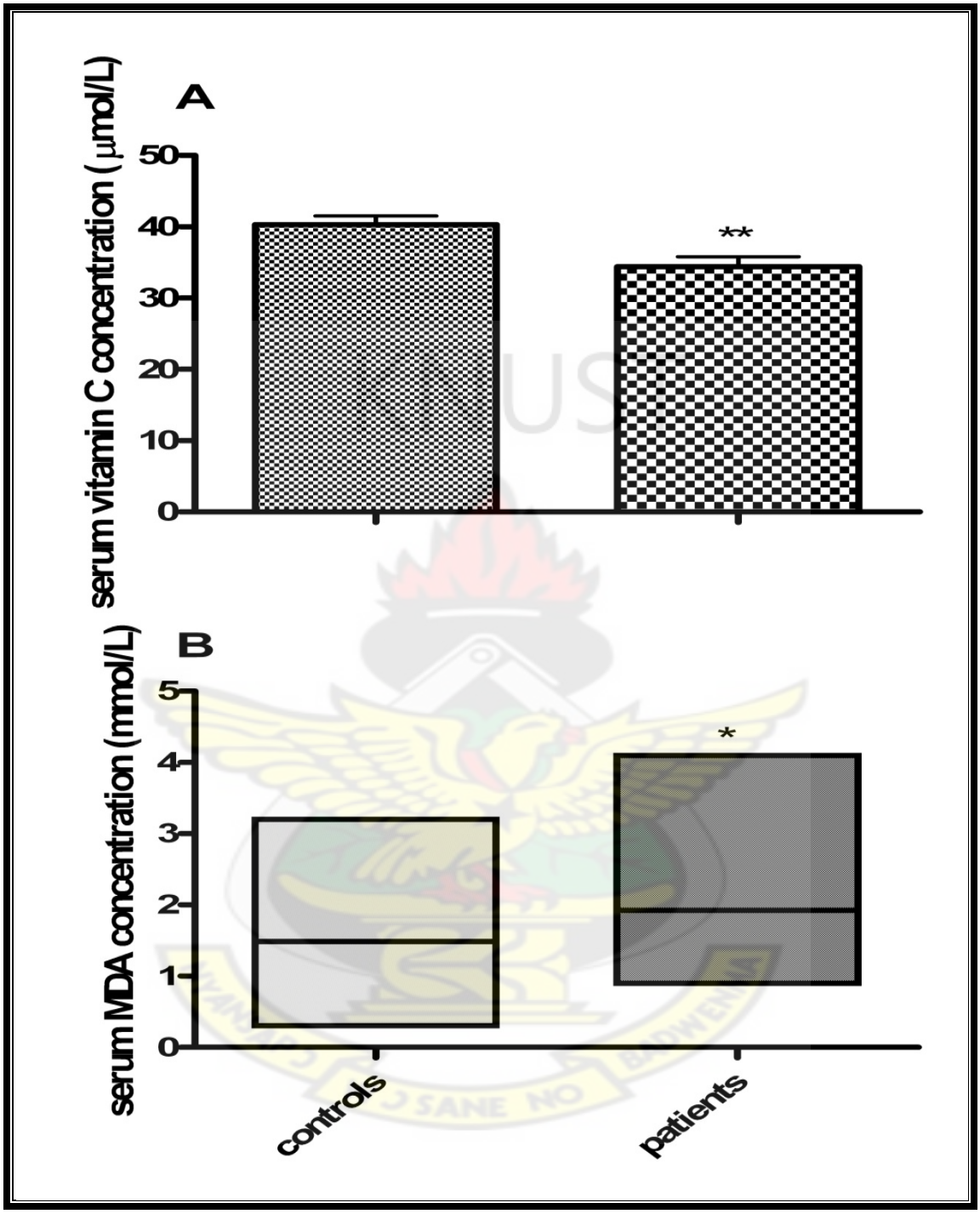

Figure 3.16 Comparison of serum malondialdehyde levels (A) and vitamin C levels (B) between patients and controls. Data are expressed as mean±SEM *Difference in means between the two groups is significant with p<0.05. ** Difference in means between the two groups is significant with p<0.001.

3.2.5 ANTHROPOMETRIC FEATURES

Table 3.6 Unpaired t-test comparison of anthropometric features of patients and controls

Parameter	Control	Patients	p Value
Weight (Kg)	83.55 ± 3.57	92.50 ± 2.22	0.0326
Height (m)	1.59 ± 0.01	1.60 ± 0.01	0.7060
WC (cm)	66.44 ± 3.31	87.44 ± 3.47	0.0001
HC (cm)	76.79 ± 5.23	92.55 ± 3.91	0.0474
BMI (Kgm^{-2})	22.40 ± 0.22	25.35 ± 0.49	0.0001
WHR	0.87 ± 0.01	0.91 ± 0.01	0.0084
WC/HC	56.30 ± 2.56	49.36 ± 2.06	0.0449

WC: Waist Circumference, HC: Hip Circumference, BMI: Body Mass Index, WHR: Waist-to-Hip Ratio. The data are expressed as Mean±SEM.

Table 3.7 Pearson's correlation coefficients between anthropometric variables for controls (lower left-hand side) and patients (upper right-hand side)

	Weight	height	WC	HC	BMI	WHR	TW
Weight		0.49***	0.53***	0.53***	0.97***	0.14	-0.20
Height	0.47*		0.17	0.15	0.25	0.14	0.15
WC	0.96***	0.43		0.99***	0.53***	0.29*	-0.01
HC	0.96***	0.42	0.89***		0.53***	0.14	0.01
BMI	0.96***	0.22	0.93***	0.93***		0.13	0.04*
WHR	-0.05	-0.05	0.16	-0.29	-0.04		0.03*

**Correlation is significant at the 0.05 level (2-tailed), **correlation is significant at the 0.01 level (2-tailed), ***correlation is significant at the level of 0.001 (2-tailed). WC: Waist Circumference, HC: Hip Circumference, BMI: Body Mass Index, WHR: Waist-to-Hip Ratio.*

3.2.5.1 Weight

The weight of patients differed significantly from those of the control subjects when the data was subjected to unpaired t-test analysis (t=2.244, p=0.0326). The mean weight of the patients was 92.50 ± 2.22 Kg while the mean weight of the control subjects was 83.55 ± 3.57 Kg as shown in Table 3.6. Pearson's correlation analysis showed that the weight of the patient had a significant positive correlation with the height, waist circumference, hip circumference and BMI but had no significant correlation with the waist-to-hip ratio for both controls and patients; or weight of tumour developed in the case of patients; as shown in Table 3.7.

3.2.5.2 Height

With a mean height of 1.60 ± 0.01 m and 1.59 ± 0.01 m for the patients and controls respectively there was no significant difference between the two groups (t=0.3788, p= 0.7060) as Table 3.6 shows. When correlated with the other anthropometric variables using Pearson's analysis, the height of the patient only had a positive correlation with the weight. The waist circumference, hip circumference, BMI and waist-to-hip ratio all had no significant correlation with the height in both patients and control groups as Table 3.7 shows. For the patients, the weight of the tumour developed did not correlate significantly with the height.

3.2.5.3 Waist Circumference

Table 3.6 shows that the Mean values for waist circumference for controls and patients were 66.44 ± 3.31 cm and 87.44 ± 3.47 cm implying a significant difference between the two means (t=4.352, p=0.0001). Within the patients waist circumference had a significant positive correlation with the weight, hip circumference, BMI and the waist-to-hip ratio but not with the height and the weight of tumour developed. For the controls there was a significant correlation between the waist circumference and, weight, hip circumference and BMI, but

there was no significant correlation for the height and the waist-to-hip ratio as shown in Table 3.7.

3.2.5.4 Hip Circumference

A significant difference(t=2.028, p= 0.0474) was revealed after unpaired t-test analysis of the data with a mean hip circumference of 76.79 ± 5.23 cm for the control group and a mean of 92.55 ± 3.91 cm for the patients as can be seen in Table 3.6. There was a significant correlation between the hip circumference and the weight, waist circumference and BMI for both groups but no significant correlation for waist-to-hip ratio and height of the subjects. The weight of the tumour developed did not also correlate significantly with the hip circumference of the patients as seen in Table 3.7.

3.2.5.5 Body Mass Index (BMI)

Comparison of BMI values for the patients and controls using unpaired t-test analysis showed a significant difference between the two groups (t=6.020, p<0.0001) with a mean BMI of 22.40 ± 0.22 and 25.35 ± 0.49for the controls and the patients respectively as shown in Table 3.6. The BMI also correlated significantly with weight, waist circumference and hip circumference of both patients and control groups but did not have a significant correlation with height and the waist-to-hip ratio. The tumour weight and the BMI had a significant correlation as shown in Table 3.7. When subjects were classified as underweight (<18.5), normal (18.5 – 24.9), overweight (25.0 – 29.9) and obese (≥30), analysis using Fisher's exact test revealed a strong association between the development of fibroid and an increase in the patients BMI; with the over weight patients having an almost two fold increased risk (OR= 1.91; exact 95% CI= 1.14 - 3.20) and the obese having far more than a two fold risk increase (OR= 2.25; exact 95% CI= 1.21 - 4.17) of developing fibroid when compared to the patients with normal BMI (Figure 3.17). The under

weight patients however had a decreased risk (OR= 0.70; exact 95% CI= 0.34 - 1.42) of developing fibroid when compared to the normal BMI patients (Table 3.10).

3.2.5.6 Waist-to-Hip Ratio (WHR)

WHR for controls and patients were significantly different (t=2.763, p= 0.0084) although both groups had high WHR with a mean WHR of 0.91 ± 0.01 for the patients and 0.87 ± 0.01 for the control group as shown in Table 3.6. Except for the waist circumference of the patients where there was a significant correlation, the WHR did not correlate significantly with any of the other anthropometric variables in both groups as seen in Table 3.7. When subjects were categorized as normal or obese and analyzed using Fisher's exact test, there was a strong association between obesity and the development of fibroid (OR= 3.60; exact 95% CI= 1.74 - 7.47) with the obese having close to a fourfold increased risk of developing fibroids as shown in Table 3.10.

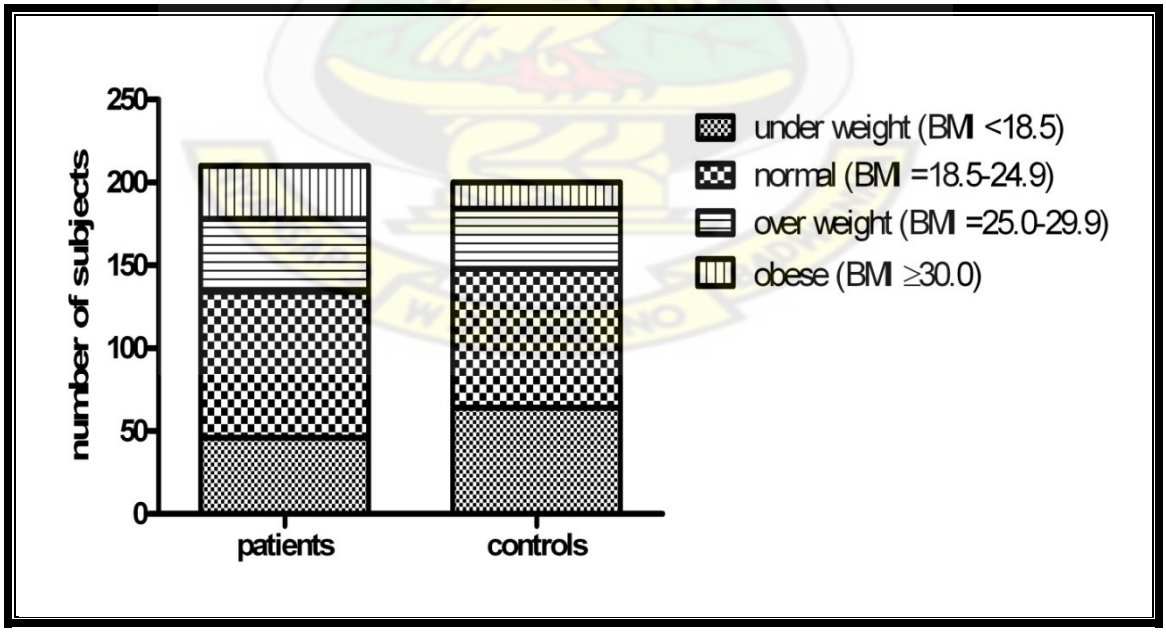

Figure 3.17 ***Chi square analyses of the different categories of BMI of patients and controls.***

3.2.6 SOCIO-DERMOGRAPHIC FEATURES

3.2.6.1 Age

The ages of patients ranged from 20 to 40 years. The data showed a significant difference between the patients and the control subjects when unpaired t-test was used for the analysis (t=2.208, P= 0.0288). The mean age of the patients was 34.62 ± 0.3381 and that of the control subjects was 33.47 ± 0.3908 as shown in Table 3.8. There was a significant positive correlation (r^2= 0.9964, p= 0.0018) between the age of the patient and the tumour size as shown in Figure 3.18.

3.2.6.2 Income

Analysis of data on income revealed a significant difference between the income earned by the patients and those earned by the control subjects (t=6.861, P<0.0001). The patients were engaged in economic ventures that earned them a mean income of 149.5 ± 6.007 Ghana cedi per month. The controls on the other hand, also had varied engagements that earned them a mean income of 85.25 ± 7.181 Ghana cedi per month as shown in Table 3.8.

3.2.6.3 Education

For the analysis of the data on education, subjects were divided into four groups; those who had no formal education, those who had only basic education, those who had up to secondary education, and those who had up to the tertiary level of formal education. Those who had basic education were then compared to the other three groups. There was a significant association with the level of education and the development of fibroids. Those who had secondary and tertiary education were at a more than two fold (OR= 2.35; exact 95% CI= 1.33 - 4.14) and three fold (OR= 3.28; exact 95% CI= 1.64 - 6.55) increased risk respectively of developing fibroids. Those with no education however had a lower risk (OR= 0.43; exact 95% CI= 0.26 - 0.73) associated with fibroid development as shown in Table 3.10.

3.2.6.4 Marital status

Subjects were divided into two groups (ever married and never married) for the analysis of data on marital status. Using Fisher's exact test to analyze the data it was revealed that there was a significant association between marriage and the development of fibroids (Table 3.10). With an odds ratio of 1.62 (exact 95% CI, 1.07 - 2.44) those who had never been married were at a higher risk of developing fibroids compared to those who had ever married.

3.2.6.5 Smoking

All patients and controls stated that they did not, in the past or as at the time of the study, smoke. Therefore there was no significant difference between the controls and the patients in terms of smoking.

3.2.6.6 Alcohol intake

For the purpose of analyzing data on alcohol consumption, subjects were divided into two groups; those who take alcohol or have ever taken alcohol, and those who have never taken alcohol. Though alcohol intake was wide spread in both controls and patients the drinkers had a significantly increased risk (OR= 1.69, exact 95% CI= 1.13 - 2.53) of developing fibroids compared to the non-drinkers (Table 3.10).

3.2.6.7 Family history of fibroids

History of fibroids in subjects was strongly associated with the development of fibroids as people with such family history had more than three fold increased risk (OR= 3.61, exact 95% CI= 2.21 - 5.90) of developing fibroids compared to people who had no such family history (Table 3.10).

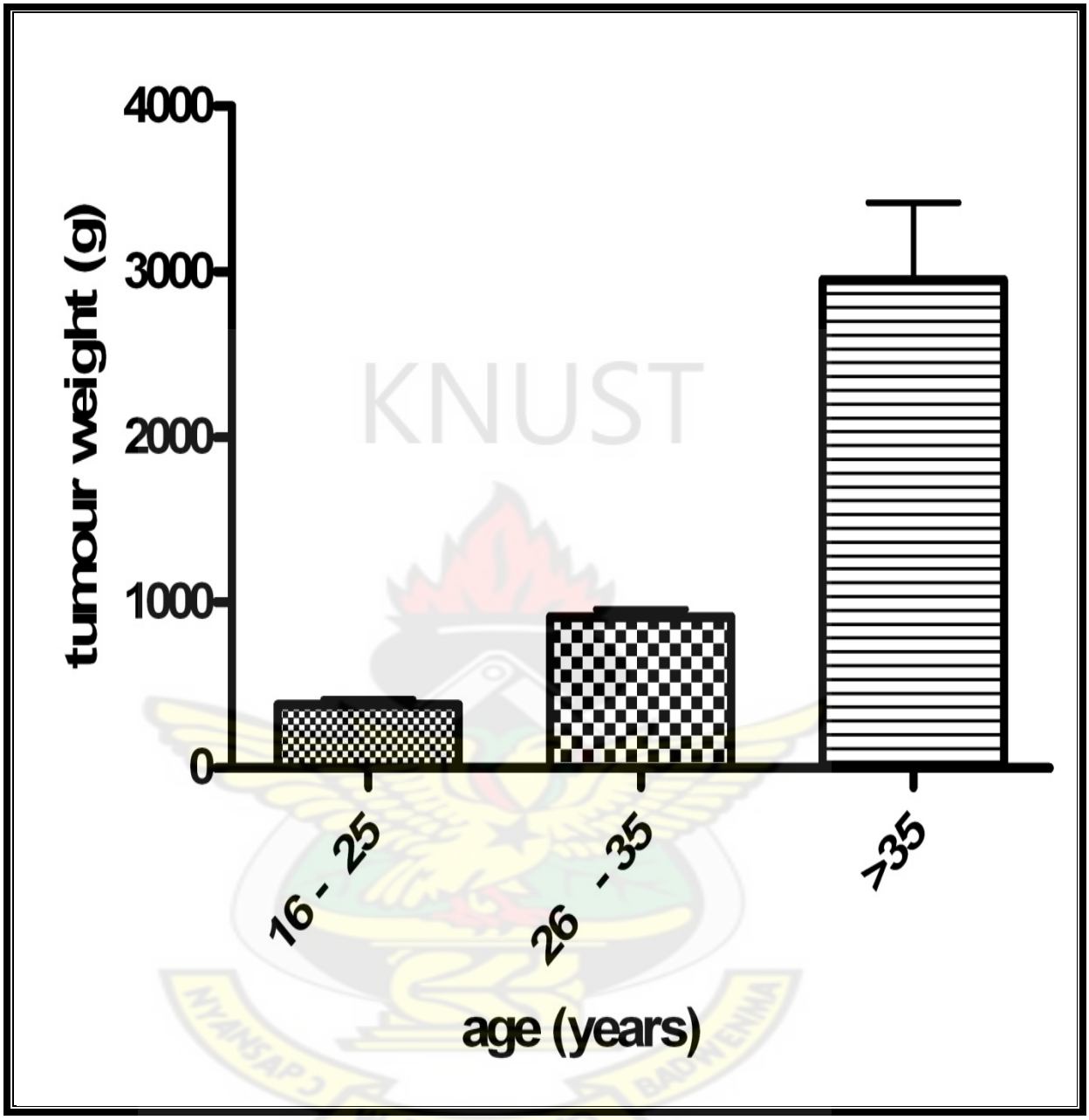

Figure 3.18 Correlation between age and the weight of tumour developed.

Table 3.8 Comparison of some demographic and gynaecologic characteristics between controls and patients.

Variable	Controls	Patients	p Value
Age (years)	33.47 ± 0.39	34.62 ± 0.34	0.0288
Income (Ghana Cedis)	85.25 ± 7.18	149.50 ± 6.01	0.0001
Age at menarche (years)	13.38 ± 0.10	12.98 ± 0.09	0.3041
Age at first pregnancy (years)	20.07 ± 0.45	21.13 ± 0.70	0.1987
Age at first birth (years)	23.40 ± 0.90	27.90 ± 0.59	0.0005
Parity	3.00 ± 0.32	1.00 ± 0.32	0.0021
Number of previous abortions	0.83 ± 0.12	1.97 ± 0.13	0.0001
Age at first abortion (years)	18.80 ± 0.23	18.19 ± 0.09	0.0099
Length of menstrual cycle (days)	28.06 ± 0.13	28.24 ± 0.10	0.2740

The data are expressed as Mean ± SEM.

Table 3.9 Pearson's correlation coefficients between demographic and gynaecologic parameters for controls (lower-left hand side) and patients (upper right-hand side)

	Age	Income	AP	AB	Parity	NPA	AFA	LMC	TW
Age		**-0.34**	**-0.67****	**0.37**	**-0.85*****	**0.76****	**-0.54***	**0.11**	**0.83*****
Income	**-0.09**		**0.22**	**-0.23**	**0.31**	**-0.41**	**0.26**	**-0.13**	**-0.34**
AP	**-0.91*****	**0.09**		**-0.63****	**0.94*****	**-0.91*****	**0.93*****	**-0.10**	**-0.61****
AB	**-0.76****	**-0.07**	**0.77****		**-0.92*****	**0.25**	**-0.31**	**0.09**	**-0.55***
Parity	**-0.87*****	**0.21**	**-0.84*****	**-0.92*****		**-0.91*****	**-0.51***	**-0.11**	**-0.47***
NPA	**0.59**	**-0.12**	**0.02**	**0.39**	**-0.34**		**-0.89*****	**0.02**	**0.82*****
AFA	**-0.20**	**0.06**	**0.44**	**-0.18**	**0.06**	**0.08**		**-0.07**	**-0.63****
LMC	**0.13**	**-0.16**	**-0.20**	**0.07**	**-0.11**	**0.09**	**0.16**		**0.20**
TW									

**Correlation is significant at the 0.05 level (two-tailed), **Correlation is significant at the 0.001 level (two-tailed), ***Correlation is significant at the 0.0001 level (two-tailed). AB: Age at first birth,*

AP: Age at first pregnancy, NPA: Number of previous abortions, AFA: Age at first abortion,

LMC: Length of menstrual cycle, TW: Tumour weight

Table 3.10 Frequency distribution and odd ratios for the association of putative risk factors for fibroid development.

Parameter	Controls No. (%)	Patients No. (%)	OR	CI
BMI				
Under Weight	**32 (16.0)**	**18 (9.0)**	**0.70**	**0.34 - 1.42**
Normal*	**64 (32.0)**	**46 (23.0)**	**1.00**	
Over Weight	**54 (27.0)**	**74 (37.0)**	**1.91**	**1.14 - 3.20**
Obese	**26 (13.0)**	**42 (21.0)**	**2.25**	**1.21 - 4.17**
W/H Ratio				
Obese	**13 (6.5)**	**45 (22.5)**	**3.60**	**1.74 - 7.47**
Normal*	**52 (26.0)**	**50 (25.0)**	**1.00**	
Education				
No Education	**88 (44.0)**	**35 (17.5)**	**0.43**	**0.26 - 0.73**
Basic Education*	**71 (36.0)**	**65 (32.5)**	**1.00**	
Secondary Education	**27 (13.0)**	**58 (29.0)**	**2.35**	**1.33 - 4.14**
Tertiary Education	**14 (7.0)**	**42 (21.0)**	**3.28**	**1.64 - 6.55**
Marital Status				
Ever Married*	**139 (69.5)**	**117 (58.5))**	**1.00**	
Never Married	**61 (30.5)**	**83 (41.5)**	**1.62**	**1.07 - 2.44**
Alcohol Intake				
Never Drank*	**132 (66.0)**	**107 (53.5)**	**1.00**	
Ever Drank	**68 (34.0)**	**93 (46.5)**	**1.69**	**1.13 - 2.53**
Family History of Fibroids				
Yes	**74 (37.0)**	**28 (14.0)**	**3.61**	**2.21 - 5.90**
No/Not aware*	**126 (63.0)**	**172 (86.0)**	**1.00**	

**Reference category. OR – Odds Ratio; CI – Confidence Interval; No. – Number of subjects*

Table 3.11 Frequency distribution and odd ratios for the association of putative risk factors for fibroid development.

Parameters	Controls No. (%)	Patients No. (%)	OR	95 % CI
Age At Menarche (Years)				
≤12	78 (39.0)	84 (42.0)	1.13	0.76 - 1.69
>12*	122 (61.0)	116 (58.0)	1.00	
Age At First Birth (Years)				
<25*	52 (26.0)	50 (25.0)	1.00	
≥25	88 (44.0)	30 (15.0)	2.82	1.60 - 4.98
Parity				
Nulliparous	46 (23.0)	132 (66.0)	6.50	4.18 - 10.10
≥1 Births*	154 (77.0)	68 (34.0)	1.00	
Abortions				
Ever Aborted	19 (9.5)	52 (26.0)	3.35	1.90 - 5.91
Never Aborted*	181 (90.5)	148 (74.0)	1.00	
Type Of Abortion				
Spontaneous*	10 (5.0)	13 (6.5)	1.00	
Induced	9 (5.0)	39 (19.5)	3.33	1.11 - 9.99
Age At First Abortion (Years)				
≤18*	6 (3.0)	31 (15.5)		
>18	16 (8.0)	21 (10.5)	3.94	1.32 - 11.71
Method Of Delivery				
Ever Had A Caesarian Section	33 (16.5)	13 (6.5)	0.87	0.42 - 1.78
Never Had A Caesarian Section*	121 (60.5)	55 (27.5)	1.00	
Length Of Menstrual Cycle (Days)				
≤26	5 (2.5)	3 (1.5)	0.58	0.14 - 2.47
27-29*	183 (91.5)	189 (94.5)	1.00	
≥30	12 (6.0)	8 (4.0)	0.65	0.26 - 1.62
Contraception				
Never Used*	108 (54.0)	83 (41.5)	1.00	
Ever Used	92 (46.0)	117 (58.5)	1.66	1.11 - 2.46
Sexually Transmitted Infections				
Never Infected*	184 (92.0)	167 (83.5)	1.00	
Ever Infected	16 (8.0)	33 (16.5)	2.27	1.21 - 4.28
Phytotherapy				
Never Used*	86 (43.0)	62 (31.0)	1.00	
Ever Used	114 (57.0)	138 (69.0)	1.68	1.11 - 2.53

**Reference category. OR – Odds Ratio; CI – Confidence Interval; No. – Number of subjects*

3.2.7 OBSTETRICS AND GYENAECOLOGIC FINDINGS

3.2.7.1 Age at menarche

The age at menarche ranged from 12 to 14 years for all subjects. There was no significant difference between the patients and the control subjects when the data was analyzed using unpaired t-test (t=2.923; p=0.3041).The mean age at menarche for the patients was 12.98 ± 0.09045 years and that of the control group was 13.38 ± 0.09835 as in Table 3.8. subjects were categorized into two groups based on whether their age at menarche was ≤12, or >12 and analyzed with Fisher's exact test to determine whether there was any risk associated with the age at menarche for the development of fibroids (Table 3.11). The analysis showed no increased risk associated with having menarche at the age of ≤12 years (OR= 1.13, exact 95% CI= 0.76 - 1.69) when compared to those who had their menarche after 12years of age.

3.2.7.2 Age at first pregnancy

The age at first pregnancy for the patients ranged from 18 to 33 years while that of the controls ranged of 16 to 28. Unpaired t-test analysis revealed an insignificant difference between the two groups with a mean age at first pregnancy of 20.07 ± 0.4547 years for the controls and 21.13 ± 0.7028 years for the patients (t=1.302, p= 0.1987) as in Table 3.8.

3.2.7.3 Age at first birth

There was a significant difference between the two groups when data on age at first birth were analyzed using unpaired t-test (t=4.200, p= 0.0005). The patients had a higher age at first birth with a mean of 27.90 ± 0.5859 years whiles the control subjects had a lower age at first pregnancy with a mean of 23.40 ± 0.8969 years as shown in Table 3.8. Fisher's exact test was used to compare subjects who have had their first birth before the age of 25 years to those who had their first birth at 25 years or after (Table 3.11). The analysis revealed that those who had their first

birth at 25 years or more had close to three fold increased risk of developing fibroid compared to those who had their first birth before the age of 25 years (OR= 2.82, exact 95% CI= 1.60 - 4.98).

3.2.7.4 Parity

There was a significant difference between the number of births recorded for the patients and the number for the controls with the controls demonstrating higher parity compared to the patients (t=8.124, p= 0.0001). The mean number of births for the patients was 1.391 ± 0.1059 and that for the controls was 2.891 ± 0.1512 (Table 3.8). Women who had a parity ≤ 1 (live + still births) were more protected compared to nulliparous women. The nulliparous women had 6.5 times increased risk of developing fibroid compared to those who had one or more births (OR= 6.50, exact 95% CI= 4.18 - 10.10) as seen in Table 3.11.

3.2.7.5 Number of Abortions

There was a significant difference between the patients and controls subjects in terms of total number of abortions when the two sets of data were compared using the t-test (t=6.477, p= 0.0001). The mean number of abortions for the patients was 1.969 ± 0.1279 and that for the control subjects was 0.8281 ± 0.1211 as shown in Table 3.8. There was a strong association between history of abortion and the development of fibroid (Table 3.11). Women who have ever had at least one abortion in the past were at a more than three times increased risk of developing fibroid compared to women who had no history of past abortions (OR= 3.35, exact 95% CI= 1.90 - 5.91). Those who had induced abortion were also at a three time or more increased risk of developing fibroid compared to those who had spontaneous abortion (OR= 3.33, exact 95% CI= 1.11 - 9.99).

3.2.7.6 Age at first abortion

There was a significant difference between the patients and the controls subjects in terms of age at which first abortion occurred when t-test was used to analyze the data (t=2.613, p= 0.0099) with the patients showing a lower age at which abortion occurred. The mean age at which first abortion occurred for the patients was 18.19 ± 0.09087 years and that for the controls was 18.80 ± 0.2348 years as Table 3.8 shows. There was a very strong association between the age at first abortion and the risk of developing fibroid. Table 3.11 shows that those who had an abortion at the age of 18 years and below were at a five fold increased risk of developing fibroid compared to those who had an abortion after the age of 18 years (OR= 5.11, exact 95% CI = 1.59 – 16.46).

3.2.7.7 Method of delivery

For the purpose of analysis of the data using Fisher's exact test, the subject births were categorized as vaginal deliveries and caesarean sections. There was no significant association between the risk for fibroid development and the method which was used to deliver them of their babies for those who had children (Table 3.11). When Subjects who were delivered using caesarian sections were compared to those who had only vaginal deliveries, they had no increased risk of or protection from developing fibroids (OR= 0.87, exact 95% CI= 0.42 to 1.78).

3.2.7.8 Length of menstrual cycle (LMC)

There was no significant difference between length of the menstrual cycles of the patients and those of the controls when t-test was used to analyze the data (t=1.098, p= 0.2740). The control subjects had a mean menstrual cycle length was 28.06 ± 0.1257 days and that of the patients was 28.24 ± 0.1010 days as shown in Table 3.8. For the purpose of analysis, the subjects were divided into those whose LMC is <26 days, those whose LMC is 27 -29 days and those whose LMC is ≥30 days. Those with LMC 27- 29 was compared to those with LMC <26 days and to those with

LMC ≥30 days. There was no risk associated with a subject's length of menstrual cycle since those with LMC <26 days had an OR= 0.58 (exact 95% CI= 0.14 to 2.47) and those who had LMC ≥30 days had an OR= 0.65 (exact 95% CI= 0.26 to 1.62) (Table 4.1).

3.2.7.9 Contraceptive use

Even though the use of contraception was widespread across patients and controls, its use was significantly higher among the patients than in the controls (Table 3.11). Analysis using Fisher's exact test revealed a significant association between the risk for developing fibroid and the use of contraceptives. Those who used contraceptives were at an increased risk of developing fibroid (OR= 1.66, exact 95% CI= 1.11 to 2.46) when compared to those who did not use contraceptives.

3.2.7.10 Phytotherapy

The use of phytotherapeutic agents was common amongst both controls and patients. Even with this widespread use, the patients significantly had a higher association with phytotherapy (OR= 1.68, exact 95% CI= 1.11 to 2.53) (Table 3.11).

3.2.7.11 Sexually transmitted infections (STIs)

Fisher's exact test for analysis of subjects on the basis of whether they have had STIs in the past and those who have not had STIs for both control subjects and patients revealed a significant association between STIs and the development of fibroids (Table 3.11). Those who have had past infections had more than double increased risk of developing fibroids compared to those who have never had infections in the past (OR= 2.27, exact 95% CI= 1.21 to 4.28).

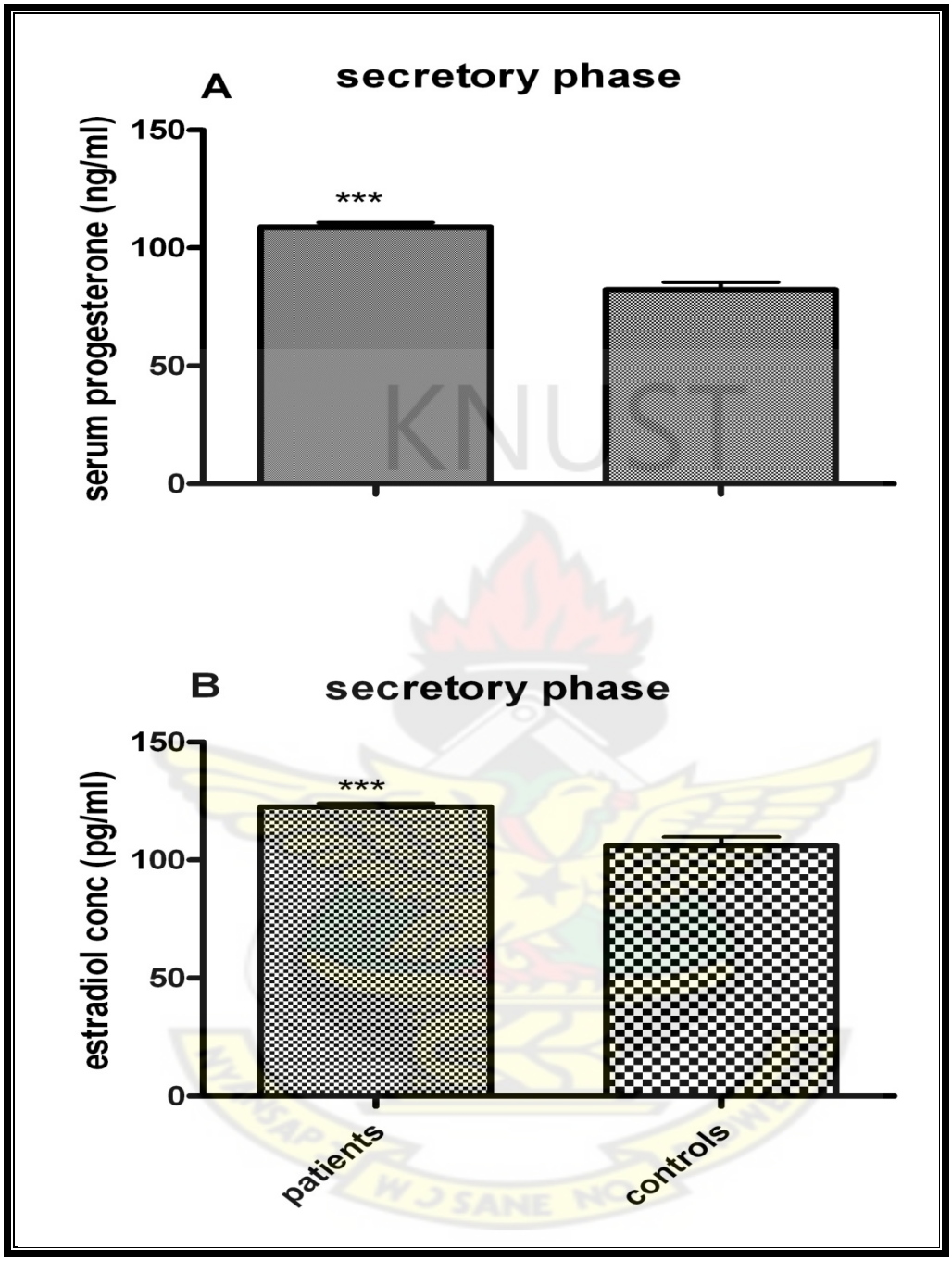

Figure 3.19 t-test comparison of the serum progesterone (A) and serum oestradiol (B) levels between the patients and controls in the secretory phase of the menstrual cycle.

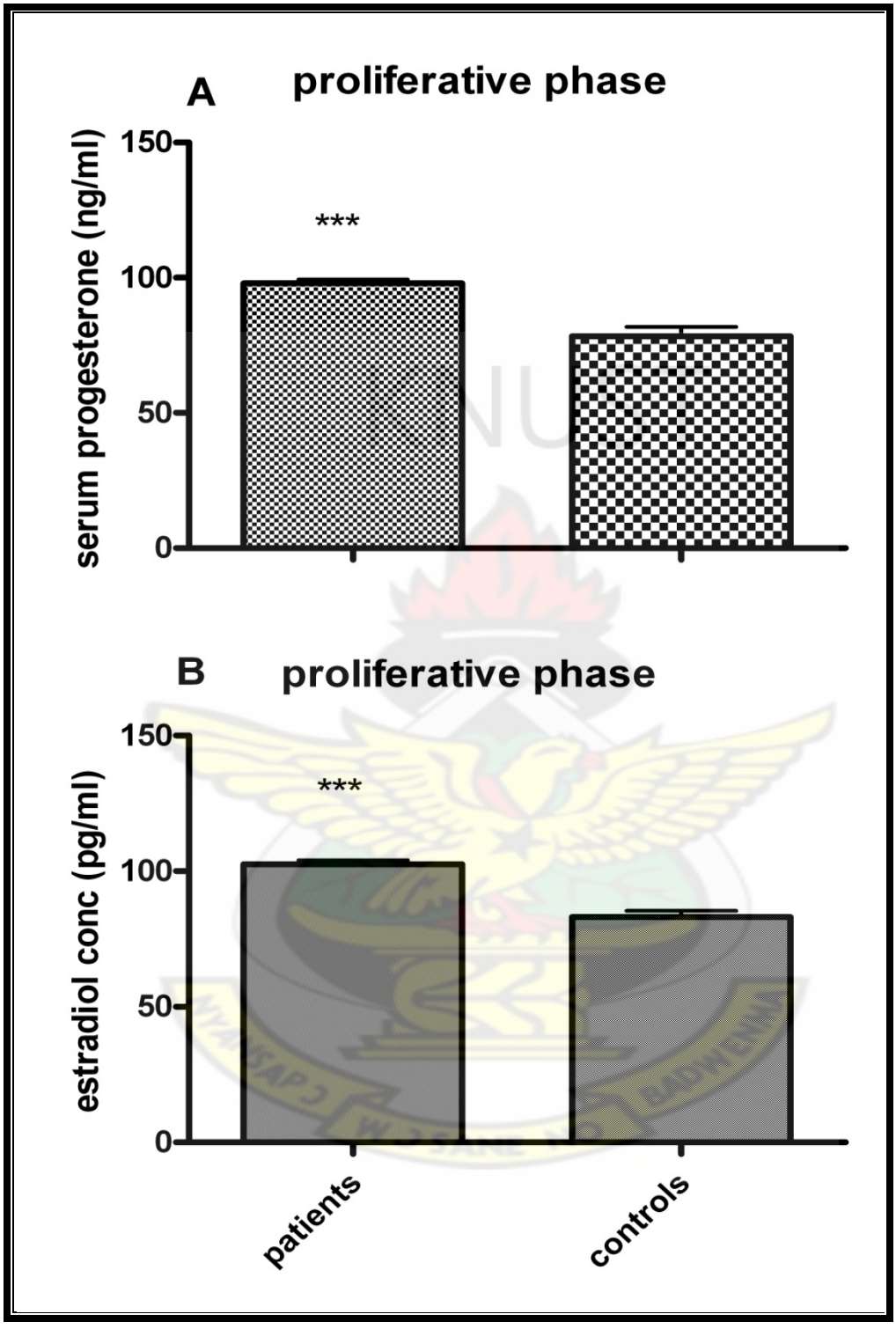

Figure 3.20 t-test comparison of the serum progesterone (A) and serum oestradiol (B) levels between the patients and controls in the proliferative phase of the menstrual cycle.

Chapter 4

DISCUSSION

4.1SOCIO-DEMOGRAPHY

Although this study sought to select women of reproductive ages for both groups, there exist significant differences between the mean ages of the two groups. The mean age of 34.62 ± 0.34 for the patients is consistent with the observation of other researches which revealed that leiomyomata mostly occur among women in their late reproductive ages (Ross *et al.*, 1986; Velebil *et al.*, 1995; Marshall *et al.*, 1997). Two reasons have been proposed for this observation. The first is the increase in the risk of new fibroids increasing rapidly in women during their late thirties. The second is that the observed increase could also result from increased growth of, or increased symptomatology from, already existing fibroids, as well as from a greater willingness of women in the later reproductive years to have gynaecologic care (Parker, 2007). If the likelihood of fibroid development and growth is actually accelerated during the late reproductive years, the hormonal factors associated with perimenopause may be important modulators; alternatively, the apparent increase in the late reproductive years may simply represent the cumulative culmination of 20-30 years of stimulation by oestrogen and progesterone. The ages of the patients was observed to correlate positively with the tumour sizes. This implies that as the ages increased the tumour size in the patient also increased. The trend suggests that the patients developed these tumours earlier in life and that these tumours increased in size as the years go by. Or, the older the woman gets the larger the tumour if she happens to develop one. Thus the age of the woman dictates the size of the tumour she develops (Wright *et al.*, 2007). But the observation that they did not occur in girls below reproductive age, suggests a more reasonable theory; that the women might have developed the tumours in their twenties and that their growth continued as they aged. This explanation is

consistent with the findings of this study which shows that the age of the patients correlated positively with the size of the tumour obtained.

This study observed a significant difference in the level of education between the two groups. This study revealed that the patients were more educated than the control group. The average level of education for the patients was senior high school and that of the control group was primary school. This study also revealed that those women with tertiary and secondary education had more than a three fold and two fold risk of developing fibroid compared to women with basic education whereas women with no formal education had a lower risk of developing fibroid. The results of this study are consistent with the findings of another study (Chiaffarino *et al.*, 1999a) which found that women with uterine myomas were more educated, than those who had no myomas. This study was however carried out on women who came in for gynaecological surgery. Social determinants of gynaecologic surgery are also well recognized. Surgery for myomas or other benign neoplastic conditions, such as benign breast disease and ovarian cysts, is more frequent in more educated women of higher social classes (Parazzini *et al.*, 1993) and may reflect the greater attention that better-educated women pay to relatively minor health problems. Thus, greater attention to health may also favor the diagnosis of myomas among these well educated women, thereby producing a false association.

High education tends to enable women acquire job positions that are sedentary and afford them little exercise. The sedentary lifestyles can be implicated in obesity related conditions such as diabetes, hypertension, some cancers and infertility.

One of the modifiable risk factors that have been reported for breast cancer and endometrial cancer, other hormonally mediated tumours, is physical activity (Lee, 2003; Matthews *et al.*, 2005).

The more educated women often have to go through the full length of their education before giving birth. This presupposes that most of them do not have children or tend to have children at a relatively late age compared to the less educated counterparts in terms of age. And since parity is known to play a protective role in the development of leiomyomata (Chen *et al.*, 2001; Parazzini, 2006) it may explain the significant difference between the two groups in terms of education. The vast disparity between the two groups in terms of income earning is attributable to the variation in the educational levels of the two groups. No literature was found associating income level with the risk of fibroid development. Patients were more educated on the average compared to their counterparts of same age in the control group. It follows, therefore, that the patients were probably engaged in jobs that earned them more income than their contemporaries in the control group. Another explanation however for the difference in income levels could be that women with high income levels had the means to access health facilities and therefore were captured more than their counterparts with low incomes who could not access the health facilities due to financial constraints.

The findings of this study suggest that unmarried women are at a higher risk of developing fibroid compared to their married counterparts. In a study in which women of various races were examined, marriage was not seen to be associated with the risk of developing fibroid (Marshall *et al.*, 1997). The significant difference between the two groups in terms of marriage in this study may be attributed to the effect of education. the observation that patients are more educated than the controls and thus had to mostly wait longer before marriage in order to complete their education means the number of patients who will be married, for the same age group, will be lower than that for the controls. This probably modified the average age of marriage up for the patients and might have accounted for most of

them not being married. In the typical Ghanaian society, women who are not married should not have children and society frowns upon such women. The result is that most of the women who are not married do not have children and since parity is known to play a protective role in the development of leiomyomata, it explains the significant difference between the two groups in terms of marriage.

Though this study did not distinguish between current and past consumption, nor the quantity of alcohol consumed, an association was observed between alcohol consumption and the presence of fibroid based on whether the woman had ever drank or not. Women with the condition responded yes more to the question compared to the control group. This can be explained in the sense that once the condition was diagnosed in the woman, she sought all kinds of treatment for it, including herbal preparations, which are mostly alcohol based extracts. Alcohol in fact plays a variety of roles in Ghanaian culture (Akyeampong, 1995) and so women, particularly the elderly, indulge freely and frequently in the act of alcohol consumption as they go through the numerous rites that they are supposed to take part in. This makes it easier for them to accept the various alcoholic herbal extracts that are advertised as being very potent drugs for fibroid. The findings of this study are consistent with those (Wise *et al.*, 2004b)which also showed that the risk of uterine leiomyomata was positively associated with years of alcohol consumption and current consumption of alcohol, particularly beer. This present study however, was not that specific on the type of alcoholic beverage. Another study also found a positive association for current alcohol consumption, but did not report effect estimates (Marshall *et al.*, 1997), while an Italian case–control study found no association (Chiaffarino *et al.*, 1999b). Alcohol consumption is a modifiable risk factors that may affect endogenous levels of hormones via changes in ovarian function of metabolism. Alcohol consumption is associated with higher

endogenous levels of oestradiol (E_2) and estrone which may promote growth of uterine leiomyomata (Reichman *et al.*, 1993; Hankinson *et al.*, 1995; Andersen, 1996b), but other studies show no such associations (Cauley *et al.*, 1989; London *et al.*, 1991; Dorgan *et al.*, 1994; Newcomb *et al.*, 1995; Coyne *et al.*, 2008). A meta-analysis of cohort studies showed that beer drinkers have the largest relative risk of breast cancer, although the beverage-specific risks were not significantly different (Smith-Warner *et al.*, 1998). Moreover, a recent study showed that the phytoestrogen in beer, 8-prenylnarigenin, stimulates the growth of MCF-7/6 breast cancer cell lines *in vitro*, which may mimic the effects of 17-E_2 (Rong *et al.*, 2001).

None of the women interviewed admitted to smoking. Thus there was no difference between the two groups. This was expected as women who smoke in Ghana are despised and looked at as "bad". Thus in most African communities, women do not. It's almost a taboo for a woman to smoke in most Ghanaian societies (Anonymous, 2005). Most epidemiologic studies done in the western countries have reported an inverse relation between risk of uterine leiomyomata and cigarette smoking (Ross *et al.*, 1986; Romieu, 1988; Lumbiganon *et al.*, 1996; Parazzini *et al.*, 1996c). The risk ranges from 20 to 50% lower among smokers, depending on the definition of smoking (e.g., current, ever). The risk among former cigarette smokers does not appear to differ from that of women who have never smoked cigarettes (Parazzini *et al.*, 1996a; Marshall *et al.*, 1998b). Two studies showed an inverse trend associated with the amount of cigarette smoking (Ross *et al.*, 1986; Romieu, 1988; Jago *et al.*, 2005), whereas one showed a reduction in risk regardless of amount smoked (Parazzini *et al.*, 1996c). These researchers believe cigarette smoking reduces the risk of developing leiomyomata. Since the majority of Ghanaian women do not smoke (Anonymous, 2005), it stands to reason that they do not gain the protection that is offered by cigarette smoking. Cigarette smoking is known to have an effect on the hormonal milieu. It has been found in

other studies that smoking for approximately 20 years or more was associated with a 40 percent decreased risk of fibroid in comparison with nonsmoking (Faerstein *et al.*, 2001). Smoking is considered to be associated with an oestrogen-deficient state, through disturbed gonadotropin release or enhanced formation of inactive oestrogen from oestradiol, among other postulated mechanisms (Baron *et al.*, 1990).

4.2 GYNAECOLOGIC FEATURES

This study found a significant difference between the two groups in terms of age at menarche. Though very close, the mean age at menarche for the controls was significantly higher than that of the patients. Again there was only a slightly higher risk associated with age at menarche of less than or equal to twelve years. There has been conflicting results from studies done in the past. Some studies have suggested a slightly increased risk of fibroids associated with early menarche, although the risk has often not been statistically significant (Parazzini *et al.*, 1988; Cramer *et al.*, 1995a; Cramer *et al.*, 1995b; Samadi *et al.*, 1996). These studies corroborate the results of this study. The findings in these studies suggest that the age at menarche is not a factor to consider as to whether a woman will develop fibroid later in life. Some other studies however suggest a significant inverse association between risk of fibroids and age at menarche (Romieu, 1988; Lumbiganon *et al.*, 1996; Marshall *et al.*, 1998a). One study (Sato *et al.*, 2000b) found that women with uterine leiomyomata more often exhibited an early normal menstrual cycle pattern, and concluded that early menstrual regularity may enhance leiomyoma growth in early reproductive life. It has been suggested that early menarche is associated with higher oestrogen levels throughout reproductive life (MacMahon *et al.*, 1982a), although direct evidence for this is lacking *(Bernstein et al., 1991)*. Again, menarche at a younger age implies earlier establishment of regular ovulation (Apter and Vihko, 1983) and thus more prolonged exposure to

both oestrogen and progesterone. The inverse association found in the Black Women's Health Study and other studies supports the hypothesis that women with an early age at menarche have, on average, increased menstrual cycling and greater lifelong exposure to bioavailable oestrogens, which are thought to promote growth of uterine leiomyomata. Early age at menarche has been correlated with higher levels of oestradiol (Windham *et al.*, 2002) and oestrone and lower levels of sex hormone binding globulin (MacMahon *et al.*, 1982a; Apter *et al.*, 1989).

Mitotic activity in the myometrium appears to be greatest during the luteal phase of the menstrual cycle *(Kawaguchi et al., 1991)*. Thus, if progesterone is an important aetiologic factor, it might be expected that a longer history of cycling might be associated with increased risk. The early onset of menstrual cycles may increase the number of cell divisions that the myometrium undergoes during the reproductive years, resulting in an enhanced chance of mutation in the genes controlling myometrial proliferation (Marshall *et al.*, 1998a). Young age of menarche (<12.5 years) has also been found to be associated with obesity (Pierce and Leon, 2005). Whether early menarche is an independent risk factor or a marker of other environmentally determined mediators of fibroid risk such as obesity, is unclear.

This study observed no significant difference between the two groups in terms of age at first pregnancy. Age at first pregnancy also correlated positively with parity and age at first abortion and negatively with the age, number of previous abortions, and the tumour size. Given that the results also show a significant disparity between the two groups in terms of age at first birth, one explanation for this observation is that the patients have a higher number of abortions compared to the controls. This tends to lower their parity which is protective for the women (Ross *et al.*, 1986; Lumbiganon *et al.*, 1996; Parazzini *et al.*, 1996b; Marshall *et al.*,

1998a). Since age at first pregnancy correlates positively with the age at first birth and parity, for the controls, it is appropriate to indicate that a lower age at first pregnancy may be protective for the women. This study has also shown that a lower age at first pregnancy may prevent or retard the development of fibroids as seen in the negative correlation between age at first pregnancy and the tumour weight obtained.

This study revealed a significant difference between the two groups of women in terms of age at first birth. The patients had their first birth at a higher age compared to the control group. The age at first birth also correlated significantly and negatively with the parity, age at first pregnancy, and the tumour weight obtained for the patients whereas the correlation was significantly negative with age, parity, and tumour weight, but positively correlated with the age at first pregnancy in the control group. This correlates well with the observation that the patients were more educated and as a consequence married late compared to their age mates who were in the control group. Since most Ghanaian women will like to complete their education and marry before having children (Anarfi and Awusabo-Asare, 1993), those who attain higher education tend to start child bearing later than their contemporaries of same age group. The realization that women who had an age of first birth of 25 years or more were at a significantly higher risk of developing fibroids is consistent with another study that found that older age at last birth appears to be inversely associated with the risk of surgically confirmed tumours *(Marshall et al., 1998a)*, although other studies have not found any association between age at first-term birth and risk of surgically confirmed uterine leiomyomata (Ross *et al.*, 1986; Lumbiganon, 1994; Parazzini *et al.*, 1996b). Another study however, reported an age-adjusted reduced risk for age at first birth after age 24 (Romieu *et al.*, 1989). It has been observed that fibroids are associated negatively with parity (Ross *et al.*, 1986; Lumbiganon, 1994; Parazzini *et al.*, 1996b;

Marshall *et al.*, 1998a). On the other hand, it has been found that there is a positive association between infertility and fibroids (Faerstein *et al.*, 2001). Thus for women who wait late into their reproductive lives before they give birth, they may develop fibroid nodules in the course of time before their first pregnancy. Since submucous tumours tend to protrude into the uterine cavity and are likely to interfere with ovum implantation and the normal progression of pregnancy (Hunt and Wallach, 1974; Buttram and Reiter, 1981), these women have great difficulty conceiving and thus reducing the parity of these women, hence increasing their risk of developing fibroid (Faerstein *et al.*, 2001). The negative correlation between the age at first birth and the tumour weight suggest that early age at first birth prevents the development or retards the growth of fibroids. The fact that there is a higher than 25 years mean age at first pregnancy also supports this assertion. Women who give birth early in terms of age tend to have higher parity compared to those who have childbirth late which is protective. Thus all put together this study suggests that, early age at first birth is protective for the women.

Results of this study have revealed a significant difference between the two groups of women in terms of the number of births. Parity was higher among the controls compared to the patients. Parity correlated negatively with age, age at first birth, age at first abortion, number of previous abortions, age at first abortion, and tumour weight, but correlated positively with age at first birth. This observation can be linked to the fact that the patients had to spend more time studying, since they were more educated formally, and thus would prefer to have children after acquiring some level of education. This may account for the difference in parity between the two groups since the control group were less educated and hence spent less years on education and therefore could marry early and bear children earlier than the patients of same age. This is supported by the observation that parity correlated positively with the age at first birth. The higher number of

previous abortions recorded among the patients may explain the low parity in this group. There was also a six and half times risk of developing fibroid among nulliparous women compared to women who have had at least one birth. This is consistent with the finding that the weight of tumour obtained correlated negatively with parity among women. Some studies have also reported the risk of uterine leiomyomata to be 20-50% lower among women who have ever given birth compared to nulliparous women and the risk appears to decrease with increasing parity (Ross *et al.*, 1986; Lumbiganon, 1994; Parazzini *et al.*, 1996b; Marshall *et al.*, 1998a). These studies corroborate the findings of this study. Several mechanisms may underlie the observed inverse associations with parity. Although endocrine factors change dramatically during pregnancy, the post-pregnancy hormonal state also differs from that in nulliparous women *(Bernstein et al., 1991; Dorgan et al., 1994)*. Furthermore, increased parity could represent a reduction in a woman's lifetime exposure to normal cycling oestrogens and progesterone. Pregnancy may also lead to a reduction in oestrogen receptor levels in myometrial tissue *(Kawaguchi et al., 1991)* that could reduce the sensitivity of these tumours to hormonal stimuli. Alternatively, childbearing may counteract the development of uterine leiomyomata through non-hormonal mechanisms. For example, myometrial hypertrophy during pregnancy could inhibit growth of small clones of transformed myometrial cells, and reductions in collagen content and smooth muscle cell apoptosis during uterine regression could eliminate or reduce the size of minute uterine leiomyomata or selectively remove cells most likely to develop into tumours. The postpartum state could also represent an intersection of hormonal and non-hormonal pathways, as loss of smooth muscle cell cytoplasm could diminish oestrogen receptor levels and thus, at least temporarily, decrease the oestrogen responsiveness of existing uterine leiomyomata (Schwartz *et al.*, 2000).

The positive association between the number of previous abortions and the weight of tumour obtained agrees with findings from other studies (Chen *et al.*, 2001)) and suggests that the protection conferred by pregnancy may relate to full-term pregnancy only. A full-term pregnancy induces significant long-term changes in levels of ovarian hormones and growth factors (Bernstein *et al.*, 1985; Musey *et al.*, 1987a; Dorgan *et al.*, 1995), including lower levels of plasma and urinary oestradiol (Bernstein *et al.*, 1985; Windham *et al.*, 2002), higher levels of sex hormone binding globulin (Bernstein *et al.*, 1985), and long-term decreases in basal levels of prolactin (Musey *et al.*, 1987b; Mora *et al.*, 1995). A full term pregnancy may cause a reduction in oestrogen receptor levels in myometrial tissue, which could reduce the sensitivity of leiomyomata to hormonal stimuli (Kawaguchi *et al.*, 1991). Again, extensive uterine tissue degradation and remodeling occurs both during and after full-term pregnancy (Walker *et al.*, 2001). Collagenases and other tissue-degrading enzymes induce apoptosis and may inhibit the growth of preneoplastic or neoplastic cells (Walker *et al.*, 2001).

The time-risk relation between parity and risk of leiomyomata is similar for uterine cancer (Lambe *et al.*, 1999), which supports the hypothesis that the protection conferred by parity may be related to pregnancy-related hormonal changes or the mechanical shedding of transformed myometrial cells.

There was a significant difference between the two groups of women in terms of age at first abortion. Although the number of previous abortions was considerably high among both controls and patients, the patients had more abortions in the past compared to the control subjects. The age at first abortion also correlated negatively with the weight of the tumour. This observation is consistent with the fact that in developing countries, abortion is a major health problem that contributes significantly to maternal mortality and morbidity (Adewole, 1992; WHO, 1996). In Ghana it is estimated that more than a third of all gynaecology

related admissions is abortion related (Ampofo, 1970; K.A.T.H., 1997). Although no studies have been done in this regard in the past, these findings correlate well with the observation that the patients were more educated and as a consequence married late compared to their age mates who were in the control group. Since most Ghanaian women will like to complete their education and marry (Anarfi and Awusabo-Asare, 1993) before having children, those who attain higher education tend to start child bearing later than their contemporaries of the same age group. Thus any pregnancy that occurs before this is terminated in order for them to achieve their educational ambitions. In a society where birth before marriage is a taboo, as in many African communities (Poulter *et al.*, 1981), such abortions are common since the girls will have to do this to protect both self and family reputation. The theory of tissue injury could be implicated in the association between fibroids and the high number of previous abortions; and between the tumour weight and the age at first abortion. These abortions may result in cellular injury or inflammation which have been proposed as mechanisms for initiation of myoma formation (Stewart and Nowak, 1998). In this case mechanical injury as a result of the abortion procedure could account for tissue injury. Infections that follow termination of pregnancies may result in tissue injury. This could then precipitate the development of fibroid.

There was no significant risk associated with method of delivery in the past. Some authors have also proposed that local uterine irritation could be the cause of uterine fibroid development (Witherspoon and Butler, 1934). This study based on this found it worthwhile exploring the role of some sources of uterine trauma such as caesarean sections. However the results of this study suggest otherwise. The results suggest that trauma as a result of caesarean section during delivery is not strongly associated with the development of fibroids. Thus it can be said, based on

the results, that trauma as a result of caesarean sections during childbirth does not play any significant role in the development of fibroids.

The length of the menstrual cycle did not differ between the two groups. The length of the menstrual cycle did not also correlate significantly with the weight of the tumour obtained. The findings of this study are consistent with those of other previous studies (Cramer *et al.*, 1995b; Sato *et al.*, 2000b; Faerstein *et al.*, 2001). Other researchers however obtained results contrary to the findings of this study (Chen *et al.*, 2001; Wegienka *et al.*, 2003) and they have suggested that tissue layer location and axial position of the fibroid may play a role in the severity of menstrual symptoms. They, however, failed to find any association between the menstrual cycle characteristics and the location of the fibroid.

The use of phytotherapeutic agents was more widespread among patients than among the control group. These phytotherapeutic agents were mostly plant based derivatives. In Ghana, the more advanced preparations are usually offered as alcoholic based extracts and traditional healers boast of a number of diseases one of such extracts is capable of treating. They are usually promoted mainly by past patrons of such agents. Women in traditional societies use phytotherapeutic agents for a variety of gynaecological purposes. For example the leaves of the guava tree in decoction are recommended for treating uterine haemorrhage. The same decoction is used as a wash for some *vaginal* and uterine infections (Michael *et al.*, 2002).

In spite of the growing availability of useful conventional medical treatments, plant-derived and other herbal remedies continue to offer a popular alternative for the treatment of various ailments in the developing world. Healers in traditional societies use herbal remedy recipes that have been handed down from one generation to another within families (Borins, 1990) and these are mostly plant derived. According to estimates, some 80% of the world's population rely

primarily on traditional medicines for their primary health care needs, and a major part of traditional therapy involves the use of plant extracts or their active ingredients (WHO, 1993). Use of herbs for healing is especially important in developing nations, where the cost of drugs is prohibitive, accessibility to drugs in rural areas is virtually nonexistent, and there is a shortage of physicians (Borins, 1990). This is particularly true for Ghana, where it is estimated that there is one (1) traditional doctor to approximately four hundred (400) people as opposed to one allopathic or orthodox doctor to every twelve thousand (12,000) people (Erah, 2008).

In this study there was a more than twice risk of developing fibroid in women who have had previous sexually transmitted infections (STIs) compared to those who have never had an STI.

Although not well documented, it is widely accepted that fibroids are much more common in American black women than American white women (Novak and Wood, 1979). One proposed explanation for this is that black women have a higher prevalence of pelvic infections than white women and that such infections cause myometrial irritation leading to abnormal uterine growth (Witherspoon and Butler, 1934).

In a similar study where researchers looked at the correlation between pelvic inflammatory disease (PID) and leiomyomata (Wyshak *et al.*, 1986), there was some indication that the frequency of PID was higher among fibroid cases than among controls. An effect of infectious agents on myometrial tissue seems plausible because of the growing body of evidence relating infectious agents to several neoplasms (Tomatis and Bartsch, 1990), and more specifically because of the observed development of smooth muscle neoplasms among children infected with human immunodeficiency virus (McClain *et al.*, 1995). A possible relation between

uterine infections and fibroid is also suggested by the association of fibroid with chlamydial infection, a common cause of PID (Benson and Pernoll, 1994), but not with infections (such as genital herpes) which affect mostly the external genitalia.

In general, the findings of this study data support the hypothesis that local uterine irritants of infectious origin may play a role in fibroid development, either because endometrial inflammation induces a myometrial response or because both endometrial and myometrial tissues are directly involved, as sometimes occurs (endometritis-myometritis) in situations such as puerperal sepsis (Benson and Pernoll, 1994).

Contraceptive usage appears to be common among Ghanaian women; be they patients or controls, as the results of this study reveals. The significantly higher use among the controls suggests some negative association between contraceptive use and fibroids and that the use of contraceptives may be protective. Most of the women used injectable contraceptives whiles a few others used oral contraceptives and intrauterine devices (IUD). Thus the protection was, to a large extent, offered by the injectable contraceptives. These injectable contraceptives were mostly progestin-only injectables.

Studies of the use of steroid contraceptives [combined oestrogen-progestin oral contraceptive pills (OCP), and progestin-only injections] and hormone replacement therapy (unopposed oestrogen or combined oestrogen-progesterone) have clearly identified the procarcinogenic and anticarcinogenic roles of oestrogens and progestins, respectively, in the endometrium (Cook and Weiss, 1999). A number of the epidemiologic studies of uterine leiomyomata have thus examined risk in relation to use of these hormonal medications as an approach to clarifying the specific contributions of steroid hormones in the development of these tumours.

The relation between OCP use and uterine leiomyomata has been studied extensively, but no clear patterns have emerged. The risk of uterine leiomyomata

among women who had ever used OCPs has been observed to be reduced (Ross *et al.*, 1986; Lumbiganon *et al.*, 1996), similar (Romieu *et al.*, 1989; Parazzini *et al.*, 1996d; Samadi *et al.*, 1996), and increased relative to never users. There is some evidence that current OCP users in particular may be at 20-60% lower risk, whereas past users have the same risk as never users (Marshall *et al.*, 1998a). Although one study suggests that long-term OCP users are at reduced risk (Ross *et al.*, 1986), other investigations do not show trends with duration or ages of use (Romieu *et al.*, 1989; Parazzini *et al.*, 1996d; Marshall *et al.*, 1998a) or suggest an increase in risk with increasing years of use of these medications *(Bernstein et al., 1991)*. One study found that uterine leiomyomata cases reported more frequent use of OCPs containing progestins that have oestrogenic properties (Ross *et al.*, 1986).

Depot medroxyprogesterone acetate (DMPA) is a progestin-only injectable contraceptive that is used extensively in developing countries including Ghana. A study in Thailand showed a strong inverse association between a history of DMPA use and the risk of surgically confirmed uterine leiomyomata *(Lumbiganon et al., 1996)*. Current use of progestin-only injectables was associated with a decreased risk of uterine leiomyomata relative to non-use of hormonal contraception. A study in Thailand showed a similar inverse relation between the use of DMPA, an injectable progestin, and surgically confirmed leiomyomata (Lumbiganon *et al.*, 1996). In that study, the reduction in risk was most pronounced for women using DMPA for 5 or more years compared to current users. In the Black Women's Health Study, the inverse association for use of progestin-only injectables remained unchanged after excluding past users. This study's results are consistent with the results of the present study and they go to support several hypotheses regarding the aetiology of uterine leiomyomata, including a direct inhibitory effect of progestins on myometrial tissue proliferation and/or a protective effect of menstrual cycle suppression.

Use of DMPA has been associated with an increase in gonadotropin-releasing hormone pulse frequency, modification of the endometrial lining, suppression of gonadotropins, and reduced secretion of oestradiol (Mishell, 1996; Clark *et al.*, 2001) and progesterone. Progestins may also down-regulate the oestrogen receptor in leiomyomata (Englund *et al.*, 1998). Oestradiol concentrations in DMPA users resemble postmenopausal levels, and the degree of oestradiol suppression may increase with longer use (Clark *et al.*, 2001).

4.3 ANTHROPOMETRICS

This study found significant difference in weights but not the heights between the subjects in the two groups. The heights of subjects in both groups were similar and did not correlate significantly with the weight of the tumour obtained from the patients. Overall, it was observed that an increase in body mass index (BMI), weight, and waist-to-hip ratio were each associated with an increase in uterine leiomyoma risk. However, it was observed that there was no association with height. This pattern suggests that body mass and weight gain increase uterine leiomyoma risk, while height does not. Although height is associated with higher follicular-phase plasma oestradiol levels in premenopausal women (Dorgan *et al.*, 1994) this study found no evidence of an association between height and uterine leiomyomata, consistent with findings from other studies (Marshall *et al.*, 1998b). A significant difference in weight between the two groups and an insignificant difference in height between them resulted in the patients recording higher BMI compared to the controls. This observation suggests an association between high BMI and leiomyomata development and/ or growth. Though other studies have come out with similar findings, it is unclear whether BMI actually plays a role in development of leiomyomata or it aids their growth; or both. Several epidemiologic studies (Ross *et al.*, 1986; Lumbiganon *et al.*, 1996; Marshall *et al.*,

1998b) have found that the risk of uterine leiomyomata increases monotonically with increasing BMI. The results of this study also showed an association between increase in BMI and the risk of developing fibroid. This finding is also consistent with the findings of some other studies. In two of these studies the heaviest women were at 2- to 3-fold greater risk than the leanest women (Ross *et al.*, 1986; Marshall *et al.*, 1998b). Data from other studies, however, do not agree with the findings of this study as they show little or no increased risk associated with elevated BMI *(Parazzini et al., 1988; Romieu et al., 1988; Samadi et al., 1996)*. In one study where uterine leiomyomata confirmed by ultrasound were included, the associations tended to be weaker than when analyses were restricted to uterine leiomyomata confirmed by hysterectomy (Marshall *et al.*, 1998b). This finding suggests that if obesity is related to uterine leiomyomata, it may be involved in enhancing the growth of uterine leiomyomata and/or promoting the development or severity of symptoms. There is some evidence from one cohort study that women who experience an increase in weight during the reproductive years are at increased risk, whereas weight at 18 years of age is not related to risk (Marshall *et al.*, 1998b). The mean BMI for the patients was 25.35 ± 0.49 which suggest that the patients were generally slightly overweight in this study whereas the control subjects were generally of normal BMI as shown by a mean BMI of 22.4 ± 0.22. The results of this study also showed a higher mean serum progesterone concentration among the patients compared to the controls. Thus, the possible association between obesity and uterine leiomyomata may reflect associated hormonal changes as well as alterations in metabolic controls that affect myometrial cell signaling through mediators such as insulin receptors, insulin-like growth factors, and peroxisome proliferating activating receptors (Jump *et al.*, 1997). Since ovarian hormones are believed to play a key role in the aetiology of uterine leiomyomata (Andersen, 1996b), body mass index which is a measure of absolute body fat (Marshall *et al.*,

1998b) may influence the risk of uterine leiomyomata through changes in steroid hormone metabolism and bioavailability (Schwartz *et al.*, 2000). Studies in premenopausal women have consistently documented an inverse association between BMI and circulating levels of sex hormone-binding globulin (Grenman *et al.*, 1986; Dorgan *et al.*, 1994; Verkasalo *et al.*, 2001). Decreases in sex hormone-binding globulin may increase the proportion of free oestrogen or the fraction available for biologic activity (Dorgan *et al.*, 1994). Obesity is associated with diminished 2-hydroxylation of estrone to catechol oestrogens and increased 16-alpha-hydroxylation of estrone to estriol, thereby producing oestrogens with greater uterotropic activity (Fishman *et al.*, 1975; Schneider *et al.*, 1983).

The relation of uterine leiomyomata to body fat distribution, as measured by waist circumference or waist-to-hip ratio (Marshall *et al.*, 1998b), has been evaluated only to a limited extent. Several studies have documented that central obesity is greater for black women than white women and is positively associated with age and nulliparity which are both increased risk factors for fibroids (Burke *et al.*, 1990). There is also evidence that women with greater upper body obesity have decreased sex hormone-binding globulin levels, altered oestrogen metabolism, and hyperinsulinaemia, factors that may promote tumour development.

In this study, evaluation of waist-to-hip-ratio showed a significant difference between the patients and the control groups, as the figures were generally higher for the patients. Again there was a positive significant correlation between the waist-to-hip ratio and the weight of the tumour obtained at surgery which implied that the waist-to-hip ratio could have an influence in the development and/or growth of the tumour. Independent of BMI, central obesity (excess fat in the upper trunk region) is associated with hormonal and metabolic changes in premenopausal women, including altered oestrogen metabolism, insulin resistance and hyperinsulinemia, and decreases in sex hormone-binding globulin levels

(Kirschner *et al.*, 1990; Falkner *et al.*, 1999; Moran *et al.*, 1999; Verkasalo *et al.*, 2001). Insulin, which is itself a mitogenic agent (Andersen, 1996a), is associated with down-regulation of sex hormone-binding globulin (Falkner et al, 1999) and up-regulation of insulin-like growth factor-1 and epidermal growth factor. These agents could influence tumour development through direct promotion of myometrial smooth muscle cell proliferation or enhanced ovarian hormone secretion (Andersen, 1996a; Schwartz *et al.*, 2000).

The underweight subjects showed a rather reduced risk of developing fibroids according to the results of this study. Therefore this study suggests that being underweight is protective for fibroid. This finding of a reduced risk among the leanest women (BMI <20 kg/m2) supports the hypothesis that uterine leiomyomata are hormone-dependent tumours (Andersen, 1996a). It has been proposed that a variety of hormones may be involved in this modification of risk, given that thin or anorexic women are found to have higher levels of sex hormone-binding globulin (Dorgan *et al.*, 1995; Verkasalo *et al.*, 2001), decreased prolactin secretion (Zumoff *et al.*, 1981), and increased hydroxylation of estrone to catechol oestrogens (Fishman *et al.*, 1975) —all of which may create an endogenous hormonal milieu with lower susceptibility to uterine leiomyomata.

4.4 HAEMATOLOGY

Not much research data is available on the haematological profile of leiomyomata patients. Analysis of collected data however showed a significant difference between the red cell indices of the patients and the controls. The patients had higher mean values of all red cell indices, except MCV, compared to the controls contrary to popular reasoning that menorrhagia, a common symptom in fibroid patients, should reduce the red cell indices of the patients. Yet in many of the patients the haematologic profile did not correlate with the severity of menorrhagia. The results of this study showed that the patients had significantly higher total iron and lower total iron binding capacity compared to those of the controls. The total WBC count and platelet counts were not significantly different between the two groups and these two indices did not correlate significantly with the weight of the tumour obtained after surgery. All the red cell indices correlated significantly with the weight of the tumour obtained except the MCV.

Other studies have also corroborated this finding. In some situations, some have reported polycythaemia associated with fibroid (Thomson and Marson, 1953). Erythrocytosis has been commonly used to describe this phenomenon instead of polycythaemia since there is only the elevation of the erythrocytes and not the pancytosis that characterizes polycythaemia (Hoffbrand *et al.*, 2006). From the data obtained the red blood cell count and the associated red cell indices were the haematologic parameters that differed significantly between the patients and the controls.

Three schools of thought have emerged as possible causes of the erythrocytosis, all based on the observation that there is complete resolution of the erythrocytosis after surgical extirpation of the leiomyomata (Fleming and Markley, 1957).

Some authors proposed intrauterine shunting as the reason behind the erythrocytosis (Fleming and Markley, 1957; Aboulafia *et al.*, 1962; Spurlin *et al.*, 1972).Their theory about the existence of intra-myomatous artrio-venous shunts is supported by histopathologic findings of marked vascularity and sinusoidal vascular spaces. These shunts allow the flow of nonoxygenated blood into the arterial vasculature, stimulating the bone marrow to increase red cell production. But this theory has been rejected for several reasons (London *et al.*, 1949; Fleming and Markley, 1957; Le Roux, 1959; Laurin *et al.*, 1960; Hertko, 1963; Spurlin *et al.*, 1972; Weiss *et al.*, 1975)

The second theory is based on myoma size and site as substantial uterine size has been linked to the syndrome (Zilliacus, 1959; Vanden Berg and Vasu, 1963). Some researchers have proposed that additional factors such as specific anatomic site, mass density, and mass chronicity should be considered in understanding the pathophysiology of the myomatous erythrocytosis syndrome since no abdominal mass other than those with uterine origin had been linked to enhanced erythropoiesis (Cohen and Rothenberg, 1962; Kline *et al.*, 1969). Impaired pulmonary function as a result of increased pressure by a large uterus on the diaphragm and extreme obesity has also been postulated to cause the erythrocytosis as the pressure may compromise ventilation and cause hypoxia (Castle, 1956-1957; Kline *et al.*, 1969). This theory has not been substantiated as little or no evidence has been uncovered with regards to oxygen desaturation. Other pulmonary tests performed did not support this theory either (Aboulafia *et al.*, 1962; Paranjothy and Vaish, 1967; Payne *et al.*, 1969; Spurlin *et al.*, 1972; Clark *et al.*, 1994). Due to the fact that the pelvic bone marrow is a site of active haemopoiesis, it is possible that a vascular supply impairment caused by a large myoma may lead to local hypoxia and increased red cell production.

Two other possible mechanisms for the erythrocytosis which are based on changes in red cell life span have been offered(Babuna *et al.*, 1959). One attributes the erythrocytosis to the prolongation of the red cell life span. The other theory links this erythrocytosis to a myomatous humoral factor causing a disruption in the blood conservation mechanism of the spleen. Inhibition of the normal removal of excess red cells results in erythrocytosis. However, these theories were disputed by some studies which showed normal red cell life span in these patients (London *et al.*, 1949). Enhanced erythropoietin production by the kidney in the presence of myoma has also been suggested as the aetiology of the erythrocytosis and increased erythropoietin activity in serum and urine has been documented (Wrigley *et al.*, 1971; Ossias *et al.*, 1973; Toyama *et al.*, 1973; Weiss *et al.*, 1975; Itoh *et al.*, 1987). Two mechanisms explaining the interrelationship of uterine leiomyomata and renal erythropoietin production have been proposed. Increased pressure within the renal parenchyma and elevated erythropoietin output could result from compression of the urinary outflow tract by a retroperitoneal tumour (Jones *et al.*, 1960; Toyama and Mitus, 1966; Lozzio *et al.*, 1971). A second mechanism could be impaired renal perfusion secondary to tumour impingement on the renal vessels thus enhancing erythropoietin release(Menzies, 1961; Rothman and Rennard, 1963; Payne *et al.*, 1969; Spurlin *et al.*, 1972). This mechanism has been discredited since the superior border of the tumour is below the level of the inferior pole of the kidney. Thus either hydronephrosis or altered renal blood flow may enhance erythropoietin production (Spurlin *et al.*, 1972; Thorling, 1972). Uterine myomas have been hypothesized as a possible source of erythropoietin production although the kidney is the primary source of production (Horwitz and Mc, 1955). The argument against this hypothesis is that much slower improvement in red cell count would be anticipated after surgery than is usually observed. But the rapid normalization of red cell indices after surgery might be due to the contribution of

blood loss before and during surgery (Laurin *et al.*, 1960). To further prove this hypothesis erythropoietin activity in myomatous tumour extracts have been investigated using various methods(Wrigley *et al.*, 1971; Ossias *et al.*, 1973; Toyama *et al.*, 1973; Weiss *et al.*, 1975). These investigations led to the conclusion that erythrocytosis is mediated through direct production of erythropoietin by the myoma tissue and by a renal erythropoietic factor.

Since this study did not evaluate erythropoietin activity of the myomatous tissue or in the serum of patients, conclusion can only be inferred from the various existing hypotheses. Looking at all the possible aetiologies for the erythrocytosis, the most plausible theory for our observation of higher mean red cell counts of patients compared to controls is the one attributing the myomatous erythrocytosis syndrome to altered erythropoietin production and release. Erythropoietin production either by the myoma itself or by the kidney secondary to the presence of a myoma, may account for the increase in red cell production. Although this study did not find any case of erythrocytosis, the theory can well be used to explain the absence of anaemia in the presence of menorrhagia. It can be projected that in the absence of the menorrhagia in some cases, there could have been erythrocytosis. The generally higher red cell indices of patients compared to the normal women supports this projection. The observation that the higher the red cell count the larger the tumour means that a larger myoma tissue produces and releases more erythropoietin.

The higher levels of blood iron and low level of iron binding capacity may be attributed to the observation that most of the patients had prescribed for them, blood tonics which contained iron supplements. Care must therefore be taken when prescribing iron containing supplements as that could lead to iron over load.

4.5BIOCHEMICAL TEST

4.5.1 Oxidative stress markers

Oxidative stress has been defined as an imbalance between oxidants and antioxidants in favor of the former, resulting in an overall increase in cellular levels of reactive oxygen species (Sies and Cadenas, 1985). Reactive oxygen species can be produced by both endogenous and exogenous sources. Potential endogenous sources include oxidative phosphorylation, P450 metabolism, peroxisomes, and inflammatory cell activation.

Increased oxidative stress is used generally to describe a condition in which cellular antioxidant defenses are inadequate to completely inactivate the reactive oxygen species (Stocker and Keaney, 2004) and reactive nitrogen species generated because of excessive production of these species, loss of antioxidant defenses, or both. A major consequence of oxidative stress is damage to nucleic acid bases, lipids, and proteins, which can severely compromise cell health and viability or induce a variety of cellular responses through generation of secondary reactive species, ultimately leading to cell death by necrosis or apoptosis. Observations in vitro and in cultured cell systems indicate that oxidative stress contributes to tumour risk by numerous mechanisms that are independent of genotoxicity (Dedon and Tannenbaum, 2004).

Patients had significantly higher levels of MDA and lower levels of vitamin C than the controls. The significant difference observed between the two groups in the levels of MDA and ascorbic acid (vitamin C) suggests that oxidative stress may be associated with the development of fibroids.

Under normal physiological conditions, cells are capable of counterbalancing the production of reactive oxygen species with antioxidants. Endogenous cellular antioxidant defenses are mainly enzymatic and include superoxide dismutase,

glutathione peroxidase, and catalase. Superoxide dismutases are localized to the cytosol and mitochondria and function to reduce superoxide anion to hydrogen peroxide and water. Glutathione peroxidases, localized in the cytosol and mitochondria, remove the majority of hydrogen peroxide, whereas catalase, located in peroxisomes, is responsible for the removal of high levels of hydrogen peroxide. Nonenzymatic antioxidants such as vitamin E, vitamin C, β-carotene, glutathione, and coenzyme Q function to quench reactive oxygen species (Clarkson and Thompson, 2000). When the redox balance is shifted in favor of cellular oxidants, oxidative damage to nucleic acids, lipids, or proteins can result and produce modification to cell function and cell viability. Many of the cellular antioxidants are however regulated in part by the redox status of the cell. Due to budget and logistic constraints only vitamin C (ascorbic acid) was analyzed. The significant difference between the two groups in terms of their serum levels of vitamin C implies the controls are likely to better manage oxidative stress than the patients.

Vitamin C (ascorbic acid) is a very important, and powerful, antioxidant that works in aqueous environments of the body, such as are present in the lungs and in the lens of the eye. Its primary antioxidant partners are Vitamin E and the carotenoids, as well as working along with the antioxidant enzymes. Vitamin C co-operates with Vitamin E to regenerate α-tocopherol from α-tocopherol radicals in membranes and lipoproteins (Carr and Frei, 1999; Kojo, 2004).

It is acknowledged that Vitamin C protects membranes against oxidation (Retsky *et al.*, 1999). The intake of very high doses of Vitamin C, suggested initially by Linus Pauling, has been a subject of intense debate for many years (Cameron and Pauling, 1976). While intake of high doses of Vitamin C (up to 2000 mg/day) has not been consistently reported to result in side effects, the benefit of a high intake

of Vitamin C has never been established. However, it has been reported that low serum levels of Vitamin C in high-risk population may contribute to the increased risk of gastric metaplasia or chronic gastritis, which are both precancerous lesions (You *et al.*, 2000) The positive effect of Vitamin C in reducing the incidence of stomach cancer is most probably due to the inhibitory action in the generation of *N*-nitroso compounds by interrupting the reaction between nitrites and amine groups. A consistent protective effect of Vitamin C has also been found in lung and colorectal cancer (Knekt *et al.*, 1991).

Some in vitro and animal supplementation studies explored the pro-oxidant properties of ascorbate (Kang *et al.*, 1998). The pro-oxidant effect of ascorbate was attributed to the release of metal ions from damaged cells. In addition, it has been reported that Vitamin C induces lipid hydroperoxide decomposition to the DNA-reactive bifunctional electrophiles 4-oxo-2-nonenal, 4,5-epoxy-2(E)-decenal and 4-hydroxy-2-non-enal (Lee *et al.*, 2001). The compound 4,5-epoxy-2(E)-decenal is a precursor of etheno-2′-deoxyadenosine a highly mutagenic lesion found in human DNA. Recent in vitro and ex vivo studies revealed that Vitamin C in plasma increases dose-dependently resistance to-lipid peroxidation, even in the presence of redox-active iron or copper and H_2O_2 (Suh *et al.*, 2003). Overall, in vitro studies have shown that Vitamin C either has no effect or inhibits transition metal (Fe, Cu)-ion dependent lipid peroxidation in plasma and other biological fluids. In contrast, Vitamin C may be able to promote metal ion-dependent hydroxyl radical formation in biological fluids, but only under unphysiological conditions (Smith *et al.*, 1997). Majority of in vivo studies showed a reduction in markers of oxidative DNA, lipid and protein damage after supplementation with Vitamin C. Even in the presence of iron, Vitamin C predominantly reduces in vivo oxidative damage, despite its well known pro-oxidant properties in vitro in

buffer systems containing Fe. Studies reporting a pro-oxidant effect for Vitamin C in human supplementation studies should be taken with caution as to their choice of biomarkers, methodology and experimental design to rule out any oxidation effects (Burton and Ingold, 1989).

Recent studies indicate the ability of ascorbic acid to regulate factors that may influence gene expression, apoptosis and other cellular functions (You *et al.*, 2000). In many studies Vitamin C protects against cell death triggered by various stimuli and a major proportion of this protection has been linked with its antioxidant ability. Studies of the anti-apoptotic activity of Vitamin C have revealed a role of Vitamin C in modulation of the immune system. Several studies reported the mechanisms by which Vitamin C regulates the AP-1 complex, including the Fos and Jun superfamilies. Ascorbate treated cells exposed to UV-B irradiation led to a 50% decrease in JNK phosphorylation (which activated AP-1), therefore inhibiting the JNK/AP-1 signalling pathways.

The observation that MDA is lower among the controls than in the patients further supports the view that the patients manage oxidative stress less efficiently compared to the controls. Aside from oxidized nucleic acids, other oxidation derived DNA adducts appear important in chemical carcinogenesis. Radical-mediated damage to cellular biomembranes results in lipid peroxidation, a process that generates a variety of products including reactive electrophiles such as epoxides and aldehydes (Janero, 1990). Malondiadlehyde (MDA), a by-product of lipid degradation, is a tautomer that is both highly electrophilic and nucleophilic. This characteristic does not only allow interreaction with cellular nucleophiles, but also enhances the formation of MDA oligomers (Golding *et al.*, 1989). MDA and MDA-MDA dimers are mutagenic in bacterial assays as well as in the mouse lymphoma assay (Riggins and Marnett, 2001). MDA was also shown to induce

thyroid tumours in chronically treated rats (Spalding, 1988). MDA reacts with several nucleic acid bases to form dG, dA, and dC adducts (Stone *et al.*, 1990). The observed MDA-DNA adducts appear to be promutagenic as they induce mutations in oncogenes and tumour suppressor genes seen in human tumours. MDA-DNA adduct levels also to correlate with altered cell cycle control and gene expression in cultured cells (Ji *et al.*, 1998). The significant difference between the two groups in terms of total cholesterol and triglyceride levels could also explain the difference between the two groups in their MDA levels.

Metabolic activation and production of reactive oxygen species by cytochrome P450 has been proposed by Parke and associates (Parke, 1994). Reactive oxygen species can be produced from several sources during metabolism, including (*a*) through redox cycling in the presence of molecular oxygen, (*b*) through peroxidase-catalyzed single-electron drug oxidations, and (*c*) through "futile cycling" of cytochrome P450 (Parke, 1994; Parke and Sapota, 1996). Through the induction of cytochrome P450 enzymes, the possibility for the production of reactive oxygen species, in particular, superoxide anion and hydrogen peroxide, arises following the breakdown or uncoupling of the P450 catalytic cycle (Parke and Sapota, 1996). P450 2E1 is involved in the oxygenation of substrates such as ethanol, and is capable of generating a prolonged burst of reactive oxygen species near the site of substrate oxidation (Eksrom and Ingleman-Sundberg, 1989). Similarly, metabolism of phenobarbital by P4502B results in the uncoupling of the catalytic and subsequent release of superoxide anion (Rice *et al.*, 1994). Thus, the correlation between induction of P450 isozymes and the subsequent reactive oxygen species production warrants consideration as a possible mechanism for the induction of oxidative stress and tumour promotion seen following exposure

to a number of chlorinated and nonchlorinated compounds such as dieldrin, TCDD, lindane, and phenobarbital (Klaunig *et al.*, 1997).

The potential for the production of oxidative species derived from peroxisomes has also been proposed. Chemicals such as peroxisome proliferators are potent inducers of cytochrome P450 4A, and induce the formation of peroxisomes, and as such, an increase in H_2O_2 production. Through peroxisome proliferation and increased peroxisomal enzyme activity, H_2O_2 will escape and shift the cellular redox balance toward the oxidative state (Rao and Reddy, 1991; Wade *et al.*, 1992).

Reactive oxygen species can be produced by a host of exogenous processes. Environmental agents including nongenotoxic carcinogens can directly generate or indirectly induce reactive oxygen species in cells (Rice-Evans and Burdon, 1993). The induction of oxidative stress and damage has been observed following exposure to xenobiotics of varied structures and activities. Chlorinated compounds, radiation, metal ions, barbiturates, phorbol esters, and some peroxisome proliferating compounds are among the classes of compounds that have been shown to induce oxidative stress and damage in vitro and in vivo (Klaunig *et al.*, 1997). 2-Butoxyethanol is an example of a chemical that produces reactive oxygen species indirectly, resulting in liver cancer in mice. Reactive oxygen species production, as evidenced by the induction of 8-hydroxyguanosine in liver, appears to result from Kupffer cell activation secondary to 2-butoxyethanol-induced haemolysis and subsequent hepatic iron deposition (Seisky *et al.*, 2002). The role of Kuppfer cell–derived reactive oxygen species and the potential importance of Kupffer cell activation to the carcinogenesis process are intriguing and yet to be clearly understood.

Reactive oxygen species can directly produce single- or double-stranded DNA breaks, purine, pyrimidine, or deoxyribose modifications, and DNA cross-links. Persistent DNA damage can result in either arrest or induction of transcription, induction of signal transduction pathways, replication errors, and genomic instability, all of which are seen in carcinogenesis.

Many forms of reactive oxygen species are capable of forming oxidized bases. The hydroxyl radical in particular has been shown to produce a number of oxidized DNA lesions (Marnett, 2000). The reactivity of the hydroxyl molecule is such that its migration in the cell is limited and thus reacts quickly with cellular components (Sies, 1985). For the hydroxyl radical to react and oxidize DNA, it must be generated adjacent to the nucleic acid material. H_2O_2, a precursor to the hydroxyl radical, is less reactive and more readily diffusible and thus more likely to be involved in the formation of oxidized bases (Guyton and Kensler, 1993; Barber and Harris, 1994). Peroxynitrite, another strong cellular oxidant, is formed from the coupling of nitric oxide and superoxide (Koppenol *et al.*, 1992; Radi, 2009). As with H_2O_2, peroxynitrite is diffusible between cells and is taken up by active transport mechanisms into cells (Radi, 2009). Equally important to the induction of mutation by reactive oxygen species is the fact that nitric oxide and superoxide are produced in activated macrophages, and as such, it is likely that peroxynitrite is formed in proximity to these cells. The DNA damaging capability of peroxynitrite may therefore help to explain the reported association between inflammation and mutation (Marnett, 2000). Results of this study indicate that the patients had recorded more STIs in the past compared to the controls. Thus it could be argued that the patients have more in inflammations than controls and thus are at a higher risk of damaging DNA and hence developing mutations which could then initiate their tumours.

A role for reactive oxygen species production and oxidative stress has again been proposed for both the stimulation of cell proliferation and for cell deletion by apoptosis (Burdon, 1995; Slater *et al.*, 1995). The mechanisms for the involvement of oxidative stress in the induction of the cell proliferation and apoptotic processes are not known, but clearly do not involve a universal mechanism. The effects of reactive oxygen species and oxidative stress within cells appear to be cell specific and dependent upon the form as well as the intercellular concentration of reactive oxygen species. Thus, the involvement of reactive oxygen species in cell growth regulation is complex, and dependent on a number of cellular and biochemical parameters.

Reactive oxygen species function to induce cell proliferation during the tumour promotion stage of carcinogenesis (Cerutti, 1985). Both H_2O_2 and superoxide anion induce mitogenesis and cell proliferation in several mammalian cell types (D'Souza *et al.*, 1993). Furthermore, a reduction in cellular oxidants via supplementation with antioxidants such as superoxide dismutase, catalase, β-carotene, and flavenoids inhibits cell proliferation in vitro (Alliangana, 1996). Oxidative stress also modulates apoptosis. High concentrations of reactive oxygen species trigger an apoptotic signaling pathway, resulting in cell loss (Dypbukt *et al.*, 1994). A number of endogenous substances (prostaglandins, and lipid hydroperoxides), redox cycling compounds (quinones, adriamycin), and growth factors (transforming growth factor β and tumour necrosis factor α) induce apoptosis via the generation of reactive oxygen species (Sandstrom *et al.*, 1994; Aoshima *et al.*, 1997). Antioxidants such as *N*-acetyl cysteine (NAC), glutathione, and dithiothreitol inhibit the apoptotic process, further supporting the link between reactive oxygen species induction and apoptosis (Sandstrom *et al.*, 1994). Xenobiotics may differentially interact with the cell to elicit biological responses. Lipophilic and

esterified compounds can freely cross the plasma membrane and produce effects within a cell; other compounds gain entry to the cell only through specific channels or energy-dependent pumps, whereas membrane-impermeable chemicals may stimulate cellular responses by acting on receptors that initiate signaling cascades within the cell. As a result, each chemical may provide a unique stimulus that sets in motion specific signaling pathways. Although no single mechanism explains the increased cell proliferation and/or inhibition of apoptosis observed following conditions that favor increased cellular oxidants, mounting evidence is emerging that links reactive oxygen species with altered expression of growth regulatory genes.

This study also demonstrated a marked difference in serum iron levels between the controls and the patients. Iron has been shown in many studies as playing a role in carcinogenesis. Links have been proposed between increased levels of iron in the body and an enhanced risk of a variety of diseases including vascular disease, cancer and certain neurological conditions (Berg *et al.*, 2001; Siah *et al.*, 2005). Iron-mediated formation of ROS leading to DNA and lipid damage appears to result from an exaggeration of the normal function of iron, which is to transport oxygen to tissues. Iron-induced free radical damage to DNA appears to be important for the development of cancer and cancer cells are known to grow rapidly in response to iron (Ullen *et al.*, 1997). Nelson and Babbs proposed that intestinal exposure to ingested iron may be a principal determinant of human colorectal cancer in highly developed, meat-eating countries (Babbs, 1990; Nelson, 1992). an alternative mechanism has been proposed in which the bile acids (deoxycholic acid), the K vitamins, iron(II) complexes and oxygen interact to induce an oncogenic effect in the colon by the generation of free radicals (Valko *et al.*, 2001).

Genetic haemochromatosis is associated with an increased risk for hepatocellular carcinoma. The association between elevated body iron stores and the development of hepatocellular carcinoma in subjects with iron overload unrelated to genetic haemochromatosis along with the experimental evidence of a co-carcinogenic role of iron strongly support the contention that iron is involved in the development of hepatocellular carcinoma (Deugnier and Turlin, 2001; Kowdley, 2004).

Occupational exposure to asbestos containing about 30% (weight) of iron is related to an increased risk of asbestosis — the second most important cause of lung cancer after smoking (Stayner *et al.*, 1996). It is generally accepted that asbestos-induced carcinogenesis is linked with the free radicals. Intramuscular injections of an iron–dextran complex, frequently used for the treatment of anaemia in humans, caused spindle cell sarcoma or pleomorphic sarcoma in rats at the site of injection (Bhasin *et al.*, 2002).

From the results of this study, it can be inferred that the increased oxidative stress in patients is attributable to a variety of factors including, high serum iron, high lipid levels (especially triglycerides), and frequent injury from STIs resulting in high rate of inflammation. These factors combine to elicit the cascade of events as discussed above.

4.5.2 Liver Function

The liver plays a major part not only in synthetic function, but also in metabolic and excretory functions, and conventional liver tests measure only a small proportion of the organ's many functions.

Serum liver biochemical testing includes measures of synthetic function (ie, serum albumin and protein), and estimates of cellular injury (ie, aspartate aminotransferase, alanine aminotransferase), cholestasis, or duct injury (ie, alkaline phosphatase, gamma-glutamyltransferase, and direct-reacting bilirubin) (Pratt and Kaplan, 2000).

Aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltransferase, alkaline phosphate, total bilirubin, indirect bilirubin were all significantly higher in the patients than in the controls. However direct bilirubin, total protein, albumin and globulin levels were not significantly different between the two groups. It was, however, observed that both groups had mean levels of all the parameters within normal ranges. Alanine transaminase (ALT) and aspartate transaminase (AST) are together referred to as the aminotransferases or transaminases. They are both intracellular enzymes which catalyse steps in gluconeogenesis. ALT is present almost exclusively in the liver, whereas AST is less specific and is also found in cardiac tissue and muscle, for example. These enzymes are released into the bloodstream when liver cells are damaged, even if this damage is not severe enough to cause cell death. Though alkaline phosphatase (ALP) is found mainly in liver and bone, in the liver, ALP is found in cells near the bile ducts and canaliculae and is, therefore, classically raised when bile drainage is obstructed (Pratt and Kaplan, 2000). Gamma glutamyl transferase (GGT) is found mainly in hepatocytes and biliary epithelial cells as a microsomal enzyme. Though a sensitive test of hepatobiliary disease its usefulness is limited by lack of specificity. Raised levels may be seen in pancreatic disease, myocardial infarction,

renal failure, chronic obstructive pulmonary disease, diabetes, certain drugs, and alcoholism (Rosalki *et al.*, 1971; Goldberg and Martin, 1975). Bile duct obstruction causing damage to cells containing the enzyme will also cause a rise in the GGT level. Serum bilirubin is normally mainly in an unconjugated form reflecting a balance between production and hepatobiliary excretion. Bilirubin production increases in haemolysis, ineffective erythropoiesis, resorption of a haematoma, and rarely in muscle injury. In all these cases the bilirubin is mainly in an unconjugated form (Lidofsky, 2006). Bilirubin is often elevated in liver disease as liver damage causes reduced function of the enzymes involved in its metabolism and excretion, leading to a block in the pathway. Common risk factors for non-communicable liver diseases, such as alcohol consumption diet and obesity (Becker *et al.*, 1996; Clarke *et al.*, 2009) were significantly different between the two groups. This may account for the difference between the two groups in the levels of the liver enzymes and bilirubin. Results of this study show the patients had higher BMI and a higher percentage of them consumed alcohol compared to the controls. The higher levels of total and indirect bilirubin in patients compared to the controls may also, apart from the issue of differences in alcohol consumption, be attributed to the higher levels of red blood cells in the patients. With the higher red cell counts, the patient's liver will have to deal with clearing more bilirubin and hence the tendency to accumulate more of it even though the liver might be in fine condition. Elevated levels of serum direct bilirubin occur in inherited or acquired defects in hepatic excretion. Direct bilirubin levels have prognostic significance in alcoholic hepatitis, primary biliary cirrhosis, and in acute liver failure. However a disproportionate rise in conjugated bilirubin has limited diagnostic value as conjugated bilirubin is excreted in urine. Conjugated bilirubin levels are rarely elevated in the absence of renal failure or haemolysis (Lidofsky, 2006). Since all the means of the liver function parameters are within normal ranges, it suggests that

both groups had no liver dysfunction. This is further corroborated by the observation that there were no significant differences between the two groups in serum protein and albumin which correlate with the prognosis in chronic liver disease. From the results it can be deduced that liver function in both groups is fine. On the basis of the results of this study, it can be inferred that the development of fibroids does not affect normal liver function.

4.5.3 Renal Function

The results of this study showed no significant difference between the control group and the patient group in the serum sodium, potassium, chloride, and blood urea concentrations. There was however a significant difference in the serum creatinine levels between the two groups. None of the markers of kidney function correlated significantly with the weight of the tumour obtained at surgery. This suggests that there is no association between kidney function and the development of fibroids. Both controls and the patients had normal kidney function from the results of this study since the means of all parameters were within normal limits. Literature on the effect of fibroids on renal function is very scanty. Those available have been reports of cases (Fong and Singh, 1999). These available literature have reported of normal kidney function in leiomyomata cases and adverse effects or otherwise of fibroids on kidney function were not found in literature. These case reports are consistent with the findings of the present research. The difference in serum creatinine between the two groups can be attributed to the fact the patients had higher BMI compared to the controls. The higher BMI in part may be attributed to higher muscle mass. Creatinine is known to be higher in people with greater muscle mass (Culleton *et al.*, 1999) and this could explain the difference between the two groups in serum creatinine. Body mass index has been reported to be associated with elevated serum creatinine levels in men, but not in women. It is generally understood that men have a greater percentage of muscle mass than

women at any level of BMI (Baumgartner *et al.*, 1995). As creatinine is formed from muscle creatine (Boorsook and Bubroff, 1947), it is not surprising that BMI was associated with higher serum creatinine values. It is plausible that a better measure of muscle mass, such as that obtained from bioimpedance or dual energy x-ray absorptiometry, would provide more significant correlations with serum creatinine values. Alternatively, the elevated serum creatinine values may be attributable to drug treatment rather than to the disease it is being used to treat. From this study patients were found to use more phytotherapeutic agents compared to their control counterparts. The chemical constituents of most of the agents are unknown and their effects on the human system are rarely assessed. It can therefore be presumed that the patients' slightly higher serum creatinine levels may be due to the use of some of these phyto agents which probably affected kidney function slightly but not enough to cause any renal impairment. With the location of the fibroid and the kidneys, it could be inferred that mechanical pressure from the growths, especially massive growths would impair the function of the kidney. This study results however show that fibroids generally do not impair renal function. Thus any renal impairment in fibroid patients could result from some other causes and can hardly be attributed to the presence of the fibroids.

4.5.4 HORMONES

The levels of both progesterone and oestradiol in patients were generally higher than those of the controls. Whiles there was no significant correlation between progesterone levels and the weight of the tumour obtained, serum oestradiol levels had a positive correlation with the weight of the tumours obtained at surgery. Both oestrogen and progesterone appear to promote the development of myomas. Myomas are rarely observed before puberty, are most prevalent during the reproductive years, and regress after menopause. Factors that increase overall lifetime exposure to oestrogen, such as obesity and early menarche, increase the

incidence. Decreased exposure to oestrogen found with exercise and increased parity is protective (Cook and Walker, 2004). Although blood levels of oestrogen and progesterone are similar in women with and without clinically detectable myomas, levels of oestradiol within myomas are higher than in normal myometrium. Consistent with this idea, myomas show a higher proliferative index than normal myometrium throughout the menstrual cycle (Cook and Walker, 2004). Biochemical, clinical, and pharmacologic evidence confirm that progesterone is important in the pathogenesis of myomas. Myomas have increased concentrations of progesterone receptors A and B compared with normal myometrium (Englund *et al.*, 1998; Nisolle *et al.*, 1999). The highest mitotic counts are found during the secretory phase at the peak of progesterone production, and mitotic counts are higher in women treated with medroxyprogesterone acetate (MPA) than in untreated controls (Kawaguchi *et al.*, 1991). Gonadotropin-releasing hormone (GnRH) agonists decrease the size of myomas, but progestin given concurrently with GnRH prevents a decrease in size. One study found that use of progestin-only injectable contraceptives was inversely associated with risk of having myomas (Wise *et al.*, 2004a). Mifepristone, a progesterone-receptor modulator, decreases myoma size (Murphy *et al.*, 1995).There is evidence that reproductive factors influence the endocrine profiles of women in the reproductive age. Higher levels of oestradiol (MacMahon *et al.*, 1982a; Apter *et al.*, 1989; Moore *et al.*, 1991) and oestrone (MacMahon *et al.*, 1982a) and lower levels of sex hormone-binding globulin (Apter *et al.*, 1989) have been observed among adult women who experienced early menarche. These reports lend credence to the findings of this study. The patients who had higher levels of oestradiol and progesterone also had earlier menarche than the controls. Likewise, oestradiol levels have been observed to be higher among nulliparous women than among parous women (Bernstein *et al.*, 1991) and to increase with age (up to 40 years) during the mid-cycle and luteal

menstrual phases among nulliparous women but to decline with age among parous women (Dorgan *et al.*, 1994). The findings of this study are consistent with the above in that most of our patients were nulliparous. The observations of increased risk among women with an early menarche and decreased risks among parous women and women of higher parity are consistent with these reports and support the hypothesis that myometrial response to the oestrogens may be important in the aetiology of these benign neoplasms (Andersen and Barbieri, 1995).

The possible effects of other steroid hormones or of other cellular processes should also be considered. For example, levels of androgens, which are aromatized to estrone and oestradiol, have been found to be lower in parous women than in nulliparous women (Musey *et al.*, 1987a). Early onset of menstrual cycles may increase the number of cell divisions that the myometrium undergoes during the reproductive years and, consequently, the chance of mutation in genes that control myometrial proliferation.

4.5.5 Lipid profile

Total cholesterol and triglycerides levels of patients were significantly higher than those of the controls. The levels of HDL cholesterol and LDL cholesterol were not significantly different between the two groups. Total cholesterol, LDL cholesterol, and Triglycerides correlated positively with the weight of the tumour obtained at surgery. There was however no significant association between the levels of HDL cholesterol and the weights of tumours. Although there is some epidemiologic evidence linking high cholesterol levels with certain types of cancer, there has been little research of its association with fibroid growth. High cholesterol levels have been reported to influence the growth of some tumours. Researchers at a Children's Hospital in Boston have demonstrated that high blood cholesterol levels accelerate the growth of prostate tumours, showing that cholesterol helps prostate

tumours survive and grow at the molecular level by altering chemical signaling patterns within tumour cells (Zhuang *et al.*, 2005). This study also came out with results that are in keeping with population studies that have linked prostate cancer with high cholesterol levels and Western diets high in cholesterol. In this study, human prostate cancer cells were injected into mice and the tumour growth observed. When the animals' blood cholesterol was raised by diet, cholesterol accumulated in the outer membranes of the tumour cells, specifically in structures called lipid rafts. Cholesterol elevation in the rafts activated a chemical "cell-survival" pathway known as Akt, thought to be a central pathway in prostate cancer progression. Activation of Akt enabled the tumour cells to resist chemical cues to commit suicide through the process known as apoptosis, thereby allowing the cancer to proliferate. Lipid rafts are structures in the outer cell membrane (Freeman and Solomon, 2004) that are dynamic and continually aggregate and disaggregate. They have naturally high concentrations of cholesterol and are believed to be important in cell signaling. Solomon and Freeman (2004) believe that cholesterol in the lipid rafts may help sequester proteins involved in cancer pathways in close proximity with each other, facilitating biochemical reactions that promote cancer growth. Some studies have also reported that increase in the risk of colorectal cancer is positively associated with serum triglycerides; decreased risk is negatively associated with serum triglycerides. The relationship between lifestyle risk factors and serum triglycerides and plasma glucose has been studied in the context of research into heart disease and diabetes, rather than in studies of cancer. However, the findings suggest that a number of factors which are positively related to the risk of colorectal cancer are also positively related to serum triglycerides; other factors negatively related to colorectal cancer risk are negatively related to these physiological characteristics (Truswell and Mann, 1972; Brown and Karmally, 1985; Mann, 1987). High simple carbohydrate diets have

long been recognized as associated with hypertriglyceridemia but diets which are high in fiber rich carbohydrates do not induce hypertriglyceridemia when fed over prolonged periods (Lewis *et al.*, 1981; Coulston *et al.*, 1983; Mann, 1987). Alcohol, like carbohydrate, has long been recognized as increasing serum triglycerides, and beer is a source of both alcohol and carbohydrates (about 30 g/liter) (Garn, 1990). Thus, alcohol increases serum triglycerides, especially when consumed in a non-fasting state. Positive associations between obesity, serum triglycerides, and blood glucose are well established (Ashley and Kannel, 1974; Garn, 1990). Clinical studies have shown that the degree of obesity is associated with production of VLDL triglyceride, and that increases in weight are associated with increases in triglycerides (Garn, 1990). Though none of these studies was done in relation to fibroids which is benign and thus different from the malignant ones that were studied, the result of our findings are in keeping with what these other studies found. Alcohol consumption and obesity, which were found in these previous studies to be associated with tumours, were also found to be higher among patients compared to the controls in this study. Again, the tendency to link these findings to those of previous studies though of tumours of different nature is appealing because of correlations between fibroids and cancers of the breast, colon, rectum, ovary, and prostate, sites which share some risk factors (Garn, 1990) and which might be influenced by biological mechanisms affecting proliferation such as those described for fibroids.

In general, data which have bearing on the relationship between some tumours and serum triglycerides are generally compatible with the hypothesis of a positive association. However, some more studies are needed to establish associations directly and to examine their molecular basis.

Chapter 5

CONCLUSION & RECOMMENDATION

5.1 CONCLUSION

The ages correlated positively with the weight of the tumour developed by the patients. Cumulative effects of the female hormones (oestrogen and progesterone) which are said to stimulate the growth of these tumours may account for the positive correlation between the ages and the sizes of the tumour.

It was observed that marriage had a negative association with the risk of developing fibroids since women who were married had a positive correlation with high parity which in turn had a negative correlation with the risk of developing fibroids. Marriage was again associated with education since women who were more educated were mostly unmarried and had lower parity which implied higher risk.

Significant difference also existed between the patients and the controls in terms of their level of formal education which could be explained by the fact that patients who were more educated were also engaged in mostly sedentary jobs that exposed them to some risk factors such as high BMI. They were also more health conscious and had the financial power to seek medical attention more often than the less educated controls.

The findings of this study were consistent with those of previous studies in that parity was observed to be negatively associated with the risk of developing fibroids. Women who had given birth may have been offered some protection through some endocrine factors since these factors change dramatically during pregnancy and during the post-pregnancy hormonal state. This results in the reduction of women's life time exposure to normal menstrual cycle levels of oestrogen and progesterone. Again childbearing may counteract the

development of uterine fibroids through non-hormonal mechanisms such as inhibiting growth of small clones of transformed myometrial cells.

Lower age at first pregnancy was protective for women since it was positively associated with age at first birth also and parity which are both protective for fibroid development.

Early age at menarche was observed to have positive association with the risk of developing fibroid. Early age at menarche is suggested to enhance fibroid growth since it is associated with higher oestrogen levels. Early age at menarche implies earlier establishment of regular ovulation and hence prolonged exposure to oestrogen and progesterone leading to greater life long exposure to bioavailable oestrogen and progesterone which values were generally higher among the women with fibroids. Again early onset of regular menstrual cycles may increase the number of cell division that the myometrium undergoes during the reproductive years resulting in an increased chance of mutation in genes controlling myometrial proliferation. Additionally, obesity which is associated with high risk of fibroid development has also been reported to be associated positively with early development of menarche.

Abortion was found to be positively associated with the risk of development of fibroid. Younger age at first abortion was found to be positively associated with the risk of developing fibroids. Cellular injury or inflammation as a result of abortion could account for the association between abortion and fibroid development. Tissue injury from abortion procedures could precipitate the development of fibroids. The positive association between the number of previous abortions suggests that protection conferred by pregnancy may relate to full-term pregnancy only which induce significant long term changes in levels of ovarian hormones and growth factors.

Progestin-only-injectables contraception was found to be negatively associated with the risk of fibroid development. A direct inhibitory effect of progestins on myometrial tissue proliferation and/or a protective effect of cycle suppression

similar to what is observed during pregnancy may account for this. Progestin have also been observed to be associated with an increased in gonadotropin-releasing hormone pulse frequency, modification of the endometrial lining, suppression of gonadotropins, reduced secretion of oestrogen and progesterone, and down-regulation of oestrogen receptors in leiomyomata; all of which are negatively associated with fibroid development.

A positive association between alcohol consumption and the risk of fibroid development was observed. This observation may be attributed to the positive correlation between alcohol consumption and higher endogenous oestradiol levels which may promote growth of fibroids.

There was a positive association between the risk of development of fibroids and the history of sexually transmitted infections. Local uterine irritants of infectious origin may play a role in the development of fibroids either because endometrial inflammation induces a myometrial response, or, both endometrial and myometrial tissues are directly involved.

BMI was found to be positively associated with leiomyomata development and/or growth. This possible association between obesity and uterine leiomyomata may reflect associated hormonal changes as well as alterations in metabolic controls that affect myometrial cell signaling through mediators such as insulin receptors, insulin-like growth factors, and peroxisome proliferating activating receptors. BMI may also influence uterine leiomyomata through changes in steroid hormone metabolism and bioavailabilty.

The findings of this study showed that the patients poorly managed oxidative stress compared to the controls as seen in the lower vitamin C levels and the higher MDA values. Patients were exposed to higher oxidative stress due to a variety of factors including high serum iron, high lipids levels (especially triglycerides), and frequent injury from STIs resulting in high rate of inflammation.

Liver function and renal function tests generally showed that patients had normal liver function and normal renal function implying the development of fibroids does not affect liver and renal function of patients.

Patients generally had higher red cell indices (except MCV) compared to the controls suggesting a positive association between high red cell indices and the growth of fibroids. Higher total iron and lower total iron binding capacity were also positively associated with the development of fibroids. Intra-uterine shunting in the form of an intra-myomatous ateriovenous shunts which allow the flow of non-oxygenated blood into the arterial vasculature, stimulating the bone marrow to increase red cell production could be one mechanism by which patients achieved these higher red cell indices. Myoma size and site may also account for this observation since specific anatomic site of the tumour, mass density, and mass chronicity have been linked to enhanced red cell indices through impaired pulmonary function as a result of increased pressure by a large uterus on the diaphragm thus compromising ventilation and causing hypoxia. Changes in cell life span due to an inhibition of normal removal of red cells through the spleen through a myomatous factor causing a disruption in the blood conservation mechanism of the spleen may also explain the high red cell indices observed. Higher blood iron levels could be attributed to iron supplements which were generally prescribed for patients.

In general, patients had some significant anthropometric, gynaecologic, and socio-economic characteristic differences compared to the controls and these were similar to what has been observed in the developed world. In spite of the menorrhagia usually associated with fibroids, prescription of iron supplements for patients must be done cautiously as most patients may not need such supplements. The tumours do not seem to affect the function of the kidney and the liver. Oxidative stress damage may also play a role in the development of fibroids. A history of sexually transmitted infections or infections as a result of abortion procedures was observed by this study to be strongly associated with

the development of fibroids. Thus for most cases of fibroids in Ghana, a combination of infections and oxidative stress damage mechanisms at the molecular level may influence the development of fibroids.

5.2 RECOMMENDATIONS

1. Animal models such as eker rats should be used to investigate the exact mechanisms at the molecular level that lead to the development of fibroids.
2. Cellular studies using conditions that mimic the conditions created by identified risk factors should be done to help understand the mechanism of fibroid development and growth.
3. Caution should be taken when prescribing iron containing food supplements for women patients as this could lead to iron overload and its consequently increase oxidative stress which may enhance the development and growth of fibroids.
4. STIs and all pelvic infections must be treated promptly and effectively to prevent the triggering of mechanism at the molecular level that might lead to the development of fibroids.

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APPENDIX

DEPARTMENT OF MOLECULAR MEDICINE
SCHOOL OF MEDICAL SCIENCES
KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

CHEMICAL PATHOLOGY OF FIBROID

GENERAL BACKGROUND INFORMATION

1. AGE

3. TRIBE

5. NATIONALITY

7. OCCUPATION

8. EDUCATIONAL STATUS

A) NEVER BEEN TO SCHOOL

C) SHS/SSS/OL/AL/VOC/TECH

E) DIPLOMA

G) POSTGRADUATE

A) MARRIED

C) DIVORCED

10. OCCUPATION OF HUSBAND

11. LENGTH OF MARRIAGE

12. LENGTH OF SEPARATION

13. LIFE STYLE

A) DO YOU SMOKE? YES [ ] NO [ ]

B) HAVE YOU EVER SMOKED? YES [ ] NO [ ]

C) DO YOU TAKE ALCOHOL? YES [ ] NO [ ]

OBSTETRIC AND GYNAECOLOGIC FINDINGS

1. AGE AT MENARHCE [ ]
2. AGE AT FIRST PREGNANACY [ ]
3. AGE AT FIRST BIRTH [ ]
4. PARITY [ ] PLUS [ ] ABORTIONS
5. NUMBER OF INDUCED ABORTIONS [ ]
6. NUMBER OF SPONTANEOUS ABORTIONS [ ]
7. AGE AT WHICH FIRST ABORTION OCCURRED [ ]
8. AGES OF CHILDREN

A) B) C)

D) E) F)

G) AGE OF LAST CHILD IF MORE THAN SIX CHILDREN [ ]

9. METHODS OF DELIVERY

A) NUMBER DELIVERED BY C/S [ ]

B) NUMBER DELIVERED NORMALLY [ ]

10. SEX OF CHILDREN

A) NUMBER OF MALES [ ]

B) NUMBER OF FEMALES [ ]

11) REASONS FOR REPORTING AT HOSPITAL/CLINIC:

12) PRELIMINARY DIAGNOSIS:

13) CONFIRMATORY DIAGNOSIS:

14) LAST MENSTRUAL PERIOD:

15) LENGTH OF MENSTRUAL CYCLE:

16) DO YOU USE ANY CONTRACEPTIVE METHODS?

17) HAVE YOU EVER USED ANY CONTRACEPTIVES? [YES] [NO]

18) IF YES SPECIFY WHICH OF THEM.

19) EVER USED ANY PHYTOTHERAPEUTIC AGENTS? [YES] [NO]

20) IF YES NAME THEM.

21) HAVE YOU ANY CHRONIC MEDICAL CONDITION?

22) ARE YOU ON ANY MEDICATION?

23) HAVE YOU EVER HAD ANY SEXUALLY TRANSMITTED INFECTION?

ANTHROPOMETRIC FINDINGS

1. WEIGHT [ ] kg

2. HEIGHT [ ] cm

3. WAIST CIRCUMFERENCE [ ] cm

4. HIP CIRCUMFERENCE [ ] cm

5. BMI [ ] kg/m^2

6. WAIST TO HIP RATIO [ ]

OPERATIVE FINDING

1. NUMBER OF FIBROID NODULES [ ]
2. TOTAL WEIGHT OF TUMOUR [ ]
3. SIZE OF UTERUS COMPARED TO A GRAVID UTERUS [ ]
4. LOCATION OF TUMOUR

A) SUBMUCOSAL [ ]
B) INTRAMURAL [ ]
C) C) SUBSEROSAL [ ]
D) D) OTHER [ ]