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Hookworm infection induces glycometabolic modulation in South Indian individuals with type 2 diabetes

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ABSTRACT

Objectives: A number of epidemiological studies have demonstrated that there is an inverse relationship between helminth infections and diabetes mellitus, suggesting that helminth infection may have a positive effect on type 2 diabetes mellitus (T2DM). However, the association between hookworm infection and T2DM has barely been studied. Hence, we aimed to investigate and analyze the interaction and association between hookworm infection and T2DM.

Methods: We examined the effect of hookworm infection on biochemical parameters, including plasma random blood glucose, glycated hemoglobin, and the plasma levels of pancreatic hormones, incretins, and adipokines in individuals with T2DM with (INF, n = 35) or without (UN, n = 35) hookworm infection. Moreover, we re-evaluated these analyte concentrations in a subset of INF individuals 6 months following anthelmintic therapy.

Results: Compared to UN individuals, INF individuals had significantly lowered levels of random blood glucose and glycated hemoglobin. INF individuals also exhibited significantly diminished levels of adiponectin, adipsin, C-peptide, insulin, and glucagon compared to UN individuals. In contrast, INF individuals displayed substantially elevated levels of visfatin and incretins compared to UN individuals. Interestingly, this effect was not seen following anthelmintic treatment.

Conclusion: Our study findings indicate that concomitant hookworm infection exerts a beneficial effect on glycometabolic parameters in T2DM.

Introduction

Hookworm infection, caused by *Necator americanus* and *Ancylostoma duodenale,* affects a large majority of the world's population and is highly prevalent in tropical and subtropical regions with lower socio-economic status [1]. Conversely, it is worthy of note that the prevalence of type 2 diabetes mellitus (T2DM) has risen dramatically in developing countries and in populations undergoing westernization, where helminths have been eliminated [2].

T2DM is a devastating metabolic disorder characterized by persistent hyperglycemia and a chronic inflammatory condition and is one of the world's foremost causes of morbidity and premature mortality. As per the statistics provided by the International Diabetes Federation, around 537 million individuals (aged 20-79) worldwide have diabetes. Furthermore, it is anticipated that by 2030 and 2045, this number will escalate to 643 million and 783 million, respectively [3]. The onset of hyperglycemia could activate beta cell dysfunction and insulin resistance, which lead to the development of T2DM [4]. The pathological hallmark of T2DM involves an array of macrovascular (cardiovascular disorders, peripheral arterial disease, and stroke) and microvascular (neuropathy, retinopathy, and nephropathy) complications, and its chronicity is associated with multi-system organ dysfunction [5].

T2DM can be coincident with helminth infections, specifically the soil-transmitted nematodes such as *Ascaris lumbricoides, Trichuris trichiura, Strongyloides stercoralis*, and hookworm species that are gaining the most research attention. Several cross-sectional investigations conducted in India, rural China, Indonesia, and among Aboriginal Australians have shown a marked decrease in the incidence of helminth infections among T2DM subjects compared to those without diabetes [6–12]. According to these studies, helminth infestations might hinder or postpone the onset of T2DM. Consequently, current deworming programs, in addition to more established risk factors like sedentary

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lifestyles and high-calorie diets, could possibly contribute to the development of T2DM in numerous low- and middle-income nations [8]. Our previous findings have demonstrated that the parasites *Strongyloides stercoralis* and *Wuchereria bancrofti* can provide a certain level of protection against T2DM-related pathology by modulating the systemic proinflammatory cytokine and chemokine milieu [6,13–19]. The association between hookworm infection and T2DM has barely been studied, and little is known about its immunomodulatory effect.

Glucose metabolism dysfunctions are associated with a low-grade chronic inflammatory condition and type 2 diabetes exhibits altered adipokine profiles leading to profound metabolic risk, and alterations in insulin sensitivity leading to insulin resistance (IR), and is a major etiological factor in meta-inflammation [20]. Hence, in the present study, we investigated the relationship between hookworm infection and T2DM comorbidity and explored the impact of hookworm infestation on crucial factors related to adipose tissue homeostasis. To this end, we evaluated the circulating levels of pancreatic hormones (C-peptide, insulin, and glucagon), incretins (ghrelin, gastric inhibitory peptide [GIP], and glucagon-like peptide-1 [GLP-1]), and adipokines (adiponectin, adipsin, resistin, leptin, visfatin, and plasminogen activator inhibitor-1 [PAI-1]) among T2DM individuals with or without concomitant hookworm infection. We further studied the effect of anthelmintic therapy on the aforesaid parameters in hookworm-infected subjects.

Material and methods

Study population

We enrolled 70 study participants with T2DM, comprised of 35 clinically asymptomatic hookworm-infected individuals (hereafter INF group), and 35 individuals without hookworm infection (hereafter UN group), from Kanchipuram and Tiruvallur Districts, Tamil Nadu, South India. All recruited participants were in the age range of 25-63 years. None of them had a previous helminth infection or prior anthelminthic treatment. The study excluded participants with histories of HIV infection, chronic renal failure, hyperbilirubinemia, and women who were pregnant or nursing.

Hematological, biochemical, and anthropometric measurements

Complete blood counts were determined from fresh venous ethylenediaminetetraacetic acid-containing blood samples using a DxH 520 hematology analyzer (Beckman Coulter, Brea, California). Biochemical assays (plasma random blood glucose [RBG], glycated hemoglobin [HbA1c], aspartate aminotransferase [AST], alanine transaminase [ALT], urea, and creatinine) were done using standardized techniques as described previously [21]. In addition, socio-demographic data (age and sex) and anthropometric parameters (height, weight, and body mass index) were analyzed.

Determination of type 2 diabetes mellitus status

T2DM was characterized by HbA1c levels (>6.4%) and RBG readings (>200 mg/dl), as per the guidelines established by the American Diabetes Association. Biochemical assays, except RBG, were evaluated after an overnight period of fasting. All diabetic individuals were newly diagnosed, not on any anti-diabetic medication at the time of blood draw, and without any known complications or co-morbidities. All individuals were referred to the primary health care center for diabetic treatment and lifestyle modification.

Parasitological examination and anthelmintic treatment

Stool samples were collected, transported to the laboratory at ambient temperature, and subjected to direct microscopic examination, and hookworm infection was confirmed by quantitative real-time polymerase chain reaction. The stool samples positive for other intestinal worms, such as *Ascaris, Strongyloides,* and *Trichuris* were excluded. Further, filarial-positive cases were ruled out by being negative for circulating filarial antigen in the TropBio Og4C3 enzyme-linked immunosorbent assay (TropBio, Townsville, QLD, Australia). Ivermectin and albendazole were administered as a single dose to all INF subjects. Follow-up samples were taken again 6 months later, and polymerase chain reaction was repeated to ensure the efficacy of anthelminthic therapy.

Measurement of adipocytokines

The plasma levels of pancreatic hormones (C-peptide, insulin, and glucagon), incretins (ghrelin, GIP, and GLP-1), and adipokines (adiponectin, adipsin, resistin, leptin, visfatin, and PAI-1) were measured by the Bio-Plex multiplex immunoassay system according to the manufacturer's instructions (Bio-Rad, Hercules, California) in INF, UN, and post-treated samples.

Statistical analyses

The central tendency was evaluated through the use of geometric means (GMs). The comparison between INF and UN was performed using Mann-Whitney U-tests with Holm's correction for multiple comparisons. The Wilcoxon signed-rank test was utilized to compare pretreatment and post-treatment parameters, while univariate logistic regression analysis was used to examine the associated factors. Statistical significance was ascertained by a P-value of ≤ 0.05. GraphPad Prism version 9.0 (GraphPad, San Diego, California) and Stata version 15 (Stata Corp., College Station, Texas) were used for the analyses, and JMP17 software was employed to plot the principal component analysis (PCA) and heat map.

Results

Study population characteristics

The demographic characteristics and hematological parameters of the study participants at baseline are illustrated in Table 1. We observed no statistically significant differences in age, gender, or body mass index between the two groups. In terms of hematological parameters, eosinophils ($P = 0.0093$) and basophils ($P = 0.0449$) were significantly higher in INF subjects compared to UN subjects. The baseline and post-treated individual demographics and hematological parameters are shown in Table 2. Following anthelmintic treatment, the counts of white blood cells, monocytes, eosinophils, basophils, and mean corpuscular hemoglobin concentration were significantly altered when compared with their baseline counts.

Altered levels of glucose indices and biochemical parameters in hookworm-infected subjects and reversal after anthelmintic therapy

To evaluate the influence of hookworm infection on glucose control indices in T2DM, we measured the levels of RBG, HbA1C, and biochemical parameters like AST, ALT, urea, and creatinine in INF and UN subjects. As can be seen from Figure 1a, INF subjects exhibited significantly diminished levels of RBG (GM of 139.4 mg/dl in INF vs 224.7 mg/dl in UN; P <0.0001) and HbA1c (GM of 7.5% in INF vs 8.8% in UN; P <0.0001) compared to UN subjects. Conversely, INF individuals had higher ALT levels (GM of 26.5 U/L in INF vs 19.7 U/l in UN; P <0.0479) than UN individuals. The concentrations of AST, urea, and creatinine were not statistically different between the groups.

After a follow-up period of 6 months, the impact of anthelmintic treatment on RBG, HbA1c, and other biochemical parameters in INF individuals was assessed. As illustrated in Figure 1b, the post-treatment

Table 1
Demographics and hematological parameters of the study population.

Parameters	With hookworm-infected (n = 35)	Without hookworm-infected (n = 35)	*P*-value
Gender (Male/Female)	18/17	12/23	
Median age (in years) (range)	45 (25-63)	45 (32-59)	
Weight, kg (range)	67.2 (48-104)	67.5 (48-103)	0.9362
Body mass index kg/m^2 (range)	27.1 (19.5-37.3)	27.5 (21.4-37.8)	0.7371
White blood cells count, x 10^3 cells/ul	86.9 (44.2-154)	83.8 (58-132.2)	0.3152
Lymphocyte count, x10^6 cells/ul	2916.2 (1475.6-4844.5)	2645.1 (1522.6-3980.4)	0.2239
Neutrophil count, cells/ul	4589.4 (1992-9933)	4352.7 (431.5-9777.1)	0.8598
Monocyte count, cells/ul	499.7 (244.8-970.2)	413.6 (33.1-711.6)	0.1435
Eosinophil count, cells/ul	391.1 (53.5-1146.7)	220.1 (16.5-3311.6)	0.0093
Basophil count, cells/ul	71 (22.0-184.8)	55.0 (23.6-107.3)	0.0449
Red blood cells, g/dl	5.1 (3.7-7.0)	4.6 (3.3-5.8)	0.1105
Hemoglobin, g/dl	13.6 (8.2-20.1)	12.7 (8.6-16.4)	0.3980
Hematocrit, %	42.7 (34.5-61.3)	39.1 (28.1-50.5)	0.4118
Mean corpuscular volume, fL	83.5 (67.1-96.3)	84.8 (65.9-95.6)	0.2545
Mean corpuscular hemoglobin, pg	27.1 (20.6-32.2)	27.5 (20-31.4)	0.5238
Mean corpuscular hemoglobin concentration, g/dl	32.5 (29.2-34.2)	32.4 (30.3-35.2)	0.6404
Red cell distribution width, %	14.2 (10.4-21.5)	15.2 (11.6-22.5)	0.1075
Platelet, 10^3/ul	256.8 (133.6-439.3)	268.3 (161.1-445.3)	0.4680

Table 2
Hematological parameters of the study population following anthelmintic treatment.

Parameters	Pre-treatment (n = 18)	Post-treatment (n = 18)	*P*-value
Gender (Male/Female)	09/09		
Median age (in years) (range)	39 (27-63)		
Weight, kg (range)	64.2 (49-98.2)	59.4 (44-87.6)	0.1961
Body mass index Kg/m^2 (range)	26.1 (19.6-32.2)	25.5 (18.5-29.4)	0.1846
White blood cells count, x 10^3 cells/ul	91.5 (67-154)	89 (64-150)	0.0429
Lymphocyte count, x 10^6 cells/ul	3015.3 (1475.6-4165.4)	2514.5 (1648-4440)	0.9661
Neutrophil count, cells/ul	4603.2 (3417-9933)	5778.5 (3072-10500)	0.1964
Monocyte count, cells/ul	607.8 (231-1161.6)	469.9 (192-2261)	0.0448
Eosinophil count, cells/ul	474.25 (190.9-3533.2)	407.8 (239.4-560)	0.0395
Basophil count, cells/ul	76.9 (29.6-184.8)	68.9 (6.4-145.6)	0.0035
Red blood cells, g/dl	5.0 (3.3-7.0)	4.7 (3.3-5.8)	0.3665
Hemoglobin, g/dl	13.8 (9.3-20.1)	12.8 (8-16.8)	0.3054
Hematocrit, %	42.6 (29.6-61.3)	39.4 (27.5-52)	0.7869
Mean corpuscular volume, fL	83.4 (67.1-97.4)	85.9(64-96.7)	0.5308
Mean corpuscular hemoglobin, pg	27.4 (20.6-32.8)	27.7 (18.7-32.6)	0.1516
Mean corpuscular hemoglobin concentration, g/dl	32.6 (29.2-34.2)	32.4 (29.1-34)	0.0017
Red cell distribution width, %	14.4 (10.4-21.5)	14.7 (11.4-22.5)	0.6079
Platelet, 10^3/ul	260.5 (133.6-439.3)	284.7 (161.1-445.3)	0.9460

(post-T) levels of RBG (an increase of 1.9 fold; P <0.0001), HbA1c (an increase of 1.3 fold; P <0.0001), AST (an increase of 1.4 fold; P = 0.0276), and ALT (an increase of 1.5 fold; P = 0.0021) were significantly altered compared with pre-treatment (pre-T) levels. Therefore, glucose indices and other biochemical parameters were altered in INF subjects with T2DM, and these alterations were partially reversed after anthelmintic medication.

Altered circulating levels of pancreatic hormones and incretins in hookworm-infected subjects and reversal after anthelmintic therapy

To investigate the effect of hookworm infestation on the circulating levels of pancreatic hormones (C-peptide, insulin, and glucagon) and incretins (ghrelin, GIP, and GLP-1), the aforementioned analytes were measured in INF and UN subjects at baseline. As depicted in Figure 2a, the levels of C-peptide (GM of 52.3 pg/ml in INF vs 292.4 pg/ml in UN; P <0.0001), insulin (GM of 212.8 pg/ml in INF vs 624.9 pg/ml in UN; P <0.0001), and glucagon (GM of 1075 pg/ml in INF vs 1443 pg/ml in UN; P <0.0001) were significantly decreased in INF subjects in comparison to UN subjects. Contrarily, the circulating levels of incretins such as ghrelin (GM of 558.1 pg/ml in INF vs 432.5 pg/ml in UN; P = 0.0142), GIP (GM of 273.2 pg/ml in INF vs 148.1 pg/ml in UN; P <0.0001), and GLP-1 (GM of 42.0 pg/ml in INF vs 35.2 pg/ml in UN; P = 0.0027) were substantially elevated in the INF subjects compared with UN subjects.

Pancreatic hormones and incretin levels were then evaluated after a 6-month follow-up period in INF subjects to ascertain the impact of anthelminthic treatment. As displayed in Figure 2b, the post-T levels of C-peptide (an increase of 2.1 fold; P <0.0001), insulin (an increase of 2.0 fold; P <0.0001), and glucagon (an increase of 1.1 fold; P <0.0040) were significantly elevated compared to pre-T levels. On the contrary, ghrelin (decrease of 0.7 fold; P <0.0001), GIP (decrease of 0.5 fold; P = 0.0010), and GLP-1 (decrease of 0.7 fold; P <0.0001) levels declined dramatically following anthelmintic therapy. Thus, INF individuals with T2DM are associated with decreased circulating levels of pancreatic hormones and increased levels of gut hormones, and their levels were reversed following anthelmintic treatment.

Altered circulating levels of adipocytokines in hookworm-infected subjects and reversal following anthelmintic treatment

The adipokines (adiponectin, adipsin, resistin, leptin, visfatin, and PAI-1) levels were measured in INF and UN subjects at baseline to evaluate the impact of hookworm infestation on the adipocytokines. Figure 3a shows that adiponectin (GM of 2,070,948 pg/ml in INF vs 2,792,696 pg/ml in UN; P = 0.0025) and adipsin (GM of 19,730 pg/ml in INF vs 66,654 pg/ml in UN; P <0.0001) levels were significantly diminished in INF subjects in comparison with UN subjects. On the contrary, visfatin (GM of 6757 pg/mL in INF vs 2653 pg/mL in UN; p < 0.0001) level was

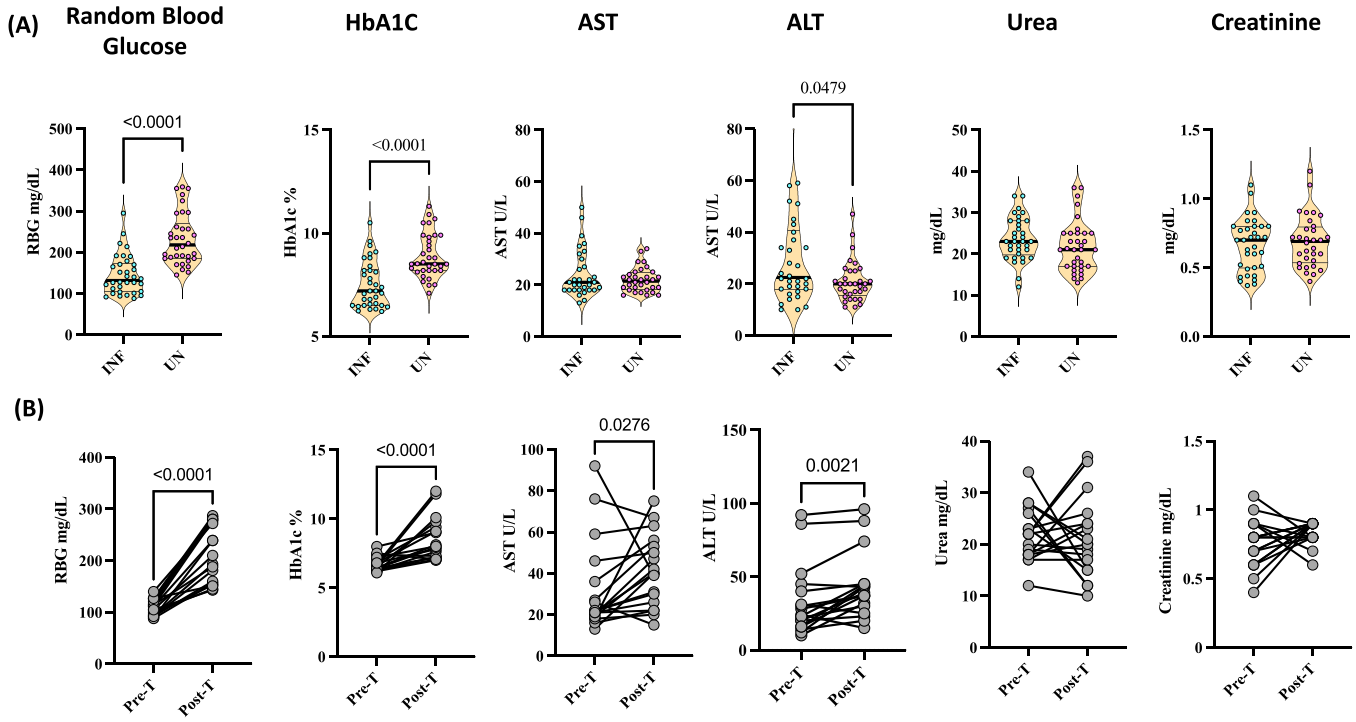

Figure 1. Altered levels of glucose indices and biochemical parameters in INF individuals and reversal following anthelmintic treatment. (a) Plasma levels of RBG levels, HbA1c, AST, ALT, urea, and creatinine in T2DM with INF (n = 35) and without UN (n = 35) individuals. Each dot is an individual subject with the bar representing the geometric mean. Mann-Whitney U-test. (b) Plasma levels of RBG levels, HbA1c, AST, ALT, urea, and creatinine in T2DM with INF Pre-T (n = 18) and 6 months post-T (n = 18) individuals. *P*-values were calculated using the Wilcoxon matched pair test.
ALT, alanine transaminase; AST, aspartate aminotransferase; HbA1c, glycated hemoglobin INF, with hookworm-infected; post-T, following treatment; Pre-T, pre-treatment; RBG, random blood glucose; T2DM, type 2 diabetes mellitus; UN, without hookworm-infected.

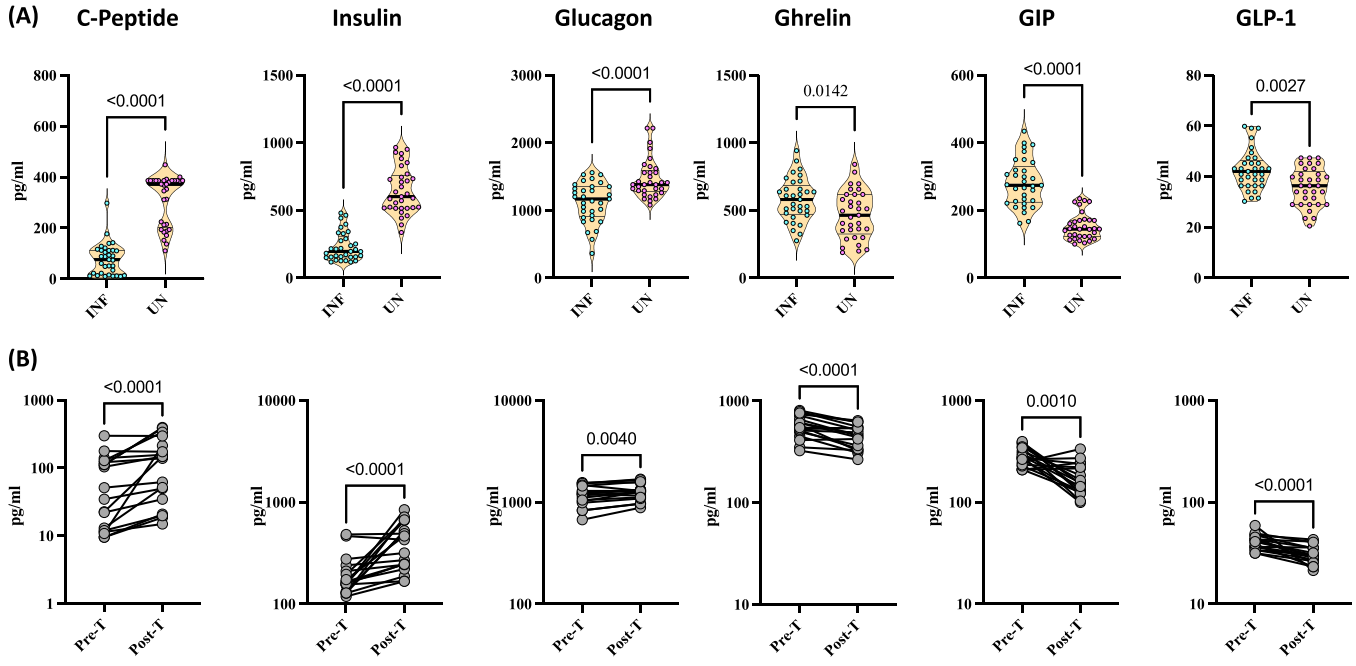

Figure 2. Altered circulating levels of pancreatic hormones and incretins in INF individuals and reversal following anthelmintic treatment. (a) Plasma levels of pancreatic hormones (C-Peptide, insulin, and glucagon) and incretins (ghrelin, GIP, and GLP-1) in T2DM with INF (n = 35) and without UN (n = 35) individuals. Each dot is an individual subject with the bar representing the geometric mean. *P*-values were calculated using the Mann-Whitney U-test. (b) Plasma levels of pancreatic hormones (C-Peptide, insulin, and glucagon) and incretins (ghrelin, GIP, and GLP-1) in T2DM with INF Pre-T (n = 18) and 6 months post-T (n = 18) individuals. *P*-values were calculated using the Wilcoxon matched pair test.
GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; INF, with hookworm-infected; post-T, following treatment; Pre-T, pre-treatment; T2DM, type 2 diabetes mellitus; UN, without hookworm-infected.

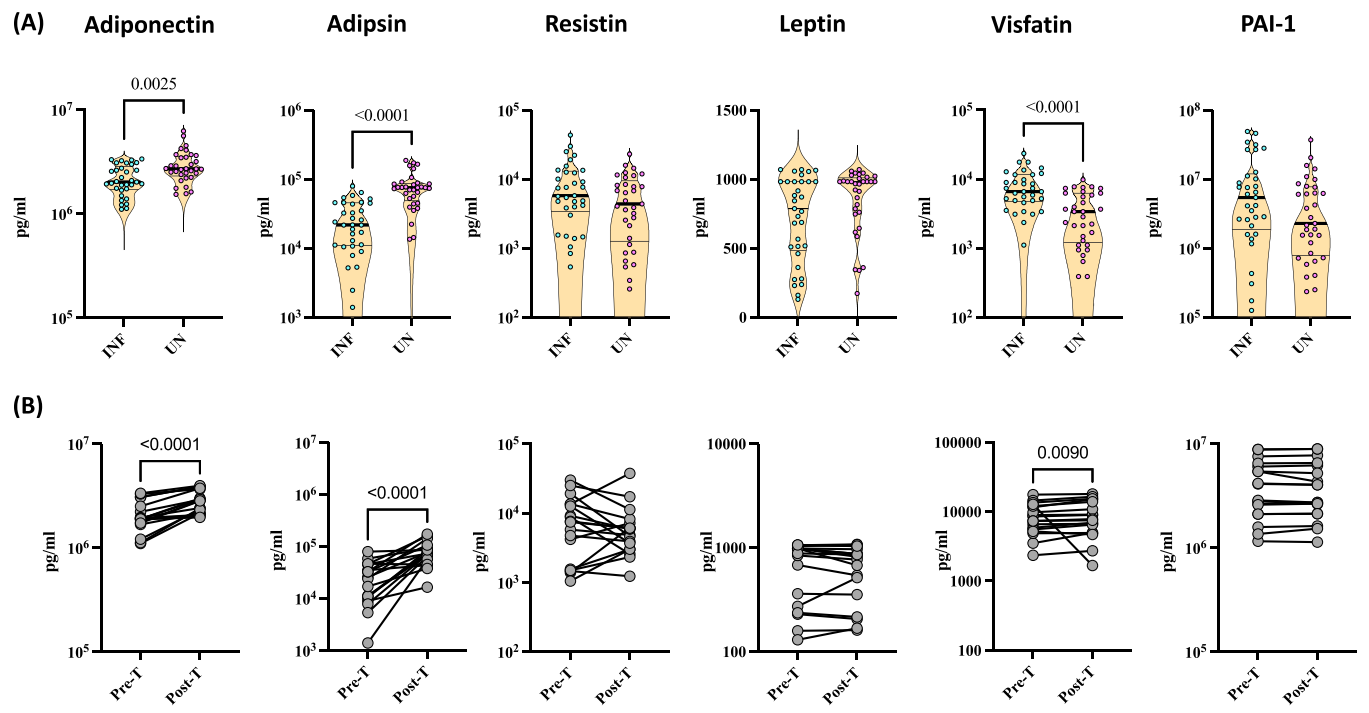

Figure 3. Altered circulating levels of adipocytokines in INF individuals and reversal following anthelmintic treatment. (a) Plasma levels of adipocytokines (adiponectin, adipsin, resistin, leptin, visfatin and PAI-1) in T2DM with INF (n = 35) and without UN (n = 35) individuals. Each dot is an individual subject with the bar representing the geometric mean. Mann-Whitney U-test. (b) Plasma levels of adipocytokines (adiponectin, adipsin, resistin, leptin, visfatin, and PAI-1) in T2DM with INF Pre-T (n = 18) and 6 months post-T (n = 18) individuals. *P*-values were calculated using the Wilcoxon matched pair test.
INF, with hookworm-infected; PAI-1, plasminogen activator inhibitor-1; post-T, following treatment; Pre-T, pre-treatment; T2DM, type 2 diabetes mellitus; UN, without hookworm-infected.

noticeably higher in INF subjects, while resistin, leptin, or PAI-I were not significantly different between the two groups.

Further, to assess the impact of anthelmintic treatment on the adipocytokines, the circulating levels of adipokines (adiponectin, adipsin, resistin, leptin, visfatin, and PAI-1) were measured in INF individuals 6 months following anthelmintic treatment. In Figure 3b, it is illustrated that, compared to the pre-T phase, there was a substantial elevation in adiponectin (increases of 1.4-fold; $P < 0.0001$), adipsin (an increase of 3.8-fold; $P < 0.0001$) and visfatin (an increase of 1.2-fold; $P = 0.0090$) levels at the post-T phase. Therefore, hookworm infection is associated with lower levels of adiponectin and adipsin and higher visfatin levels in INF subjects with T2DM, and these levels were partially reversed following anthelmintic treatment.

Univariate regression analysis

Univariate regression analysis was performed to evaluate the impact of hookworm infestation on several variables evaluated in T2DM subjects. As illustrated in Supplementary Table 1, the levels of RBG, insulin, glucagon, C-peptide, ghrelin, GIP, GLP-1, PAI-1, visfatin, adiponectin, and adipsin were significantly influenced by hookworm infestation. Therefore, our data demonstrate that hookworm infection has a profound impact on various crucial variables in subjects with T2DM, including the levels of RBG, pancreatic hormones, gut hormones, and adipocytokines.

Principal component analysis and heat map analysis reveal trends in glycometabolic parameters

We performed heat map and PCA analyses to display variations among the clusters formed on the entire dataset. After the data were log2 transformed and plotted, heat map analysis was done to show the clustering pattern of HbA1c, RBG, C-peptide, glucagon, insulin, adiponectin, adipsin ghrelin, GIP, GLP-1, and visfatin in T2DM subjects with or without hookworm infection. As depicted in Supplementary Figure 1a, the blue color (HbA1c, RBG, C-peptide, glucagon, insulin, adiponectin, and adipsin) depicts the lower levels, and the red color (GIP, GLP-1, and visfatin) depicts the higher levels. Additionally, we used data sets from two groups to do a hierarchical clustering analysis utilizing HbA1c, RBG, C-peptide, glucagon, insulin, adiponectin, adipsin, ghrelin, GIP, GLP-1, and visfatin. For glycometric parameters, a heatmap and dendrogram are shown. Further, we performed PCA analysis with HbA1c, RBG, C-peptide, glucagon, insulin, adiponectin, adipsin, ghrelin, GIP, GLP-1, and visfatin. Following the elimination of factors with similarities below 0.5, PCA-1 (HbA1c, RBG, C-peptide, glucagon, insulin, adiponectin, and adipsin) and PCA-2 (GIP, visfatin, GLP-1, and ghrelin) were assessed. Supplementary Figure 1b, shows that glucose indices, pancreatic hormones, incretins, and adipokine clusters varied between the INF and UN subjects. The first two principal components of the score plot accounted for a total variance of 46.8% and 14%, respectively.

Discussion

To the best of our knowledge, this is the first report on the association between hookworm infection and glycometabolic parameters in T2DM. Our study explores the impact of hookworm infestation on parameters related to glycemic control in T2DM subjects. Also, we examined the effect of anthelmintic treatment on glycemic control. We observed that 1. INF individuals exhibited elevated levels of eosinophils, and basophils, and following anthelmintic treatment, the counts of eosinophils, monocytes, and basophils were significantly reduced. 2. INF individuals exhibited decreased levels of pancreatic hormones, increased levels of incretins, and decreased levels of adipocytokines (adiponectin, adipsin), all of whose levels were at least partially reversed following anthelmintic treatment.

Growing epidemiological and experimental data have shown that helminth-associated immunomodulation might prevent the onset of T2DM, as it tends to regulate adverse inflammatory responses and promote glucose homeostasis [2,7,9–11,15,16]. Also, several reports have revealed that helminth infections have a positive impact on insulin resistance, dyslipidemia, and the pathology associated with T2DM [6,9,12,15,16]. Anthelmintic therapy might remove this helminth-mediated beneficial effect in T2DM and other metabolic diseases [22]. There is a paucity of information concerning the relationship between hookworm and T2DM, and the hookworm infection-T2DM interface needs to be explored.

Emerging evidence has demonstrated that eosinophils play a crucial role in the innate immune response in parasitic infections due to their pro and anti-inflammatory properties. Helminth infection was associated with elevated blood levels of eosinophil granule proteins, suggesting a possible role in tissue remodeling [23]. Recent data revealed that an increased eosinophil proportion was linked to a reduced likelihood of developing T2DM and insulin resistance [24]. Vozarova *et al.* [25] reported that leukocytosis was associated with a worsening of insulin sensitivity. In line with earlier reports, we observed that INF individuals exhibited significantly elevated numbers of eosinophils and basophils. Following anthelmintic treatment, the counts of white blood cells, monocytes, eosinophils, and basophils significantly decreased. T2DM is an inflammatory disorder in which many immune cells are involved in the pathology of chronic inflammation either directly or by secreting inflammatory cytokines [26]. Eosinophils and basophils are a class of immune cells often associated with hypersensitivity reactions and parasitic infestations that modulate the activation status of macrophages in mammalian adipose tissue and could have a significant impact on glucose homeostasis. Our data suggest that immune cells have a vital role in preventing the progression and pathogenesis of T2DM perhaps due to the effect of eosinophils in augmenting the browning of white adipose tissue, and indirectly through macrophages which enhance energy expenditure and thermogenesis and decrease adiposity, thereby preventing the onset of insulin resistance [27].

Next, we investigated the effect of hookworm infestation on glycemic control indices in T2DM subjects. RBG and HbA1c were significantly impacted by hookworm infection. Following anthelmintic treatment, both HbA1c and RBG levels worsened. Our findings imply that hookworm infestation ameliorates hyperglycemia and perhaps insulin resistance. Insulin deficits develop in T2DM when pancreatic islet cells are unable to produce enough insulin to overcome insulin resistance or to replenish lost islet cells due to islet cell death [28]. A recent meta-analysis of 14 studies and earlier studies implied that helminth-infected individuals had considerably diminished levels of fasting blood glucose and HbA1c and a reduced prevalence of metabolic syndrome and T2DM [29]. Our data corroborate these findings and extend them to hookworm infection.

T2DM is associated with a clinical spectrum of liver abnormalities, and AST is a marker of liver damage. We observed no significant changes at baseline. Following anthelmintic treatment, the levels of ALT and AST were significantly elevated in people with a diagnosis of T2DM. However, there was no apparent relationship between infection burden and biochemical variables in our investigation.

The pancreatic hormones are released in an endocrine manner, and each of the hormones has distinct functions. The adipokine pro- and anti-inflammatory ratio may be out of balance, which could result in insulin resistance. The most prevalent adipokine in plasma, adiponectin, is an anti-inflammatory adipokine. Adiponectin has recently received attention for its ability to control inflammation in several disorders [30]. In general, there is a positive association between circulating adiponectin levels and insulin sensitivity. According to a study, measuring adipsin levels can be used to diagnose people who are most likely to experience accelerated diabetes and pancreatic islet cell failure [31]. In our study, the INF group exhibited a substantial reduction in the plasma levels of pancreatic hormones (C-peptide, insulin, and glucagon) and adipokines, namely adiponectin and adipsin, when compared to the UN group. Nevertheless, this effect was not observed after the follow-up period. The decreased levels of the aforementioned analytes are in accordance with our prior studies on *Strongyloides stercoralis* (*Ss*) and filarial-infected individuals [6,10,11,13–16,18]. In the present study, plasma levels of resistin, leptin, and PAI-I did not differ between the two groups. These results imply that hookworm infestation could impact glucose homeostasis and insulin resistance in T2DM perhaps by decreasing the levels of pancreatic hormones and adipocytokines.

Helminth infection influences immunoendocrine activities, but the precise mechanism by which it does so is unclear. Incretin hormones show clinical promise for the treatment of T2DM. Hence, in recent decades, much research has been focused on developing incretin-based therapies for the treatment of T2DM [32]. Our earlier studies indicate that helminth infections may stimulate the secretion of incretins, thereby increasing the intricacy of the relationship between the helminths and their host [6,13,15,18]. Visfatin has recently been reported to exert pro-inflammatory, immunomodulatory, and insulin-sensitizing, or mimetic properties [33]. In the present study, gut-derived hormones (ghrelin, GIP, and GLP-1) and visfatin levels were considerably elevated in INF subjects in comparison with UN subjects. Following anthelmintic treatment, these levels were found to have significantly dwindled. The heightened levels of incretins are in agreement with a recent investigation on subjects with metabolic syndrome and filarial infection [34]. Thus, our data suggest that a mechanistic underpinning of the hookworm-diabetes interface involves the upregulation of incretins, which have the propensity to augment the proliferation of pancreatic beta cells [35]. These results indicate that incretins play a pivotal role in promoting hookworm-related favorable effects on insulin resistance. Consequently, our study suggests a plausible biological mechanism through which concurrent hookworm infestation can potentially modulate T2DM pathogenesis. Also, protection from T2DM seems to be largely due to the upregulation of gut hormones that tend to improve insulin sensitivity. Furthermore, PCA and heat map analysis demonstrate that there was clear discrimination in the levels of glucose indices, pancreatic hormones, gut hormones, and adipocytokines between the INF and UN subjects.

In summary, our study reveals that hookworm infestation may confer protection against the pathology associated with T2DM by alleviating the altered levels of glucose indices, pancreatic hormones, incretins, and adipocytokines. Our study has some limitations: it is not a randomized controlled trial; there are no direct measures of insulin resistance, oral glucose tolerance tests for diabetes, or protozoan tests, and we have not studied the effect of antidiabetic drugs on the post-treated samples. Nonetheless, our study sheds new insight into the hookworm-mediated regulatory networks, which appear to have a notable bystander effect on immune responses to T2DM. Our data also extends this observation to show that this regulatory effect in T2DM subjects is directly associated with hookworm infection since this effect was mostly abrogated following anthelmintic therapy. Furthermore, our results highlight the potential of worms as a new therapeutic strategy to conquer T2DM by regulating host immunity.

Declarations of competing interest

The authors have no competing interests to declare.

Authors' contributions statement

Conceptualization: S.B.; Data curation: B.D, A.R.; Formal analysis: B.D, A.R.; Funding acquisition: S.B.; Investigation: B.D, A.R and S.M.; Methodology: B.D., A.R and S.M.; Statistical Analysis: F.A., B.D., and A.R., Project administration: S.B. ; Resources: P.A.M.; Software: S.B.; Supervision: S.B.; Validation: S.B,. B.D., and A.R.; Visualization: B.D., A.R and S.B.; Writing – original draft: B.D., and A.R.; Writing – review & editing: S.B and S.N.

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Human ethics statement

Ethical approval for this study (NCT00342576, NCT01547884, and NCT04526613) was obtained from the Institutional Review Boards of the National Institute of Allergy and Infectious Diseases (USA) and the National Institute for Research in Tuberculosis (India) (approval nos. NIRT-IEC 2013 001, NIRT-IEC-2011 013, and NIRT-IEC 2020 005). Formal written consent was obtained from all study participants.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2023.08.009.

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