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ORIGINAL RESEARCH

New-Onset of Crohn's Disease Is Associated with Antistreptolysin O Positive Titers

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Objective: Different infectious agents have been presumed to be candidates acting as an etiologic factor or trigger of Crohn's disease (CD). Group A *Streptococcus* (GAS) is a common human infection agent that can also trigger post-infectious immune-mediated conditions. The current study aimed to examine whether the immunogenic activity induced by GAS may trigger new-onset of CD.

Methods: Data for antistreptolysin O (ASO) level, throat culture for GAS, and history of streptococcal infection were collected from 91 patients with CD that were divided into three groups including; new-onset CD, CD in remission and active CD. The data were compared with the control group.

Results: All participants had negative results of throat culture for GAS and had no history of documented streptococcal infection in the past year. Our results indicate that new-onset CD, but not CD in remission or active CD, is associated with significantly increased positive ASO compared to controls. Half of the patients in the new-onset CD group were ASO positive, which was significantly higher compared to the control group in a univariant (OR: 4.00; 95% CI 1.27–12.58; P=0.02) and multivariant analysis (OR: 4.41; 95% CI 1.35–14.37; P=0.014).

Conclusion: Our study is the first to focus on ASO levels in patients with CD and to demonstrate a significant association between ASO and new-onset of CD. Large prospective randomized controlled studies are needed to confirm the validity of this data and to further clarify the clinical significance of our findings.

Keywords: inflammatory bowel disease, IBD, Crohn's disease, new-onset, group A *Streptococcus*, antistreptolysin O, ASO

Introduction

Crohn's disease (CD) is a chronic inflammatory disease of the gastrointestinal (GI) tract. The pathology in CD causes inflammation of all bowel wall layers and can attack any part of the digestive tract.[1] Although the precise etiology is unknown,[2] the most widely accepted hypothesis purports CD as an immune-mediated condition in genetically susceptible individuals. Often, disease onset is triggered by environmental factors that perturb the mucosal barrier, alter the healthy balance of the gut microbiota and abnormally stimulate gut immune responses.[3] Antibodies to several specific antigens have been reported in the sera of patients with CD and include microbial antibodies like Anti-Saccharomyces cerevisiae antibody (ASCA),[4,5] the Anti-outer-membrane porin C (OmpC) antibody[6,7] and Anti-flagellin (Cbir1) antibody.[8]

Antistreptolysin O (ASO) is an antibody against streptolysin O that is generally produced by Group A *Streptococcus* (GAS). GAS causes a diverse range of human infections, like pharyngitis, cellulitis, scarlet fever, and others. Moreover, a GAS

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infection can trigger postinfectious immune-mediated disorders, including acute poststreptococcal glomerulonephritis (APSGN), acute rheumatic fever (ARF) and rheumatic heart disease (RHD).[9] Interestingly, other diseases with suggested immunological components in their pathogenesis including: narcolepsy,[10] ankylosing spondylitis with uveitis,[11] Henoch-Schoenlein purpura[12] and psoriasis[13,14] were reported having positive levels of ASO.

We hypothesized that the immunogenic activity induced by GAS may trigger new-onset of CD. This prospective study aimed to examine the ASO level in patients with CD.

Materials and Methods

A single-center case–control prospective study was conducted at Shamir (Assaf Harofeh) Medical Center in Israel between Jun 2015 and Jul 2019. Ninety-one patients with CD were divided into three groups: active CD, CD in remission, and new-onset CD. CD remission was defined as CD activity index (CDAI) scores of ≤150. Active disease was defined as CDAI >150. The new-onset CD group included patients who were diagnosed with CD within the last six months. The control group included personnel of the medical center or patients of our clinic with functional disorders like gastrointestinal reflux disease (GERD) and without a recent history of infectious disease. None of the controls had inflammatory bowel disease (IBD).

Following informed consent, blood samples for assessment of ASO and throat swab for culture were collected. Patients also answered the questionnaire that included demographics (gender, age), information regarding recent streptococcal infection and data necessary for CDAI assessment.

The determination of serum ASO level was performed by using the Rheumajet ASO, Latex Agglutination Kit for the detection of ASO (BioKit, A Werfen Company). An ASO titer greater than 200 IU/mL was considered a positive test. For diagnosis of GAS, the throat swab was incubated for 48 hours on a sheep blood agar plate.

This study was approved by the Ethics Committee of Shamir (Assaf Harofeh) Medical Center (decision number: 0155-16-ASF). All patients gave written informed consent before participation in the study which was performed according to the Declaration of Helsinki.

Statistical Analysis

Data are expressed as mean ± standard deviation (SD) for normally distributed variables, or as median with interquartile range (IQR) for variables that did not follow a normal distribution, or as frequencies for categorical data.

Differences between the patient's groups were analyzed by analysis of variance (ANOVA) using one-way ANOVA or chi-square test, as appropriate.

Multivariate logistic regression was used to determine whether a significant association between positive ASO and new-onset CD remained significant after adjustments for age, gender, and history of sore throat. The univariate and multivariate logistic regression analyses are presented as (OR; CI).

All statistical tests were two-sided, with a value for $p<0.05$ defining significance.

All statistical analyses were performed using SPSS software, version 18.0 (SPSS Inc, Chicago, IL).

Results

One hundred twenty-one patients were included in our study. The new-onset CD, CD in remission, and control groups consisted of 30 patients each. There were 31 patients in the active CD group. Demographic characteristics and ASO positivity are summarized in Table 1.

There were no significant differences in gender, disease location, disease phenotype and smoking between the groups. However, the patients with new-onset CD were significantly younger than patients with known active CD (27.6 ± 13.3 vs 41.5 ± 15.8 years, p=0.002) and younger than patients in remission (27.6 ± 13.3 vs 38.7± 16.6 years, p=0.026), but not significantly younger than patients in control group (27.6 ± 13.3 vs 33.8 ± 12.9 years, p=0.65).

Half of the patients in the new-onset CD group were ASO positive and this was significantly higher compared to the control group in a univariant analysis (OR: 4.00; 95% CI 1.27–12.58; P=0.02) and in a multivariant analysis adjusted for age and gender as well (OR: 4.41; 95% CI 1.35–14.37; P=0.014) (Table 2). In contrast, there was no significant difference in ASO positivity between either the active CD group or remission CD group when compared to the control group.

All participants had negative results of throat culture for GAS and had no history of skin infection in the past year. Five patients in the new-onset group, 4 in the active CD groups and 5 in the remission group had a history of sore throat in the past year without documented streptococcal infection (Table 1). This data did not change the significant difference in ASO positivity between the groups (Table 2).

Discussion

The idea for the current study came from several positive ASO results in CD patients that were accidentally performed

Table 1 Demographic Features and ASO Positive Percent of All Groups

Variable	(1) CD New-Onset n=30	(2) CD Active CD n=31	(3) CD Remission n=30	(4) Control n=30	P-value
Mean age (years)	27.6 ± 13.3	41.5 ± 15.8	38.7± 16.6	33.8 ± 12.9	0.002
Male [%]	19 [63.3]	17 [54.8]	14 [46.7]	12 [40]	0.3
Disease location[a]					
L1	19 [63.3]	16 [51.6]	15 [50]	-	0.74
L2	2 [6.7]	4 [12.9]	4 [13.3]	-	
L3	9 [30]	10 [32.3]	11 [36.7]	-	
L4	0	1 [3.2]	0	-	
Perianal disease	3 [10]	2 [6.5]	2 [6.7]	-	0.85
Disease phenotype[a]					
B1	24 [80]	21 [67.7]	24 [80]	-	0.433
B2	0	3 [9.7]	1 [3.3]	-	0.172
B3	6 [20]	7 [22.6]	5 [16.7]	-	0.84
Smoking [%]	11 [36.7]	7 [22.6]	6 [20.0]	5 [18.5][b]	0.35
ASO positive [%]	15 [50]	7 [22.6]	7 [23.3]	6 [20]	0.033
Sore throat [%]	5 [17.2]	4 [12.9]	5 [16.7]	0	0.13

Notes: [a]According to Montreal Classification. [b]Data are missing for 3 patients.
Abbreviations: ASO, antistreptolysin O; CD, Crohn's disease.

Table 2 Association Between Groups of Patients and Positive ASO Titer According Logistic Regression

	New-Onset CD (1) OR (95% CI)	Active Disease (2) OR (95% CI)	CD Remission (3) OR (95% CI)
Univariate	4.00 (1.27–12.58) p=0.02	1.17 (0.34–3.98) p=0.81	1.22 (0.35–4.17) p=0.75
Multivariate[a]	4.41 (1.35–14.37) p=0.014	1.23 (0.35–4.34) p=0.75	1.24 (0.36–4.33) p=0.73
Multivariate[b]	4.44 (1.31–15.1) p=0.017	1.15 (0.32–4.16) p=0.83	1.15 (0.32–4.11) p=0.83

Notes: [a]Adjusted for age and gender. [b]Adjusted for age, gender and sore throat.
Abbreviations: ASO, antistreptolysin O; CD, Crohn's disease.

instead of ASCA tests. Indeed, our results indicate that new-onset CD, but not CD in remission or active CD, is associated with significantly higher positive ASO levels compared to controls (OR: 4.41; 95% CI 1.35–14.37; P=0.014) (Table 2).

GAS is a common bacterium that causes acute or subclinical streptococcal pharyngitis[15,] impetigo, and other skin infections. ASO is positive in only 80-85% of streptococcal infections and the level starts to rise in 1–3 weeks after infection with peaks in 3–5 weeks, and then goes back to an insignificant level over 6–12 months.[16] One of the major questions is whether the serum ASO titer reflects a present or recent streptococcal infection. All the participants in our study had negative results of throat culture for GAS with no history of recent skin infection. Although some patients had a history of a sore throat in the past year, the etiology of these was unknown. Nevertheless, the association between positive ASO and new-onset CD remained significant even after adjustments for history of sore throat (Table 2).

Previous studies indicate that ASO titers may be high in healthy persons without evidence of streptococcal infection and vary with age, season and geography.[17] We found only one study from our geographic region showing 8% positive ASO results (among 60 healthy adults) which is significantly lower from our results (20% in controls).[18] Moreover, we know from previous works that the presence

of elevated ASO antibodies does not necessarily reflect recent exposure to GAS and that ASO antibodies may cross-react with antigens from other infective agents, such as *Mycoplasma*[18] or *Streptococcus* group C and G[19]. Conditions like tuberculosis, active viral hepatitis, or monoclonal gammopathy that can cause false-positive ASO tests were not present in our cohort. By contrast, many patients with a new CD diagnosis or active CD had taken antibiotics and steroids that may have actually resulted in false-negative ASO results.[19]

Crohn's disease (CD) is a chronic inflammatory bowel disease with undefined etiology. Different infectious agents that were isolated from patients with CD have been supposed to be candidates acting as an etiologic factor or trigger for CD, among them: *Mycobacterium avium paratuberculosis (MAP)*[20] adherent-invasive *Escherichia coli (AIEC)*,[21] *Listeria*[22] and measles virus.[23] In addition, CD is associated with serum antibodies against a large variety of microorganisms, including commensal bacteria normally present in the human body, probably due to the altered intestinal permeability. Since the appearance of serum antibodies (such as anti-Saccharomyces cerevisiae antibody (ASCA) IgG and IgA, pANCA, anti-OmpC and anti-CBir1) months or years before the development of CD was found to be highly predictive for CD, several groups suggested that this may be used as a diagnostic tool for CD.[4,8,24-26] Moreover, besides their predictive association, anti-microbial antibodies have also been shown to bear prognostic information associated with complications, such as obstruction, penetrating complications or surgery.[27–30] However, until now, the association of CD with these infections was not confirmed. Moreover, the recently published ECCO-ESGAR guideline for diagnostic assessment in IBD recommends that serological testing may be used to support a diagnosis but not as a routine diagnostic test of CD.[31]

Why is positive ASO titer more prevalent in new diagnosed CD? There are several possible hypotheses to explain these findings. It may be that a sub-clinical streptococcal infection or exposure to other Streptolysin O producing *Streptococci*, such as *Streptococcus* groups C and G, cause the immune-mediated response which triggers CD. Another explanation is that ASO antibodies reflect an aberrant immune response rather than direct effector involved in the pathogenesis of CD.

Though our study is the first to focus on ASO levels in patients with CD, the sample size is relatively small. Another limitation is that this is a single center study.

In conclusion new-onset CD, but not active CD or CD in remission, is the only clinical diagnosis correlated with positive titers of ASO with no recent overt streptococcal infection. Large prospective randomized controlled studies are needed to confirm the validity of this data and to further clarify the clinical significance of our findings.

Abbreviations

CD, Crohn's disease; GAS, Group A *Streptococcus*; ASO, Antistreptolysin O; GI, Gastrointestinal; ASCA, Anti-Saccharomyces cerevisiae antibody; OmpC, Anti-outer-membrane porin C; APSGN, Acute poststreptococcal glomerulonephritis; ARF, Acute rheumatic fever; RHD, Rheumatic heart disease; CDAI, Crohn's disease activity index; GERD, Gastrointestinal reflux disease; IBD, Inflammatory bowel disease; SD, Standard deviation; IQR, Interquartile range; ANOVA, Analysis of variance; MAP, Mycobacterium avium paratuberculosis; AIEC, Adherent-invasive *Escherichia coli.*

Ethics Approval

This study was approved by the Ethics Committee of Shamir (Assaf Harofeh) medical center on 28 June 2016. All clinical investigations were conducted according to the ethical guidelines of the Declaration of Helsinki. (decision number: 0155-16-ASF).

Informed Consent

Informed consent was obtained for each patient.

Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Lennard-Jones JE. Classification of inflammatory bowel disease. *Scand J Gastroenterol*. 1989;24(sup170):2–6. doi:10.3109/00365528909091339
2. Gomollón F, Dignass A, Annese V, et al. 3rd European evidence-based consensus on the diagnosis and management of Crohn's Disease. Part 1: diagnosis and medical management. *J Crohns Colitis*. 2016;11(1):3–25. doi:10.1093/ecco-jcc/jjw168
3. Boyapati R, Satsangi J, Ho GT. Pathogenesis of Crohn's disease. *F1000 Prime Rep*. 2015;7:44. doi:10.12703/P7-44

4. Main J, McKenzie H, Yeaman GR, et al. Antibody to Saccharomyces cerevisiae (bakers' yeast) in Crohn's disease. *BMJ*. 1988;297 (6656):1105–1106. doi:10.1136/bmj.297.6656.1105
5. Reese GE, Constantinides VA, Simillis C, et al. Diagnostic precision of anti-Saccharomyces cerevisiae antibodies and perinuclear antineutrophil cytoplasmic antibodies in inflammatory bowel disease. *Am J Gastroenterol*. 2006;101(10):2410–2422. doi:10.1111/j.1572-0241.2006.00840.x
6. Joossens S, Colombel JF, Landers C, et al. Anti-outer membrane of porin C and anti-I2 antibodies in indeterminate colitis. *Gut*. 2006;55 (11):1667–1669. doi:10.1136/gut.2005.089623
7. Ferrante M, Henckaerts L, Joossens M, et al. New serological markers in inflammatory bowel disease are associated with complicated disease behaviour. *Gut*. 2007;56(10):1394–1403. doi:10.1136/gut.2006.108043
8. Lodes MJ, Cong Y, Elson CO, et al. Bacterial flagellin is a dominant antigen in Crohn disease. *J Clin Invest*. 2004;113(9):1296–1306. doi:10.1172/JCI200420295
9. Walker MJ, Barnett TC, McArthur JD, et al. Disease manifestations and pathogenic mechanisms of Group A *Streptococcus*. *Clin Microbiol Rev*. 2014;27(2):264–301.
10. Aran A, Lin L, Nevsimalova S, et al. Elevated anti-streptococcal antibodies in patients with recent narcolepsy onset. *Sleep*. 2009;32 (8):979–983. doi:10.1093/sleep/32.8.979
11. Sun L, Wu R, Xue Q, Wang F, Lu P. Risk factors of uveitis in ankylosing spondylitis: an observational study. *Medicine*. 2016;95 (28):e4233. doi:10.1097/MD.0000000000004233
12. Al-Sheyyab M, Batieha A, El-Shanti H, Daoud A. Henoch-Schonlein purpura and streptococcal infection: a prospective case-control study. *Ann Trop Paediatr*. 1999;19(3):253–255. doi:10.1080/02724939992329
13. Sato H, Ishikawa H. Unspecific increase of antistreptolysin-O titer in acute guttate psoriasis. *J Dermatol*. 1977;4(5):187–191. doi:10.1111/j.1346-8138.1977.tb01036.x
14. Telfer NR, Chalmers RJ, Whale K, Colman G. The role of streptococcal infection in the initiation of guttate psoriasis. *Arch Dermatol*. 1992;128(1):39–42. doi:10.1001/archderm.1992.01680110049004
15. Gupta R, Prakash K, Kapoor AK. Subclinical group A streptococcal throat infection in school children. *Indian Pediatr*. 1992;29:1491–1494.
16. Hammad T, Staros EB. Antistreptolysin O Titer [Online, 29 June 2019]; 2014. Emedicine.medscape.
17. Rantz LA, Randall E, Rantz HH, Antistreptolysin O. A study of this antibody in health and in hemolytic *Streptococcus* respiratory disease in man. *Am J Med*. 1948;5(1):3–23. doi:10.1016/0002-9343(48)90003-5
18. Ben-Chetrit E, Moses AE, Agmon-Levin N, Block C, Ben-Chetrit E. Serum levels of anti-streptolysin O antibodies: their role in evaluating rheumatic diseases. *Int J Rheum Dis*. 2012;15(1):78–85. doi:10.1111/j.1756-185X.2011.01668.x
19. Geerts I, De Vos N, Frans J, Mewis A. The clinical-diagnostic role of Antistreptolysin O antibodies. *Acta Clin Belg*. 2011;66(6):410–415. doi:10.2143/ACB.66.6.2062604
20. Chiodini RJ, Van Kruiningen HJ, Merkal RS, Thayer WR, Coutu JA. Characteristics of an unclassified Mycobacterium species isolated from patients with Crohn's disease. *J Clin Microbiol*. 1984;20 (5):966–971. doi:10.1128/JCM.20.5.966-971.1984
21. Darfeuille-Michaud A. Adherent-invasive *Escherichia coli*: a putative new *E. coli* pathotype associated with Crohn's disease. *Int J Med Microbiol*. 2002;292(3–4):185–193. doi:10.1078/1438-4221-00201
22. Liu Y, Van Kruiningen HJ, West AB, Cartun RW, Cortot A, Colombel JF. Immunocytochemical evidence of *Listeria, Escherichia coli*, and *Streptococcus* antigens in Crohn's disease. *Gastroenterology*. 1995;108(5):1396–1404. doi:10.1016/0016-5085(95)90687-8
23. Wakefield AJ, Ekbom A, Dhillon AP, Pittilo RM, Pounder RE. Crohn's disease: pathogenesis and persistent measles virus infection. *Gastroenterology*. 1995;108(3):911–916. doi:10.1016/0016-5085(95)90467-0
24. Mitsuyama K, Niwa M, Takedatsu H, et al. Antibody markers in the diagnosis of inflammatory bowel disease. *World J Gastroenterol*. 2016;22(3):1304–1310. doi:10.3748/wjg.v22.i3.1304
25. Israeli E, Grotto I, Gilburd B, et al. Anti-Saccharomyces cerevisiae and antineutrophil cytoplasmic antibodies as predictors of inflammatory bowel disease. *Gut*. 2005;54:1232–1236. doi:10.1136/gut.2004.060228
26. van Schaik FD, Oldenburg B, Hart AR, et al. Serological markers predict inflammatory bowel disease years before the diagnosis. *Gut*. 2013;62:683–688. doi:10.1136/gutjnl-2012-302717
27. Dubinsky MC, Kugathasan S, Mei L, et al. Increased immune reactivity predicts aggressive complicating Crohn's disease in children. *Clin Gastroenterol Hepatol*. 2008;6:1105–1111. doi:10.1016/j.cgh.2008.04.032
28. Amre DK, Lu SE, Costea F, Seidman EG. Utility of serological markers in predicting the early occurrence of complications and surgery in pediatric Crohn's disease patients. *Am J Gastroenterol*. 2006;101:645–652. doi:10.1111/j.1572-0241.2006.00468.x
29. Gupta N, Cohen SA, Bostrom AG, et al. Risk factors for initial surgery in pediatric patients with Crohn's disease. *Gastroenterology*. 2006;130:1069–1077. doi:10.1053/j.gastro.2006.02.003
30. Choung RS, Princen F, Stockfisch TP, et al. Serologic microbial associated markers can predict Crohn's disease behaviour years before disease diagnosis. *Aliment Pharmacol Ther*. 2016;43:1300–1310. doi:10.1111/apt.13641
31. Maaser C, Sturm A, Vavricka SR, et al. ECCO-ESGAR guideline for diagnostic assessment in IBD Part 1: initial diagnosis, monitoring of known IBD, detection of complications. *J Crohns Colitis*. 2019;13 (2):144–164K.