ORIGINAL ARTICLE

HOTAIR Long Non-Coding RNA Expression in Genital Warts Patients in Suez Canal Area

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Abstract

Background: ***Anogenital warts (AGWs) are a prevalent, highly transmittable illness induced by the human papillomavirus (HPV), with elevated rates of recurrence leading to direct medical costs, diminished productivity, and heightened psychosocial effects.***

Objectives: ***This research aimed to assess the level of expression of HOTAIR Long noncoding RNA in genital wart cases with an emphasis on its diagnostic values.***

Patients and Methods: ***This case control research has been conducted on sixty persons that have been recruited from the Dermatology and Andrology Outpatient Clinic of the Suez Canal University Hospitals. Only those with anogenital warts (AGW) have been involved. They have been separated into two groups: Group A (case group): 30 cases with genital warts, Group B (control group): 30 healthy volunteers.***

Results: ***A statistically significant distinction (p-value less than 0.05) has been observed among HOTAIR Long noncoding RNA expression in lessional tissue and normal tissue. Correlation between the size of the lesion and HOTAIR gene expression level in the diseased tissue (p-value <0.05) may be used as a marker for the severity of AGW.***

Conclusion: ***Our findings revealed that the expression of the HOTAIR gene, an oncogenic long noncoding RNA, is significantly higher in AGWs tissue relative to adjacent normal tissue. This suggests that circulating HOTAIR is a potential biomarker for the early diagnosis of AGWs, and this lncRNA may serve as a promising treatment target.***

Keywords: Genital warts; sexually transmitted diseases; human papillomaviruses

1. Introduction

Genital warts (condyloma acuminatum) are the clinical symptoms of an infection that is transmitted sexually induced by human papillomavirus. Around ninety percent of cases exposed to human papillomavirus who become infected will not manifest genital warts. Merely ten percent of those affected may transmit the virus. There are more than one hundred recognized varieties of human papillomavirus viruses.[1]

Numerous human papillomavirus types have been related to carcinogenic illnesses, specifically types 16, 18, 31, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82. HPV16 is the most dangerous type of human papillomavirus, accounting for fifty percent of all cervical cancer cases.[2]

Condyloma acuminatum is typically transmitted by sexual intercourse. It can also be spread through non-penetrative sexual activity, but this is less prevalent.[3]

In human papillomavirus-16-positive cells, the E6 and E7 viral genes are integrated into the host genome and expressed. However, E6/E7 overexpression may be absent in certain human papillomavirus16-infected cells. Additionally, E6/E7 overexpression is observed in cells infected by various human papillomavirus varieties.[4]

Accepted 05 January 2025.
Available online 31 January 2025

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https://doi.org/10.21608/aimj.2025.446467

HOTAIR functions as a carcinogen in cervical cancer by enhancing the proliferation of cells, invasion, autophagy, and migration while suppressing apoptosis, improving angiogenesis, accelerating cell cycle progression, and triggering the transition of epithelial-mesenchymal. In cervical cancer cases, increased HOTAIR concentrations are significantly correlated with poor prognosis.[5] HOTAIR (HOX transcript antisense RNA) is a human-long noncoding RNA situated among HOXC12 & HOXC11 on chromosome 12. It is the inaugural case of an RNA expressed on one chromosome that was identified to affect the transcription of HOXD cluster posterior genes located on chromosome 2.[6]

This research aimed to assess the level of expression of HOTAIR long noncoding RNA in genital wart cases with an emphasis on its diagnostic values.

2. Patients and methods

This case-control research has been conducted to measure the expression concentration of HOTAIR long noncoding RNA in serum, lesional, and non-lesional tissues of genital warts patients, to correlate serum concentration of long noncoding RNA with its lesional level and to correlate the expression level of HOTAIR long noncoding RNA with genital warts severity. Sixty persons were recruited from the Dermatology and Andrology Outpatient Clinic of the Suez Canal University Hospitals. They have been separated into 2 groups: Group A (case group): 30 cases with genital warts; the following samples were taken from each patient in this group: blood sample shaved biopsy from the genital wart tissue, shaved biopsy from the healthy tissue surrounding the genital wart lesion and Group B (control group): 30 healthy volunteers of matched age and gender at Suez Canal University Hospitals not suffering from any infection, malignancies or autoimmune diseases. A blood sample was taken from each participant in the group.

Ethical consideration

In compliance with the guidelines of the Helsinki Declaration, we conducted our study, in addition to the approval from the investigation ethics committee and the institutional review board of Suez Canal University, Faculty of Medicine, Ismailia, Egypt, with code number 4633#. Written informed consent was obtained from all participants prior to enrollment in the study.

Inclusion criteria: Cases with genital warts of age above eighteen years, both sexes and cases with one or more anogenital warts were included in the research.

Exclusion criteria: Patients who had systemic, inflammatory, or autoimmune diseases (e.g., Vitiligo, Psoriasis, SLE, autoimmune urticaria, multiple sclerosis), cases with previous premalignant skin lesions or cancer in the skin or taking immunosuppressive medications like methotrexate and cases under treatment with other modality during the time of the investigation or within the last three months.

All cases have been exposed to the following:

Full history taking, local and general inspection, dermatological examination for the diagnosis of genital warts by Clinical assessment Cutaneous WARTS (CWARTS) diagnostic tool (10), laboratory investigations including assessment of serum and tissue expression level of HOTAIR long noncoding RNA.

Molecular Study: Trizol reagent & miRNeasy kit (Cat.no. 217204, QIAGEN, United States of America) was used to extract RNA from plasma of patients and their matched control group, lesional and non-lesional tissues followed by reverse-transcription to convert RNA to cDNA utilizing miScript II RT Kit (Cat. no. 218161, QIAGEN, United States of America) according to the manufacturer's guidelines. Subsequently, qRT-PCR has been conducted to quantify the levels of expression of long noncoding RNAs utilizing the StepOne real-time polymerase chain reaction instrument (Applied Biosystems, CA, United States of America) as well as the QuantiNova® SYBR® Green RT-PCR Kit (Cat. no. 208152, QIAGEN, United States of America), along with specific primers for the target long noncoding RNA (HOTAIR) and U6 primers as a housekeeping gene for the quantitative polymerase chain reaction. Table 1 below presents the primer sequences. The polymerase chain reaction conditions for the experiment were: initial denaturation at ninety-five degrees Celsius for fifteen minutes, followed by forty cycles consisting of denaturation at ninety-five degrees Celsius for fifteen seconds, annealing at fifty-eight degrees Celsius for thirty seconds. & extension at seventy-two degrees Celsius for twenty seconds. The HOTAIR expression level in each sample has been standardized to the internal control U6, and the relative levels of expression of HOTAIR were computed using the $2-\Delta\Delta Ct$ technique .[7]

Table 1: Primer sequence of HOTAIR and U6 long noncoding RNA

GENE	PRIMER SEQUENCE		SIZE BY BASE PAIR (BP)
HOTAIR	Forward: GGAAAGATCCAAATGGGACCA-3	5'-	297 bp
	Reverse: CTAGGAATCAGCACGAAGCAAA-3'	5'-	
U6	Forward: GCTTCGGCAGCACATATACTAAAAT-3'	5'-	89 bp
	Reverse: CGCTTCACGAATTTGCGTGTCAT-3'	5'-	

Statistical analysis

The data were input into the computer and analyzed utilizing IBM SPSS software version 20.0 (Armonk, NY: IBM Corp). The utilized statistical tests included the chi-square test for categorical variables to compare various groups, the student t-test for normally distributed quantitative variables to compare two examined groups, the paired t-test for normally distributed quantitative variables to compare lesional and non-lesional tissue genes, and the Pearson coefficient to assess the correlation among both normally distributed quantitative variables.

3. Results

An insignificant variation has been observed among both groups regarding baseline characteristics p-value more than 0.05. (Table 2)

Table 2. Distribution of both examined groups according to baseline characteristics

	GROUP A (NUMBER = THIRTY)		GROUP B (NUMBER = THIRTY)		SIG TEST	P
	No.	%	No.	%		
SEX						
MALE	30	100.0	30	100.0	–	–
AGE (YEARS)						
MIN. – MAX.	22.0 – 42.0		22.0 – 39.0		t=	0.159
MEAN ± SD.	30.0 ± 5.39		28.17 ± 4.53		1.426	
SMOKING						
SMOKING	24	80.0	17	56.7	c^2=	0.052
NON-SMOKING	6	20.0	13	43.3	3.774	

16.7% of participants were single, 76.7% married, and 6.7% were divorced. As regard site of the lesions were at the groin, scrotum, penis, and perianal area. The mean number of lessions was 9.07 ± 9.49. (Table 3)

Table 3. Distribution of the examined cases regarding marital status, site of lesions and number of lesions (n = 30)

	NO.	%
MARITAL STATUS		
SINGLE	5	16.7
MARRIED	23	76.7
DIVORCED	2	6.7
SITE OF LESIONS		
GROIN	9	30.0
SCROTUM	6	20.0
PEINS	24	80.0
PERIANAL	2	6.7
NUMBER OF LESIONS		
≤10	20	66.7
>10	10	33.3
MIN. – MAX.	1.0 – 46.0	
MEAN ± SD.	9.07 ± 9.49	

Twenty cases (66.7%) began with gradual onset and 10 cases (33.3%) began with sudden onset. As regard course, 13 patients (43.3%) had progressive course and 17 patients (56.7%) had stationary course. (Table 4)

Table 4. Distribution of the examined cases regarding onset and course (n = 30)

	NO.	%
ONSET		
GRADUAL	20	66.7
SUDDEN (INSIDIOUS)	10	33.3
COURSE		
PROGRESSIVE	13	43.3
STATIONARY	17	56.7

As regard duration and recurrence of the disease, about 28 patients (93.3%) had new lesion and only 2 patients (6.7%) had recurrent lesion, while mean of duration was 3.20 ± 1.58 month. This table shows the morphology of the lesion, about 24 patients (80%) were papules while 8 patients (26.7%) were condylomas. (Table 5)

Table 5. Distribution of the examined cases regarding recurrence, morphology and duration (n = 30)

	NO.	%
RECURRENCE		
NEW	28	93.3
RECURRENT	2	6.7
MORPHOLOGY		
PAPULE	24	80.0
CONDYLOMA	8	26.7
DURATION (MONTHS)		
MIN. – MAX.	1.0 – 6.0	
MEAN ± SD.	3.20 ± 1.58	

HOTAIR gene expression in lessional tissue ranged from min-max 2.10 – 6.80 with a mean of 3.80 ± 1.02 while in normal tissue it ranged from min-max 1.0 – 1.0 with a mean of 1.0 ± 0.0. A statistically significant variation (p-value less than 0.05) has been observed among HOTAIR gene expression in lessional tissue and normal tissue. (Table 6)

Table 6. Comparison between Diseased & normal tissue regarding HOTAIR gene expression level

HOTAIR EXPRESSION LEVEL	LESIONAL TISSUE (N = 30)	NON-LESIONAL TISSUE GENE (N = 30)	T	P
MIN. – MAX.	2.10 – 6.80	1.0 – 1.0	15.076*	<0.001*
MEAN ± SD.	3.80 ± 1.02	1.0 ± 0.0		

HOTAIR gene expression in patient serum ranged from min-max 2.25 – 9.57. A statistically significant variation (p-value less than 0.05) has been observed among HOTAIR gene expression in diseased serum and control serum. (Table 7)

Table 7. Comparative analysis among both examined groups according to HOTAIR gene expression

HOTAIR EXPRESSION IN SERUM	GROUP A (NUMBER = THIRTY)	GROUP B (NUMBER = THIRTY)	T	P
MIN. – MAX.	2.25 – 9.57	1.0 – 1.0	14.456*	<0.001*
MEAN ± SD.	5.82 ± 1.83	1.0 ± 0.0		

The size of the lesion and HOTAIR gene expression level in lessional tissue can indicate AGW severity p<0.05, while serum HOTAIR gene expression level was insignificant. (Table 8)

Table 8. Correlation between HOTAIR gene expression level in lessional tissue and serum with the size of lesion in patient group (n = 30)

	HOTAIR GENE LEVEL IN LESSIONAL TISSUE		HOTAIR GENE LEVEL IN SERUM	
	r	P	r	p
SIZE OF LESION (CM)	0.881*	<	0.009	0.962

r : Pearson coefficient Statistically significant as p ≤ 0.05

4. Discussion

Genital warts, often referred to as anogenital warts or condylomata acuminate, are elevated lesions that arise on the mucous membranes and skin following infection with certain forms of human papillomavirus, a virus spread through sexual contact.[8]

Long noncoding RNAs (lncRNAs) can influence gene expression at various levels, including chromatin structure, transcriptional processes, and following transcriptional modifications. Furthermore, variants within certain LNCRNAs were demonstrated to modify the probability of different human conditions. MiRNAs primarily influence gene expression at the post-transcriptional level by binding to the 3' UTR of transcripts, leading to diseases.[9] SNHG8, HOTAIR, SNHG12, SOX2OT, SOX21-AS1, GABPB1-AS1, HOST2, DINO, FAM83H-AS1, CCDST, TMPOP2, & CCEPR are cases of lncRNAs that participate in this process.[10]

HOX transcript antisense RNA, also known as HOTAIR, is a trans-acting long noncoding RNA (lncRNA) that has 6 exons in humans. It is transcribed from the antisense strand of the homeobox gene C cluster. This long noncoding RNA also serves as a modular framework for the ubiquitination of proteins and the silencing of genes.[11]

The purpose of this controlled research was to measure the expression level of HOTAIR long noncoding RNA in lesional and non-lesional tissues and serum of the study group. Sixty males have been involved in this research. They have been separated into two groups: Group A (case group): thirty cases with genital warts & Group B (control group): 30 healthy volunteers of matched age and gender. They have been recruited from the Dermatology and Andrology Outpatient Clinics of the Suez Canal University Hospitals.

In the current research, in the patient group, the mean age was 30.0 ± 5.39 years, with a minimum age of twenty-two years and a maximum age of forty-two years, while in the control group, the mean age was 28.17 ± 4.53 years with a minimum age 22 and maximum age 39. Regarding sex, both studied groups were males. As regards smoking, about 24 patients (80%) were smoking, and 6 patients (20%) were nonsmoking, while control group, 17 (56.7%) were smokers and 13(43.3%) were non-smoking.

In the current study, about 5 patients (16.7 %) were single, 23 patients (76.7%) were married, and 2 patients (6.7%) were divorced. Multiple variables of sexual conduct were related to elevated incidences of anogenital warts.[12] The presence of several sexual partners is a significant factor related to the elevated incidence of anogenital warts development.

In the present study, as regard the site of the lesion, 9 patients (30%) were at the groin, 6 patients (20%) were at the scrotum, 24 patients (80%) were at the penis, and 2 patients (6.7%) were at the perianal area. As regards the number of lesions, 20 patients (66.7%) had less than 10 lesions, 10 patients (33.3%) had more than 10 lesions, and the mean of the number of lesions was 9.07 ± 9.49 lesions.

Park et al.[13] found that genital warts were frequently situated on the penile shaft (65.2 percent of cases), pubic region (12.1 percent), the base of the penis (31.8 percent), & coronal sulcus (9.8 percent). Lisboa et al.[1] found that the majority of the AGWs were located on the pubis/ penile shaft (254/541; 47.0%). Perianal warts were present in 32.7 (177/541) of the study population. AGW can develop as individual growths or may occur in a cluster. Often, EGWs tend to grow in both number and size. A patient may initially develop one warty lesion, but others may develop subsequently.[14] This finding was consistent with El-Hamd & Aboeldahab,[15] who stated that genital warts often manifested on the moist tissues of the anogenital region.

As regard onset, 20 cases (66.7 percent) began with gradual onset and 10 cases (33.3 percent) started with sudden onset. As regard course, 13 patients (43.3%) had progressive course and 17 patients (56.7%) had stationary course. As regard recurrence, 28 patients (93.3%) developed new

AGWs while 2 patients were recurrent AGWs (6.7%). The mean disease duration was 3.20 ± 1.58 months.

HPVs tend to be transient. Approximately one-third of AGWs will resolve spontaneously, typically regressing within four months of infection, while over fifty percent of cases will self-resolve between four to six months. Over 90% of patients with AGWs experience complete clearance within 2 years, with or without treatment. Sometimes, the virus that causes AGW can remain dormant and undetected for long periods of time. Nia et al.[16] reported that the mean length of GWs was 19.92 ± 20.45 months.

As regards the morphology of the lesion, 24 patients (80%) were papules, and 8 patients (26.7 %) were condylomas. Lowhagen et al.[17] showed that high-risk human papillomavirus types have been identified in eight percent of acuminate lesions, twenty-four percent of popular lesions, and fifty-six percent of macular lesions. In the current research, a statistically significant distinction (p-value less than 0.001*) has been observed among Hotair gene expression in lessional tissue (mean ± SD = 3.80 ± 1.02) versus adjacent normal tissue (mean± SD = 1.0 ± 0.0). Likewise, a statistically significant distinction (p-value less than 0.001*) has been observed among Hotair gene expressions in diseased serum (mean ± SD = 5.82 ± 1.83) versus control serum (mean ± SD =1.0 ± 0.0), but a statistically insignificant association has been discovered among Hotair gene expression in lesional tissue and serum of AGWs patients (r= -0.060, P= 0.752).

In the current research, a statistically significant distinction (p-value less than 0.001*) has been observed among the size of the lesion and hot air gene level in lesional tissue, which may suggest being used as a marker for severity of AGW, while there was statistically insignificant between hot air gene level in diseased serum and the size of the lesion (p = 0.962).

Recent investigations have shown that circulating HOTAIR serves as a significant prognostic factor in cases with gastric or breast cancer.[18,19] In accordance with these results, increased plasma HOTAIR concentrations correlate with tumor recurrence and worse overall survival.[20]

To our knowledge, this is the first report that assesses the expression of HOTAIR long noncoding RNA in serum, lesional, and non-lesional tissues of genital warts patients. There were no previous studies about the role of HOTAIR in the diagnosis, prognosis, and treatment of genital warts to compare our results.

4. Conclusion

Our study demonstrated that HOTAIR gene expression, as an oncogenic LNCRNA, is significantly raised in AGWs tissue compared to adjacent normal tissue. Also, its serum level significantly increased in AGWs patients versus controls. This indicates that circulating HOTAIR is a potential biomarker for the early identification of AGWs. The elevated expression of Hotair at the gene level in lesional tissue may represent a prospective therapeutic target.

Disclosure

The authors have no financial interest to declare in relation to the content of this article.

Authorship

All authors have a substantial contribution to the article

Funding

No Funds : Yes

Conflicts of interest

There are no conflicts of interest.

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