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ORIGINAL ARTICLE

# Expression Profile of Long Non Coding RNA PVT1 in Patients with Ulcerative Colitis

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***Key words:***
***Ulcerative colitis (UC), long non coding RNA, PVT1***

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## ABSTRACT

***Background:*** *Ulcerative colitis (UC) is a chronic progressive inflammatory bowel disease, many long non coding RNA (lncRNAs) have been studied to have a role in the pathogenesis of Ulcerative colitis.* ***Objective:*** *is to evaluate expression level of long noncoding RNA PVT1 in ulcerative colitis and its association with the severity of the disease.* ***Methodology:*** *Sixty ulcerative colitis patients and 60 subjects were enrolled as controls. LNCRNA PVT1 relative expression level was tested using miScript SYBR Green PCR Kit.* ***Results:*** *Results showed significant differences between the patients with ulcerative colitis and controls as regard the median of the relative expression level of LNC PVT1 (P<0.0001). Also, there were positive significant correlations between the expression level of LNC PVT1 and AST(r=0.398, p=0.002), WBC(r=0.473, p<0.0001) in UC patients. The ROC curve analysis of LNC PVT1 revealed; LNC PVT1; AUC=0.784, P<0.0001, cut off point 1.06, sensitivity 73.3%, specificity 83.3%.* ***Conclusion:*** *Serum Lnc PVT1 could be used as a potential biomarker for UC diagnosis and prognosis*

## INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory bowel disease and it is one of two major forms of inflammatory bowel disease (IBD) the other one is Crohn's disease (CD), it characterized by development of multiple ulcers through inflammation of mucosa and submucosa of the colon[1].

The exact etiology of UC is not clear but many risk factors may have a role in the pathogenesis as genetic predisposition, environmental factors and disturbed immune system[2]. Common symptoms include chronic bloody diarrhoea, abdominal pain, weight loss, the condition may be complicated by impairment of intestinal movement, disturbed anorectal function and increase the risk of development of colorectal cancer .Many studies demonstrated the role of long noncoding RNAs (lncRNAs) in the pathogenesis of inflammatory bowel disease, Its effect mainly through interactions with chromatin, transcription factors, RNA, and proteins[3- 5]

Plasmocytoma variant translocation 1 (PVT1) is a long non coding RNA that located on human chromosome 8q24, 57 kb downstream of c-MYC locus contains 21 exons leading to 25 transcript variants (Ensemble Genome Browser). It was reported in many types of cancers as ovarian cancer[6], cervical cancer[7] and gastro intestinal tract[8], Moreover a recent study reported by Águeda et al showed the oncogenic role of PVT1 in colorectal and gastric cancer and its role in chemotherapeutic resistant[9]. Recently, researchers studied the role of PVT1 in autoimmune disorders as rheumatoid arthritis[10] and systemic lupus erythromatosis[11]

We aimed to detect the relative expression level of LNC PVT1 in patients with ulcerative colitis and its association with the severity of the disease

## METHODOLOGY

### Subjects

Our study included 120 subjects divided into 60 as healthy subjects considered as control (26 male and 34female) and 60 patients (28 male and 32 female) with ulcerative colitis. Patients were enrolled from Department of Internal Medicine, Fayoum University Hospital, Egypt. Written informed consent was obtained from all patients. All human studies have been revised and approved by Ethics Committee at Faculty of Medicine, Fayoum University.

Complete physical and laboratory investigations were done to all subjects. The diagnosis of UC depends

on the combination of patient history, clinical symptoms, standard radiographic and endoscopic criteria followed by histopathological examination, with exclusion of potential differential diagnoses[12-13]. Two biopsies of the inflamed and non-inflamed areas were collected for histopathological examination by the colonoscopy according to European Crohn's and Colitis Organization (ECCO) guidelines (two biopsies for the five segments of the colon and terminal ileum)[14]. Endoscopic parameters of assessment of severity include mucosal vascular pattern (MVP), friability and mucosal damage.

The Mayo Score wase used for grading the severity. The Score based on four parameters: stool frequency, rectal bleeding, Endoscopic findings and Physician rating of disease activity[15]

**Total criteria point count:**

Scores range from 0 to 12, with higher scores indicates more severe disease.

***Stool pattern***

Patient reports a normal number of daily stools (0 points), 1 to 2 more stools than normal (1 point), 3 to 4 more stools (2 points), 5 or more stools (3 points)

***Rectal bleeding***

None (0 points), Blood streaks seen in the stool less than half the time (1 point), all stools contain blood (2 points), Presence of pure blood (3 points)

***Endoscopic findings***

Normal or inactive colitis seen (0 points), Mild colitis: mild erythema, decrease in vascularity (1 point), Moderate colitis: marked erythema, erosions seen (2 points), Severe colitis: spontaneous bleeding (3 points)

*Physician rating of disease activity*

**Normal (0 points), Mild colitis (1 point), Moderate colitis (2 points), severe colitis (3 points)**

**Samples collection*:***

Five milliliter whole blood samples were drawn from all participants and collected in 2 tubes one of them contain EDTA for CBC, the second plain tubes were centrifuged at 2000 Xg for 5 minutes ,Sera were collected for determination of all serological tests and molecular biology analysis.

***Routine tests:***

Liver biochemical profile (using Biosystem kits), renal function tests: Urea, Creatinine (using Reactivos GPL).

**RNA extraction:**

By using (Qiagen, Valenica, CA, USA) extraction kits according to data supplied by the manufacturer

**Reverse transcription reactions:**

RNAs were reversed transcribed into cDNAs using (Qiagen, Valenica, CA) RT-PCR kit. LNC PVT1 expression level was determined by using the miScript SYBR Green PCR Kit (Qiagen, USA). The primer used to amplify the gene expression of LNCPVT1was: Forward 5′- TGAGAACTGTCCTTACGTGACC -3′ and Reverse 5′- AGAGCACCAAGACTGGCTCT-3′ (Invitrogen). Gene expression of LNCPVT1 was normalized to GADPH expression. Real-time PCR was done using Rotor gene Q System (Qiagen) with the PCR was performed under the following cycling conditions: 94°C for 10min (initial denaturation), followed by 30 cycles of 94°C for 30s, 65°C for 30s (annealing) and 72°C for 7min (final extension). The relative expression fold changes of RNA were calculated by the 2-ΔΔCt method [16]

*Statistical analysis*

SPSS software statistical computer package version 18 (SPSS Inc, USA) was used for analyzing data. For quantitative data, the mean, median, standard deviation (SD), and range were calculated. For quantitative non parametric data Mann-Whitney test was used for comparing two independent groups, Pearson correlation to test association between LNC PVT1 with study parameters. ROC curve was used to demonstrate LNC PVT1 as a predictor in differentiating between control and UC patients. Significance was adopted at $P \leq 0.05$

## RESULTS

Demography and laboratory characteristics of the study groups: Table 1 showed that there was a highly statistically significantdifference between patients with UC when compared to control subjects as regards the mean values ± SD of AST, ALT, Hb and WBCx1000 (P<0.0001) for each, Platlatesx1000 (p=0.002) (Table1).

**Table 1: Basic & laboratory characteristics of study groups**

| | | Cases (N=60) | | Controls (N=60) | | P-value |
|---|---|---|---|---|---|---|
| | | Mean | ± SD | Mean | ± SD | |
| **Age (years)** | | 38.8 | 5.9 | 39.6 | 5.5 | 0.484 |
| **Sex** | Male | 28 | 46.7% | 26 | 43.3% | 0.714 |
| | Female | 32 | 53.3% | 34 | 56.7% | |
| **DM** | No | 48 | 80.0% | | | |
| | Yes | 12 | 20.0% | | | |
| **HTN** | No | 48 | 80.0% | | | |
| | Yes | 12 | 20.0% | | | |
| **AST** | | 22.5 | 7.7 | 27.5 | 3.6 | **<0.0001*** |
| **ALT** | | 23 | 8.9 | 27.7 | 4.5 | **<0.0001*** |
| **Albumin** | | 4 | 0.6 | 4 | 0.4 | 0.884 |
| **Total bilirubin** | | 0.6 | 0.2 | 0.7 | 0.2 | 0.611 |
| **Hb g/dl** | | 11.4 | 1.4 | 12.2 | 1.2 | **<0.0001*** |
| **WBCx1000** | | 7.5 | 3.1 | 5.4 | 1.4 | **<0.0001*** |
| **Platlatesx1000** | | 240.9 | 66.4 | 207.9 | 42.7 | **0.002*** |
| **Urea** | | 27.4 | 9.9 | 25.6 | 8.9 | 0.299 |
| **Serum creatinine** | | 0.7 | 0.2 | 0.8 | 0.2 | 0.101 |
| **Diarrhoea** | Present | 46 | 76.7% | | | |
| | Absent | 14 | 23.3% | | | |
| **Rectal bleeding** | Present | 42 | 70.0% | | | |
| | Absent | 18 | 30.0% | | | |
| **Severity** | Mild | 14 | 23.3% | | | |
| | Moderate | 36 | 60.0% | | | |
| | Severe | 10 | 16.7% | | | |

Abbreviations: HTN , Hypertension ; DM, Diabetes mellitus; Hb gl/dl , Haemoglobin ; WBC,White blood cell ; AST ,aspartate transaminase; ALT,alanine transaminase ;

Statistical analysis is performed by Mann-Whitney U- test

*Significant **Comparison between cases and controls as regards LNC PVT1**

The results showed that there was a significant difference in relative expression level of LNC PVT1 between control and patients with ulcerative colitis groups (Table 2, figure 1)

**Table 2: Comparison between ulcerative colitis patients and control groups as regards the relative expression level of LNC PVT1**

| | Cases (N=60) | | Controls (N=60) | | P-value |
|---|---|---|---|---|---|
| | Median | IQR | Median | IQR | |
| **LNC PVT1** | 3.24 | (0.58-5.27) | 0.77 | (0.49-0.93) | **<0.0001*** |

Statistical analyses were performed by the Mann-Whitney U- test

*Significant

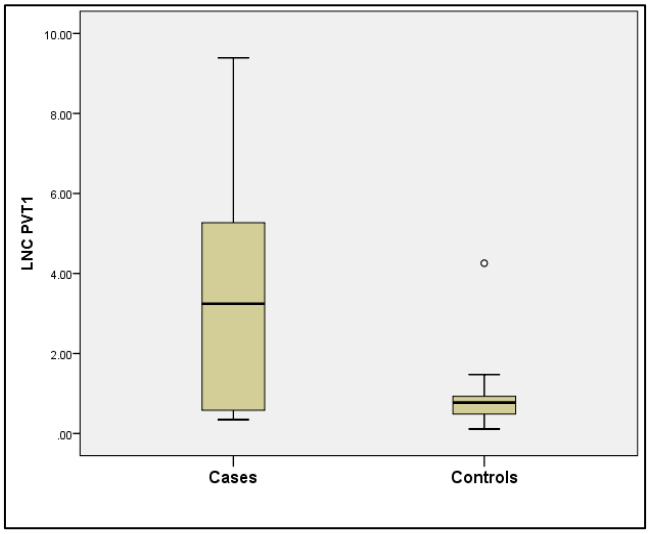


**Fig. 1: Comparison between ulcerative colitis patients and control groups as regards the relative expression level of LNC PVT1**

**Relation between LNC PVT1 and clinical characteristics**

Table (3), showed a strong association between LNC PVT1 and clinical characteristics of the patient as regards Diarrhea (p<0.0001), Rectal bleeding (p <0.0001) and Severity of the disease (Mild vs. Moderate p =0.007Moderate vs. Severe p =0.001Mild vs. Severe (p <0.0001)

**Table 3: Relation between expression level of LNC PVT1 and clinical characteristics**

| | | LNC PVT1 | | | P-value |
|---|---|---|---|---|---|
| | | Median | IQR | | |
| **Sex** | Male | 2.98 | 0.53 | 6.35 | 0.722 |
| | Female | 3.49 | 1.39 | 5.12 | |
| **DM** | No | 3.23 | 0.86 | 5.81 | 0.882 |
| | Yes | 3.78 | 0.42 | 4.81 | |
| **HTN** | No | 3.21 | 0.86 | 5.12 | 0.657 |
| | Yes | 3.78 | 0.53 | 6.87 | |
| **Diarrhoea** | Present | 4.33 | 2.7 | 6.87 | **<0.0001*** |
| | Absent | 0.53 | 0.42 | 1.64 | |
| **Rectal bleeding** | Present | 4.33 | 2.8 | 6.87 | **<0.0001*** |
| | Absent | 0.51 | 0.42 | 1.14 | |
| **Severity** | Mild | 0.53 | 0.42 | 1.64 | **0.007*@** |
| | Moderate | 3.24 | 2.15 | 4.81 | **0.001*#** |
| | Severe | 7.96 | 7.39 | 8.7 | **<0.0001*$** |

*Significant

@ Mild vs. Moderate, # Moderate vs. Severe, $ Mild vs. Severe

**Correlation of expression level of LNC PVT1 with study parametersamong cases**

Table (4) showed that there was a positive significant correlation between the expression level of LNC PVT1 and AST (p=0.002), WBCx1000 (p <0.0001).

**Table 4: Correlation of expression level of LNC PVT1 withthe study parameters among cases**

| | | LNC PVT1 |
|---|---|---|
| **Age** | r | -0.073 |
| | P-value | 0.579 |
| **AST** | **r** | **0.398** |
| | **P-value** | **0.002*** |
| **ALT** | r | 0.056 |
| | P-value | 0.673 |
| **Albumin** | r | -0.024 |
| | P-value | 0.853 |
| **Total bilirubin** | r | -0.154 |
| | P-value | 0.241 |
| **HbGL** | r | -0.149 |
| | P-value | 0.256 |
| **WBCx1000** | **r** | **0.473** |
| | **P-value** | **<0.0001*** |
| **Pltltsx1000** | r | -0.199 |
| | P-value | 0.128 |
| **Urea** | r | 0.017 |
| | P-value | 0.895 |
| **Serum creatinine** | r | -0.110 |
| | P-value | 0.405 |

Statistical analyses were performed by Spearman correlation
*Significant

ROC curve of Sensitivity and Specificity LNC PVT1 as a marker for diagnosis of ulcerative colitis Figure (2) illustrates the ROC curve of LNC PVT1 in patients with UC, showing the diagnostic value of LNC PVT1 as a predictor in differentiating between cases of UC and control. It was found to be significant at Cut off point=1.06 with sensitivity 73.3%, specificity 83.3% AUC=0.784 and P<0.0001.

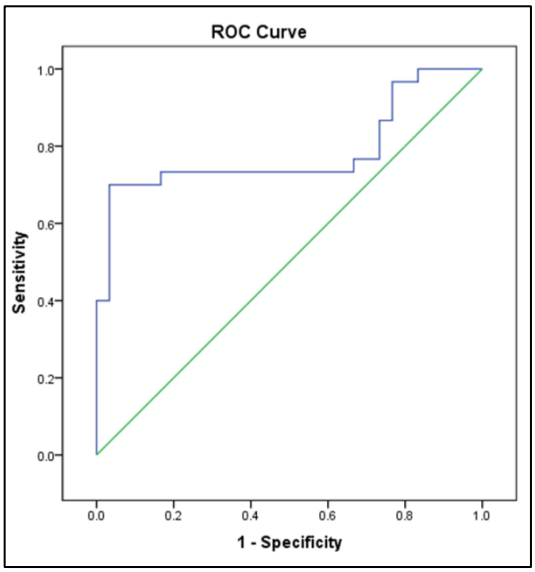


**Fig. 2:** ROC curve of Sensitivity and Specificity of LNC PVT1 between ulcerative colitis patients and control

Cut off point=1.06, sensitivity 73.3%, specificity 83.3% AUC=0.784 and P<0.0001.

## DISCUSSION

Long non-coding RNAs (lncRNAs) play an important role in the pathogenesis of autoimmune and inflammatory diseases, such as IBD [17-18]. In the inflammatory reaction they act as regulators in physiologic and pathologic processes[19].

The role played by lncRNAs in regulation of the inflammatory response is through activation and inhibition of immune genes [e.g., lincRNA-Cox2] and T cell differentiation programs [e.g., lincRNA-MAF-4][20-21]. LncRNAs in IBD is linked with the severity, intestinal permeability, and cell-intrinsic functions such as apoptosis and proliferation[22- 24]

Also Yufei et al[25] found that the expression of PVT1 is upregulated in osteoarthritic chondrocytes**.** PVT1 binds to the Sirtuin 6 (sirt6) promoter region. Sirts are regulators of various cellular and molecular processes including cell survival, gene transcription, and inflammation[26]**.** Furthermore Mirza et al performed the first transcriptomic profiling of lncRNAs in IBD patients[27] Additionally, Wu et al[22] also reported a profiling analysis of lncRNAs differentially expressed in UC patients versus controls**.**

Our results showed that the relative expression level of LNC PVT1 was significantly up regulated in the serum of UC patients compared to controls (P <0.001). A study done by Feng et al[28] demonstrated LNCPVT1 as an inflammatory regulator, they found that LNCPVT1 down regulation reduced levels of inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-10, IL-17, and IFN-γ), and inhibit MAPK/NF-kB pathway**.**

Furthermore, Diagnostic performance analysis of LNC PVT1 showing its diagnostic value to differentiate between UC patients from healthy control subjects as follows: Cut off point=1.06 with sensitivity 73.3%, specificity 83.3% AUC=0.784 and P<0.0001.

## CONCLUSION

Serum Lnc PVT1 could be used as a potential biomarker for UC diagnosis and prognosis

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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