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Vitamin D Deficiency and Risk of Uterine Leiomyoma among Congolese Women. A Hospital-Based Case-Control Study

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Abstract

The aim of the present study was to assess the relationship, between Vitamin D deficiency and uterine leiomyoma in Congolese women.

From April 1 to October 31, 2014, 216 patients with ultrasound diagnosis of uterine leiomyoma (cases) and 216 women without this condition (controls) recruited in six medical facilities in Kinshasa were enrolled in the present study. A single blood sample was obtained from all participants to assess serum17β-estradiol and progesterone concentration using RIA and 25(OH) D by IRMA. Vitamin D deficiency was defined as 25(OH) D levels <4 ng/mL and <12 ng/mL using local and IOM cut-off levels, respectively. Chi square, Student t and Mann Whitney tests were used for group comparison. Logistic regression analysis was used to identify factors associated with Vitamin D deficiency. Vitamin D deficiency was observed in 17.1% and 47.7% of patients with ULM using local and IOM criteria defining different steps of vitamin D respectively. Compared to controls, the difference was statistically significant only when using local criteria (17.7% vs 10.2%; p = 0.028).

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ULM main risk factors were age ≥35 years (aOR=2,974; 95%CI 1,702-5,139; p = 0,001); null parity (aOR=3,951;95%CI 2,311-6,754; p= 0,001). Familial history of ULM (aOR=2,619; 95% CI 1,376-4,986; p =0,003) personal history of ULM a(OR3,776; 95% CI 1,885-7,565; p=0,001); absence of menopause(OR5,502; 95% CI 2,615-11,517; p= 0,001); high serum progesterone levels (aOR 2,320 95% CI1,136-4,711; p= 0,021), alcohol consumption (aOR0,295; 95 % CI 0,150-0,580; p= 0,001) and Vitamin D deficiency(aOR2,153; 95% CI 1,035-4,517; p= 0,040). Vitamin D deficiency was a common finding in patients with ULM and emerged as one of the main risk factors. However, this relationship need to be confirmed with a representative sample of women with ULM.

Key Words: Vitamin D Deficiency; Uterine Leiomyoma; Congolese Women.

1. Introduction

Uterine leiomyoma (ULM) is the most common benign gynecologic condition worldwide [1,2]. ULM has-been reported to be three times more common among women of African descent and characterized by earlier age of onset, greater severity of symptoms, and different response to treatment as well as frequent treatment complications [3]. By the age of 50 years, the cumulative incidence of ULM is approximately 70% in white women and more than 80% in black women based on ultrasound detection of fibroids [1]. The main complications of ULM are pelvic pain, hemorrhage, early pregnancy loss and infertility [1,3]. ULM is the number one gynecological reason of hospitalization and the first indication of hysterectomy [4,5].Except hysterectomy, medical treatment and myomectomy are associated with high risk of recidivism [6]. Furthermore, direct costs for ULM and related care are high and represent a major burden on the health-care system [7]. For all the above mentioned reasons, prevention, early diagnosis and management appear as the appropriate and effective strategy to alleviate the health, social and economic burden of ULM leiomyoma, especially in at-risk black women. However, the implementation of such strategy implies the prior identification and control of potential associated risk factors [3].

The pathogenesis of ULM remains is poorly understood and requires the continuous search of molecular mechanisms underlying ULM development and the potential risk factors as well as the influence of race [8]. In this regard, the rapid progression of ULM during reproductive years and its regression after menopause indicate that estrogen and progesterone play a key role as a growth-promoting factor that modulates the expression of genes involved in cellular differentiation, growth, apoptosis and extracellular matrix metabolism [8]. Besides genetic predisposition, the main risk factors associated with ULM include race, age, earlier menarche, parity, oral contraception and traditional risk factors such as obesity, hypertension, alcohol intake, smoking and diabetes mellitus [3, 8]. However, all these risk factors do not fully explain the increasing risk of leiomyoma highlighting the need for the search for new potential risk factors contributing to the development and progression of ULM.

In this regard, several in vitro studies have shown that vitamin D [(25(OH) D] can inhibit the proliferation of uterine leiomyoma [8, 9, 10, 11, 12]. Recent epidemiological studies reported an association between low vitamin D levels and the risk of ULM [13, 14, 15, 16]. Recent data showed significant association between

lower serum vitamin D levels and ULM among different ethnic groups, especially in black women [14,15]. Further analysis showed negative correlation between serum vitamin D levels and ULM size ($r = -0.42$; $p < 0.001$) among black individuals [17]. Vitamin D deficiency is estimated to be 10 times more common in African American women (40%) compared with white (4%) and could partially explain the white-black differences in the pathogenesis and clinical manifestations of ULM [15].Bearing in mind the above findings, one can expect vitamin D supplementation to be a one of the means for preventing or at least, for improving the course of ULM [18]. ULM as well as low vitamin D levels are prevalent in black women [3, 19] however, studies assessing the relationship between these two conditions are scarce.

In the Democratic Republic of the Congo (DRC), ULM is a condition frequently encountered in the daily gynecological practice and is one of the main indications of hysterectomy [4]. If the relationship between vitamin D levels and the risk of breast cancer has already been established [20], this is not the case for ULM. The present case-control study aimed to establish, if any, the relationship between vitamin D status and the risk of ULM among black women seeking care in the hospital settings in Kinshasa, the Democratic Republic of Congo.

2. Materials and Methods

From April 1 to October 3, 2014, 432 participants were recruited from six hospitals in Kinshasa, the capital City of the Democratic Republic of Congo (DRC) to evaluate the association between low level of 25(OH) D and risk of uterine leiomyoma. Kinshasa is a city located in the NORTHERN WESTERN region of DRC (latitude 4°19, 39" SOUTH and longitude 15°18, 48" EAST). The current study was performed during the dry season, during which time the city has an average of 5 hours of daily sunlight and mean temperature of 24, 4 degrees.

A sample size of 216 cases and 216 controls was assessed to provide more than 80% power to detect a significant difference ($\alpha = 0.05$) across groups in circulating 25(OH) D concentrations. Informed oral consent was obtained from all participants. All women aged 15 years or older with an ultrasound diagnosis of ULM (cases) and a sample women free of ULM (controls) were enrolled in the present study. Pregnant women and patients with chronic disorders (hepatic or renal) known to affect vitamin D metabolism were excluded.

The following variables were taken into account : lifestyle (smoking, alcohol intake, physical activity), medical history [personal history of ULM (PH-ULM) and family history of ULM (FH-UL), personal and family history of hypertension (FH-HT), personal and family history of diabetes mellitus (FH-DM), menopause, parity, oral contraception, age, age at menarche, age at first and last birth,] .Questionnaire was administered to each participant during the clinic visit to determine participant characteristics. Height and weight were measured using a calibrated beam scale with a height scale. Body mass index (BMI) was calculated as weight (Kg)/height (m) ². Excessive weight as overweight or obesity were defined as BMI $\geq$ 25 Kg/m² and>29 Kg/m², respectively [21].

A blood sample was obtained from all 432 women (216 cases and 216 controls) to determine serum 25(OH) D, estradiol and progesterone concentrations. All tubes were protected from light and the specimens were

centrifuged at 2500 rpm for 10 minutes. The serum was separated and stored at -20°C until analyzed at the Regional Center of Nuclear Study of Kinshasa/University of Kinshasa. Serum 25(OH) D concentrations were measured using a local immunoradiometric assay (IRMA) method at the Regional Center of Nuclear Study laboratory of Kinshasa; this method has been described elsewhere [22]. Briefly, serum 25(OH) D was particularly captured by a homemade polyclonal antibody during specimen incubation in RIA tube during two hours at the room temperature (25°C); captured 25(OH) D then reacted with a commercial monoclonal anti 25(OH) D antibody labeled with Iodine-125. Using local laboratory cutoffs, vitamin D insufficiency and deficiency was defined as 25(OH)D values of 4-14 ng/ml and < 4 ng/ml, respectively; vitamin D sufficiency as ≥15ng/ml [23]. Institute of Medicine (IOM) criteria were also used to defined vitamin D deficiency, insufficiency and sufficiency as 25(OH)D values of <12 ng/mL, 12–19 ng/mL and ≥20 ng/ml, respectively [24].

Radioimmunoassay was used to determine serum estradiol (17β-estradiol) and progesterone levels at the Division of Hormonology of the same laboratory. Normal range estradiol and progesterone levels were defined according to patient's reproductive life cycle and 17β estradiol and progesterone kit maker cut-off points as follows: for 17β-Estradiol: follicular phase (57-227pg/ml); ovulation phase (127-476 pg./ml); luteal phase (77-476pg/ml); menopause (15-75pg/ml) [25] and progesterone: follicular phase(0-1,3ng/ml);ovulation phase(0-1,4ng/ml);luteal phase(3,4-23,5ng/ml);menopause(15-75ng/ml) [26].

Statistical analyses were performed using Excel 12.0 and SPSS 21 statistical software's. Demographic, clinical and biological characteristics for cases and controls were evaluated using descriptive statistics: mean, standard deviation (SD), median and interquartile range and frequencies, as appropriate. Chi square, Student t and Mann Whitney tests were used to compare categorical, continuous normally and non-normally distributed variables, respectively. Stepwise logistic regression analysis was used to identify correlated of ULM. Were considered for inclusion into the model the following variables: age, parity, personal and family history of uterine leiomyoma, personal and family history of hypertension, personal and family history of diabetes mellitus, alcohol intake, menstrual cycle, body mass index (BMI), 17β-estradiol and progesterone levels, and vitamin D level. All variables associated with the outcome in univariate analysis were included in a multivariate analysis. P value ≤ 0.05 defined the level of statistical significance. The study received the clearance of the Ethical Committee of Kinshasa School of Public Health/University of Kinshasa (ESP/CE/N°028/2013).

3. Results

Table 1: Demographic, and clinical characteristics of the study population as a whole and according to uterine leiomyoma status.

Characteristic N	All (432)	Cases	Controls	P value
Age (years)	37,48 ± 10,52	37,78 ± 8,22	37,20 ± 12,36	0,576
Age at Menarche	13,60 ± 2,55	13,66± 2,34	13,66±2,75	0,995
Age at first delivery	20,11± 8,27	21, 31 ± 8,25	19,39 ± 8,23	0,088
Age at last delivery	29,59 ± 7,27	29,66 ± 6,90	29,56± 7,51	0,920

Education (%)[3]				0,000
Primary or less	5, 6	3, 7	7, 4	
Secondary	54,4	49,1	60,6	
University	38,1	46,3	31,9	
Parity	3(2-3)	2(2-3)	3(2-3.5)	0.046
0	182	8	64	0,000
≥1	235	93	142	
Menopause (%)				0,000
Yes	7,2	1,9	12,5	
No	92,8	98,1	87,5	
History of ULM (%)				
In mother	5,8	5,1	6,5	0,681
In sister	19,0	25,0	13,0	0,001
Personal	6,9	27,3	6,5	0,000
History of HT (%)				
In parent	35,4	34,3	36,6	0,687
In brother or sister	24,3	25,5	23,1	0,654
Personal	16,9	19,0	14,8	0,250
History of DM (%)				
In parent	15,0	17,1	13,0	0,282
In brother or sister	11,3	13,0	9,7	0,363
Personal	4,2	4,2	4,2	1,000
OP[7] oral contraception				
Yes (%)	6,7	5,1	8,3	0,248
Current smoking (%)				
Yes (%)	0,7	-	-	-
Alcohol intake (%)				
Yes (%)	17,1	8,3	25,9	0,000
Physical Activity (%)				
Yes (%)	3,9	2,3	5,6	0,135
BMI (kg/m^2)	25,22± 5,19	25,81± 5,18	24,66 ±5,16	0,025
Excess weight, %				
Yes	42,1	37,5	46,8	
No	41,7	45,8	46,8	

Data are expressed as mean ± SD or relative frequency in percent. Abbreviations: ULM, uterine leiomyoma; HT, hypertension; DM, diabetes mellitus; BMI, body mass index.

The table below show that the mean age of the study population was 37 ± 10 years while the BMI mean was 25.2 ± 5 Kg/m², Their median parity was 3(2-3). 7,2% of women in the sample were already at the menopause stage Oral contraception, personal and family history of leiomyoma were reported by 6, 7%;6, 9% and 24, 8%

of them.

Compared to controls, cases (BMI = 25.8±5, 18 Kg/m^2) had higher BMI than controls (BMI=24 ± 5 Kg/m²; p = 0.025) and lower parity [2(2-3)] vs [3(2-3.5), p=0,046]. Personal history of uterine leiomyoma was higher in cases than controls (27, 3% vs 6, 5%, p=0,000), even for history of uterine leiomyoma in sister (25 vs 13%, p=0,001) and high education (46, 3% vs 31, 9%). Menopause was lower in cases than controls (1, 9% vs 12, 5%, p=0,000). Alcohol intake was lower incases than controls (8, 3% vs 25, 9%, p=0,000).The differences observed in others variables were not statistically significant.

Tableau2: Biologicals characteristics of the study population as a whole and according to uterine leiomyoma status.

Characteristic N	**ALL (n=432)**	**CASES (n=216)**	**CONTROLS (n=216)**	**P value[2]**
25(OH)D, ng/ml	9, 05(8-12)	9(7-13)	9, 10(8-13)	
Vit D Cat/CRENK				0,028
Deficiency	13,7	17,1	10,2	
Insufficiency	48,4	42,6	54,2	
Sufficiency	29,9	31,5	28,2	
Vit D Cat/IOM				0,419
Deficiency	48,4	47,7	49,1	
Insufficiency	35,4	33,8	37,0	
Sufficiency	8,1	9,7	6,5	
17β-estradiol, pg/ml	93,5(85,5-108)	115(92-133)	81(68-94)	
17β-estradiol (%)				0,053
Elevated	12,7	12,5	13,0	
Normal	57,9	59,3	56,5	
Low	19,7	22,2	17,1	
Progesterone, ng/ml	0,6(0,6-0,8)	0,9(0,6-1,1)	0,4(0,3-0,6)	
Progesterone (%)				0,047
Elevated	14,4	19,0	9,7	
Normal	68,5	67,1	69,1	
Low	7,7	8,3	6,9	

Data are expressed as mean ± SD or relative frequency in percent. Abbreviations: Vit, vitamin Cat; category IOM, institute of medicine; CRENK, Kinshasa Regional Nuclear Energy Center.

From the table 2 the mean levels of serum 25(OH) D, 17ß-estradiol and progesterone in the whole group were 9, 05 ng/ml (8-12); 93, 5pg./ml (85, 5-108); 0, 6 ng/ml (0, 6-0, 8) respectively. Vitamin D insufficiency and deficiency using local criteria defining different steps of vitamin D were observed in 48.4% and 13.7% of patients, respectively. These proportions using IOM criteria defining different steps of vitamin D were 35.4%

and 48.4%, respectively. Compared to controls (10.2%) when using local criteria, there were more women with vitamin D deficiency among cases [(17.1%) p =0,028]. Difference in proportion of vitamin D insufficiency and deficiency by using IOM criteria were not statistically significant.

Table 3: Multivariate independent determinants of the risk of uterine leiomyoma

Variable	**Cases**	**Controls**	**Crude OR 95% CI**	**p**	**Adjusted OR 95% CI**	**p**
	n = 216	**n = 216**				
Age						
<35years	73	98	1		1	
≥35years	143	118	1.627(1,103-2,400)	0.014	**2.974**(1,722-5,139)	0.000
Parity						
≥1	93	142	1		1	
0	118	64	2.815(1,885-4,205)	0.000	**3.951**(2,311-6,754)	0.000
FH-ULM/sister						
No	161	179	1		1	
Yes	55	28	2.184(1.321-3.609)	0.002	**2.619**(1,376-4,986)	0.003
PH- ULM						
No	156	192	1		**1**	
Yes	59	15	4.851(2.646-8.864)	0.000	**3.776** (1,885-7,565)	0.000
Alcohol intake						
No	198	155	1		**1**	
Yes	18	50	0.282 (0.158-0.502)	0.000	**0.295**(0,150-0,580)	0.000
Menopause						
Yes	5	26	1		**1**	
No	211	190	4.142(2.247-7.633)	0.000	**5.502**(2,615-11,517)	0.000
Progesterone, ng/ml						
Normal	174	196				
Elevated	42	20	2, 176(1,238-3,824)	0.007	**2,320**(1,136-4,711)	0.021
25(OH) D, ng.ml						
Sufficiency			1		**1**	
Insufficiency			1.509(0,803-2,835)	0.201	1.390 (0,632-3,055)	0.413
Deficiency	37	22	2.139(1,181-3,875)	0.053	**2.163**(1,035-4,517)	0.040

Abbreviations: FH, familial history PH, personal history ULM, uterine leiomyoma OH, hydroxyl D, vitamin D.

Table 3 shows that the age, parity, sister's history of ULM, personal history of ULM, absence of menopause, alcohol intake, progesterone and vitamin D status emerged as the main independent determinants of the risk of having ULM. Age ≥35 years (aOR= 2.974; 95%CI 1.722-5.139; p=0,001), null parity (aOR= 3.951; 95%CI

2.311-6.754; p=0,010), ULM history in the sister (aOR= 2.619; 95%CI 1. 376-4.986; p=0,003), personal history of ULM (aOR= 3.776; 95% CI 1.885-7.565; p=0,001), absence of menopause (aOR= 5.502; 95% CI 2.615-11.517; p=0.001), high serum progesterone levels (aOR= 2.320; 95% CI 1.136-4.711; p=0,021) and vitamin D deficiency (aOR= 2.163; 95%CI 1.035-4.517; p=0,040) were associated with 1.62, 2.81, 2.18, 4.85, 4.14, 2.17, 1.50 and 2.13 fold increased risk of having ULM whereas alcohol intake (aOR= 0.295; 95%CI 0.150-0.580; p=0,001) conferred 29% lower risk.

4. Discussion

The main findings of the present case-control study can be summarized as follows. First, whatever the criteria used, vitamin D deficiency was frequently seen among patients with ULM with significantly higher proportions in comparison to controls when using local criteria. Second, increased age, nulliparity, personal and sister history of ULM, alcohol intake, menopause, high serum progesterone levels and vitamin D deficiency emerged as the main determinants of the risk of having ULM.

Mean levels of Vitamin D levels were similar in cases and controls in the present study. This finding contrasts with data from previous studies reporting low Vitamin D levels in women with ULM. In this regard, studies from Italy [14], United States of America [15] and Egypt [17] reported lower Vit D levels in women with ULM than controls. This disparity could be explained by the small sample size used in the present study.

Age, nulliparity, familial history of ULM, absence of menopause, high serum progesterone levels, and serum Vitamin D deficiency were associated with increased risk of ULM whereas alcohol intake conferred less risk. It has been reported that many genetic, hormonal and biological factors contribute to the development and the progression of ULM [27]. It is also assumed that the impact of each factor is related to its interference with the levels and metabolism of sex steroids and their metabolites [27]. The finding of increased risk of ULM associated with age, nulliparity, absence of menopause and familial history of ULM is consistent with that of previous reports [27, 28]. Alcohol intake appears to be paradoxically protective suggesting that a moderate consumption of alcohol could be protective as women are likely not be heavy consumers.

Increased serum progesterone levels were associated with increased risk of ULM. While early research has mostly focused on the role of estrogen in ULM, more recent evidence points to a significant role of progesterone [28] Kawagushi et al [29] demonstrated that mitotic activity in ULM is significantly higher in secretory (progesterone-dominant) phase than in proliferative (estrogen-dominant) phase of the menstrual cycle. Estrogen has been reported to induce the expression of progesterone receptor in ULM. It is now admitted that progesterone is at least as important as estrogen for ULM development [28]. Mechanisms of progesterone on ULM development include cross-talk with estrogen, interaction with growth factor signaling by down regulating the expression of insulin like growth factor 1 (IGF-1) and up regulating that of proliferating cell nuclear antigen (PCNA) and epidermal growth factor (EGF), known regulator of leiomyoma cells proliferation [28].

Vitamin D deficiency conferred increased risk of ULM in the present study. Recent evidence from 3 independent research groups in population from North Africa, East USA and Central Europe have demonstrated

an association between Vitamin D and increased risk of ULM. The Al-Hendy group reported first the association between serum Vitamin D levels and increased susceptibility to ULM in 2012 using a cohort of black and white women in North Africa [17, 30]. This finding was observed in other major studies including Baird and his colleagues , in a cohort of women from eastern USA [15] and Paffioni and his colleagues in Italian women [14]. This association does suggest that Vitamin supplementation could prevent or, at least, slow the progression of ULM. In this regard, Blauer and his colleagues [31] confirmed the effects of Vitamin D on the inhibition of human myometrial and fibroid cell growth. The anti-proliferative effect of Vitamin D is thought to rely upon the reduction of the expression of cell cycle regulator proteins, such as CKD1, CKD2 and CDK4, the activation of the caspase signaling, the reduction of the expression of the anti-apoptotic BCL2 and BCL2 protein expression in fibroid tumors and an anti-fibrotic role in leiomyoma through modulation TGF-β signaling [32, 33, 34, 35, 36, 37].

The interpretation of the results of the present study should take in account of some limitations.

The case-control design being more prone to recall and selection biases, some of the association found could be spurious. Assuming that misclassification could have been non-differential, we think that some associations found could be stronger than observed.

5. Conclusion

Vitamin D deficiency was a common finding in women with ULM and emerged as one of the main risk factors associated with ULM. However, this finding needs to be confirmed with a representative sample of women with ULM.

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Appendix*: author contributions*

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

ALPHONSE TSHIBAND, performed biological analysis and reviewed the manuscript

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

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

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

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

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