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Comprehensive study of associated factors with Type 2 Diabetes in Mexico: protocol

Estudio integral de factores asociados a diabetes tipo 2 en México: protocolo

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Abstract

Background: Recent studies on type 2 diabetes (T2D) include the analysis of inflammatory molecules, metabolic factors, and complex genetic approaches. Analyzing these factors, in conjunction with clinical variables and lifestyle, is essential to improve glycemic control and prevent the development of complications.
Objective: To describe the protocol design for the comprehensive (molecular, genetic, and clinical) evaluation of patients with T2D, considering glycemic control and its complications.
Material and methods: A cross-sectional, observational, and multicenter study was conducted in three primary care units from the states of Coahuila, Jalisco, and Veracruz. To determine clinical factors associated with the link between molecular profiles and glycemic dysregulation, particularly considering the glomerular filtration rate, clinical and biochemical parameters, lifestyle habits, physical condition, type of treatment, treatment adherence, and nutritional aspects were evaluated. Additionally, RNA-Seq was performed to identify the genes that best stratify and distinguish patients with glycemic dysregulation, metabolic alterations, and diabetic nephropathy through principal component analysis and ROC curves based on relative RNA expression. Finally, multiple logistic regression models were developed to identify a pathological molecular signature characteristic of the disease.

Resumen

Introducción: los estudios recientes sobre diabetes tipo 2 (DT2) incluyen el análisis de moléculas inflamatorias, factores metabólicos y abordajes genéticos complejos. Se propone analizar estos factores, en conjunto con variables clínicas y estilo de vida, para mejorar el control glucémico y evitar el desarrollo de complicaciones.
Objetivo: describir el diseño del protocolo para la evaluación integral (molecular, genética y clínica) de los pacientes con DT2, considerando el control glucémico y sus complicaciones.
Material y métodos: estudio transversal, observacional y multicéntrico en tres unidades de atención primaria en los estados de Coahuila, Jalisco y Veracruz. El descontrol glucémico se evaluó mediante la hemoglobina glicosilada, mientras que la nefropatía diabética se determinó calculando la tasa de filtrado glomerular. Asimismo, se evaluaron parámetros clínicos, bioquímicos, estilo de vida, condición física, tipo de tratamiento, adherencia terapéutica y aspectos nutrimentales. Adicionalmente, se realizó RNA-Seq para identificar los genes que mejor estratifican a los pacientes con descontrol glucémico, alteraciones metabólicas y nefropatía diabética, mediante el análisis de componentes principales y curvas ROC basadas en la expresión relativa de RNA. Para determinar los factores clínicos implicados en la asociación entre los perfiles moleculares y el descontrol glucémico, se desarrollaron modelos de regresión logística múltiple para identificar una huella molecular patológica característica de la enfermedad.

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Introduction

In the past decades has been growing evidence of the inflammatory processes involved in a wide range of chronic-degenerative diseases, including type 2 diabetes (T2D), hypertension, ischemic heart disease, cancer, cerebrovascular disease, and chronic kidney disease.[1]

Diabetes is one of the leading causes of death worldwide, and, particularly in Mexico, it ranks as the second leading cause of death and is considered the primary disease associated with the loss of healthy life years. It is estimated that 60% to 80% of deaths among individuals with diabetes are attributable to cardiovascular diseases. In 2016, diabetes was a public health emergency in Mexico. According to the 2022 National Health and Nutrition Survey, the prevalence of T2D was 18.3% (12.6% previously diagnosed and 5.8% undiagnosed), and 62.8% of individuals with T2D had poor glycemic control.[2]

T2D is considered a chronic low-grade inflammatory state, characterized by elevated levels of inflammatory mediators such as cytokines and adipokines,[1] largely due to increased adipose tissue, particularly abdominal obesity. Additional contributing factors to chronic systemic inflammation include physical inactivity, high-fat and hypercaloric diets, intestinal dysbiosis, psychological stress, sleep disturbances, environmental pollutants, and smoking.[1]

Adipose tissue produces various adipokines, including leptin, resistin, and adiponectin, which have endocrine, paracrine, and autocrine functions. Leptin and resistin promote insulin resistance, while adiponectin has protective effects.[3] Additionally, inflammation driven by proinflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), coupled with oxidative stress from reactive oxygen species,[1] and the depletion of intracellular antioxidants, contributes to insulin resistance and pancreatic β-cell dysfunction—both key mechanisms in the development and progression of T2D.[4]

In this context, Mirza *et al.* conducted a cross-sectional study in a Mexican American population to explore the relationship between diabetes and inflammation. The study found elevated levels of IL-6, leptin, C-reactive protein, and TNF-α in individuals with diabetes. Moreover, poor glycemic control was positively and linearly associated with increased IL-6 and leptin levels, with statistically significant associations even after adjusting for body mass index (BMI) and age. However, no association was observed between TNF-α and glycemic control.[5]

Inflammation and oxidative stress are also implicated in the development of chronic complications such as retinopathy, neuropathy, nephropathy, and cardiovascular disease.[6] Among individuals with T2D, diabetic nephropathy is a common complication frequently associated with cardiovascular morbidity, and in Mexico, it is a significant cause of mortality.[7] Regarding diabetic kidney disease, non-modifiable risk factors include genetics, sex, age at T2D onset, and disease duration, while modifiable risk factors include glycemic control, physical inactivity, blood pressure, lipid profile, and smoking.

Anti-inflammatory effects of physical activity in patients with T2D

Physical activity has demonstrated multiple benefits in individuals with T2D, including improved insulin sensitivity, body composition, physical condition, and anti-inflammatory effects as well as.[8,9] Despite these benefits, it is estimated that only 28.2% of individuals with T2D in the United States meet the recommended levels of physical activity.

It has been proposed that contracting skeletal muscle releases cytokines with autocrine, paracrine, or endocrine functions. For instance, a single bout of exercise can elicit a potent anti-inflammatory response by enhancing insulin sensitivity and suppressing IL-1β activity, which may help protect against IL-1-mediated beta cell destruction.[8,9] In 2017, a study involving 43 women with T2D evaluated the effects of aerobic training on anthropometric measures and inflammatory markers. Following the intervention, improvements were observed in functional capacity (measured via a 10-minute walk test), waist circumference, and BMI. However, no significant changes were detected in IL-6, IL-10, or TNF-α levels.[10]

In Mexico, the physical condition of the population with T2D remains unknown, due to the difficulty of evaluating it using the gold standard: determining the maximum oxygen consumption (VO2 max). Nevertheless, there are surrogate methods to evaluate sub-maximal oxygen consumption, such as the six-minute walk. This test reflects the ability to perform activities of daily living, measuring the maximum distance an individual can walk for six minutes. When compared to the gold standard (VO2 max), this test shows a correlation of 0.73.[11,12] Besides it has been used as a prognostic indicator in heart failure, pulmonary fibrosis, and chronic obstructive pulmonary diseases, however, its utility in patients with T2D is barely explored.

Transcriptomic Studies in Diabetes

In recent years, various "*omics*" approaches have been employed to study diseases such as cancer and autoimmune

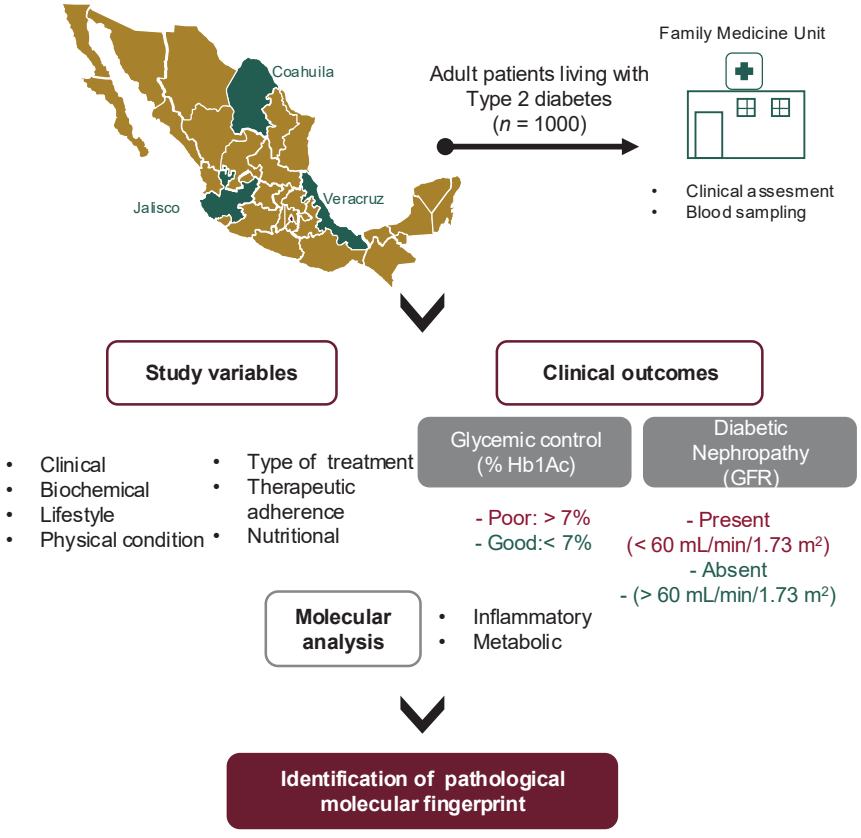
disorders.[13] These approaches include genomics, transcriptomics, proteomics, epigenomics, and metabolomics, which respectively analyze DNA, RNA, proteins, histone modifications, and metabolites. These technologies provide insights into pathophysiological mechanisms and help identify candidate molecules for potential use as prognostic markers.[14,15] In diabetes, mutations and differential gene expression profiles have been identified, indicating unique molecular signatures in affected individuals.[16,17] While most transcriptomic studies in diabetes have focused on tissues such as the pancreas, adipose tissue, and kidney, there is a lack of research on peripheral blood transcriptomics.[18,19] The present study aimed to describe the protocol design for a comprehensive assessment of patients with T2D, focusing on the identification of factors influencing glycemic control and the development of complications.

Methods

Study population and design

This cross-sectional, observational, analytical, and multicenter study was conducted in three Family Medicine Units (FMU): FMU No. 181 in Jalisco, FMU No. 30 in Veracruz, and FMU No. 10 in Coahuila. The study population consisted of patients diagnosed with T2D, with or without hypertension. The study spanned 12 months, and data were collected from clinical records and direct interviews and recorded in a standardized data sheet. Blood samples were collected for the determination of glycated hemoglobin (HbA1c), as well as for biochemical, inflammatory, and metabolic analyses (figure 1).

Figure 1 Design of the study for the comprehensive study of type 2 diabetes in Mexico



This observational study recruited adult patients from three regions of Mexico (Coahuila, Jalisco, and Veracruz) with a total sample size of 1000 participants. The study was carried out in one Family Medicine Unit from each region, where patients underwent a clinical evaluation and blood sample collection. The study will assessed eight variables, including two primary clinical outcomes: glycemic control (poor control defined as HbA1c > 7%) and diabetic nephropathy (defined as GFR < 60 mL/min/1.73 m²). In addition, inflammatory and metabolic factors were analyzed using molecular techniques in patients living with Type 2 diabetes. Once data collection was complete, multivariate analysis and principal component analysis were performed to identify the pathological molecular fingerprint associated with poor metabolic control and complications.

HbA1c: Glycated hemoglobin; GFR: Glomerular filtration rate

Selection criteria

Patients aged 18 years or older with a diagnosis of T2D were included, provided they had not experienced any conditions associated with elevated levels of proinflammatory molecules within one month prior to enrollment (Table I). To avoid the inclusion of young individuals with other forms of diabetes, patients with Maturity-Onset Diabetes of the Young (MODY) were identified using the Exeter MODY Probability Calculator and excluded from the study.[20]

This calculator considers variables such as age at diagnosis, sex, current treatment (insulin or oral hypoglycemic agents), time to insulin initiation (for those on insulin), body mass index (BMI), glycated hemoglobin (HbA1c, %), current age, parental history of diabetes, and ethnicity.

Additionally, patients with C-peptide concentrations below 0.6 ng/mL were classified as having autoimmune diabetes (despite not undergoing testing for beta-cell autoantibodies) and were excluded from the study.

Recruitment process

Patients with T2D from each FMU were contacted by telephone and invited to attend their medical unit for a comprehensive evaluation. They were informed about the possibility of participating in a clinical study, with an explanation of its purpose and assurance that their participation would not interfere with their routine clinical care. An appointment was scheduled, specifying the need to arrive after eight hours of fasting and to wear comfortable clothing and footwear.

Upon arrival at the FMU, patients were referred to the nursing service, where they were reinvited to participate in the study and screened for eligibility criteria. Interested patients were then evaluated by clinicians, who provided a detailed explanation of the study. At that time, the informed consent form was provided for review, allowing sufficient time for careful reading. Nursing and medical staff will addressed any questions the patient may had. For participants who were unable to read, the clinical staff read the form aloud and provided clarifications. If a potential participant preferred to take the consent form home to discuss it with family members, they were allowed do so and return in the following days to submit it and be included in the study.

If a patient chose not to participate, they proceeded with their scheduled evaluation, including point-of-care HbA1c and glucose measurements, vital signs assessment, and routine clinical follow-up. If the patient agreed to participate, they will proceeded to the laboratory for blood sample collection, followed by the assessment of clinical variables.

Clinical variables

Medical staff involved in the study will collect the following data from each participant, to be recorded in both the electronic medical record and a standardized data collection form:

- General data: age, sex, occupation, education level
- Family history: diabetes and other comorbidities
- History of the disease: age at diagnosis, time of evolution, presence of comorbidities (hypertension, dyslipidemia, obesity), presence of chronic (neuropathy, nephropathy, retinopathy) or acute complications (hypoglycemia, ketoacidosis, hyperosmolar state), and current treatments for each condition

Table I Criteria for inclusion, exclusion, and elimination of patients living with Type 2 Diabetes

Inclusion criteria	Exclusion criteria	Elimination criteria
• Patients over 18 years with a diagnosis of T2D • Patients willing to participate and that sign the informed consent letter	• Pregnant of lactating mother • Current use of steroids • Concomitant illness with inflammatory or autoimmune component (rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, psoriatic arthritis, gout) • Tobacco use (in the last 3 months) • History of cerebrovascular disease, heart failure or kidney failure that required hospitalization in the previous month • History of infection in the previous 10 days (Respiratory tract infection, gastroenteritis, urinary tract infection, soft tissue infection) • Severe obesity, with BMI> 40Kg/m^2 • History of bariatric surgery	• Those with probability of having MODY >36%, determined by the Exeter MODY Diabetes calculator[21] • C-peptide less than 0.6ng/mL[22] • Incomplete clinical record • Patients who decide withdrawing from the protocol

Following the medical evaluation, nursing staff collected the following:

- Anthropometric data: weight, height, BMI, waist and hip circumference, waist-to-hip ratio, systolic and diastolic blood pressure
- Hb1Ac was determined using a point-of-care test and capillary blood glucose monitoring by glucometer.
- Once the sample was taken, the nursing staff performed the six-minute walk test to determine physical condition.

Lifestyle assessment

The Measuring Lifestyle in People with Diabetes (IMEVID) test was used to assess lifestyle.[23] This tool evaluates seven domains: nutrition, physical activity, tobacco and alcohol use, diabetes-related knowledge, emotional health, and therapeutic adherence (Supplementary Table 1). It comprises 25 items and categorizes lifestyle as:

- **Good:** > 80 points
- **Fair:** 60 - 80 points
- **Poor:** < 60 points

Dietary assessment

Dietary intake was evaluated using the Mini-ECCA v.2 Food Consumption Quality Mini-Survey,[24] consisting of 14 Likert-type items (from *"never"* = 1 to "*always*" = 4) supported by food quantity photographs.[25] This tool is validated for the Mexican population, demonstrating adequate reproducibility (κ = 0.422-0.662).[24] Higher scores reflect better dietary quality. The survey also identified three dietary patterns:

1. Healthy intake: increased consumption of water, vegetables, fruits, healthy fats, legumes, meats
2. Habits for improvement: low fish intake with excessive unhealthy fat intake
3. Unhealthy intake: frequent consumption of sugary beverages, fast food, processed foods, refined grains, and alcohol (Supplementary Table 2)

Therapeutic adherence assessment

The 4-item Morisky-Green scale was applied to assess adherence.[26] Participants were considered adherent if they responded correctly to all four items (No/Yes/No/No):

1. Do you ever forget to take your diabetes medication?
2. Do you take your medication at the scheduled times?
3. When you feel better, do you sometimes stop taking your medication?
4. If you feel ill, do you stop taking it?

If necessary, the nursing or administrative staff will rephrase the questions in simpler terms to ensure patient understanding.

Physical condition assessment: Six-Minute walk Test

The six-minute walk test was conducted to assess physical capacity.[27] Patients were asked to walk as far as possible along a 30-meter corridor for six minutes. The test evaluated integrated responses from pulmonary, cardiovascular, and muscular systems. Heart rate, respiratory rate, dyspnea, and oxygen saturation were recorded at the start and end of the test. The timer was set for six minutes, during which laps were tracked. Patients were encouraged to continue walking throughout. At the conclusion, vital signs and oxygen saturation were re-evaluated, and patients rested for 10 minutes. The test ended once the patient was stable and free of alarming symptoms. The main outcome was the total distance walked (in meters).

Contraindications included: capillary glucose > 250 mg/dL or < 70 mg/dL, unstable angina or recent myocardial infarction (within one month), recent acute foot injury, diabetic foot, resting heart rate > 120 or < 60 bpm, blood pressure > 180/100 mmHg or < 90/60 mmHg, oxygen saturation < 89%, or impaired functional capacity assessed by affirmative responses to any of the following:

1. Do you experience chest pain?
2. Do you feel breathless while sitting, walking, or exerting yourself?
3. Do you experience shortness of breath while lying down or sleeping?
4. Have you fainted or lost consciousness?

The test was not conducted if any of these were reported. It was performed in a location equipped for emergency

management and cardiorespiratory resuscitation. The test was interrupted in case of chest pain, severe dyspnea, leg pain, diaphoresis, cyanosis, pallor, or signs of exhaustion.

Laboratory studies

Blood samples were collected in the FMU laboratory for genetic material (RNA), complete blood count (CBC), and biochemical analysis. A 6 mL vacutainer tube was centrifuged at 3150 G for 15 minutes to obtain serum.

Commercial kits were used for the quantification of glucose (Glucose Kit, Cat. No. GLU0102), cholesterol (Total Cholesterol Kit, Cat. No. TC0102), HDL-c, and triglycerides (Triglycerides Kit, Cat. No. TG0102) (Mindray, Nanshan, Shenzhen, P.R. China). HbA1c was measured via fluorescent immunoassay (iChroma HbA1c kit, Cat. No. CFPC-38, Boditech Med Inc., Chuncheon-si, Republic of Korea). LDL-c was calculated using the Friedewald formula.

RNA isolation

Total RNA was extracted using the miRNeasy Mini Kit (Qiagen Inc., CA, USA). Quantification was performed with a Nanodrop 2000, and RNA integrity was assessed with RNA Nanochips 6000 using an Agilent 2100 Bioanalyzer. Transcriptome libraries were prepared with the TruSeq Stranded Total RNA Library Prep with Ribo-Zero Gold kit (Illumina, San Diego, CA, USA) and quantified using the Qubit dsDNA HS assay kit (Invitrogen, Carlsbad, CA, USA). Library size was analyzed with the Standard S2 DNA Cartridge for QSep 400 (BiOptic, Taiwan), and sequencing was performed on a NextSeq 2000 (Illumina) using 150 bp paired-end reads.

Preprocessed fragments were aligned to the human reference genome (hg38) using STAR. Gene expression quantification was performed with the DESeq2 package, generating a count table for differential gene expression analysis.

Statistical Analysis

Sample Size Calculation

To evaluate the independent association between inflammatory and metabolic factors and various clinical variables, the sample size was calculated using the Events Per Variable (EPV) formula for multivariate analysis. A glycemic control rate of 16.3% and 15 potential confounding variables associated with poor metabolic control were considered.

EPV=10 xk/n

Where:

k = number of variables (15)

n = frequency of the outcome (0.163)

10×150/0.163

A total of **880 participants** was required to compensate for missing information or deleted data. Therefore, **1,000 patients** were included in the study to ensure robustness.

Descriptive analysis

For quantitative variables (age, disease duration, BMI, HbA1c, and inflammatory mediators), the distribution type was assessed using the Kolmogorov-Smirnov test. Variables with a p-value > 0.05 were considered normally distributed. Mean and standard deviation were reported for normally distributed variables, while median and interquartile ranges (25% - 75%) were determined for quantitative variables with free distribution. Qualitative variables were presented as frequencies and percentages.

Principal Component Analysis (PCA)

PCA was used to for simplifying the complexity in data sets while preserving the original variability as possible. It can generate as many dimensions as there are original variables. To explore the relationship between inflammatory and metabolic factors with glycemic control and chronic complications, a PCA was performed using data from patients with and without adequate glycemic control. A loading factor threshold of ±0.30 was used to determine variable contributions to each component.

For the transcriptomic analysis, complete RNA sequencing was used to analyze all protein-coding transcripts (approximately 36,000 per individual). PCA reduced dimensionality by retaining the two or three dimensions that best captured variability, enabling identification of inflammatory and metabolic molecules associated with poor glycemic control in patients with T2D.

Receiver Operating Characteristic (ROC) curve for RNAs

ROC curves were used to define cut-off points for relative RNA expression levels of inflammatory and metabolic

components associated with poor glycemic control and nephropathy. The optimal cut-off will maximized sensitivity, specificity, and the **Youden Index** (sensitivity - false positive rate). The **area under the curve** (AUC) and **95% confidence intervals** (CI) were calculated. Molecules with an AUC ≥ 0.70 and a 95% CI between 0.60 and 0.80 were considered components of the proposed inflammatory and metabolic fingerprint.

Bivariate analysis

To compare participant characteristics by the IMMF expression group, the Student's t-test or Mann-Whitney *U* test was used for quantitative variables, depending on distribution. For qualitative variables, the Chi-squared test or Fisher's exact test was applied. Differences in inflammatory mediators across levels of glycemic control (HbA1c less than 5.7%, from 5.7 to 6.5% and higher than 6.5%) were analyzed using ANOVA or the Kruskal-Wallis test, as appropriate.

Multivariate analysis

A **multiple logistic regression model** was constructed to identify factors associated with poor metabolic control and chronic complications. Beta coefficients and 95% confidence intervals were reported. The model was adjusted for gene expression and potential confounders, including diet, lifestyle, obesity level, disease duration, age, and physical condition.

Ethics approval and consent to participate

This study protocol was approved by the Research and Ethics Committee of the Mexican Social Security Institute (IMSS) (CNIC registry number: R-2024-785-007). All procedures adhere to local ethical regulations, the Mexican General Health Law for Health Research, institutional policies, and the Declaration of Helsinki (1975, and subsequent amendments). The study also complies with current international standards for Good Clinical Practice. Informed consent was obtained from all participants. Patients were personally invited during their routine outpatient visits. Consent forms were signed by the principal or co-investigators. Participants were informed of their right to withdraw consent at any stage. The study was sponsored by the IMSS.

Discussion

Type 2 diabetes is a leading cause of morbidity and premature mortality worldwide. According to the Mexican National Health and Nutrition Survey, 68.2% of patients with T2D have poor glycemic control, which is linked to an increased risk of chronic complications that diminish quality of life, reduce work productivity, and elevate healthcare costs and mortality rates. Furthermore, hyperglycemia promotes low-grade inflammation and oxidative stress, contributing to the onset and progression of T2D.[2]

Despite the implementation of pharmacological strategies, education programs, screening tools, and public health initiatives such as nutritional labeling,[28,29] many individuals with T2D in Mexico continue to exhibit poor glycemic control. This observation suggests the involvement of intrinsic factors within the Mexican population, potentially with significant economic repercussions.[30,31]

Multiple studies have aimed to identify genetic and epigenetic determinants of diabetes development and metabolic control. Inflammatory molecules -including cytokines from adipose tissue (adipokines), muscle (myokines), and liver (hepatokines)- alongside oxidative products and lipid metabolites, have been implicated in disease mechanisms.[32] However, these studies often focus on limited genetic information, such as single nucleotide polymorphisms. This protocol proposes a comprehensive approach to evaluating inflammatory and metabolic factors associated with T2D and its complications. Through integration of clinical, biochemical, and transcriptomic data, it seeks to identify a **molecular fingerprint** associated with poor glycemic control and diabetic kidney disease—both highly prevalent in T2D patients.

The main advantage of this approach lies in its ability to detect all differentially expressed molecules between patients with and without metabolic control and those with or without microvascular complications. The proposed sequencing technique enables analysis of the full transcriptome in an unsupervised manner, facilitating the discovery of novel inflammatory and metabolic biomarkers and their correlation with clinical parameters.

To our knowledge, this is the first protocol to comprehensively investigate the inflammatory and metabolic molecular landscape associated with glycemic control and complications in patients with T2D. The findings could contribute to improved clinical decision-making and inform public health policies aimed at better management of T2D.

Conflict of interest disclosure: The authors have completed and sent the Spanish-translated form of the Declaration for Potential Conflicts of Interest of the International Committee of Medical Journal Editors, and no conflicts of interest were reported related to this article.

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Supplementary Table I Lifestyle evaluation in patients with diabetes with the IMEVID survey

Instrument to evaluate lifestyle in patients with Diabetes (IMEVID)			
1. How often do you eat vegetables?	Every day of the week	Some days	Hardly ever
2. How often do you eat fruits?	Every day of the week	Some days	Hardly ever
3. How many pieces of bread do you eat a day?	0 - 1	2	3 or more
4. How many tortillas do you eat a day?	0 - 3	4 - 6	7 or more
5. Do you add sugar to your food or drinks?	Hardly ever	Sometimes	Frequently
6. Do you add salt to the foods?	Hardly ever	Sometimes	Almost always
7. Do you eat food between meals?	Hardly ever	Sometimes	Frequently
8. Do you eat food outside home?	Hardly ever	Sometimes	Frequently
9. When you finish eating the initially portion served, do you ask for more?	Hardly ever	Sometimes	Almost always
10. How often do you do at least 15 minutes of exercise?	Frequently	Sometimes	Hardly ever
11. Do you keep busy aside your usual work activities?	Almost always	Sometimes	Hardly ever
12. What do you do in your free time?	Take a walk	Work at home	Watch TV
13. Do you smoke?	I do not smoke	Sometimes	I smoke daily
14. If so, how many cigarettes do you smoke a day?	None	1 to 5	6 or more
If you smoke, indicate the length of time in years you have been smoking:			
15. Do you drink alcohol?	Never	Seldom	1 time or more per week
16. How many alcoholic drinks do you drink on each occasion?	None	1 to 2	3 or more
17. How many talks for people with diabetes have you attended?	4 or more	1 to 3	None
18. Do you try to get information about diabetes?	Almost always	Sometimes	Hardly ever
19. Do you get angry easily?	Hardly ever	Sometimes	Almost always
20. Do you feel sad?	Hardly ever	Sometimes	Almost always
21. Do you have pessimistic thoughts about your future?	Hardly ever	Sometimes	Almost always
22. Do you do your best to keep your diabetes under control?	Almost always	Sometimes	Hardly ever
23. Do you follow a diet to manage your diabetes?	Almost always	Sometimes	Hardly ever
24. Do you forget to take your diabetes medications or take your insulin?	Hardly ever	Sometimes	Frequently
25. Do you follow properly the medical instructions?	Almost always	Sometimes	Hardly ever

Supplementary Table II Mini-ECCA2: Mini Food Consumption Quality Survey

No.	Questions	Answers
1	Do you drink at least 1.5 liters of **water**every day (Monday to Sunday)?	1) Never ☐ 2) Sometimes ☐ 3) Almost always ☐ 4) Always ☐
2	Do you consume at least 200 g of **cooked or raw vegetables** every day(Monday to Sunday)?	1) Never ☐ 2) Sometimes ☐ 3) Almost always ☐ 4) Always ☐
3	Do you consume at least 200 g of **fresh or frozen fish (not canned)** in a week?	1) Never ☐ 2) Sometimes ☐ 3) Almost always ☐ 4) Always ☐
4	How many times a week do you consume one or more cans (or glasses) of **sugary drinks**?	1) Never ☐ 2) 1-3 times ☐ 3) 4-6 times ☐ 4) Daily ☐
5	Do you consume, at least 200 g of **fruit** everyday (Monday to Sunday)?	1) Never ☐ 2) Sometimes ☐ 3) Almost always ☐ 4) Always ☐
6	What **oil** do you use more frequently to prepare your food? (Weekly)	1) A Olive or canola oil ☐ 2) B Mayonnaise, corn oil, sunflower or soy oil, dressings ☐ 3) C Butter, cream, cream cheese ☐ 4) D I don't know ☐
7	Do you consume at least 30 g of **oilseeds** or half a piece of **avocado** everyday (Monday to Sunday)?	1) Never ☐ 2) Sometimes ☐ 3) Almost always ☐ 4) Always ☐
8	Do you eat **foods prepared out of home** 3 or more times a week?	1) Never ☐ 2) Sometimes ☐ 3) Almost always ☐ 4) Always ☐
9	What type of **meat** do you consume the most in a week?	1) A Red meat (beef or pork) ☐ 2) B Chicken ☐ 3) C Fish ☐
10	Do you eat **processed foods** (fried foods,sausages, fast food) 2 or more times per week?	1) Never ☐ 2) Sometimes ☐ 3) Almost always ☐ 4) Always ☐
11	Do you consume **desserts** (cookies, flans, rice with milk, cakes) or sweets (candy,lollipops, chocolates) 2 or more times per week?	1) Never ☐ 2) Sometimes ☐ 3) Almost always ☐ 4) Always ☐
12	Do you consume, at least 300 g of **legumes** a week?	1) Never ☐ 2) Sometimes ☐ 3) Almost always ☐ 4) Always ☐
13	What **cereals do you** consume most frequently in the week?	1) A Corn, tortillas, oats and amaranth ☐ 2) B Rice, pasta, bread, potatoes ☐ 3) C Cereal, flour tortillas, toasts, canned corn ☐
14	For men: Do you drink 2 or more **alcoholic beverages in a** day? For women: Do you drink 1 or more **alcoholic beverages in** a day?	1) Never ☐ 2) Sometimes ☐ 3) Almost always ☐ 4) Always ☐