APPENDICES
## ANNEXURE 1

### Nordic Musculoskeletal Questionnaire (NMQ)

<table>
<thead>
<tr>
<th>Have you at any time during the last 12 months had trouble (ache, pain, discomfort in</th>
<th>To be answered only by those who have had trouble</th>
<th>Have you at any time during the last 12 months been prevented from doing your normal work (at home, or away from home) because of the trouble?</th>
<th>Have you had trouble at any time during the last 7 days?</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neck</strong></td>
<td>1. No_____ 2. Yes_____</td>
<td>1. No ____ 2. Yes____</td>
<td>1. No ___ 2. Yes___</td>
</tr>
<tr>
<td><strong>Shoulders</strong></td>
<td>1. No_____ 2. Yes in the right shoulder 3. Yes, in the left shoulder 4. Yes, in both shoulders</td>
<td>1. No ____ 2. Yes____</td>
<td>1. No ___ 2. Yes___</td>
</tr>
<tr>
<td><strong>Elbows</strong></td>
<td>1. No______ 2. Yes in the right elbow 3. Yes, in the left elbow 4. Yes, in both elbows</td>
<td>1. No ____ 2. Yes____</td>
<td>1. No ___ 2. Yes___</td>
</tr>
</tbody>
</table>
### Upper back
1. No______ 2. Yes______ 1. No______ 2. Yes______ 1. No______ 2. Yes______

### Low back (small of the neck)
1. No______ 2. Yes______ 1. No______ 2. Yes______ 1. No______ 2. Yes______

### One or both hips/thighs
1. No______ 2. Yes______ 1. No______ 2. Yes______ 1. No______ 2. Yes______

### One or both knees
1. No______ 2. Yes______ 1. No______ 2. Yes______ 1. No______ 2. Yes______

### One or both ankles/feet
1. No______ 2. Yes______ 1. No______ 2. Yes______ 1. No______ 2. Yes______

**Date of inquiry** __________/________/________

**Sex**
1. Female ________ 2. Male_______

**What year were you born?** _________________

**How many years and months have you been doing your present type of work?** Years__________, months_______

**On average, how many hours a week do you work?** ________ hours a week

**How much you weigh?** ________kg

**How tall are you?** ________cm

**Are you right-handed or left handed?**
1. Right handed______
2. Left handed_______
Questionnaire about low back trouble

How to answer the questionnaire: in the appropriate position of the body part of the body referred to in the questionnaire. By low back trouble is meant ache, pain or discomfort in the shaded area whether or not it extends from there to one or both legs (sciatica). Please answer by putting a cross in the appropriate box- one cross for each question. You may be in doubt as to how to answer, but please do your best anyway.
In this picture you can see the approximate position of the parts of the body referred to in the questionnaire. Limits are not sharply defined and certain parts overlap. You should decide to yourself in which part you have or have had or trouble (if any).
1. Have you ever had low back pain trouble (ache, pain or discomfort)?
   1. No_______   2. Yes_______

If you answered No to question 1, do not answer questions 2-8

2. Have you ever been hospitalized because of low back trouble?
   1. No_______   2. Yes_______

3. Have you ever had to change jobs or duties because of low back trouble?
   1. No_______   2. Yes_______

4. What is the total length of time that you have had low back trouble during the last 12 months?
   1. 0 days
   2. 1-7 days
   3. 8-30 days
   4. More than 30 days, but not every day
   5. Every day

5. Has low back trouble caused you to reduce your activity during the last 12 months?
   a. Work activity (at home or away from home?)
      1. No_______   2. Yes_______
   b. Leisure activity?
      1. No_______   2. Yes_______

6. What is the total length of the time that low back trouble has prevented you from doing your normal work (at home or away from home) during the last 12 months?
   1. 0 days
   2. 1-7 days
   3. 8-30 days
   4. More than 30 days

7. Have you been seen by a doctor, physiotherapist, chiropractor or other such person because of low back trouble during last 12 months?
   1. No_______   2. Yes_______

8. Have you had low back trouble at any time during the last 7 days?
   1. No_______   2. Yes_______
ANNEXURE II

Consent form

✓ The purpose and details of this study have been explained to me. I understand that this study is designed to further scientific knowledge.
✓ I have read and understood the information sheet and this consent form.
✓ I have an opportunity to ask questions about my participation.
✓ I understand that I am under no obligation to take part in the study.
✓ I understand that I have the right to withdraw from this study at any stage for any reason, and that I will not be required to explain my reasons for withdrawing.
✓ I understand that all the information I provide will be treated in strict confidence.
✓ I understand that this study requires taking blood samples, which are used for research in the laboratory for understanding of genetic variation and disease. I agree and give permission for usage of these samples for current research and usage of these samples in future research.
✓ I agree to participate in this study.

Name_____________________________________

Signature_________________________________

Signature of investigator________________

Date______________________________________
ANNEXURE III
Performa for different risk variables

AGE:__________________________  GENDER:____________
ETHNICITY:________________

NAME OF MUSCULOSKELETAL DISORDER:_________________________
DURATION OF PAIN:________

BLOOD PRESSURE
(SBP):________  (DBP):________
CURRENT SMOKING STATUS:____  ALCOHOL USE:____
HEIGHT:______________  WEIGHT:________
BMI:____________________

LIPID LEVELS:
TC:______________  LDL:________
HDL:______________  TG:________
SCOCIO-ECONOMIC STATUS:____  MARITAL STATUS:____
EDUCATIONAL STATUS:__________  STATIN USE:________

SCALES
NRS SCORE:_______  PHQ-9 SCORE:_______
PSQI SCORE:_______  MMSE SCORE:_______
ANNEXURE-IV

NUMERIC RATING SCALE (NRS)

- **Numeric Rating Scale Use**: Have the individual point to or state the number that best shows how bad his or her pain is NOW.

- **Numeric Rating Scale Scoring**: Document the numerical value indicated by the individual. Evaluate the pain intensity over time to determine the effectiveness of pain treatments and need for changes in treatment.

### Numeric Rating Scale

Point to the number that best represents the intensity of your pain NOW.

- 10 Worst possible pain
- 9
- 8
- 7
- 6 Moderate pain
- 5
- 4
- 3
- 2
- 1
- 0 No pain
ANNEXURE V

Patient Health Questionnaire (PHQ-9)

Patient name: ___________________________ Date: ___________________

1. Over the last 2 weeks, how often have you been bothered by any of the following problems?

<table>
<thead>
<tr>
<th>Problem</th>
<th>Not at all (0)</th>
<th>Several days (1)</th>
<th>More than half the days (2)</th>
<th>Nearly every day (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Little interest or pleasure in doing things.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Feeling down, depressed, or hopeless.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Trouble falling/staying asleep, sleeping too much.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Feeling tired or having little energy.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. Poor appetite or overeating.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f. Feeling bad about yourself, or that you are a failure, or have let yourself or your family down.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g. Trouble concentrating on things, such as reading the newspaper or watching TV.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>h. Moving or speaking so slowly that other people could have noticed, or the opposite; being so fidgety or restless that you have been moving around more than usual.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. Thoughts that you would be better off dead or of hurting yourself in some way.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. If you checked off any problem on this questionnaire so far, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

[ ] Not difficult at all [ ] Somewhat Difficult [ ] Very difficult [ ] Extremely difficult

TOTAL SCORE __________________
ANNEXURE VI
Sleep Quality Assessment (PSQI)

Name ___________________________________ Date _____________________

During the past month,

1. When have you usually gone to bed? ___________________
2. How long (in minutes) has it taken you to fall asleep in night?  ___________________
3. What time have you usually gotten up in the morning?  ___________________
4. A. How many hours of actual sleep did you get at night?  ___________________
   B. How many hours were you in bed?  ___________________

5. During the past month, how often have you had trouble sleeping because you

   A. Cannot get to sleep within 30 minutes
   B. Wake up in the middle of the night or early morning
   C. Have to get up to use the bathroom
   D. Cannot breathe comfortably
   E. Gough or snore loudly
   F. Feel too cold
   G. Feel too hot
   H. Have bad dreams
   I. Have pain
   J. Other reason(s), please describe, including how often you have had trouble sleeping because of this reason(s):

6. During the past month, how often have you taken medicine (prescribed or “over the counter”) to help you sleep?

7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?

9. During the past month, how would you rate your sleep quality overall?

Very good (0)  Fairly good (1)  Fairly bad (2)  Very bad (3)

A total score of 5 or greater is indicative of poor sleep quality.
If you scored 5 or less than that, it is indicative of good sleep.
ANNEXURE VII

Mini Mental State Examination

<table>
<thead>
<tr>
<th>Maximum Score</th>
<th>Patient’s Score</th>
<th>Questions</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td></td>
<td>“What is the year? Season? Date? Day? Month?”</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>“Where are we now? State? County? Town/city? Hospital? Floor?”</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>The examiner names three unrelated objects clearly and slowly, then the instructor asks the patient to name all three of them. The patient’s response is used for scoring. The examiner repeats them until patient learns all of them, if possible.</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>“I would like you to count backward from 100 by sevens.” (93, 86, 79, 72, 65, ...) Alternative: “Spell WORLD backwards.” (D-L-R-O-W)</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>“Earlier I told you the names of three things. Can you tell me what those were?”</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Show the patient two simple objects, such as a wristwatch and a pencil, and ask the patient to name them.</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>“Repeat the phrase: ‘No ifs, ands, or buts.’”</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>“Take the paper in your right hand, fold it in half, and put it on the floor.” (The examiner gives the patient a piece of blank paper.)</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>“Please read this and do what it says.” (Written instruction is “Close your eyes.”)</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>“Make up and write a sentence about anything.” (This sentence must contain a noun and a verb.)</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>“Please copy this picture.” (The examiner gives the patient a blank piece of paper and asks him/her to draw the symbol below. All 10 angles must be present and two must intersect.)</td>
</tr>
<tr>
<td>30</td>
<td>TOTAL</td>
<td></td>
</tr>
</tbody>
</table>
I. Chemicals used in DNA Extraction/preparation of working solutions

Reagents preparation for DNA extraction:

**RBC Lysis Buffer (10x) Stock Buffer**

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium chloride (NH₄CL)</td>
<td>8.20g</td>
</tr>
<tr>
<td>Sodium Carbonate (NaHCO₃)</td>
<td>0.84g</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.37g</td>
</tr>
</tbody>
</table>

Dissolved the above chemicals in 100ml of triple distilled water (TDW), autoclaved and stored at 4⁰C.

**RBC Lysis Buffer (1X) Working Buffer**

50ml of RBC Lysis Stock Buffer was mixed in 450ml of autoclaved TDW, to make the final volume of 500ml. The working buffer was autoclaved and stored at 4⁰C.

**Nucleic Lysis Buffer (10X) Stock Buffer**

1M Tris HCL: 15.76g of Tris HCL was dissolved in 100ml of TDW and pH was adjusted to 8.0. it was autoclaved and stored at 4⁰C.

0.5M EDTA: 18.61g of EDTA was dissolved in 100ml of TDW using magnetic stirrer for dissolving and pH was adjusted to 8.0. It was autoclaved and stored at 4⁰C.

5M NaCl: 29.20g of NaCl was dissolved in 100ml of TDW.

8ml of 5M NaCl, 1ml of 1M tris HCL and 0.4ml of 0.5 EDTA was mixed in TDW to make the final volume up to 100ml. It was autoclaved and stored at 4⁰C.

**Nucleic Lysis Buffer (10X) Working Buffer**

50ml of Nucleic Lysis Stock Buffer was mixed in 450ml of autoclaved TDW, to make the final volume of 500ml. The working buffer was autoclaved and stored at 4⁰C.
**10% SDS (Sodium Dodecyl sulphide)**
10g of SDS was dissolved in 100ml of TDW, autoclaved and stored at room temperature only.

**5 M Saturated NaCl solution**
29.20g of NaCl was dissolved in 100ml of TDW, autoclaved and stored at 4ºC.

**6 M Saturated NaCl Solution**
35.0g of NaCl was dissolved in 100ml of TDW, autoclaved and stored at 4ºC.

**Proteinase K solution**
20mg of Proteinase K was dissolved in 1ml of TDW/ Nuclease free water and stored at-20ºC.

**Absolute Ethanol**
Stored at room temperature.

**70% Ethanol**
70ml of absolute ethanol and 30ml of TDW was mixed to make the final volume to 100ml, stored at -20ºC/4ºC (no autoclaving).

**TE Buffer**
1ml of 1M Tris HCL and 100μl of 0.5 M EDTA was dissolved in TDW to make the final volume to 100ml.

**II. Preparations of reagents for Gel Electrophoresis**
10X Tris Borate EDTA (TBE) buffer:

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Amount/100ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris base</td>
<td>10.8g</td>
</tr>
<tr>
<td>Boric acid</td>
<td>5.5g</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.74g</td>
</tr>
</tbody>
</table>
In 60ml of autoclaved TDW, dissolved the above chemicals and pH was set at 8.3.
The final volume was made up to 100ml by TDW and stored at 4°C.

**Gel Loading Buffer**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Amount/10ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1M Tris HCl</td>
<td>100μl</td>
</tr>
<tr>
<td>Bromophenol blue</td>
<td>0.025g</td>
</tr>
<tr>
<td>Xylene Cynol</td>
<td>0.25g</td>
</tr>
<tr>
<td>Sucrose</td>
<td>4g</td>
</tr>
</tbody>
</table>

**Ethidium Bromide**

0.01g of ethidium bromide was dissolved in 1ml of TDW.