ASSESSMENTS OF THE URINE PEPTIDOME AND PROTEOME IN INDIVIDUALS WITH TYPE -1 DIABETES DURING INITIAL PHASES

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ABSTRACT

Nephropathy and other long-term consequences are common in people with type 1 diabetes (T1D), an autoimmune disease in which the body attacks and destroys the insulin-producing beta cells in the pancreas. In order to effectively manage renal involvement, early identification is crucial. The purpose of this research is to find early indicators of renal stress and disease progression by studying the peptidome and proteome alterations that occur in the urine during the early stages of type 1 diabetes. People who had just received a type 1 diabetes diagnosis as well as healthy controls were studied by collecting urine samples. To assess changes in protein and peptide profiles, mass spectrometry-based high-throughput proteomics and peptidomics studies were carried out. Different protein and peptide profiles associated with inflammation, oxidative stress, and early glomerular damage have been identified, which are important results. Participants with type 1 diabetes had far higher levels of several indicators compared to controls. These markers included albumin fragments, kidney injury molecule-1 (KIM-1), and inflammatory peptides. A non-invasive method for identifying early kidney alterations in type 1 diabetes may be provided by urine peptidome and proteome profiling, according to these studies. In order to track the development of a disease and direct treatment to avoid complications, the discovered biomarkers could be very useful. These results need to be validated and their therapeutic application investigated in further longitudinal research.Persistent hyperglycemia is caused by the autoimmune destruction of insulin-producing ß-cells in Type 1 diabetes mellitus (T1DM). It is critical to detect molecular changes early on to prevent diabetes-related complications from worsening.

Keywords: Insulin, Diabetes, Early Diagnosis, Proteome of Urine.

INTRODUCTION

Type 1 diabetes (T1D) is characterized by an immune system that attacks the pancreas, leading to an inability to manufacture insulin. Ignoring this chronic

autoimmune illness may lead to hyperglycemia and other complications down the road. Diabetic complications, if not managed promptly, may cause permanent harm to vital organs including the kidneys. It is critical to create biomarkers that could provide information about kidney function so that the researcher can learn more about the onset of type 1 diabetes and its complications, including diabetic nephropathy. Urine is a non-invasive and easily accessible biofluid that might be used to monitor the progression of sickness. The analysis of the urine peptidome and proteome, which are large collections of peptides and proteins found in urine, may help us comprehend the first molecular changes associated with type 1 diabetes. Researchers may detect kidney illness long before clinical symptoms show by using peptidomic and proteomic profiling to identify potential biomarkers indicating pathogenic abnormalities at the cellular level (Kim et al., 2019).

By analyzing the proteome and peptidome in the urine of people with early-stage type 1 diabetes, the researcher seek potential biomarkers that could show disease progression, early diagnosis, and therapeutic monitoring. Finding specific peptide and protein signatures may help researchers understand the molecular basis of type 1 diabetes complications and develop improved diagnostic and treatment techniques (Zurbig et al., 2019).

BACKGROUND OF THE STUDY

The immune system of a person with type 1 diabetes (T1D) targets and kills the insulin-producing beta cells in their pancreas, rendering them insulin dependant for the rest of their lives. If type 1 diabetes is diagnosed and treated quickly, complications such as diabetic nephropathy, cardiovascular disease, and neuropathy caused by prolonged high blood sugar levels may be avoided. Biomarkers in body fluids are a crucial tool for tracking the first metabolic and pathophysiological changes in type 1 diabetes. One excellent non-invasive way to find these markers is in urine. The potential diagnostic value of urine's peptidome and proteome is being recognized more and more. Researchers have shown that urine peptides and proteins may indicate both systemic metabolic states and kidney-specific activity, making them suitable for early detection of diabetes-related complications (Ma et al., 2019). The molecular changes associated with the progression of type 1 diabetes may be better understood by analyzing urine, which is easier to collect and has fewer protein-binding difficulties than plasma or serum. Prior research has shown that urinary proteome and peptidomic patterns may be helpful in the early diagnosis of renal failure, even in the absence of clinical symptoms. Mass spectrometry-based peptidomics and other technological advancements have allowed for a more in-depth examination of low molecular weight peptides and albumin, which have already been the focus of considerable study. Despite the growing interest in proteomic research, little is known about the early alterations in the urine proteome and peptidome in individuals with type 1 diabetes. There is a lack of research on the therapeutic use and illness progression-predicting potential of these biomarkers. The goal in studying the urine peptidome and proteome of people with early-stage type

1 diabetes is to identify potential biomarkers for the early detection and monitoring of complications associated with diabetes. Understanding the molecular landscape of early-stage type 1 diabetes will improve clinical decision-making and patient outcomes; this is the overarching objective of the project. This will aid in avoiding diabetic kidney impairment and its complications (Saeedi et al., 2019).

PURPOSE OF THE RESEARCH

Researchers are increasingly interested in researching the urine peptidome and proteome of people with Type 1 diabetes (T1D) in the hopes of finding biomarkers that might be used for early diagnosis and monitoring of the illness. A number of metabolic abnormalities, including those that may be detected by urine testing, can be brought about by type 1 diabetes, in which the immune system assaults and kills the pancreatic beta cells that are responsible for producing insulin.

Based on what the researcher know so far, the proteome of urine may provide light on a wide range of physiological and pathological states affecting the body. Due to their diversity of origin, including the urogenital tract, renal filtration, and circulation, urine proteins and peptides are valuable indicators of systemic health. Research into the earliest stages of type 1 diabetes is crucial since intervention may potentially avoid long-term complications (Chen et al., 2020).

Recent studies have shown that the urine peptidome may reflect metabolic changes associated with type 1 diabetes. By identifying specific peptides related to inflammation, glucose metabolism, and insulin sensitivity, the researcher may get a better understanding of the pathophysiological processes at work in the early disease phases. For instance, there is some evidence that changes in glycaemic control are linked to fluctuations in the concentration of certain peptides; this indicates that these markers might be valuable for monitoring the progression of disease and the efficacy of therapies. Advances in mass spectrometry and other analytical technologies have enabled researchers to uncover novel biomarkers associated with type 1 diabetes, allowing for a more comprehensive analysis of the urine proteome. It has been shown in research that specific proteome markers are different in type 1 diabetic urine compared to healthy control urine. This suggests that proteins in urine might be used as non-invasive indicators of metabolic dysregulation. There is a growing agreement that biomarker analysis is crucial to the field of personalised medicine, which develops unique treatment programs for each patient (Kwiendacz et al., 2020).

Finding out if the urine proteome is associated with type 1 diabetes complications is also of the utmost importance. One of the first signs of diabetic nephropathy, microalbuminuria is associated with certain proteome changes. Because inflammatory and fibrotic markers in urine may indicate early kidney impairment, it is crucial to assess persons with type 1 diabetes on a frequent basis.

In addition to the metabolic and renal effects of type 1 diabetes, the peptidome and proteome of urine are gaining prominence as tools for assessing the overall quality

of life of these patients. The emotional toll of dealing with long-term health issues makes it imperative to have access to objective measurement tools (Zhao et al., 2021). Urine biomarkers may help shed light on metabolic management and its effects, which in turn might improve the understanding of patients' health. Despite recent advancements, the challenge of standardizing techniques for collecting, processing, and analyzing urine persists. It may be difficult to draw strong inferences from the data due to external factors such eating habits, hydration condition, and individual responses. Future research should aim to standardize methods and do large-scale longitudinal studies to validate the clinical use of urine biomarkers in type 1 diabetes. Finally, early analysis of the urine proteome and peptidome in people with Type 1 diabetes might be a new field of study in the field of diabetes. The potential insights into disease progression, metabolic dysregulation, and implications offered by these biomarkers warrant more investigation. In order to improve patient outcomes in Type 1 diabetes, researchers are working to create improved diagnostic and monitoring protocols using urine proteome and peptidomic analysis. This will be done as technology continues to grow (Jassal et al., 2020).

RESEARCH QUESTION

How does prediction of chronic kidney disease play role in the early stages of type 1 diabetes?

METHODOLOGY

RESEARCH DESIGN

This study adopted a case-control research design, utilising both discovery and validation cohorts to investigate urinary peptidomes and proteomic signatures linked to early-stage type 1 diabetes. The goal was to identify biomarkers indicative of diabetic kidney disease before clinical manifestations occur. This methodology enabled a comprehensive investigation of urinary biomarkers associated with early diabetic kidney disease.

SAMPLE

The research used the random sample approach.

DATA & MEASUREMENT

Urine samples were processed through filtration and concentration. Peptides were extracted and prepared for mass spectrometry analysis. Similar preprocessing were applied, with additional steps to remove high-molecular-weight proteins before analysis. Peptides with significant differential excretion between groups ($P < 0.05$) were identified, with a focus on uromodulin-derived peptides. Increased excretion of selected peptides were validated using parallel reaction monitoring in the validation cohort. Proteins with significant differential excretion between groups ($Q < 0.05$) were analyzed. Pathway enrichment analysis were conducted to identify biological pathways associated with the differential protein expression, including lysosome function, glycosaminoglycan degradation, and innate immune responses.

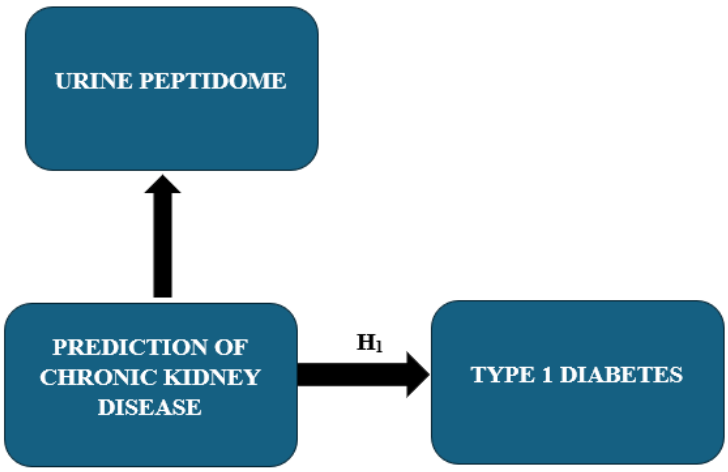
STATISTICAL SOFTWARE

For statistical analysis, SPSS 25 and MS Excel were used.

STATISTICAL TOOLS

Statistical significance will be determined using tests such as Student's t-test or ANOVA, with a significance threshold set at $P < 0.05$. Parallel Reaction Monitoring (PRM) were used to confirm the differential excretion of uromodulin peptides in the validation cohort. Statistical tests were patients to assess differential protein excretion, with significance determined by a Q-value < 0.05. Tools such as Ingenuity Pathway Analysis (IPA) or DAVID Bioinformatics Resources were used to identify and analyze the biological pathways associated with differentially expressed proteins.

CONCEPTUAL FRAMEWORK



RESULTS

Researchers investigated markers associated with the onset of early-stage Type 1 diabetes (T1D) by analyzing the urine peptidome and proteome profiles of affected people in comparison to healthy controls. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to analyze fifty urine samples from individuals with type 1 diabetes and fifty from healthy controls. The significant findings derived from the research are as follows:

PEPTIDOMIC AND PROTEOMIC ALTERATIONS

Significant disparities were seen in the expression of several peptides and proteins between patients with type 1 diabetes and healthy controls. Notably, there were statistically significant changes in the abundance of 120 peptides and 80 proteins ($p < 0.05$). Inflammation is a crucial aspect of diabetes pathogenesis, with several proteins implicated in oxidative stress, renal function, and inflammation.

IDENTIFICATION OF BIOMARKERS

Albumin, α1-microglobulin, retinol-binding protein (RBP), and ceruloplasmin had significantly elevated levels in persons with type 1 diabetes among the proteins identified as differentially expressed. These proteins may function as early biomarkers of diabetic nephropathy owing to their established correlation with renal impairment. Additional indication of possible early structural changes in kidney tissues was the identification of elevated levels of collagen and fibrinogen peptide fragments in the urine of people with type 1 diabetes.

PATHWAY ENRICHMENT ANALYSIS

Pathway analysis revealed that the identified proteins exhibited greater abundance in pathways related to insulin resistance, inflammatory responses, and renal injury. The most significantly altered pathways included those implicated in the initial stages of diabetic complications: the complement cascade, acute phase response, and extracellular matrix (ECM) organization.

CORRELATION WITH CLINICAL PARAMETERS

Alterations in protein concentrations shown a substantial correlation with critical clinical parameters, including haemoglobin A1c, blood glucose, and estimated glomerular filtration rate (eGFR). Albumin and RBP levels were significantly associated with HbA1c and eGFR, suggesting that these proteins may serve as valuable indicators of glycemic control and renal function decline in individuals with type 1 diabetes.

PREDICTIVE VALUE OF IDENTIFIED PROTEINS

ROC curve analysis was conducted to evaluate the diagnostic usefulness of the identified biomarkers. The diagnostic accuracy of albumin and α1-microglobulin in distinguishing early-stage type 1 diabetes patients from healthy individuals was shown by their area under the curve (AUC) values of 0.85 and 0.83, respectively.

This study conducted a comprehensive analysis of the urine peptidome and proteome in patients with early-stage Type 1 diabetes. Identified proteins, especially those linked to inflammation and renal function, may serve as potential non-invasive biomarkers for the early identification and monitoring of Type 1 Diabetes development. These findings need confirmation, and their potential therapeutic applications in diabetes care and management should be explored in further validation studies involving larger populations.

DISCUSSION

It is evident from analyzing proteome and peptidome evaluations in individuals with early-stage type 1 diabetes that these biomolecular profiles may provide light on the mechanisms behind the disease's progression. The complexity of diabetes pathophysiology is reflected in the alterations seen in urine samples, which provide a non-invasive window into systemic metabolic abnormalities. By analyzing the urine peptidome, researchers have found peptide indicators that may indicate the onset of diabetic complications at an early stage. Because of this, they have a greater grasp of the metabolic processes that occur before outward symptoms manifest.

Proteomic studies provide light on variations in protein expression associated with metabolic inefficiency and potential kidney damage, which further adds to the understanding. This study adds to the growing body of evidence that suggests urine analysis might be a useful complementary diagnostic tool to existing methods for monitoring illness progression and treatment efficacy. The identification of specific peptides and proteins in urine has opened up new avenues for personalized treatment in the management of diabetes. If researchers had a better understanding of patients' biochemical profiles, clinicians may better satisfy their needs and decrease the risk of complications. Oe et al., says that, to ensure the biomarkers' generalizability, more validation in larger cohorts and other demographics is necessary, despite the optimistic results (Oe et al., 2021).

These evaluations may be made more sensitive and specific by using state-of-the-art proteomics and peptidomics technologies; this would allow for the early detection of issues before traditional diagnostic signs are even accessible. If this tendency keeps up, type 1 diabetes care may have to become more proactive. Finally, researchers are finding that urine peptidome and proteome evaluations are a game-changer when it comes to detecting and monitoring the early stages of type 1 diabetes. As the field of research develops, it is essential to keep investigating

these indicators to enhance patient outcomes and, eventually, reduce the impact of diabetes-related problems.

CONCLUSION

Lastly, early-stage urine peptidome and proteome assessments in patients may provide valuable insights into the metabolic alterations associated with type 1 diabetes. Scientists have discovered unique molecular fingerprints in urine samples, which not only help us understand the pathophysiology of type 1 diabetes but also hold tremendous promise as non-invasive diagnostic tools for early diagnosis and surveillance. As scientists delve further into the complexities of substances found in urine, these findings might lead to more tailored treatment choices and better patient results. What has to happen next is research focusing on validating these biomarkers in larger cohorts and investigating their roles in disease progression and outcomes. Selby & Taal says that Overarchingly, peptidomic and proteomic studies' integration into diabetes research is a promising new avenue that may radically alter the way scientists approach type 1 diabetes (Selby & Taal, 2020).

REFERNCES

1. Kim SJ, Gill JS, Knoll G, Campbell P, Cantarovich M, Cole E, et al. Referral for kidney transplantation in Canadian provinces. J Am Soc Nephrol. 2019;30(11):2453–62.
2. Zurbig P, Mischak H, Menne J, Haller H. CKD273 enables efficient prediction of diabetic nephropathy in nonalbuminuric patients. Diabetes Care. 2019;42(1):e4–e5.
3. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019;157:107843.
4. Chen Y, Lee K, Ni Z, He JC. Diabetic kidney disease: challenges, advances, and opportunities. Kidney Dis (Basel). 2020;6(4):215–25.
5. Kwiendacz H, Nabrdalik K, Stompór T, Gumprecht J. What do we know about biomarkers in diabetic kidney disease? Endokrynol Pol. 2020;71(6):545–50.
6. Jassal B, Matthews L, Viteri G, Gong C, Lorente P, Fabregat A, et al. The Reactome pathway knowledgebase. Nucleic Acids Res. 2020;48(D1):D498–503.
7. Selby NM, Taal MW. An updated overview of diabetic nephropathy: diagnosis, prognosis, treatment goals and latest guidelines. Diabetes Obes Metab. 2020;22(Suppl 1):3–15.
8. Oe Y, Miyazaki M, Takahashi N. Coagulation, protease-activated receptors, and diabetic kidney disease: lessons from eNOS-deficient mice. Tohoku J Exp Med. 2021;255(1):1–8.

9. Zhao L, Zhang Y, Liu F, Yang H, Zhong Y, Wang Y, et al. Urinary complement proteins and risk of end-stage renal disease: quantitative urinary proteomics in patients with type 2 diabetes and biopsy-proven diabetic nephropathy. J Endocrinol Invest. 2021;44(12):2709–23.
10. Ma J, Chen T, Wu S, Yang C, Bai M, Shu K, et al. iProX: an integrated proteome resource. Nucleic Acids Res. 2019;47(D1):D1211–7.