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Research Article: Interest of hTERT Expression as a Prognostic Marker in Precancerous and Cancerous Lesions of the Cervix in the Departments of Niari and Bouenza, Congo

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ABSTRACT

Introduction: Overexpression of human telomerase reverse transcriptase (hTERT) plays an important role in the progression of cancer, which is predicted to have a poor prognosis. This work show the interest of the expression of the hTERT subunit of telomerase as a prognostic marker in the evolution of precancerous and cancerous lesions of the cervix in women in the departments of Niari and Bouenza.

Method: A total of 98 dry tube blood samples collected between October 2020 and November 2021were tested to investigate hTERT expression. The assay was performed by third-generation Elisa enzyme immunoassay using the Human telomerase reverse transcriptase (hTERT) ELISA Kit; Sunlong Biotech Co., Ltd". A study of the cytological profile by cervico-vaginal smear and pool genotyping by Xpert-HPV® had also been undertaken.

Results: The average age of women was 43.26±11.52 yearswith extremities ranging from 24 to 73 years. The cytological profile of women subjected to hTERT expression was as follows: 87.65% of normal cytology, 10.11% of pre-cancerous lesions and 2.24% of patients with invasive cervical cancer.
The prevalence of HPV-HR was 81.82% (9/11) of which 66.67 (6/11) was HPV16.
The expression of hTERT in the general population was as follows: 44.94% of patients without expression, 48.31% of patients with expression and 6.75% with overexpression. Depending on cytology, overexpression of hTERT was observed in all patients with pre-cancerous and cervical cancer lesions.
hTERT was overexpressed in 28.57% and expressed in 71.43% of ASCUS patients. 48.72%ofwomen with normal cytology also expressed hTERT.
Among the 9 HPV positive patients, hTERT overexpression was observed in 44.45% of patients and expression in 33.33% of patients. HPV16 carriers also overexpressed and expressed hTERT in 33.33% and 22.22% respectively.

Conclusion: The telomerase subunit hTERT has been shown to be a biomarker of poor prognosis whose expression is proportional to the stage of evolution of cervical lesions.

Keywords: HPV-HR, expression hTERT, Cervical cancer, Congo

Introduction:

Cervical cancer is a tissue neoformation due to excessive, anarchic and autonomic cell proliferation with the production of metastases [1].
Globally, cervical cancer is the fourth most frequently diagnosed cancer and the fourth leading cause of cancer death in women, with approximately 604,000 new cases and 342,000 deaths worldwide in 2020 [1]. Cervical cancer is the most commonly diagnosed cancer in 23 countries and is the leading cause of cancer death in 36 countries around the world, the vast majority of which are in sub-Saharan Africa, Melanesia, South America and Southeast Asia[2].
In Africa, the age-standardized incidence, estimated at 29.3 per 100,000 inhabitants per year, varies considerably over the years between regions [3].In 2018, 21.7% of all cancer deaths among women in Africa were attributed to cervical cancer, making it the most common cause of cancer death in the region [4].
In Congo, according to data from the cancer registry, cervical cancer comes in

second place among female cancers after breast cancer, with a frequency of 26.6% and is the second leading cause of cancer death in women [5].

It is currently well established that human papillomavirus (HPV) is the main pathogen of cervical cancer. Similarly, other sexual and non-sexual factors act as cofactors in the progression of HPV infection to cervical cancer [6-7]; cervical cancer is preceded by a precancerous phase that can last several years before the onset of clinical symptoms, the major sign of which is genital hemorrhage [8].

However, there are certain factors that may accelerate tumor progression during these precancerous lesions, such as the human telomerase retro transcriptase (hTERT) subunit of telomerase which, once activated by the action of oncoproteins E6 and E7 of high-risk human papillomavirus (HPV-HR), leads to the neoplastic transformation of cervical cells infected with this virus into tumors [9].

Indeed, some studies have shown significant expression of hTERT telomerase and high activity levels in different types of cancers such as lung, pancreatic, liver, prostate, skin, certain gastrointestinal tumors and malignant cell lines, while hTERT activity levels were very low in healthy tissues [9-12]. In Africa and particularly in Congo, there are no data that have evaluated the expression of the telomerase hTERT subunit in precancerous and cancerous lesions of the cervix. It is in this approach that the present study participatesin order to know the levels of expression of hTERT in cervical lesions.

Materials And Methods

Type and study population

This was a cross-sectional descriptive study that took place over a period of 14 months from October 2020 to November 2021. The women included in this study were recruited from Dolisie and Nkayi General Hospitals. A total of 98 women underwent a cervico-vaginal smear, HPV genotyping and hTERT expression analysis.

Collection of cervical cells

Samples were taken using a cytobrush of the cervix during a speculum examination. After scraping, the cells were suspended in collection vials containing 10 ml of BD Sure Path solution ™ and stored at -20°C. These samples were used for cytological and molecular analyses.

Blood Collection

After obtaining informed consent from the patient, a tourniquet was attached to the elbow crease and the puncture area was disinfected. A frank puncture with a suitable vacuitener needle was performed on a tube without anticoagulant. A total of 4ml of blood was taken and a code guaranteeing anonymity was assigned. The samples were then centrifuged, aliquoted and the resulting serum was stored at -20 ° C until use.

Cytological Study

After sampling in the different departments, the samples were sent to the pathology laboratory of the Loandjili General Hospital (HGL) in Pointe-Noire for the cytopathological study. A cervical vaginal smear was performed using the Papanicolaou technique. The examination was performed by a pathologist. The results were rendered according to the Classification of the Bethesda 2001 System [13]: Normal or benign reactive cell changes (Normal/BRCC, involving any cervicitis not related to malignancy), atypical squamous cells of undetermined significance (ASCUS), low-grade intraepithelial squamous cell lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL) and invasive cervical cancers (ICC).

Hpv Detection and Genotyping

From the rest of the cytological products, pool detection and genotyping was performed on patients with pre-cancerous and cervical cancerous lesions.

A total of 11 patients were investigated by real-time PCR using GeneXpert technology (CEPHEID, USA) from the Xpert® HPV kit.

The Xpert HPV Assay test is an automated test for the qualitative detection and differentiation of HPV DNA by pooled molecular typing, including high-risk oncogenic HVVs. This detection was done bydifferentiation of the presence of an HPV-16 genotype alone, by grouped genotyping of HPV-18/45 types, grouped genotyping of types 16/18/45 and by detection of high-risk HPV types other than 16, 18, and 45.Extraction, amplification and detection are automated and last 60 minutes.

Expression de hTERT

The sandwich ELISA technique allowed us to evaluate the expression of hTERT in the 89 patients in this study.

This expression was evaluated using the Human telomerase reverse transcriptase (hTERT) ELISA Kit; Sunlong Biotech Co., Ltd". The hTERT ELISA kit uses the "sandwich" ELISA method in which polystyrene micropuit strips are pre-coated with hTERT-specific monoclonal antibodies. The sample serum is added to the microchips with a second monoclonal antibody conjugated to horseradish streptavidin-peroxidase (streptavidin-HRP).

During incubation, the specific immunocomplex formed by the presence of hTERT in the sample is captured on solid phase. After washing to remove the unbound sample, the chromogenic solution containing tetramethylbenzidine (TMB) is added to the wells, resulting in a blue-colored solution. A solution of sulfuric acid is then added to the wells to stop the reaction and yellows the solution. The intensity of the developed yellow color is directly proportional to the concentration of hTERT in the sample. The hTERT levels are thus quantified by measuring the absorbance at 450 nm in 15min and comparing it to the concentration generated from the standard curve.

Statistical analyses

We used Microsoft Excel 2013 software to build the database and develop the graphs. Quantitative variables were expressed as an average ± standard deviation and qualitative variables were expressed as a percentage.

The statistical analysis was carried out using Epi-Info V.7 software. (wwwn.cdc.gov/epiinfo). The Chi² test and the exact Fisher test were used to compare the proportions in order to establish the different associations between the parameters studied. Two variables studied were considered statistically significant when $p<0.05$.

RESULTS

Socio-demographic characteristics

A total of 89 cervical samples collected from the cities of Dolisie and Nkayi were analysed.

The most represented age group was between 40 and 50 years old or 32.58%.

The average age of women was 43.26 ± 11.52 years with extremes ranging from 24 to 73 years.
Socio-demographic characteristics are shown in Table I. The main local risk factors for HPV infection studied in the women in our study were: age, age of first sexual intercourse, number of sexual partners and pregnancies, taking oral contraceptives, smoking.

Cytological profile
Of the 89 female samples collected in our study, the following cytological profile was defined (Table II): 87.65% (78/89) of normal cytology cases, 10.11% of pre-cancerous lesions [7.87% (7/89) ASCUS cases, 1.12% (1/89) LSIL cases, 1.12% (1/89) HSIL cases] and 2.24% (2/89) of patients with invasive cervical cancer.

ASCUS: Atypical squamous cells of undetermined significance. LSIL: Low-grade squamous intraepithelial lesion. HSIL: High-grade scaly intraepithelial lesion. CHF: invasive cervical carcinoma.

Génotypage HPV- HR
Of the 89 patients in this study, 11 had cytological lesions and had received genotyping for HPV of which 9 were positive for HPV-HR or 81.82%. Among the 9 HPV-HR positive patients, HPV 16 was the genotype mostly represented with 66.67%. Multiple infections ranged from two to more than three strains of HPV. The genotypic distribution by department is shown in Table III.

Expression de hTERT
The expression of hTERT (Figure 1) among the 89 patients in our study was as follows: 44.94% (40/89) of patients without expression, 48.31% (43/89) of patients with expression and 6.75% (6/89) with overexpression.

Bivariate analysis
Expression of hTERT according to cytology
The results of hTERT expression according to cytological statuses in this study reported the following(Figure 2): The non-expression of hTERT exclusively in the category of women with normal cytology or 51.28% (40/78) cases. Expression in women with normal cytology in 48.72% (38/78) of cases and ASCUS in 71.43% (5/7) of cases. However, overexpression of hTERT was observed in 28.57% (2/7) of ASCUS, 100% (1/1) of LSIL; 100% (1/1) of HSIL and 100% (2/2) of CCI. In all lesion stages, statistical differences were highly significant (p-value< 0.001).

Expression of hTERT according to HPV-HR genotypes
In our study, we determined HPV-HR only in patients with cytological abnormalities. Of these patients, 2 (18.18%) were HPV-HR negative, and 1 showed an average hTERT expression of 50%.
Of the 9 HPV-HR positive patients, 2 had not expressed hTERT or 22.22%; on the other hand, 3 had expressed the hTERT on average or 33.33% and 4 had overexpressed the hTERT or 44.45%. The HPV 16 genotype was mainly represented in our study population or 66.67% (6/9) of cases.
Among these 6 HPV 16 positive patients, 1 patient was observed who had not expressed hTERT or 16.67%; 2 had expressed the hTERT on average or 33.33% and 3 had overexpressed the hTERT or 50%. A statistically significant difference was observed between hTERT expression and HPV genotyping (p-value = 0.012).The expression of hTERT according to the genotypes of HPV-HR is shown in Table IV.

Discussion
The objective of this work was to show the interest of the expression of the hTERT subunit of telomerase as a prognostic marker in the evolution of precancerous and cancerous lesions of the cervix in women in the departments of Niari and Bouenza.
The average age of the women participating in this study was 43.26 ± 11.52 years with age intervals ranging from 24 to 73 years.
Our results are similar to those of Boumba et al. (2015) and Loubanouet al. (2020) who reported respectively the average ages of 43.6 ± 9.5 years and 43.74 ± 10.30 years [14.15].
In contrast, Mehender et al. (2011) in India reported a significantly higher result of 47.9 ± 1.8 years [16]. This similarity of ages in this study could be explained by the fact that women in these age groups are sexually active and could contract the infections that can lead them to consult hospital services. In addition, the minor difference observed could be explained by the small size of our study population.
The majority of women who participated in this study had a secondary level of education or 74.15%; this result is similar to most of the work carried out in particular by Loubanou et al. (2020), Mwenzeet al. (2019) and Antaonet al.(2021) which reported 53.10%, 65.2% and 74.67% of secondary school women respectively [15, 17, 18]. These results could be explained by certain socio-economic conditions that prevent some women from being able to pursue higher education.
The present study reported that 69.66% of women had at least 5 pregnancies. This result is similar to that of Olivier et al. (2005) who obtained 59.6% in an epidemiological and cytopathological profile study of dysplastic lesions of the cervix in Congo-Kinshasa [19]. 78.65% of women had their first sexual intercourse before the age of 18. This result is slightly lower than that of Catarinoet al. (2016) in Cameroon and Arora et al. (2005) in England who reported the ages of 19 years [20, 21]. On the other hand Shin et al. (2019) reported an average age of 17 years in India [22]. This age of first sexual intercourse (18 years) which correlates to the highest risk in our study is a consequence of the socio-economic level of the country, which is one of the lowest in Africa, but also of the fact that it is a risk factor for HPV infection.
The cytological profile was as follows: 87.65% of normal cytology, cytological abnormalities were found in 12.35% of cases including: 7.87% of ASCUS, 1.12% of LSIL; 1.12% HSIL and 2.24% CCI.
87.65% (78/89) of normal cytology cases, 10.11% of pre-cancerous lesions [7.87% (7/89) ASCUS cases, 1.12% (1/89) LSIL cases, 1.12% (1/89) HSIL cases] and 2.24% (2/89) of patients with invasive cervical cancer.
Our results are superimposed on those of Loubanou et al. (2020) who obtained 0.77% for HSIL and 3.08% for ICC in a study carried out in 2020 in Pointe-Noire and Dolisie [15]. Similarly, Kasapet al. (2011) in Turkey and Takamatsu et al. (2017) in Nigeria reported abnormal FCV rates of 10.85% and 14.57% respectively [23.24]. On the other hand, Vjosaet al. (2017) reported a much higher prevalence of 49.93% in Macedonia [25]. This low rate of abnormal cytology (12.35%) in our study is explained by the fact that we have a young population in our study and that the immune response being more effective would better eliminate the viruses responsible for cervical lesions.

HPV-HR genotyping was performed in women who had abnormalsmears. 81.82% of women with cytological abnormalities were positive for HPV-HR of which HPV 16 was predominant with 66.67%. These results are close to those of Boumbaet al. (2015) 89.6% and Mwenzeet al.92.7% (2019) who had studied HPV-HR in cervical intraepithelial lesions and cervical cancers [14,17].

The genotype of HPV 16 was the majority in our study, this predominance is consistent with the literature which shows an oncogenic role of this genotype among high-risk HPV found in cervical lesions [25]. Similarly, Boumba et al.(2015), Mwenzeet al. (2019) and Loubanouet al. (2020) also reported the same prevalences of HPV 16 in their study populations [14, 15, 17].

Of the 89 patients in this study, 40 (44.94%) patients had not expressed hTERT. However hTERT was expressed in 48.31% (43/89) of cases and its overexpression was observed in 6.75% (6/89) of cases.

Our numbers are lower than those of Moreno-Acosta et al. (2020) who had obtained 31% non-expression, 52.9% mean expression and 16.1% overexpression of hTERT in a study to show the expression of the hTERT protein and its association with HPV infections in cervical cancer patients in Bogota, in Colombia [26]. On the other hand, Brancaet al. (2006) had obtained significantly higher numbers: 30.3% of patients with an unexpressed rate; 56.6% for an expressed rate and 13.1% with an overexpressed rate in a study on the regulation of hTERT in cervical lesions in Italy [27].

These minor differences observed in our results could be explained by the size and types of studies used by the authors. However, the youthful nature of our population and the low rate of cytological abnormalities observed could also explain the low rate of expression and overexpression of hTERT in our study.

The analysis of hTERT reported according to cytological status is the following: The non-expression of hTERT exclusively in the category of women with normal cytology or 51.28% (40/78) cases. Expression in women with normal cytology in 48.72% (38/78) of cases and in patients with ASCUS in 71.43% (5/7) of cases. However, an overexpression of hTERT was observed in 28.57% (2/7) of ASCUS, 100% (1/1) of LSIL; 100% (1/1) of HSIL and 100% (2/2) of CCI. The statistical difference was significant with a p-value of <0.001.

Our results are similar to those obtained by Vjosa et al. (2017) in Macedonia with an average level of expression of hTERT in 60% of women with ASCUS and an overexpression in 70% of LSIL, 100% of HSIL and 100% of CCI [28]. Similarly, studies by Kailash et al. (2006) in England and Wang et al. (2015) in China, reported an overexpression of hTERT in 100% of cases of cervical carcinomas [29, 30].

In contrast, Branca et al.(2006) reported the absence of 100% hTERT expression in women who had normal cytology; hTERT was expressed in 7.89% of LSIL, overexpressed in 5.62% of HSIL and nearly 23% of cervical cancer [27]. This difference from our results could be explained by the sensitivity and specificity of the hTERT detection technique used in this study.

In the present study, the expression of hTERT showed an exponential increase with the progression of cervical lesions. This increase was greater in CCI.

Conclusion

Based on our results, HPV 16 remains the most prevalent genotype in our study population. The telomerase subunit hTERT has been shown to be a biomarker of poor prognosis in the course of precancerous and cancerous lesions of the cervix whose expression is proportional to the stage of evolution of cervical lesions.

However, additional studies on a large sample size and even in other types of progressive tumors, would confirm the interest of the expression of hTERT in the Congolese population.

Table I: Socio-demographic characteristics

Socio-demographic characteristics	Total	
	Effective (n)	Frequency (%)
Age group (years)		
< 30	13	14.61
30-40	22	24.72
40-50	29	32.58
> 50	25	28.09
First sexual intercourse age (years)		
< 18	70	78.65
≥ 18	19	21.35
Number of Sexual partners		
< 5	65	73.03
≥ 5	24	26.97
Number of pregnancies		
None (0)	2	2.25
< 5	25	28.09
≥ 5	62	69.66
Level of Education		
Primary	6	6.74
Secondary	66	74.15
University	17	19.11
Marital Status		
Singles	6	43.82
Married	66	46.07
Divorced	17	5.62
Widows	4	4.49
Current Smoke		
Yes	7	7.87
No	82	92.13
Oral Contraception use		
Yes	38	42.69
No	51	57.31

Table II: Distribution of the cytological profile

Cytology	Effective (n)	Frequency (%)
Normal	78	87.65
ASCUS	7	7.87
LSIL	1	1.12
HSIL	1	1.12
ICC	2	2.24

ASCUS: Atypical squamous cells of undetermined significance. LSIL: Low-grade squamous intraepithelial lesion. HSIL: High-grade scaly intraepithelial lesion. CHF: invasive cervical carcinoma.

Table III: Prevalence of HPV types

Genotype HPV	Effective (n)	Frequency (%)
HPV -	2	18.18
HPV+	9	81.82
16	6	66.67
18/45	1	11.11
16/18/45	1	11.11
16/Other	1	11.11
Other	0	0

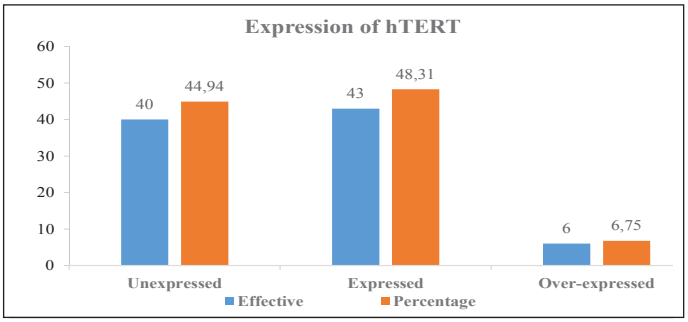

Figure 1: Expression of hTERT

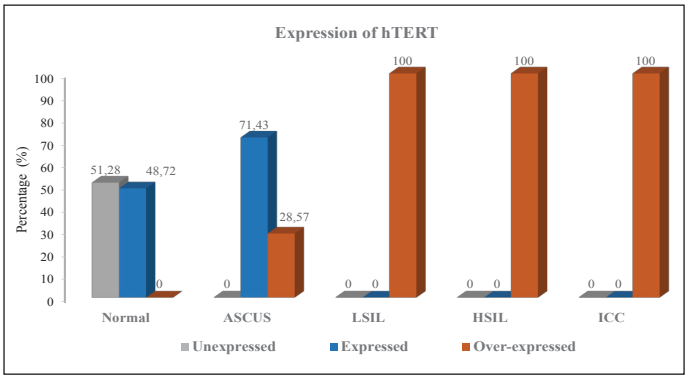

Table IV: hTERT Results by HPV-HR Genotypes

HPV-HR	hTERT					
	Un expressed		Expressed		Over-expressed	
	N	%	n	%	n	%
HPV -	1	50	1	50	0	0
HPV+	2	22.22	3	33.33	4	44.45
16	1	11.11	2	22.22	3	33.33
18/45	1	11.11	0	0	0	0
16/18/45	0	0	1	11.11	0	0
16/Other	0	0	0	0	1	11.11
Other	0	0	0	0	0	0

p = 0,012

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