Changes in Intestinal Microbiota and Their Significance in Pediatric Henoch-Schönlein Purpura: Postprint

Authors: Zhang Li, Zhang Jianjiang, Dou Wenjie, Zeng Huiqin, Wang Qin

Date: 2022-09-13T00:00:00+00:00

Abstract

Background Currently, there are some studies on gut microbiota changes in children with Henoch-Schönlein purpura compared to healthy children both domestically and internationally, but the number of studies is limited, and no relevant reports have been published on the gut microbiota in the early disease stage of children with Henoch-Schönlein purpura nephritis.

Objective To investigate the changes in gut microbiota in children with Henoch-Schönlein purpura and explore the role of these changes in the occurrence and development of the disease.

Methods Thirty-seven newly diagnosed children with Henoch-Schönlein purpura admitted to the Department of Pediatrics, First Affiliated Hospital of Zhengzhou University from July 2019 to September 2019 were selected as the study group (Group A), and 12 healthy children were selected as the control group (Group B). The children with Henoch-Schönlein purpura were followed up for 6 months and grouped according to the presence or absence of renal injury: children without renal injury were designated as Group A1, and children with renal injury were designated as Group A2, i.e., the Henoch-Schönlein purpura nephritis group. High-throughput sequencing technology was used to sequence and analyze the gut microbiota of all enrolled subjects, and LEfSe analysis was employed to identify species with significant differences between groups.

Results PCoA analysis revealed differences in gut microbiota community structure between Group A and Group B, and Adonis analysis indicated that the difference in microbiota community structure between these two groups was statistically significant (R2=0.044, P=0.005). PCoA analysis showed differences in gut microbiota community structure among Groups A1, A2, and B, and Adonis analysis indicated that the difference in microbiota community structure between Group A1 and Group B was statistically significant (R2=0.086,

P=0.006), the difference between Group A2 and Group B was statistically significant (R2=0.061, P=0.006), and the difference between Group A1 and Group A2 was statistically significant (R2=0.043, P=0.045). LEfSe analysis showed that when comparing Group A and Group B, the abundances of Blautia, Chryseobacterium, Agathobacter, and Roseburia were significantly decreased, while those of Megamonas and Enterococcus were significantly increased in Group A (LDA>4.0). When comparing Group A1 and Group A2, the abundances of Christensenella and Bacteroides were significantly increased in Group A1, while those of Lactobacillus and Rothia were significantly increased in Group A2 (LDA>4.0).

Conclusion Children with Henoch-Schönlein purpura exhibit gut microbiota dysbiosis, characterized by decreased beneficial butyrate-producing bacteria and increased opportunistic pathogens, which may be associated with the pathogenesis of Henoch-Schönlein purpura. In the early disease stage, the gut microbiota of children with Henoch-Schönlein purpura nephritis already differed from those of children with Henoch-Schönlein purpura without renal injury, suggesting that gut microbiota dysbiosis in the early disease stage may be associated with the development of Henoch-Schönlein purpura nephritis.

Full Text

Changes and Significance of Intestinal Flora in Children with Henoch-Schönlein Purpura

DOI: 10.12114/j.issn.1007-9572.2022.0656
Authors: Zhang Li, Zhang Jian-Jiang, Dou Wen-Jie, Zeng Hui-Qin, Wang Qin

Funding: 2020 Henan Provincial Medical Science and Technology Research Project (SBGJ202003050); Henan Provincial Higher Education Key Scientific Research Project (21A320070)

Affiliation: Department of Pediatrics, The First Affiliated Hospital of Zhengzhou University, Henan Provincial Clinical Research Center for Pediatric Nephrology, Zhengzhou 450052, Henan, China

Corresponding Author: Zhang Jian-Jiang, Chief Physician; E-mail: zhangjianjiang10@163.com

Clinical Trial Registration Number: 2019-KY-268

Abstract

Background: While several studies have investigated differences in intestinal flora between children with Henoch-Schönlein purpura (HSP) and healthy controls, the body of research remains limited, and no reports have specifically

examined the intestinal microbiota of children with Henoch-Schönlein purpura nephritis (HSPN) in the early disease stage.

Objective: This study investigated alterations in the intestinal flora of children with HSP and explored the role of these changes in disease pathogenesis and progression.

Methods: Thirty-seven newly diagnosed HSP patients admitted to the Department of Pediatrics at The First Affiliated Hospital of Zhengzhou University between July and September 2019 were enrolled as the study group (Group A). Twelve healthy children served as controls (Group B). All HSP patients were followed for six months and stratified based on renal involvement: those without renal injury comprised Group A1 (n=13), while those with renal injury (HSPN) comprised Group A2 (n=24). High-throughput sequencing was performed to characterize and analyze the intestinal microbiota, and LEfSe analysis was used to identify significantly different species between groups.

Results: (1) PCoA analysis revealed distinct intestinal microbial community structures between Group A and Group B, with Adonis analysis confirming statistically significant differences (R^2=0.044, P=0.005). Among Groups A1, A2, and B, PCoA showed distinct community structures for all pairwise comparisons. Adonis analysis demonstrated significant differences between A1 and B (R^2=0.086, P=0.006), A2 and B (R^2=0.061, P=0.006), and A1 and A2 (R^2=0.043, P=0.045). (2) LEfSe analysis comparing Groups A and B showed significant reductions in *Blautia*, *Chryseobacterium*, *Agathobacter*, and *Roseburia*, along with significant increases in *Megamonas* and *Enterococcus* in Group A (LDA>4.0). Comparison of Groups A1 and A2 revealed significant increases in *Christensenella* and *Bacteroides* in A1, while *Lactobacillus* and *Rothia* were significantly elevated in A2 (LDA>4.0).

Conclusions: (1) Children with HSP exhibit intestinal dysbiosis characterized by decreased butyrate-producing beneficial bacteria and increased opportunistic pathogens, suggesting that microbial imbalance may contribute to HSP pathogenesis. (2) In the early disease stage, the intestinal microbiota of HSPN patients already differs from that of HSP patients without renal involvement, indicating that early microbial dysbiosis may be associated with HSPN development.

Keywords: Children; Henoch-Schönlein purpura; Henoch-Schönlein purpura nephritis; Intestinal flora; High-throughput sequencing technology

---

Henoch-Schönlein purpura (HSP) is the most common systemic small-vessel leukocytoclastic vasculitis in childhood, with an incidence of approximately 10-20 per 100,000 children [1]. Long-term prognosis depends primarily on renal involvement and the degree of kidney damage. Between 20% and 80% of children with HSP develop renal involvement, known as Henoch-Schönlein purpura nephritis (HSPN), and approximately 1% progress to end-stage renal disease

(ESRD), accounting for 1-2% of pediatric ESRD cases [2].

The human microbiome colonizes the skin, oral cavity, gastrointestinal tract, respiratory tract, and urogenital tract [3], with the majority residing in the digestive tract as the intestinal flora [4]. The gut microbiota comprises over ten times more cells than the human body and encodes 150 times more genetic information than the human genome. Its diversity is remarkable, typically represented by *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Verrucomicrobia* [5, 6]. As a critical component of human health, disruption of intestinal flora composition and activity can lead to various diseases [7]. Although some studies have compared intestinal flora between HSP children and healthy controls, the literature remains limited, and no reports have examined the early-stage intestinal microbiota in HSPN patients.

Therefore, this study focused on characterizing early-stage intestinal flora changes in pediatric HSP and HSPN to investigate the role of microbial alterations in disease pathogenesis and progression.

1.1 Study Subjects

We prospectively enrolled 37 newly diagnosed HSP patients admitted to our pediatric department between July and September 2019 as the study group (Group A). Patients were followed for six months and classified according to renal involvement: those without renal injury comprised Group A1 (n=13), while those with renal injury (HSPN) comprised Group A2 (n=24). Twelve healthy children served as the control group (Group B).

Inclusion criteria for the study group: (1) HSP diagnosis met the 2013 evidence-based guidelines from the Immunology Group of the Chinese Pediatric Society [8], and HSPN diagnosis met the 2016 evidence-based guidelines from the Nephrology Group of the Chinese Pediatric Society [9]; (2) No gastrointestinal disease within 30 days prior to admission (except HSP-related abdominal pain); (3) No use of antibiotics, probiotics, glucocorticoids, or immunosuppressants within 30 days prior to admission; (4) No sudden changes in living environment or dietary structure within 30 days prior to admission; (5) No malnutrition, immunodeficiency, or congenital inherited metabolic disorders.

Inclusion criteria for the control group: (1) No gastrointestinal disease within 30 days; (2) No history of allergic diseases such as asthma or allergic cough; (3) No use of antibiotics, probiotics, glucocorticoids, or immunosuppressants within 30 days; (4) No sudden changes in living environment or dietary structure within 30 days; (5) No organic disease.

This study was approved by the Ethics Committee of The First Affiliated Hospital of Zhengzhou University, and informed consent was obtained from all participants' guardians (2019-KY-268).

1.2.2 Experimental Methods and Procedures

Sample collection: At least 1 g of clean midstream feces was collected in a routine stool container and immediately stored at -80°C.

DNA extraction and sequencing: Genomic DNA was extracted using the CTAB method. PCR products were purified with the GeneJET Gel Extraction Kit (Thermo Scientific) and libraries were constructed using the Ion Plus Fragment Library Kit 48 rxns (Thermo Fisher). Sequencing was performed on the Ion S5™ XL platform.

1.3 Statistical Methods

Data were analyzed using SPSS 24.0. Normally distributed quantitative data are presented as mean ± standard deviation, while non-normally distributed data are expressed as median (P25, P75). Categorical data are presented as frequencies and percentages. Comparisons between two groups were performed using independent samples t-test for normally distributed data and Mann-Whitney U test for non-normally distributed data. For comparisons among multiple groups, one-way ANOVA was used for normally distributed data with homogeneity of variance, and Kruskal-Wallis test for non-normally distributed data, with post-hoc LSD-t test for pairwise comparisons. Categorical variables were compared using continuity-corrected chi-square test or Fisher' s exact test. Statistical significance was set at $P<0.05$.

1.4 Intestinal Flora Analysis Methods

Alpha diversity analysis assesses within-sample microbial community diversity, reflecting richness and diversity through indices such as Chao1 and ACE (for richness) and Shannon (for diversity). Principal Coordinates Analysis (PCoA) extracts principal components from multidimensional data through eigenvalue and eigenvector sorting; samples with closer distances indicate more similar species composition and community structure, tending to cluster together. PCoA was used to explore differences in community structure between groups, with significance testing performed to validate observed differences. Linear discriminant analysis Effect Size (LEfSe) identifies statistically significant biomarkers between groups, serving as a tool for discovering high-dimensional biological signatures and revealing genomic features.

2.1 General Characteristics

Group A comprised 37 cases and Group B comprised 12 cases, all of Han ethnicity. No statistically significant differences were observed between groups in sex, age, or BMI ($P>0.05$) . Among Groups A1 (n=13), A2 (n=24), and B, no significant differences were found in sex, age, or BMI ($P>0.05$) .

2.2.1 Sequencing and Quality Control

Raw sequencing data contain a proportion of interference sequences. To ensure accurate and reliable results, raw data were assembled and filtered to obtain valid sequences. Raw Reads refer to sequences after filtering low-quality bases, while Clean Reads represent sequences after chimera removal for final analysis. Groups A and B yielded 4,112,856 Raw Reads, which optimized to 3,877,216 Clean Reads.

2.2.2 Dilution Curves

Dilution curves for Groups A, B, A1, and A2 all plateaued, indicating adequate sequencing depth for subsequent analysis [Figure 1: see original paper] [Figure 2: see original paper].

2.2.3 Alpha Diversity Indices

No statistically significant differences were observed between Groups A and B in Shannon, Chao1, or ACE indices ($P>0.05$) , suggesting comparable microbial richness and diversity. Similarly, no significant differences were found among Groups A1, A2, and B in these indices .

2.2.4 PCoA Analysis and Inter-group Community Structure Differences

PCoA revealed distinct intestinal microbial community structures between Group A and Group B [Figure 3: see original paper], with Adonis analysis confirming significant differences ($R^2=0.044$, $P=0.005$). Among Groups A1, A2, and B, PCoA showed distinct structures for all groups [Figure 4: see original paper]. Adonis analysis demonstrated significant differences between A1 and B ($R^2=0.086$, $P=0.006$), A2 and B ($R^2=0.061$, $P=0.006$), and A1 and A2 ($R^2=0.043$, $P=0.045$).

2.2.5 Characteristic Microorganism Analysis (LEfSe)

Comparing Groups A and B at the genus level, *Blautia*, *Chryseobacterium*, *Agathobacter*, and *Roseburia* were significantly reduced in Group A, while *Megamonas* and *Enterococcus* were significantly increased (LDA>4.0). At the species level, *Bacteroides_{coprocola}* and *Candidatus_{Chryseobacterium}_{massiliae}* were decreased, whereas *Enterococcus_{faecalis}* was increased in Group A [Figure 5: see original paper].

Comparing Groups A1 and A2, *Christensenella* and *Bacteroides* were significantly increased in A1 at the genus level, with *Christensenella_{minuta}*, *Enterococcus_{faecalis}*, and *Bacteroides_{vulgatus}* increased at the species level. In A2, *Lactobacillus* and *Rothia* were significantly increased at the genus level, with *Lactobacillus_{salivarius}* increased at the species level (LDA>4.0) [Figure 6: see original paper].

The human gastrointestinal tract harbors trillions of microorganisms comprising 9.9 million microbial genes. This host-microbe mutualism plays crucial roles in immune maturation and regulation, pathogen suppression, vitamin and neurotransmitter synthesis, and xenobiotic detoxification. Given these diverse functions, the gut microbiome has become a research focus in inflammatory and autoimmune diseases [10]. HSP is an immune-mediated disorder, and intestinal flora significantly influences immune regulation.

Traditional culture methods fail to detect 80% of gut microorganisms. High-throughput sequencing, or next-generation sequencing, overcomes this limitation and better reflects digestive tract microbial diversity. Targeted sequencing of 16S rRNA hypervariable regions is the most widely used method for identifying bacterial taxa and their relative abundances [11].

Our LEfSe analysis revealed that HSP children had significantly reduced *Roseburia*, *Blautia*, *Chryseobacterium*, and *Agathobacter* compared with healthy controls, along with increased *Megamonas* and *Enterococcus*. *Roseburia* is among the most prevalent and active bacteria in healthy human gut microbiota [19]. As a member of *Firmicutes*, *Roseburia* produces short-chain fatty acids (SCFAs) including acetate, propionate, and butyrate by fermenting indigestible carbohydrates. SCFAs regulate intestinal physiology and immune homeostasis through anti-inflammatory properties that suppress pro-inflammatory cytokine responses [12].

Blautia is a Gram-positive bacterium that produces metabolites such as acetate and butyrate through polysaccharide degradation [13]. *Agathobacter* is a Gram-positive anaerobe that primarily produces butyrate and acetate in culture [14]. Butyrate serves as the main energy source for colonic epithelial cells and suppresses pro-inflammatory cytokine mRNA expression in mucosa by inhibiting nuclear factor-kB (NF-kB) activation. Butyrate-induced activation of G-protein coupled receptors (GPCRs) and downstream phosphorylated P38 mitogen-activated protein kinase (p-P38 MAPK) signaling modulates inflammatory pathways. Butyrate also reduces systemic inflammation by maintaining intestinal epithelial barrier integrity. While butyrate does not increase tight junction protein expression, it enhances epithelial electrical resistance and localizes tight junction proteins to cellular perimeters, thereby protecting and repairing the intestinal barrier. Additionally, butyrate plays important roles in immune defense [12, 15]. Butyrate induces development of colonic Treg cells; its reduction impairs Treg formation and causes immune imbalance [16]. Treg cells exert anti-inflammatory effects by releasing interleukin-10 (IL-10) and transforming growth factor-$ 1($TGF-$ $1) [17].

Chryseobacterium comprises Gram-negative rods that are opportunistic pathogens. Common isolates include *Chryseobacterium indologenes* and *Chryseobacterium meningosepticum*, which can cause meningitis, bacteremia, endocarditis, and other diseases [18]. Our finding of increased *Megamonas* and *Enterococcus* in HSP children aligns with studies by Wang X et al. [19] and Chen Peng-de et al. [20]. *Megamonas* belongs to *Firmicutes*, though its

specific role in the gut remains unclear [21]. Zhao et al. [22] reported increased *Megamonas* in children with autism spectrum disorder compared with healthy controls. *Enterococcus* comprises Gram-positive bacteria ubiquitous in soil, surface water, and seawater. As pathogenic gut commensals, they are important opportunistic pathogens causing various infections including urinary tract, soft tissue, and device-related infections [23]. In summary, HSP children exhibit intestinal dysbiosis characterized by reduced butyrate-producing beneficial bacteria and increased opportunistic pathogens, which may be closely related to HSP pathogenesis.

LEfSe analysis also revealed that in the early disease stage, HSP children without renal injury had significantly higher *Christensenella* and *Bacteroides* compared with HSPN patients, while HSPN children showed increased *Lactobacillus* and *Rothia*. *Christensenella* may influence gut microbial composition by promoting colonization of certain hydrogenotrophic bacteria and providing acetate to other butyrate-producing bacteria [24]. *Bacteroides* is the predominant anaerobe in the gut. Beneficially, it forms symbiotic relationships with the host, senses available nutrients to adjust metabolism accordingly, and controls other pathogens through host immune system interactions. However, *Bacteroides* is also a clinically important opportunistic pathogen involved in most anaerobic infections, including bacteremia and abscess formation [25]. *Lactobacillus* can stimulate immune cells, suppress pro-inflammatory cytokine secretion, and induce production of anti-inflammatory cytokines [26]. *Rothia* comprises Gram-positive cocci that are opportunistic pathogens causing various serious infections, primarily in immunocompromised hosts [27]. In conclusion, HSPN patients already show distinct intestinal microbiota in the early disease stage compared with HSP patients without renal injury, suggesting that early microbial dysbiosis may be closely associated with HSPN development. No relevant reports exist domestically or internationally, and our relatively small sample size necessitates further validation and mechanistic investigation.

In summary, children with HSP exhibit intestinal dysbiosis characterized by reduced butyrate-producing beneficial bacteria and increased opportunistic pathogens. HSPN patients show distinct early-stage intestinal microbiota compared with HSP patients without renal injury, suggesting that intestinal dysbiosis may be intimately involved in HSP pathogenesis and progression.

Author Contributions: Zhang Li collected and organized specimens and data and drafted the manuscript; Zhang Jian-Jiang conceived the study, supervised quality control, and takes overall responsibility; Dou Wen-Jie contributed to study conception and manuscript revision; Zeng Hui-Qin participated in manuscript revision; Wang Qin participated in manuscript revision.

Conflict of Interest: The authors declare no conflict of interest.

References: [1] Di Pietro G M, Castellazzi M L, Mastrangelo A, et al. Henoch-Schonlein Purpura in children: not only kidney but also lung[J]. Pediatr Rheumatol Online J, 2019,17(1):75. [2] Hetland L E, Susrud K S, Lindahl

K H, et al. Henoch-Schonlein Purpura: A Literature Review[J]. Acta Derm Venereol, 2017,97(10):1160-1166. [3] Ruan W, Engevik M A, Spinler J K, et al. Healthy Human Gastrointestinal Microbiome: Composition and Function After a Decade of Exploration[J]. Dig Dis Sci, 2020,65(3):695-705. [4] Xing P Y, Pettersson S, Kundu P. Microbial Metabolites and Intestinal Stem Cells Tune Intestinal Homeostasis[J]. Proteomics, 2020:e1800419. [5] Collins S L, Patterson A D. The gut microbiome: an orchestrator of xenobiotic metabolism[J]. Acta Pharm Sin B, 2020,10(1):19-32. [6] Miqdady M, Al M J, Azaz A, et al. Prebiotics in the Infant Microbiome: The Past, Present, and Future[J]. Pediatr Gastroenterol Hepatol Nutr, 2020,23(1):1-14. [7] Oliphant K, Cochrane K, Schroeter K, et al. Effects of Antibiotic Pretreatment of an Ulcerative Colitis-Derived Fecal Microbial Community on the Integration of Therapeutic Bacteria In Vitro[J]. mSystems, 2020,5(1). [8] Immunology Group, Pediatrics Branch of Chinese Medical Association, Editorial Board Chinese Journal of pediatrics. Recommendations on evidence-based diagnosis and treatment of Henoch-Schönlein purpura in children[J]. Chinese Journal of Pediatrics, 2013,51(7):502-507. [9] Nephrology Group, Pediatrics Society of Chinese Medical Association. Evidence-based guidelines for diagnosis and treatment of Henoch-Schönlein purpura nephritis(2016)[J]. Chinese Journal of Pediatrics, 2017,55(9):647-651. [10] Lynch S V, Pedersen O. The Human Intestinal Microbiome in Health and Disease[J]. N Engl J Med, 2016,375(24):2369-2379. [11] Macke L, Schulz C, Koletzko L, et al. Systematic review: the effects of proton pump inhibitors on the microbiome of the digestive tract-evidence from next-generation sequencing studies[J]. Aliment Pharmacol Ther, 2020,51(5):505-526. [12] Tamanai-Shacoori Z, Smida I, Bousarghin L, et al. Roseburia spp.: a marker of health?[J]. Future Microbiol, 2017,12:157-170. [13] Li X, Yin J, Zhu Y, et al. Effects of Whole Milk Supplementation on Gut Microbiota and Cardiometabolic Biomarkers in Subjects with and without Lactose Malabsorption[J]. Nutrients, 2018,10(10). [14] Rosero J A, Killer J, Sechovcova H, et al. Reclassification of Eubacterium rectale (Hauduroy et al. 1937) Prevot 1938 in a new genus Agathobacter gen. nov. as Agathobacter rectalis comb. nov., and description of Agathobacter ruminis sp. nov., isolated from the rumen contents of sheep and cows[J]. Int J Syst Evol Microbiol, 2016,66(2):768-773. [15] McNabney S M, Henagan T M. Short Chain Fatty Acids in the Colon and Peripheral Tissues: A Focus on Butyrate, Colon Cancer, Obesity and Insulin Resistance[J]. Nutrients, 2017,9(12). [16] Zhao J, Nian L, Kwok L Y, et al. Reduction in fecal microbiota diversity and short-chain fatty acid producers in Methicillin-resistant Staphylococcus aureus infected individuals as revealed by PacBio single molecule, real-time sequencing technology[J]. Eur J Clin Microbiol Infect Dis, 2017,36(8):1463-1472. [17] Li B, Ren Q, Ling J, et al. The change of Th17/Treg cells and IL-10/IL-17 in Chinese children with Henoch-Schonlein purpura: A PRISMA-compliant meta-analysis[J]. Medicine (Baltimore), 2019,98(3):e13991. [18] Izaguirre-Anariba D E, Sivapalan V. Chryseobacterium indologenes, an Emerging Bacteria: A Case Report and Review of Literature[J]. Cureus, 2020,12(1):e6720. [19] Wang X, Zhang L, Wang Y, et al. Gut microbiota dysbiosis is associated with

Henoch-Schonlein Purpura in children[J]. Int Immunopharmacol, 2018,58:1-8. [20] Chen PD, Lin Y, Yang J, et al. Study on the structure and diversity of intestinal flora in children with allergic purpura[J]. Chinese Maternal and Child Health Study, 2017,28(7):747-752, 757. [21] Morotomi M, Nagai F, Sakon H. Genus Megamonas should be placed in the lineage of Firmicutes; Clostridia; Clostridiales; 'Acidaminococcaceae' ; Megamonas[J]. Int J Syst Evol Microbiol, 2007,57(Pt 7):1673-1674. [22] Zhao RH, Zheng PY, Liu SM, et al. Correlation between intestinal microflora and behavioral symptoms in children with autism spectrum disorder[J]. Chinese Journal of Current Pediatrics, 2019,21(7):663-669. [23] Garcia-Solache M, Rice L B. The Enterococcus: a Model of Adaptability to Its Environment[J]. Clin Microbiol Rev, 2019,32(2). [24] Ruaud A, Esquivel-Elizondo S, de la Cuesta-Zuluaga J, et al. Syntrophy via Interspecies H2 Transfer between Christensenella and Methanobrevibacter Underlies Their Global Cooccurrence in the Human Gut[J]. mBio, 2020,11(1). [25] Wexler H M. Bacteroides: the good, the bad, and the nitty-gritty[J]. Clin Microbiol Rev, 2007,20(4):593-621. [26] Sun J, Chen H, Kan J, et al. Anti-inflammatory properties and gut microbiota modulation of an alkali-soluble polysaccharide from purple sweet potato in DSS-induced colitis mice[J]. Int J Biol Macromol, 2020. [27] Bejjanki H, Koratala A. Rothia mucilaginosa bacteremia in end-stage renal disease and solid organ transplant: the need for raised awareness[J]. Infez Med, 2019,27(1):82-84.

Note: Figure translations are in progress. See original paper for figures.