STUDY OF SERUM LONG NON CODING RNAINTERFERON GAMMA ANTISENSE-1 AS BIOMARKER OF ACTIVITY IN CROHN'S DISEASE

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INTRODUCTION

Inflammatory bowel diseases are group of chronic in flammatory disorders that affect the gastrointestinal tract .There are two major forms: Crohn's disease and ulcerative colitis. The etiology of IBD is a result of complex interactions between genetics, immune dysregulation, and environmental factors. Crohn's disease occurs in attacks of remission and relapse and characterized by patchy transmural inflammation that affect all layers of the intestine in any part of the GIT. It is presented clinically by chronic diarrhea with or without blood, mucus and may be associated with extrain testinal manifestations.

Long non coding RNA (LncRNAs) are transcripts that are longer than200 nucleotides. Dysregulation of lncRNA expression is linked to the development of various diseases. Evolving studies show that lncRNAs play important roles in the pathogenesis of IBD. Several genes were significantly upregulated specially the lncRNAs interferon gamma antisense 1 (IFNG-AS1), while others are down regulated in active cases of Crohn's disease.

AIM OF THE WORk

The aim of the work was to assess the role of serum lncRNA (IFNG-AS1) as a marker of disease activity in patients with Crohn's disease.

SUBJECTS AND METHODS

SUBJECTS: The study will include fifty subjects that will be divided as follow:

Group I: will include thirty patients with Crohn's disease and will be divided into 2 subgroups:

- **Group Ia:** includes 15 patients with active Crohn's disease.
- **Group Ib:** includes 15 patients with Crohn's disease in remission.

Group II: Is a control group and includes 20 patients with normal colonoscopy who are indicated for colonoscopy for reasons other than IBD.

METHODS: *All patients will be subjected to the following:*

1. Detailed history taking with emphasis on gastrointestinal symptoms .
2. Thorough systemic physical examination including abdominal examination with stress on abdominal tenderness, palpable organs or masses, peri-anal fistula and extra-intestinal manifestations of IBD.
3. Laboratory investigations will include:
   A. Routine lab investigations: Complete blood picture (CBC), Erythrocyte sedimentation rate (ESR), quantitative C reactive protein (CRP), Serum albumin, renal function test and liver function test.
   B. Fecal markers: quantitative assessment of fecal calprotectin by ELISA.
   C. Determination of circulating lncRNA IFNG-AS1 using real time quantitative RT-polymerase chain reaction (qRT-PCR).
4. Imaging: CT Entero-colonography if needed.
5. Ileocolonoscopy will be done for all subjects and tissue specimens for histopathology confirmation of diagnosis and assessment of activity of disease will be taken.
6. Assessment of disease activity in Crohn's disease patients clinically by CDAI and endoscopically by SES-CD.

RESULTS

Table 1: Comparison of IFN-G AS1 (2-cT) levels between group I and group II.

IFN-G AS1 ($2^{-\Delta\Delta cT}$)	Group I (n = 30)	Group II (n = 20)	U	P
Min. – Max.	0.03 – 625.99	0.24 – 1.38	187.0*	0.025*
Mean ± SD.	70.10 ± 150.55	0.70 ± 0.34		
Median (IQR)	1.43 (0.52 –5.65)	0.74 (0.41 –0.92)		

IQR: **Inter quartile range** SD: **Standard deviation** **U: Mann Whitney test**
p: p value for comparing between the studied groups *: Statistically significant at $p \leq 0.05$
Group I: Patients with Crohn's disease **Group II: Control group with normal colonoscopy**

Table 2: Comparison between the two studied subgroups according to IFN-G AS1 ($2^{-\Delta\Delta cT}$).

IFN-G AS1 ($2^{-\Delta\Delta cT}$)	Group Ia (n = 15)	Group Ib (n = 15)	U	p
Min. – Max.	1.57 – 625.99	0.03 – 1.30	0.000*	<0.001*
Mean ± SD.	139.64 ± 191.28	0.55 ± 0.40		
Median (IQR)	5.65(2.48 –198.1)	0.52 (0.22 – 0.76)		

IQR: **Inter quartile range** SD: **Standard deviation** **U: Mann Whitney test**
p: p value for comparing between the studied groups *: Statistically significant at $p \leq 0.05$
Group Ia: Patients with active Crohn's disease **Group Ib: Patients with Crohn's disease in remission**

Table 3: Validity (AUC, sensitivity, specificity) for IFN-G AS1 (2-ΔΔcT) to discriminate Patients with active Crohn's disease (n = 15) from Patients with Crohn's disease in remission (n = 15).

	AUC	p	95% C.I	Cut off#	Sensitivity	Specificity	PPV	NPV
IFN-G AS1	1.000	<0.001*	1.000 – 1.000	>1.3	100.0	100.0	100.0	100.0

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

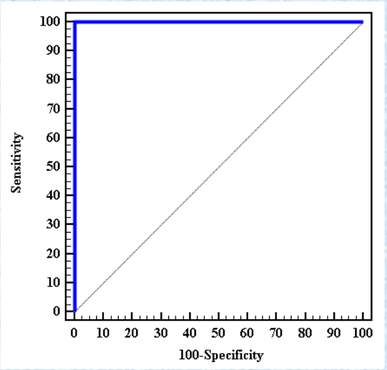

Figure 1:
ROC curve for IFN-G AS1 (2-ΔΔcT) to discriminate Patients with active Crohn's disease (n = 15) from Patients with Crohn's disease in remission (n = 15).

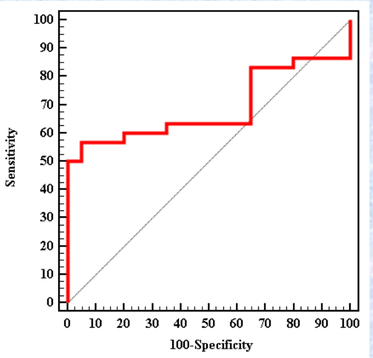

Figure 2:
ROC curve for IFN-G AS1 (2-ΔΔcT) to discriminate patients with Crohn's disease (n = 30) from Control group with normal colonoscopy (n = 20).

CONCLUSION

We conclude that IFNG AS-1 measurement provides a unique reliable biomarker useful to diagnose and monitor the degree of mucosal affection in CD. Studies on a broader sample of CD patients with varied disease behaviours and ethnic groupings are needed to validate the results, and it will provide a new important marker of disease activity. There is a need for more research into the usefulness of serum IFNG AS-1 in terms of predicting future episodes of relapse and assessing treatment response.