MATERIAL AND METHODS
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The present study was carried out in Ramanagara, a town and a city municipal council in Bangalore Rural district in the Indian State of Karnataka. Ramanagara is located at 12.72° N 77.3° E. It is approximately 50 km southwest of Bangalore. It has an average elevation of 747 metres (2450 feet). Ramanagara cocoon market is the largest commercial cocoon market in India and the market attracts largest number of farmers and reelers by providing better price to farmers, larger facility and easier accessibility to reelers. All religious communities are involved with silk industry that dominates the area, which involve silkworm rearing, silk reeling and twisting activities. The silk reeling sector has about 1256 charakas, 1339 filatures and 52 multiend reeling units providing direct employment to over 6100 people.

3.1. Respondent's Profile

The data for the present study was collected from the primary source by canvassing prestructured questionnaires (appendix 1) specially prepared for the study. The sample size considered for the survey was 500, which included men, women and children working in the reeling units. Respondents were drawn from all the three types of reeling units viz., charaka, cottage basin and multiend, as all these types of machines are prevalent in Ramanagara. The following information was obtained to ascertain various aspects of their working life.

1) Age group of the respondents
2) Educational level of the respondents
3) Martial status of the respondents
4) Religion of the respondents
5) Personal habit of the respondents
6) Worker of working units of the respondents
7) Nature of Work
8) Working hours per day
9) Work experience
10) Health related problems

The results obtained based on sex and age groups are presented in tables 1-10.

3.2 Clinical Study

A clinical study was conducted on 106 workers selected randomly from the 500 respondents representing charaka, cottage basin and multiend reeling units. Peak expiratory flow rate, Immunoglobulin E and Blood cell count was determined for all the subjects.

3.2.1 Peak Expiratory Flow Rate (PEFR)

The peak flow meter measures peak expiratory flow rate, which is the fastest speed at which a person can blow air out of his / her lungs after taking as big a breath as possible. PEFR was recorded on the Wright peak flow meter. Calibration of the meter was checked with a standard rotameter as well as a healthy individual whose PEFR values were used as known constants. Before the test was carried out, the purpose and technique of measurement were explained to each subject. After a practical demonstration, the subject was asked to inhale maximally and
then exhale forcefully into the peak flow meter. At least 4-5 readings were taken after several practice trials and the mean of three highest values was recorded (Wright and McKerrow, 1959).

**Collection of blood samples**

5 ml of blood samples was collected intravenously from each of the workers considered for the study into heparinised tube and stored at -20°C for further use. An experienced laboratory technician from a diagnostic center was accompanied for the collection of blood samples from the subjects. The tests were conducted to obtain the following parameters in the laboratory of Medinova Diagnostic Service, Bangalore.

- a. Immunoglobulin E
- b. Haemoglobin
- c. Red cell count
- d. Packed Cell Volume (PCV)
- e. Mean Corpuscular Volume (MCV)
- f. Mean Corpuscular Hemoglobin (MCH)
- g. Mean Corpuscular Hemoglobin Concentration (MCHC)
- h. Platelet count
- i. Total White Cell Count (TWCC) and Differential white cell count
  - a. Neutrophils
  - b. Lymphocytes
  - c. Monocytes
  - d. Eosinophils
  - e. ESR Westergren method
  - f. ABS Eosinophil count


3.2.2. Immunoglobulin E

The IgE Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-IgE antibody for solid phase (microtiter wells) immobilization and another anti-IgE antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen (serum) is added to the IgE antibody coated microtiter wells and incubated with the Zero Buffer. If human IgE is present in the specimen, it will combine with the antibody on the well. The well is then washed to remove any residual test specimen, and IgE antibody labeled with horseradish peroxidase (conjugate) is added. The conjugate will bind immunologically to the IgE on the well, resulting in the IgE molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation at room temperature, the wells are washed with water to remove unbound labelled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped and the color is changed to yellow with the addition of 2N hydrochloric acid. The extent of color development is measured spectrophotometrically at 450 nm. The concentration of analyte is directly proportional to the color intensity of the test sample.

3.2.3 Haemoglobin (Hb)

Haemoglobin was estimated by Oxyhaemoglobin method (Bell et. al., 1945). Briefly, 20 mg-diluted blood was mixed with 5 ml of 0.004% of ammonia solution. The development of reddish pink colour was measured at 625 nm.
3.2.4 Red Cell Count

Red cell count was determined by visual Haemocytometer method (Eric Ponder et. al., 1934). Briefly the anti-coagulated blood was diluted with Hayem's fluid and filled up to the mark in the RBC pipette and mixed well for 3 minis. The Neubauer's chamber was charged after discarding 1 or 2 drops of the mixture from the RBC pipette. The cells were allowed to settle down for 2 mins, and the RBCs were counted under low power in the four large corner squares.

3.2.5 Packed Cell Volume (PCV)

It was estimated by Wintrobe's method (1929). Briefly, the anti-coagulated blood was filled into a Wintrobe's tube up to 10 cm and centrifuged at 2000-3000 rpm for 30 minutes. After centrifugation, layers were separated, as uppermost layer of plasma, thin white layer of platelets, greenish pink layer of leucocytes, lower most the layer of RBCs and grey white layer of leucocytes and platelets interposed between plasma above and packed RBCs, below which is called buffy coat. The lowermost height of column layer of RBC was noted and expressed as percentage.

3.2.6 Mean Corpuscular Volume (MCV)

MCV is calculated using the equation

\[
\text{MCV} = \frac{\text{PCV in L/L}}{\text{RBC count/L}}
\]
3.2.7 Mean Corpuscular Haemoglobin (MCH)

MCH is calculated using the equation
\[
\text{MCH} = \frac{\text{Hb}}{\text{RBC count}} \text{L/L}
\]

3.2.8 Mean Corpuscular Haemoglobin Concentration (MCHC)

MCHC is calculated using the equation
\[
\text{MCHC} = \frac{\text{Hb}}{\text{PCV in L/L}}
\]

3.2.9 Total White Cell and Platelet Count (TWCC) and (PLC)

TWCC and PLC were determined by visual Hemocytometer method (Eric Ponder et al., 1934). Briefly, the anti-coagulated blood was diluted with diluting fluid and filled up to the marking in the WBC pipette and mixed well for three minutes. The Neubauer’s chamber was charged after discarding 1 or 2 drops of the mixture from the WBC pipette. The cells were allowed to settle down for 2 minutes, and the WBCs and PLCs were counted under low power in the four large corner squares.

3.2.10 Differential White Cell Count (DWCC)

Differential White Cell Count was obtained by slide method. Briefly, a drop of blood was placed in the center of a clean glass slide and then it was spread uniformly using smooth edge of another slide. The slide was dried and stained with Giemsa stain.
for 15-20 min, washed in distilled water, examined under oil immersion for different types of WBCs. Counts were expressed in terms of percentage of Neutrophils, Lymphocytes, Monocytes, and Eosinophils.

3.2.11 Erythrocyte Sedimentation Rate (ESR)

Erythrocyte Sedimentation Rate was estimated by Westergren method (1921). Briefly, 1.6 ml of the blood was mixed with 0.4 ml of anti-coagulant and filled in Westergren pipette up to the zero mark and allowed into stand for one hour in a vertical rack, after which reading was taken at the upper meniscus of the RBCs.