ROLE OF CIRCULAR RIBONUCLEIC ACID COILED-COIL DOMAIN CONTAINING 66 AND SERINE/THREONINE KINASE-2 IN PATHOGENESIS OF CROHN'S DISEASE

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Introduction

Crohn's disease (CD) is a chronic and relapsing inflammatory bowel disease affecting GIT. Circular RNAs (CircRNAs) are non-coding RNAs produced in mammals through back splicing. They have advantages over linear RNAs in stability and resistance, so they have the potential to be used as both therapeutic targets and diagnostic biomarkers. CircRNAs are thought to regulate autophagy and inflammation. Circular RNA CCDC66 (circCCDC66) in CD are not well characterized, and their functions are not well understood. Thus, it is crucial to investigate its role in this disease. CircCCDC66 is thought to regulate inflammation and it has been identified as a novel oncogenic circRNA. CircCCDC66 is thought to contribute to colorectal cancer via upregulating the expression of autophagy related serine / threonine kinase 2 (AKT2) by sponging micro RNA-510-5p and regulate autophagy. Because autophagy may play a role in CD pathogenesis, circCCDC66 may also contribute to CD development by regulating AKT-2.

Aim of the Work

The aim of this study was to evaluate the role of circCCDC66 and circulating AKT-2 in the pathogenesis of CD, as well as to identify the relationship between circCCDC66 expression and circulating autophagy-related AKT-2 and disease activity of CD patients. Additionally, it aimed to test the possibility of using circulating circCCDC66 as a potential promising non-invasive biomarker for CD.

Subjects and Methods

This study included 50 subjects divided as follows:
Group I: included 34 patients with Crohn's disease and was divided into 2 subgroups:
• Group Ia: included 17 patients with active Crohn's disease.
• Group Ib: included 17 patients with inactive Crohn's disease.
Disease activity was assessed clinically by Crohn's disease activity Index (CDAI) and endoscopically by the simple endoscopic activity score in Crohn's disease (SES-CD).
Group II: included 16 sex-and age-matched healthy subjects as a control group.
All patients included in the current study were subjected to history taking, clinical examination and endoscopy. Routine laboratory investigations (as CBC, liver enzymes, albumin, kidney function tests, CRP and ESR), fecal calprotectin, relative expression of circular RNA circCCDC66 by quantitative real time reverse transcriptase polymerase chain reaction (qRT-PCR) and determination of plasma level of AKT2 protein by enzyme-linked immunosorbent assay (ELISA) were performed in all subjects.

Results

Table 1: Diagnostic performance for different parameters to discriminate group I (a +b) (Crohn's disease) patients (n = 34) from group II (Control group) (n = 16)

	AUC	p	95% C.I	Cut off	Sensitivity%	Specificity%	PPV %	NPV %
Calprotectin (mg/kg)	0.824	<0.001*	0.712 – 0.937	>33	76.47	81.25	89.7	61.9
$2^{-\Delta\Delta ct}$ (CCDC66)	0.750	0.005*	0.606 – 0.894	>1.014#	79.41	62.50	81.8	58.8
AKT2 (pg/ml)	0.706	0.020*	0.550 – 0.862	>2853	64.71	62.50	78.6	45.5

AUC: Area Under a Curve
NPV: Negative predictive value
*: Statistically significant at $p \leq 0.05$
p value: Probability value
PPV: Positive predictive value
#Cut off was choose according to Youden index
CI: Confidence Intervals

Table 2: Diagnostic performance for different parameters to discriminate inactive group (group Ib) patients (n = 17) from control group (n = 16)

	AUC	p	95% C.I	Cut off	Sensitivity%	Specificity%	PPV %	NPV %
Calprotectin (mg/kg)	0.700	0.049*	0.515 – 0.885	>28	64.71	62.50	64.7	62.5
$2^{-\Delta\Delta ct}$ (CCDC66)	0.798	0.004*	0.641 – 0.955	>1.206	76.47	68.75	72.2	73.3
AKT2 (pg/ml)	0.739	0.019*	0.557 – 0.921	>3067	70.59	87.50	85.7	73.7

AUC: Area Under a Curve
NPV: Negative predictive value
p value: Probability value
PPV: Positive predictive value
CI: Confidence Intervals
*: Statistically significant at $p \leq 0.05$

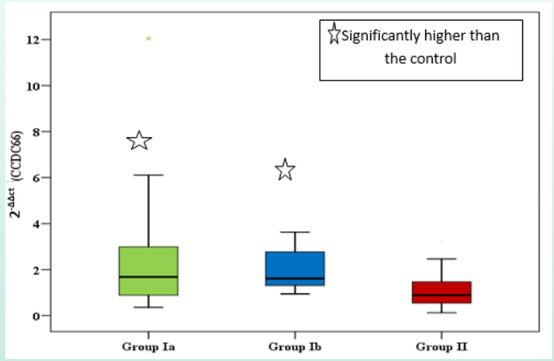

Figure: Plasma circCCDC66 relative expression level in the 3 studied groups.

Conclusion

Plasma circCCDC66 plays an important role in pathogenesis of CD and might act as a promising non-invasive diagnostic biomarker in CD. Concomitant measurement of plasma circCCDC66 and plasma AKT2 can differentiate CD patients in remission state from healthy control.
Neither circCCDC66 nor AKT2 is regarded as a prominent marker for CD activity and only the faecal calprotectin could discriminate patients with active disease from those with inactive disease.