REVIEW

Mining of protein based biomarkers for type 2 diabetes mellitus

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Abstract: Diabetes mellitus in general and Type 2 Diabetes (T2D) in particular, are very complex diseases with heterogeneous etiology. T2D results from the impaired secretion or action of the insulin with a clinical phenotype of persistent hyperglycemia in the uncontrolled subjects. The disease clinical features appear after several years of latent preclinical asymptomatic conditions. Thus, when the disease is full blown, there are limited chances to reverse the disease phenotype; however, it can be managed by controlling diet, changing life style and using medicines. Understanding pathological mechanisms whereby genetic and/or environmental factors contribute to the development of diabetes is important for the prevention and treatment of the disease. In this regard, approaches such as genomics, transcriptomics, proteomics and metabolomics are being applied to identify more specific biomarkers for T2D for its early detection, management and devising new therapies. The emphasis of the present review is to provide updates on the applications of proteomics in addressing T2D with a focus on protein based biomarker discovery. Moreover, the idea of personalized medicine and intervention is also discussed for diabetes treatment in proteomics perspective.

Keywords: Diabetes, proteomics, biomarker, serum, 2DGE, LC-MS.

INTRODUCTION

Diabetes Mellitus (DM)

Diabetes mellitus (DM) is a complex multifactorial metabolic disorder which is characterized by defects in insulin secretion/action or both. Insulin resistance and impairment of insulin secretion lead to hyperglycemia, dyslipidemia and perturbed gene expression and protein functions (Ross *et al*., 2004, Sundsten and Ortsater, 2009). Person, who has fasting plasma glucose (FPG) between ranges of 100 mg/dl (5.6 mmol/l) to 125 mg/dl (6.9 mmol/l) is having impaired fasting glucose (IFG) and is called pre-diabetic whereas, the level above this range is an indicator of clinical diabetes (A.D.A., 2012). The severity and complications of this disease depends upon its uncontrolled duration, which subsequently appears in the form of micro- and macro-vascular complications (Brownlee, 2001). Diabetes as a silent killer cripples 9.5% of the adult population worldwide (Danaei *et al*., 2011). For the last two decades this disease accelerated exponentially and 246 million victims were estimated till 2007. This number is expected to increase up to 380 million by the year 2025 (van Dieren *et al*., 2010, Maris *et al*., 2008). The diabetes prevalence and its adverse health consequences seem similar in South Asian regions as in other parts of the world (Ghaffar *et al*., 2004). According to an estimate, the prevalence of diabetes along with pre-diabetes is about 22-25% in Pakistan (Basit and Shera, 2008). The increasing prevalence of diabetic individuals may be due to the increase of prevalence of obesity and physical inactivity (Sullivan *et al*., 2005, Hossain *et al*., 2007). Once diabetes is developed, it is hard to manage, due to its chronic nature and severity of complications, which require high healthcare costs. Globally, diabetes healthcare costs have been estimated about 232 billion dollars in 2007, which will exceed to 302.5 billion dollars by 2025 (Maris *et al*., 2008).

Major types of diabetes mellitus (DM)

The diabetes is mainly divided into two major types; type 1 diabetes mellitus (T1D) and type 2 diabetes mellitus (T2D). In the case of T1D, destruction of pancreatic beta cells (β-cells) occurs due to autoimmunity (Husebye and Anderson, 2010). Whereas, T2D is a dual disease which may result from insulin resistance (IR) and β-cells dysfunction (Butler *et al*., 2003). The average onset of this T2D is 40 years of age; but it also appears in obese-young adults, under the age of 19 years old (Weill *et al*., 2004, Hatunic *et al*., 2005, Koopman *et al*., 2005). The incidence of this disease is continuously increasing due to change in life style patterns, physical inactivity and high dietary fat intake (Hu *et al*., 2001, Wild *et al*., 2004, Villegas *et al*., 2006). Besides these factors, genetics and epigenetics are also involved in the pathobiology of T2D (Kleivi, 2006, Meigs *et al*., 2008, Ling and Groop, 2009). It is responsible for more than 90% of all cases of diabetic individuals and its earliest sign is a high level of glucose in blood plasma (Steinberger and Daniels, 2003, A.D.A., 2012). The establishment of liver, pancreas, skeletal muscles and adipose tissue roles in the regulation and

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pathogenesis of diabetes is a great milestone in the history of diabetes research (Ross *et al*., 2004, Rosen and Spiegelman, 2006, Suh *et al*., 2007, Donath and Shoelson, 2011). Liver involves in the clearance of hormone insulin and storage of excess glucose in the form of glycogen, whereas pancreas produces insulin, which opens the channels of skeletal muscle cells for glucose uptake (Poy *et al*., 2002, Kotronen *et al*., 2008, Sundsten and Ortsater, 2009). In the case of insulin resistance and/or deficiency, glucose stays in the blood, instead of going into the target cells (Van den Berghe, 2004, Sundsten and Ortsater, 2009). Consequently, hyperglycemia occurs, which predisposes to chronic complications that adversely affect eyes, kidneys, blood vessels and nerves (Brownlee, 2001). Many investigations indicate that the onset of T2D can be regulated by its early detection and intervention by medication and lifestyle (Tuomilehto *et al*., 2001, Knowler *et al*., 2002). In general practice, prediabetes/impaired glucose tolerance and diabetes are diagnosed by a fasting plasma glucose test (FPGT), an oral glucose tolerance test (OGTT), random blood glucose tests (RBGT), glycated hemoglobin tests (HbA1c) etc., (Bonora and Tuomilehto, 2011, Cowie *et al*., 2006, Bennett *et al*., 2007, Saudek *et al*., 2008). These diagnostic tests are late makers where the disease progressed and passed a latent preclinical asymptomatic phase (Okada *et al*., 2010). Once an individual becomes pre-diabetic or diabetic, it is hard to reverse the disease phenotype with available therapies (Maynard *et al*., 2007, Waugh *et al*., 2007, Saudek *et al*., 2008). However, it seems possible to reverse and regulate diabetes development by identifying the signatures of disease at preclinical asymptomatic states (Hanash, 2003, Koehn and Krapfenbauer, 2010). In this context, besides other approaches such as genomics, transcriptomics and metabolomics, biomedical scientists have applied a post-genomic approach, called proteomics in order to uncover factors involved in the development of diabetes (Lim and Elenitoba-Johnson, 2004, Maris *et al*., 2008).

Proteomics

Proteomics is the study of proteome, which intends to cover the entire set of expressed proteins in various tissues of an organism (Anderson, 2005, Hu *et al*., 2006). Every protein is a product of a specific gene. Post translational modifications of proteins alter their conformations leading to the changes of protein structures and functions (Pieper *et al*., 2003, Ordovas and Corella, 2004, Kleivi, 2006). Therefore, only a deoxyribonucleic acid (DNA) sequence is not enough to determine protein structures and functions in the human body. Similarly, protein expression levels may or may not correlate with messenger ribonucleic acid (mRNA) levels due to post transcription regulatory events. The alteration of protein structure, function and interaction is the underlying mechanism of many diseases including diabetes (Lim and Elenitoba-Johnson, 2004, Scott *et al*., 2005, Sundsten and Ortsater, 2009). Proteomics offers detailed studies of various aspects of protein expression, post-translational modifications, their interactions and functions at a global scale, because protein modifications and interactions cannot be detected at genome or transcriptome levels. For these reasons it is argued that most of the diseases can be better explained through proteomic approaches relative to genomic or transcriptomic studies (Aebersold and Mann, 2003, Scott *et al*., 2005). The ultimate goal of proteomics is to identify potential physiological and pathological factors valuable for diagnosis and treatment of diseases (Borrebaeck and Wingren, 2009). Proteins ranging from 300 to 2377 different types have been reported for the physiological and pathophysiological conditions using 2DGE/MS and LC-MS/MS approaches (Pieper *et al*., 2003, Tirumalai *et al*., 2003, Li *et al*., 2008). Furthermore, different proteomic approaches have been developed in order to discover new protein-based biomarkers in various biofluids under different pathophysiological states (Pisitkun *et al*., 2006, Grus *et al*., 2007, Rao *et al*., 2007, Rao *et al*., 2009, Naseeb *et al*., 2008). Concisely, proteomics is a high-throughput approach which provides a chance to look at numerous proteins simultaneously (Issaq *et al*., 2007).

Technical challenges and serum proteomics

Proteomic studies can be divided into three major phases; disease biomarker discovery, validation and development of novel interventional drug therapies. However, biomarker discovery/validation is complicated, as it depends on the maintenance of sample integrity. Thus, to ensure consistent, reliable and reproducible biomarker(s) results, standardized protocols for the collection, handling, storage and processing of samples are pre-requisites for the downstream proteomic analyses (Rai *et al*., 2005, Rai and Vitzthum, 2006). Moreover, broad and dynamic spectrum of serum proteins ranging from milligrams to picograms per milliliter is overwhelming, which makes it extremely challenging to characterize the serum proteome, especially for the low abundant proteins. As in most of the pre-clinical asymptomatic stages, potential protein biomarkers are present only in low abundance (see fig. 2, reviewed by Herder *et al*., 2011). Moreover, low abundant serum protein biomarker(s) are overlapped by the highly abundant proteins such as albumin, immunoglobulin G (IgG), transthyretin, transferrin, alpha-antitrypsin, complement C3, and retinol binding proteins (RBP) etc, (Tirumalai *et al*., 2003). One strategy to overcome this problem is to unmask low abundance proteins by delipidation and depleting highly abundant proteins before proteomic analysis (Merrell *et al*., 2004). Various physical and chemical methods can be used for removing lipids while preserving proteins in the serum. These methods include organic solvents based extractions, lipids absorbents and centrifugation. Centrifugation can effectively remove chylomicrons and very low density lipoproteins (VLDLPs) (Ferraz *et al*., 2004, Fu *et al*., 2005), whereas various chromatographic techniques such as immuno-depletion, ion exchange chromatography, etc have been employed to pre-fractionate highly abundant proteins; thus, reducing the complexity of the samples and allowing the detection of less abundant proteins more convenient (Echan *et al*., 2005, Mischak *et al*., 2007). Moreover, serum protein separation and identification depends upon the application of serum protein depletion columns which remove highly abundant serum proteins and the use of LC-MS technology (Thakur *et al*., 2011, Rathmann and Giani, 2004). However, the disadvantage of this depletion method is the unwanted loss of proteins that are in conjugation with highly abundant carrier proteins. Albumin is a carrier for many lipoproteins, hormones and molecules that are important for biological functions. Thus, the depletion of albumin without prior disruption of linked proteins causes the loss of potential protein based biomarker(s). It has been reported that treatment of serum samples with acetonitrile based buffer is more effective to separate less abundant low molecular proteins from albumin, by reducing the possible loss of potential biomarkers (Kay *et al*., 2008) as shown in fig. 1.

Tools and technologies for proteomic studies

In a broader sense, proteomics is often coupled with a

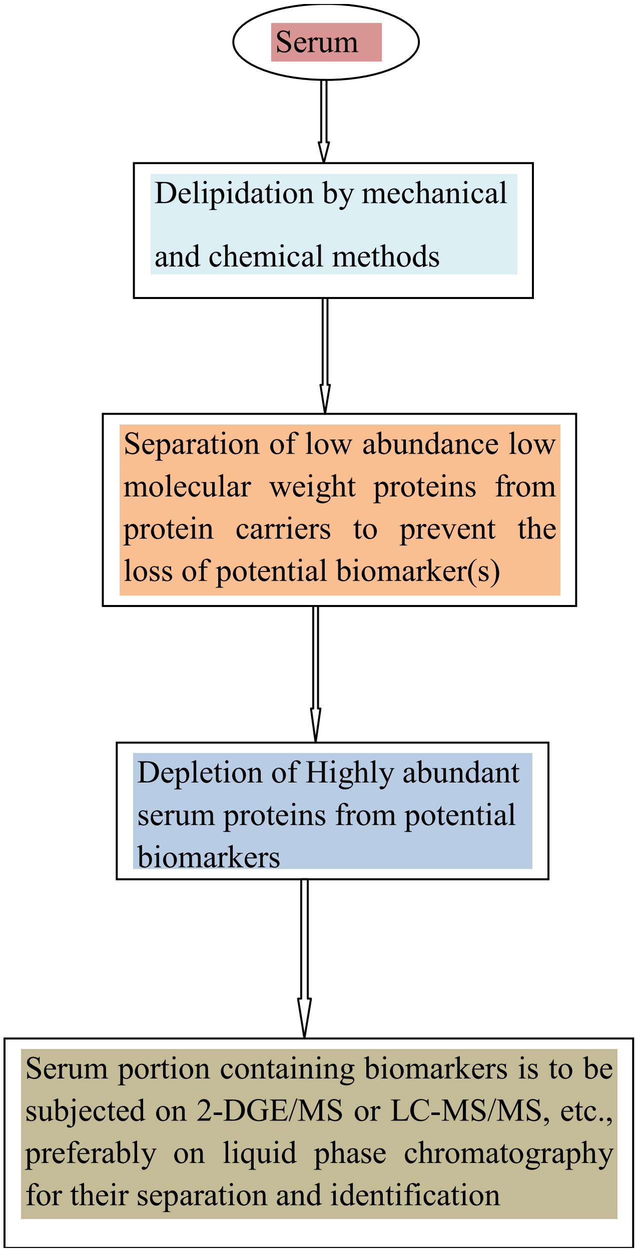

Fig. 1: Schematic work flow for blood serum processing for discovery of protein biomarkers in the less abundant/low molecular weight protein range.
This scheme was adapted from these papers (Fu *et al.,* 2005, Mischak *et al*., 2007, Kay *et al*., 2008)

variety of analytical techniques to analyze protein profiles in biological samples. These technologies include two Dimensional Gel Electrophoresis/Mass Spectrometry (2DGE/MS), and Liquid Chromatography/Mass Spectrometry (LC/MS), LC/MS with Isotope Coded Affinity Tag (ICAT)/Isotope Tags For Relative and Absolute Quantification (ITRAQ), Capillary Electrophoresis-Mass Spectrometry (CE-MS), Matrix Assisted Laser Desorption Ionization-Time Of Flight-Mass Spectrometry (MALDI-TOF-MS), and Surface Enhanced Laser Desorption Ionization-Time Of Flight-Mass Spectrometry (SELDI-TOF-MS), etc. These techniques differ from each other in their detection method, sensitivity, reproducibility, and cost per sample analysis (Miller *et al*., 2001, Aebersold and Mann, 2003, Duncan and Hunsucker, 2005). These mass spectrometric based techniques permit the analysis of hundreds of proteins in a given time with very small sample size from microgram to picogram per milliliter with high sensitivity (Mann *et al*., 2001, Lim and Elenitoba-Johnson, 2004, Tammen *et al*., 2005). Two separating approaches, 2DGE and LC are commonly employed in order to separate the various types of proteins in biofluids or other tissue samples prior to introducing them into MS for their identification (Apweiler *et al*., 2009). These techniques have their own pros and cons; however, both facilitate better understanding of disease pathogenesis (Grebe and Singh, 2011, Marko-Varga and Fehniger, 2004, Tang *et al*., 2005, Mischak *et al*., 2007).

2DGE and LC-MS

Two Dimension Gel Electrophoresis (2DGE) is a gel-based technique for proteins separation in which intact proteins are separated in the first dimension on the basis of their isoelectric points (PI) using thin strips, and then the same gel strips are inverted onto Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), in order to separate proteins in the second dimension according to their molecular masses. Hundreds of proteins can be resolved on one gel and visualized by chemical staining. Differentially expressed protein spot(s) as potential biomarker(s) are then excised, trypsinized and identified with MS.

Liquid Chromatography – Mass Spectrometry (LC-MS) is a gel-free system in which proteolytically digested complex mixture of proteins is separated by liquid chromatography (LC), then on the basis of protein/peptide's physical and chemical characteristics such as pH, hydrophobicity, charges, etc. these peptides are identified by MS or MS/MS. LC-MS is more powerful than 2DGE as it can provide a lot of information based on mass spectra from a complex mixture of proteins for further proteome mining. Moreover, for additional sophisticated analysis of proteins, LC-MS is coupled with isotope labeling strategies such as ICAT, iTRAQ, and stable isotope labeling by amino acids in cell culture (SILAC) etc, allowing for quantification of protein expression levels in different samples. For more technical details of these approaches, these papers are recommended (Rabilloud, 2002, Ahmed *et al*., 2003, Pieper *et al*., 2003, Lim and Elenitoba-Johnson, 2004, Qian *et al*., 2006, Tucholska *et al*., 2009) as a detailed account of these technologies is beyond the scope of present review.

Protein biomarkers

About 0.1 million proteins have been attributed to different cell types, biofluids and organs in the body, which are responsible to perform various types of physiological functions (Wang *et al*., 2006). Fluctuations in their concentrations reflect the health status of an individual. For example, an acute phase protein, called C-reactive protein (CRP), is a marker of inflammation which rises during the course of inflammatory events in the body and an elevated level of leptin is a well-known and established marker of obesity (Shamsuzzaman *et al*., 2004, Bahceci *et al*., 2005, Martin *et al*., 2008). Biomarker(s) can be studied by detecting differences in the patterns of proteins expressed in healthy subjects relative to disease ones. As each tissue is related to a specific function, analysis of the tissue-specific protein expression is important to get insight into the human health and disease (Scott *et al*., 2005). Type 2 diabetes is a multi-organs disease, so it is important to identify circulating proteins involved in communication between various types of body tissues/organs, such as the pancreas, liver, muscle, fat, heart and brain ((Herder *et al*., 2011, Ross *et al*., 2004). It is also speculated that the concentrations of serum proteins may be more useful source in order to detect type 2 diabetes earlier, before the appearance of clinical symptoms (Zhang *et al*., 2010, Festa *et al*., 2002, Koehn and Krapfenbauer, 2010). Identification of differentially expressed serum proteins could be potentially important for the prevention and treatment of T2D by pharmacological intervention of the molecular targets, which trigger the disease onset even before its progression to clinical symptoms (Gahagan and Silverstein, 2003, Colagiuri and Davies, 2009).

Recent findings and therapies for type 2 diabetes using protein based approaches

A few recent reports have described the association of protein biomarkers with type 2 diabetes using a single marker (Cho *et al*., 2006, Choi *et al*., 2008, Ix *et al*., 2008, Erikstrup *et al*., 2009). However, in some further studies diabetes risk has been evaluated using multiple biomarker approaches rather than conventional risk assessment. In one study termed as Inter99, four serum proteins, adiponectin, CRP, ferritin and interleukin-2 receptor agonist (IL-2RA) were determined in addition to glucose and insulin. By using various types of statistical approaches, a diabetes risk model was developed, which incorporated these six markers. This study explored that rather than single biomarker, multi-marker approach using both protein and non-protein based biomarkers are more useful to predict the development of diabetes. In this way a diabetes risk was identified, which can predict a five year risk of modulation in type 2 diabetes. The results of four proteins excluding glucose and insulin in the percent (%) fold differences calculated are shown in table 1 (Kolberg *et al*., 2009).

Similarly, Salomaa and colleagues conducted two independent human population based follow-up cohort studies; in the FINRISK97 study, 31 biomarkers were evaluated, and 10 from these considered in the Health 2000 – a follow-up study; in order to validate the execution of the biomarker score. The studies confirmed adiponectin, apolipoprotein-B (apoB), CRP, interleukin-1 receptor agonist (IL-RA) and ferritin as strongest predictors of incidental type 2 diabetes (Salomaa *et al.*,

Table 1: A summary of differentially expressed proteins in human type 2 diabetes

Sample type	Subjects & numbers	Differential expression of proteins (% fold change in disease relative to controls)	Approach/method	Paper reference
Blood serum	Among 3046 subjects; Non-converters* =472 vs converters** =160	Adiponectin (↓12.16), CRP(↑37.5), ferritin (↑44.92), IL-2RA(↑2.53)	Immunoassays	(Kolberg *et al*., 2009)
Blood serum	Two independent cohort studies: FINRISK97 & Health 2000 study consisted of 417 & 179 incidental diabetes	Adiponectin (↓), CRP (↑), ferritin (↑), IL-1RA (↑)	Immunoassays	(Salomaa *et al*., 2010)
Blood serum	Participants=3189, NFG(n=1897) versus IFG (n=858) & T2D (n=434)	*[NFG vs IFG & NFG vs T2D]* Adiponectin (↓16.8 & ↓32.2), RBP4 (↑5.3& 5.3↑), PA1 (↑43.38 & ↑49.77), Resistin (↑0 & ↑0.38), CRP (↑27.8 & ↑41.23), IL-6 (↑16 & ↑23.8), Ferritin (↑20 &↑33.33), TNF-α (↓ 0.61 & ↓1.22)	Immunoassays	(Wu *et al*., 2011)
Blood serum	Control (n=21) versus T2D (n=29) & T2D with complications (n=21)	*[C vs T2D & C vs T2D+complications]* CRP (↑69.81 & ↑85.58), Cp (↑26.8 & ↑39.81), transferrin (↓11.24 & ↓8.58)	Nephlometry	(Memisogullari and Bakan, 2004)
Blood serum	NGT (N=3) versus 3 T2D (n=3)	Apo-C3 (↑), TTR (↑), Alb (↓), TRA(↓)	SELDI-TOF-MS, & Protein Chip	(Sundsten *et al*., 2006)
Blood serum	NGT(n=20) vs IGT & T2D (n=20 each)	Apo-C3 (↑), Alb (↓), TTR (↓) in family history of diabetes	SELDI-TOF-MS	(Sundsten *et al*., 2008a)
Blood serum	NGT(n=10) vs T2D+ high EIR; NGT vs T2D +T2D+low EIR (n=10) (EIR: early insulin response)	α & β chains of Hb (↓), TTR (↓) in T2D + high EIR; Apo-H↓ in T2D+ low EIR (EIR: early insulin response)	SELDI-TOF-MS	(Sundsten *et al*., 2008b)
Blood plasma	NGT(n=50) & T2D (n=150)	Apo-E (↑802), leptin (↑842), CRP (↑872), Apo-A-1 (↓6.4).	MALDI-TOF-MS	(Riaz *et al*., 2010a)
Muscle biopsies	Non-diabetic lean control versus non-diabetic obese & T2D subjects (n=8 in each group)	15 proteins are differentially expressed including; HSP90 Co-Chaperone-CDC37 (↑), Desmin (↑), α-actinin (↑), TCP-1 (↓), Myosin15 (↑), Isomerase A3 precursor (↑), Cullin homolog-5(↑), etc.	GE & MS/MS	(Hwang *et al*., 2010)
Blood serum	Non-diabetics (n=5) vs T2D (n=50)	ficolin-3 (↑), Apo-A1 (↑), A11 (↑), C-11 (↑), C-111 (↑), Paraoxanase-1 (↓)	LC-MS/MS with LSPAD approach	(Li *et al*., 2008)
Saliva	IGT (n=30) vs T2D (n=10)	A1AT (↑324), CysC (↑222), A2MG (↑223), TTR (↑240), RBP (↑215), FABP (↓255), Complement-C6 (↑475), Carbonic – anhydrase (↑384), Glycogen- phosphorylase (↑347)	2D-LC-MS/MS	(Rao *et al*., 2009)
Urine	NGT (n=9) vs Diabetic Nephropathy (n=33)	VDBP (↑1110), A1-glycoprotein 1 (↑597), A1AT (↑217), Apo-A11 (↑200), PRBP (↓256), A1-antichymotrypsin (↑514), Complement factor B (↑227), Apo-A1 (↓253), Cp-precursor (↓637)	2D-DIGE & LC-MS	(Rao *et al*., 2007)
Urine	Controls (n=50) vs T2D (n=100)	Hp-precursor (↓81.45), alb (↑486.5), AMPB (↓55.25), RBP4 (↑100), TTR (↓30.8), Zn α2 glycoprotein (↑29.23),E-cadherin (↑693) transthyretin, (↓30.8), alpha-1-microglobulin / bikunin precursor (↓55.2)	2D-HPLC & MALDI-TOF-MS	(Riaz *et al*., 2010b)

Symbols and abbreviations: ↑= up-regulation, ↓=down-regulation, n=number, α= alpha, β=beta, C=controls, T2D= type 2 diabetes, NGT=normal glucose tolerance, IFG=impaired fasting glucose, Non-converters* are individuals who did not change as diabetics and converters** are those who developed type 2 diabetes during the five years of follow-up, CRP=C-reactive protein, IL-2RA=interleukin-2-receptor agonist, IL-1RA= interleukin-1-receptor agonist, RBP=retinol binding protein, PA1= plasma angiotensin-1, IL-6 =interleukin-6, TNF-α=tumor necrosis factor- α, Cp= ceruloplasmin, Apo-= apolipoprotein-, TTR=transthyretin, Alb=albumin, TRA=transferrin, FHD= symbol of genetic back history, A1AT= α1-antitrypsin, CysC=cystatin-C, A2MG=α2-macroglobulin, FABP=fatty acid binding protein, VDBP=vitamin D binding protein, A1=alpha-1, AMBP= alpha-1-microglobulin /bikunin precursor, Hp=heptoglobulin, Zn=zinc, PRBP=plastid RNA-binding protein, TCP-1=T-complex protein-1, Hb=hemoglobin, EIR=early insulin response. GE=gel electrophoresis

2010). Wu's group through various immunoassays and biomarker risk scores successfully demonstrated that out of eight, four proteins: adiponectin, plasma angiotensin-1 (PA-1), IL-6 and ferritin were strongly associated with type 2 diabetes (Wu *et al*., 2011).

In this review, percent fold differences of proteins were calculated from previously published literature as biomarkers for diabetes and added in table 1. Hwang's group conducted a study to assess mitochondrial and cytoplasmic patterns of protein sets in skeletal muscles biopsies of lean non-diabetic controls, obese non-diabetic subjects and T2D patients (n=8 in each group). They successfully demonstrated differential expression of fifteen proteins such as desmin and alpha-actinin significantly decreased, conversely, chaperonin subunit (TCP-1), and proteosomes subunits markedly increased in T2D patients and obese subjects, compared to the lean non-diabetic control group. From these results, they found that these proteins have differential expression, which are the signals for different pathways implicated in insulin resistance development (Hwang *et al*., 2010). In another study, three proteins: ceruloplasmin (Cp), transferrin (Trf) and C-reactive protein (CRP) expression was studied in blood serum of human healthy controls (n = 21), T2D patients without complications (group I: n = 29) and T2D with complications (Group II: n =21). Cp and CRP significantly increased in T2D patients with and without complications relative to healthy controls. Conversely, transferrin (Trf) decreased in two disease groups than the control subjects (Memisogullari and Bakan, 2004). The calculated percent fold difference is shown in table 1.

Although protein analysis by conventional techniques improve the understanding regarding T2D pathogenesis, but due to concerns about specificity and sensitivity, proteomics is rapidly moving towards MS based biomarker discovery. Therefore, in the present review, we have also summarized findings from published reports about proteomics applications in diabetes, which were conducted using advanced MS based techniques. As in a proteomic study conducted on 125 T2D patients and 50 non-diabetic healthy age-matched control subjects using ELISA, reverse phase HPLC and MALDI-TOF revealed that Apolipoprotein A1 was 6.4% decreased in T2D patients, whereas Apolipoprotein E, leptin and CRP raised 802, 842 and 872 percent respectively, in blood serum of diabetics relative to the control subjects (Riaz *et al*., 2010a).

Another study was conducted on blood serum samples of three T2D patients and three normal glucose tolerant (NGT) subjects, using Protein Chip arrays and surface enhanced laser desorption-ionization time of flight (SELDI-TOF/MS). The analysis of mass spectra of fifteen proteins showed that five of them decreased; whereas, five protein levels were increased. Subsequently, using peptide mass finger printing (PMF), four differentially expressed proteins were identified. Among these, applipprotein-C3, and transthyretin were up-regulated, whereas albumin and transferrin were down-regulated in T2D patients (Sundsten *et al*., 2006). These researchers also analyzed serum samples from 60 subjects for another proteomic study (Sundsten *et al*., 2008a). Among them 20 were NGT and 20 had IGT and 20 were T2D patients. In this study, 13 proteins showed differential expressions in T2D relative to NGT subjects. Alterations occurred in the levels of three proteins: Apolipoproteins-C3, albumin and transthyretin in serum of T2D patients. Perturbations in the expression levels of former two proteins were due to genetic effects, whereas the latter one altered without the influence of genetic history.

In another study by Li's group LC-MS/MS technique was used, which identified 1377 proteins in five type 2 diabetic patients relative to five age-matched non-diabetic subjects. Furthermore, these were analyzed by using a localized statistics of protein abundance distribution (LSPAD) approach and manifested that out of 1377 proteins, 147 were related to metabolic pathways. By further selection of these proteins, they clearly revealed that Apolipoproteins- A1, A11, C-11 and C-111 (apo-A1, A11, C-11 and C-111) were significantly increased, whereas six proteins including, regulatory factor paraoxanase-1 are required in lipid metabolism, and was significantly decreased in type 2 diabetic subjects. They have also validated that complement activating protein ficolin-3 is activated and up-regulated in serum of diabetes, compared to non-diabetic control individuals (Li *et al*., 2008).

In search for finding non-invasive biomarkers for diabetes, a human study was conducted on saliva of 40 individuals; from these, ten subjects had T2D and 30 were IGT/pre-diabetic subjects. In this study, it was reported that alpha-1-antitrypsin (A1AT), cystatin C (Cys C), alpha-2-macroglobulin (A2MG), transthyretin (TTR), retinol binding protein (RBP), complement-6, carbonic anhydrase and glycogen phosphorylase were up-regulated 324, 222, 223, 240, 215, 475, 384 and 347 percent folds, respectively. Whereas, fatty acid binding protein (FABP) was down-regulated: 255% in saliva of type 2 diabetes compared to pre-diabetic subjects (Rao *et al*., 2009). An earlier study by the Rao's group on type 2 diabetic patients having nephropathy analyzed urine samples of 33 diabetic subjects and 9 healthy controls. In this case differential in gel electrophoresis (DIGE) was used, which identified that seven proteins were up-regulated and four proteins showed down-regulation in urine of diabetic nephropathy patients relative to non-diabetic control subjects (Rao *et al*., 2007). A summary of protein differential expression for this study is given in table 1.

Likewise, a urinary proteomic study was conducted on a Pakistani population consisting of 50 healthy non-

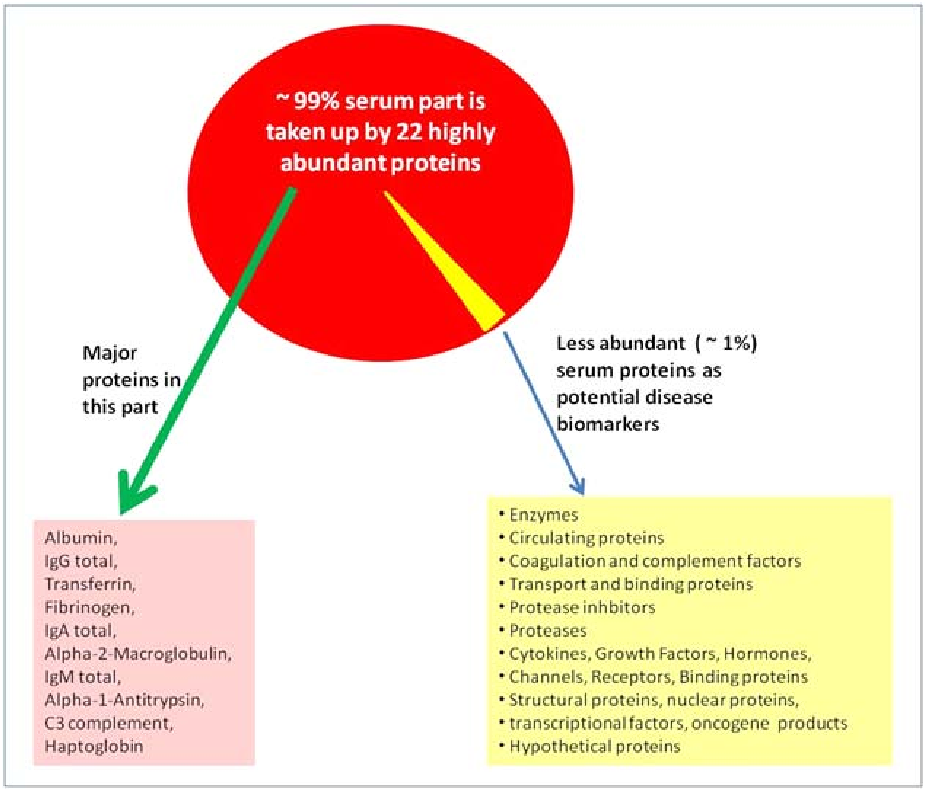

Fig. 2: Serum proteome as a source for potential disease biomarkers.
[~99% serum proteome is constituted by 22 major proteins, while ~1% part contains low abundant proteins which can be mined for disease biomarker discovery. This picture is adapted from (Tirumalai *et al*., 2003).

diabetics and 100 T2D patients. In this study, it was shown that transthyretin (TTR), α-1-microglobulin and heptoglobulin precursor concentrations are decreased by 30.8%, 55.2%, and 81.45% respectively in T2D patients compared to non-diabetic healthy controls. On the other hand, albumin, RBP4, zinc α2-glycoprotein, and E-cadherin were elevated in diabetes relative to healthy control subjects (Riaz *et al*., 2010a). Subsequently, Riaz, *et al* demonstrated thiamine therapeutic effect on the levels of these urinary proteins. The results indicated that albumin decreased significantly up to 34%, whereas TTR, alpha-1-microglobulin/bikunin precursor (AMBP), haptoglobin precursor, RBP4, zinc α2-glycoprotein, and E-cadherin decreased non-significantly in urine of T2D patients (Riaz *et al*., 2011). Goldfine, *et al* summarized various therapeutic strategies for type 2 diabetes regulation (Goldfine *et al*., 2011).

Moreover, in addition to regular physical activity and particular meal patterns, various anti-diabetic drugs, such as sulphonylureas, metformin, rosiglitazone, acarbose, insulin modalities, etc, are prescribed to treat diabetic patients (Moller, 2001). Furthermore, new treatments in the form of incretins, liraglutide, exendin-4 and its derivatives, etc, are also proposed for glucose regulation (Kolterman *et al*., 2003, Madsbad *et al*., 2004, Inzucchi and McGuire, 2008) and summarized in fig. 3. The comparative differential expression of proteins in various biofluids and/or tissues of type 2 diabetic human subjects have also been enlisted in table 1. An analytical look on the summary of aforementioned data show that multiple protein biomarker profiles depend on various parameters, such as genetic family history, age, obesity, gender, etc. In other words, analyses of the results from table 1 lead the idea of individualized therapies. As it is well established that type 2 diabetes is heterogeneous and multi-organs disorder and it is not necessary that all T2D patients respond to given treatments at equal extent (Malandrino and Smith, 2011). Therefore, the concept of personalized medicine seems to be useful to prescribe and design therapies for a particular diabetic group (Klonoff, 2008). In addition to this, cumulative published results as reviewed in this article provide rationale for assessing proteomics as predictive and preventive technology for type 2 diabetes. The approach of multiple biomarker discovery may lead towards the exploration of many new pathways which are involved in the disease pathogenesis. Research based on multi-protein differential expressions can be a useful resource for potential biomarkers, which subsequently paves the way for the development of rationale anti-diabetic medications. Furthermore, the concept of multiple biomarkers with a high throughput

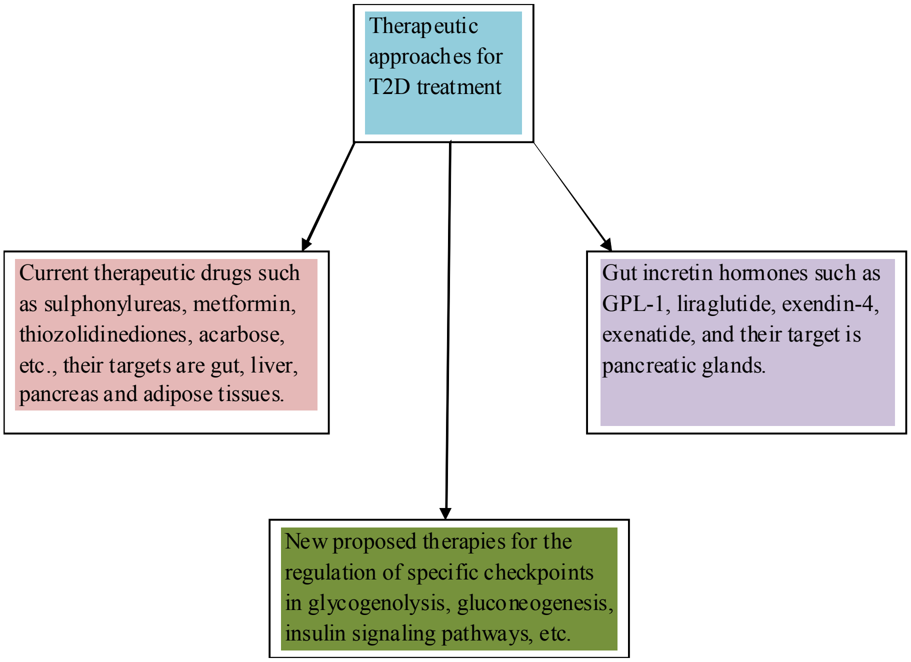

Fig. 3: Overview of current and proposed drugs and therapeutic approaches for type 2 diabetes
Data compiled from these references (Moller, 2001, Kolterman *et al*., 2003, Madsbad *et al*., 2004, Inzucchi and McGuire, 2008).

approach will open new avenues of personalized interventional therapies, as this has also been described for diabetes (Klonoff, 2008, Herder *et al*., 2011).

Drug Discovery for diabetes based on proteomics findings

In expression proteomics, proteins that show variations and modifications in T2D patients relative to non-diabetic controls could become the predictors of disease pathogenesis and be helpful to design specific drugs (Ahn and Simpson, 2007, Wang *et al*., 2009). For example, previous studies demonstrated that two blood serum proteins, vascular cell adhesion molecule-1 (VCAM-1) and resistin are biomarkers of vascular endothelial dysfunction in hypertensive T2D patients (Lozano-Nuevo *et al*., 2011). Similarly, another study showed increased blood serum level of fetuin-A protein in incidental older diabetic individuals. Fetuin-A is secreted by liver and inhibits insulin action by binding with insulin-receptors (Ix *et al*., 2008). Rossing, *et al* found that collagen-derived peptide was consistently decreasing in diabetic individuals, who also had nephropathy (Rossing *et al*., 2008). In nutshell, evidences clearly indicate that new protein based biomarker discovery have the potential to create novel opportunities for the early detection of diabetes, its complications and disease therapeutic targets. However, the protein marker level in the blood is too low, and in order to detect it, ultra-sensitive technology such as MS is required (Liu *et al*., 2005, Ackermann and Berna, 2007). Currently, the MS, along with other separation techniques, 2DGE, LC, etc, are being commonly used for the analysis of proteins in blood serum to identify protein based biomarker(s), which can be translated into clinical and drug discovery (Chevalier, 2010, Apweiler *et al*., 2009). Briefly, the early detection of alteration in serum/plasma protein differential expression at preclinical states will provide a better chance to cure an individual with early intervention (Okada *et al*., 2010). Furthermore, due to the heterogeneous nature of diabetes the drug discovery and development is rapidly turning towards personalized poly-pharmacotherapy (Ouzounian *et al*., 2007).

Future directions

Diabetes is becoming an alarming threat for human health at a global scale. Therefore, in a worldwide scenario, its prevention and management has become the top priority of physicians and scientists. Diagnosis is the first step for screening T2D patients among non-diabetic healthy individuals. Existing diagnostic tests such as HbA1c, OGTT and FBG, etc, are relatively late stage markers where the disease has already progressed to cause substantial damage to the human body. However, before the onset of clinical symptoms, diabetes passes through an asymptomatic phase, which is called preclinical state. This pre-symptomatic period prior to the disease onset provides a number of opportunities for disease prevention if diagnosed accurately using molecular markers. Available therapies are not enough to prevent and reverse the disease phenotype once it has passed the asymptomatic phase, and the appearance of clinical features such as elevation of fasting blood glucose, etc. However, it seems possible that early detection of diabetes at an asymptomatic stage might be useful to prevent or delay T2D development. Once an individual is diagnosed early, before the onset of diabetes, the magnitude of anti-diabetic drugs efficacy may be increased and enough to prevent type 2 diabetes pathogenesis. In addition to this, early prediction of T2D may open new lines for early interventions. In this regard, researchers use different approaches such as genomics, transcriptomics, proteomics and metabolomics to unravel the enigma of early diabetes detection. Among these –omics technologies; proteomics could offer a great deal for this purpose, because proteins accomplish diversified biological activities and physiological functions. Aforementioned summary of previous proteomic investigations clearly indicate the potential of proteomics to uncover disease biomarkers, drug targets and designing of therapeutic molecules for diabetes.

Investigators used different biological samples for biomarkers discovery. Among these sample types, blood serum seems exceptional and an ideal specimen, because of its constant circulation and perfusion in almost all body parts. Due to this, it seems to hold numerous disease biomarkers in the form of various proteins, and most likely mediates overall body metabolic perturbations; whereas, other sample types are tissue-specific and may reflect pathogenesis of a particular tissue type. Despite these facts; the complex nature of serum proteome holds major issues, including reproducibility of results between laboratories regarding biomarkers discovery and validation. This issue may be resolved by adopting uniform protocols from sample collection to proteomic analyses. On the basis of previously published studies, it can be inferred that the challenges and problems while doing serum proteomic studies can be overcome by; (1) Blood serum delipidation; (2) Disruption of low abundant proteins from high molecular weight carriers, such as albumin, by reducing the possible loss of disease biomarker(s); (3) Depletion of highly abundant high molecular weight serum proteins; and (4) Serum portion containing disease biomarkers is to be subjected to 2-DGE/MS or LC-MS/MS, etc., preferably on liquid phase chromatography for their separation and characterization. This method is summarized in fig. 1.

To the best of our knowledge, yet proteomic based biomarker(s) is/are not routinely used in clinical laboratory tests and translating research findings into reliable diagnostic assays hold a lot of future activity in this area. This indicates that there are still some unresolved issues and challenges for them to be valid, reliable, reproducible biomarkers and early detection of diabetes via, the proteomic approach. Pertinent to this, more large scale prospective follow-up proteomic studies are needed. These studies would be beneficial to developing early interventional therapies for individuals who are at risk to develop diabetes in the future. Since, protein expressions are modified by various factors such as, age, race, genetic history, diet, gender etc., therefore, personalized interventional therapies specific to a patient or particular group would be more effective rather generalizing treatment for the whole population of complex disorders like diabetes.

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REFERENCES

ADA (2012). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, **35**(Suppl 1): S64-S71.

Ackermann BL and Berna MJ (2007). Coupling immunoaffinity techniques with MS for quantitative analysis of low-abundance protein biomarkers. *Expert Rev. Proteomics*, **4**: 175-186.

Aebersold R and Mann M (2003). Mass spectrometry-based proteomics. *Nature*, **422**: 198-207.

Ahmed N, Barker G, Oliva K, Garfin D, Talmadge K, Georgiou H, Quinn M and Rice G (2003). An approach to remove albumin for the proteomic analysis of low abundance biomarkers in human serum. *Proteomics*, **3**: 1980-1987.

Ahn SM and Simpson RJ (2007). Body fluid proteomics: Prospects for biomarker discovery. *Proteomics Clin. Appl.*, **1**: 1004-1015.

Anderson L (2005). Candidate-based proteomics in the search for biomarkers of cardiovascular disease. *J. Physiol.*, **563**: 23-60.

Apweiler R, Aslanidis C, Deufel T, Gerstner A, Hansen J, Hochstrasser D, Kellner R, Kubicek M, Lottspeich F, Maser E, Mewes HW, Meyer HE, Mullner S, Mutter W, Neumaier M, Nollau P, Nothwang HG, Ponten F, Radbruch A, Reinert K, Rothe G, Stockinger H, Tarnok A, Taussig MJ, Thiel A, Thiery J, Ueffing M, Valet G, Vandekerckhove J, Verhuven W, Wagener C, Wagner O and Schmitz G (2009). *In*: *Clin. Chem. Lab. Med.*, **47**: 724-744.

Bahceci M, Tuzcu A, Ogun C, Canoruc N, Iltimur K and Aslan C (2005). Is serum C-reactive protein concentration correlated with HbA1c and insulin resistance in Type 2 diabetic men with or without coronary heart disease? *J. Endocrinol. Invest.*, **28**: 145-150.

Basit A and Shera AS (2008). Prevalence of metabolic syndrome in Pakistan. *Metab. Syndr. Relat. Disord.*, **6**: 171-175.

Bennett CM, Guo M and Dharmage SC (2007). HbA(1c) as a screening tool for detection of Type 2 diabetes: A systematic review. *Diabet. Med.*, **24**: 333-343.

Bonora E and Tuomilehto J (2011). The pros and cons of diagnosing diabetes with A1C. *Diabetes Care*, **34**(Suppl 2): S184-S190.

Borrebaeck CA and Wingren C (2009). Transferring proteomic discoveries into clinical practice. *Expert Rev. Proteomics*, **6**: 11-13.

Brownlee M (2001). Biochemistry and molecular cell biology of diabetic complications. *Nature*, **414**: 813-820

Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA and Butler PC (2003). Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes*, **52**: 102-110.

Chevalier F (2010). Highlights on the capacities of "Gel-based" proteomics. *Proteome Sci.*, **8**: 23.

Cho YM, Youn BS, Lee H, Lee N, Min SS, Kwak SH, Lee HK and Park KS (2006). Plasma retinol-binding protein-4 concentrations are elevated in human subjects with impaired glucose tolerance and type 2 diabetes. *Diabetes Care*, **29**: 2457-2461.

Choi SH, Kwak SH, Youn BS, Lim S, Park YJ, Lee H, Lee N, Cho YM, Lee HK, Kim YB, Park KS and Jang HC (2008). High plasma retinol binding protein-4 and low plasma adiponectin concentrations are associated with severity of glucose intolerance in women with previous gestational diabetes mellitus. *J. Clin. Endocrinol. Metab.,* **93**: 3142-3148.

Colagiuri S and Davies D (2009). The value of early detection of type 2 diabetes. *Curr. Opin. Endocrinol. Diabetes Obes.*, **16**: 95-99.

Cowie CC, Rust KF, Byrd-Holt DD, Eberhardt MS, Flegal KM, Engelgau MM, Saydah SH, Williams DE, Geiss LS and Gregg EW (2006). Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population: National Health And Nutrition Examination Survey 1999-2002. *Diabetes Care*, **29**: 1263-1268.

Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, Lin JK, Farzadfar F, Khang YH, Stevens GA, Rao M, Ali MK, Riley LM, Robinson CA and Ezzati M (2011). National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. *Lancet,* **378**: 31-40.

Donath MY and Shoelson SE (2011). Type 2 diabetes as an inflammatory disease. *Nature Review Immunology,* **11**: 98-107.

Duncan MW and Hunsucker SW (2005). Proteomics as a tool for clinically relevant biomarker discovery and validation. *Exp. Biol. Med.* (Maywood), **230**: 808-817.

Echan LA, Tang HY, Ali-Khan N, Lee K and Speicher DW (2005). Depletion of multiple high-abundance proteins improves protein profiling capacities of human serum and plasma. *Proteomics*, **5**: 3292-3303.

Erikstrup C, Mortensen OH, Nielsen AR, Fischer CP, Plomgaard P, Petersen AM, Krogh-Madsen R, Lindegaard B, Erhardt JG, Ullum H, Benn CS and Pedersen BK (2009). RBP-to-retinol ratio, but not total RBP, is elevated in patients with type 2 diabetes. *Diabetes Obes. Metab.,* **11**: 204-212.

Ferraz TP, Fiuza MC, Dos Santos ML, Pontes De Carvalho L and Soares NM (2004). Comparison of six methods for the extraction of lipids from serum in terms of effectiveness and protein preservation. *J. Biochem. Biophys. Methods*, **58**: 187-193.

Festa A, D'Agostino R, Jr., Tracy RP and Haffner SM (2002). Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes*, **51**: 1131-1137.

Fu Q, Garnham CP, Elliott ST, Bovenkamp DE and Van Eyk JE (2005). A robust, streamlined, and reproducible method for proteomic analysis of serum by delipidation, albumin and IgG depletion, and two-dimensional gel electrophoresis. *Proteomics*, **5**: 2656-2664.

Gahagan S and Silverstein J (2003). Prevention and treatment of type 2 diabetes mellitus in children, with special emphasis on American Indian and Alaska Native children. American Academy of Pediatrics Committee on Native American Child Health. *Pediatrics*, **112**: e328.

Ghaffar A, Reddy KS and Singhi M (2004). Burden of non-communicable diseases in South Asia. *BJM,* **328**:807-10.

Goldfine AB, Fonseca V and Shoelson SE (2011). Therapeutic approaches to target inflammation in type 2 diabetes. *Clin. Chem.,* **57**: 162-167.

Grebe SK and Singh RJ (2011). LC-MS/MS in the Clinical Laboratory - Where to From Here? *Clin. Biochem. Rev.,* **32**: 5-31.

Grus FH, Joachim SC and Pfeiffer N (2007). Proteomics in ocular fluids. *Proteomics Clin. Appl.,* **1**: 876-888.

Hanash S (2003). Disease proteomics. *Nature*, **422**: 226-232.

Hatunic M, Burns N, Finucane F, Mannion C and Nolan JJ (2005). Contrasting clinical and cardiovascular risk status between early and later onset type 2 diabetes. *Diab. Vasc. Dis. Res.,* **2**: 73-75.

Herder C, Karakas M and Koenig W (2011). Biomarkers for the prediction of type 2 diabetes and cardiovascular disease. *Clin. Pharmacol. Ther.,* **90**: 52-66.

Hossain P, Kawar B and El Nahas M (2007). Obesity and diabetes in the developing world – A growing challenge. *N. Engl. J. Med.,* **356**: 213-215.

Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG and Willett WC (2001). Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *N. Engl. J. Med.,* **345**: 790-797.

Hu S, Loo JA and Wong DT (2006). Human body fluid proteome analysis. *Proteomics*, **6**: 6326-6353.

Husebye ES and Anderson MS (2010). Autoimmune polyendocrine syndromes: Clues to type 1 diabetes pathogenesis. *Immunity*, **32**: 479-487.

Hwang H, Bowen BP, Lefort N, Flynn CR, De Filippis EA, Roberts C, Smoke CC, Meyer C, Hojlund K, Yi Z and Mandarino LJ (2010). Proteomics analysis of human skeletal muscle reveals novel abnormalities in obesity and type 2 diabetes. *Diabetes*, **59**: 33-42.

Inzucchi SE and McGuire DK (2008). New drugs for the treatment of diabetes: part II: Incretin-based therapy and beyond. *Circulation*, **117**: 574-584.

Issaq HJ, Xiao Z and Veenstra TD (2007). Serum and plasma proteomics. *Chem. Rev.,* **107**: 3601-3620.

Ix JH, Wassel CL, Kanaya AM, Vittinghoff E, Johnson KC, Koster A, Cauley JA, Harris TB, Cummings SR and Shlipak MG (2008). Fetuin-A and incident diabetes mellitus in older persons. *JAMA,* **300**: 182-188.

Kay R, Barton C, Ratcliffe L, Matharoo-Ball B, Brown P, Roberts J, Teale P and Creaser C (2008). Enrichment of low molecular weight serum proteins using acetonitrile precipitation for mass spectrometry based proteomic analysis. *Rapid Commun. Mass Spectrom.,* **22**: 3255-3260.

Kleivi K (2006). Advances in the genetics and epigenetics of gene regulation and human disease. *Genome Biol.,* **7**: 325.

Klonoff DC (2008). Personalized medicine for diabetes. *J. Diabetes Sci. Technol.,* **2**: 335-341.

Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA and Nathan DM (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N. Engl. J. Med.,* **346**: 393-403.

Koehn J and Krapfenbauer K (2010). Advanced proteomics procedure as a detection tool for predictive screening in type 2 pre-Diabetes. *The EPMA Journal*, **1**: 19-31

Kolberg JA, Jorgensen T, Gerwien RW, Hamren S, McKenna MP, Moler E, Rowe MW, Urdea MS, Xu XM, Hansen T, Pedersen O and Borch-Johnsen K (2009). Development of a type 2 diabetes risk model from a panel of serum biomarkers from the Inter99 cohort. *Diabetes Care*, **32**: 1207-1212.

Kolterman OG, Buse JB, Fineman MS, Gaines E, Heintz S, Bicsak TA, Taylor K, Kim D, Aisporna M, Wang Y and Baron AD (2003). Synthetic exendin-4 (exenatide) significantly reduces postprandial and fasting plasma glucose in subjects with type 2 diabetes. *J. Clin. Endocrinol. Metab.,* **88**: 3082-3089.

Koopman RJ, Mainous AG, 3rd, Diaz VA and Geesey ME (2005). Changes in age at diagnosis of type 2 diabetes mellitus in the United States, 1988 to 2000. *Ann. Fam. Med.,* **3**: 60-63.

Kotronen A, Juurinen L, Tiikkainen M, Vehkavaara S and Yki-Jarvinen H (2008). Increased liver fat, impaired insulin clearance, and hepatic and adipose tissue insulin resistance in type 2 diabetes. *Gastroenterology*, **135**: 122-130.

Li RX, Chen HB, Tu K, Zhao SL, Zhou H, Li SJ, Dai J, Li QR, Nie S, Li YX, Jia WP, Zeng R and Wu JR (2008). Localized-statistical quantification of human serum proteome associated with type 2 diabetes. *PLoS One,* **3**: e3224.

Lim MS and Elenitoba-Johnson KS (2004). Proteomics in pathology research. *Lab. Invest.,* **84**: 1227-1244.

Ling C and Groop L (2009). Epigenetics: a molecular link between environmental factors and type 2 diabetes. *Diabetes*, **58**: 2718-2725.

Liu J, Carrillo B, Yanofsky C, Beaudrie C, Morales F and Kearney R (2005). A novel approach to speed up peptide sequencing via MS/MS spectra analysis. *Conf. Proc. IEEE Eng. Med. Biol. Soc.,* **4**: 4441-4444.

Lozano-Nuevo JJ, Estrada-Garcia T, Vargas-Robles H, Escalante-Acosta BA and Rubio-Guerra AF (2011). Correlation between circulating adhesion molecules and resistin levels in hypertensive type-2 diabetic patients. *Inflamm. Allergy Drug Targets,* **10**: 27-31.

Madsbad S, Schmitz O, Ranstam J, Jakobsen G and Matthews DR (2004). Improved glycemic control with no weight increase in patients with type 2 diabetes after once-daily treatment with the long-acting glucagon-like peptide 1 analog liraglutide (NN2211): A 12-week, double-blind, randomized, controlled trial. *Diabetes Care*, **27**: 1335-1342.

Malandrino N and Smith RJ (2011). Personalized medicine in diabetes. *Clin. Chem.,* **57**: 231-240.

Mann M, Hendrickson RC and Pandey A (2001). Analysis of proteins and proteomes by mass spectrometry. *Annu. Rev. Biochem.,* **70**: 437-473.

Maris M, Overbergh L and Mathieu C (2008). Type 2 diabetes: Gaining insight into the disease process using proteomics. *Proteomics Clin. Appl.,* **2**: 312-326.

Marko-Varga G and Fehniger TE (2004). Proteomics and disease – The challenges for technology and discovery. *J. Proteome Res.,* **3**: 167-178.

Martin SS, Qasim A and Reilly MP (2008). Leptin resistance: A possible interface of inflammation and metabolism in obesity-related cardiovascular disease. *J. Am. Coll. Cardiol.,* **52**: 1201-1210.

Maynard JD, Rohrscheib M, Way JF, Nguyen CM and Ediger MN (2007). Noninvasive type 2 diabetes screening: Superior sensitivity to fasting plasma glucose and A1C. *Diabetes Care*, **30**: 1120-1124.

Meigs JB, Shrader P, Sullivan LM, McAteer JB, Fox CS, Dupuis J, Manning AK, Florez JC, Wilson PW, D'Agostino RB, Sr. and Cupples LA (2008). Genotype score in addition to common risk factors for prediction of type 2 diabetes. *N. Engl. J. Med.,* **359**: 2208-2219.

Memisogullari R and Bakan E (2004). Levels of ceruloplasmin, transferrin, and lipid peroxidation in the serum of patients with Type 2 diabetes mellitus. *J. Diabetes Complications*, **18**: 193-197.

Merrell K, Southwick K, Graves SW, Esplin MS, Lewis NE and Thulin CD (2004). Analysis of low-abundance, low-molecular-weight serum proteins using mass spectrometry. *J. Biomol. Tech.,* **15**: 238-248.

Miller KJ, Bowsher RR, Celniker A, Gibbons J, Gupta S, Lee JW, Swanson SJ, Smith WC and Weiner RS (2001). Workshop on bioanalytical methods validation for macromolecules: Summary report. *Pharm. Res.,* **18**: 1373-1383.

Mischak H, Julian BA and Novak J (2007). High-resolution proteome/peptidome analysis of peptides and low-molecular-weight proteins in urine. *Proteomics Clin. Appl.,* **1**: 792.

Moller DE (2001). New drug targets for type 2 diabetes and the metabolic syndrome. *Nature*, **414**: 821-827.

Naseeb U, Shafqat J, Jagerbrink T, Zarina S, Alvestrand A, Jornvall H and Axelsson J (2008). Proteome patterns in uremic plasma. *Blood Purif*, **26**: 561-568.

Okada S, List EO, Sankaran S and Kopchick JJ (2010). Plasma protein biomarkers correlated with the development of diet-induced Type 2 diabetes in mice. *Clin. Proteomics*, **6**: 6-17.

Ordovas JM and Corella D (2004). Nutritional genomics. *Annu. Rev. Genomics Hum. Genet.,* **5**: 71-118.

Ouzounian M, Lee DS, Gramolini AO, Emili A, Fukuoka M and Liu PP (2007). Predict, prevent and personalize: Genomic and proteomic approaches to cardiovascular medicine. *Can. J. Cardiol.,* **23**(Suppl A): 28A-33A.

Pieper R, Gatlin CL, Makusky AJ, Russo PS, Schatz CR, Miller SS, Su Q, McGrath AM, Estock MA, Parmar PP, Zhao M, Huang ST, Zhou J, Wang F, Esquer-Blasco R, Anderson NL, Taylor J and Steiner S (2003). The human serum proteome: Display of nearly 3700 chromatographically separated protein spots on two-dimensional electrophoresis gels and identification of 325 distinct proteins. *Proteomics,* **3**: 1345-1364.

Pisitkun T, Johnstone R and Knepper MA (2006). Discovery of urinary biomarkers. *Mol. Cell Proteomics,* **5**: 1760-1771.

Poy MN, Yang Y, Rezaei K, Fernstrom MA, Lee AD, Kido Y, Erickson SK and Najjar SM (2002). CEACAM1 regulates insulin clearance in liver. *Nat. Genet.,* **30**: 270-276.

Qian WJ, Jacobs JM, Liu T, Camp DG, 2nd and Smith RD (2006). Advances and challenges in liquid chromatography-mass spectrometry-based proteomics profiling for clinical applications. *Mol. Cell Proteomics*, **5**: 1727-1744.

Rabilloud T (2002). Two-dimensional gel electrophoresis in proteomics: Old, old fashioned, but it still climbs up the mountains. *Proteomics*, **2**: 3-10.

Rai AJ, Gelfand CA, Haywood BC, Warunek DJ, Yi J, Schuchard MD, Mehigh RJ, Cockrill SL, Scott GB, Tammen H, Schulz-Knappe P, Speicher DW, Vitzthum F, Haab BB, Siest G and Chan DW (2005). HUPO Plasma Proteome Project specimen collection and handling: Towards the standardization of parameters for plasma proteome samples. *Proteomics*, **5**: 3262-3277.

Rai AJ and Vitzthum F (2006). Effects of preanalytical variables on peptide and protein measurements in human serum and plasma: Implications for clinical proteomics. *Expert Rev. Proteomics*, **3**: 409-426.

Rao PV, Lu X, Standley M, Pattee P, Neelima G, Girisesh G, Dakshinamurthy KV, Roberts CT, Jr. and Nagalla SR (2007). Proteomic identification of urinary biomarkers of diabetic nephropathy. *Diabetes Care*, **30**: 629-637.

Rao PV, Reddy AP, Lu X, Dasari S, Krishnaprasad A, Biggs E, Roberts CT and Nagalla SR (2009). Proteomic identification of salivary biomarkers of type-2 diabetes. *J. Proteome Res.,* **8**: 239-245.

Rathmann W and Giani G (2004). Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care*, **27**: 2568-2569 (author reply 2569).

Riaz S, Alam SS and Akhtar MW (2010a). Proteomic identification of human serum biomarkers in diabetes mellitus type 2. *J. Pharm. Biomed. Anal.,* **51**: 1103-1107.

Riaz S, Alam SS, Srai SK, Skinner V, Riaz A and Akhtar MW (2010b). Proteomic identification of human urinary biomarkers in diabetes mellitus type 2. *Diabetes Technol. Ther.,* **12**: 979-988.

Riaz S, Skinner V and Srai SK (2011). Effect of high dose thiamine on the levels of urinary protein biomarkers in diabetes mellitus type 2. *J. Pharm. Biomed. Anal.,* **54**: 817-825.

Rosen ED and Spiegelman BM (2006). Adipocytes as regulators of energy balance and glucose homeostasis. *Nature*, **444**: 847-853.

Ross SA, Gulve EA and Wang M (2004). Chemistry and biochemistry of type 2 diabetes. *Chem. Rev.,* **104**: 1255-1282.

Rossing K, Mischak H, Rossing P, Schanstra JP, Wiseman A and Maahs DM (2008). The urinary proteome in diabetes and diabetes-associated complications: New ways to assess disease progression and evaluate therapy. *Proteomics Clin. Appl.,* **2**: 997-1007.

Salomaa V, Havulinna A, Saarela O, Zeller T, Jousilahti P, Jula A, Muenzel T, Aromaa A, Evans A, Kuulasmaa K and Blankenberg S (2010). Thirty-one novel biomarkers as predictors for clinically incident diabetes. *PLoS One*, **5**: e10100.

Saudek CD, Herman WH, Sacks DB, Bergenstal RM, Edelman D and Davidson MB (2008). A new look at screening and diagnosing diabetes mellitus. *J. Clin. Endocrinol. Metab.,* **93**: 2447-2453.

Scott EM, Carter AM and Findlay JB (2005). The application of proteomics to diabetes. *Diab. Vasc. Dis. Res.,* **2**: 54-60.

Shamsuzzaman AS, Winnicki M, Wolk R, Svatikova A, Phillips BG, Davison DE, Berger PB and Somers VK (2004). Independent association between plasma leptin and C-reactive protein in healthy humans. *Circulation*, **109**: 2181-2185.

Steinberger J and Daniels SR (2003). Obesity, insulin resistance, diabetes, and cardiovascular risk in children: an American Heart Association scientific statement from the Atherosclerosis, Hypertension, and Obesity in the Young Committee (Council on Cardiovascular Disease in the Young) and the Diabetes Committee (Council on Nutrition, Physical Activity, and Metabolism). *Circulation*, **107**: 1448-1453.

Suh SH, Paik IY and Jacobs K (2007). Regulation of blood glucose homeostasis during prolonged exercise. *Mol. Cells*, **23**: 272-279.

Sullivan PW, Morrato EH, Ghushchyan V, Wyatt HR and Hill JO (2005). Obesity, inactivity, and the prevalence of diabetes and diabetes-related cardiovascular comorbidities in the U.S., 2000-2002. *Diabetes Care*, **28**: 1599-1603.

Sundsten T, Eberhardson M, Goransson M and Bergsten P (2006). The use of proteomics in identifying differentially expressed serum proteins in humans with type 2 diabetes. *Proteome Sci*., **4**: 22.

Sundsten T and Ortsater H (2009). Proteomics in diabetes research. *Mol. Cell Endocrinol.,* **297**: 93-103.

Sundsten T, Ostenson CG and Bergsten P (2008a). Serum protein patterns in newly diagnosed type 2 diabetes mellitus – Influence of diabetic environment and family history of diabetes. *Diabetes Metab. Res. Rev.,* **24**: 148-154.

Sundsten T, Zethelius B, Berne C and Bergsten P (2008b). Plasma proteome changes in subjects with Type 2 diabetes mellitus with a low or high early insulin response. *Clin. Sci.* (Lond), **114**: 499-507.

Tammen H, Schulte I, Hess R, Menzel C, Kellmann M, Mohring T and Schulz-Knappe P (2005). Peptidomic analysis of human blood specimens: Comparison between plasma specimens and serum by differential peptide display. *Proteomics*, **5**: 3414-3422.

T ang HY, Ali-Khan N, Echan LA, Levenkova N, Rux JJ and Speicher DW (2005). A novel four-dimensional strategy combining protein and peptide separation methods enables detection of low-abundance proteins in human plasma and serum proteomes. *Proteomics*, **5**: 3329-3342.

Thakur SS, Geiger T, Chatterjee B, Bandilla P, Frohlich F, Cox J and Mann M (2011). Deep and highly sensitive proteome coverage by LC-MS/MS without prefractionation. *Mol. Cell Proteomics*, **10**: M110 003699.

Tirumalai RS, Chan KC, Prieto DA, Issaq HJ, Conrads TP and Veenstra TD (2003). Characterization of the low molecular weight human serum proteome. *Mol. Cell Proteomics*, **2**: 1096-1103.

Tucholska M, Bowden P, Jacks K, Zhu P, Furesz S, Dumbrovsky M and Marshall J (2009). Human serum proteins fractionated by preparative partition chromatography prior to LC-ESI-MS/MS. *J. Proteome Res*., **8**: 1143-1155.

Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V and Uusitupa M (2001). Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N. Engl. J. Med.,* **344**: 1343-1350.

Van den Berghe G (2004). How does blood glucose control with insulin save lives in intensive care? *J. Clin. Invest.,* **114**: 1187-1195.

van Dieren S, Beulens JW, van der Schouw YT, Grobbee DE and Neal B (2010). The global burden of diabetes and its complications: an emerging pandemic. *Eur. J. Cardiovasc. Prev. Rehabil.,* **17**(Suppl 1): S3-S8.

Villegas R, Shu XO, Li H, Yang G, Matthews CE, Leitzmann M, Li Q, Cai H, Gao YT and Zheng W (2006). Physical activity and the incidence of type 2 diabetes in the Shanghai women's health study. *Int. J. Epidemiol.,* **35**: 1553-1562.

Wang J, Li D, Dangott LJ and Wu G (2006). Proteomics and its role in nutrition research. *J. Nutr.,* **136**: 1759-1762.

Wang Z, Park K, Comer F, Hsieh-Wilson LC, Saudek CD and Hart GW (2009). Site-specific GlcNAcylation of human erythrocyte proteins: Potential biomarker(s) for diabetes. *Diabetes*, **58**: 309-317.

Waugh N, Scotland G, McNamee P, Gillett M, Brennan A, Goyder E, Williams R and John A (2007). Screening for type 2 diabetes: Literature review and economic modelling. *Health Technol. Assess.*, **11**: iii-iv, ix-xi, 1-125.

Weill J, Vanderbecken S and Froguel P (2004). Understanding the rising incidence of type 2 diabetes in adolescence. *Arch. Dis. Child*, **89**: 502-504.

Wild S, Roglic G, Green A, Sicree R and King H (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*, **27**: 1047-1053.

Wu H, Yu Z, Qi Q, Li H, Sun Q and Lin X (2011). Joint analysis of multiple biomarkers for identifying type 2 diabetes in middle-aged and older Chinese: A cross-sectional study. *BMJ Open*, **1**: e000191.

Zhang SX, Sun H, Sun WJ, Jiao GZ and Wang XJ (2010). Proteomic study of serum proteins in a type 2 diabetes mellitus rat model by Chinese traditional medicine Tianqi Jiangtang Capsule administration. *J. Pharm. Biomed. Anal.,* **53**: 1011-1014.