**Table 1**: Analytical and assessment characteristics

|  |  |  |
| --- | --- | --- |
| **NO.** | **ASSESSMENT** | **EXPLAINATION** |
| 1. | Blood sample | **Blood collection**  - 10 ml of venous blood were taken by a trained nurse.  - The whole blood will be collected into plain polyethylene tube until blood clot formation.  - The clots will be separated from the wall of tube using a wooden applicator stick.  - The serum will be separated by centrifugation 10,000g at 4°C for 15 min to eliminate debris and cellular matter.  **Blood test**  **1) Substance P**  - The blood serum will be tested using Human Substance P EIA kit (Cusabio Biotech)  **2) Cytokines**  - The blood serum will be tested individually using their Elisa kits to investigate  a) TNF-α  b) IL-1β  c) IL-6  d) IL-8  e) IL-10  c) monocyte chemotactic protein-1(MCP-1)  d) The soluble form of intercellular adhesion molecule (Sicam-1). |
| 2. | Saliva sample | **Saliva collection**  - 3 to 4 ml of unstimulated (resting) whole saliva was  collected 2 minutes after the patients had rinsed his  mouth several times with tap water.  - The accumulated saliva in the floor of the mouth was  drawn by a plastic disposable pipette and collected into  a plastic polyethylene tube.  - The collected saliva was centrifuged 10,000g at 4°C for 15 min to eliminate debris and cellular matter.  **Blood test**  **1) Substance P**  - The blood serum will be tested using Human Substance P EIA kit (Cusabio Biotech)  **2) Cytokines**  - The blood serum will be tested individually using their Elisa kits to investigate:  a) TNF-α  b) IL-1β  c) IL-6  d) IL-8  e) IL-10  c) monocyte chemotactic protein-1(MCP-1)  d) The soluble form of intercellular adhesion molecule (Sicam-1). |
| 3. | Visual Analogue Scale (VAS) | - Pain intensity was measured using VAS by asking patient’s pain level before and after treatment. |
| 4. | Rolland-Morris Disability questionnaire | - Functional disability was measured using Rolland-Morris Disability questionnaire. |