**Supplementary Information**

**Materials and methods:** This prospective non-randomized control trial was conducted between 2018 – 2019 and was approved by the Institutional ethical committee, AIIMS Bhubaneswar. **Patient population:** 19 patients were included in this pilot study, after the exclusion of candidates who did not conform to inclusion and exclusion criteria. The patients who were included in the study as per the inclusion and exclusion criteria. Details on patient recruitment, sample collection, and Follow-up were performed as depicted in Figure-1, CONSORT. Inclusion criteria: (1) more than 18 years, (2) having features of AtR in terms of symptoms and clinical examination, and (3) patients having the disease bilateral. The exclusion criteria for this study were (1) those having a history of nasal surgery, (2) having a history of previous nasal trauma or irradiation, (3) those who had the characteristic systemic or nasal granulomatous disease,(4) those who had any history of allergy to honey, (5) known diabetic patients and (6) pregnant and lactating female patients and (7) those lost to follow-up, (8) those unwilling to be part of the study, (8) any nasal douches that he/she might be taking for this condition. **Patient assessment:** Patients were recruited following the inclusion criteria and exclusion criteria. A complete medical history was taken along with an examination. Special emphasis was given as per the duration of main symptoms, other associated symptoms, any drug history, diabetes, douches that he/she must be using, multivitamins that he/she must be taking, and history of head and neck operation, trauma, any radiation history. Thereafter, patients were investigated: Blood Complete Blood count, ESR, HbA1c, Chest X-ray, Mantoux test, VDRL, Nasal endoscopy, and nasal mucosal biopsy, CT scan nose, and PNS. They were then confirmed as primary AtR. Further patients who did not give a prior history of diabetes but were found to have HbA1c more than 6.5 were excluded from the study. The rest of the patients fulfilling the criteria for enrolment patients were enrolled for the study with their consent. They were told to not use any other nasal treatment for this condition other than what was being applied topically in the study. They were advised to stop any form of multivitamins if they were taking one week before start for the duration of the study. The evaluation parameters were taken just before the intervention, and at the end of 8 weeks. Then these parameters were compared statistically.

**Procedure:** Nasal endoscopy was done crusts were removed and a nasal mucosal biopsy was taken from both sides on day 1 of the study. The right side of the nasal cavity of each patient was considered as case and the left side was considered as control. After taking the biopsy the right nasal cavity(case) was sprayed with freshly prepared manuka honey solution and the left nasal cavity(control) was sprayed with normal saline. The spraying of honey preparation was done twice a week for 8 weeks. During this period, they were told not to apply anything else in their nose or take any medications without information from the Principle Investigator. After 8 weeks of therapy, the nasal mucosa was again biopsied, from the right and left sides of the patients. At the beginning of the study, a score was assigned depending on some clinical featuresthat could be noted by doing nasal endoscopy. The score was assigned as per the following clinical features: **Crusting:** Gross – 2; Minimal – 1; Nil – 0; **Discharge:** Thick – 2; Thin – 1; Absent – 0; **Nasal mucosa:** Congested – 2; Not congested – 1; **Atrophic turbinate:** Present – 1; Not Present – 0; **Size of Nasal cavity:** Roomy – 2; Not roomy – 1

At the end of the study duration following the intervention, a repeat endoscopic sore was assigned to the nasal cavities (both test and control sides).

***Honey preparation:*** A concentration of 10 gm honey was dissolved in 100ml normal saline as the test solution (1:10 concentration). It was prepared freshly each day and sprayed with an applicator in the right nasal cavity after removal of crust. The left nasal cavity was sprayed with normal saline with a separate applicator.

Honey was subjected to **(FTIR-ATR) spectroscopy.**

***Histological preparation:*** Hematoxylin-Eosin stain slides prepared from the paraffin-embedded tissue were assessed for evaluating the histological parameters. The slides were semi-quantitatively assessed for different histological parameters. including granulation tissue, the thickness of the basement membrane, fibrosis, mucus gland, and bacterial colonies. Histologically all the parameters were graded as absent (0), mild (1), moderate (2), and marked (3). The assessment was done at a single point of time and during the assessment, the pathologist was blinded to all clinical data. The grades for each parameter were then compared for initial and post-treatment biopsies. Improvement in the grade by at least 2 was regarded as an improvement. The cases which had a score of 0 for any given parameter in both initial as well as post-treatment biopsies were excluded from the analysis.

***I***mmunohistochemistry***:*** 3-4 μm thick sections were cut from the paraffin block and mounted on poly-L-lysine coated slides followed by fixation in a hot plate. The slides are then deparaffinized by xylene and rehydrated with a graded concentration of alcohol. Antigen retrieval was done in a 600-watt microwave oven for 30 min using Tris EDTA buffer (pH 9.0). Peroxidase blocking was performed with 3% H2O2 in methanol for 30 minutes. The sections were then incubated with anti-GPR 43 antibody (Invitrogen, Dilution 1:200) in a humidity chamber for 2 hours. Sections were then washed in Tris-buffer and treated with the biotin-labeled secondary antibody for 60 minutes. The sections were washed and then incubated with peroxidase-conjugated streptavidin for 30 minutes at room temperature. Then the slides were rinsed with three changes of Tris-HCl buffer and followed by diaminobenzidine staining under microscopic control. The slides were then washed with distilled water, counterstained in hematoxylin for 1 min, and mounted. Skeletal muscle tissue was used as a positive control.

**DNA extraction:** Tissues were processed paraffin-embedded tissues using routine pathological laboratory procedure. 10 µm of sections were cut from each paraffin-embedded tissue and were placed into 2ml microcentrifuge tubes and kept at room temperature until analysis. The First 2 to 3 sections were discarded as samples have been exposed to air. DNA was extracted using QIAGEN’s QIAamp DNA FFPE Tissue kit following the manufacturer’s guidelines for DNA Purification from Tissue. In brief, 10 µm of 8-10 sections from each sample were used for the DNA extraction. Added 1ml of xylene into the sample vial and mixed vigorously for 30 seconds and centrifuged at full speed for 2min. The supernatant was discarded and added 1ml of ethanol to the pellet and mixed thoroughly using a vortex. A pipette was used to remove the supernatant from the vial and incubated at 37°C until all residual ethanol evaporated completely. Pellet was resuspended using an appropriate buffer and added proteinase K and incubated at 56°C for 3hrs and we then proceed according to the manufacture instructions. The elution volume for DNA was 100µl using with nuclease-free water and stored in a -20°C freezer for long term storage until amplification and sequencing analysis. DNA was quantitated using Nanodrop and 1% agarose gel electrophoresis for the quality check.**Sequencing Methodology:** 25 ng of DNA was used to amplify 16S rRNA hypervariable region V3-V4. The reaction includes the KAPA HiFi HotStart Ready Mix and 100 nm final concentration of modified 341F and 785R primers. The PCR involved an initial denaturation of 95°C for 5 min followed by 25 cycles of 95°C for 30s, 55°C for 45s and 72°C for 30s, and a final extension at 72°C for 7 min. The amplicons were purified using Ampure beads to remove unused primers. Additional 8 cycles of PCR were performed using Illumina barcoded adapters to prepare the sequencing libraries. The libraries were quantitated using Qubit DNA HS quantitation assay (Thermo Scientific) which specifically quantitates dsDNA assay.**V3-V4 primer sequences.** V3V4F-5'CCTACGGGNGGCWGCAG 3', V3V4R- 5'GACTACHVGGGTATCTAATCC3' and an **Adapter Sequence** P7 adapter read1 AGATCGGAAGAGCACACGTCTGAACTCCAGTCAP5 adapter read2 AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT**. Sequence Data QC:** The sequence data were generated using Illumina MiSeq. Data quality was checked using FastQC and MultiQC software. The data was checked for base call quality distribution, % bases above Q20, Q30, %GC, and sequencing adapter contamination. All the samples have passed the QC threshold (Q20>95%). **Data Analysis:** The reads were trimmed (20bp) from the 5’ end to remove the degenerate primers. The trimmed reads were processed to remove adapter sequences and low-quality bases using Trimgalore. The QC passed reads were imported into Mothur, and the pairs were aligned with each other to form contigs. The contigs were screened for errors, and only those between 300bp and 532bp were retained. Any contig with ambiguous base calls was rejected. The high-quality contigs were checked for identical sequences, and duplicates were merged. Although the primers for the experiment were designed for 16s bacterial rRNA, there are good chances for nonspecific amplification of other regions. To correct for this, we align the contigs to a known database for 16s rRNA. Depending on the variable region being amplified, most of the contigs will align to their respective region on the database. Any ambiguous contigs aligning to other regions on the database were discarded. After this process, the gaps and the overhang at the ends from the contigs were removed and processed for chimera removal which may have formed due to PCR errors. UCHIME algorithm was used to flag contigs with chimeric regions. A known reference of all the chimeric sequences was used to identify and remove possible chimeric sequences. The filtered contigs were processed and classified into taxonomical outlines based on the GREENGENES v.13.8-99 database. The contigs were then clustered into OTUs (Operational Taxonomic Unit). After the classification, OTU abundance was estimated. PICRUSt was used to predict gene family abundance. PICRUSt program was designed to estimate the gene families contributing to a metagenome by bacteria or archaea identified using 16S rRNA sequencing. The metagenomes were predicted using the predict\_metagenomes.py script. The predicted pathways were collapsed into higher categories. OTU contributions for the particular functions were estimated by metagenome\_contributions.py script. **Statistical analysis:** Continuous variables have been presented as Mean ±S.D. and categorical variables as a percentage. Comparison of means of continuous variables (parametric) between two groups has been done using a two-sided unpaired *t*-test. Fisher’s exact test has been used for categorical variables. The histopathological feature pre and post-operative period is expressed in median and Interquartile range.Statistical analyses were performed using statistical software SPSS 22.0 (IBM, NY USA) considering a significance level of *P* < 0 ·05.

The alpha diversity is found to be increased post-treatment on the test (right) side compared to the control side.





The beta diversity of the samples collected before and after treatment in the test and control side.





Network analysis: In community-analyzer these abundance values of the taxonomic group are then normalized with respect to the before and after treated right and left nostrils of individuals samples. A hierarchical clustering methodology is used to rank  the taxonomic groups according to their pairwise correlations. Based on the relative abundances  of various microbial groups in each metagenomic sample, a hooke's law based algorithm is applied in order to place sample on the same graphical layout. though Statistically not significant.



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From FTIR-ATR Spectroscopy, presence of complex carbohydrates in manuka honey were identified, and the predominant carbohydrates were: Zymosan A from Saccharomyces, Laminarin from L digitata, Cellotriose, Cellulose, Milk sugars like Lacto-N-Fucopentaose I & Lacto-N-DiFucohexaose II and Cyclohexylmethyl-Beta-D- Maltoside.

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