Development of Innovative technologies to detect long-term diabetes using Human toenail by Vibrational spectroscopy

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Aim and Justification

- The aim and objective of the study is to investigate the extracellular damage in diabetes and compared in normal subjects using toenail and its impact on human body is determined by various aspects of biochemical parameters to be correlated.

- The justification of the spectral studies is non invasive and we can predict before encountering (prone chances of diabetes) diabetes and metabolic syndromes.

- Found significant changes in prolong diabetic specimens.

Risk and Outcome

- There is no risk to patients during sampling and whole study. The name of patients and clinical data's are undisclosed to general public. After coding it will be utilized for publication and other purposes.

- The outcome benefits to the subjects about their health status and prone chances to diabetes are avoided by changing diet and physical exercise and counseling will be given to stressed individuals to avoid the SI-diabetes.

- The statistical data of this study will help the epidemiological and preventive measures of the diabetes nationwide.

Specific Objective

- Identification of structural symptoms in long-term diabetes.
- Investigation of the blood parameters and structural changes in toenails with reference to diabetes in multi system disorder.
- Management of diabetes in early stages by diet and physical exercise.

Materials and methods

- Source of data: The prospective study includes diabetic and nondiabetic patients of
  - ESI – Vaniyambadi Dispensary
  - ESI – Ambur Dispensary
  - ESI – Pernambut and Gudiyattam Dispensary.

Duration: May 2014 – May 2015.

However this work is permitted by Tamilnadu Govt. Letter No. 13577/F1/2014-1, health and Family Welfare Department, dt-28.05.2014.

DMS- ESI, Ref No. 167/ESI/P1/2014.

Recommended by IEC, CSIR-CLRI (IEC/2010-2015/001)

Sample size: Multicentre together with 400 cases and extended to get Statistical Significance of each group.

Methodology:

- The protocol is designed by gender wise with different age group with three categories.
- Male and female with age group between 18 - 60 and above.
- The toenail sample collection is quite simple by scraping the nail using sterile blades and It is utilized for spectroscopic analysis.
- The biochemical and structural parameters of the normal, diabetic and prone to diabetic to be correlated with spectroscopic revealed data.

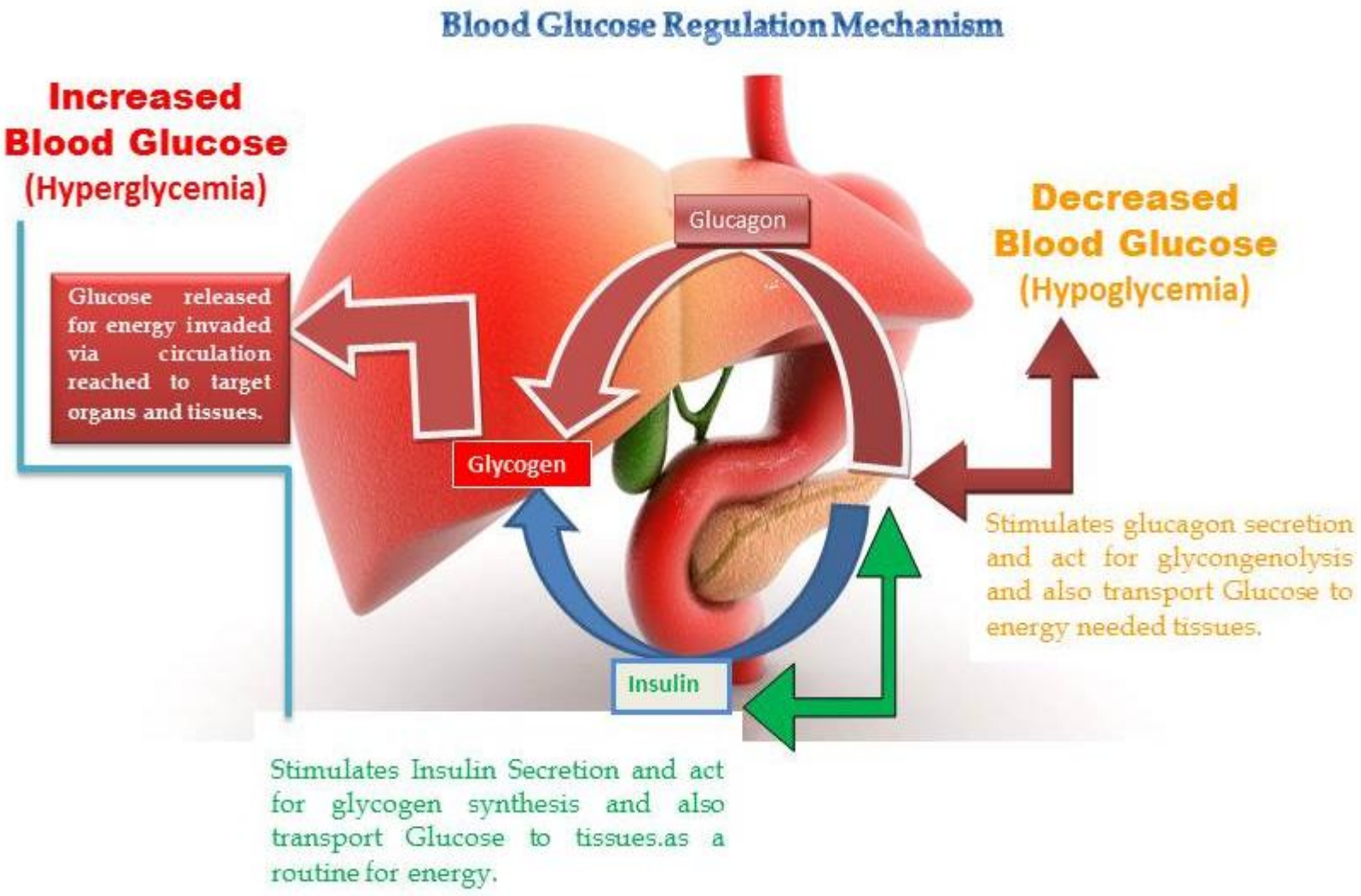
- This presentation covers Diabetic and non-diabetic specimens with FBS, PPBS, $HbA_{1c,}$ Urea, Creatinine, Albumin, Triglycerides and Total Cholesterol were studied.

Inclusion and Exclusion Criteria

- Inclusion Criteria:
  - Diabetic Patients with different age group
  - Acute and chronic Diabetic patients.
  - Non-diabetic patients with all parameters within normal limits.

- Exclusion Criteria:
  - Consent not interest to participate in this study.
  - Patients age less than 18.
  - Old age people unable to fit to give samples.

Introduction Diabetes



Background of study:

- What is diabetes?
- Types of diabetes?
- How To Determine Whether You Have Diabetes, Prediabetes or Neither.
- Normally, hemoglobin A1 (HbA1), glycosylated hemoglobin, has been clinically used as an indicator of long term control of blood glucose in diabetic patients.

- Significant changes in human fingers nails during diabetes

  is possible to use long-term indication of diabetes mellitus.

Human Nails: Introduction

- In human anatomy, a nail is a hornlike envelope covering the dorsal aspect of the terminal phalanges of fingers and toes.
- Nails are similar to claws in mammals and birds.
- Fingernails and toenails are made of a tough protein called keratin, as are animals' hooves and horns.
- The human nail plate is one of the most impervious of biological structure and composed of hard α – keratin, which is the substance forming stratum corneum.
- Hard α-keratin has a high cystine content compared to soft α- keratin.
- The α-keratin contains α-helical polypeptides, which are organized into intermediate filaments (IFs).
- The IF polypeptides are richest in those amino acids favoring an α-helix formation, namely lysine, aspartic acid, glutamic acid and leucine, and comparatively poor in half-

Nail in disease condition

- The condition of Human nail was used as a prediction of the probable course and outcome of a disease. i.e. Diagnostic tool.

- The condition of nails, such as yellow discoloration and side flutes, reflects systemic diseases of the
- Kidney
- Thyroid and liver
- systemic lupus erythematosus (SLE)
- Human immunodeficiency virus (HIV)-infection
- Diabetes mellitus, which cause chronic fatigue, are known to induce abnormality of the nails.

Some of our early results

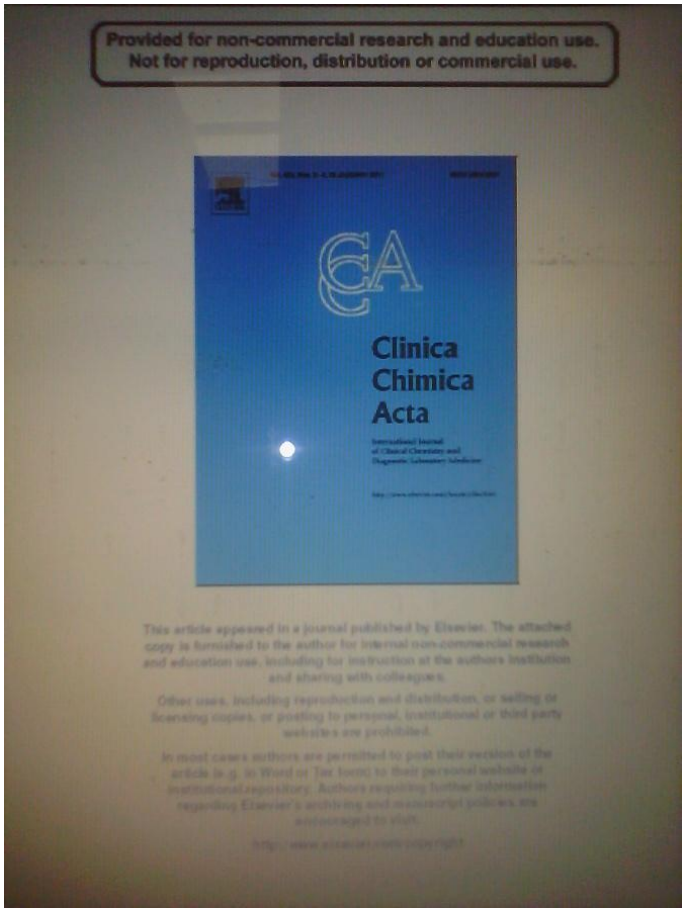


Fig.1. IR Spectra of nails of normal subjects, there is no peak at 468 cm^{-1}.
Fig.2. IR Spectra of nails of Diabetic patients, appearance of peak at 468 cm^{-1} is seen.

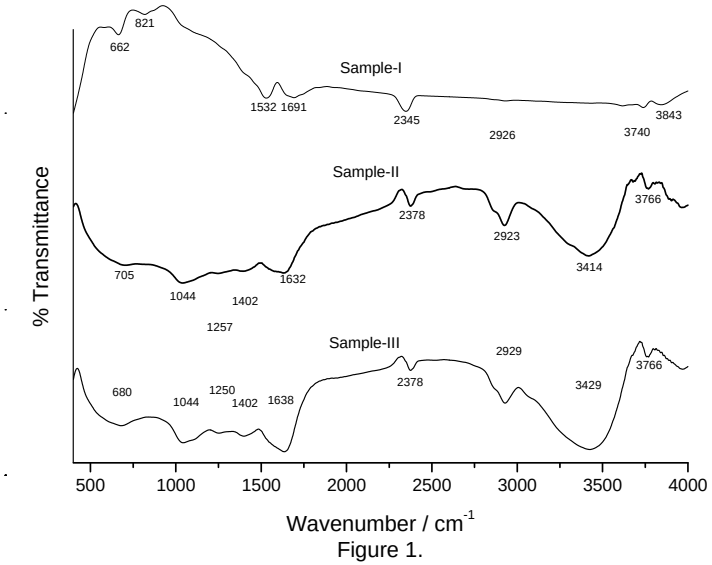


Figure 1.

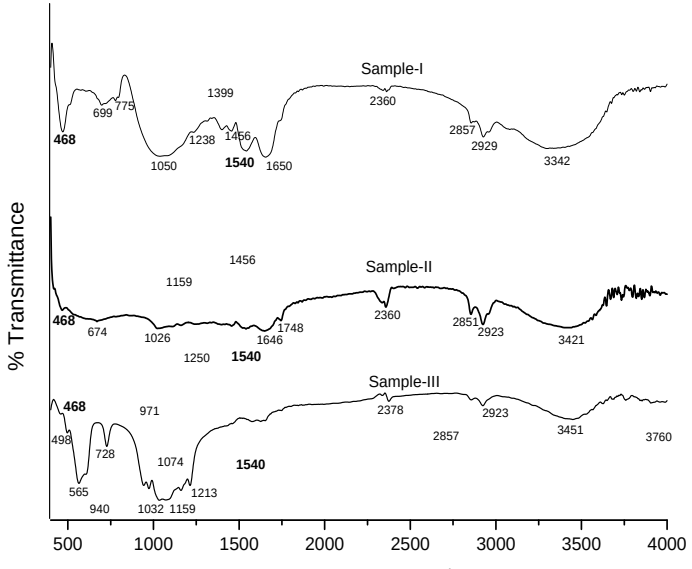


Figure 2.

DHN: Amide I band at 1645- 1659 cm^{-1}, where 1650 cm^{-1} corresponds to amide I of α- helical structures. Amide II bands are observed around 1540 cm^{-1}. Amide III bands are observed around 1259cm^{-1}

NDHN: Amide I band is observed below 1640 cm^{-1}.the bands are observed like 1626, 1632 and 1638 cm^{-1}.The amide III band is observed as such at 1250 cm^{-1}.

Spectral Wave numbers and its configurations

Infrared absorption bands from proteins and peptides:

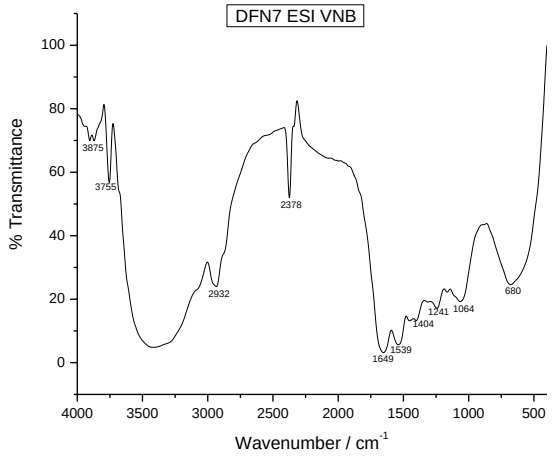
Amide A	**3,300**	**NH stretching**
Amide B	**3,100**	**NH stretching**
Amide I	**1,600–1,690**	**C=O stretching**
Amide II	**1,480–1,575**	**CN stretching; NH bending**
Amide III	**1,229–1,301**	**CN stretching; NH bending**
Amide IV	**625–767**	**OCN bending**
Amide V	**640–800**	**Out-of-plane NH bending**
Amide VI	**537–606**	**Out-of-plane C=O bending**
Amide VII	**200**	**Skeletal torsion**

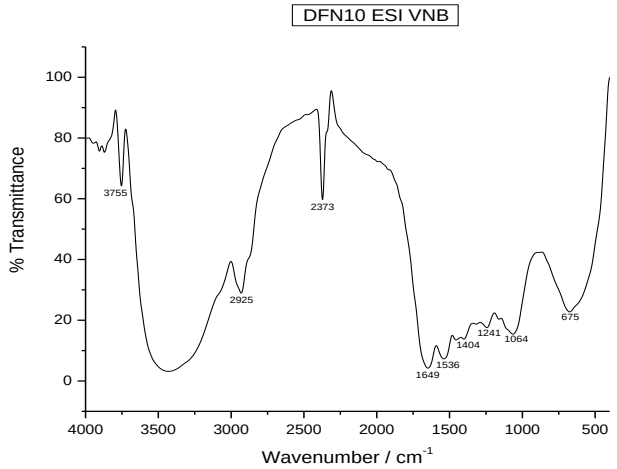
Amide I frequencies assigned to protein secondary structure

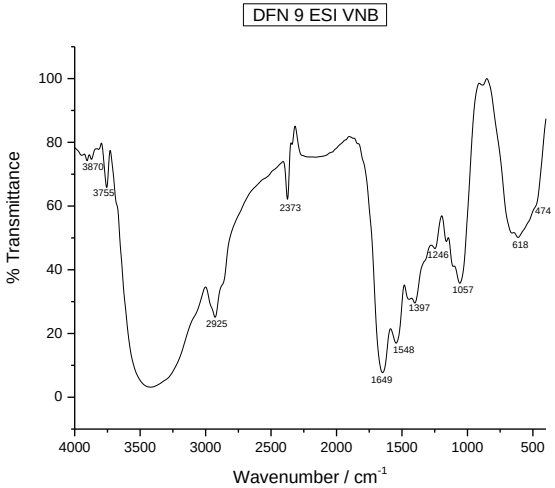
Secondary structure	Range	Average
A-helix	1,648–1,660	1,654
aα-helix turns	1,630	1,630
βα-sheet	1,612–1,641	1,625
	1,626–1,640	1,633
	1,670–1,694	1,682
Turns	1,662–1,684	1,673
Random coil	1,640–1,650	1,645

Based on experimental data and assignments available from the literature (Goormaghtigh et al. 1994; Mantsch and Chapman 1996; Pelton and McLean 2000)

a According to Murayama and Tomida (2004)


DFN7 ESI VNB
% Transmittance
Wavenumber / cm-1
3875
3755
2932
2378
1649
1539
1404
1241
1064
680


DFN10 ESI VNB
% Transmittance
Wavenumber / cm-1
3755
2925
2373
1649
1536
1404
1241
1064
675


DFN 9 ESI VNB
% Transmittance
Wavenumber / cm-1
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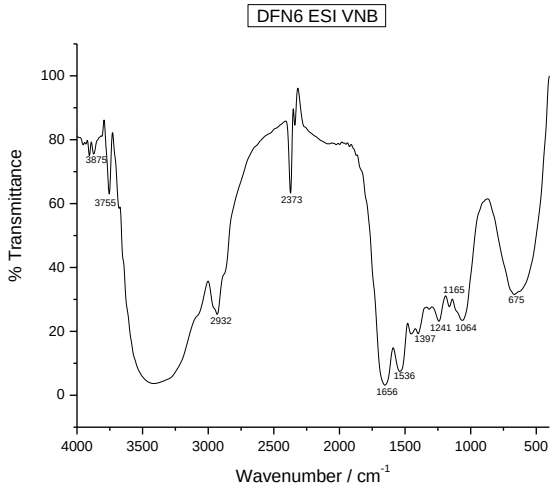
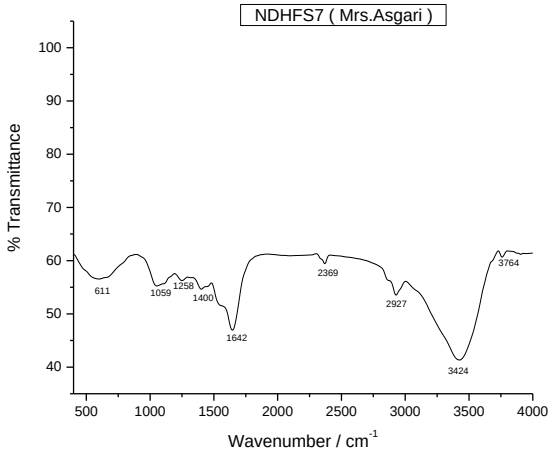
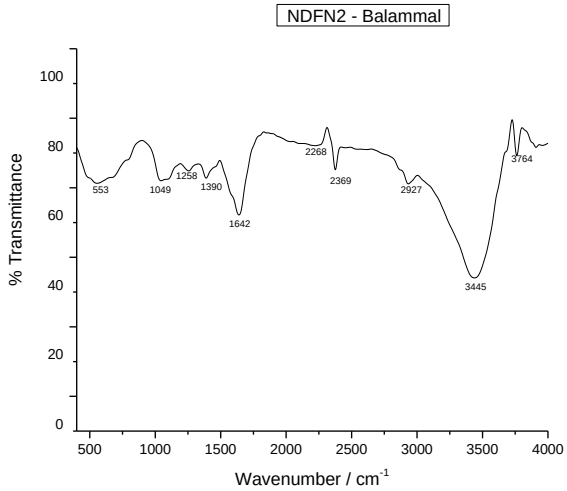
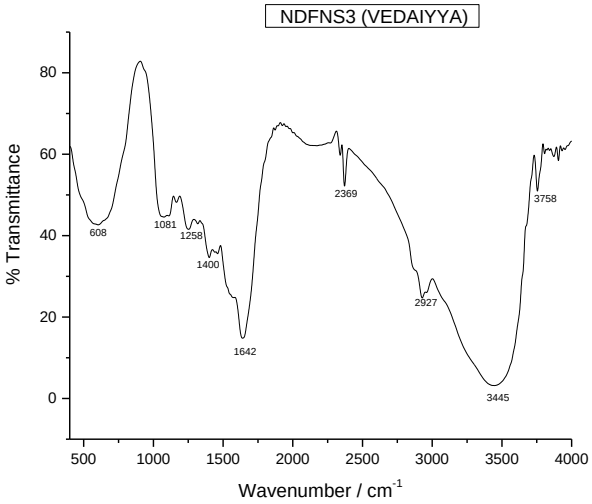
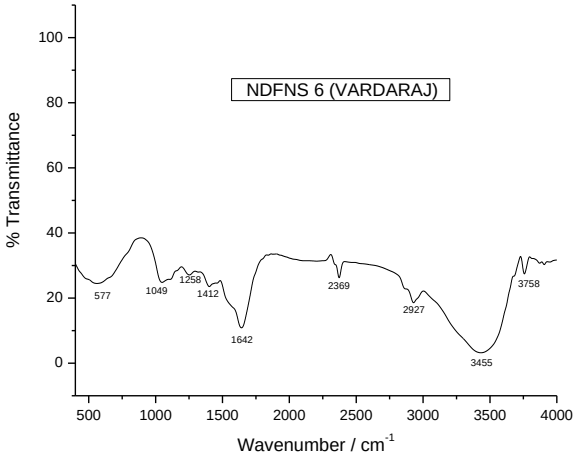
3755
2925
2373
1649
1548
1397
1246
1057
618
474

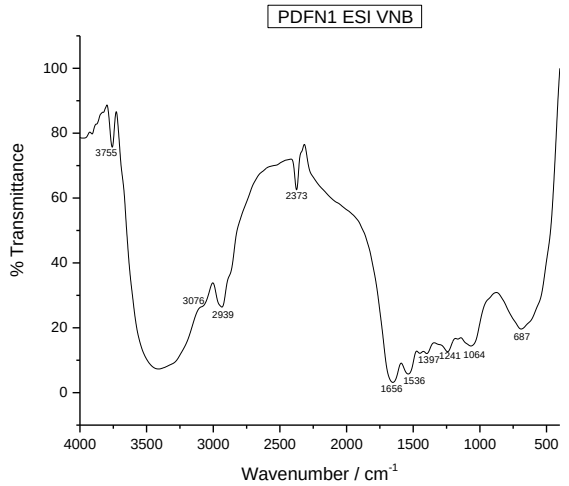

DFN6 ESI VNB
% Transmittance
Wavenumber / cm-1
3875
3755
2932
2373
1656
1536
1397
1241
1165
1064
675

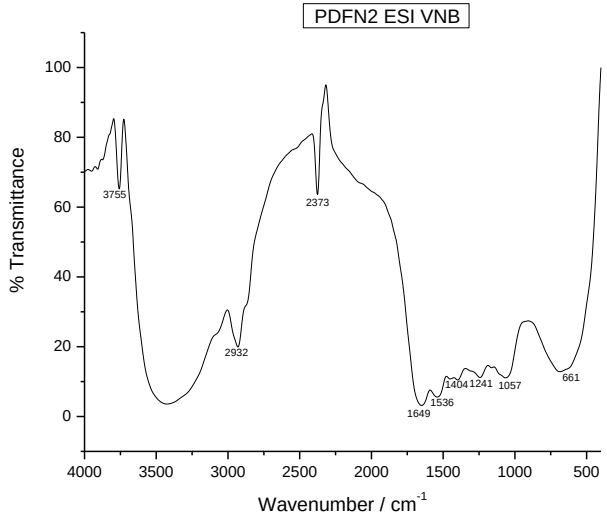

NDHFS7 (Mrs.Asgari)
% Transmittance
Wavenumber / cm-1
611
1059
1258
1400
1642
2369
2927
3424
3764


NDFN2 - Balammal
% Transmittance
Wavenumber / cm-1
553
1049
1258
1390
1642
2268
2369
2927
3445
3764


NDFNS3 (VEDAIYYA)
% Transmittance
Wavenumber / cm-1
608
1081
1258
1400
1642
2369
2927
3445
3758


NDFNS 6 (VARDARAJ)
% Transmittance
Wavenumber / cm-1
577
1049
1258
1412
1642
2369
2927
3455
3758


PDFN1 ESI VNB
% Transmittance
Wavenumber / cm-1
3755
3076
2939
2373
1656
1536
1397
1241
1064
687


PDFN2 ESI VNB
% Transmittance
Wavenumber / cm-1
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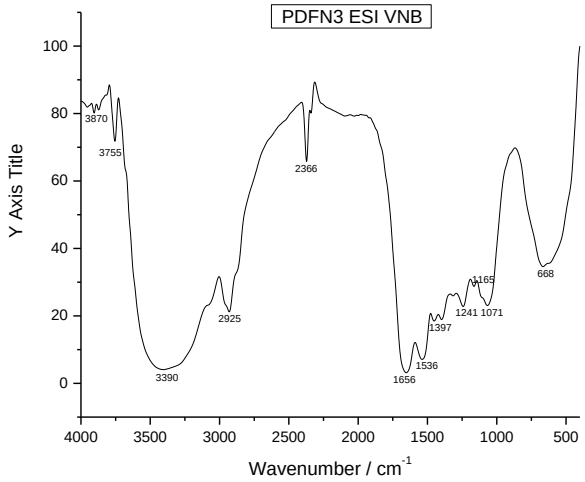
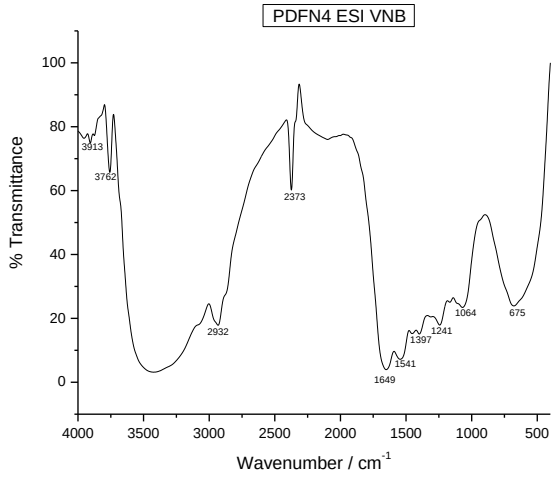
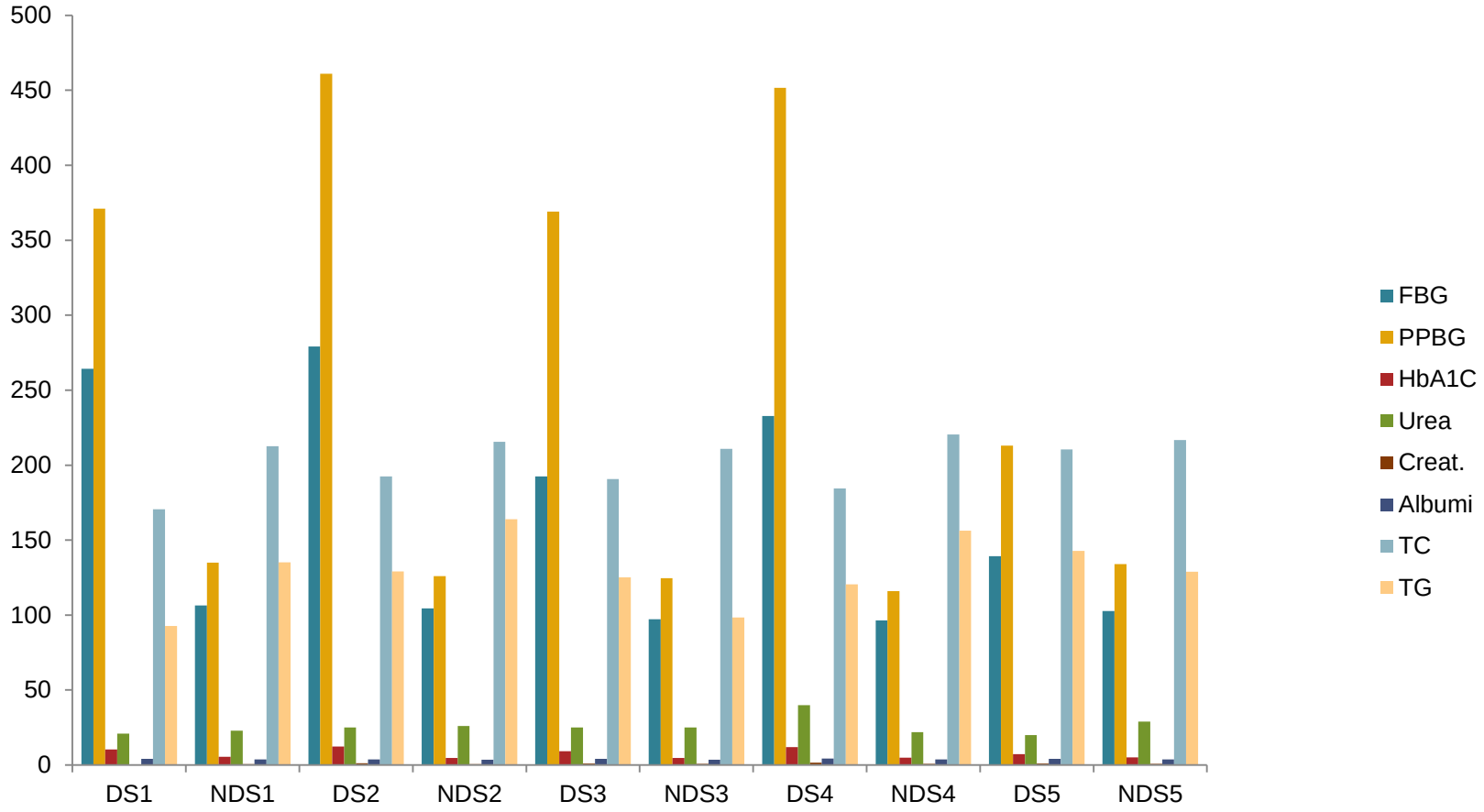
2932
2373
1649
1536
1404
1241
1057
661

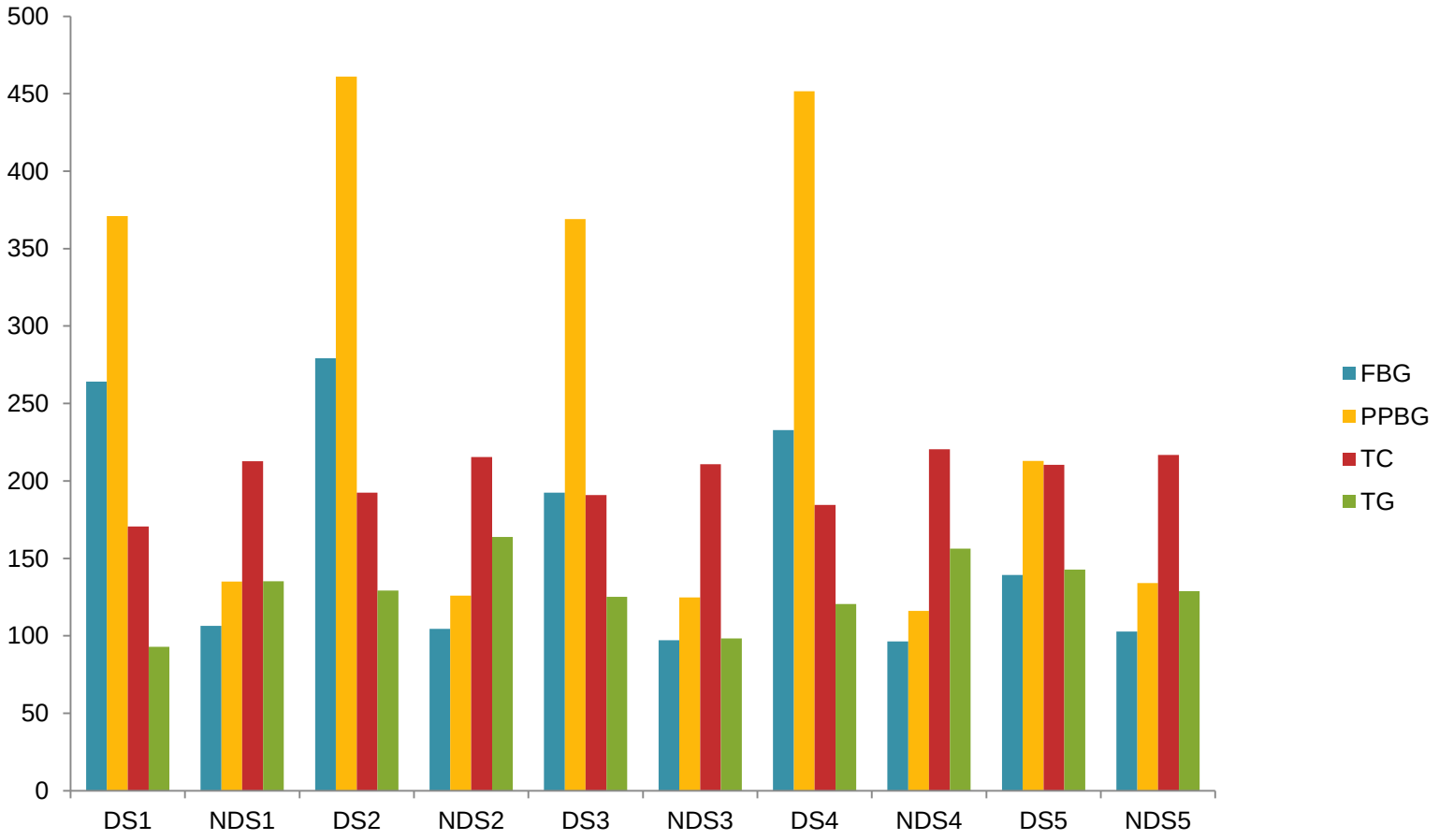

PDFN3 ESI VNB
Y Axis Title
Wavenumber / cm-1
3870
3755
3390
2925
2366
1656
1536
1397
1241
1165
1071
668


PDFN4 ESI VNB
% Transmittance
Wavenumber / cm-1
3913
3762
2373
2932
1649
1541
1397
1241
1064
675

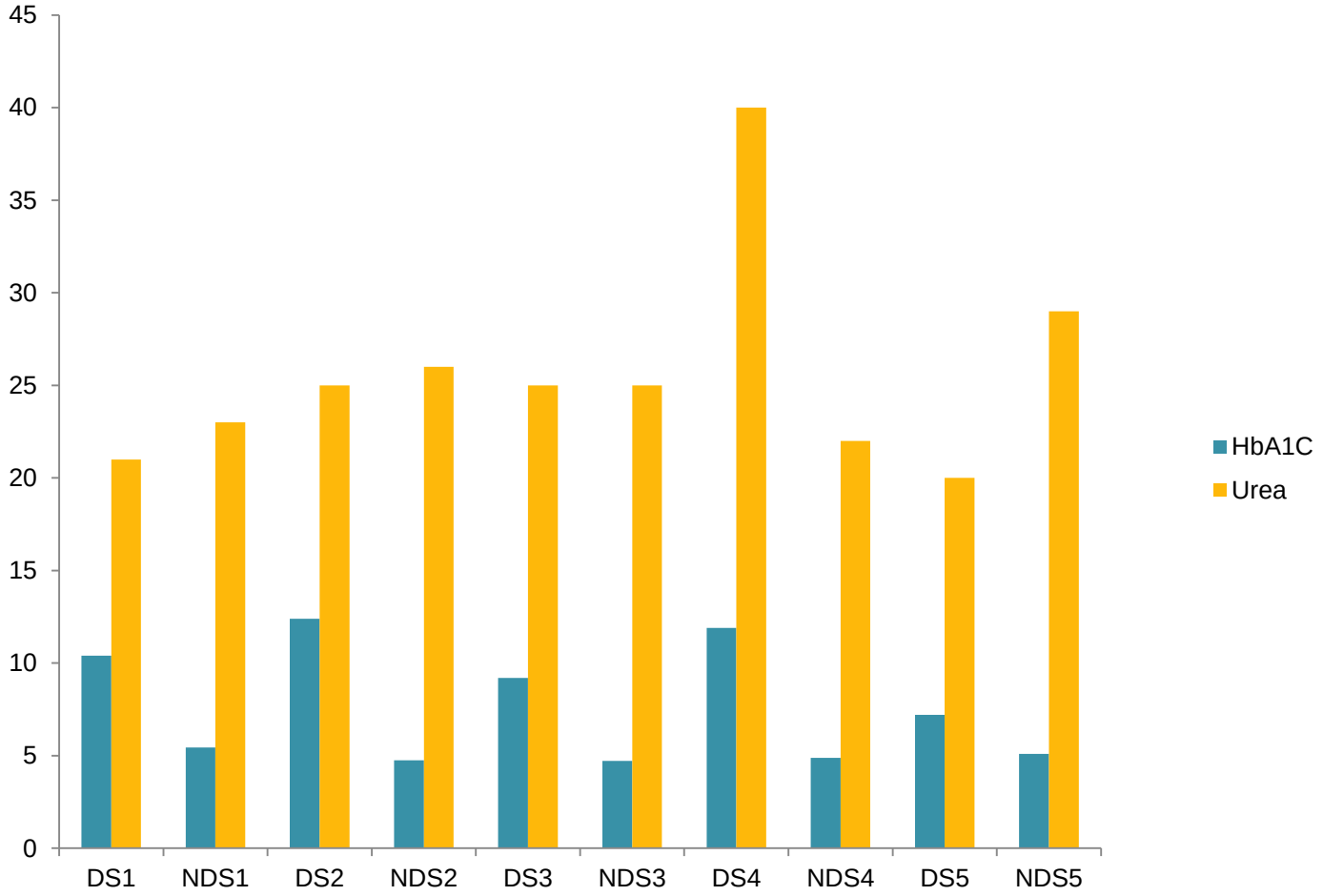
Graph shows over all Data of Blood Glucose Fasting & Postprandial, HbA_{1c}, Urea, Creatinine, Albumin, Total Cholesterol and Triglycerides for Both Diabetic and Normal Subjects:



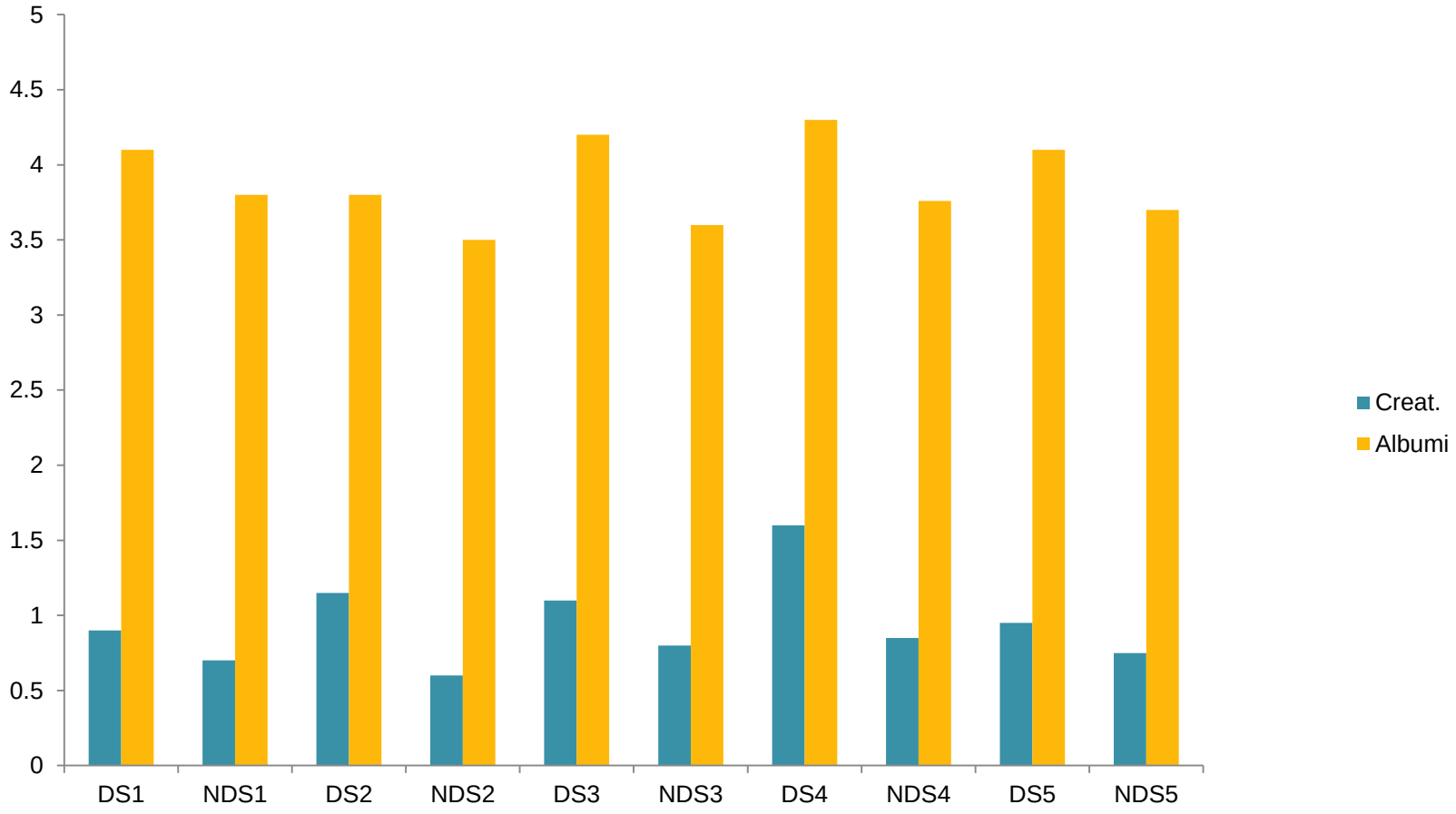
Graph shows Data of Blood Glucose Fasting & Postprandial, Total Cholesterol and Triglycerides for Both Diabetic and Normal Subjects:



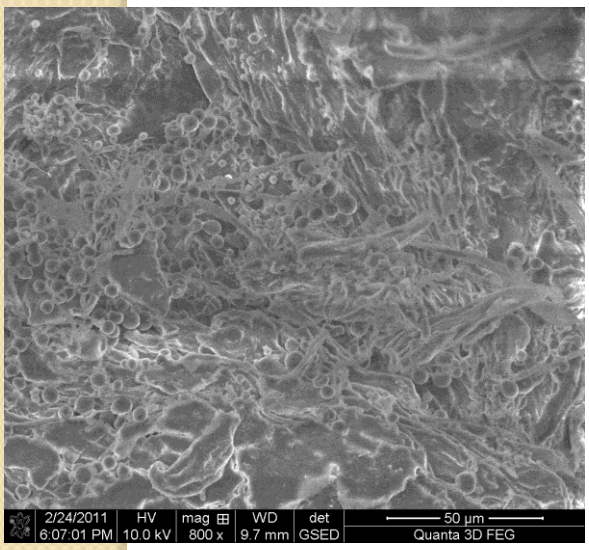
Graph shows Data of HbA_{1c} and Urea for Both Diabetic and Normal Subjects:

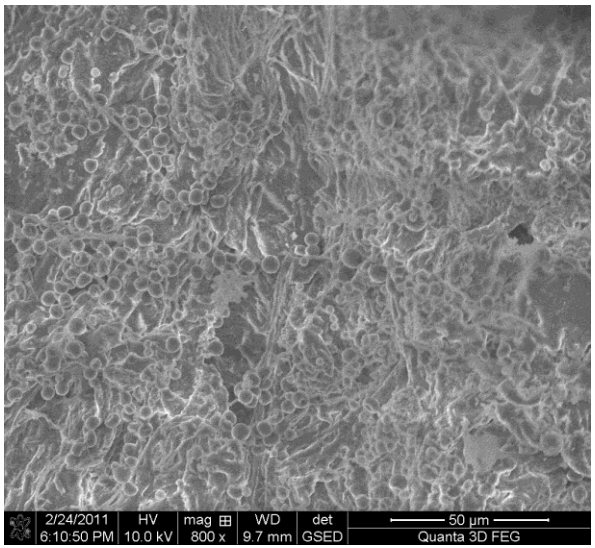


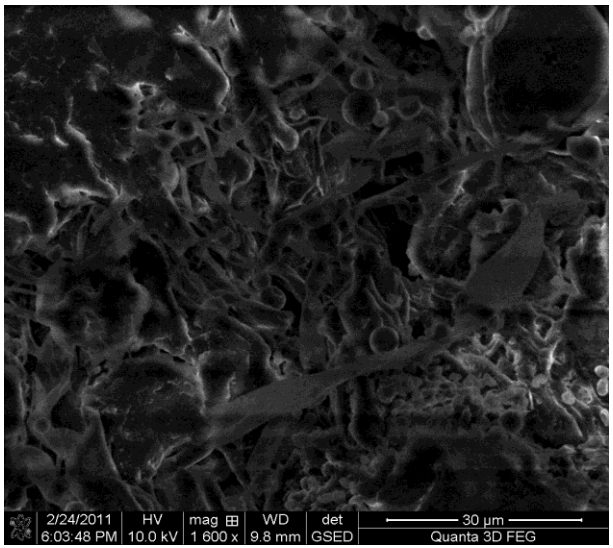
Graph shows Data of Creatinine and Albumin, for Both Diabetic and Normal Subjects:

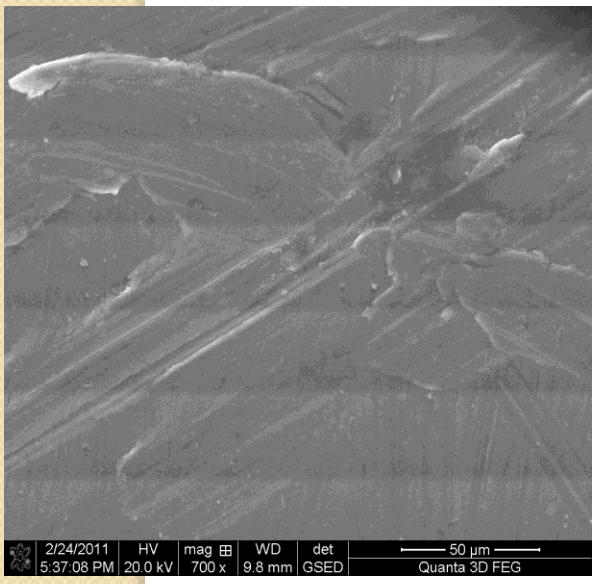


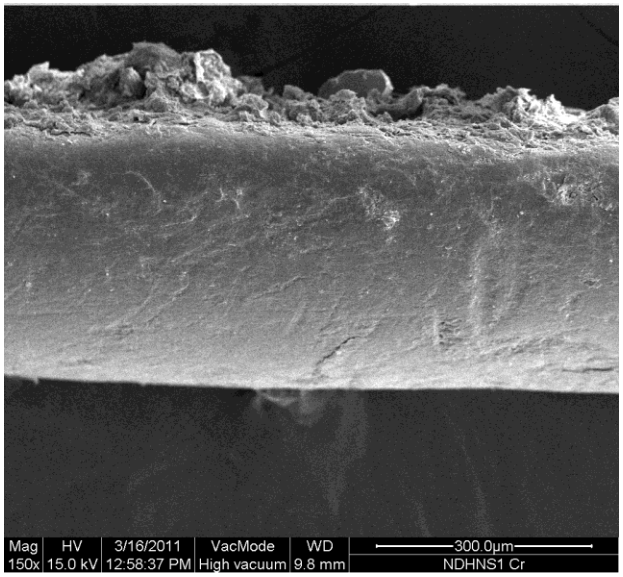
SEM Images of DHN and NDHN

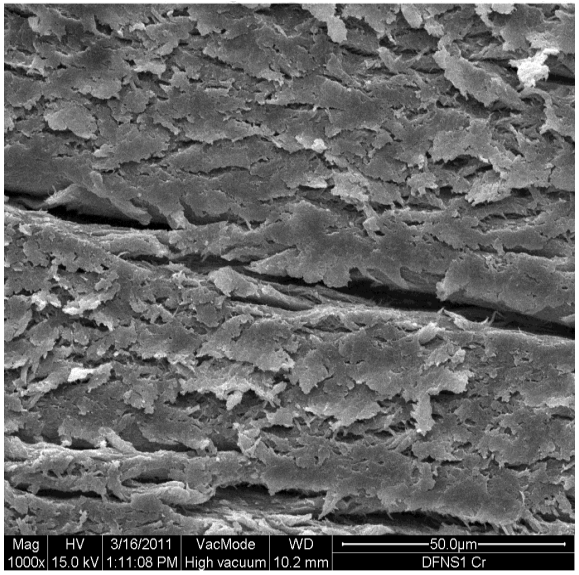


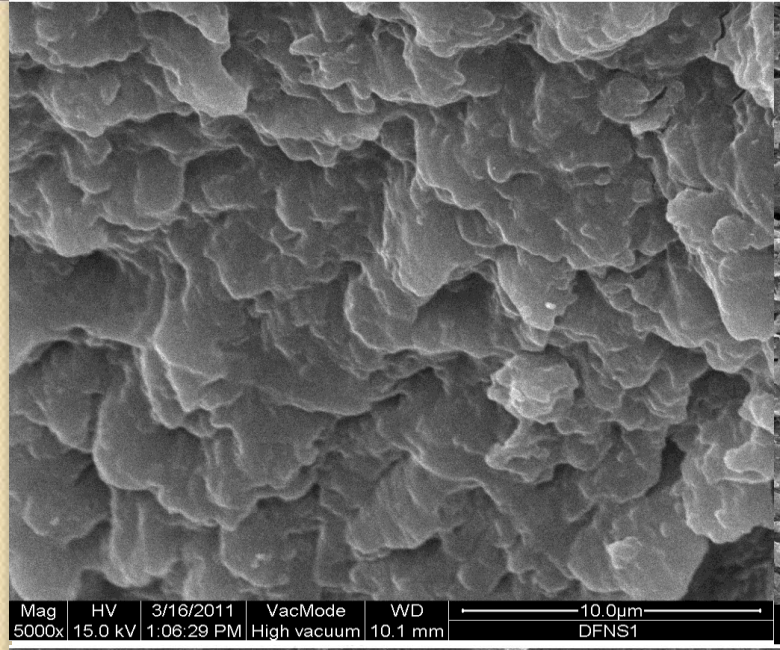


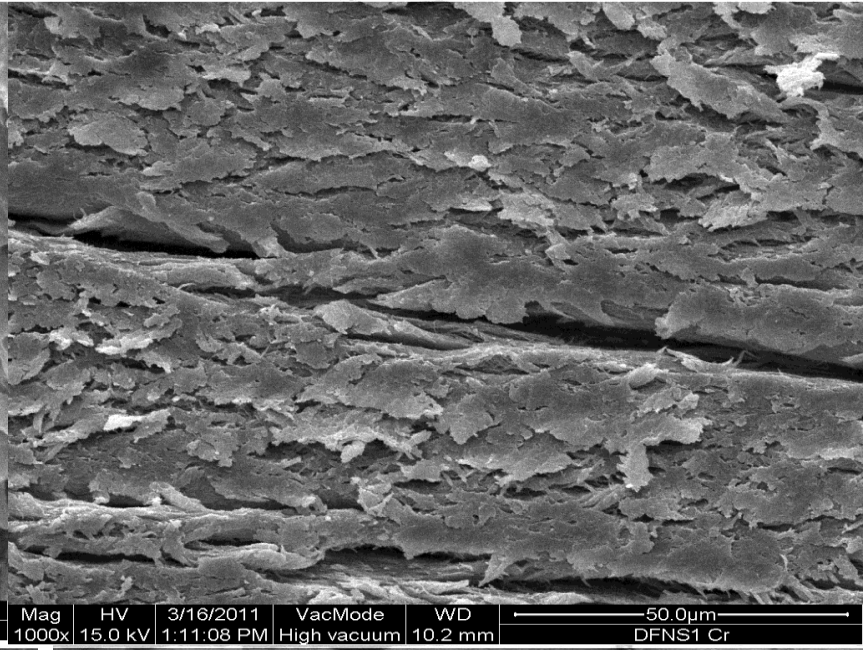









Mag
5000x
HV
15.0 kV
3/16/2011
1:06:29 PM
VacMode
High vacuum
WD
10.1 mm
10.0µm
DFNS1


Mag
1000x
HV
15.0 kV
3/16/2011
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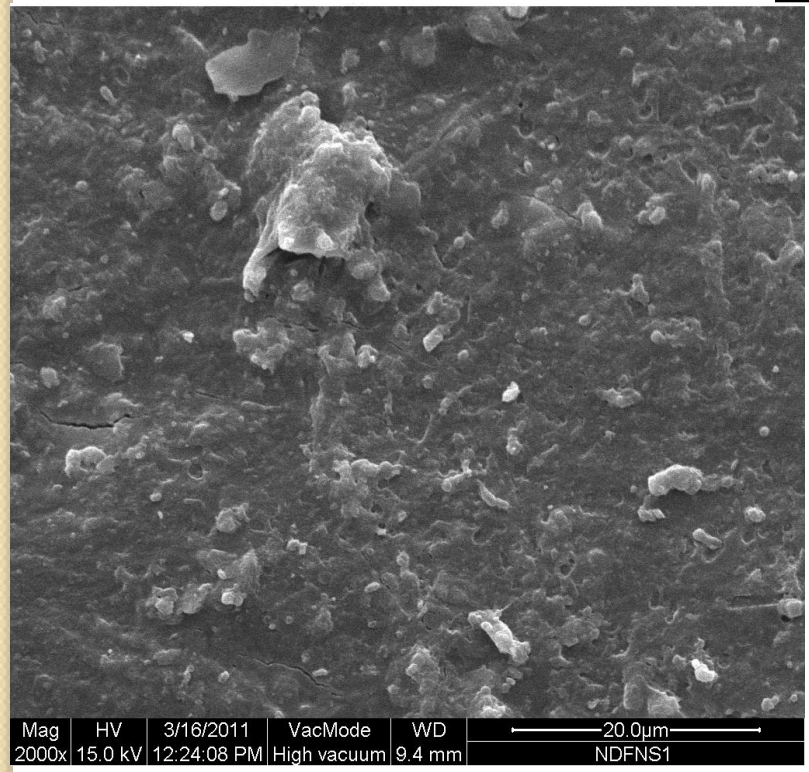
VacMode
High vacuum
WD
10.2 mm
50.0µm
DFNS1 Cr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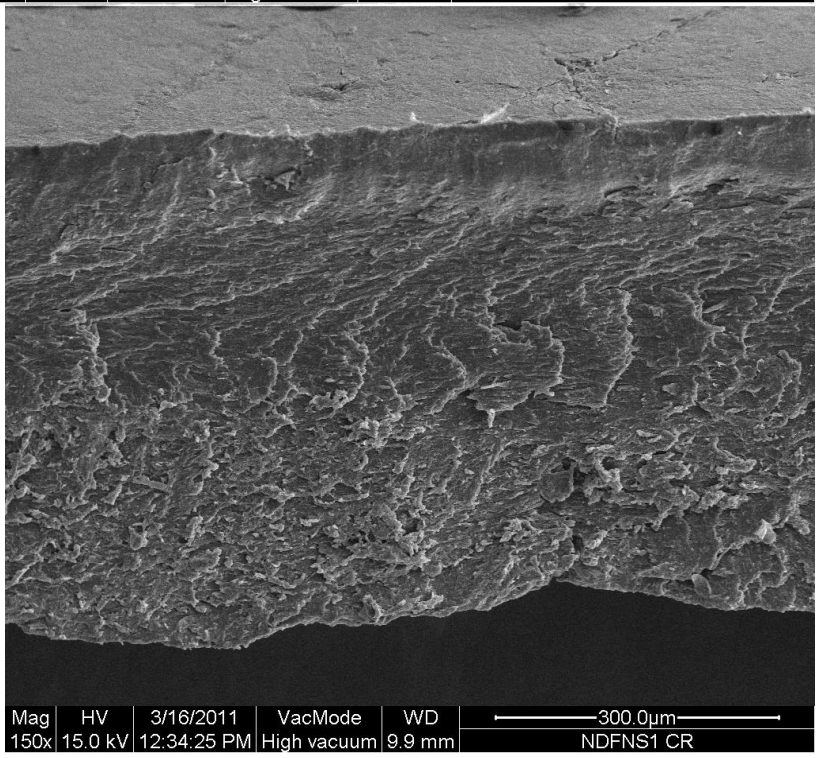
2000x
HV
15.0 kV
3/16/2011
12:24:08 PM
VacMode
High vacuum
WD
9.4 mm
20.0µm
NDFNS1


Mag
150x
HV
15.0 kV
3/16/2011
12:34:25 PM
VacMode
High vacuum
WD
9.9 mm
300.0µm
NDFNS1 CR

Conclusion

- The significant change in nail is assed by spectroscopically and confirmed by number of multiple and duplicated samples.
- This can be further confirmed with patients of diabetes with multi system disorders.
- In future we correlate the spectral changes of nails of non-diabetic, diabetic, prone to diabetic human specimen with biochemical, structural and functional changes in diabetic nephropathy, cardiopathy, and retinopathy.

Thank you