American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)

ISSN (Print) 2313-4410, ISSN (Online) 2313-4402

http://asrjetsjournal.org/

Bcl2, Bax and LMP1 Genes Expression in Uterine Leiomyoma Tissue According to Vitamin D Status among Congolese Women

P. Ingala[a*], B. Lebwaze[b], J. Mboloko[c], F. Lepira[d], P Kayembe[e], N. Pakasa[f], A. Tshiband[g], A. Mputu[h]

[a,c,h]*Department of Obstetrics and Gynecology, University of Kinshasa Hospital,Kinshasa,00243,Démocratic Republic of Congo*

[b,,f]*Department of Pathology, University Of Kinshasa,Kinshasa,00243,Démocratic Republic of Congo*

[d]*Department of Internal Medicine, University of Kinshasa ,Kinshasa,00243,Démocratic Republic of Congo*

[e]*Public Health School of Kinshasa ,Kinshasa,00243,Democratic Republic of Congo*

[g]*Division of radio immunologie,Regional Center of Nuclear study of Kinshasa,Kinshasa,00243,Democratic Republic of Congo*

Abstract

The objective of this paper is to describe genes expression patterns of the anti-apoptotic Bcl2 and pro apoptotic Bax as well as EBV infection marker LMP1 in, leiomyoma tissue according to patients vitamin D status. To investigate the relationship between alterations of genes expression patterns and serum vitamin D levels, samples from 105 women undergoing surgery for uterine leiomyoma in 6 hospitals in Kinshasa from April 1 to October 31, 2014 were obtained for genes expression analysis of Bcl-2, Bax and LMP-1 genes. Genes expression in leiomyomas was measured by immunohistochemistry. Serum vitamin D levels were determined by IRMA. Association between vitamin D status and genes expression was assessed using logistic regression analysis. From 105 women providers of leiomyoma tissues examined, 41 were sufficients in vitamin D, 36 were insufficients and 28 deficients. In uterine leiomyoma tissues obtained Bcl2 was expressed in 96 (91.4%) of samples with low, moderate and high expression observed in 33.3%, 32.4% and 25.7%, respectively. The differences in Bcl2 expression between the three subgroups of vitamin D categories were not statistically significant.

---

* Corresponding author.

As a mirror of Bcl2 expression, Bax protein was not expressed in the majority (n = 95, 90.5%) of samples. Similar to Bcl2, the differences in Bax expression between the three subgroups of vitamin D categories did not reach the level of statistical significance. Of the 105 leiomyoma tissue examined, LMP1 was observed in 30 (28.6%) of samples and showed a tendency towards increased expression with the decline in vitamin D levels; however, the tendency was fairly not statistically significant.

In multivariate analysis, predictors of Bcl2 expression were age, parity, and overweight/obesity and insufficiency/deficiency vitamin D; however, the differences observed were not statistically significant. The same predictors were also associated with Bax expression but once again the observed differences were not statistically significant. Vitamin D deficiency, the only predictor significantly associated with LMP1 expression, conferred a 3.9 fold greater risk (aOR 3.9; 95% CI 1.068-14.242; p = 0.039) of expressing LMP1 in leiomyoma tissue.

In the present study, Bcl2 gene expression in ULM tissues tended to increase with the decline in vitamin D levels but observed differences were not statistically significant. In contrast, LMP1 gene expression was significantly associated with vitamin D deficiency. In spite of methodological limits, these findings do suggest a role of Vitamin D deficiency in the development and progression of ULM.

Keys words: Bcl 2; Bax; and LMP1 genes expression; Uterine leiomyoma tissue; vitamin D status.

1. Introduction

Uterine leiomyomas (ULM) are the most common benign tumors of the female genital tract resulting in menstrual abnormalities, recurrent pregnancy loss and other serious gynecological disorders [1]. They are 3-4 higher in black women than in white women [2] and occur in 20-25% of premenopausal women [3, 4]. Indeed, ULM have been reported to appear at the start of the reproductive age, grow under the influence of sex steroids and regress after menopause indicating the ovarian steroid-dependent growth potential [2]. Sex steroids estrogen and progesterone are thought to lead to tumor by stimulating a modest rate of cellular proliferation and the production of high amounts of extracellular matrix (ECM) proteins mainly collagen [5]. Although the role of steroids sex hormones estrogen and progesterone remain incompletely understood, the neoplastic transformation of a myocyte is likely due to some sort of cellular insult, although the exact etiology of this transforming event is currently unknown. Among potential cellular insults, viral infection by cytomegalovirus (CMV) and Epstein Barr virus (EBV) has been the focus of several studies [6, 7]. An association between latent membrane protein-1 (LMP-1), a marker of EBV infection [8] and breast cancer as well as leiomyosarcomas of some soft tissues in immunocompromised young people [9] has been already found. The transformation of uterine smooth muscle cells to leiomyomas has been reported to probably involve somatic mutations of genes playing roles in proliferation and apoptosis in a normal myometrium and in complex interactions between sex steroids and growth factors [4]. With regards to apoptosis, ULM are associated with altered apoptosis by overexpression of the anti-apoptotic protein Bcl2 or by decreased expression of the pro-apoptotic protein Bax [10].

The mainstay treatment option of ULM is surgery, in the form of myomectomy or hysterectomy [11]. However,

these procedures can be associated with substantial morbidity and mortality [11]. Therefore, it appears rationale to search for novel nonsurgical alternatives for the management of symptomatic ULM. In this regard, apart from GnRH agonists, aromatase inhibitors and selective-progesterone receptor modulators (SPRMs), there is encouraging research on the role of supplements such as vitamin D as potential treatment for ULM [12]. Recent clinical evidence indicates an association between serum vitamin D deficiency and increased risk of ULM [13, 14, 15]. Furthermore, many experimental studies showed vitamin D to exert anti-proliferative effects on ULM cells through the reduction of the expression of cell proliferation markers proliferating cell nuclear antigen (PCNA), cyclin dependent kinase-1 (CDK-1) and Ki67 nuclear antigen [16]. Vitamin D also reduces the expression of the anti-apoptotic proteins Bcl2 and Bcl 2I1 [Bcl-X] [16]. Vitamin D has been also reported to reduce the expression of estrogen receptor (ER) [17]. In addition, vitamin D deficiency, a known immune dysfunction risk factor, could promote viral infection that can trigger neoplastic myocyte transformation [18].

In Democratic Republic of the Congo (DRC), ULM are commonly seen in gynecological daily practice and remain one the most common causes of hysterectomy and subsequent infertility [19]. Despite recent evidence on the role abnormal vitamin D homeostasis and ULM, no study has evaluated the relationship between vitamin D status and ULM cells expression of markers of apoptosis and EBV infection. Therefore, the aim of the present study was to assess the relationship, if any, between serum vitamin D status and expression of markers of apoptosis and EBV infection in uterus removed from women with ULM in clinical settings in Kinshasa, Democratic Republic of the Congo.

2. Material et methods

The study protocol was approved by the Ethical Committee of Kinshasa School of Public Health, University of Kinshasa (ESP/CE/028/2013).

Clinical data

In total, 105 leiomyoma tissue samples and blood samples were obtained from 105 women undergoing surgery for uterine leiomyoma in 6 hospitals in Kinshasa from April 1 to October 31, 2014 to study gene expression of Bcl-2, Bax and LMP-1. Gene expression in leiomyomas was measured by immunohistochemistry. Serum vitamin D levels were determined by IRMA. Clinical parameters (age, parity, and menstrual cycle, personal history of uterine leiomyoma (ULM), hypertension, diabetes mellitus, and body mass index(BMI) were retrieved from medical chats of patients.

The size (m) and the weight (kg) have been measured by a weights sick with spacer .The results obtained allowed the obtaining of the body mass index of each woman made by the report: weight(kg)/size (m)². The overweight and obesity have been defined respectively by BMI ≥25 kg/m² and >29.9 kg/(m)^2. The two states have been considered as excess weight.

Vitamin D measurement

10 ml of blood were collected from each patient before surgery act for the determination of vitamin D status .

The tubes of blood collected were protected from the light and then centrifuged at 2500 revolutions during 10minutes after coagulation. Serum obtained after this centrifugation was kept at -20°C in the Regional Center in nuclear energy at the University of Kinshasa .The evaluation of the Vitamin D has been carried out by the radio immunological method (IRMA) (20).

With the method IRMA, vitamin D contained in the serum of each patient was first captured by a poly clonal antibody. Then the vitamin D captured has then reacted with a commercial mono clonal antibody coupled to the Iodine 125 for its revelation. The amount of vitamin D that is obtained is proportional to the radio activity recorded by the gamma counter.

In agreement with the local values of the vitamin D, the results obtained were enrolled in the three tranches below: sufficiency (≥15ng/ml), insufficiency deficit (4-14 ng/ml) and deficiency (< 4 ng/ml).

Histopathology

A levy biopsy, on the fabric of ULM, has been carried out at each made during the course of the procedure. The samples were then fixed to the buffered formalin and routed on the same day to the laboratory for pathology anatomy of the Faculty of Medicine of the University of Kinshasa for analysis.

A histopathological analysis was conducted on each of these levies to confirm or refute the diagnosis of uterine leiomyomas. For its realization the fabrics have been treated in six following successive stages: macroscopy external and internal; Dehydration successively in the alcohol at 100°, then in the xylol; Coating for obtaining paraffin blocks with fabrics biopsies; cut paraffin blocks to the microtome in strips of 2 to 3µ thickness and construction of blades; coloring with haematoxylin and eosin; and mounting of the blades.

The reading of the blades was then made within 15 minutes using the microscope ocular bi Leica brand DMRB with digital camera DCM integrated 900 (9 m pixel).

Immunohistochemistry

Immunohistochemical analysis was also made using paraffin blocks already availables. The analysis focused on the evaluation of the expression of proteins Ki 67, Bcl-2, Bax, and LMP1.Each block has allowed the construction of four blades of immunohistochemistry corresponding to the four proteins to assess. The blades were then kept in an oven for 24 hours at 56°C before to undergo the dewaxd. Blades have then suffered the pre EDTA treatment then marking: the first marking, the antigens on the blades have reacted with 100 µl of primary poly clonal antibody. The second marking, blades have reacted with the secondary mono clonal antibody. Each blade was then submitted to a chromogenic, DAB and then to a counter coloration with haematoxylin.

The reading of the blades has been made by the microscope ocular Leica brand DMRB with digital camera DCM integrated 900 (9M pixel). The expression of the protein Ki 67 was sought in the cell nucleus. The expression of Bcl-2, and Bax were sought in the cell cytoplasm and that of LMP1 at membrane. The expression

was either positive or negative. In the case of positivity three levels were differenced: low expression (expression < 10%); moderate expression (expression, 10% to 30%); high expression (expression > 30%).The following table shows the different mono clonal antibodies used to test expressions of proteins Bcl-2, Bax, Ki 67, and LMP1

Table 1: Mono clonal antibodies anti Bcl 2, Bax and LMP1

ANTIBODY	**CLONE**	**TARGET**	**PRE TRAITEMENT**	**DILUTION**
Ki67	SP6/MM FRANCE	Nuclear	CITRATE (pH : 6)	1/300
LMP1	MRQ-19/MM FRANCE	Membranaire	CITRATE (pH : 6)	1/100
Bcl-2	100/D5/MM FRANCE	Cytoplasmic	CITRATE (pH : 6)	1/100
Bax	2D2/MM FRANCE	Cytoplasmic	CITRATE (pH : 6)	1/100

Statistical analysis

SPSS for Windows version 21.0 was used to perform the statistical analysis of the data. Normally distributed and skewed continuous variables were expressed as mean ± SD or median (range), respectively. Patients were divided in three categories according to vitamin D status: sufficiency, insufficiency and deficiency. Comparison of normally distributed and skewed variables between the three subgroups were made using one ANOVA and Kruskall Wallis, respectively; for the comparison of categorical variables, chi square test was used. Logistic regression analysis was used to assess the association between gene expression and vitamin D status adjusting for age, parity, menstrual cycle, personal history of ULM, hypertension and diabetes mellitus, BMI. P value < 0, 05 defined the level of statistical significance.

3. Results

Data are expressed as mean ± standard deviation, absolute frequency (relative frequency in percent). Abbreviations: PH, personal history ULM; HT, hypertension; DM, diabetes mellitus; BMI, body mass index. p< 0.05

The table above shows that one hundred and five women were included in the present study; their mean age and

median vitamin D levels were 37.6 ± 7.5 years and 27.4 (24.8-27.3??) ng/ml, respectively. Nearly all of the patients were still having menses and more than half of them were nulliparous. Personal history of ULM, hypertension and diabetes was present in characterized 28.6%, 18.1% and 6.7% of patients, respectively. More than half of them had abnormal body weight with overweight and obesity observed in 35.2% and 21%, respectively.

Apart from vitamin D levels, the differences observed in other variables of interest between the three subgroups did not reach the level of statistical significance.

Table 2: General characteristics of the study population according to serum vitamin D status.

Variable	All	Vitamin D status			p
		Sufficient	Insufficient	Deficiency	
	n = 105				
		n = 41	n =36	n = 28	
Age, years	37.6 ± 7.5	36.5 ± 7.1	38.3 ± 8.7	38.0 ± 6.6	0.566
Parity, n (%)					
Null parity	58(55.2)	23(56.1)	21 (58;3)	14(50.0)	0.315
≥1	47(44.8)	18(43.9)	15 (41.7)	14(50.0)	0.758
Menstrual cycle, n (%)					
Yes	104(99)	41(100)	35(97.1)	28(100)	0.295
No	01(1)	-	1(2.8)	-	
PH-ULM, n (%)					
Yes	30(28.6)	15(36.6)	10(27.8)	5(17.9)	0.082
No	75(71.4)	26(63.4)	26(72.2)	23(82.1)	0.887
PH-HT, n (%)					
Yes	19(18.1)	7(17.1)	6(16.7)	6(21.4)	0.949
No	86(81.9)	34(82.9)	30(83.3)	22(78.6)	0.272
PH-DM, n (%)					
Yes	7(6.7)	3(7.3)	1(2.8)	3(10.7)	0.565
No	98(93.3)	38(92.7)	35(97.2)	25(89.3)	0.242
BMI, Kg/m²	19.3 ± 7.7	26.4 ± 4.4	26.8± 5.4	25.1± 4.1	0.356
Normal, n (%)	46(43.8)	18(43.9)	15 (41.7)	13(46.4)	0.662
Overweight, n (%)	37(35.2)	13(31.7)	12(33.3)	12(42.3)	0.973
Obesity, n (%)	22(21)	10(24.4)	9(25)	3(10.7)	0.142
Serum 250HD, ng/ml	27.4	16,50	9.00	2.50	<0.0001
	(24.8-29.9)	(16.0-18.5)	(7.0-11.0)	(2.0-3.0)	

Table 3: Gene expression of apoptotic markers Bcl2, Bax and of EBV infection marker LMP1 in uterine leiomyoma according to serum vitamin D status.

Variable		All	Vitamin D status			p
			Sufficient	Insufficient	Deficiency	
		n = 105	n = 41	n = 36	n = 28	
Bcl2 expression, n (%)						
Positive:	Low	35(33.3)	12(29.3)	12(33.3)	11(39.3)	0.972
	Moderate	34(32.4)	15(36.6)	12(33.3)	7(25.0)	0.237
	High	27(25.7)	10(24.4)	10(27.8)	7(25.0)	0.717
Negative		9(8.6)	4(9.8)	2(5.6)	3(10.7)	0.717
Bax expression n (%)						
Positive:	Low	4(3.8)	3(7.3)	-	1(3.6)	0.617
	Moderate	4(3.8)	2(4.9)	1(2.8)	1(3.6)	0.779
	High	2(1.9)	1(2.4)	1(2.8)	-	0.480
Negative		95(90.5)	35(85.4)	34(94.4)	26(92.9)	0.464
LMP1 expression n (%)						
	Yes	30(28.6)	15(36.6)	5(13.9)	10(35.7)	0.082
	No	75(71.4)	26(63.4)	31(86.1)	18(64.3)	0.180

Abbreviations: Bcl, B cell lymphoma; Bax, B cell associated X protein; LMP1, lymph membrane protein 1. P< 0.05.

According to the table 3, of the 105 leiomyoma tissue examined, Bcl2 was expressed in 96 (91.4%) of samples with low, moderate and high expression observed in 33.3%, 32.4% and 25.7%, respectively. The differences in Bcl2 expression between the three subgroups of vitamin D categories were not statistically significant.

As a mirror of Bcl2 expression, Bax protein was not expressed in the majority (n = 95, 90.5%) of samples. Similar to Bcl2, the differences in Bax expression between the three subgroups of vitamin D categories did not reach the level of statistical significance.

Of the 105 leiomyoma tissue examined, LMP1 was observed in 30 (28.6%) of samples and showed a tendency towards increased expression with the decline in vitamin D levels; however, the tendency was fairly not statistically significant.

The result on the table 4 shows that in multivariate analysis, predictors of Bcl2 expression were age, parity, overweight/obesity and insufficiency/deficiency; however, the differences observed were not statistically significant (Data not shown). The same predictors were also associated with Bax expression but once again the observed differences were not statistically significant (Data not shown). Vitamin D deficiency, the only

predictor significantly associated with LMP1 expression, conferred a 3.9 fold greater risk (aOR 3.9; 95% CI 1.068-14.242; p = 0.039) of expressing LMP1 in leiomyoma tissue (Table 4).

Table 4: Multivariate determinants of LMP1 expression among the study population.

VARIABLES	**aOR**	**CI95%**	**p**
Age (years)	2,079	0,666-6,493	0.208
Parity	1,147	0,444-2,964	0,777
Menstrual cycle	NS	NS	NS
PH- ULM	0.552	0,193-1,580	0.268
PH-ULM	0.989	0,303-3,222	0.985
PH -MD	0.607	0.114-3,231	0.559
BMI	1	-	-
Overweigh	0,653	0,176-2,415	0.523
Obesity	1,703	0,426-6,808	0,451
Vitamin D	1	-	-
Insufficiency	1,004	0,335-3,011	0,994
Deficiency	3,900	1,068-14,242	0,039

Abbreviations: FH-ULM, familial history of uterine leiomyoma; PH-ULM, personal history of uterine leiomyoma; PH-MD, personal history of diabetes mellitus.

4. Discussion

The present study has concerned the patients whose average age is 37.56 ± 7,548 years. Bang et al. (21), Okogbo et al. (22) respectively have found 34.9 ±5.3 years and 39.4± 7.3 years. Recently Cham et al. (23) and Muhindo (24) have found 34.5 ± 7.5 years and 35.3 ± 4,9ans. These results comparable to each other is an argument which confirms the opinion according to which the uterine leiomyomas is a pathology of the elderly

woman of childbearing age.

The Low Parity (average: 2.49 ± 1,804) and the Overweight (41.5%) as well as Obesity (21.0%) which predominate in these patients corroborate the high levels of risk always charged to these two factors (25, 26).

A single case of menopause has been obseved. It is a patient aged 55years.This case shows the importance of sex hormonal excitations in the birthness and growth of uterine leiomyomas (27).

The anti-apoptotic protein Bcl-2 was more expressed in ULM tissues than the pro apoptotic protein Bax in the present study. This finding agrees with results of previous reports on gene expression in human ULM tissues (28, 29, 30, 31). Using RT-PCR technique in case control study, Csatlos et al (28) found that Bcl2 gene was overexpressed in leiomyoma group compared with the control group although there was not such a difference in the gene expression of Bax. They concluded that the overexpression of the anti-apoptotic Bcl-2 gene appeared to be an important factor in the development of ULM whereas the pro apoptotic Bax gene did not seem to play a substantial role in the process. A similar finding was reported by Dixon et al (30) who found an altered apoptosis by overexpression of Bcl2 and decreased expression of Bax in human ULM tissues. While it is thought that the initial events that trigger leiomyoma tumor genesis involve somatic mutations, it is evident that the development and growth of ULM depends on ovarian steroid hormones (32, 33). Although the evidence points to the role of these hormones, progesterone in particular plays a major role in ULM development and growth (32; 33). Studies have shown that progesterone can increase the expression of the anti-apoptotic Bcl-2 gene (33, 34). Indeed, direct binding of liganded progesterone receptor to Bcl-2 promoter enhances its transcription in primary leiomyoma cells (32, 33).

Bcl-2 expression tended to increase with the decline in Vit D levels in the present study but this association did not reach the level of statistical significance. Although contradictory in appearance to findings from previous studies (16, 34), the observed lack of association between Vitamin D levels and Bcl-2 expression in ULM tissues could be due to methodological issues relating to the small sample used. Nevertheless, an association between Vitamin D levels and apoptosis regulating protein has been already reported (34),Indeed, Vitamin D has been reported to exert anti-apoptotic effects on ULM cells by controlling members of Bcl-2 protein family that consists of both anti-apoptotic proteins such as Bcl-2, Bcl-xl, and Bcl-w and pro-apoptotic including Bax, Bak and Bad (34). Antonsson et al (35) showed repression of Bcl-2 and Bcl-w in human leiomyoma cell upon treatment with Vitamin D. They concluded that the growth inhibitory effect of Vitamin D is also mediated by blocking the expression of anti-apoptotic Bcl-2 and Bcl-w genes (34).

In addition, Vitamin D apoptosis inhibitory effect has been reported to rely upon the suppression of cathecol-O-methytransferase (COMT) expression and activity in human ULM cells (34) COMT enzyme catalyzes the transfer of a methyl group from S adenosyl-methionine to one of the phenolic groups of a variety of cathecols including cathecolestrogens and cathecholamin neurotransmitters (34). COMT is known to convert the cathecolestrogens, 2- and 4-hydroxyestrogen, to 2- and 4-methoxyestrogen, respectively (34) 2-hydroxyestrogen can act as estrogen antagonists in a variety of cells including human myometrial and leiomyoma cells while their methylated counterpart, 2-methoxyestrogen, as estrogen antagonists (34) Vitamin D has been reported to

reduce COMT mRNA and protein expression as well as the enzyme activity in human ULM cells(34) Therefore, Vit D deficiency may be an important risk factor in growth and progression of ULM (34).

LMP1 expression was significantly associated with Vitamin D deficiency. Vitamin D has been implicated in the host defense against infection as immune modulator through down-regulation of the pro-inflammatory response (36). It also has antimicrobial effects by inducing activation of macrophages with subsequent production of IFNϒ (36). Therefore, Vitamin D deficiency and subsequent relative immune dysfunction could facilitate latent or reactivated EBV infection to trigger tumor genesis in ULM cells (37). The pathophysiological relationship and molecular mechanisms of EBV-mediated tumor genesis have not yet been fully elucidated. EBV-induced tumors expressed three EBV-encoded proteins with latent membrane protein-1 (LMP-1) as the central player in the tumor genesis process (37). LMP1, an EBV-encoded primary oncogene, functions as a viral mimic of TNF receptor (TNFR) family member, CD40 and engages in a number of signaling pathways that induce morphological and phenotypic alterations in epithelial cells (37). LMP1 has been also reported to up regulate epithelial-mesenchymal transition (EMT) with subsequent malignant transformation of epithelial cells (37). Other EBV-encoded proteins such as BarmHI-A rightward frame 1 (BART1) can modulate apoptosis and host immune mechanisms (37).

The interpretation of the results of the present study should take in account of some limitations. First, the cross-sectional design of the study precludes the establishment of causal or temporal relationship. Second, the small sample size could not allow much power to statistical tests to detect additional associations between variables of interest. Third, vitamin D levels were determined once with the risk of overestimation of values obtained given the variability characterizing biologic parameters.

5. Conclusion

In the present study, Bcl2 gene expression in ULM tissues tended to increase with the decline in vitamin D levels but observed differences were not statistically significant. In contrast, LMP1 gene expression was significantly associated with vitamin D deficiency. In spite of methodological limits, these findings do suggest a role of Vitamin D deficiency in the development and progression of ULM.

Acknowledgement

The authors gratefully thank Medical team of Saint Joseph hospital, university clinics of Kinshasa, Monkole medical center, Ngaliema clinic, Chinese-Congolese hospital and clinic of angelus for their invaluable help for the measurement of biological parameters. The authors remain deeply indebted to MODIA ANTOINE and SMITH MPAKA.

References

[1]. C.Rongieres.Epidemiologie du leiomyome uterin .J. Obstetrics and gynecology board review medical .1999:28:701-706.

[2]. Catherino WH, Eltoukhi HM, Al-Hendy. Racial and ethnic differences in the pathogenesis and clinical

manifestation of uterine leiomyoma.SeminReprod Med.2013.September:31151:370-379.doi:10-1055[i-0033-1348896]

[3]. Marshall LM & al. Variation in the incidence of uterine leiomyoma among premenopausal women by age and race.Obstet.gyencol.1997; 90; 967-9736b

[4]. Elugwaraonu O;Okojie A.I.O,Okhia O,Oyadoghan G.P.The incidence of uterus fibroid among reproductive age women a five years review of cases at ISTA,IRRUA,EDO,NIGERIA.Intrenational Journal of Basic, Applied and innovative research JJ,2013,2(3):55-60.

[5]. Cavattini A.,Di Giuseppe J.,Stortoni P.,Mntik N.,Giannubilo SR et al.Uterine Fibrosis :Pathogenesis and Interactions with Endometrium and Endomyometrial doi.org/10.1155/2013/17 3184.

[6]. Iwakiri Mechanisms of Epstein-Bar Virus Mediated Oncogenesis.Gan To Kagaku Ryoho.2015 Oct;42(10):1133-6.

[7]. Smirnova K V,Diduk SV,Senyuta NB,Gurtsevitch VE.Molecular biological properties of the Epstein-Bar virus LMP1gene:structure,function and polymorphism. Vopr Virusol.2015;60(3):5-13.

[8]. Ann K.Richardson,Margaret J.Curie,Bridget A.Robinson,Helen Morrin,Yen Phung,John F.Pearson,Trevor P.Anderson,Jon D.Potter and Logan C.Walker.Cytomegalovirus and Epstein barr virus in breast cancer .Plos one 2015,10(2);e0118989.

[9]. Kenneth.McClain,M.D.,Ph.D,Charles T.leach,M.D.,Hal B.Jenson,M.D.,Vijav V.joshi.M.D.,PHD,Brad H.Pollock,M,P,H.,Ph.D.,Richard T.Parmley,M.D.,Frederick J.DiCarlo.,Ellen Gould Chadwick,M.D.,and Sharon B,Murphy,M.D.Association of Epstein -Barr virus with Leiomyosarcomas in young people with AIDS.N Engl J Med 1995;332:12-18N January 5,1995.

[10]. Soumia,Braka.MD.,Justin S.Diamond,BS., A yman Al-Hendy,MD.,PhD., Michael P.Diamond,MD.,Sumilk.Holder,PhD.Role of vitamin D in uterine fibroid Biology.Fertility and Sterility,September 2015 Volume 104.Issue 3,Pages 698-706.

[11]. Adama Hongebla A, Mondji S, Aboubakari A,Wonegouk,Akpadza Aspects épidémiologiques et chirurgicaux de fibromes utérines opérés à l'hôpital du district n°3 de Lomé.J.rech.Sci.Univer.Lomé.2012,serie D,14(1):131-137

[12]. Sabry M; Al-Hendy A.(2012)oral Innovative Treatments of uterine leiomyoma.Obstet.Gynecol.Int.2012:943635

[13]. Paffioni A,et al. Vitamin D satus in women with uterine leiomyomas.J.Clin.Endocrinol.Metab.2013; 98:e1374-8.

[14]. Sabry M,et al. Serum vitamin D3 level volume in different ethnic groups:a cross-sectional observational study.Int.J.Womens Health.2013;5:93-100.

[15]. Baird DD,Hill MC,Schectman JM,Hollis BW. Vitamin D and the risk of uterine fibroids.epidemiology.2013; 24:447-53.

[16]. Halder SK,Sharan C,Al-Hendy A.1,25-dihydroxyvitamine D3 treatment shrinks uterine leiomyoma tumors in the Eker rat model.Biol Reprod 2012 Apr 19: 86(4) 116 doi 10.1095/biolreprod 111 098145 Print 2012 A pr.

[17]. Davoodi F,Brenner RY,Evans SR,Schumaker LM,Shabahang M,Nauta RJ,Buras RR.Modulation of vitamin D receptor and estrogen receptor by 1,25$(OH)_2$-vitamin D_3mT-47Dhuman breast cancer cells.J. Steroid Biochem Mol Biol. 1995 Aug:54(3-4):147-53.

[18]. Robyn M.Lucas,Shelley Gorman,Sian Geldenhuys and Prue H.Hart.F1000 Prime Reprots 2014,6:118／doi:10.12703／P6-118).

[19]. Nzau.Do, Mboloko.E, Tandu-Umba NFB, Lokengo LD. Hysterectomy to the university clinic of Kinshasa: 2002-2010.Medicine of the Black Africa. April 2012, Vol.59, No. 4, pp221-230.

[20]. Tshiband A, MputuL,Tozin R, Ingala , Kiampa Solid-Phase Immuno Radio Metric Assay(IRMA) of 25-(OH) D and displacement from serum binding proteins for Resource –Limited Settings. Journal of Biomedical Engineering and Medical Devices.2015, 1, 1.

[21]. Bang ntamack JA, Mayitsongs S, Sina ole B, Meye JF. Pregnancy after myomectomy performed.Clin mother child heaith.2009; Vol 6 No. 2, 1101-1106.

[22]. Okogbo FO, Ezechi OC, Loto OM, Ezeobi PM. Uterine leiomyomata in south western Nigeria: a clinical study of Presentations and Management outcome.African Health Sciences Vol 11No. 2 June 2011,pp 271-278.

[23]. Cham LC, Mwembo TNA, Kabulu KA, Otshudiongo TS, Kalenga MKP. Study of the risk factors associated with the onset of uterine fibromyomes in Lubumbashi. Great Lakes Medical Review Flight, 1, March2013:12-18.

[24]. Muhindo S. profile of patients with uterine myomas in a population of infertile women in Kinshasa. Memory of end of specialization; 2013; Faculty of Medicine University of Kinshasa.

[25]. Parazzini F,Negri E,La Vecchia C,Charenoud The,Ricci E,Guarnerio Reproductive factors and risk of uterine fibroids. Epidemiology 1996.7: 440- 442.

[26]. R.K.Ross, M.C, Pike and M.P.Verssey, risk factors for uterine fibroids: reduced risk associated with oral contraceptives; the British Medical Journal.1986; Vol.293, no.6543, pp.359-362.

[27]. Andrea Ciavatini,Jacopo Di Giuseppe,Piergiorgio Stortoni,Nina Montik,Nina Montik,Stefano R, Giannubilo, Pietro The ITTA,Md.Soriful Islam,Andrea The Tranquilli,Fernando Mr. Reis, and Pasquapina Ciarnela.uterine fibroids: pathogenesis and interaction with endometrium and Endomyometrial junction. Obstetrics and Gynecology Volume International 2013(2013), Article ID 173184, 11pages. http:// dx.doi.org/10.1155/2013/173184.

[28]. Csatlós É, Máté S, Laky M, Rigó J Jr, Joó JG. Role of Apoptosis in the Development of Uterine Leiomyoma: Analysis of Expression Patterns of Bcl-2 and Bax in Human Leiomyoma Tissue With Clinical Correlations. Int J Gynecol Pathol. 2015 Jul;34(4):334-9.

[29]. Zhang ZL1, Zhang Y, Zhou JH. [Expression of bcl-2 and bax protein in uterine leiomyosarcomas and leiomyomas]. [Article in Chinese]. Zhong Nan Da XueXueBao Yi Xue Ban. 2005 Apr; 30(2):183-6.

[30]. Dixon D1, Flake GP, Moore AB, He H, Haseman JK, Risinger JI, Lancaster JM, Berchuck A, Barrett JC, Robboy SJ. Cell proliferation and apoptosis in human uterine leiomyomas and myometria.Virchows Arch. 2002 Jul;441(1):53-62. Epub 2002 Mar 23.

[31]. Wu X1, Blanck A, Olovsson M, Henriksen R, Lindblom B. Expression of Bcl-2, Bcl-x, Mcl-1, Bax and Bak in human uterine leiomyomas and myometrium during the menstrual cycle and after menopause. J Steroid Biochem Mol Biol. 2002 Jan;80(1):77-83.

[32]. Kim JJ, Sefton EC. Role of the progesterone signaling in the pathogenesis of uterine leiomyoma. Mol

Cell Endocrinol 2012 July 25; 358(2): 223-231.

[33]. Plewka A, Plewka D, Madej P, Nowaczyk G, Sieron-Stoltony K, Jakubiec-Bartulk B. Process of apoptosis and cell proliferation in uterine myomas originating from reproductive and perimenopausal women. Folia Histochemica et Cytobiologica 2011; 49(3): 398-404.

[34]. Sharan C, Halder SK, Thota C, Jaleel J, Nair S, Al-Hendy A. Vitamin D inhibits proliferation of human leiomyoma cells via cathecol-O-methyl transferase. Fertil Steril 2011 January; 95(1): 247-35.

[35]. Antosson B, Martinou JC. The Bcl-2 protein family. Exp Cell Res 2000; 256: 50-57.

[36]. White JH. Vitamin D signaling, infectious diseases and regulation of innate immunity. Infect Immune 2008; 76(9): 3837-42.

[37]. Hayashi T, Horuichi A, Sano K, Hiraoka N, Ichimora T, Sudo T, et al. Diagnostic biomarkers: differential expression of LMP2/ß1 and cyclin B1 in human sources. Tumori 2014 Jul-Aug; 100(4): 99e

Annexes

P INGALA, participated in protocol elaboration, data collection and analysis and drafted the manuscript.

A TSHIBAND, performed biological analysis and reviewed the manuscript

J MBOLOKO, participated in protocol elaboration conception and data analysis, reviewed the manuscript.

F LEPIRA, participated in protocol elaboration conception and data analysis, reviewed the manuscript.

P KAYEMBE, performed statistical analysis of data and reviewed the manuscript.

B LEBWAZE, participated in protocol elaboration conception and data analysis, reviewed the manuscript.

N PAKASA, reviewed the manuscript.

ARSENE LOBOTA MPUTU, conceived the study, participated in data analysis and reviewed the manuscript.