# THYROID RISK EVALUATION IN SELECTED BEEDI WORKERS POPULATION IN TIRUNELVELI

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Manonmaniam Sundaranar University in partial fulfilment of the requirements for the award of the degree of
Doctor of Philosophy

By

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# CERTIFICATE

This thesis entitled **"THYROID RISK EVALUATION IN SELECTED BEEDI WORKERS POPULATION IN TIRUNELVELI"** submitted by **K.M.SYED ALI FATHIMA** for the award of Degree of Doctor of Philosophy in Zoology of Manonmaniam Sundaranar University is a record of bonafide research work done by her and it has not been submitted for the award of any degree, diploma, associateship, fellowship of any University or Institution.

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# DECLARATION

I hereby declare that the thesis entitled **"THYROID RISK EVALUATION IN SELECTED BEEDI WORKERS POPULATION IN TIRUNELVELI"** submitted by me for the Degree of Doctor of Philosophy in Zoology is the result of my original and independent research work carried out under the guidance of **Dr.S.MARIA SEBASTIAN,** Associate Professor, Department of Zoology, St. Xavier's College (Autonomous), Palayamkottai- 627 002 and it has not been submitted for the award of any degree, diploma, associateship, fellowship of any University or Institution.

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Date :

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**K.M. SYED ALI FATHIMA**

# CONTENTS

# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| ALP | - | Alkaline Phosphate |
| AMA | - | Anti Microsomal Anibody |
| ATG | - | Anti Thyroglobulin Antibody |
| B | - | Beedi rollers |
| B | - | Blank |
| C | - | Control |
| C | - | Degree Centigrade |
| CAP | - | College of American Pathology |
| CRP | - | C – Reactive Protein |
| D $H_2O$ | - | Deionized water |
| DMR | - | Diacetyl Monoxime |
| ELISA | - | Enzyme Linked Immuno Sorbent Assay |
| ESR | - | Erythrocyte Sedimentation Rate |
| Fig | - | Figure |
| $FT_3$ | - | Free Triiodothyonine |
| $FT_4$ | - | Free Thyroxine |
| g | - | gram |
| g/dl | - | gram per deciliter |
| g/L | - | gram per liter |
| GGT | - | Gamma Glutamitate Transaminase |
| Hb | - | Haemoglobin |
| HCL | - | Hydrochloric acid |
| HRP | - | Horse radish peroxidase |
| I.D | - | Identity Card |

| | | |
|---|---|---|
| Ig | - | Immunoglobulin |
| IU | - | International Unit |
| IU/L | - | International unit per liter |
| m L | - | mole liter |
| M mol | - | Millimoles |
| Min | - | Minute |
| $mm^3$ | - | Cubic millimeter |
| N B | - | Non Beedi rollers |
| N.S | - | Non Smokers |
| ng/d L | - | nanograms per deciliter |
| OD | - | Optical Density |
| OP | - | Out patient |
| PCV | - | Packed Cell Volume |
| pg/ml | - | pictograms per milliliter |
| RB | - | Reagent Blank |
| RBC | - | Red Blood Corpuscles |
| REE | - | Resting Energy Expenditure |
| Rpm | - | Rotation per minute |
| S | - | Smokers |
| Sec/min | - | second per minute |
| SGOT | - | Serum Glutamic Oxaloacetic Transaminase |
| SGPT | - | Serum Glutamic Pyruvic Transaminase |
| SHS | - | Second Hand Smoke |
| Std | - | Standard |
| T | - | Test |

| | | |
|---|---|---|
| T.Bilurubin | - | Total Bilurubin |
| T.Cholesterol | - | Total Cholesterol |
| T.Protein | - | Total Protein |
| $T_3$ | - | Total Triiodothyronine |
| $T_4$ | - | Total Thyroxine |
| TEC | - | Total Erythrocyte Count |
| TLC | - | Total Leucocyte Count |
| TMB | - | Tetra Methyl Benzidine |
| TSC | - | Thio Semi Carbozide |
| TSH | - | Thyroid Stimulating Hormone |
| U/L | - | Unit per liter |
| WBC | - | White Blood Corpuscles |
| < | - | greater than |
| % | - | Percent |
| µ IU/ml | - | micro-international unit per milliliter |
| µg/ d L | - | micrograms per deciliter |

**Syed Ali Fathima K.M.** 2012 Thyroid Risk Evaluation in Selected Beedi Workers Population in Tirunelveli Ph.D Thesis, Manonmaniam Sundaranar University,Tirunelveli,Tamil Nadu, India.

# CHAPTER - 1
# REVIEW OF LITERATURE

Tobacco cultivation has a history of about 8000 years (IIIM, 2012). Europeans were introduced to tobacco when Columbus landed in America in 1492 (www.tobacco.org). Portuguese traders introduced it in India in the late 16$^{th}$ or nearly 17$^{th}$ century (Din prakash *et al*.,2010).

Beedi industry is spread across the country,concentrated in the states of MadhyaPradesh,Maharashtra,Tamilnadu,Karnataka,AndhraPradesh,WestBengal,Gujarat, Kerala, Orissa, Bihar, Rajasthan, Assam,Tripura and Delhi (Giriappa, 1987; Prasad and Prasad, 1985). Beedi manufacturing is the second largest industry in India (Shimkhada *et al*.,2003).

Beedi industry provides employment to millions of women and children mostly from the poor socio economic strata (Shimkhada *et al*.,2003; Aghi, 2003). Among community groups the backwards caste and muslims dominate in beedi work (Koli,1990; Jhabvala*et al*.,1985; Mohandas,1980; Gopal, 1997).Women's involvement in beedi rolling has been linked to the ease of learning the skill, its manual operations, the fact that work can be carried out at home and so on. However, there are references to women and children being better at the job,especially girl children (Nair, 1990; Pande,1990; Karunanidhi,1997; Datar *et al*.,1985). Most of the beedi workers belong to the poor, landless (Koli,1990; Mohandas,1980; Gopal,1997).

A descriptive report on exploiting women and children, focused on various problems faced by women and children employed in beedi industries. According to government estimates, beedi rolling employs nearly 4.45 million people, of whom 65% are women (Samsherganj, 1990) and 15 to 25% are children. Women often face discrimination and are paid less than men. Children are even worse with no wage structure and usually get paid the least. Most families in the beedi industry live below the poverty line. Bhuvana (2000) records 6 lakh women in nellai district of Tamilnadu itself.

The production of beedi under the home based system using women and children is quite old. The Royal Commission on Labour (1931) has mentioned that beedi rolling is carried out in the dwelling of workers as well. Lakshmidevi (1985) has also mentioned that outwork and contract system has been prevalent since 1930s. Beedi manufacturing on a commercial basis is about a century old, although beedi making for own consumption must have been practiced even earlier. Till today, the range of beedi manufacturing varies from individual self-employed beedi workers to the large branded beedi companies (Rustagi, 1991).

Even within the factory system, women workers predominate in the task of beedi rolling. Although beedi rolling has been identified as a hazardous occupation, the health and working conditions of beedi workers has not been in the forefront of public consciousness. The reasons for this could be the lack of mobilization among beedi workers themselves. Beedi workers eke out a living like helpless puppets amid unspeakable poverty, unemployment and hunger, unable to raise their voices (Sudarshan *et al*., 1999).

Beedi rolling is just inhalation of tobacco dust that causes health problems, but other factors that affect their health like lack of nutritious food, clean environment and

pollution and working in a dark, ill ventilated room affects health adversely (Rao,1992). Unlike the other areas of diseases especially occupational health hazards no research is done on diseases caused by exposure to tobacco. Women work during advanced stage of pregnancy also affect the unborn child (Suman *et al.,*2007). Occupational health hazards have been recently given more importance because of the increase in occupational disease. For instance, the beedi workers are affected by diseases like tuberculosis, chronic bronchitis, nutritional anaemia, back pain, head-ache and eye irritation. It is reported that children engaged in beediworkers are often objects to respiratory infections (Aghi, 2001).

A project report by Karunanidhi (1997) discloses that since the children engaged in the beediworkers have malnutrition, they are highly susceptible to the respiratory infections resulting in frequent cold and cough. The children suffering from these health problems, are often suspected to have the problem of chronic bronchitis. Beedi making inherently poses tremendous health risks for the workers who are constantly exposed to tobacco dust and fumes. The risk is even more in the case of children both as workers and as household members since the living and working places are the same for homebased workers. Two factors that cause health hazards are first, the raw materials, especially tobacco and secondly the nature of work, working conditions and the workplace (Bharathi,2010). The nature of work involves prolonged sitting with forward trunk bend, the excessive use of fingers and the constant high tension levels to meet targets cause a number of health problems. The sitting postures leads to a number to a static construction of back muscle, resulting in head, neck, leg and back aches as these is no body movement. Workers also suffer from piles and rheumatism (Dharmalingam,1993). The long hours of work and the sitting posture while rolling beedis are said to cause back pain and certain gynaecological problems to

women beedi workers (Rajasekhar *et al.,* 2002).The nature of beedi work is such that it causes severe strains to the eyes of the beedi workers resulting in loss of impairement of eyesight, as they grow older (Avachat, 1978).

The Factory Advisory Services and Labor Institute in Bombay, a unit of the Labour Ministry of India, found the incidence of bronchial asthma and tuberculosis to higher among beedi workers than any other group in the general population. Mittal *et al*.,(2006) found that respiratory impairments like restrictive, obstructive and combined restrictive and obstructive type among exposed workers as off whole were much higher (23.53%) compared to control(3.56%).Ignacak *et al*., (2002)revealed that occupational chronic exposition to the dust of tobacco leaves is associated with significant increase in the occurrence of chronic obstructive ventilatory disturbances like COPD and asthma. Barman (2007)revealed that continuous exposure to the tobacco processing environment reduced the workers lung volume and peak expiratory flow rates. Kolarzyk *et al*.,(2006) revealed that, exposure to tobacco dust increases the risk of chronic obstructive pulmonary diseases.

Sen (2011) found that beedi workers experienced pain in limbs and shoulders, headache, backache and continuous cold, allergy, eye problems, gas trouble and asthma. Beedi industry in India, found that the beedi workers in indore suffered from dizziness, pain in the abdomen, menstrual problems, leucorrhoea and anaemia. Sen (2011) found that beedi workers head aches and pain, cough, stomach pain, piles, urinary burning, white discharge, swelling, fever, palpitation and wheezing. International Labour Office Geneva (2003) found that beedi workers has allergic growth of boils in the mouth. Bharathi (2010) found that beedi rollers are at a higher risk for genotoxic hazards due to occupational exposure to unburnt tobacco.

Kumar *et al.,*(2010) have recorded musculoskeletal problems,respiratory complications, eye irritation, dermatitis and malnutrition. Kumar *et al.,*(2010) showed that majority of the women were suffering from hazardous health problems like backache, spondilitis, asthma, tuberculosis. Srinivasulu (1997) reported that 90% of beedi workers are women. When beedis are stored in the house, food spoils quicker and family members experience nausea and headache (Panchamukhi, 2000). Popescu *et al.,* (1964) reported that exposure to tobacco leaves may cause allergic toxic and possibly irritative effects. Ghosh *et al.,*(1980) observed occupational illness among 89% of tobacco farmers due to the handling of green tobacco leaves. Ghosh *et al.,*(1985) described non respiratory occupational health complaints among tobacco processing workers such as vomiting, giddiness and headache that were associated with high urinary nicotine and cotinine levels. Kjaergaard *et al.,* (1989) Nakkeeran *et al.,*(2010) reports complaints and objective changes of the conjunctiva in tobacco workers. Sen (2011) reported that the women beedi workers were affected by aches and pains, coughs, giddiness, stomach related pains.

Kaur and Ratna (1999); Aghi and Gopal (2001)reported induration of the hands and complications of pregnancy in women beedi rollers. Bagwe *et al*.,(1992);Bhisey and Bagwe (1995); Mahimkar and Bhisey (1995) and Umadevi *et al*.,(2003)researched on the cytogenetic toxicity caused by occupational exposure to tobacco. Although a number of occupational health problems have been reported for the women beedi rollers. Khanna (1993) discovered large amount of nicotine, a tobacco specific compounds found its way into bloodstream of beedi rollers. A compound formed when nicotine is broken down in the liver found in the urine and saliva of beedi rollers who did not consume tobacco. Ranjithsingh and Padmalatha (1995) reviewed that beedi rollers were affected by respiratory disorders, skin diseases, gastrointestinal illness,

gynaecological problems, lumbosacral pain and are susceptible to fungal diseases, peptic ulcer, haemorrhoids and diarrhoea, numbness of fingers, breathlessness and stomach pains including cramps and gas, have also been reported in beedi rollers (Dikshit and kanhere (2000); Kuruvila *et al.,*(2002) and Mittal *et al.,*(2006) found that postural pain, eye problems and burning sensations in the throat are common ailments in women beedi rollers.

Bhisey *et al*.,(2006) recorded that inspirable dust of tobacco factory was associated with chronic bronchitis in workers. Considering the high content of nicotine and other chemicals in beedi tobacco these workers are at an extremely high risk of developing systematic illness (Malson *et al*.,2001).

The constituents of tobacco get absorbed into the body, get bioactivated and result in increased risk of developing ailments for which tobacco consumption is a major risk factor, including chronic obstructive pulmonary disease, cardiovascular system abnormality, carcinomas and premature death (U.S Department of Health and Human services, 2004).

Kuruvila *et al.,*(2002) found that dermatological observations included callosities seen in beedi workers on fingers and feet, nail changes like pigmentation, paronchia, dystrophy which were more prominent on the right index finger, fungal infections and eczema and callosities and localized nail changes can be considered to be occupational marks in beedi rollers correlated to use of scissors for cutting leaves and use of gum and artificial metallic nail for rolling beedis. Schievelbein *et al*.,(1984) observed occupational groups exposed to carbon monoxide(co) and from experiments with animals chemically treated with co(or)nicotine, the conclusion can be drawn that

neither (or) nor nicotine is likely to play a role in the development and progression of coronary heart disease in these concentration normally found in passive smokers.

Ghosh *et al*.,(2005) reported that beedi workers have most frequent pain in shoulder followed by back pain and neck pain. Apart from that knee, aches, elbow and wrist pain have also been reported. Yasmin *et al*.,2010, Kumar *et al.,* (2010)found that beedi rollers suffered from eye, gastrointestinal, neurons, respiratory and osteological problems. Jadhau (2012) recorded that thousands of beedi rollers in Solapur suffer from occupational hazards like gastrointestinal and nervous problems, throat burning, cough and respiratory problems.

Voluntary Health Association of India reported that beedi rollers are constantly exposed to tobacco dust and hazardous chemicals. They experience exacerbation of tuberculosis, asthma, anemia, giddiness, postural and eye problems and gynaecological difficulties. A study conducted by the National Institution of Occupation Health (NIOH), Ahmedabad revealed that the main hazard in the beedi industry is tobacco dust, burning of the eyes, conjunctivitis, bronchitis and emphysema (Mittal*et al*.,2008).

Beedi workers are highly prone to respiratory problems. Most of them suffer from tuberculosis, chronic bronchitis, asthma and so on. Most beedi workers eventually die of one of these ailments (Avachet, 1978). Many studies report 20-30 percent or less workers having these diseases, while all highlight the high likelihood of workers suffering from them (Datar, 1990; Gopal, 1997; Pande,1990; LabourBureau, 1996;Karunanidhi,1997).

In India workers engaged in the processing of tobacco for the manufacture of beedis are chronically exposed to tobacco flakes and dust via the cutaneous and morphological routes (Mahimkar *et al.,* 1995).They receive massive chronic exposure

to unburnt tobacco, mainly by the cutaneous and nasopharyngeal routes which may develop pulmonary function impairments among the workers exposed to the environment (Bagwe *et al*.,1992). According to Bagwe and Bhisey(1991) and Swami *et al.,*(1995) beedi rollers are exposed to unburnt tobacco mainly through the cutaneous and nasopharyngeal route. Viegi *et al.,* (1986) also reported that tobacco workers experienced a decrease in forced and expiratory flows associated with work duration. Lorenzo*et al.,*(1988) found that 13%of the tobacco workers diffuse intestinal pulmonary fibrosis or clinical symptoms of occupational asthma. Mukhtar *et al.,*(1991) studied tobacco workers and found that tobacco dust may cause the constriction of smaller airway. Chattopadhyay *et al.,(*2006) Popovic *et al.,* (1992) and Yanev (1987) reported lower results of lung function tents mostly of the obstructive type in tobacco workers. Behera *et al*.,1995 studied $T_4$,$T_3$ and their ratio were significantly lower in both beedi and cigarette smokers. Circulating TSH and $T_4$ and $T_3$ were lower in both beedi and cigarette smokers. Beedi rolling is just inhalation of tobacco dust that causes health problems, but other factors that affect their health like, lack of nutritious food, clean environment, pollution,working in a dark and illventilated room affects health adversely (Rao,1992).

Nicotine deposit primarily in the mouth and upper respiratory tract. To use extent that ammonia increases the deposition of nicotine into the arterial blood stream and to the central nervous system (Seeman *et al*.,2008).Nictotine consumption increases a resting heart rate, as soon as 30 minutes after puffing and the higher the nicotine consumption the higher the heart rate (Rose, 2002). Smokers heart have to work harder than consumption non smokers heart. Nicotine consumption increases blood pressure (Ross, 2001) older male smokers have been found to have higher systolic blood pressure than non smoking men do. Nicotine, one of the harmful

constituents of Tobacco, is metabolized rapidly and extensively in the liver,70% is converted to cotinine,which has a half life of approximately 16 hours. The presence of cotinine in serum is considered the best marker of smoking and is preferred to other markers such as carboxy haemoglobin or thiocyanate (Jeemon *et al*.,2010).

Nicotine increases the amount of bad fats (LDL triglycerides, cholesterol) circulating in the blood vessels and decreases the amount fats (HDL) available (Mitchell, 1999). These silent effects begin immediately and greatly increase the risk for heart disease and stroke. In fact, smoking 1-5 cigarettes per day presents a significant risk for a heart attack (Mitchell, 1999).

There are reveal factors that can accelerates artherosclerosis. Nicotine and other toxic substances from tobacco smoke are absorbed through the lungs into the blood stream and are circulated throughout the body. These substances damage the blood vessel walls which allow plaques to form at a faster rate they would in a non smoker (Mitchell, 1999).

In this way, smoking increase the risk of heart diseases by hardening artherosclerosis. In addition a recent study in Japan showed a measurable decrease in the elasticity of the coronary arteries of non smokers.

Smoking increases the number of circulating oxidants it also increase the consumption of existing antioxidants (Goldman, 2000). Smoking immediately causes oxidant stress in blood while the anti oxidant potential is reduced because of this stress (Durak, 2000). In addition a National Cancer Institute study found that beta carotene supplements, which contain precursors of vitamin A1 mostly increase of lung cancer and overall mortality in cigarette smokers (Albanes, 1999).

Component of tobacco smoke hasten the breakdown of some blood thinners, anti depressants and anti seizure medications and tobacco smoke also decreases the effectiveness of certain sedatives, painkillers, heart, ulcer and asthma medicines (Behrman, 2000).

Some studies have shown a link between ETS in childhood and a higher prevalence of asthma in adulthood. Children exposed to ETS are at increased risk of many infections, most commonly middle ear and respiratory infections patients with sickle cell anaemia who smoke and known to have increased incidence of Acute Chest Syndrome.

**Syed Ali Fathima K.M.** 2012 Thyroid Risk Evaluation in Selected Beedi Workers Population in Tirunelveli Ph.D Thesis, Manonmaniam Sundaranar University,Tirunelveli,Tamil Nadu, India.

## 1.1GENERAL INTRODUCTION

Tobacco related industry is a major commercial enterprise around the world (Shimkhada *et al*.,2003). Production of tobacco leaf increased by 40% between 1971, during which 4.2 million tons of leaf were produced in 1997, during which 5.9 million tons of leaf were produced (FAO, 2003). According to the Food and Agriculture Organisation of the UN, tobacco leaf production was expected to hit 7.1 million tons by 2010. This number is a bit lower than the record high production of 1992, during which 7.5 million tons of leaf were produced (FAO,2010). The production growth was almost entirely due to increased productivity by developing nations, where production increased by 12.8% (Jacobs *et al*.,2000). During that same time period, production in developing countries actually decreased (FAO,2010).

China's increase in tobacco production was the single biggest factor in the increase in world production. China's share of the world market increased from 17% in 1971 to 47% in 1997. This growth can be partially explained by the existence of a high import tariff on foreign tobacco entering China. While this tariff has been reduced from 64% in 1991 to 10% in 2004 (Mao *et al*.,2006) it still has led to local, Chinese cigarettes being preferred over foreign cigarettes because of their cost. Every year 6.7 million tons of tobacco are produced throughout the world. The top producers of tobacco are china (39.6%), India (8.3%), Brazil (7.0) and the United states (4.6%) (CBFTS,2005).

India's Tobacco Board is headquartered in Guntur in the state of Andhra Pradesh. India has 96,865 registered tobacco farmers (Shoba *et al*.,2002) and many more who are not registered. Around 0.25% of India's cultivated land is used for tobacco production (CTRI,1999). Since 1947, the Indian government has supported growth in the tobacco industry. India has seven tobacco research centers that are located in Chennai, Andhra Pradesh, Punjab, Bihar, Mysore, West Bengal, and Rajamundry (Shoba *et al*.,2002). Rajamundry houses the core research institute. The government has set up a Central Tobacco Promotion Council, which works to increase exports of Indian tobacco.

India accounts for only 4 percent of the world exports of raw tobacco. Total export earnings from both leaf tobacco and manufactured products in India have increased almost 25 fold, from Rs 326 million at the beginning of the 1970s to about Rs 8100 million in 1998/99 (kulkarni, 2001). This was partly due to the general increase in tobacco prices at world level. During the last decade, tobacco export earnings increased almost three fold, from Rs 1900 million to Rs 6300 million. However, for manufactured tobacco products they rose by only 145 experiment, from Rs 699 million to Rs 1700 million. Among tobacco products, exports of hookah paste accounts for around 80 percent in volume but only 40 percent in value terms. The share of cigarettes has declined since 1993 /94, following the reduced demand from the former Soviet Union, with which Indian had a bilateral trade agreement.

Nearly two thirds of exports of raw tobacco were to European countries, with Russia being a leading importer. Saudi Arabia is the single largest importer of hookah paste. Bahrain, Oman, Singapore and the United Arab Emirates import beedis from India. Russia has been the largest importer of Indian made cigarettes.

India is the world's third largest producer of leaf tobacco. It is also a very large consumer of tobacco products. Tobacco is one of the important cash crops in the country, and makes a significant contribution to the Indian economy in terms of employment, income and government revenue (Kulkarni, 2001). It generates nearly Rs 20 billion of income per annum. The economic importance of the crop can be considered at three levels; farm households engaged in tobacco growing and processing; major tobacco producing states; and central government level. Tendu leaf accounts for 74 percent by weight of beedi. Dark and sundried tobacco varieties are used in beedi production. Almost 80 percent of beedi tobacco comes from Gujarat, and the rest comes from Karnataka. Beedis account for over 50 percent of total tobacco use, compared with less than 20 percent by the cigarette segment. There are an estimated 2,90,000 growers of beedi tobacco. The collection of tendu leaf that is used to wrap beedis forms an important link for the industry. Tendu leaf is almost wholly grown on government owned forestlandwith around 62 percent of tendu leaf being grown in Madhya Pradesh.

Annual production of tendu leaf in 1994/95 had an estimated value of Rs 14,700 million. About 2 million people are engaged in leaf collection, while another 4.4 million people are employed directly for beedi rolling. Beedi rolling is concentrated in the states of Madhya Pradesh, Andhra Pradesh, Tamil Nadu, Uttar Pradesh and West Bengal. Beedis are manufactured largely in the independent smallscale and cottage industry sector. There are a few large manufactures of branded beedi, which tend to be closely-held, family–run businesses. The beedi industry is estimated to have used 2,68,000 tonnes of tobacco in 1998/99, 54.4 percent of the total apparent tobacco use (Indian Market Research Bureau Report, 1996).

Beedi is a leaf-rolled cigarette made of coarse uncured tobacco, tied with a string at one end. Beedis dominate the smoking market of India for every cigarette ten beedis are smoked (Emil, 2008).

There is no historical record of the exact period during which the practice of smoking tobacco rolled in leaves started in India. The cultivation of tobacco started in southern Gujarat in the late 17$^{th}$ century. Beedis were developed soon after, possibly around the Kheda and Panchamahal districts of Gujarat, where cultivation of tobacco was high. Labourers would roll leftover tobacco in leaves of astra tree and smoke at leisure. Communities across India experimented using leaves of Mango, Jack fruit, banana, Sal, Pandancus and Palash (Anon, 1942). Initially, communities in Gujarat made beedis only for their own consumption but their increasing popularity inspired some to make it into a home base business. Soon beedis made locally became more popular than hookahs, largely because beedis overcome the obstacle of sharing the hookah, as individual could smoke without hurting caste and religious sentiments and also because they were portable and did not require assembling and extensive preparation to light up (Anon, 1879). The early business model of the beedi industry in Gujarat involved the businessmen and their workers rolling their own beedis putting them in a tray and selling them along with tobacco and matches in local markets. Gujarati families that had settled down in Bombay saw the potential of the beedi business and soon started manufacturing beedis on a large scale. Beedi has penetrated into other parts of the country outside of Bombay, but until 1900 beedi manufacturing was largely restricted to Bombay and southern Gujarat (Lal,2009).

Beedi tobacco growing is concentrated in Kaira in Gujarat, and Belgaum and Nipani in Karnataka, while most manufacturing clusters are in Mangalore, Mysore and

Nipani (Karnataka); Pune and Nashik (Maharashtra); Jabalpur, Damoh and Sagar (Madhya Pradesh MP); Raipur (Chhattisgarh); Tirunelveli and Chennai (Tamil Nadu); Cannore (Kerala) and Nizamabad, Karimnagar, and Warangal (Andhra Pradesh) (Shoba, 2008). By the mid 1970s, production of beedis had reached nearly the same level as of today, between 800 billion and 1.2 trillion beedis. While new clusters were being created rapidly, several old clusters like Vidharbha dissipated. Even strong holds like Madhya Pradesh, which accounted for more than half the beedis produced till the 1980s, have lost out to new epicenters of beedi rolling like West Bengal, Bihar and Orissa, where labour is cheaper (Desai, 1997).

According to government estimates quoted by international labour organisation, there are close to 5 million workers involved in rolling of beedis in India.Tamilnadu is the major hub for beedi industry. There are nearly 6 lakhs beedi workers in Tirunelveli district of Tamilnadu (Bhuvana, 2000). These individuals work in small industries or at house hold based enterprise in an environment laden with tobacco dust, of which 90% are home based women workers (Prasad and Prasad, 1985).

It is estimated that over 2.3 million persons depended on this sector for their livelihood. The annual wage bill in these enterprises averaged Rs 4300 million and annual wages per worker varied from Rs 8400 in beedi factories to Rs 55,730 in cigarette, cigar and cheroot factories. The total net value added by all enterprises averaged Rs 15,000 million per annum, of which beedi factories contributed 41.2 percent, and cigarette and allied industries 34.3 percent. The total annual wage bill in the cigarette and allied industries, despite wages per worker being substantially higher, was only 4 percent of its gross value of output, compared to 16 percent in the beedi factories, because beedi manufacturing is more labour intensive. Beedi manufacturing

is estimated to provide employment to more than 4.4 million workers, a large number of whom are women and children. If the forward and backward economic linkages are taken into account, beedis generated 1310 million workdays, whereas cigarettes generated 340 million workdays (Indian Market Research Bureau Report,1996).

Over 3 million Indians are employed in the manufacture of beedis, a cottage industry that is typically done by women in their homes. Workers roll an average of 500-1000 beedis per day, handling 225-450 grams of tobacco flake and inhaling tobacco dust and other volatile components present in the work environment (Ghosh *et al*.,2007;Mahimkar *et al*.,1995). Studies have shown that cotinine levels in the body fluids of beedi workers are elevated even among those who do not use tobacco (Khan *et al.,*2002). By the middle of the 20$^{th}$ century beedi manufacture had grown into a highly competitive industry. This stage of commercial production at the height of the beedi's popularity saw the creation of many new beedi brands as well as beedi factories employing upwards of one hundred primarily male beedi rollers (Cornell International Report, 1998). In India there are large number of beedi industries spread across the length and breadth of the country (Suman *et al*.,2007). Majority of these workers are women and these women belong to low socioeconomic status which compels them to work and earn money to supplement their family income (Ghosh, 2005).

It is estimated that 3,25,000 children work rolling beedis despite beedi manufacture being classified by the Indian Child Labour Prohibition and Regulation Act as hazardous work (American Medical Association, 2000).

Beedi making is a skilled job. Beedis are made from processed tobacco wrapped in the tendu leaves. The leaves are moistened by soaking them in water

overnight. The wet leaves are then cut into pieces roughly rectangular in shape and in size depending on the length of the beedi. The processed tobacco in powdered form is thoroughly mixed by hand and then rolled on a piece of tendu leaf. A thread is then tied around it towards the narrower and to maintain the shape of beedi (Chattopadhyay *et al*.,2006).

Major problems faced by the beedi workers were peripheral poverty, very low wages, no wage bargaining system, no access to entitlement benefits and gratuity, delayed payments, illiteracy, hidden child labour, constant exposure to tobacco dust, postural pain and induration of the hands. Hazards of beedi workers remained neglected since long time and there is a need to study health hazards among beedi workers and to reduce it, if not eliminate the hazards of beedi workers for greater safety and health(Bagwe *et al.,*1993).

Children who work for hours sitting crosslegged making beedis often suffer from backaches and knee problems. Initially tobacco dust often makes them feel giddy, and over the long term, many develop chronic bronchitis, asthma and even tuberculosis. Some complain of a burning sensation in the eyes and throat. They also suffer from rheumatic syndromes besides allergies, stomach troubles and piles (Chauhan, 2001; Aghi *et al*.,2001).

Suffering from green tobacco sickness form of nicotine poisoning when wet leaves are handled, nicotine from the leaves gets absorbed in the skin and causes nausea, vomiting and dizziness. Children were exposed to 50 cigarettes worth of nicotine through direct contact with tobacco leaves. The level of nicotine in children can permanetly alter brain structure and function (ILO, 2003).

Currently, 1.3 billion people worldwide smoke or use other tobacco product and nearly 5 million die as a result. Eighty four percent of the world's tobacco users live in countries with developing or transitional economics. An average men who smoke beedi the popular hand rolled cigarettes that contain about one quarter as much tobacco as a full sized cigarette shorten their lives by about six years. There are approximately 120 million smokers in India about 37 percent of all men and 5 percent of all women between the ages of 30 and 69. The projected increase in smoking related deaths in India is part of a global trend, according to the World Health Organization (WHO) which estimates that smoking related death worldwide will suppress 9 million annually by 2020 with 7 million of those deaths occurring in developing nations. Most beedi smokers are illiterate and malnourished which makes them more vulnerable to smoking related morbidity and mortality (Rahman *et al*.,2000). Tobacco smoke contains over 4000 chemicals in the form of particles and gases (United States Environemental Production Agency, 1992). Many potentially toxic gases are present in higher concentrations in side stream and nearly 85% of the smoke in a room results from side stream smoke. Smoking has numerous immediate health effects on the brain and on the respiratory, cardiovascular, gastrointestinal, immune and metabolic systems. Smokers brain cells especially brain cell receptors have been shown to have dopamine receptors (Dagher, 2000).

Occupational exposure to unburnt tobacco may occur during tobacco manufacture particularly in beedi factories in India which are often small scale industries that have poor working conditions. The workers are mainly women and are exposed to tobacco by dermal contact and also have air borne exposure to tobacco dust and volatile components. Studies of such industries have mainly reported in the concentration of tobacco dust and particulate matter in the ambient air in the factories

as well as biomonitoring of the workers, but no epidemiological studies on tobacco related health risks in these workers have been carried out.

Eventhough occupational hazards and other health hazards such as, pulmonary, skin problems and cancer ailments were prevalent among the beedi worker population especially among women and children much reports were not available based on the haematological parameters. Blood profile analysis of beedi workers regarding thyroid dysfunction was not reported eventhough few reports are available in smokers (Behera *et al*.,1995). Since, women constitute a major role of beedi rollers and are susceptible to thyroid disorders a detailed clinical examination and tests for thyroid profile was carried out in the respondents. Screening of serum TSH level $T_3$ and $T_4$ and $FT_3$, $FT_4$, AMA, and ATG were measured and analysed .Quite apart from the health impacts of smoking or chewing tobacco are the health hazards of working with tobacco. The nicotine inhaled from smoking or absorbed through the skin when harvesting tobacco, leading to a condition called "Green tobacco sickness (GTS). GTS has been reported to occur in 1-10% of us tobacco workers and younger workers are at higher risk. Symptoms of GTS include weakness, headache, nausea, vomiting, dizziness, abdominal cramps, breathing difficulty, abdominal temperature, paller, diarrhoea, chills, fluctuation in blood pressure or heart rate and increased persiperation and salivation (Mc Bride *et al*.,1998).

Many people are convinced that other factors are at paly in the development of thyroid disease. Some suggest it might be linked to stress. Others suggested it might be the result of viral or bacterial infection, or the result of physical trauma to the thyroid gland (Shomon, 2012).

Some studies have also reported smoke related changes in thyroid hormone levels; that is, increased $T_3$ and free $T_4$, $FT_4$, as well as decreased TSH levels (Ericsson *et al*.,1991, Fisher *et al*.,1997 and Knudsen *et al*.,2002), yet published data are inconsistent. Iodine status appears to play a role in the relationship between active smoking and thyroid function, whereas some studies reported no effects of smoking on $T_3$, $FT_4$ and TSH (Ericsson *et al*., 1991; Fisher *et al*.,1997 and Muller *et al.,*1995). Concomitantly, it is unclear whether changes in thyroid hormone levels are associated with the a fore mentioned smoke induced increase in resting energy expenditure (REE) It was reported that the exposure to passive smoke was accompanied by a statistically significant increase in $T_3$ and $FT_4$ levels. TSH showed a non significant decrease in both conditions.

Active and passive exposure to cigarette tobacco smoke is associated with a mild inhibitory effect on the thyroid reflected in higher serum $T_4$ and $T_3$ in nonsmokers compared to smokers in this cohert women. The relation between tobacco smoking and thyroid function is not well understood.

Secondhand smoke (SHS) induced effects on thyroid function is characterized by a mild inhibition of thyroid hormone levels (Soldin *et al*.,2009). Neither the components in tobacco that may cause the thyroid effects nor their mechanisms of action are clear. Nicotine causes sympathetic activation, which can increase thyroid secretion (Robert *etal*.,1998).

Eventhough, the impact of tobacco smoke containing many components, cause anamolies of thyroid function with respect to active and passive smoking are available, continuous exposure to unburnt tobacco and its constituents on thyroid function it practically unavailable. More studies are required to confirm the minimal data published and clearly identify the long term effects of unburnt tobacco dust and

theirconstituents in beedi rollers. As a pioneer study which is undertaken presently to determintal effects of nicotine of tobacco dust on the thyroid function in beedi workers is evaluated to assess the risk of thyroid disorders. Since blood parameters serve as very good indices in the assessment of the health profile, efforts were undertaken in the present study to evaluate the health status of beedi worker population based on the blood profiles such as electrolyte level, liver function test, lipid profile with special emphasis to thyroid profiles and related parameters. The study may enlighten about the impact of handling tobacco during beedi rolling on the health status of a selected beedi worker population in Melapalayam, Tirunelveli District.

**OBJECTIVES**

- To interrogate with a proper questionnaire among a selected beedi worker and to know the health status, along with their socioeconomic background population in Tirunelveli.
- To identify the occupational hazards of beedi rolling age wise due to working postures through contact and inhalation.
- To assess the impact of dermal contact and inhalation of raw tobacco dust among the beedi worker community.
- To investigate the health condition of beedi workers through haematological indices with a special emphasis to thyroid profile and compare with normal population.
- To analyse the thyroid abnormalities due to inhalation tobacco smoke by smokers and compare the risk of thyroid disorders in women beedi rollers due to the inhalation of raw tobacco dust and highlight the impact of tobacco.

**Syed Ali Fathima K.M.** 2012 Thyroid Risk Evaluation in Selected Beedi Workers Population in Tirunelveli Ph.D Thesis, Manonmaniam Sundaranar University,Tirunelveli,Tamil Nadu, India.

# CHAPTER - 2

# SOCIO ECONOMIC STATUS OF THE BEEDI WORKERS IN MELAPALAYAM, TIRUNELVELI.

## 2.1 Introduction

Health is an integral part of socio-economic development of any nation. As there is no direct method of measuring health, it can be measured indirectly by finding the incidence of ill health (Anil *et al*., 2012). Factors that influence the health of the population are housing, water, sewage and waste disposal, nutrition and education. Apart from this the health of any worker is also influenced by the nature of work and working environment. The commercial Indian beedi industry saw a rapid growth in 1930's (Cornell International Report, 1998) probably driven by an expansion of tobacco cultivation at that time (International Labour Office, 2003). Further Gandhi's support on Indian industry and Indian products promoted beedi rolling as a cottage industry (Census of India, 1931). Perhaps due to this even educated classes in India preferred beedis over cigarettes (Lal, 2009). Beedi workers in India is the third largest component of the work force after agriculture and textile workers and it provides full and part time employment to nearly 75 lakhs people out of which 90% are women (Anil *et al*., 2012)

Beedi workers are densely populated in the state of Uttar Pradesh, Madhya Pradesh, Karnataka, Andhra pradsh and Tamilnadu. The workers are mainly women and are exposed to tobacco by dermal contact and also have airborne exposed to tobacco dust and volatile components. Studies of such industries have mainly reported

on the concentration of tobacco dust and particulate matter in the ambient air in the factories. Biomoniterring in the workers and the epidemiological studies on tobacco related health risks in these workers have not been carried out. Rolling beedi is an indigenous technique of producing handmade cigarettes which is the major occupation of Melapalayam in Tirunelveli. It provides employment to many inhabitants of this area, who have a poor socio-economic background. Beedi rolling is taken up by women and children at home and it is regarded as primarily women's work. A cross sectional study was carried out through a questionnaire which gave information regarding their social and economic background which includes their monthly salary, educational background, dwelling environment, working hours, revenue etc. This pioneer study formed first phase of the present investigation, collecting general information and individual observation of the beedi workers.

**2.2 Materials and Methods**

As the first phase of present study, a survey of beedi worker population was carried out in Melapalayam of Tirunelveli District during August 2010 to December 2010 through oral questioning and semi structured questionnaire (Annexure 1). Data regarding general information about the family, occupation, annual income, educational background and specific information regarding beedi rolling and working environment were collected(Plate: 1).After analyzing the data a sample of three hundred female beedi workers was selected and a primary data was generated by interviewing the selected respondents. Data were coded and analysed using statistical packages such as Microsoft Excel and SPSS version 16.0. Analysis included tabulation and diagramatic presentation. For statistical test Independent sample t-test (2-tailed) $P < 0.05$ was taken as the level of significance.

**PLATE : 1**

Fig 1: Raw tobacco dust.

Fig 2: Tendu leaf used in beedi making.

Fig 3&4: Cut leaf pieces and rolled beedis.

Fig 5&6: Process of rolling beedis by women.

## PLATE - 1

**Figure 1**

**Figure 2**

**Figure 3**

**Figure 4**

**Figure 5**

**Figure 6**

**PLATE : 2**

Fig 7: School children.

Fig 8: Middle age.

Fig 9: Aged.

Fig 10:Aged.

## PLATE - 2

**Figure 7**

**Figure 8**

**Figure 9**

**Figure 10**

**PLATE: 3**

Fig 11: A women residing in thatched house involved in beedi rolling.

Fig 12: A women occupying a house with only one room a roll beedis.

Fig 13: Women using a small sit out to roll beedies.

Fig 14: A beedi rolling women living in a house which not completely constructed.

## PLATE - 3

**Figure 11**

**Figure 12**

**Figure 13**

**Figure 14**

**PLATE : 4**

Fig 15: A single room in which all members reside and use for cooking dining, sleeping and also rolling.

Fig 16: Elderly women in beedi rolling occupation.

Fig 17: Women rolling beedi along with collecting drinking water.

Fig 18: Unhealthy working environment while beedi rolling.

## PLATE - 4

**Figure 15**

**Figure 16**

**Figure 17**

**Figure 18**

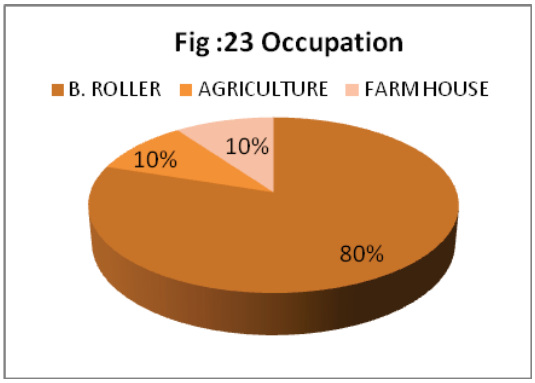
## 2.3 Results

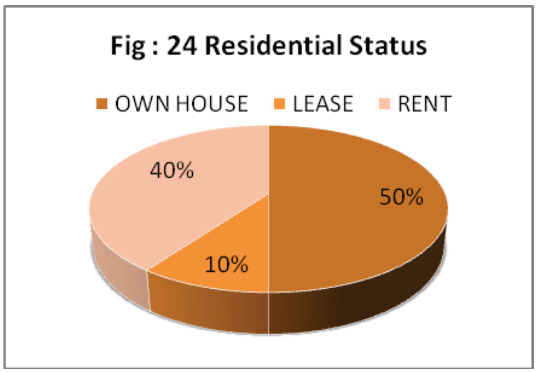
Most of the respondents randomly selected were largely poor and illiterate. 80% women in the age group of 18-60 who were involved in beedi rolling (Plate: 2)(Fig:23) earn only a meagre amount as a contribution to the total income of the family. Men of these of selected families worked mostly as a coolie in the beedi industry indulged in sorting, leveling and packing. Thus the monthly income Rs 2000-2500. Beedi work was found to be by and blending of tobacco. The residential environment was very poor eventhough they occupy their own houses 50% of the population explored, lived in own houses which mostly consisted of one or two small rooms only. 40% of the respondents occupied rented houses and remaining 10% occupied houses purchased on lease (Fig: 24). Houses were not well planned and the construction was irregular with poor infrastructure. Some respondents lived in that shed houses and the environmental background and situation were very poor. Poor hygiene prevailed throughout their dwellings, where garbage and beedi wastes and tobacco dust scattered (Plate: 3). The environment in which they sat and worked for prolonged hours had poor and unhealthy sanitary conditions with water spills and wastes. Most of them live in one small room where they do the beedi work as also cook and sleep(Plate: 4).

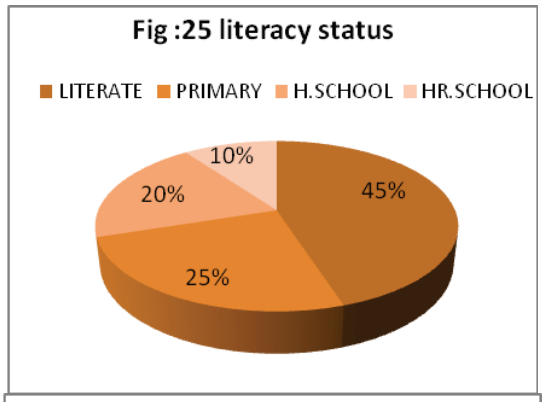
Thus these women beedi rollers with a low socio-economic status had a working environment which was confined to a small area and unventilated houses.

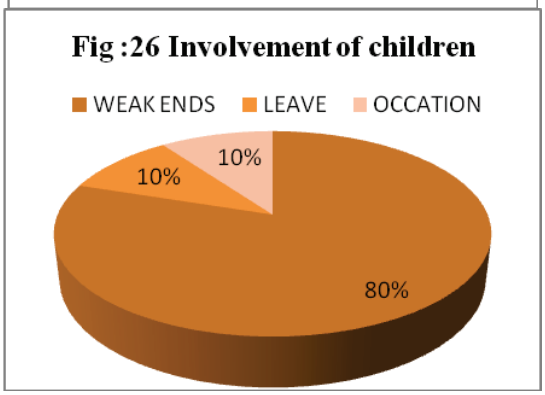
The literacy level of these population was shown in (Fig:25) 45% of the respondents were illiterates, 25% had only primary education, 20% with secondary education and only 10% had higher secondary education. Women constitute a major percentage of beedi manufacturing. Yet, children helped their mothers in the profession

**Socio economic background of beedi rollers**



in the selected families of the present study. 80% of the children engaged themself in beedi rolling during vacation and weekends. 10% of children worked during their holidays and another 10% involved occasionally (Fig: 26).

Working hours among the respondents also differ from 5-8 hours/day to 1-2 hours/day and regarding the number of beedis rolled per day among the respondents also varied. 90% of the respondents rolled 800-1000 beedis/day, whereas 5% of the respondents rolled 500-800 beedis/day and remaining 5% less than 500 beedis. Thus the general information provides the socio economic background of these population and forms the pilot study for the health assessment of these beedi rollers.

**2.4 Discussion**

The beedi manufacturing industry in India in an age old industry in which a huge population is engaged for their two square meals (Ghosh *et al*.,2005). Previously beedi rolling was a household occupation but presently it is not only confined to the houses of the workers but also co-operative societies have come up wherein beedi rollers are working together as an industry. The main problem of beedi workers now-a-days is their poor socio economic status, education and living which forces them to work in unsafe environmental conditions and improper working postures. Government of India has provided various schemes for the beedi workers like welfare measures, Health schemes, Education schemes, Housing schemes, Social security etc. Inspite of various welfare measures, the socio economic status of the beedi workers remain at low level since the welfare measures are insufficient in comparison to the numbers of beedi workers in India.

Due to the poor socio economic status, the beedi workers are forced to work continuously for hours in improper working postures and beyond their normal working capacities which lead to the development of serious physiological manifestations.

As per the status report of UNICEF 1995 Tirunelveli and Thoothukoodi district comprise of 5,50,000 women beedi rollers which is very high when compared to other districts(Table:1). According to Kumar *et al.,* (2010) beedi industry provides employment to millions of women and children mostly from the poor socio economic strata. The socio economic status is largely poor and only 50% of beedi rollers population possessed their own house for their dwelling.

**Table : 1 Distribution of Women Beedi workers in Tamilnadu**

| District | No.of Women |
|---|---|
| Tirunelveli and tuticorin | 5,50,000 |
| Vellore | 1,50,000 |
| Tiruchirapalli | 15,000 |
| Chennai | 15,000 |
| Chengalpattu | 10,000 |
| Salem | 5,000 |
| Erode | 2,000 |

**Source**: A Status report of UNICEF, Madras 1995. Beedi industry in Rural Areas: An overview P.Anandha Rajakumar.

As per the data explored in the study area 80% of the population depend on the beedi industry for their livelihood. Aghi (2001) has reported there are approximately 4.4 million full time workers and another 4 million beedi industry related jobs and most of them are largely poor and illiterate.

Beedi work was found to be by and large female dominated especially in the rolling of beedi and blending of tobacco (Ghosh *et al*.,2005). The all India Beedi, Cigar and Tobacco workers Federation estimation comprise 90-95% of total employees in beedi manufacture. They are primarily beedi rollers and typically operate from homes. (Jadhavala, 2000). Suman *et al*.,2007 has reported that in Solapur around 45,000 women are employed in theseindustry and majority of them belong to low socio-economic status which compels to work and earn money to supplement a family income. In the present investigation women beedi rollers who work in confined and unventilated houses under poor sanitary and hygienic conditions, predispose chronic inhalation of tobacco dust and accompanying biohazards. Shukla (2011) has reported a similar opinion that working condition is an important factor that contributes to the occupational hazards in the rollers, which is reflected in workers working in confirmed environmental conditions. Further he claims that these beedi rollers who work is such unsafe situation are prone to significantly high genotoxic effects. Srinivasulu (1997) reported that when beedis are stored in the house, food spoils quicken and family members experience nausea and headaches. Anil *et al*., (2012) who have studied the morbidity pattern of female beedi workers in Mangalore have reported the literacy status. It shows that only 19% are illiterate, 60% had primary education, 17% secondary education and only 4% have higher secondary education. In the present investigation in Melapalayam shows a similar data which comprises of 45% illiterate, 25% possessing primary education, 20% having higher education and 10% of higher secondary education. In the present study 80% of children involved themself in beedi rolling especially during week ends and holidays whereas the remaining 20% work during leave and occasionally. This tendency of children involvement is due to the fact that they want to help their mothers who are engaged in beedi making. On school days

they roll beedis before reaching school and again continue after returning back. Franke (2005) have given a similar report in socialist scholar conference in 1997. Figures relating to full time employment of children is unreliable due to the fact that they are afraid of disclosing because of the Child Labour Act of 1956.

According to Economic and Political Weekly (2002) considerable variation regarding the number of beedi rolled per day among the respondents. About 34 percent were rolling 501-800 beedis, 31 percent 301-500 beedis and 22 percent more than 800 beedis per day. The number of beedis rolled per day was less than 300 in the case of remaining 13 percent respondents.

Ghosh *et al*.,(2005) has studied the socio-economic status of the beedi workers of Vidharbha region in Nagpur and reported the average family monthly income Rs.1719 where the minimum number of earning member was one. Thus it can be conducted that beedi rollers are exposed to xenobiotics in the course of their occupation due to poor socio-economic status, which includes Housing, Education and Working environment.

Varma and Sasikumar (2005) in their study on impact of anti-tobacco legislation on the livelihood of beedi rollers in West Bengal have reported that most beedi workers are illiterates (42.8%) or literates with formal schooling (14.2%) and of those who have attended school majority have attained primary level of education (20.4%).Gills after their primary or secondary education were not sent to school for further studies since they are forced to take beedi rolling due to their family circumstances. According to Ghosh *et al* (2005) entry to this profession starts from 15 to 16 years of age for both the sexes. Iqbal and Ghosh (2005) has opined that beedi workers start their work at tender age.

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**CHAPTER -3**

# SURVEY OF OCCUPATIONAL HEALTH HAZARDS IN WOMEN BEEDI ROLLERS IN MELAPALAYAM, TIRUNELVELI.

## 3.1 Introduction

Occupational safety and health in an unorganized sector, especially in the homework segment is increasingly demanding the attention of every one. Occupational health aims at the promotion and maintainace of the highest degree of physical, mental and social well being of workers. The beedi manufacturing industry in India is an age old industry and a large rural population is engaged. The poor economic status of these workers renders them incapable of accessing health services. Low income is not the only problem faced by the beedi rollers but the working conditions threaten their physical well being as well (Anil *et al*.,2012). The beedi workers lack education and training and because of their poor socioeconomic condition they are forced to work continuously in improper work postures which lead to the development of serious physiological manifestations (Ghosh *et al*.,2005). It is estimated that around four million workers, mostly women and children are employed in beedi making (www.vhai.org;www.rctfi.org). It is a labour intensive task because each beedi is rolled individually. According to Yasmin *et al*.,(2010) women are predominantly involved in beedi rolling since the work can be done generally from home and they can do it while at the same time attending to their children and their household activities. Further, their deft fingers are more suited to the work of beedi rolling. Workers who

roll an average of 125-450 beedis per day handle about 100-250 grams of tobacco flake and inhale tobacco dust and other volatile components present in the environment.

The occupational health hazards of beedi workers remained neglected since long time and there is a need to study the health hazards predominant in beedi workers. It will be a difficult task to eliminate the hazards of the beedi workers, but at the same time we can reduce the ill effects to some extent after a careful study. Therefore it was necessary to identify the occupational health problems associated with the beedi workers and to suggest medical measures in order to bring about safety, health and proper ergonomic conditions. Since the beedi rollers working in small factories or at household based enterprises laden with tobacco dust for long working hours are likely to inhale swallow and exposed their skin and mucous surface to significant amount of unburnt tobacco dust.

These constituents of tobacco get absorbed into the body, get bio activated and results in increased risk of developing ailment like chronic obstructive pulmanory diseases, cardio vascular system abnormalities, carcinomas and other occupational hazards like back pain, head ache, neck pain etc. Eventhough number of occupational hazards were described in literature Bhisey *et al*.,(1999) and Bagwe and Bhisey (1993) the most common ailments among the beedi rollers are still under study. Beedi rollers handle tobacco flakes and inhale tobacco dust as well as volatile components of tobacco which put them at a high risk of many health problems. Nicotine gets absorbed through the skin while rolling tobacco and has been demonstrated in the blood of beedi rollers who do not smoke (Ghosh *et al*.,2005).Nicotine is a major component of tobacco and has potential adverse health consequences. The objective of the present second phase of study was to obtain information about the probable genotoxic effects of long

time exposure of tobacco dust and the occupational disorders encountered by the beedi rollers in their day to day life.

### 3.2 Materials and Methods

Occupational health profile was evaluated through questionnaire and interview separately with the beedi wokers and also from the authorities working in Melapalayam Beedi Workers Welfare Fund Dispensary. A survey of occupational disorders from the year 2007 to 2012 August were obtained from the medical records available in the dispensary. A random sample of thousands women beedis rollers were interviewed separately and their responses were recorded and categorized. Data was subjected to statistical analyses through Microsoft Excel and SPSS version16.0. For statistical test $P < 0.05$ was taken as a significance.

### 3.3 Result

A descriptive study conducted in Melapalayam of Tirunelveli district on the health hazards of beedi industry workers through clinical report obtained from Melapalayam Beedi Workers Welfare Fund Dispensary (Table:2) and with direct interview with a questionnaire among thousand women beedi rollers revealed that the health hazards was created due to two factors

1) Working posture
2) Direct contact and inhalation of tobacco dust

As per the survey, number of occupational health hazards have been reported by the beedi workers. Hazards may be attributed due to improper working postures (Plate:5).

**PLATE: 5**

Fig 19: Pregnant women.

Fig 20: Adults.

Fig 21: Boys arounding.

Fig 22: Young lady with small children.

## PLATE - 5

**Figure 19**

**Figure 20**

**Figure 21**

**Figure 22**

**Table :2Occupational hazards clinical Report of health hazards among beedi rollers in Melapalayam, Tirunelveli district.**

| 2007 | JAN | FEB | MAR | APR | MAY | JUNE | JULY | AUG | SEP | OCT | NOV | DEC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Respiratory disease | 1106 | 1226 | 1260 | 1072 | 986 | 892 | 873 | 954 | 893 | 974 | 1613 | 1243 |
| Gastro intestines disease | 142 | 102 | 119 | 106 | 132 | 96 | 112 | 142 | 113 | 138 | 292 | 461 |
| Cardio vascular disease | 52 | 67 | 52 | 49 | 38 | 42 | 73 | 70 | 62 | 57 | 70 | 52 |
| Musculo skeletal disease | 1176 | 1213 | 1243 | 1083 | 1009 | 867 | 901 | 963 | 1017 | 1102 | 1449 | 1370 |
| Neurological disease | 189 | 130 | 117 | 99 | 86 | 74 | 69 | 73 | 54 | 44 | 76 | 58 |
| Skin disease | 67 | 71 | 94 | 68 | 412 | 365 | 276 | 198 | 176 | 163 | 194 | 217 |
| Genito urinary tract disease | 64 | 59 | 110 | 113 | 96 | 102 | 164 | 119 | 210 | 242 | 247 | 292 |
| Miscellaneous | 293 | 336 | 306 | 192 | 271 | 310 | 477 | 574 | 407 | 851 | 662 | 668 |
| TOTAL | 3089 | 3204 | 3301 | 2782 | 3030 | 2748 | 2945 | 3083 | 2932 | 3571 | 4563 | 4361 |

| 2008 | JAN | FEB | MAR | APR | MAY | JUNE | JULY | AUG | SEP | OCT | NOV | DEC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Respiratory disease | 1092 | 1107 | 1098 | 1012 | 1128 | 1017 | 12791 | 1094 | 1046 | 1012 | 1076 | 1292 |
| Gastro intestines disease | 570 | 632 | 643 | 536 | 811 | 692 | 543 | 329 | 520 | 397 | 402 | 437 |
| Cardio vascular disease | 64 | 79 | 62 | 51 | 49 | 56 | 75 | 67 | 53 | 49 | 37 | 62 |
| Musculo skeletal disease | 1279 | 1290 | 1284 | 1167 | 1072 | 1103 | 1218 | 1320 | 1373 | 1210 | 1261 | 1371 |
| Neurological disease | 61 | 53 | 67 | 69 | 93 | 77 | 163 | 112 | 118 | 96 | 83 | 197 |
| Skin disease | 332 | 402 | 390 | 410 | 236 | 2411 | 283 | 336 | 292 | 232 | 240 | 312 |
| Genito urinary tract disease | 218 | 198 | 242 | 336 | 260 | 270 | 267 | 215 | 310 | 271 | 297 | 253 |
| Miscellaneous | 468 | 573 | 596 | 256 | 499 | 662 | 875 | 789 | 483 | 349 | 426 | 581 |
| TOTAL | 4086 | 4334 | 4382 | 3837 | 4148 | 4118 | 4703 | 4262 | 4188 | 3616 | 3822 | 4555 |

| 2009 | JAN | FEB | MAR | APR | MAY | JUNE | JULY | AUG | SEP | OCT | NOV | DEC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Respiratory disease | 1174 | 1092 | 1089 | 1012 | 1672 | 1127 | 1476 | 1019 | 767 | 1072 | 1094 | 1127 |
| Gastro intestines disease | 4379 | 267 | 258 | 199 | 346 | 532 | 439 | 372 | 179 | 159 | 1160 | 113 |
| Cardio vascular disease | 54 | 75 | 92 | 58 | 97 | 77 | 45 | 30 | 21 | 127 | 18 | 22 |
| Musculo skeletal disease | 1290 | 1112 | 1120 | 1127 | 1390 | 1095 | 1248 | 1313 | 718 | 867 | 873 | 1310 |
| Neurological disease | 110 | 97 | 79 | 62 | 73 | 89 | 97 | 85 | 12 | 11 | 19 | 97 |
| Skin disease | 408 | 319 | 323 | 293 | 541 | 341 | 218 | 276 | 97 | 104 | 131 | 44 |
| Genito urinary tract disease | 213 | 320 | 346 | 420 | 412 | 276 | 124 | 141 | 56 | 69 | 58 | 52 |
| Miscellaneous | 543 | 671 | 676 | 445 | 445 | 5418 | 401 | 246 | 60 | 246 | 323 | 100 |
| TOTAL | 4174 | 3953 | 3983 | 3216 | 5176 | 4078 | 4048 | 3482 | 1910 | 2555 | 2676 | 2865 |

| 2010 | JAN | FEB | MAR | APR | MAY | JUNE | JULY | AUG | SEP | OCT | NOV | DEC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Respiratory disease | 1219 | 986 | 1078 | 832 | 1089 | 425 | 572 | 421 | 515 | 285 | 215 | 219 |
| Gastro intestines disease | 410 | 72 | 311 | 146 | 154 | 312 | 490 | 380 | 282 | 120 | 180 | 175 |
| Cardio vascular disease | 36 | 46 | 27 | 19 | 21 | 172 | 201 | 472 | 113 | 415 | 320 | 350 |
| Musculo skeletal disease | 1921 | 810 | 975 | 570 | 598 | 198 | 229 | 413 | 315 | 310 | 216 | 230 |
| Neurological disease | 271 | 11 | 30 | 38 | 36 | 150 | 221 | 112 | 150 | 215 | 251 | 305 |
| Skin disease | 277 | 24 | 178 | 86 | 92 | 210 | 212 | 78 | 240 | 250 | 217 | 288 |
| Genito urinary tract disease | 82 | 42 | 19 | 27 | 24 | 115 | 211 |  | 240 | 275 | 235 | 313 |
| Miscellaneous | 299 | 281 | 214 | 177 | 132 | 558 | 105 |  | 325 | 243 | 407 | 362 |
| TOTAL | 4516 | 2272 | 2832 | 1895 | 2146 | 2080 | 2241 | 2203 | 2180 | 2113 | 2030 | 2242 |

| 2011 | JAN | FEB | MAR | APR | MAY | JUNE | JULY | AUG | SEP | OCT | NOV | DEC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Respiratory disease | 227 | 255 | 274 | 296 | 364 | 312 | 312 | 214 | 310 | 253 | 450 | 480 |
| Gastro intestines disease | 272 | 312 | 287 | 200 | 276 | 350 | 225 | 260 | 331 | 232 | 412 | 177 |
| Cardio vascular disease | 351 | 217 | 187 | 225 | 213 | 214 | 89 | 332 | 102 | 273 | 422 | 124 |
| Musculo skeletal disease | 224 | 225 | 248 | 86 | 128 | 153 | 240 | 250 | 315 | 98 | 415 | 212 |
| Neurological disease | 302 | 250 | 223 | 185 | 191 | 201 | 350 | 225 | 162 | 102 | 188 | 275 |
| Skin disease | 268 | 258 | 245 | 86 | 39 | 221 | 340 | 195 | 374 | 242 | 102 | 163 |
| Genito urinary tract disease | 240 | 210 | 178 | 185 | 95 | 1250 | 356 | 215 | 259 | 252 | 312 | 345 |
| Miscellaneous | 331 | 311 | 295 | 80 | 19 | 260 | 345 | 227 | 325 | 361 | 350 | 253 |
| TOTAL | 2215 | 2038 | 1937 | 1294 | 1525 | 1961 | 2257 | 1918 | 2178 | 1813 | 2651 | 2209 |

| 2012 | JAN | FEB | MAR | APR | MAY | JUNE | JULY | AUG |
|---|---|---|---|---|---|---|---|---|
| Respiratory disease | 282 | 232 | 440 | 217 | 152 | 220 | 514 | 367 |
| Gastro intestines disease | 231 | 212 | 222 | 120 | 172 | 222 | 210 | 218 |
| Cardio vascular disease | 102 | 95 | 163 | 102 | 95 | 150 | 95 | 192 |
| Musculo skeletal disease | 111 | 100 | 110 | 95 | 172 | 125 | 97 | 188 |
| Neurological disease | 225 | 210 | 220 | 125 | 215 | 110 | 12 | 150 |
| Skin disease | 132 | 222 | 232 | 170 | 120 | 265 | 222 | 144 |
| Genito urinary tract disease | 225 | 282 | 292 | 112 | 140 | 178 | 227 | 143 |
| Miscellaneous | 255 | 122 | 132 | 253 | 261 | 211 | 323 | 153 |
| TOTAL | 1533 | 1475 | 1553 | 1194 | 1327 | 1481 | 1700 | 1555 |

Courtesy :Beedi workers welfare fund DispensaryMelapalayam.

- Sitting on the floor in cross legged position.
- Sitting on the floor in one leg folded and another leg in extended position(Plate: 6).
- Sitting on the floor with both the legs in extended position (plate: 7)
- Sitting on the floor with both the legs folded behind at the knee.
- Squatting posture (Plate: 8).
- Stooping posture.
- Sitting on the chair/stool/box with less or no leg room.
- Sitting on the chair/stool/box with improper back support.
- Sitting on the chair/stool/box with no arm rent.
- Downward bending of head and neck during work.

**PLATE: 6**

Fig 27: Sitting on the floor without improper back support.

Fig 28: Sitting on a small stool without back support and legs bent.

Fig 29: Sitting on a bench with improper back support

Fig 30: Cross legged sitting posture.

## PLATE - 6

**Figure 27**

**Figure 28**

**Figure 29**

**Figure 30**

**PLATE: 7**

- Fig 31: Sitting on the floor in one leg folded and another leg in extended position.
- Fig 32: Sitting on the floor in cross legged position.
- Fig 33: Sitting on a cot without proper back support.
- Fig 34: Cross legged posture.

- **PLATE -7**

**Figure 31**

**Figure 32**

**Figure 33**

**Figure 34**

**PLATE: 8**

- Fig 35: Sitting on the bench with both legs in extended position.
- Fig 36: Stooping posture.
- Fig 37: Squatting posture.
- Fig 38: Downward bending of head and neck during work.

- **PLATE – 8**

**Figure 35**

**Figure 36**

**Figure 37**

**Figure 38**

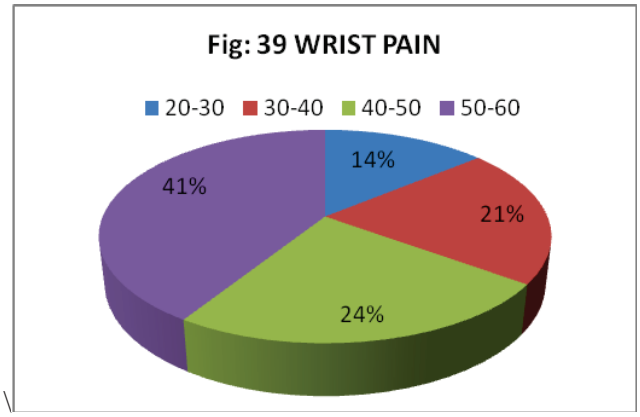


Fig: 39 WRIST PAIN
20-30
30-40
40-50
50-60
14%
21%
24%
41%

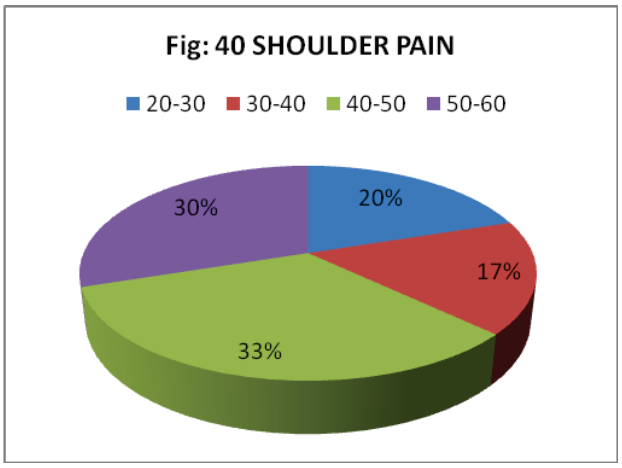


Fig: 40 SHOULDER PAIN
20-30
30-40
40-50
50-60
20%
17%
33%
30%

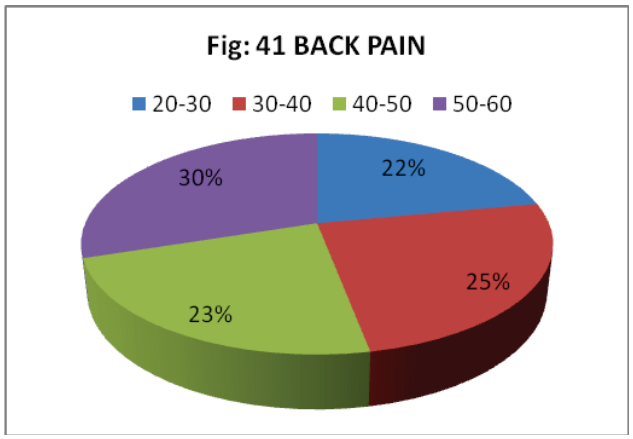
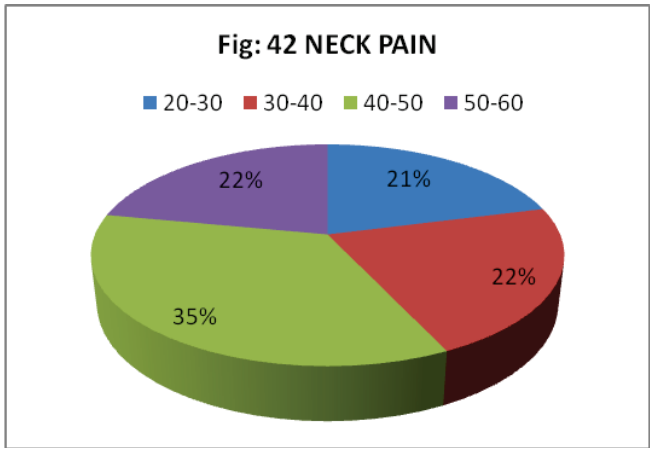
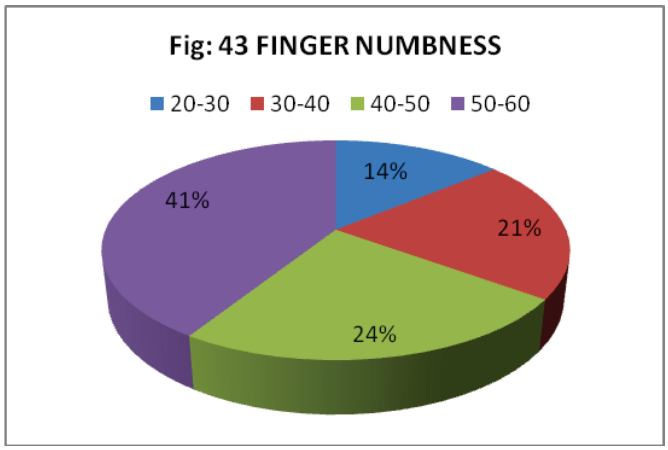
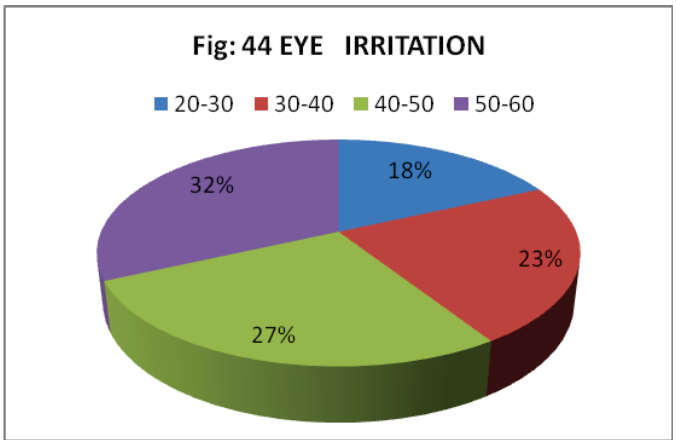


Fig: 41 BACK PAIN
20-30
30-40
40-50
50-60
22%
25%
23%
30%

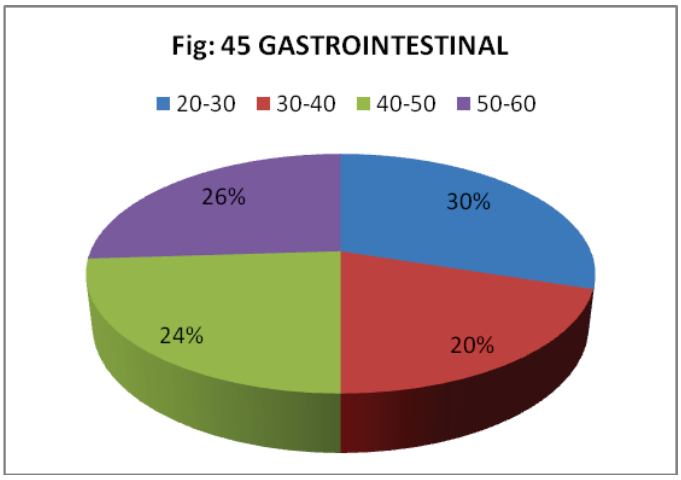
It has been observed that almost all the workers developed pain in various part of the body and they can be categorized into wrist pain, shoulder pain, neck pain and back pain.Women beedi rollers in the age group of 50-60 recorded wrist pain in pain large numbers (41%) followed by the age group of 40-50 (24%) and 30-40 21% and finally in the age group of 20-30 (14%) (Fig: 39). Shoulder pain was maximum in women beedi rollers belonging to the age group of 40-50 and only 17% of women beedi rollers in the age group of 40-50 recording 35% and 22% in 30-40 and 50-60 age groups and 21% 20-30 age groups(Fig: 40). Eventhough back pain was prevalent in all the age groups comparatively higher percentage (30%) was observed in the group of 50-60 in 20-30, 30-40 and 40-50 age groups back pain was reported by 22%, 25% and 23% of women beedi rollers(Fig: 41). Thus these musculoskeletal pains or aches were prevalent in the women beedi rollers irrespective of age. However, the age factor and working duration may be the reasons for the variation in the percentage of these disorders due to working postures(Fig: 42).

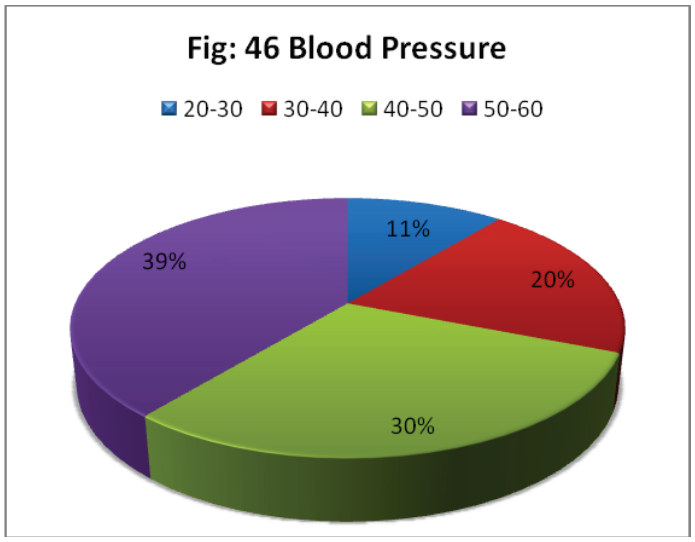





Continuous contact with the tobacco dust, beedi leaves and the act of rolling may lead to finger numbness. Women at the age group of 50-60 who said for a long period and rolling beedis showed a higher percentage of finger numbness(41%). As the age group comes down from 40-50, 30-40 and 20-30 the percentage of report with finger numbness also decreases from 24%, 21% and 14% respectively (Fig: 43). A similar trend was also observed where women beedi rollers reported eye irritation of the age. A report of 32%, 27%, 23% and 18% was observed in the age group of 50-60, 40-50, 30-40 and 20-30 respectively (Fig: 44).




As per the data collected women beedi rollers in Melapalayam complained of respiratory ailments, gastro intestinal disorder and cardiac vascular manifestation such as blood pressure or hypertension. Gastro intestinal disorders frequently reported by the respondents were burning sensation in the stomach. A percentage of 30,20,24 and 26 was observed in the age group of 20-30, 30-40,40-50 and 50-60 respectively 39% of beedi rollers in the age group of 40-50(Fig: 45). In the age group of 30-40 20% had hypertension and 11% in the age group of 20-30. An analysis of the wrist pain observed among beedi rollers at various age group is shown in fig: 46.

### 3.4 Discussion

The present study to identify the occupational hazards of beedi rollers helps to understand the risk of beedi rolling. In our study we have reported postural pains as a major ailment of beedi rollers. Women beedi rollers in Melapalayam complained that they have frequent shoulder pain, neck pain, wrist pain and back pain owing to the posture during their work. Headaches and leg pains also responded by a few aged women. Our observations agree with the reports of Bagwe and Bhisey (1991) and Swami*et al*.,(1995). Padmaladha (1995) reviewed that beedi rollers were affected by lumbosacral pain. Dikshit and Kanhere (2000) and Mittal *et al*.,(2008) have also reported postural pain among the beedi rollers. Ghosh *et al*., (2005) has evaluated the working postures of the workers and the most commonly reported health hazards among the beedi rollers in Vidharbha region in Nagpur, Mangalore in Karnataka and Kannor of Kerala and Jabalpur in Madhya Pradesh and Kanpur in U.P was muscloskeletal pain. Accordingly, 75% of the respondents reported frequent pain in shoulder, neck, wrist and on the back. High intensity of shoulder, back and neck pain is due to sitting in forward leaning posture and bending head and neck downward for prolonged hours without any back support and arm rest.Ghosh *et al*.,(2005) has claimed that the reason is less tolerance and less physiological working capacity in females and may be also due to improper diet and malnutrition. Rajasekhar and Sridhar (2002) reported 25% of back ache among of beedi workers in Dakshina Kannada. Gopal (2000) reported 65% of beedi workers had aches and pains, Sen (2007) who has investigated the health conditions of beedi workers in Sagar District of Madhya Pradesh has also claimed that 62.8% suffer from headache 51.43% from back pain and 20% from eye pain. Thus the present study agrees with majority of easier reports available in the literature.

The other possible ill effect of beedi rolling is due to contact 70% of beedi rollers suffered from eye irritation (Kumar *et al*.,2010). Tobacco dust mainly contains nitrosamines which are readily observed by the body tissues like skin, respiratory epithelium, mucous membranes of mouth, nose and intestine (Rahman, 2009).

Dikshit and Kanhere (2000) and Mittal*et al.,*(2008) found eye problem and burning sensation in women beedi rollers in a study in Bhopal. Mittal *et al.,*(2008) have studied the ocular manifestation in beedi industry workers and have reported common ocular symptom like defective vision and eye irritation. In the present study also women beedi rollers have reported eye irritation as a consequence of occupational exposure of tobacco dust. The above findings agrees with Mittal *et al*.,(2008) who have given the opinion that the tobacco dust contains nitrosamines, polycyclic aromatic hydrocarbons, radioactive elements and cadmium. This view is also agreed by an earlier work of Robert (1988). Eyes get involved secondary to generalized toxic levels of nitrosamines and polycyclic aromatic hydrocarbon or from the direct exposure of ocular surface to the dust laden environment. Direct exposure may lead to painful stimulation of conjunctival and corneal nerve endings (Mahimker and Bhisey 1995) and Solberg *et al*., 1998.

Women beedi rollers experienced numbness of the finger tips after continuous exposure to tobacco during their work. A few reported that their fingertips became thin and became inefficient to roll beedis after a particular age. Similar reports were given by Dikshit and Kanhere (2000) and Mittal *et al.,*(2008).

Clinical reports of the health hazards among beedi rollers of Melapalayam has been reviwed from the records since Jan 2007 till date (Auguest 2012) to enumerate respiratory diseases, gastro intestinal disorders, cardio vascular diseases, muscular

diseases, skin diseases and genitourinary tract diseases. It is inferred from the data that in all the years a high incidence of respiratory diseases were noted. Beedi workers also have registered musculoskeletal diseases as the next health hazards in this dispensary. The above data has given the evidence that inhalation of tobacco dust may be responsible for respiatory ill effects and postural defects leading to musculoskeletal pain.

Respondents of beedi workers also complaint of respiratory, gastro intestinal and cardio vascular disease manifestation. Yasmin *et al*.,(2010) reported 50% of beedi rollers suffered from respiratory problems. According of Gopal (2000) 8.4 % of beedi workers suffered from stomach related problems. Anil *et al*.,(2012) who studied the morbidity pattern of female beedi workers in Mangalore reported gastro intestinal disorders in 13% of the population. According to Bagwe and Bhisey (1991) and Swami*et al*.,(1995) beedi rollers were exposed to unburnt tobacco mainly through cutaneous and nasopharyngeal route. Ranjith Singh and Padmalatha (1995) reviewed that the beedi rollers were affected by respiratory disorders, skin diseases, gastro intestinal illness and gynaecological problems. Kumar *et al*.,(2010) in his study revealed that 28% of beedi rollers possessed skin diseases, 32% had anaemic problems and more than 70% suffered from gastro intestinal problems and more than 50% had respiratory ailments. Shetty (2009) is of the opinion that beedi workers get exposed to unburnt tobacco mainly through cutaneous and nasopharyngeal route, since, they do not make use of any protective wear. According to Umadevi *et al*.,(2003) exposure to tobacco dust is known to affect that respiratory tract in human. Mahimker and Bhisey (1995), Bagwe and Bhisey (1993) investigated workers in the tobacco industry and reported that unburnt tobacco may enter through the cutaneous and nasopharyneal routes and cause skin and pulmonary function impairements. Mittal *et al*.,(2008) has

identified that beedi rollers work in a small factories or at household based enterprises laden with tobacco dust. Hence individuals who work for 6-10 hrs/day, swallow and expose their skin and mucous surface to significant amount of particulate tobacco. The constitutents of tobacco get absorbed in the body, get bio-activated and results in increased risk of developing ailments like pulmonary diseases, gastro intestinal disorders and cardio vascular system abnormality. Umadevi *et al*.,(2003) is of the view that tobacco dust containing nitrosamines were readily absorbed by the body tissues like skin respiratory epithelium and mucous membrane of mouth, nose and intestines. Thus it is evident that beedi workers face a number of health hazards because of their occupation and prolonged exposure to the unburnt tobacco dust in an unhygenic environment.

**Syed Ali Fathima K.M.** 2012 Thyroid Risk Evaluation in Selected Beedi Workers Population in Tirunelveli Ph.D Thesis, Manonmaniam Sundaranar University,Tirunelveli,Tamil Nadu, India.

**CHAPTER -4**

# ROUTINE HAEMATOLOGICAL PARAMETERS AND THYROID PROFILE IN WOMEN BEEDI ROLLERS IN MELAPALAYAM, TIRUNELVELI.

## 4.1Introduction

Occupational exposure to unburnt tobacco may occur during beedi making by women folk who are largely involved. Their ill health is due to the contact and inhalation of raw tobacco dust and volatile components. The health hazards which the women employees who are rolling beedis encounter are enormous. Biomonitoring of these women workers and health assessment is major hurdle in the improvement of their wellbeing. So far no epidemiological studies on tobacco related health risks in these workers have been carried out. Very few reports are available in literature based on the responses revealed by the volunteers. Tobacco related health hazards are mainly attributed to the various constituents such as nicotine cotinine and nitrosomines. These constituents may have diverse effects on the physiology of humans owing to its toxic nature. Contact and inhalation of raw tobacco dust may have systemic effect and lead to many disorders including hormone defects. According to Gupta (2001) smokeless tobacco poses a major risk to women and to the children born to them. The next phase of study aims to explore the health hazards experienced by women beedi rollers in Melapalyam based on the blood parameters and clinical findings of the volunteers. Since blood parameters serves as very good indices in assessment of health profile, steps were undertaken in the present study to evaluate the health status of women beedi rollers. Routine analysis of blood like TEC, TLC, Platelet count, haemoglobin count,

erythrocyte sedimentation rate and specific tests analyzing the trace elements were enumerated. Liver function tests which may be an indicator of the detoxification mechanism of toxic chemicals were also carried out. A special emphasis to the thyroid profile of the women beedi rollers were analysed in order to evaluate the possible thyroid dysfunction, prevalent in women subjects. Hence, this phase of work will deal with the health assessment of women beedi rollers apart from their occupational hazards which are symptomatic.

## 4.2 Materials and methods

### 4.2.1 Enumeration of total Total Erythrocyte Count

**Materials**

Haemocytometer, Haeyem's fluid, spirit, pin. Haemocytometer contain Naubauer's counting chamber, TEC pipette and TLC pipette.

**Composition of TEC fluid**

| | | |
|---|---|---|
| 1.Trisodium citrate | - | 3.13g |
| 2. Commercial alhochol | - | 1 ml |
| 3. Distilled water | - | 100 ml |

**Procedure**

1. Blood is taken up to 0.5 mark, in the R.B.C.
2. Pipette and diluting fluid up to 101 mark.
3. It is mixed well and kept for 3 minutes.
4. 2-3 drops of fluid is discarded, from the pipette, which does not contain any cells.

5. The counting chamber is charged after 3 minutes. (to allow the cells to settle).
6. The RBC in the squire are 'R' in figure under high power. R.B.C. count is then calculated.

**Calculation**

$$\text{TLC} = \frac{\text{NO. OF CELLS COUNTED} \times 10 \times 20}{4}$$

**4.2.2Enumeration of total count of Total LeucocyteCount**

**Materials**

Naubauer's double counting chamber, TLC pipette, TLC diluting fluid, spirit, needle and microscope.

**Reagent Preparation**

TLC diluting fluid composition glacial acetic acid 2 cc gentian violet 1 cc, distilled water 100 cc.

**Procedure**

1. Clean the finger tip with sprit and make a deep prick. Such blood upto 0.5 marks. then such diluting fluid up to the mark 11.
2. Mix the blood thoroughly by rolling pipette horizontally in the palms.
3. Discard 2-3 drops, clean the counting chamber and fill the chamber with prepared solution.
4. Air bubbler should avoided, focus the field under low power, objective and count the TLC in all the corner squares.

5. So that the TLC in 64 squares are counted.

**Calculation**

$$TLC = \frac{\text{NO. OF CELLS COUNTED} \times 10 \times 20}{4}$$

**4.2.3 Estimation of Platelets and estimation of Erythrocyte Sedimentation Ratio**

**Method**

Westergrein's method

**Procedure**

Blood is collected and mixed with anti coagulant in the ratio 1:4. The temperature should be 25-28$^0$C. Blood is drawn up to the mark 0. The tube is kept exactly vertical for 1 hour. The height of plasma column after one hour is noticed.

**4.2.4 Estimation of Haemoglobin**

**Method**

Sahli's method

**Materials**

By using pipette add 1mlin the haemoglobinometer up to the lowest marking. Drop blood up to 20ul in the sahli's pipette. Adjust the blood column carefully without bubbles. Wipe excess of blood on the sides of the pipette by using a dry piece of cotton blow the blood into the acid solution in the graduated tube, rinse the pipette well. Mix the reaction mixtures and allow the mixture to stand at room temperature of 10 minutes.

Dilute the solution with distilled water by adding few drop of water carefully and mixing the reaction mixture until the color matches the color in the comparator. The lower meniscus of the fluid is noted and reading is noted.

### 4.2.5 Estimation of Packed Cell Volume

**Materials**

Wintrob's haematocrit tube filter, oxalated venous blood. Wintrob's haematocrit tube is a glass tube having 110 mm length and 3mm diameter, closed at one end. It is graduated 0-100cm at one side and 100-0 on another side.

**Procedure**

In Wintrob's haematocrit tube, oxalated venous blood is taken without air bubbler. It is closed with cotton and kept in centrifuge, and rotated at 3000 rev/min for half an hour. Take out the test tube and not the blood celles packed at the bottom and buffy coat above it. Above the buffy coat, clean plasma is seen. The packed cell volume can be directly read out from the haematocrit tube normally buffy coat is of 0.5 – 1cm thickness. Wintrob's haematocrit tube is also used for determination of ESR.

### 4.2.6 Determination of Serum Sodium and Potassium

**Method**

Flame photometry

**Specimen**

Serum or heparinized plasma.

**Requirements**

1. Test tubes (15 x 125 mm)

2. Dispenser or 10 ml volumetric pipette
3. 10 ml beakers or bulbs
4. 50 or 100 micro-liter push button pipette.
5. Flame photometer.

**Mixed standards are prepared by using following two stock standards**

1. Stock standard for sodium : 1000 mEq/l: It is prepared by dissolving 5.85 g of analar garde sodium chloride in glass distilled water and diluted to 100ml by using a volumetric flask.

2. Stock standard for potassium : 100 mEq/l : It is prepared by dissolving 0.740 g of potassium chloride (AR) in glass distilled water and diluted to 100ml by using a volumetric flask.

Mixed working standards are prepared as follows :

1. Sodium/potassium : 120/2.0 mEq/l: It contains 120mEq of sodium and 2.0 mEq of potassium per liter of distilled water. It is prepared by mixing 12ml of stock standard1 and 2.0ml of stock standard. 2, in 86 ml of glass distilled water.

2. Sodium/potassium : 140/4.0 mEq/l : It is prepared by mixing 14 ml of stock standard I and 4.0 ml of stock standard. 2, in 82 ml of glass distilled water.

3. Sodium/potassium : 160/6.0 : mEq/l : It is prepared by mixing 16 ml of stock standard 1 and 6.0 ml of stock standard 2, in 78ml of distilled water.

**Procedure**

Pipette in the tubes labeled as follows

| | | Test | Std :1 | Std :2 | Std :3 |
|---|---|---|---|---|---|
| 1. | Glass distilled water, ml | 10 | 10 | 10 | 10 |
| 2. | Serum or plasma, ml (heparinized) | 0.1 | - | - | - |
| 3. | Std : 120/2.0 ml | - | 0.1 | - | - |
| 4. | Std : 140/4.0 ml | - | - | 0.1 | - |
| 5. | Std : 160/60, ml | - | - | - | 0.1 |

Mix and transfer to beakers or bulbs for the flame photometric determination

Operation of a flame photometer

1. Put on the main switch.
2. Put on air compressor and adjust the required air pressure, by adjusting the knob meant for air.
3. Introduce glass distilled water, through automiser.
4. Put on gas and control the flame by adjusting the knob meant for gas.(It is adjusted till the flame is divided into five sharp cones)
5. Adjust the proper filters for the simultaneous determination of sodium and potassium (digital display)
6. Make zero adjustment by introducing distilled water.
7. Introduce the standard 120/2.0 and by using the knob meant for sodium the digits 120.0 and by using the knob meant for potassium the digits 2.0 are adjusted.

8. Introduce standard, 140/4.0 If the standards are accurately prepared the digital display will indicate exact concentration for both sodium and potassium.

9. Introduce standard, 160/6.0 and confirm accuracy of standardization.

10. Now introduce the test and record the readings for sodium and potassium.

**4.2.7 Determination of Cholrides**

**Method**

Schales and Schales.

**Requirements**

1. Test tubes : 15 x 125 mm
2. 0.2 ml and 2.0 ml graduated pipettes.

**Reagents**

1. Mercuric nitrate reagent : Dissolve 2.9-3.0g of mercuric nitrate in about 800 ml of distilled water, add 20 ml of 2N nitric acid and make up to one liter. It is stable at room temperature in an amber colored bottle.

2. Diphenyl carbazone indicator : 100 mg/dl in 95% (v/v) ethanol. It is stable in an amber colored bottle at 2-8$^0$C.

3. Chloride standard : 100 mEq/l : It is prepared by dissolving 5.85 g of analar grade sodium chloride in one liter of glass distilled water. It is stable at 2-8$^0$C.

**Additional Reagents**

4)2/3 N sulfuric acid

5)10 g/dl, sodium tungstate.

**Specimen**

Serum

**Procedure**

I. Prepare protein free filtrate of the serum sample as follows :

In a centrifuge tube, pipette

a) 4.0 ml distilled water

b) 0.5 ml serum

c) 0.25ml 2/3 N $H_2SO_4$

d) 0.25 ml 10 g/dl sodium tungstate

Mix thoroughly and centrifuge at 3000 R.P.M. for 10 minutes. Next procedure is as follows :

a) Pipette in a test tube, 2.0ml of protein free filtrate.

b) Add one drop of the indicator.(0.05ml)

c) Titrate against mercuric nitrate reagent. (End point : colorless to violet-blue color)

d) Note the titration reading : Xml.

e) Dilute standard 1:10 by using glass distilled water.

f) Pipette 2.0 ml of diluted standard in a test tube, and titrate it against mercuric nitrate reagent by using diphenylcarbazone indicator.

g) Note the titration reading : Y ml

**Calculation**

$$\text{Serum chlorides, mEq/L} = \frac{\text{Xml}}{\text{Yml}} \times 100$$

**4.2.8 Estimation of blood Sugar**

**Method**

Trindie's method

**Reagent Composition**

Reagent I – glucose

| | | |
|---|---|---|
| Glucose oxidase (aspergillin) | - | 20,000 Iu/l |
| Peroxidase (horse radish) | - | 3,350 Iu/l |
| 4 amino antipyrine | - | 0.52 m mol/l |
| 4 hydroxy benzoic acid | - | 10 m mol/l |
| Phosphate buffer | - | 110 m mol/l |
| Also contains non reactive filter and stabilizers. | | |
| $P^H$ | - | $7 \pm 0.2$ at $25^0$ C. |

Reagent II – glucose standard

Glucose standard – 100 mg/dl or 5.55 m mol/l

**Reagent Constitution**

Allow the vial to obtained room temperature. Dissolve the contents of each vial using glucose diluted with special with special lipid cleaning agent. Make up the volume to 200 ml or 500 ml transfer into a clear and dry amber colored bottle.

**Procedure**

Take approximately 1ml of blood mixed with anticoagulant and centrifuged it for 3 minutes. Thus we can separate plasma from blood. Take 3 test tube marked T,B,S (Test Blank and Standard) add 100ml working reagent to whole of them. Add 100 ml of distilled water to test tube marked as B and 100ml sample to test tube marked as T. Mix well and incubate for minutes at $37^0$ C. Read the absorbance of standard and each sample tube against reagent blank at 510 mm. (500-540) or 510/630 mm as brichromatic analyser.

### 4.2.9 Determination of total cholesterol

**Reagent composition**

Monoreagent PIPES 200 mmol/l pH 7.0 sodium cholate 1 mmol/l, cholesterol esterase > 250 U/l, cholesterol oxidase > 250 U/l, Peroxidase > 1 KU/l, 4-aminoantipyrine 0.33 mmol/l, phenol 4 mmol/l, non-ionic tensioactives 2 g/l (w/v). Biocides.Cholesterol standard.Cholesterol 200mg/dl (5.18mmol/L). Organic matrix based primary standard.

**Reagent preparation**

The Monoreagent and the Standard are ready-to-use.

**Samples**

Serum, EDTA or heparinized plasma free of hemolysis.

Cholesterol in serum or plasma is stable up to 5 days at 2-$8^0$C and for a few months at -$20^0$C.

**Materials required**

- Photometer or colorimeter capable of measuring absorbance at 500 ±10nm.
- Constant temperature incubator set at $37^0$C
- Pipettes to measure reagent and samples.

**Procedure**

1. Bring reagents and samples to room temperature.
2. Pipette into labelled tubes.

| Tubes | Blank | Sample | Cal. Standard |
|---|---|---|---|
| R1. Monoreagent | 1.0 ML | 1.0 ml | 1.0ml |
| Sample | - | 10 $\mu l$ | - |
| Cal. Standard | - | - | 10 $\mu l$ |

3. Mix and inclubate the tubes 10 minutes at room temperature or 5 minutes at 370C
4. Read the absorbance (A) of the samples and the standard at 500 nm against the reagent blank.

The color is stable for at least 30 minutes protected from light.

**Calculations**

$$\frac{A \text{ Sample}}{A \text{ Standard}} \times C_{\text{Standard}} = \text{mg/dl total cholesterol}$$

Samples with concentrations higher than 600 mg/dl should be diluted 1:2 with saline and assayed again. Multiply the results by .

If results are to be expressed as SI units apply :

mg/dl x 0.0259 = mmol/l

**4.3 Deterrmination of Urea**

**Name of the method**

Diacetyl monoxime method.

**Materials requirement**

1. Test tubes : (15x125mm)
2. 10ml pipette, dispenser or burette.
3. Push button pipette or 0.1 ml serological pipette
4. measuring cylinder of 100ml
5. Water-bath
6. Stopwatch
7. Photometer

**Sample material**

Serum.

**Preparation & Stability of the reagents**

1. Reagent 1 : (DMR) : It contains 0.2 g/dl, diacetyl monoxime in distilled water. The reagent is stable at room temperature ($25^0C \pm 5^0$) for one year.

2. Reagent 2 : (TSC) : It contains 40 mg/dl. thiosemicarbazide in distilled water. The reagent is stable at room temperature ($25^0C \pm 5^0$) for 6 months.

3. Reagent3:(Acid): It contains 60 ml of conc. sulfuric acid, 10 ml of orthophosphoric acid and 10ml of 1 gm/dl ferric chloride in orthophosphoric acid in one liter of the reagent prepared in distilled water. This reagent is stable at room temperature for one year.

4. Urea nitrogen standard : 20 mg/dl : It contains 42.8 mg of urea in 100ml of saturated benzoic acid. This standard is stable for one year when refrigerated.

**Preparation of working reagent**

It is prepared fresh by mixing one part of reagent 1, one part of reagent 2, and two parts of reagent 3.This reagent should be prepared fresh for each batch of the determination.

**Procedure**

Pipette in the tubes labeled as follows

| | Test | Standard | Blank |
|---|---|---|---|
| Working reagent, ml | 5.0 | 5.0 | 5.0 |
| Serum/plasma, ml | 0.05 | - | - |
| Standard 20 mg/dl, ml | - | 0.05 | - |
| Distilled water, ml | - | - | 0.05 |

Mix the contents of the tubes thoroughly and place them in a boiling, water bath for exactly 15 minutes. Cool immediately by using tap water and after 5 minutes measure the intensities of the test and standard against blank at 520 nm (green filter).

**Calculation**

$$\text{urea mg/dl} = \frac{\text{O.D. Test}}{\text{O.D. Std.}} \times 20$$

### 4.3.1 Determination of Serum Creatinine

**Method**

Alkaline-picrate method

**Reaction**

Jaffe' reaction

**Specimen**

Serum (or plasma)

**Requirements**

1. Test tubes : 15 x 125 mm
2. 5.0ml serological pipettes
3. 1.0 ml & 2.0 ml volumetric pipettes
4. Test-tube stand
5. Centrifuge tubes or test tubes, 100 x 10 mm
6. Centrifuge
7. Photometer

**Preparation of the reagents**

- ❖ Picric acid reagent : 0.91 gm/dl (0.04M)
- ❖ 10/dl, sodium hydroxide
- ❖ Working creatinine standards, 1 mg/dl, 5 mg/dl and 10 mg/dl.

These standards are prepared in 0.01N hydrochloric acid by using stock creatinine standard 100 mg/dl.

**Stability of the reagent**

Reagent 1 and 2 stable at room temperature ($25^0C \pm 5^0$). The working standards are stable at 2-$8^0$C.

**Preparation of the alkaline picrate reagent**

It is prepared fresh by mixing 4 parts of reagent 1 & 1 part of reagent, 2. This working reagent is stable for one day.

**Procedure**

Pipette in the tubes labeled as follows :

| | Test(in a centrifuge tube) | Std. |
|---|---|---|
| Distilled water, ml | 3.0 | 4.0 |
| Serum, ml | 1.0 | - |
| Standard 1 mg/dl, ml | - | 1.0 |
| 2/3N Sulfuric acid, ml | 0.5 | - |
| 10 g/dl sodium tungstate ml | 0.5 | - |

Centrifuge the contents in the test and get clear filtrate. Pipette in the tubes labeled as follows

| | Test | Std : 1 | Blank |
|---|---|---|---|
| Distilled water, ml | 3.0 | 3.0 | 5.0 |
| Filtrate. Ml | 2.0 | - | - |
| Diluted Std. 1 mg/dl ml. | - | 2.0 | - |
| Alkaline picrate reagent. ml. | 1.0 | 1.0 | 1.0 |

Mix and keep at room temperature (($25^0C \pm 5^0$C) for 20 minutes.

Read intensities of test and standard at 520 nm (green filter) by setting blank to 100%T.

**Calculation**

$$\text{Serum creatinine, mg/dl} = \frac{\text{O.D. Test}}{\text{O.D. Std}} \times 1.0$$

**4.3.2 Estimation of Serum Bilirubin**

**Method**

Malloy and Evelyn

**Requirements**

1. Test-tubes : 15 x 125 mm
2. 5.0, 0.2 ml serological pipettes
3. Stop watch
4. Test tube stand
5. Photometer

**Preparation**

1. Diazo 'A' : It is prepared by mixing 0.1 g of sulfanilic acid in 100 ml of 1.5% (v/v) hydrochloric acid.
2. Diazo 'B' : It is prepared by mixing 0.5 g of sodium nitrite in 100 ml of distilled water.
3. Diazo blank reagent : (1.5% hydrochloric acid) : It is prepared by adding 1.5 ml of concentrated hydrochloric acid to about 90 ml of distilled water in a 100 ml volumetric flask. Distilled water is added upto the mark.
4. Methanol

5. 10 mg/dl artificial bilirubin standard : It is prepared as follows

a) Stock standard : It is prepared by mixing 0.29 g of methyl red in100 mlof glacial acetic acid.

b) Working standard : 0.1 ml of stock standard 0.5 ml of glacial acetic acid and 1.44 g of sodium acetate are mixed in distilled water and diluted to 100 ml by adding distilled water.

**Stability of the reagents**

Reagent 1,3,4& 5 are stable at room temperature ($25^0C \pm 5^0C$) for one year. Reagent 2 is stable at $2\text{-}8^0C$ in an amber colored bottle.

**Test Procedure**

Prepare fresh diazo mixture by mixing 5.0 ml of Diazo A and 0.15 ml of Diazo B. This mixture is stable only for a day.

Pipette in the tubes labeled as follows :

| | Total Test | Total Blank | Direct Test | Direct Blank |
|---|---|---|---|---|
| Distilled water, ml | 1.8 | 1.8 | 1.8 | 1.8 |
| Serum, ml | 0.2 | 0.2 | 0.2 | 0.2 |
| Diazo mixture, ml | 0.5 | - | 0.5 | - |
| Diazo blank reagent ml | - | 0.5 | - | 0.5 |
| Methanol, ml | 2.5 | 2.5 | - | - |
| Distilled water, ml | - | - | 2.5 | 2.5 |

Keep in dark for 30 minutes. Read the intensities at 540 nm (green filter).

Read O.D. of the artificial bilirubin standard (undiluted) by transferring the standard solution in a dry cuvette at 540 nm (or green filter).

**Calculations**

O.D. of total Bilirubin = O.D. of total test – O.D. of total Blank.

O.D. of direct Bilirubin = O.D. of direct test – O.D. of direct blank.

$$\text{Total bilirubin mg/dl} = \frac{\text{O.D. of total bilirubin}}{\text{O.D of std}} \times 10$$

$$\text{Direct bilirubin mg/dl} = \frac{\text{O.D. of total bilirubin}}{\text{O.D of std}} \times 10$$

Indirect Bilirubin, mg/dl = Total Bilirubin, mg/dl – Direct Bilirubin, mg/dl.

**4.3.3 Alkaline Phosphate Determination**

**Method**

Visible – Kinetic

**Sample Material**

Serum, heparinized plasma (Free from hemolysis).

**Reagents**

1. AMP buffer (pH : 10.3)
2. Magnesium chloride reagent : 30 mg/dl
3. P-nitrophenyl phosphate : (PNP) : Prepare fresh as follows :

**Preparation and stability of the reagent : (PNP)**

Dissolve 42.5 mg PNP in 0.5 ml of reagent No.2 This mixture is stable at 0-4$^0$C for 4 weeks.

**Procedure**

Wavelength : 405 nm

Cuvette 1 cm : light path

Temperature : 30 or $37^0$C

Pipette into cuvette

| | |
|---|---|
| AMP Buffer, ml | 2.7 |
| PNP, ml | 0.2 |
| Serum (or plasma, ml) | 0.1 |

Mix, read initial absorbance and start stop watch at the same time. Repeat readings after exactly 1, 2 and 3 minutes. Determine mean absorbance change per minute (ΔA/min).

**Calculations**

Serum alkaline phosphate :

IU ($30^0$, $37^0$C) = 1595 x ΔA 3 405/min.

**4.3.4 Determination of Gamma G.T.**

**Method**

End point reaction method.

**Sample Material**

Serum (Free from hemolysis)

**Reagents**

1. Substrate : It is prepared by mixing 250 mg of L-gamma-glutamyl – paranitroanilide, 872 mg of glycyl–glyeine & 672 mg of magnesium chloride in 300 ml of AMP buffer, pH : 8.6 (0.05M)

2. Sodium hydroxide reagent : 0.0075 M.

3. P-nitroaniline standard : 12.4 mg/dl (0.9 mM/ml).

**Stability of the reagents**

Substrate is stable at 0-4$^0$C for 3 months

Reagent 3 is stable at 2-8$^0$C for several months.

Reagent 2 is stable at room temperature (25$^0$C $\pm$ 5$^0$C) for several months.

**Procedure**

Wavelength : 405 nm (violet filter)

Temperature : 37$^0$C

Pipette into the tubes labeled as follows

| | Test | Blank |
|---|---|---|
| Substrate, ml | 1.0 | 1.0 |
| Serum, ml | 0.2 | - |
| Incubate at 37$^0$C for 45 min | - | - |
| Sodium hydroxide reagent, ml | 5.0 | 5.0 |
| Serum, ml | - | 0.2 |

Read absorbances at 405 nm (Violet filter) against blank. Refer the standard graph for the calculations of enzyme units.

**Standardization for the determination of gamma G.T.**

**Pipette in the tubes labeled as follows**

| | 1 | 2 | 3 | 4 | 5 | BL |
|---|---|---|---|---|---|---|
| Substrate, ml | 0.9 | 0.7 | 0.5 | 0.2 | 0.1 | 1.0 |
| P-nitroaniline std | 0.1 | 0.3 | 0.5 | 0.8 | 0.9 | 0.0 |
| Sodium hydroxide R, ml | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| γ-GT, γU | 10 | 30 | 50 | 80 | 90 | 00 |

Prepare a standard curve by plotting O.D. on Y-axis and IU on X-axis.

**4.3.5 Determination of Serum Glutamate Pyrruvate Transaminase (SGPT) and Serum Glutamate Oxaloacetate Transaminase (SGOT)**

**Method**

End point reaction

**Name of the method**

Reitman and frankel's method.

**Enzyme method**

**Karmen units**

These are expressed as the unit of activity which produces change in O.D. of 0.001 per minute by enzyme present in 1.0 ml of serum.

**Requirements**

1. Test tubes : 15 x 125 mm
2. 5.0 ml serological pipettes
3. 0.1 ml serological pipettes
4. Constant temperature water bath

5. Stopwatch
6. Photometer.

**Preparation of the reagents**

1. SGPT substrate : It contains 1.78 g of alanine, 30mg of alpha – keto – glutaric acid, 0.5 ml of 1.0 N sodium hydroxide in phosphate buffer pH. 7.45 (M/15). The final quantity is adjusted to 100 ml by using the phosphate buffer. pH of this substrate should be 7.45.

2. SGOT substrate : It contains 2.66 g of aspartic acid, 30 mg of alpha – keto – glutaric acid, 20 ml of IN sodium hydroxide in the phosphate buffer, pH, 7.45 (M/15). The final volume of the substrate is adjusted to 100ml by using the phosphate buffer. pH of this substrate should be 7.45.

3. DNPH reagent : It contains 200 mg of dinitrophenyl hydrazine & 85 ml of conc. hydrochloric acid in distilled water; final volume should be adjusted to one liter bysing distilled water.

4. 0.4 N sodium hydroxide

5. 22 mg/dl, sodium pyruvate standard.

**Stability of the reagents**

The reagents 1,2,3 and 5 are stable at 2-8$^0$C. Reagent 4 is stable in a polyethylene contain for several months at room temperature (25$^0$C $\pm$ 5$^0$C).

**Sample material**

Serum (Hemolysis interferes with the test). Use fresh serum.

**Procedure**

Wavelength : 546 nm (green filter, 530-550 nm)

Incubation temperature : $37^0$C

Incubation time

- SGPT 30 minutes.
- SGOT 60 minutes.

**Procedure** (SGPT Determination):

**Pipette in the tubes labeled as follows:**

| | Test | Blank |
|---|---|---|
| Substrate, ml | 0.5 | 0.5 |
| Incubate at $37^0$C for 5 minutes | | |
| Serum, ml | 0.1 | - |
| Incubate at $37^0$C for 30 minutes | | |
| DNPH, ml | 0.5 | 0.5 |
| Serum, ml | - | 0.1 |
| Mix thoroughly, keep at room temperature for ($25^0$C $\pm$ $5^0$C) 20 minutes | | |
| 0.4 N NaOH, ml | 5.0 | 5.0 |

Mix and keep at room temperature for 10 minutes. Afterwards, read intensity of test by setting blank at 100% T (540 nm: green filter)

1. **Procedure** (SGOT Determination):

**Pipette in the tubes labeled as follows**

| | Test | Blank |
|---|---|---|
| Substrate, ml | 0.5 | 0.5 |
| Incubate at $37^0$C for 5 minutes | | |
| Serum, ml | 0.1 | - |
| Incubate at $37^0$C for 30 minutes (1 hr.) | | |
| DNPH, ml | 0.5 | 0.5 |
| Serum, ml | - | 0.1 |
| Mix thoroughly, keep at room temperature for ($25^0$C $\pm$ $5^0$C) 20 minutes | | |
| 0.4 N NaOH, ml | 5 | 5 |

Mix and keep at room temperature for 10 minutes. Afterwards, read intensity of test by setting blank at 100% T (540 nm, green filter).

### 4.3.6 Total Proteins determination

**Name of the method**

Biuret method

**Requirements**

1. Test-tubes : 15 x 125 mm,
2. Serological pipette, 5ml
3. Test-tube stand
4. Push button pipette of 0.05 ml or serological pipette of 0.1ml
5. Photometer

**Specimen**

Serum

**Preparation of the reagents**

Stock Biuret reagent : Dissolve 45 g of Rochelle salt in about 400 ml of 0.2 N sodium hydroxide and add 15 g of copper sulfate by stirring continuously until the solution is complete. Add 5g of potassium iodide and make upto a liter with 0.2 N sodium hydroxide.

1. Protein reagent (ready to use) : (working Biuret reagent). Dilute 200ml of stock reagent to a liter with 0.2 N sodium hydroxide which contains 5 g of potassium iodide per liter

2. Protein standard : 6.0 g/dl : 6g of bovine albumin dissolved in 100ml of normal saline, containing 0.1 g/dl, sodium azide.

**Additional reagents**

3. Sample blank reagent : 9.0 g of Rochelle salt & 5 g of potassium iodide dissolved in one liter of 0.2 N sodium hydroxide.

**Stability of the reagents**

Reagents 1 and 3 are stable at room temperature ($25^0C \pm 50^0C$) for one year. Reagent 2 (Protein standard) is stable at $2\text{-}8^0C$ for one year.

**Procedure**

Mono-step method

**Pipette in three-tubes labeled as follows :**

| | Test | Std. | Blank |
|---|---|---|---|
| Protein in Reagent, ml | 5.0 | 5.0 | 5.0 |
| Serum, ml | 0.05 | - | - |
| Protein std 6 g/dl ml | - | 0.05 | - |
| Distilled water, ml | - | - | 0.05 |

Mix thoroughly and keep at room temperature ($25^0C \pm 5^0C$) for exactly 10 minutes. Measure the intensities of the test and standard by setting blank at 100% T, by using 530nm (green filter).

### 4.3.7 Determination of Serum Albumin

**Name of the method**

Bromocresol green method.

**Requirements**

Test-tubes : 15 x 125 mm

1. Serological and graduated pipettes, 10ml, 5ml
2. Test-tube stand
3. Push-button pipette of 0.05 ml or serological pipette of 0.1ml.
4. Photometer
5. Serum

**Preparation of the reagents**

1. Albumin reagent (ready to use) : It is prepared by mixing following chemicals in 900 ml distilled water.

a) Succinic acid : 8.85 g.

b) Bromocresol green : 108 mg

c) Sodium azide : 100 mg

d) Brij-35 : 4.0 ml

pH of this solution is adjusted by using 1N sodium hydroxide to 4.1 Final volume is made to one liter by using distilled water.

1. Albumin std 4.0 g/dl : Bovine albumin 4.0 g in 100 liter of normal saline containing 0.1 g/dl sodium azide.

2. Sample blank reagent : It contains 0.885 g of succinic acid, 10 mg of sodium azide & 0.4 ml of Brij-35 pH of this solution is adjused to 4.1

**Stability of the reagents**

Reagents 1 and 3 are stable at room temperature ($25^0C \pm 5^0C$) for one year.

Reagent : 2 (albumin standard) is stable at $2\text{-}8^0C$ for one year.

**Procedure**

Mono-step method

Pipette in three-tubes labeled as follows –

| | Test | Std | Blank |
|---|---|---|---|
| Albumin reagent, ml | 5.0 | 5.0 | 5.0 |
| Serum, ml | 0.05 | - | - |
| Albumin standard, ml | - | 0.05 | - |
| Distilled water, ml | - | - | 0.05 |

Mix thoroughly and keep at room-temperature ($25^0C \pm 5^0C$) for exactly 10 minutes. Measure the intensity of the test and standard by setting blank at 100% T, by using 640 nm (red filter).

**Calculations**

$$\text{Serum albumin, g/dl} = \frac{\text{O.D. Test}}{\text{O.D. Std}} \times 4$$

**4.3.8 Determination of Globulin**

Serum globulin= Total Protein-Albumin

**PLATE: 9**

Fig 47-52: Blood samples of beedi rollers are analysed for haematological parameters using auto analyser.

## PLATE - 9

**Figure 47**

**Figure 48**


**Figure 49**

**Figure 50**

**Figure 51**

**Figure 52**

### 4.3.9 Triiodothyronine ($T_3$)

**Method**

Enzyme Linked Immunosorbent Assay

**Sample**

Serum

**Materials provided with this kid**

1. Anti-$T_3$ Antibody-Coated Microtiter Wells, 96 wells.
2. $T_3$ HRPO Conjugate Concentrate, 0.8 ml.
3. $T_3$ HRPO Conjugate Diluent, 15 ml
4. Reference Standard, 1 set. Ready to use.
5. TMB Substrate, 12 ml
6. Stop Solution,12 ml
7. Wash Buffer Concentrate (50X),15ml

**Materials required**

1. Distilled water.
2. Precision pipettes: 0.05~ 0.2ml,1.0ml
3. Disposable pipette tips.
4. Microtiter well reader.
5. Vortex mixer or equivalent.
6. Absorbent paper.
7. Graph paper

**Reagent preparation:**

1. All reagents should be allowed to reach room temperature (18-22°C) before use.

2. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

3. To prepare T3-HRPO Conjugate Reagent, add 0.1 ml of $T_3$-HRPO Conjugate Concentrate to 2.0 ml of $T_3$ Conjugate Diluent (1:20 dilution), and mix well. The amount of conjugate diluted is depend on your assay size. The Conjugate Reagent is stable at 4°C at least for two weeks.

**Procedure**

1. Secure the desired number of coated well in the holder. Make data sheet with sample identification.

2. Dispense 50 µl of standard, samples, and controls into appropriate wells.

3. Thoroughly mix for 10 seconds,then dispense 100 µl of Enzyme Conjugate Reagent into each well.

4. Thoroughly mix for 30 seconds. It is important to have complete mixing in this step.

5. Incubate at room temperature for 60 minutes.

6. Remove the incubation mixture by flicking plate contents into a waste container.

7. Rinse and flick the microtiter wells 5 times with washing buffer(1X).

8. Strike the wells sharply onto absorbent paper to remove residual water droplets.
9. Dispense 100 µl TMB solution into each well. Gently mix for 5 seconds.
10. Incubate at room temperature in the dark for 20 minutes without shaking.
11. Stop the reaction by adding 100µl of Stop Solution to each well.
12. Gently mix for 15 seconds.
13. Read OD at 450 nm with a microtiter reader within 15 minutes.

**Calculation of results**

1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of $T_3$ in ng/ml from the standard curve.

**4.4 Thyroxine($T_4$)**

**Method**

Enzyme Linked Immunosorbent Assay

**Sample**

Serum

**Materials provided with the kid :**

Antibody-coated microtiter wells. 96 wells per bag.

1.Reference standard set, ready to use.

2. $T_4$ HRPO Conjugate Diluent, 15 ml.

3. $T_4$ HRPO Conjugate Concentrate, 0.8 ml

4. TMB Substrate, 12 ml.

5. Stop Solution, 12 ml.

6. Wash Buffer Concentrate(50X),15ml

**Materials required**

- Precision pipettes: 40µl~200µl and 1.0ml
- Disposable pipette tips.
- Distilled water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter well reader.

**Reagent preparation**

1. All reagents should be brought to room temperature (18-22°C) before use.

2. To prepare $T_4$-HRPO Conjugate Reagent, add 0.1 ml of $T_4$-HRPO Conjugate Concentrate to 2.0 ml of $T_4$ Conjugate Diluent (1:20 dilution), and mix well. The amount of conjugate diluted is depend on your assay size. The Conjugate Reagent is stable at 4°C at least for two weeks.

The best temperature condition for this assay is from19$^0$C to 22°C. If, in the environmental assay condition, the temperature is higher than expected, we recommend increasing the $T_4$ conjugate dilution up to 1:40

3. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into distilled water to prepare 750 ml of washing buffer (1x). Mix well before use

**Procedure**

1. Secure the desired number of coated wells in the holder. Make data sheet with sample identification.
2. Dispense 50µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 10 seconds. It is very important to have complete mixing in this step.
5. Incubate at room temperature (18-22°C) for 60 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with washing buffer (1X)..
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100µl of TMB solution into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 20 minutes without shaking.

11. Stop the reaction by adding 100µl of stop solution to each well. Gently mix for 5 seconds

12. Read optical density at 450nm with a microtiter well reader.

**Calculation of results**

1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.

2. Constructa standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in µg/dl on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

3. Using the mean absorbance value for each sample, determine the corresponding concentration of $T_4$ in µg/dl from the standard curve.

**4.4.1 Thyroid Stimulating Hormone(TSH)**

**Method**

Enzyme Linked Immunosorbent Assay

**Sample**

Serum

**Materials provided with the kits**

- Anti-TSH antibody coated microtiter wells.
- Set of Reference Standards: 0, 0.1, 0.5, 2, 5, and 10µIU/ml - 1.0 mL/vial.
- Enzyme Conjugate Reagent, 12 ml.
- TMB Substrate, 12 ml.

- Stop Solution, 12 ml.
- Wash Buffer Concentrate(50X),15ml

**Materials required**

- Precision pipettes**:** 50µl, 100µl, 200µl, and 1.0ml.
- Disposable pipette tips.
- Distilled water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter plate reader.

**Reagent preparation**

- All reagents should be brought to room temperature (18-22°C) before use.
- Dilute 1 volume of Wash Buffer (50x) with 49 voumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into distilled water to prepare 750 ml of washing buffer (1x).

**Procedure**

1. Secure the desired number of coated wells in the holder.
2. Dispense 100µl of standards, specimens, and controls into appropriate wells.
3. Dispense 100µl of enzyme conjugate reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to mix completely.

5. Incubate at room temperature (22 2°C) for 120 minutes.

6. Remove the incubation mixture by flicking plate contents into a waste container.

7. Rinse and flick the microtiter wells 5 times with Washing Buffer (1X).

8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.

9. Dispense 100µl of TMB solution into each well. Gently mix for 5 seconds.

10. Incubate at room temperature for 20 minutes.

11. Stop the reaction by adding 100µl of stop solution to each well.

12. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.

13. Read absorbance at 450nm with a microtiter well reader within 30 minutes.

**Calculation of results**

1. Calculate the mean absorbance value (A450) for each set of reference standards, controls and patient samples.

2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in µIU/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.

3. Use the mean absorbance values for each specimen to determine the corresponding concentration of TSH in µIU/ml from the standard curve.

**PLATE: 10**

Fig 53-58: Blood samples of women beedi rollers are tested using thyroid kit (omega) to analyse thyroid profile in auto analyser.

## 7. PLATE - 10

**Figure 53**

**Figure 54**

**Figure 55**

**Figure 56**

**Figure 57**

**Figure 58**

**8.**

### 4.4.2 Free Triido thyronine($FT_3$)

**Method**

Competitive Enzyme Immunoassay – Analog Method for Free $T_3$

**Sample**

Serum

**Reagents**

**A. Human Serum References -- 1ml/vial - Icons A-F**

Six (6) vials of human serum based reference calibrators for free triiodothyronine at approximate concentrations of 0 (A), 1.0 (B), 3.0 (C), 5.0 (D), 8.0 (E) and 16.0 (F) pg/ml. Store at 2-8°C. A preservative has been added. Exact levels are given on the labels on a lot specific basis.

For SI units: 1pg/ml x 1.536 = pmoI/L

**B. $fT_3$ –Enzyme Reagent - 13ml/vial - Icon –E**

One (1) vial of triiodothyronine-horseradish peroxidase (HRP) conjugate in a bovine albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C.

**C.$T_3$ Antibody Coated Plate 96 wells – Icon**

One 96-well microplate coated with sheep anti-triiodothyronine serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

**D.Wash Solution 20ml - Icon**

One (1) vial containing a surfactant in phosphate buffered saline. A preservative has been added. Store at 2-30°C.

**E.SubstrateA –7 ml/vial - Icon**

One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F.**Substrate** B**–7 .0ml/vial - Icon**

One (1) bottle containing hydrogen peroxide ($H_2O_2$) in buffer. Store at 2-8°C.

G.**Stop Solution – 8ml/vial- Icon STOP**

One (1) bottle containing a strong acid (1N HCI) Store at 2-30$^0$C

**Materials required**

1. Pipette capable of delivering 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.300ml volumes with a precision of better than 1.5%.
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

**Specimen collection and preparation**

The specimens shall be blood serum in type and the usual precautions in the Collection of venipuncture samples should be observed. The blood should be collected in a plain red-top venipuncture tube without additives or gel barrier. Allow blood to clot. Centrifuge the specimen to separate the serum from the cells. Specimen may be refrigerated at 2-8°C for a maximum period of 48 hours. If the specimen(s) cannot be assayed within 48 hours, the sample(s) may be stored at temperatures of -20°C for up to 30 days. When assayed in duplicate, 0.100ml of the specimen is required.

**Reagent preparation**

**1. Wash Buffer**

Dilute contents of wash Concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Store at room temperature 20-27°C for up to 60 days.

**2. Working Substrate Solution**

Pour the contents of the amber vial labeled Solution A into the clear vial labeled Solution B. Place the yellow cap on the clear vial for easy identification. Mix and Label accordingly. Store at 2 -$8^0$C

**Procedure**

1. Format the microplate wells for each serum reference, control and specimen to be assayed in duplicate.
2. Pipette 0.050 ml (50 µl) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.100 ml (100µl) of $fT_3$-enzyme reagent solution to all wells.

4. Swirl the microplate gently for 20-30 seconds to mix and cover.

5. Incubate 60 minutes at room temperature.

6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

7. Add 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two additional times for a total of three washes.

8. Add 0.100 ml (100µl) of working substrate solution to all wells .

9. Incubate for 15 minutes at room temperature

10. Add 0.050ml (50 µl) of stop solution to each well and gently mix for 15-20 seconds.

11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within 30 minutes of adding the stop solution.

**Calculation of results**

A dose response curve is used to ascertain the concentration of free triiodothyronine in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader.

2. Plot the absorbance for each duplicate serum reference versus the corresponding $fT_3$ concentration in pg/ml on linear graph paper.

3. Draw the best fit curve through the plotted points.

4. To determine the concentration of $FT_3$ for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve and read the concentration in pg/ml from the horizontal axis of the graph.

### 4.4.3 Free Thyroxine (Free $T_4$ )

**Method**

ELISA

**Sample**

Serum

**Reagents :**

**A. Human Serum References -- 1 ml/vial - Icons A-F**

Six (6) vials of human serum based reference calibrators for free thyroxine at approximate concentrations of 0 (**A),** 0.40 **(B**), 1.25 **(C),** 2.10 **(D),** 5.00 **(E)** and 7.40 **(F**) ng/dl. Store at 2-8°C. A preservative has been added.

For SI units: ng/dL x 12.9 = pmol/L

**B. $fT_4$- Enzyme Reagent – 13 ml/vial**

One (1) vial of thyroxine-horseradish peroxidase (HRP) conjugate in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

**C. Antibody Coated Microplate** -- 96 wells One 96-well microplate coated with anti-thyroxine serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

**D. Wash Solution Concentrate** -- 20ml One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-30°C.

**E**. **Substrate A –7 ml/vial** One (1) bottle containing tetramethylbenzidine (TMB) in acetate buffer. Store at 2-8°C.

**F**. **Substrate B – 7 ml/vial** One (1) bottle containing hydrogen peroxide (H2O2) in acetate buffer. Store at 2-8°C.

**G**. **Stop Solution** – 8 ml/vial One (1) bottle containing a strong acid (1N HCl). Store at 2-8 ° C.

**Materials required**

1. Pipette capable of delivering 50µl & 100µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.300ml volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

**Specimen collection and preparation**

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. The blood should be collected in a plain redtop venipuncture tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells. Specimen(s) may be refrigerated at 2-8°C for a maximum period of 48 hours. If the specimen(s) cannot be assayed within 48 hours, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100 ml of the specimen is required.

**Reagent preparation**

**1.Wash Buffer**

Dilute contents of Wash solution to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature 20-27°C for up to 60 days.

**2.Working Substrate Solution**

Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly Store at 2-8 ° C.

**Procedure**

1. Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate.
2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.100 ml (100µl) of $fT_4$-Enzyme Reagent to all wells.

4. Swirl the microplate gently for 20-30 seconds to mix and cover.

5. Incubate 60 minutes at room temperature.

6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

7. Add 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes**.**

8. Add0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section).

9. Incubate at room temperature for fifteen (15) minutes.

10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells

11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

**Calculation of results**

1. Record the absorbance obtained from the printout of the microplate reader.

2. Plot the absorbance for each duplicate serum reference versus the corresponding Cortisol concentration in µg/dl on linear graph paper.

3. Connect the points with a best –fit curve.

4. To determine the concentration of cortisol for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in µg/dl ) from the horizontal axis of the graph .

### 4.4.4 AntiThyroglobin Antibody

**Method**

Enzyme Linked Immunosorbent Assay

**Sample**

Serum

**Reagents**

1. Thyroglobulin antigen coated microassay plate: 96 wells, configured in twelve 1x8 strips, stored in a foil pouch with desiccant. (96T: one plate)

2. Serum Diluent Type III: Ready to use. Contains buffer, BSA and Tween-20, and proclin (0.1%) as a preservative. (96T: one bottle, 30 ml)

3. High Positive Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The High Positive Control is utilized to control the upper dynamic range of the assay.(96T: one vial, 0.4 ml)

4. Calibrator: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with kit specific factor printed on vial label. The Calibrator is used to calibrate the assay to account for day-to-day fluctuations in temperature and other testing conditions. (96T: one vial, 0.4 ml)

5. Negative Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Negative Control is utilized to control the negative range of the assay. (96T: one vial, 0.4 ml)

6. Low Positive Control: Human serum or defibrinated plasma. Sodium azide(< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Low Positive Control is utilized to control the range near the cutoff of the assay. (96T: one vial, 0.4 ml)

7. Horseradish-peroxidase (HRP) Conjugate: Ready to use. Goat anti-human IgG, IgA, and IgM containing proclin (0.1%) and gentamicin as preservatives. (96T: one bottle, 15 mL)

8. Wash Buffer Type II (20X concentrate): Dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBS, Tween-80 and proclin (0.1%) as a preservative. (96T: one bottle, 50 mL)

9. Chromogen/Substrate Solution Type I: Tetramethylbenzidine (TMB), ready to use. The reagent should remain closed when not in use. If

allowed to evaporate, a precipitate may form in the reagent wells. (96T: one bottle, 15 ml)

10. Stop Solution: Ready to use, contains a 1N $H_2SO_4$ solution. (96T: one bottle, 15 ml)

**Materials**

1. Wash bottle, automated or semi-automated microwell plate washing system.
2. Micropipettes, including multichannel, capable of accurately delivering 10-200 µl volumes (less than 3% CV).
3. One liter graduated cylinder.
4. Paper towels.
5. Test tube for serum dilution.
6. Reagent reservoirs for multichannel pipettes.
7. Pipette tips.
8. Distilled or deionized water ($dH_20$), CAP (College of American Pathology) Type 1 or equivalent (17,18).
9. Timer capable of measuring to an accuracy of +/- 1 second (0 – 60 minutes).
10. Disposal basins and 0.5% sodium hypochlorite (50 ml bleach in 950 ml dH20).
11. Single or dual wavelength microplate reader with 450 nm filter.

**Procedure**

1. Place the desired number of strips into a microwell frame. Allow six (6) Control/Calibrator determinations (one Negative Control, three Calibrators, one High Positive Control and one Low Positive Control) per run. A reagent blank (RB) should be run on each assay.

2. Dilute test sera, Calibrator and Control sera 1:21 (e.g., 10 µL + 200 µL) in Serum Diluent. Mix Well.

3. To individual wells, add 100 µL of the appropriate diluted Calibrator, Controls and Experimental sera. Add 100 µL of Serum Diluent to reagent blank well.

4. Incubate each well at room temperature (21° to 25° C) for 30 minutes +/- 1 minute.

5. Aspirate or shake out liquid from all wells. If using semi-automated or automated washing equipment add 250-300 µL of diluted Wash Buffer to each well. Aspirate or shake out and turn plate upside down and blot on paper toweling to remove all liquid. Repeat the wash procedure two times. After the final wash, blot the plate on paper toweling to remove all liquid from the wells.

6. Add 100 µl Conjugate to each well, including reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.

7. Incubate each well at room temperature (21° to 25° C) for 30 minutes +/- 1 minute.

8. Repeat wash as described in Step 5.

9. Add 100 µl Chromogen/ Substrate Solution (TMB) to each well, including the reagent blank well, maintaining a constant rate of addition across the plate.

10. Incubate each well at room temperature (21° to 25° C) for 15 minutes +/- 1 minute.

11. Stop reaction by addition of 100 µL of Stop Solution (1N $H_2SO_4$) following the same order of Chromogen/Substrate addition, including the reagent blank well. Tap the plate gently along the outsides, to mix contents of the wells. The plate may be held up to 1 hour after addition of the Stop Solution before reading.

12. The developed color should be read on an ELISA plate reader equipped with a 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm.

### 4.4.5 Anti Microsomal Antibody (AMA)

**Method**

Enzyme Linked Immunosorbent Assay

**Sample**

Serum

**Reagents**

1. Microsomal antigen coated microassay plate: 96 wells, configured in twelve 1x8 strips, stored in a foil pouch with desiccant. (96T: one plate)

2. Serum Diluent Type III: Ready to use. Contains buffer, BSA and Tween-20, and proclin (0.1%) as preservative. (96T: one bottle, 30 mL)

3. High Positive Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives.

4. Calibrator: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives.

5. Negative Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives.The Negative Control is utilized to control the negative range of the assay. (96T: one vial, 0.4 ml)

6. Low Positive Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added. The Low Positive Control is utilized to control the range near the cutoff of the assay. (96T: one vial, 0.4 ml)

7. Horseradish-peroxidase (HRP) Conjugate: Ready to use. Goat anti-human IgG, IgA,and IgM containing proclin (0.1%) and gentamicin as preservatives. (96T: one bottle, 15 ml)

8. Wash Buffer Type II (20X concentrate): Dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBS, Tween-80 and proclin (0.1%) as a preservative. (96T: one bottle, 50 ml)

9. Chromogen/Substrate Solution Type I: Tetramethylbenzidine (TMB), ready to use. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells. (96T: one bottle, 15 ml)

10. Stop Solution: Ready to use, contains a 1N $H_2SO_4$ solution. (96T: one bottle, 15 ml)

**Additional requirements**

1. Wash bottle, automated or semi-automated microwell plate washing system.
2. Micropipettes, including multichannel, capable of accurately delivering 10-200 µl volumes (less than 3% CV).
3. One liter graduated cylinder.
4. Paper towels.
5. Test tube for serum dilution.
6. Reagent reservoirs for multichannel pipettes.
7. Pipette tips.
8. Distilled or deionized water ($dH_20$).
9. Timer capable of measuring to an accuracy of +/- 1 second (0 – 60 minutes).
10. Disposal basins and 0.5% sodium hypochlorite (50 ml bleach in 950 ml $dH_20$).
11. Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm.

1. Place the desired number of strips into a microwell frame. Allow six (6) Control/Calibrator determinations (one Negative Control, three Calibrators, one High Positive Control and one Low Positive Control) per run. A reagent blank (RB) should be run on each assay.

2. Dilute test sera, Calibrator and Control sera 1:21 (e.g., 10 µl + 200 µl) in Serum Diluent. Mix Well.

3. To individual wells, add 100 µl of the appropriate diluted Calibrator, Controls and patient sera. Add 100 µl of Serum Diluent to reagent blank well. Check software and reader requirements for the correct reagent blank well configuration.

4. Incubate each well at room temperature (21° to 25° C) for 30 minutes +/- 1 minute.

5. Aspirate or shake out liquid from all wells. If using semi-automated or automated washing equipment add 250-300 µL of diluted Wash Buffer to each well. Aspirate or shake out and turn plate upside down and blot on paper toweling to remove all liquid. Repeat the wash procedure two times. After the final wash, blot the plate on paper toweling to remove all liquid from the wells.

6. Add 100 µl Conjugate to each well, including reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.

7. Incubate each well at room temperature (21° to 25° C) for 30 minutes +/- 1 minute.

8. Repeat wash as described in Step 5.

9. Add 100 µl Chromogen/Substrate Solution (TMB) to each well, including the reagent blank well, maintaining a constant rate of addition across the plate.

10. Incubate each well at room temperature (21° to 25°C) for 15 minutes +/- 1 minute.

11. Stop reaction by addition of 100 µl of Stop Solution (1N $H_2SO_4$) following the same order of Chromogen/Substrate addition, including the reagent blank well. Tap the plate gently along the outsides, to mix contents of the wells. The plate may be held up to 1 hour after addition of the Stop Solution before reading.

12. The developed color should be read on an ELISA plate reader equipped with a 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm.

**4.5Results**

The health assessment of beedi rollers of various age groups through blood parameters has indicated a negative impact on the population .

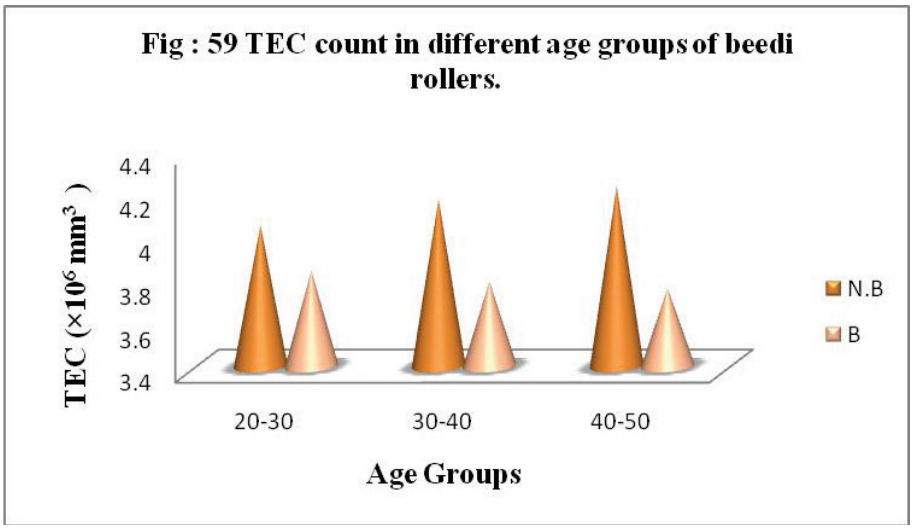
**4.5.1.Total Erythrocyte Count (TEC)**

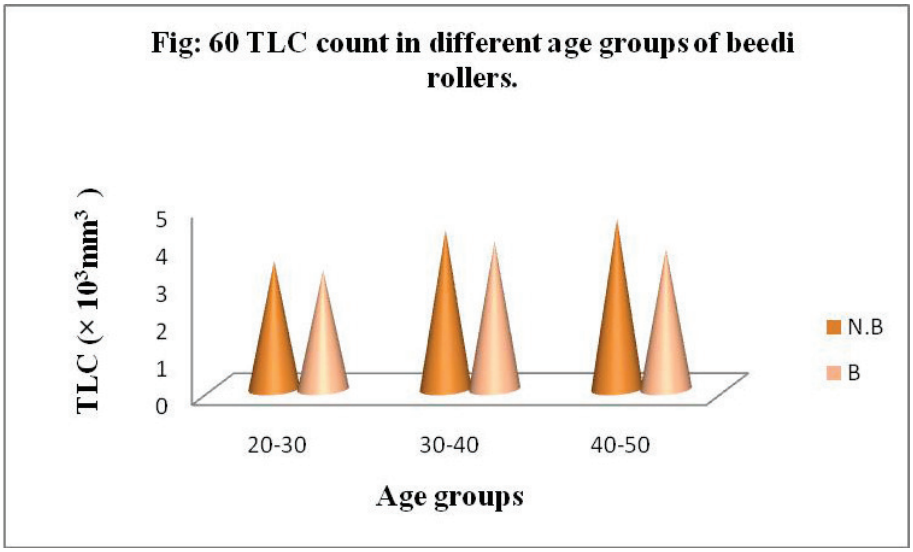
The total erythrocyte count in the control women population ranged from $4.05 \pm 0.452\times10^6/mm^3$ $4.23 \pm 0.660 \times10^6/mm^3$ in the age group of 20-30 to 40-50, which was within normal range (4 to 5 $\times10^6/mm^3$). But the beedi rollers at various age groups exhibited a decrease in TEC. In the age group of 20-30 the women beedi workers had a decrease in the TEC count ($3.84 \pm 0.583\times10^6/mm^3$) which was highly significant statistically. The same trend was observed in the age groups of 30-40 and 40-50 also (Fig: 59).

**4.5.2.Total Leucocyte Count (TLC)**

Total Leucocyte Count in the the control population exhibited near the normal lower limit (4.3 to $10.0\times10^{3/}mm^3$). At the age group of 20-30, 30-40 and 40-50 TLC

was 3.19± 0.39, 3.97± 0.50, 3.77 ± 0.58 respectively. On the otherhand, beedi rollers at the age group of 20-30, 30-40 and 40-50 showed TLC of a 3.44 ± 0.80, 4.27 ± 0.138, 4.57 ± 0.181 respectively. Significantdecreased levels (P< 0.05) of TLC was determined in the women beedi rollers in all age groups compared to the non beedi rollers (Fig: 60).



### 4.5.3 Platelet Count

There was a decrease in the platelet counts among the beedi worker population. Sampling at the age group of 20 to 30 showed 1.65 ± 0.64 thousandcells/$mm^3$when compared with normal individuals who have a count of 2.70 ± 0.883thousand cells/$mm^3$.Similar observations was observed in the other age groups of beedi rollers. Eventhough platelet count was well within normal range of 1.5-4.0 thousand cells/$mm^3$ they showed a decreasing a trend reflecting the health states of beedi workers(Fig: 61).

### 4.5.4 Haemoglobin content (Hb)

The normal range of haemoglobin content in females in 12.1 to15.1 g/dl, 10.2 ±1.15 and 10.5 ± 1.22 g/dl in the age group 20-30, 30-40, and 40-50 respectively. But the haemoglobin content of beedi workers population exhibited a decrease. It is 8.67 ± 0.162 g/dl in the age group of 20 to 30 and 8.89 ± 0.181g/dl in 30-40 and 8.36 ± 0.176 g/dl in 40 to 50. Statistical analysis of the data showed highly significant values ($P<0.001$) in the age to group of 20 to 30 and 40 to 50 and significant value ($P<0.05$) in the age group of 30 to 40(Fig: 62).

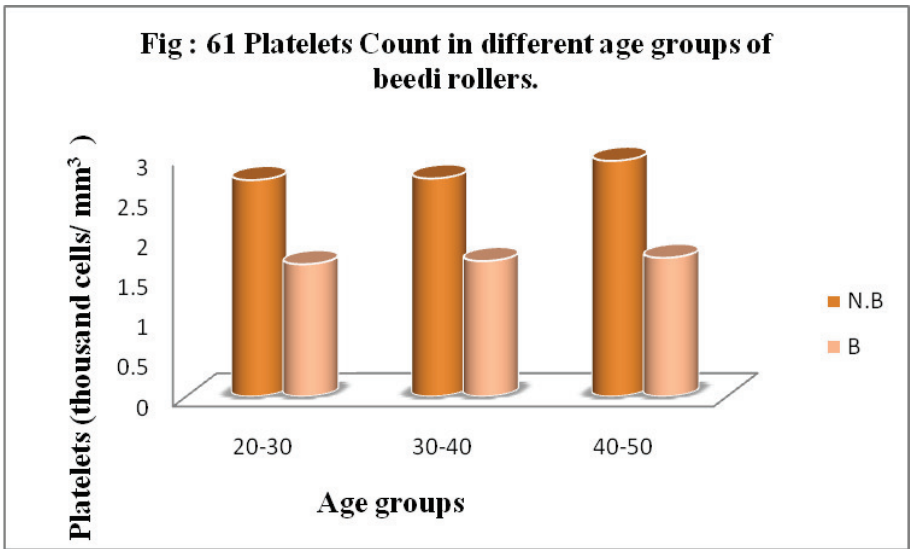



**Fig : 61 Platelets Count in different age groups of beedi rollers.**

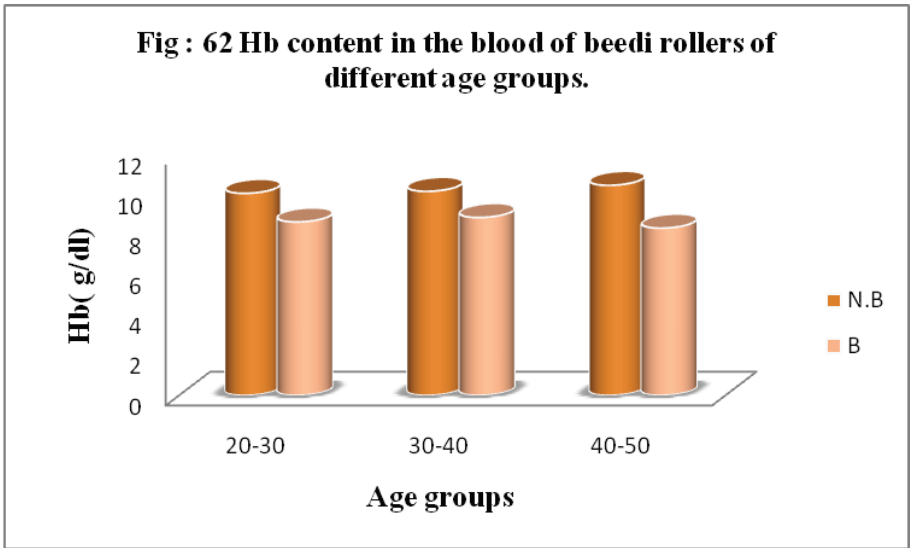



**Fig : 62 Hb content in the blood of beedi rollers of different age groups.**

### 4.5.5 Erythrocyte Sedimentation Rate (ESR)

ESR of the experimental subjects showed significantly high values, while the females have a value which lies within the normal range (0-20 mm/hr). The ESR of beedi workers at the age group 20-30 was 23 ± 2.94 mm/hr whereas that of control people in 16.8 ±1.38 mm/hr. The same trend is observed in all beedi workers at the age group of 30-40 and 40-50(Fig: 63).

### 4.5.6 Packed Cell Volume(PCV)

Significant decrease (P<0.05) in PCV was observed in beedi rollers in all age groups. The control population exhibited a PCV within normal range (36-48%). At the age group of 20-30, 30-40 and 40-50 a PCV was 35.4 ± 2.14%, 35.2±3.48% and 35 ± 3.23% respectively. On the otherhand, beedi rollers at the age group of 20-30, 30-40, 40-50 showed a PCV of 30.4 ± 2.91% 30.2±2.84% and 31.0 ±2.59% respectively(Fig: 64).

**Fig : 63 ESR content in the blood of beedi rollers of different age groups.**

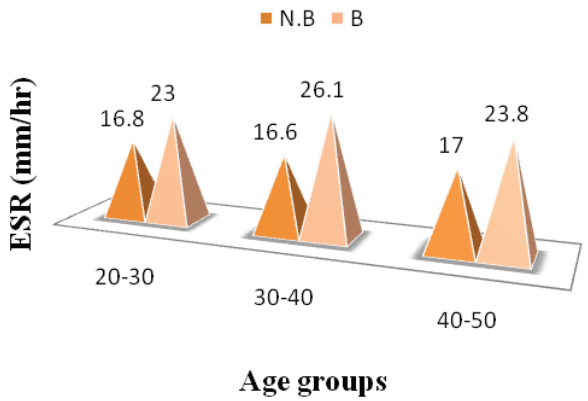
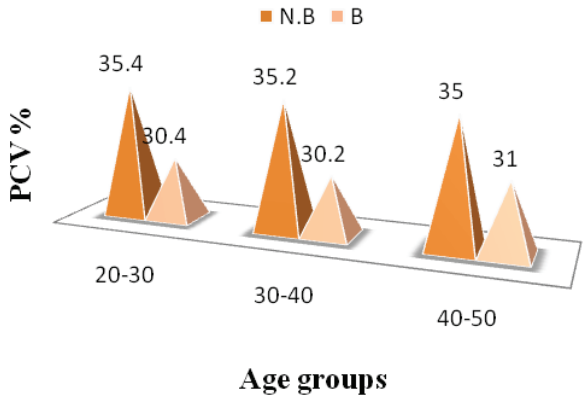



**Fig : 64 PCV in the blood of beedi rollers of different age groups**



**Trace Elements**

Trace elements Sodium, Potassium and Chloride in the blood of beedi rollers were estimated and compared with normal population. Eventhough significant increase was noted they were within the normal range.

### 4.5.7 Sodium

The sodium level of the normal female is 135 to 145 mmol/l. Elevated levels of sodium was found in all age group of beedi rollers (128 ± 6.39 mmol/l to 129 ± 4.64 mmol/l) compared to non beedi rollers (126 ± 4.60 mmol/l to 128±4.47mmol/l) respectively(Table: 3a).

### 4.5.8 Potassium

A highly significant increase ($P<0.001$) in potassium level was observed in the beedi rollers among the age group of 40-50 (5.13 ± 0.72 mmol/l.This data slightly higher then the normal potassium level of normal healthy female (3.5 -5.0 mmol/l). In the age group of 20-30 and 30-40 the potassium level in beedi rollers was 3.18±0.18 and 3.20 ±0.27 mmol/l respectively.But in beedi rollers the age group of 20-30 and 30-40 exhibited the potassium level of 3.57 ±0.43 mmol/l and 4.03 ± 0.78 mmol/l respectively (Table: 3b).

### 4.5.9 Chloride

Eventhough highly significant increase ($P<0.001$) in the chloride levels among the beedi rollers irrespective of the age groups was identified, it was within range when compared to non beedi rollers. The chloride level in normal females ranges from 98-108 mmol/l. An observation of table indicates elevated level among the beedi rollers population compared to non beedi rollers (Table: 3c).

**Table 3: Trace Elements in the beedi rollers population of various age groups.**

| PARAMETERS | AGE | | MEAN ± SD | SE | t |
|---|---|---|---|---|---|
| a) SODIUM (135-145 mmol/l) | 20-30 | N.B | 127 ± 3.49 | 2.53 | |
| | | B | 129 ± 6.13 | 1.85 | 2.27** |
| | 30-40 | N.B | 128 ± 4.47 | 2.60 | |
| | | B | 129 ± 4.64 | 1.85 | 2.30** |
| | 40-50 | N.B | 126 ± 4.60 | 1.62 | |
| | | B | 128 ± 6.39 | 1.15 | 1.82** |
| b) POTASSIUM (3.5-5.0 mmol/l) | 20-30 | N.B | 3.18 ± 0.18 | .02 | |
| | | B | 3.57 ± 0.43 | .06 | 5.39** |
| | 30-40 | N.B | 3.20 ± 0.27 | .03 | |
| | | B | 4.03 ± 0.78 | .42 | 1.61 |
| | 40-50 | N.B | 3.20 ± 0.22 | .03 | |
| | | B | 5.13 ± 0.72 | .77 | 1.85** |
| c) CHLORIDE (98-108 mmol/l) | 20-30 | N.B | 93.5 ± 2.54 | 0.39 | |
| | | B | 95.4 ± 3.67 | 1.50 | 2.93** |
| | 30-40 | N.B | 93.8 ± 2.60 | 1.35 | |
| | | B | 96.0 ± 4.46 | 0.50 | 3.19** |
| | 40-50 | N.B | 93.8 ± 2.73 | 1.37 | |
| | | B | 94.8 ± 3.08 | 0.75 | 1.24 |

**** - highly significant. * Significant**

**4.6 Sugar**

The random sugar estimated in the blood of non beedi rollers and beedi rollers showed a significant decrease ($P<0.001$).Non beedi rollers had an uniformely high sugar level of 175 ± 32.8 mg/dl to 176 ± 39.8 mg/dl against the normal sugar level of

80-120 mg/dl. On the otherhand,when compared to above range beedi rollers exhibited a significant decrease ranging from 132 ± 22.8 to 138 ± 19.7 mg/dl(Fig: 65).

### 4.6.1 Total Cholesterol

Total Cholesterol was found to be above the normal limit in beedi workers population irrespective of age it was 262 ± 33.7, 256 ± 38.6 and 251± 30.2 mg % in 20-30, 30-40 and 40-50 age group respectively (Fig: 66).

**Fig : 65 Sugar in various age groups of beedi rollers.**

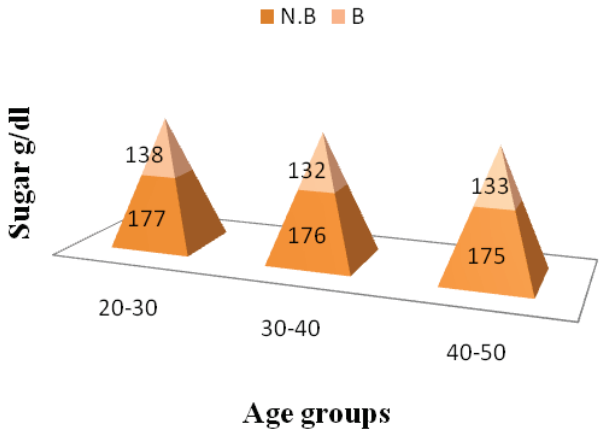
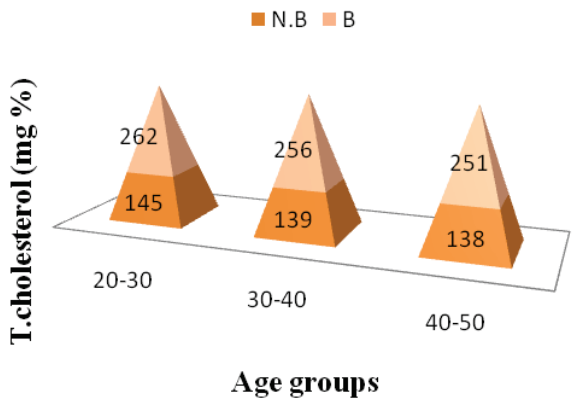



**Fig :66 Total Cholesterol in various age groups of beedi rollers.**

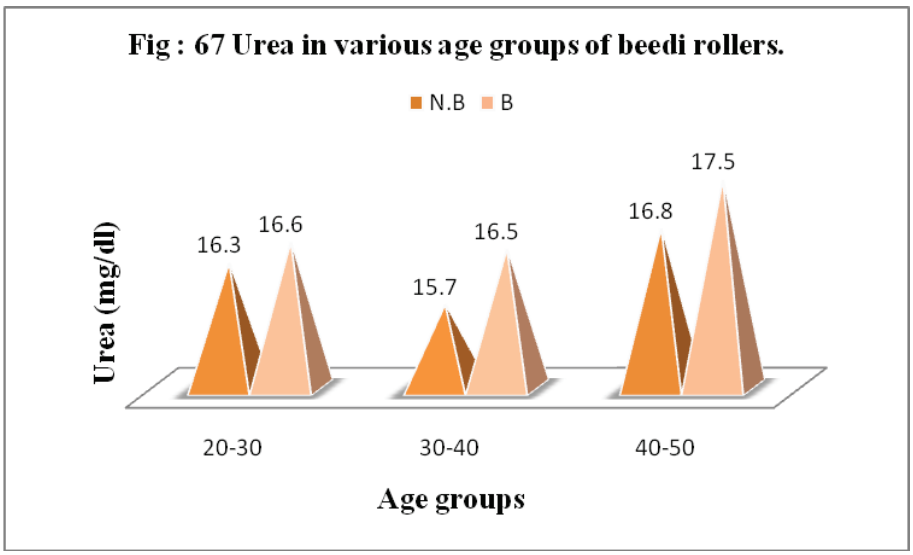


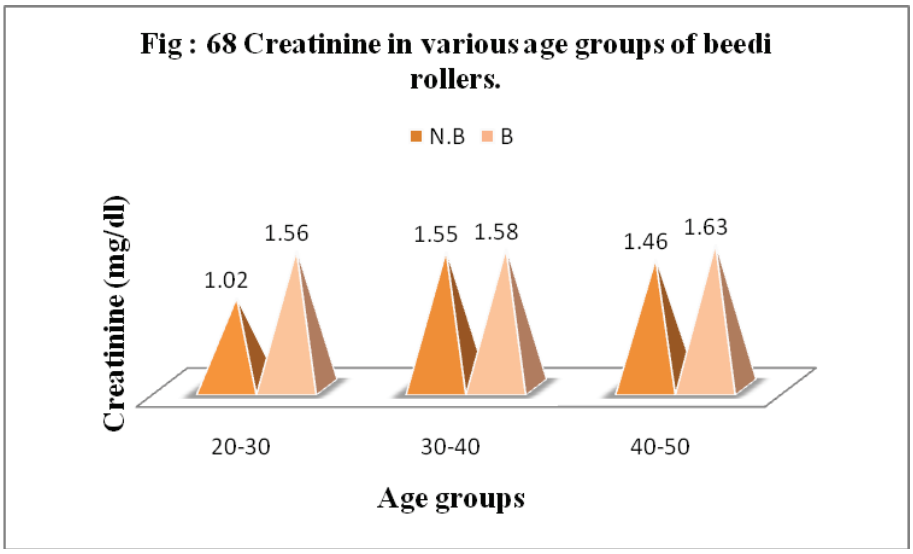
### 4.6.2 Urea

The possible level of urea in an adult female is 7-25 mg/dl. Analysis of blood urea in beedi rollers and non beedi rollers were within the normal range. However the beedi rollers exhibited a marginal increase in the blood urea level(Fig: 67).

### 4.6.3 Creatinine

Reports recording the creatinine level showed a highly significant increase ($P< 0.001$) in the beedi rollers population in the age group of 20-30 and 30-40 when compared to non beedi rollers (Fig: 68).






**Liver Function Test**

Liver is the vital organs in intermediary metabolism, indetoxification and in the elimination of toxic substance. Since the liver as considerable functional reserve, damage to the organ may not affect its activity. The results of the biochemical test provide a precise diagnostic and reflect the basic pathological process common to many condition. The liver function test is helps to scripe the beedi rollers exposed to potential heptotoxic materials if any in theindustry.The routine liver function test includes determination of serum Total Bilurubin and enzymes test such as Alkaline Phosphate(ALP), Gamma Glutaminate Transferase(GGT), Serum Glutamic Pyruic Transaminase (SGOT), Serum Glutamitc Oxaloacetic Transaminase (SGPT or AST) and Total Proteins, Albumin, Globulin.

**4.6.4 Total Bilurubin**

A measure of bile pigments and the bile salts in the blood can be determine by analyzing thetotal bilurubin.A highly significant increase in total bilurubin could be observed in beedi rollers in the age group of 20-30 (1.28 ± 0.385 mg/dl) In the age

group of 40-50 there is a significant decrease (P<0.05) in the total bilurubin level (1.20 ± 0.38 mg/dl) compared to non beedi rollers (1.36±0.395 mg/dl) (Table: 4a).

**Table 4 : Total Bilurubin, ALP, GGT of a Liver function test in the beedi rollers population of various age groups.**

| PARAMETERS | AGE | | MEAN ± SD | SE | t |
|---|---|---|---|---|---|
| a) TOTAL BILURUBIN (1.0 mg/dl) | 20-30 | N.B | 1.20 ± 0.386 | .052 | |
| | | B | 1.28 ± 0.385 | .056 | .963** |
| | 30-40 | N.B | 1.37 ± 0.381 | .053 | |
| | | B | 1.29 ± 0.400 | .042 | 1.15 |
| | 40-50 | N.B | 1.36 ± 0.395 | .054 | |
| | | B | 1.20 ± 0.382 | .081 | 1.52* |
| b) ALP (60-170u/dl) | 20-30 | N.B | 139 ± 3.60 | 1.55 | |
| | | B | 132 ± 4.43 | 2.08 | 7.21** |
| | 30-40 | N.B | 120 ± 20.6 | 1.55 | |
| | | B | 134 ± 4.83 | 2.61 | 35.8** |
| | 40-50 | N.B | 1.21 ± 19.8 | 2.80 | |
| | | B | 134 ± 4.43 | 1.54 | 18.87** |
| c) GGT (15-45 u/dl) | 20-30 | N.B | 39.14 ± 2.62 | 2.70 | |
| | | B | 33.38 ± 5.09 | 1.01 | 6.64** |
| | 30-40 | N.B | 27.98 ± 7.50 | 2.40 | |
| | | B | 33.65 ± 4.87 | 1.70 | 5.29** |
| | 40-50 | N.B | 27.01 ± 8.05 | 1.02 | |
| | | B | 34.00 ± 5.69 | 2.54 | 3.47* |

**** - highly significant. * Significant**

### 4.6.5 Alkaline Phosphate (ALP)

Changes in the enzyme Alkaline Phosphate determined in beedi rollers and non Beedi rollers did not deviate much from the normal level of 60-170 U/dl. However, the Alkaline Phosphate level decreased in beedi rollers in the age group of 20-30 and showed an increasing trend in other two age groups compared to non beedi rollers (Table: 4b).

### 4.6.6 Gamma Glutamitate Transaminase (GGT)

The enzyme GGT also exhibit a similar trend as that of ALP. Beedi rollers in the age group of exhibit a significant low level of GGT compared to non beedi rollers. But the GGT level in the age group 30-40 and 40-50 showed a significant increased 33.65 ±4.87 U/dl compared to 27.98 ±7.50 U/dl non beedi rollers and 34.0 ± 5.69 U/dl compared to 27.01±8.05 U/dl (Table: 4c).

### 4.6.7 Serum Glutamic Oxaloacetic Transaminase(SGOT)

Elevated levels of SGOT was determined in the women beedi rollers in all the age groups compared to the non beedi rollers. The non beedi rollers had a SGOT content of 39 ± 3.60 IU/l in 20-30 age group, 39.25 ± 3.07 IU/l in 30-40 age group and 38.35 ± 3.34 IU/l which was within the normal upper limit (5-40 IU/l). But in beedi rollers the SGOT range was 46.73 ±7.79 IU/l in 20-30 age group, 44.51 ±1.26 IU/l in 30-40 and 43.57 ± 6.12 IU/l which was higher than the normal value (Table: 5a).

### 4.6.8 Serum Glutamic Pyruiv Transaminase (SGPT)

The present observation of SGPT showed an increase in beedi rollers in the age group of 20 to 30 and 30-40. It was 47.80 ± 6.64 IU/l and 46.09 ± 8.80 IU/l

respectively, when compared to non beedi rollers who possessed 39.14±2.62 IU/l and 38.83 ± 2.78 IU/l in 20-30 and 30-40 age groups respectively. However in the age group of 40-50 the non beedi rollers had a high SGPT level than the beedi rollers (Table: 5b).

**Table 5 : SGOT and SGPT levels of beedi rollers populations in different age groups.**

| PARAMETERS | AGE | | MEAN ± SD | SE | t |
|---|---|---|---|---|---|
| a) SGOT (5-40 IU/l) | 20-30 | N.B | 39.00 ± 3.60 | 0.55 | |
| | | B | 46.73 ± 7.79 | 0.08 | 5.92** |
| | 30-40 | N.B | 39.25 ± 3.07 | 1.41 | |
| | | B | 44.51 ± 1.26 | 1.42 | 2.98** |
| | 40-50 | N.B | 38.35 ± 3.34 | 1.45 | |
| | | B | 43.57 ± 6.12 | 0.40 | 4.63** |
| b) SGPT(5-40 IU/l) | 20-30 | N.B | 39.14 ± 2.62 | 1.40 | |
| | | B | 47.80 ± 6.64 | 1.92 | 10.4** |
| | 30-40 | N.B | 38.83 ± 2.78 | 0.37 | |
| | | B | 46.09 ± 8.80 | 1.99 | 10.2** |
| | 40-50 | N.B | 48.72 ± 2.68 | 0.36 | |
| | | B | 47.89 ± 8.02 | 0.83 | 8.72** |

**** - highly significant. * Significant**

### 4.6.9 Total Protein

The normal level of serum of total protein was 6-8 g/dl in non beedi rollers it amounts to 6.55 ± 0.291 g/dl to 6.75 ± 0.182 g/dl which was around the lower limit of the normal value. In beedi rollers there was a highly significant increase (P< 0.001) in

all age groups. It was 6.97 ± 0.383 g/dl, 6.87 ± 0.310 g/dl and 7.20 ± 0.339 g/dl in 20-30, 30-40 and 40-50 age groups respectively (Table: 6a).

**Table 6 : Total Protein, Albumin and Globulin Content in various age groups of beedi rollers.**

| PARAMETERS | AGE | | MEAN ± SD | SE | t |
|---|---|---|---|---|---|
| a) T. PROTEIN (6-8 g/dl) | 20-30 | N.B | 6.73 ± 0.875 | 1.09 | |
| | | B | 6.97 ± 0.383 | 1.30 | 5.37** |
| | 30-40 | N.B | 6.75 ± 0.182 | 1.31 | |
| | | B | 6.03 ± 0.310 | 1.53 | 7.21** |
| | 40-50 | N.B | 6.55 ± 0.291 | 2.11 | |
| | | B | 7.20 ± 0.339 | 0.34 | 10.4** |
| b) ALBUMIN (3.5-5.0g/dl) | 20-30 | N.B | 5.28 ± 1.62 | 1.09 | |
| | | B | 3.47 ± 1.22 | 1.16 | -8.69** |
| | 30-40 | N.B | 5.46 ± 2.36 | 1.04 | |
| | | B | 3.84 ± 1.16 | 1.13 | -9.87** |
| | 40-50 | N.B | 5.42 ± 2.55 | 1.07 | |
| | | B | 4.23 ± 2.03 | 1.46 | -3.94** |
| c) GLOBULIN (1.5-5.0 g/dl) | 20-30 | N.B | 1.45 ± 0.290 | 1.13 | |
| | | B | 3.50 ± 0.390 | 1.54 | 8.20** |
| | 30-40 | N.B | 1.28 ± 0.904 | 0.12 | |
| | | B | 3.02 ± 0.337 | 1.38 | 10.0** |
| | 40-50 | N.B | 1.13 ± 0.439 | 0.12 | |
| | | B | 3.58 ± 0.391 | 0.89 | 11.3** |

**** - highly significant. * Significant**

**4.7 Albumin**

Albumin decreased in the beedi rollers irrespective of the age. They possessed albumin level near the lower limit of the normal value (3.5-5.0 g/dl).The non beedi

rollers in the age group of 20-30 had an albumin level of 5.28 ± 1.62 g/dl whereas that of beedi rollers of the same age group showed a value of 3.47 ±1.22 g/dl. A similar decreasing in trend was observed in the other two age groups also (Table: 6b).

### 4.7.1 Globulin

Serum Globulin range from 1.5 to5.0 g/dl in normal women. While the non beedi rollers exhibited a lower limit of normal Globulin value, the beedi rollers showed a highly significant increase ($P< 0.001$) in all age categorise globulin level of beedi rollers was 3.50 ± 0.390 g/dl, 3.02 ± 0.337 g/dl and 3.5 ± 0.391 g/dl in 20-30, 30-40 and 40-50 age groups. This were very high values exceeding even the lower limit of the normal value (5.0 g/dl) (Table: 6c).

### 4.7.2 Thyroid profile

Analysis of Thyroid hormones such as $T_3$ , $T_4$, TSH, $FT_3$, $FT_4$, AMA and ATG in the blood of beedi rollers may help in the health assessment.

### 4.7.2 Total Triiodothyronine( $T_3$)

$T_3$ levels of a normal healthy women ranges from 60-200 ng/dl. In the present study the beedi rollers exhibited decreased levels of $T_3$ compared to non beedi rollers. In the age group of 20-30 the beedi rollers had a $T_3$ level of 68.80 ± 6.74 ng/dl compared to 95.23 ± 5.04 ng/dl among non beedi rollers. Similar observations was found among the age group of 30-40 and 40-50 which was much below than the normal lower limit. This decrease in $T_3$ level was highly significant ($P < 0.001$) in beedi rollers indicating hormone deficiency (Table: 7a).

**Table 7 : Thyroid Profile in beedi rollers showing $T_3$, $T_4$ and TSH levels.**

| PARAMETERS | AGE | | MEAN ± SD | SE | t |
|---|---|---|---|---|---|
| a) $T_3$ (60-200 ng/dl) | 20-30 | N.B | 95.23 ± 9.12 | 1.40 | |
| | | B | 68.80 ± 6.74 | 3.80 | -5.97** |
| | 30-40 | N.B | 95.35 ± 5.04 | 1.41 | |
| | | B | 62.20 ± 9.19 | 2.24 | -10.5** |
| | 40-50 | N.B | 94.90 ± 8.16 | 1.50 | |
| | | B | 57.52 ± 7.12 | 2.85 | -12.2 |
| b) $T_4$ (4.5-12 µg/dl) | 20-30 | N.B | 8.86 ± 0.194 | 0.30 | |
| | | B | 4.43 ± 1.175 | 0.24 | -11.5 |
| | 30-40 | N.B | 8.66 ± 0.204 | 0.27 | |
| | | B | 5.75 ± 0.247 | 0.61 | -3.72** |
| | 40-50 | N.B | 8.94 ± 0.209 | 0.28 | |
| | | B | 4.88 ± 0.189 | 0.43 | -7.42* |
| c) TSH (0.3-5.5 µIU/ml) | 20-30 | N.B | 3.09 ± 0.81 | 0.12 | |
| | | B | 4.44 ± 0.95 | 0.13 | 7.25** |
| | 30-40 | N.B | 3.06 ± 0.89 | 0.12 | |
| | | B | 4.39 ± 0.10 | 0.11 | 7.58 |
| | 40-50 | N.B | 2.96 ± 0.95 | 0.12 | |
| | | B | 4.55 ± 0.11 | 0.27 | 5.86** |

**** - highly significant. * Significant**

### 4.7.3 Total Thyroxine ($T_4$)

A decreasing trend in the $T_4$ content was observed in the beedi rollers. While non beedi rollers had a value within the normal limit of $T_4$ (4.5-12.5 µg/dl) beedi rollers possessed low level of $T_4$ data observed in 20-30, 30-40 and 40-50 age groups beedi rollers were 4.43 ± 0.175 µg/dl, 5.75 ± 0.547 µg/dl and 4.88 ± 0.189 µg/dl respectively

whereas in non beedi rollers it was 8.86 ± 0.194 µg/dl, 8.66 ± 0.204 µg/dl and 8.94 ± 0.209 µg/dl (Table: 7b).

### 4.7.4 Thyroid Stimulating Hormone(TSH)

TSH level in beedi rollers and non beedi rollers were within the normal range (0.3-5µIu/ ml). However in beedi rollers the TSH level very near the upper normal limit in all age groups. Statistically highly significant results were observed in 20-30 age group where the beedi rollers showed 4.44 ± 0.95 µIu/ ml of TSH compared to 3.09 ± 0.81 µIu/ ml. In the age groups of 40-50 also the beedi rollers recorded 4.55 ± 0 .11 µIU/ ml of TSH against 2.96 ±0.95 µIU/ ml in non beedi rollers (Table: 7c).

### 4.7.5 Free Triiodo Thyronine ($FT_3$)

A decrease in the $FT_3$ level was observed in the beedi rollers which was synonymous with the $T_3$ level. Eventhough there was statistically highly significant decrease ($P<0.001$) all age groups of beedi rollers it remained within the normal limit of $FT_3$ level (Table: 8a).

### 4.7.6 Free Thyroxine ($FT_4$)

A corresponding decrease of $FT_4$ could be inferred from the data in the Beedi rollers.In the age group of 20-30 and 40-50 the $FT_4$ level went below the normal lower limit (0.7ng/dl) and it was 0.69 ± 0.21 mg/dl and 0.66 ± 0.16 ng/dl respectively (Table: 8b).

### 4.7.7 Anti Microsomal Antibody (AMA)

The Antimicrosomal antibodies in beedi rollers showed statistically significant values ($P< 0.05$) in the age group of 30-40 (48.66 ± 27.6 U/ml) and highly significant

**Table 8 : $FT_3$, $FT_4$, AMA and ATG levels of thyroid profile in beedi rollers population.**

| PARAMETERS | AGE | | MEAN | ± | SD | SE | t |
|---|---|---|---|---|---|---|---|
| a) $FT_3$ (1.7-4.2 pg/ml) | 20-30 | N.B | 2.70 | ± | 0.76 | 0.11 | |
| | | B | 1.42 | ± | 0.54 | 0.07 | -9.51** |
| | 30-40 | N.B | 2.60 | ± | 0.83 | 0.11 | |
| | | B | 1.48 | ± | 0.55 | 0.06 | -9.27** |
| | 40-50 | N.B | 2.64 | ± | 0.70 | 0.09 | |
| | | B | 1.33 | ± | 0.52 | 0.12 | -7.39 |
| b) $FT_4$ (.7-1.80 ng/dl) | 20-30 | N.B | 1.20 | ± | 0.37 | 0.05 | |
| | | B | 0.69 | ± | 0.21 | 0.02 | -8.38** |
| | 30-40 | N.B | 1.51 | ± | 0.46 | 0.19 | |
| | | B | 1.34 | ± | 0.53 | 0.59 | -2.34* |
| | 40-50 | N.B | 1.21 | ± | 0.42 | 0.05 | |
| | | B | 0.66 | ± | 0.16 | 0.36 | -5.41** |
| c) AMA (36-49 U/ml) | 20-30 | N.B | 38.52 | ± | 4.08 | 1.63 | |
| | | B | 45.15 | ± | 6.95 | 2.96 | 5.45 |
| | 30-40 | N.B | 38.57 | ± | 3.77 | 1.51 | |
| | | B | 48.66 | ± | 27.6 | 2.11 | 2.65* |
| | 40-50 | N.B | 39.55 | ± | 3.80 | 1.51 | |
| | | B | 45.47 | ± | 1.21 | 1.27 | 6.63** |
| d) ATG (225-325 IU/ml) | 20-30 | N.B | 255 | ± | 14.8 | 4.14 | |
| | | B | 287 | ± | 20.4 | 2.83 | 1.76* |
| | 30-40 | N.B | 268 | ± | 34.0 | 4.62 | |
| | | B | 281 | ± | 15.4 | 1.73 | 2.95** |
| | 40-50 | N.B | 259 | ± | 28.6 | 3.90 | |
| | | B | 277 | ± | 11.8 | 2.72 | 1.21 |

**** - highly significant. * Significant**

in the age group of 40-50 (45.47± 1.21 U/ml). While the non beedi rollers showed the AMA level near the normal lower limit (36 to 49 U/ml). The AMA level in the beedi worker population on the otherhand, were near the upper normal limit indicating elevated levels of AMA (Table: 8c).

### 4.7.8 Anti Thyro Globulin (ATG)

ATG in beedi rollers showed a marginal increase than the non beedi rollers and was found within the normal level (225-325 IU/ml). The increase in ATG was found to be statistically highly significant ($p< 0.001$) in 20-30 and 30-40 of beedi rollers population (Table: 8d).

### 4.8 Discussion

The present study dealing with the health assessment of women beedi rollers in Melapalayam has revealed that most of them suffer from pulmonary, gastrointestinal and cardiac disorders apart from various occupational hazards. Routine analysis of the blood parameters of 150 women beedi rollers from the age group of 20-50 and 150 non beedi rollers were carried out in Vimta Laboratory, Vannarpettai Tirunelveli. Total RBC (TEC) in beedi rollers were significantly lower in comparision to the control subjects. Yasmin *et al*., (2010) has reported that the TEC level of women beedi rollers of Patna in Bihar was significantly low than the control. In another comparable study, Karafakioglu *et al*.,(2009) found a significant fall in RBC count in women workers who harvest tobacco, after the harvest as compared to the count before the harvest. Metin *et al*.,(2004) and Rajesekhar *et al.,*(2007) found a reduction in RBC count in smokeless tobacco consumers. Adeniyi and Ghazal (2006) studied the effects of tobacco leaf extracts on Wister rats and found a significant reduction in RBC count. According to the doctors of Indian Medical Association Karim Nagar, majority of the women beedi rollers were suffering from anaemia and they have low count of RBC and WBC they added. They said that the beedi rolling was very high among the womenfolk of Sircilla and Jagtial revenue divisions such as Karimnagar town, Hushabad, Chigurumaidi Mandals (The Hindu, 2011). Total WBC count of beedi rollers was also significantly

lower as compared to control. These findings are in accordance with the earlier reports ofYasmin *et al*., (2010). Decrease in WBC count may be due to exposure to tobacco dust. Metin *et al*.,(2004) and Rajasekhar *et al*.,(2007) also found a reduction in WBC count in smokeless tobacco consumers. Valenca *et al*.,(2008) observed a reduction in leucocyte count in Wistar rats treated with oral nicotine. Rahman(2000) had also reported a drop in white blood cell count among beedi production workers.

In the present study haemoglobin levels were also lower among the beedi rollers. Rajesekhar *et al*.,(2007) also found a reduction in haemoglobin percentage in smokeless tobacco consumers. Adeniyi and Ghazal (2006) found a reduction in haemoglobin level of Wister rats in response to extract of tobacco leaves. Karafakioglu *et al*.,(2009) found a significant decrease in haemoglobin level in women workers who harvest tobacco, after the harvest as compared to the level before the harvest. Low haemoglobin levels with or without an absolute decrease of RBCs may lead to symptoms of anaemia. The platelet count of beedi rollers was also significantly lower as compared to the controls. Metin *et al*.,(2004) also found a reduction in platelet count in consumers of Maras powder (smokeless tobacco). Adeniyi and Ghazal (2006) found a significant reduction in platelet count in Wister rate as a result of exposure to tobacco leaf extracts. Increased ESR encounted in beedi rollers can be also be attributed to the inflammatory responses due to the inhalation of the toxic chemicals released by the raw tobacco dust Gupta *et al*., (2005). It is evident that inflammatory changes may be due to the proteins in the red blood cells causing them to bind one another clumps, making them denser then normal red blood cells. Thus the beedi rollers experienced inflammatory disorders causing stiffness and pain usually in the shoulder, neck, upper arms, hip and thigh (Sood,2002).

Other blood parameters such as trace elements also showed fluctuations in beedi rollers compared to the non beedi rollers. A decreased concentration of sodium reported as hyponatremiais prevalent among the beedi rollers. However, the level was not too little to be fatel. This condition occurs whenever there is a relative increase in amount of body water relative to sodium. This happens to occur with some diseases of liver and kidney and the patients with burn victims and numerous other conditions hyperkalemia (increased potassium level) has been observed in beedi rollers. Conditions of hyperkalemia may lead to irregular heart beats,since potassium is found to regulate the heart beat in the body. Chloride level shows an increasing trend in beedi roller which may lead to the condition of acitosis. There is no evidence of the status of blood urea and creatinine among the beedi rollers due to raw tobacco dust, eventhough increased creatinine level has been observed, which may be mainly due to the muscle degeneration caused by the ergonomic postures during beedi rolling.

The overall health status of a person can be assesed by infering results of the liver function test, since the liver plays a role in the removal of toxins. Differences in total bilurubin, ALP, GGT, SGOT, SGPT, and proteins may serve as indices of the health status. Bilurubin is the by product of the normal breakdown of red blood cells. Elevated bilurubin levels may be related to haemolytic anaemia among beedi rollers which may be correlated to a significant decrease in their total RBC content. ALP and GGT level are indicative of liver dysfunction. Beedi rollers exhibit fluctuation in these levels compared to non beedi rollers which may be caused due to nicotine toxicity (Cashman *et al*.,1992).

SGOT and SGPT are two important enzymes of the liver and other body tissues which are found to play a role in processing protein. Elevated levels in beedi rollers

indicate liver inflammation due to the inhalation of raw tobacco dust and its constituent nicotine. Similar observation was reported by Yasmin *et al*., (2010). It is suggested that nicotine may be absorbed through the skin of the fingers and palm and throughout the body. It is finally metabolized by the liver (Cashman *et al*., 1992 ; Snyder and Sheafor 1999 ; Neurath 1994). Karafakioglu *et al* (2009) also found a significant rise in SGPT level of women workers who are involved in the harvest of tobacco.

Liver function test include measuring albumin and globulin, the major blood protein produced by the liver.Changes in the amount of albumin and globulin indicate abnormality of the plasma protein produced by the liver. In the present study the albumin level has decreased in the beedi rollers while globulin elicit a significant increase. Clinical significance of these findings prove that albumin decrease and globulin increase in advance liver disease (Godkar,2007)

Thyroid diseases occur about five times more frequently women than in men (Srinivasulu,1997).Smoke exposure may affect various metabolic and biological processes including hormone biosynthesis and secretion, inferfere with thyroid hormone release, binding, transport, storage and clearance, associated with adverse effects on the thyroid resulting in changes in circulating hormone concentration. A comprehensive approach to assess the interrelationship between a raw tobacco dust and its constituents on the thyroid hormone levels revealed symptoms of physiological disorders in women beedi rollers.Behera *et al* (1995)Yet published data are inconsistent to provide adequate evidence that unburnt tobacco smoke may affect beedi rollers. Free thyroid hormone ($FT_3$ and $FT_4$) represent a more useful index of thyroid status then total thyroid hormone because $FT_3$is influence by variations of thyroid hormone binding protein (Thyroid binding globulin TBG) (Bartalena *et al*.,1995). In the present

investigation detailed analysis of thyroid hormone such as TSH, $T_3$, $T_4$, $FT_3$,$FT_4$, AMA and ATG employing innovative modern hormone analysis techniques predict notable changes in the hormone levels which affects the beedi rollers when compared to normal subjects.

In the present study the $T_3$ and $T_4$ levels were significantly low in the beedi rollers, compared to non beedi rollers. Circulating TSH was found to be higher in beedi rollers. These results agree with the earlier findings of Behara *et al*.,(1995) who reported alternations in circulating $T_3$ and $T_4$ levels in Indian smokers. Sepkovic *et al*.,(1984) found reduced serum $T_3$ and $T_4$ levels in heavy smokers. Christensen *et al*.,(1984) and Nystrom *et al*.,(1993) have reported reduced T3 level in smokers. A danish study reported positive association between tobacco smoke and hypothyroidism. Vestergaard *etal*.,(2000) Fukata *et al*.,(1996) suggest that tobacco smoke may increase the risk of hypothyroidism in humans. However, high levels of $T_3$ and $T_4$ as well as decreased TSH levels. Melander (1981), Eden *et al*.,(1984), Hegedus *et al*.,(1985), Ericsson and Lindgarde *et al*.,(1991), Petersen *et al.,* (1991), Fisher *et al*.,(1997) and Kundsen (2002), Vestergaard (2002) opined that association of tobacco smoke and hypothyroidism is controversial and previous results are not homogenous.

Pontikides and Krassas( 2002 ) in their review reports serum thyroxine level and serum $T_3$ level may remained unchanged or elevated due to tobacco dust. Hegetus *et al*.,(1985 and 1992) in two analogous studies found no different in Total $T_3$, $T_4$ and $FT_3$ levels in relation to tobacco smoke.

Exposure to passive smoke was accompanied by a statistically significant increased in $T_3$ and $FT_4$ levels. Whereas, TSH showed a non significant decrease. These findings are line with previous results (Greenspan *et al*.,1986, Fisher *et al*.,1997,

Ericssion *et al*.,1991) and provide support to the notion that the increases in $T_3$ and $T_4$ seen in our study were not attributed to TSH secretion but to the probably short term effect of tobacco dust that cannot be identified with the current data.

Active and passive exposure to tobacco smoke is associated with mild inhibitory effect on the thyroid reflected in higher serum $T_4$ and $T_3$. Soldin *et al*.,(2009) recently documented mild inhibitory effect of secondhand smoke on serum $T_3$ and $T_4$ among women chronically exposed to it and further mean TSH level significantly low. Anti microsomal antibodies, Anti thyroglobulin estimated in beedi rollers also showed fluctuations indicating thyroid dysfunction. Thiocyanate in tobacco smoke may influence the thyroid gland positively or negatively and cause imbalance in the hormone levels. (Bertelsen and Hegedus, 1994).

Beedi rollers handle tobacco flakes and inhale tobacco dust as well as volatile components of tobacco dust as well as volatile components of tobacco which put them at a high risk of many health problems. Women beedi rollers face numerous health problems possibly due to direct inhalation of tobacco flakes and dust (Yasmin *et al*., 2010). Nicotine get absorbed through the skin while rolling tobacco and has been demonstrated in the blood of beedi rollers who do not smoke (Ghosh, 2005). Nicotine is readily absorbed from the respiratory tract, buccal mucosa membrane and skin. While preparing beedi ingredients tobacco is likely to be absorbed through intact skin of the hands and inhaled as fine dust (Sumen *et al*.,2007).

Scientists discovered large amount of nicotine a tobacco specific compound, found in its way into the blood stream of beedi rollers. The average level of cotinine a compound found when nicotine is broken down in the liver, found in the urine and

saliva of beedi rollers who did not consume tobacco, was eight times that found in passive smokers (Khanna,1993).

The constituents of tobacco get absorbed into the body, get bioactivated and result in increased risk of developing ailments for which tobacco consumption is a major risk factor (Mittal *et al*., 2008). Investigations also show that these tobacco processors are exposed to extremely high levels of inspirable tobacco particulars. Considering the high content of Nicotine and other chemicals in beedi tobacco, these workers are at extremely high risk of developing systemic illness (Malson *et al*., 2001). From the above study its found that tobacco use is a critical health concern for women. “Protecting and promoting the health of women is cruciel to health and development not only for the citizen of today but also for those of future generations’’.

**Syed Ali Fathima K.M.** 2012 Thyroid Risk Evaluation in Selected Beedi WorkersPopulation in Tirunelveli Ph.D Thesis, Manonmaniam Sundaranar University,Tirunelveli,Tamil Nadu, India.

**CHAPTER – 5**

# COMPARISON OF HAEMATOLOGICAL PARAMETERS AND THYROID PROFILE OF WOMEN BEEDI ROLLERS AND SMOKERS IN MELAPALAYAM, TIRUNELVELI.

## 5.1 Introduction

Tobacco causes a wide spectrum of fatel and nonfatel diseases. Although tobacco is smoked and used in a variety of ways all over the world, the epidemiological evidence of specific harmful effects of tobacco is based largely on studies who smoke either beedis or cigarettes (Gupta, 2001). Unburnt tobacco, which the beedi rollers come across poses a major risk to women who are involved in it (Gupta, 2001). Similarly few respondents are available with women who harvest tobacco and come in contact with this unburnt tobacco. Karafakioglu *et al*.,(2009) has studied the impact of smokeless tobacco in women who harvest tobacco. However just like tobacco smoke and smokeless tobacco may cause serious ill effects on man. Smoke exposure may affect various metabolic and biological process including hormone biosynthesis, secretion, interfere with thyroid hormone release, binding, transport, storage and clearence, associated with adverse effect on thyroid resulting in changes in circulating hormone concentration. Environmental tobacco smoke or secondhand smoke has been found to cause adverse health effects in people of all ages. Tobacco also contain nicotine which is a highly addictive psychoactive chemical and contributes to a number of threats to the health. Inhalation of tobacco smoke causes several immediate responses within the heart and blood vessels. Smoking is also considered to cause about

2-3 times greater nicotine inhalation. Although nicotine plays a role in acute diseases including pulmonary and cardiac disorders increased risk of cancer has been reported by many authors. Nicotine is an amine composed pyridine and pyrolidine rings (Yildiz, 1998). Nicotine has been shown to affect of wide variety of biological function from gene expression, regulation of hormone, secretion and enzyme activity. The objective of this study was to overview the biological effects of tobacco smoke and health risks associated with that and to compare the same parameters with that of tobacco workers (beedi rollers). The study was also aimed at to assess the risk of thyroid disorders in tobacco workers in comparision with the smoker population.

**5.2 Materials and methods**

The blood parameters like TEC, TLC, platetets, haemoglobin, erythrocyte sedimentation rate, sodium, potassium,chloride, sugar, urea, creatinine, total cholesterol, liver function tests, thyroid tests were analysed following the methods given in chapter 3 in a control group, comprising of 150 volunteers, 150 women beedi rollers and 150 smokers of the age group of 20-50. The inclusion criteria adapted for selecting the control subjects were non smoking, clinically healthy individuals. The inclusion criteria adapted for the smoker population was randomized with mild smokers, moderate smokers and heavy smokers with no serious clinical reports.

**5.3 Results**

In order to assess sevearity of beedi rolling occupation the haematological parameters of women beedi rollers compared with a random group of smokers. On the whole, remarkable correlation between the smokers and beedi rollers was observed. Several parameters analysed with regard to route haematology, Liver function and Thyroid profile infer that beedi rollers are liable to health risks caused by the injurious

effect unburnt tobacco that can be encounted by the smokers who are exposed to tobacco smoke. A comparison of beedi rollers and smokers with that of normal population is explained in this chapter.

### 5.3.1 Total Erythrocyte Count (TEC)

The TEC in the samples of normal persons showed a value closed to that of the upper limit of RBC in the peripheral blood (5 to $6\times10^6/mm^3$). A decrease in TEC count was found in both the smokers and beedi rollers. But a comparison between the two could highlight a lower count in the beedi rollers ranging from $3.76 \pm 0.53 \times10^6/mm^3$, $3.84 \pm 0.58 \times10^6/mm^3$and a slightly higher count, ranging from $4.12 \pm 0.60\ 10^6/\ mm^3$ in smokers irrespective of the age groups. This decrease was found to be statistically highly significant ($P<0.001$) in both smokers and beedi rollers at the age group of 20-30 and in the smokers at the age group of 30-40 and 40-50(Fig: 69).

### 5.3.2 Total Leucocyte Count (TLC)

In the age group 20-30, 30-40 and 40-50 the smokers exhibited a WBC count which was lower than the normal lower limit ($4.3 \times10^3/mm^3$) which was $3.79 \pm 0.49$, $3.89\pm 0.559$ and $3.69 \pm 0.187 \times10^3\ /mm^3$,respectively. On the otherhand, the beedi rollers in the age group of 30-40 and 40-50 showed values $4.27 \pm 0.138$ and $4.57 \pm 0.18 \times10^6/mm^3$ respectively, which was within the normal range but closed to the lower limit when compared to the normal population which possessed $7.61 \pm 0.22\times10^6/\ mm^3$(Fig: 70).

**Fig : 69 TEC Count in different age groups of Non Smokers, Smokers, Beedi Rollers**

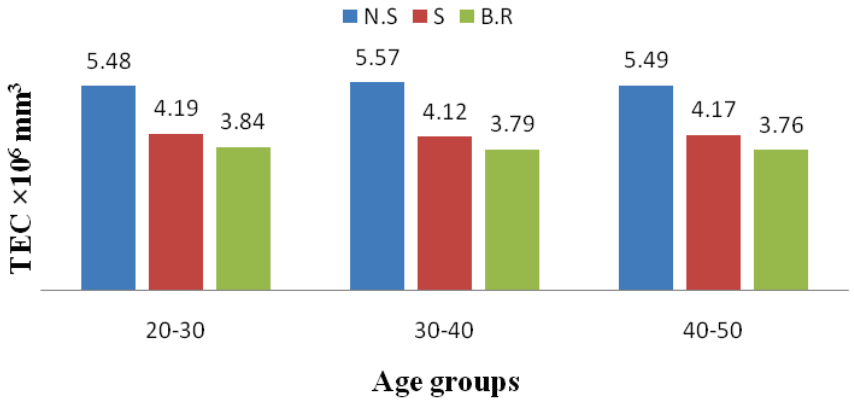
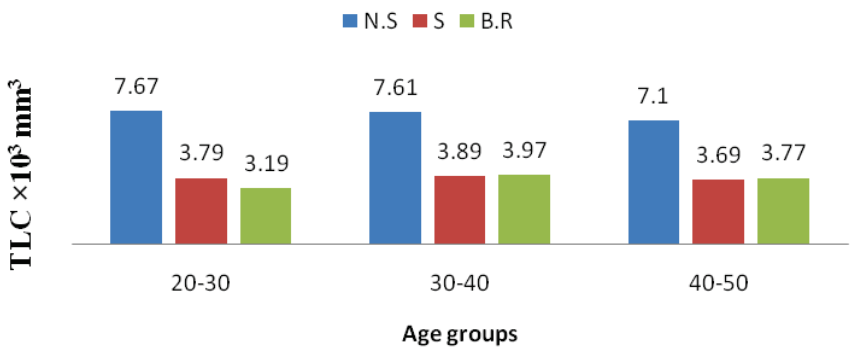



**Fig :70 TLC Count in different age groups of Non Smokers, Smokers, Beedi Rollers**

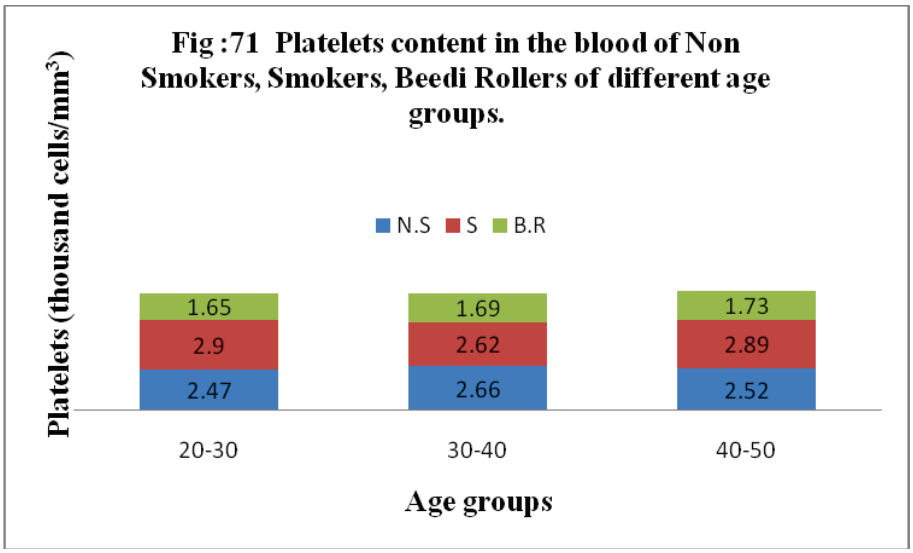


### 5.3.3 Platelet count

The platelet count in the samples of non smokers, smokers and beedi rollers were well within the normal range of normal values. However, a significant ($P< 0.05$) decrease was noted in the beedi rollers especially in the age groups of 20-30 (Fig: 71).

### 5.3.4 Haemoglobin(Hb)

A decreased haemoglobin content could be observed in beedi rollers which was lower even than that of the smokers. While the non smokers had a normal content of haemoglobin (14.4 ± .97 to 14.7 ± 1.0 g/dl). From the table it could be inferred that the beedi rollers were much affected and were susceptible to tobacco dust(Fig: 72).



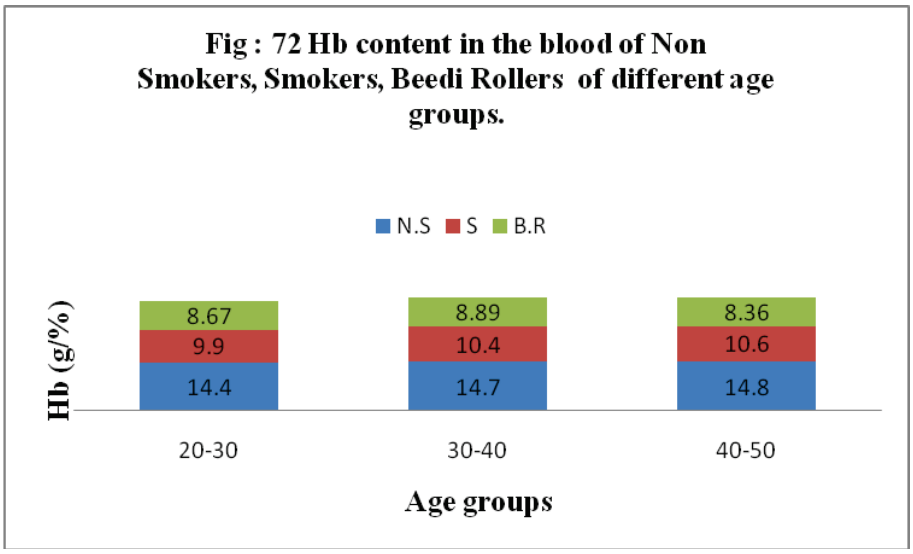



**Fig : 72 Hb content in the blood of Non Smokers, Smokers, Beedi Rollers of different age groups.**

### 5.3.5 Erythrocyte Sedimentation Rate (ESR)

ESR showed much increase in the smokers and even higher among beedi rollers irrespective of the age group. While the non smokers population, considered to be the control exhibited an ESR within the normal range (0-20 mm/hr), smokers and beedi rollers showed significantly high ($P< 0.001$). ESR ranging from $16.6 \pm 1.29$ to $17 \pm 1.31$ mm/hr and $23.8 \pm 2.10$ to $26.1 \pm 2.15$ mm/hr respectively(Fig: 73).

### 5.3.6 Packed Cell Volume(PCV)

A decreasing trend in the PCV was evident an observation of the table. The normal people showed a PCV of 43.4 % to 43.8 %, smokers 35.0 to 35.9 % and beedi rollers 30.4 % to 32.1 %. The decrease was found to be statistically significant ($P < 0.05$). (Fig: 74).

**Fig : 73 ESR content in the blood of Non Smokers, Smokers, Beedi Rollersof different age groups.**

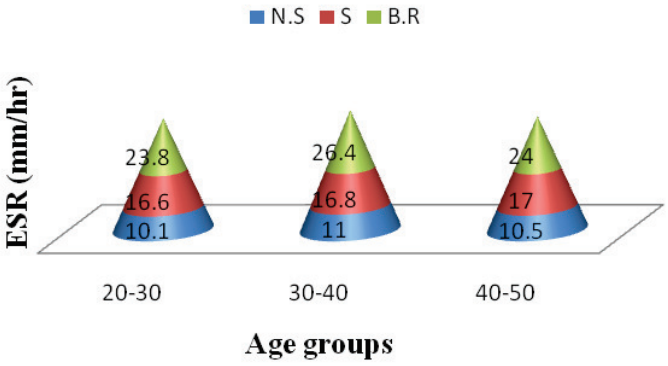
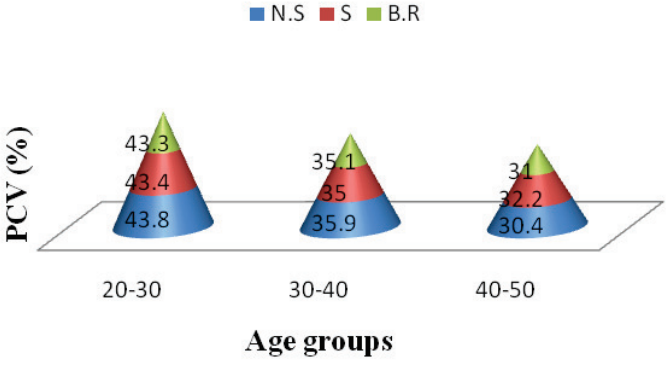



**Fig : 74 PCVcontent in the blood of Non Smokers, Smokers, Beedi Rollers of different age groups**




### 5.3.7 Trace Elements

**Sodium**

The reference value of sodium was 135-145 mmol/l. In the present analysis control population in the age group of 20-30, 30-40 and 40-50 exhibited a value of 146 ± 3.20, 143 ± 4.01 and 144 ± 4.11 mmol/l respectively.Which were closer to the upper normal limit. But the smokers and beedi rollers had a decreased. Sodium level in all age groups. It was 127 ± 3.14 to 128 ± 7.83 mmol/l in the smokers and 128 ± 9.39 to 129 ± 6.44 mmol/l in the case of beedi rollers (Table: 9a).

**Table 9 : Trace Elements in the non smokers, smokers and beedi rollers population of various age groups.**

| PARAMETERS | AGE | | MEAN ± SD | SE | T |
|---|---|---|---|---|---|
| a) SODIUM (135-145 m mol/l) | 20-30 | N.S | 146 ± 3.20 | 1.60 | |
| | | S | 127 ± 3.14 | 2.47 | .988** |
| | | B.R | 129 ± 6.13 | 1.85 | .326* |
| | 30-40 | N.S | 143 ± 4.01 | 2.67 | |
| | | S | 127 ± 4.90 | 1.66 | .577** |
| | | B.R | 129 ± 6.44 | 1.85 | .476* |
| | 40-50 | N.S | 144 ± 4.11 | 1.83 | |
| | | S | 128 ± 7.83 | 1.06 | .814** |
| | | B.R | 128 ± 9.39 | 2.15 | .832* |
| b) POTASSIUM (3.5-5.0 m mol/l) | 20-30 | N.S | 3.56 ± 0.32 | 0.04 | |
| | | S | 3.15 ± 0.15 | 0.02 | 8.21** |
| | | B.R | 3.57 ± 0.43 | 0.06 | 7.89 |
| | 30-40 | N.S | 3.64 ± 0.34 | 0.04 | |
| | | S | 3.22 ± 0.27 | 0.03 | 7.28* |
| | | B.R | 4.03 ± 0.78 | 0.42 | 8.98 |
| | 40-50 | N.S | 3.56 ± 0.34 | 0.05 | |
| | | S | 3.22 ± 0.24 | 0.03 | 5.50** |
| | | B.R | 5.13 ± 0.72 | 0.77 | 4.58 |
| c) CHLORIDE (98-108 m mol/l) | 20-30 | N.S | 90.4 ± 7.76 | 1.03 | |
| | | S | 93.7 ± 2.48 | 1.37 | 2.65** |
| | | B.R | 95.4 ± 3.67 | 1.50 | 3.28 |
| | 30-40 | N.S | 89.9 ± 7.81 | 1.03 | |
| | | S | 93.6 ± 2.59 | 1.35 | 3.31** |
| | | B.R | 96.0 ± 4.46 | 1.50 | 4.31 |
| | 40-50 | N.S | 90.3 ± 7.31 | 0.20 | |
| | | S | 93.9 ± 2.72 | 1.37 | 3.35** |
| | | B.R | 94.8 ± 3.08 | 1.75 | 4.23 |

**** - highly significant. * Significant**

### 5.3.8 Potassium

The smokers tend to be affected much by possessing low potassium level compared to the control and beedi rollers population whose potassium level was found to be within the reference value (3.5 -5.0 mmol/l) (Table: 9b).

### 5.3.9 Chloride

The chloride level did not seem to affect beedi rollers much, since it ranges within the normal limits. An analysis of chloride level indicate a higher range in the case of beedi rollers ranging from 94.8 ± 3.08 to 96.0 ± 4.46 mmol/l (Table: 9c).

## 5.4 Sugar

The random blood sugar level was found to be very high among the smokers, which was 181 ± 39.8, 176 ± 36.3 and 162 ± 43.0 mg/dl in 20-30, 30-40 and 40-50 age groups respectively. The beedi rollers also exhibited a slightly higher blood glucose level the reference value of 80-120 mg/dl. But it was lower than that of the smoker in the age group of 20-30 a sugar level 138 ± 19.7 mg/dl and in 30-40 it was 132 ± 22.8 mg/dl and 40-50 it was 133 ± 23.8 mg/dl (Fig: 75).

### 5.4.1 Cholesterol

Cholesterol level did not showed any correlation among the smokers and rollers population and even the control population, Since it could also governed by other factors. A clear information couldnot be arrived as the smokers in the age group of 20-30 showed a low cholesterol content of 137 ± 13.6 mg % and 256 ± 34.0 mg % in the age group 40-50. However a uniformely high cholesterol level was observed among the beedi rollers in all age groups(Fig: 76).

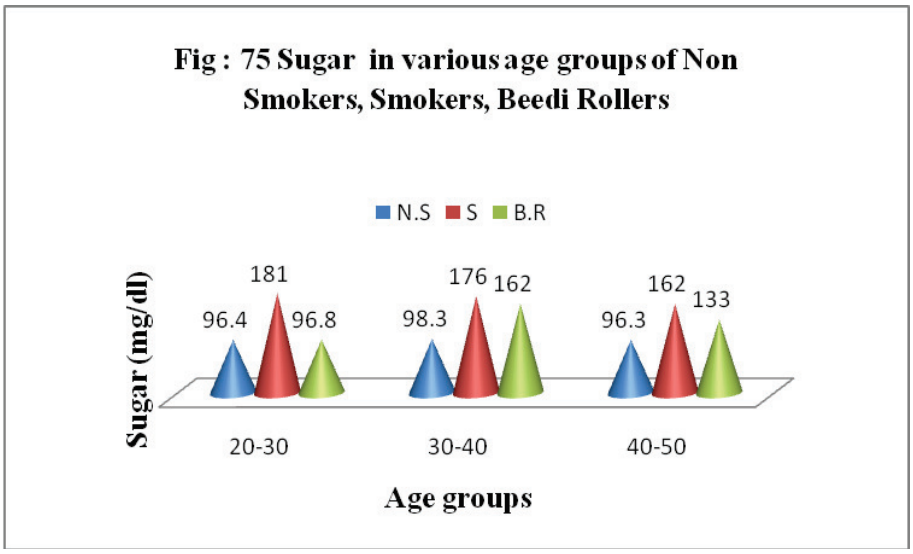



**Fig : 75 Sugar in various age groups of Non Smokers, Smokers, Beedi Rollers**

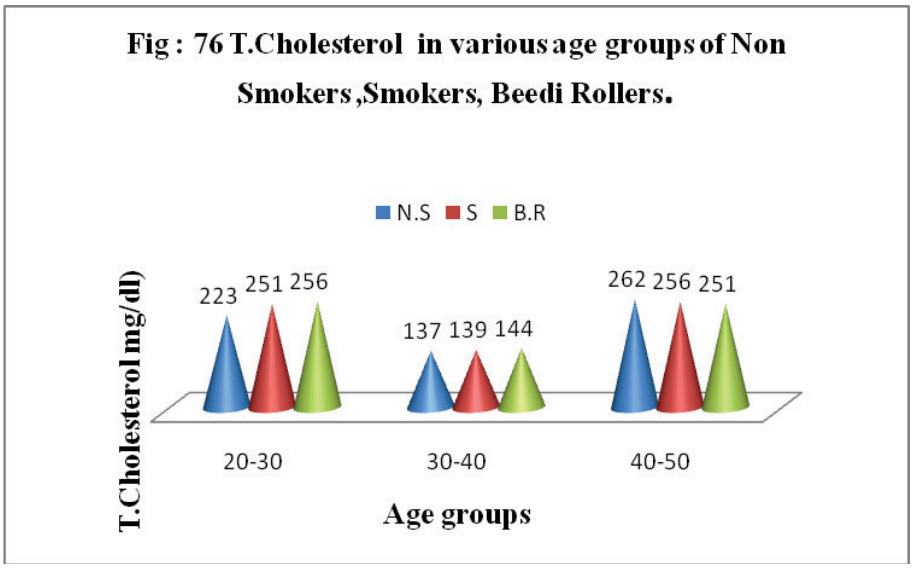



**Fig : 76 T.Cholesterol in various age groups of Non Smokers ,Smokers, Beedi Rollers.**

### 5.4.2 Urea

The urea level of both smokers and beedi rollers were found to be within the normal limit (7-25 mg/dl) but higher than that of the normal non smoker population. Compared to smokers in the age group of 30-40 and 40-50 the beedi rollers possessed a slightly higher urea level(Fig: 77).

### 5.4.3 Creatinine

The creatinine level of beedi rollers was found to exceed the normal upper limit of 1.4 mg/dl it was 1.56 ± 0.24, 1.31 ± 0.23, and 1.63 ± 0.21 mg/dl in the age group of 20-30, 30-40 and 40-50 respectively. However, these data were found to be higher than compared with the normal and smokers population(Fig: 78)

**Fig : 77 Urea in various age groups of Non Smokers, Smokers, Beedi Rollers**

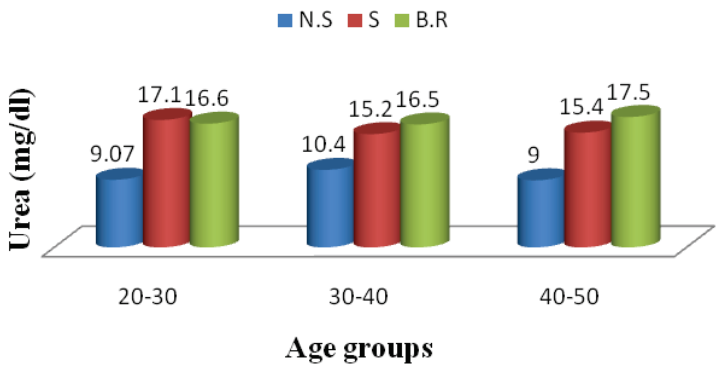
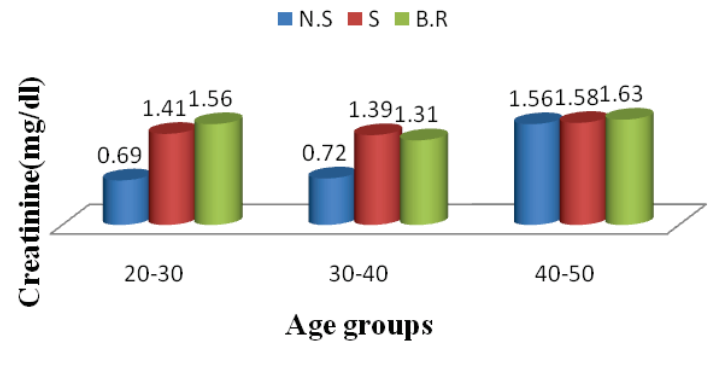



**Fig : 78 Creatinine in various age groups of Non Smokers ,Smokers, Beedi Rollers.**




### 5.4.4 T.Bilurubin

Total Bilurubin did not elicit much change among the beedi rollers compared to the normal. But the smokers had a slightly higher level of total bilurubin ranging from 1.40 ± 0.33 mg/dl to 1.44 ± 0.30 mg/dl(Table: 10a).

**Table 10 : Total Bilurubin, ALP, GGT of a Liver function test in the non smokers, smokers and beedi rollers population of various age groups.**

| PARAMETERS | AGE | | MEAN ± SD | SE | t |
|---|---|---|---|---|---|
| a) TOTAL BILURUBIN (1.0 mg/dl) | 20-30 | N.S | 1.28 ± 0.37 | .046 | |
| | | S | 1.44 ± 0.30 | .049 | 2.21** |
| | | B.R | 1.28 ± 0.85 | .052 | 3.28** |
| | 30-40 | N.S | 1.02 ± 0.37 | .045 | |
| | | S | 1.40 ± 0.33 | .047 | 1.87* |
| | | B.R | 1.29 ± 0.04 | .045 | 2.43** |
| | 40-50 | N.S | 1.27 ± 0.39 | .652 | |
| | | S | 1.43 ± 0.28 | .038 | 2.22* |
| | | B.R | 1.20 ± 0.82 | .087 | 3.21 |
| b) ALP (60-170U/dl) | 20-30 | N.S | 124 ± 12.8 | 2.51 | |
| | | S | 75.0 ± 18.2 | 4.52 | 10.0** |
| | | B.R | 132 ± 14.4 | 2.88 | 9.87 |
| | 30-40 | N.S | 123 ± 29.6 | 2.53 | |
| | | S | 76.5 ± 19.1 | 5.28 | 8.18* |
| | | B.R | 134 ± 11.3 | 6.10 | 12.3 |
| | 40-50 | N.S | 116 ± 18.4 | 3.32 | |
| | | S | 82.4 ± 20.2 | 4.76 | 5.28** |
| | | B.R | 134 ± 14.3 | 5.48 | 10.9 |
| c) GGT (15-45 U/dl) | 20-30 | N.S | 27.0 ± 6.70 | 5.89 | |
| | | S | 50.0 ± 9.53 | 1.45 | 14.1** |
| | | B.R | 33.38 ± 5.09 | 1.01 | 8.79* |
| | 30-40 | N.S | 26.9 ± 6.68 | 4.88 | |
| | | S | 50.1 ± 9.94 | 1.36 | 14.4* |
| | | B.R | 33.65 ± 4.87 | 3.70 | 12.3* |
| | | N.S | 28.4 ± 7.56 | 1.24 | |
| | | S | 49.12 ± 9.70 | 1.32 | 10.8 |
| | | B.R | 34.00 ± 5.69 | 4.54 | 11.9* |

**** - highly significant. * Significant**

### 5.4.5 Alkaline Phosphate (ALP)

The level of alkaline phosphate was within the reference values (60-170 μ/d L). But considering the three population the beedi rollers possessed high ALP level (132 ± 4.43 and 134 ± 11.3μ/dl) compared to control population who neither smoke nor beedi rollers 116 ± 18.4 to 124 ± 12.8 μ/dl). On the contrary smokers contained low ALP level (75.0 ± 18.2 to 82.4 ± 20.2 μ/dl) which was highly significant ($p < 0.001$) among the age group of 20-30 and 40-50 (Table: 10b).

### 5.4.6 Gamma Glutamitate Transaminase (GGT)

The normal value of GGT is 15-45 U/dl. The control population showed a GGT which was within the normal limit in all age groups. It was as low as 27 ± 6.70, 26.9 ± 6.68 and 28.4 ± 7.56 U/dl in 20-30, 30-40 and 40-50 age groups respectively. Beedi rollers exhibited slightly higher level within the normal limit when compared to that of normal people. It was 33.38 ± 5.09, 33.65 ± 4.87 and 34.0 ± 5.69 U/dl in age groups respectively. Smokers showed still higher level to GGT well about the upper normal level (45 U/dl). The GGT level in 20-30, 30-40 and 40-50 age groups was 50.0 ± 9.53, 50.1 ±9.94, 50.1 ± 9.94 and 49.12 ± 9.70 U/dl respectively (Table: 10c).

### 5.4.7 Serum Glutamic Pyruiv Transaminase (SGPT)

SGOT also enumerated high levels in beedi rollers than normal range (5-40 IU/l). SGOT was 46.73 ± 7.79, 44.51 ± 12.6 and 43.57 ± 6.12 IU/l. Compared to normal population containing SGOT level of 31.2 ± 7.44, 30.2 ± 7.60 and 30.8 ± 8.24 and a smoker population containing SGOT level of 39.0 ± 2.67, 38.8 ± 3.26 and 38.7 ± 2.87 IU/l in 20-30, 30-40 and 40-50 age group respectively (Table: 11a).

**Table 11: SGOT and SGPT levels of non smokers, smokers and beedi rollers populations in different age groups.**

| PARAMETERS | AGE | | MEAN ±SD | SE | t |
|---|---|---|---|---|---|
| a) SGOT (5-40 IU/l) | 20-30 | N.S | 31.2 ± 7.44 | 3.99 | |
| | | S | 39.0 ± 2.67 | 3.40 | 6.55** |
| | | B.R | 46.73 ± 7.79 | 1.08 | 7.89* |
| | 30-40 | N.S | 30.2 ± 7.60 | 1.90 | |
| | | S | 38.8 ± 3.26 | 2.44 | 7.64** |
| | | B.R | 44.51 ± 12.6 | 1.42 | 8.98 |
| | 40-50 | N.S | 30.8 ± 8.24 | 1.35 | |
| | | S | 38.7 ± 2.87 | 4.39 | 6.54** |
| | | B.R | 43.57 ± 6.12 | 1.40 | 8.71 |
| b) SGPT (5-40 IU/l) | 20-30 | N.S | 28.5 ± 6.04 | 3.80 | |
| | | S | 38.8 ± 3.11 | 2.47 | 10.1** |
| | | B.R | 47.80 ± 6.64 | 2.92 | 9.87** |
| | 30-40 | N.S | 30.5 ± 5.40 | 4.71 | |
| | | S | 38.7 ± 3.54 | 2.48 | 9.32** |
| | | B.R | 46.09 ± 8.80 | 5.99 | 11.3 |
| | 40-50 | N.S | 31.0 ± 4.32 | 2.71 | |
| | | S | 38.8 ± 2.58 | 4.35 | 10.8** |
| | | B.R | 47.89 ± 8.02 | 1.83 | 12.3 |

**** highly significant. * Significant**

### 5.4.8 Serum Glutamic Pyruiv Transaminase (SGPT)

Elevated levels of SGPT was evident among the beedi rollers. They had a statistically highly significant ($P < 0.001$) increase ranging from 46.09 ± 8.80 to 47.89 ± 8.02 IU/l which was higher than the prescribed range of 5-40 IU/l. Smokers on the

other hand were very closer to the normal upper limit of 40 IU/l. They exhibited a range of 38.7 ± 3.54 to 38.8 ± 2.58 IU/l (Table: 11b).

### 5.4.9 T.Protein

Elevated levels of Total protein could be observed in Beedi rollers irrespective of the age. When compared to low non smokers and smokers. In beedi rollers the total protein ranged from 9.37 ± 1.39 to 9.97 ± 1.83 g/dl, which was higher than the normal upper limit of the reference value (8 g/dl). A quite contradictory data was observed among the smokers (5.38 ± 1.72 to 5.61 ± 187 g/dl) which was lower than the normal lower limit of the reference value (6 g/dl ) (Table: 12a).

### 5.5 Albumin

In the age group of 20 to 30 Albumin level was (3.47 ± 1.22 g/dl) lower in beedi rollers than the normal population (5.43 ± 1.578 g/dl). Smokers also exhibited a decrease but it was higher than that of beedi rollers. A similar trend was observed in the age groups of 30-40. But in the age group of 40-50, the beedi rollers had a higher level of albumin than the smokers (Table: 12b).

### 5.5.1 Globulin

An abnormally high level of globulin was observed among beedi rollers in all age groups. The normal reference value of globulin was found to be 1.5-5.0 g/dl. While the smokers and control groups had low normal levels of globulin, the beedi rollers very high level even higher than the lower normal limit of 5.0 g/dl. It was 3.50 ± 3.90, and 3.58 ± 3.91 g/dl in 20-30, 30-40 and 40-50 age groups respectively (Table: 12c).

**Table 12 : Total Protein, Albumin and Globulin Content in various age groups of non smokers, smokers and beedi rollers.**

| PARAMETERS | AGE | | MEAN ±SD | SE | t |
|---|---|---|---|---|---|
| a) T. PROTEIN (6-8 g/dl) | 20-30 | N.S | 6.76 ± 1.91 | 0.12 | |
| | | S | 5.55 ± 0.68 | 0.10 | 7.18* |
| | | B.R | 06.97 ± 1.83 | 0.30 | 8.12 |
| | 30-40 | N.S | 6.44 ± 1.74 | 0.09 | |
| | | S | 5.61 ± 1.87 | 0.12 | 5.39** |
| | | B.R | 9.87 ± 2.10 | 0.53 | 7.12* |
| | 40-50 | N.S | 6.03 ± 1.78 | 0.12 | |
| | | S | 5.38 ± 1.72 | 0.09 | 9.44** |
| | | B.R | 7.2 ± 1.39 | 0.34 | 12.3 |
| b) ALBUMIN (3.5-5.0g/dl) | 20-30 | N.S | 5.43 ± 1.57 | 0.07 | |
| | | S | 3.82 ± 1.64 | 0.09 | 1.31 |
| | | B.R | 3.47 ± 1.22 | 0.16 | 1.14** |
| | 30-40 | N.S | 5.41 ± 1.48 | 0.06 | |
| | | S | 3.94 ± 1.60 | 0.08 | 1.40** |
| | | B.R | 3.84 ± 1.16 | 0.13 | 1.82 |
| | 40-50 | N.S | 5.27 ± 1.70 | 0.11 | |
| | | S | 3.76 ± 2.50 | 0.06 | 1.18** |
| | | B.R | 4.23 ± 2.03 | 0.46 | 3.24 |
| c) GLOBULIN (1.5-5.0 g/dl) | 20-30 | N.S | 1.32 ± 1.81 | 0.10 | |
| | | S | 1.73 ± 2.99 | 0.15 | 2.24 |
| | | B.R | 3.50 ± 3.90 | 0.54 | 9.80 |
| | 30-40 | N.S | 1.03 ± 1.73 | 0.09 | |
| | | S | 1.66 ± 1.01 | 0.13 | 3.7** |
| | | B.R | 3.02 ± 3.37 | 0.38 | 2.78 |
| | 40-50 | N.S | 1.62 ± 1.95 | 0.15 | |
| | | S | 1.62 ± 2.79 | 0.10 | 4.8 |
| | | B.R | 3.58 ± 3.91 | 0.89 | 3.2 |

**** highly significant. * Significant**

**Table 13: Thyroid Profile in non smokers, smokers and beedi rollers showing $T_3$, $T_4$ and TSH levels.**

| PARAMETERS | AGE | | MEAN ± SD | SE | t |
|---|---|---|---|---|---|
| a) $T_3$ (160-200 ng/dl) | 20-30 | N.S | 172.1 ± 15.4 | 2.06 | |
| | | S | 195.1 ± 12.4 | 1.89 | 7.97** |
| | | B.R | 68.80 ± 27.4 | 3.80 | 3.54** |
| | 30-40 | N.S | 165.2 ± 10.8 | 1.43 | |
| | | S | 196.3 ± 13.2 | 1.81 | 13.5* |
| | | B.R | 62.20 ± 19.9 | 2.24 | 10.2** |
| | 40-50 | N.S | 198.3 ± 10.2 | 1.69 | |
| | | S | 165.4 ± 7.71 | 1.05 | -17.3 |
| | | B.R | 57.52 ± 8.43 | 2.85 | -9.7* |
| b) $T_4$ (4.5-12 µg/dl ) | 20-30 | N.S | 5.11 ± 2.73 | 0.36 | |
| | | S | 9.21 ± 1.87 | 0.28 | 8.43** |
| | | B.R | 4.43 ± 1.75 | 0.24 | 12.4* |
| | 30-40 | N.S | 4.28 ± 1.64 | 0.21 | |
| | | S | 9.61 ± 1.65 | 0.22 | 16.9 |
| | | B.R | 5.75 ± 5.47 | 0.61 | 11.1* |
| | 40-50 | N.S | 8.88 ± 2.11 | 0.34 | |
| | | S | 4.01 ± 6.90 | 0.12 | -15.1** |
| | | B.R | 4.88 ± 1.89 | 0.43 | -18.8** |
| c) TSH (0.3-5.5 µ IU/ml) | 20-30 | N.S | 5.53 ± 1.89 | 0.25 | |
| | | S | 3.59 ± 5.25 | 0.80 | -2.55 |
| | | B.R | 4.44 ± 3.95 | 0.13 | -3.21* |
| | 30-40 | N.S | 5.93 ± 1.29 | 0.17 | |
| | | S | 2.81 ± 1.01 | 0.13 | -13.9 |
| | | B.R | 4.39 ± 1.06 | 0.11 | -9.87** |
| | 40-50 | N.S | 2.70 ± 1.76 | 0.12 | |
| | | S | 6.13 ± 1.18 | 0.16 | 15.47** |
| | | B.R | 4.55 ± 1.18 | 0.27 | 11.9* |

**** highly significant. * Significant**

### 5.5.2Total Triidothyronine($T_3$)

Smoker population exhibited normal $T_3$ levels (160-200 mg/dl), Beedi rollers showed a decline. It was as low as 68.80 ± 27.4 mg/dl in the age groups 20-30, 62.20 ± 19.9 mg/dl 30-40 and 57.52 ± 8.43 mg/dl. These findings were statistically higher significant ($P < 0.001$) (Table: 13a).

### 5.5.3 Total thyroxine($T_4$)

The $T_4$ in normal subjects ranged from 4.5-12 mg /dl. The results of $T_4$varied in different age groups. In the age groups of 20-30 the beedi rollers exhibited a low level of $T_4$ (4.43 ±1.75 ng/dl). But in the age group of 30-40 and 40-50 it was 5.75 ± 2.47 and 4.88 ± 1.89 ng/dl. Smokers possessed high $T_4$ level especially in 20-30 and 30-40 age groups, 9.21 ± 1.87 and 9.61 ± 1.65 mg/dl respectively (Table: 13b).

### 5.5.4 Thyroid Stimulating Hormone (TSH)

There was not much variations in the level of TSH (Table: 13c).

### 5.5.5 Free Triiodo Thyronine($FT_3$)

Free $T_3$ level exhibited a range of 1.4 ± 0.54 pg/ml. Which was highly significant ($P< 0.001$).When compared to control group who exhibited 2.33 ± 0.8 µg/ml in the age group of 20-30. But in the age group of 30-40 and 40-50 significantly ($P< 0.05$) high decrease was observed in the smoker population (1.10 ± 0.54 µg/ml). Beedi rollers in the age groups 30-40 and 40-50 also showed a decrease which was not significant as that of smokers however the amount of Free $T_3$ was considerably low when compared to the normal lower limit of the reference value (1.7-4.2 µg/ml) (Table: 14a).

**Table 14: $FT_3$, $FT_4$, AMA and ATG levels of thyroid profile in non smokers, smokers and beedi rollers population.**

| PARAMETERS | AGE | | MEAN ± SD | SE | t |
|---|---|---|---|---|---|
| a) $FT_3$ (1.7-4.2 pg/ml) | 20-30 | N.S | 2.33 ± .81 | 0.12 | |
| | | S | 1.58 ± .80 | 0.10 | .516 |
| | | B.R | 1.42 ± .54 | 0.07 | .787** |
| | 30-40 | N.S | 2.32 ± .97 | 0.13 | |
| | | S | 1.10 ± .54 | 0.13 | 8.15** |
| | | B.R | 1.48 ± .55 | 0.06 | 9.89 |
| | 40-50 | N.S | 2.50 ± .76 | 0.12 | |
| | | S | 1.10 ± .47 | 0.06 | .07** |
| | | B.R | 1.33 ± .52 | 0.12 | -.089 |
| b) $FT_4$ (0.7-1.8 ng/dl) | 20-30 | N.S | 1.27 ± 0.41 | 0.06 | |
| | | S | .93 ± 0.39 | 0.05 | .04 |
| | | B.R | 0.69 ± 0.21 | 0.02 | .047 |
| | 30-40 | N.S | 1.87 ± 0.32 | 0.04 | |
| | | S | 1.24 ± 0.39 | 0.05 | .052** |
| | | B.R | 1.34 ± 0.32 | 0.59 | .076** |
| | 40-50 | N.S | 1.82 ± 0.70 | 0.44 | |
| | | S | 0.77 ± 0.78 | 0.03 | .028** |
| | | B.R | 0.66 ± 0.16 | 0.036 | .067* |
| c) AMA (36-49 U/ml) | 20-30 | N.S | 45.8 ± 4.69 | 0.62 | |
| | | S | 36.6 ± 4.14 | 0.63 | .53 |
| | | B.R | 45.15 ± 6.95 | 0.96 | .89 |
| | 30-40 | N.S | 47.5 ± 2.78 | 0.36 | |
| | | S | 36.8 ± 3.52 | 0.48 | .17* |
| | | B.R | 48.66 ± 27.6 | 0.31 | .14 |
| | 40-50 | N.S | 47.5 ± 3.01 | 0.41 | |
| | | S | 37.5 ± 4.05 | 0.66 | .13** |
| | | B.R | 45.47 ± 1.21 | 0.27 | .11 |
| d) ATG 225-325 (IU/ml) | 20-30 | N.S | 288 ± 28.3 | 3.78 | |
| | | S | 260 ± 29.9 | 4.59 | 4.85 |
| | | B.R | 287 ± 20.4 | 2.83 | 7.78 |
| | 30-40 | N.S | 280 ± 16.7 | 2.21 | |
| | | S | 261 ± 31.4 | 4.31 | 4.03* |
| | | B.R | 281 ± 15.4 | 1.73 | 7.54 |
| | 40-50 | N.S | 285 ± 29.6 | 4.87 | |
| | | S | 261 ± 19.6 | 1.30 | 7.93** |
| | | B.R | 277 ± 11.8 | 2.72 | 6.89 |

** highly significant. * Significant

### 5.5.6 Free Thyroxine ($FT_4$)

FT4 level of normal sample was 1.27 ± 0.41, 1.87 ± 0.32 and 1.82 ± 0.70 (ng/d l) in 20-30, 30-40 and 40-50 age group respectively. Both beedi rollers and smokers showed a decrease in $FT_4$ level. But significant differences could be observed at the age group of 20-30 and 40-50. It was 0.93 ± 0.39 ng/dl in smokers and 0.69 ± 0.21 ng/dl in beedi rollers in 20-30 age groups and it was 0.77 ± 0.78 ng/dl and 0.66 ± 0.16 ng/dl in the smokers and beedi rollers respectively at the age group of 40-50(Table: 14b).

### 5.5.7 Anti Microsomal Antibody (AMA)

Antimicrosomal Antibodies did not elicit any remarkable variation in the beedi roller population. They were found to be within the normal range 36-49 U/ml. However, the smokers exhibited a significant decrease ($P < 0.05$) within the normal range in the age group 30-40 (36.8 ± 3.5 U/ml). A highly significant decrease ($P < 0.001$) was noted in the age age group of 40-50 (37.5 ± 4.05 U/ml) compared to the normal subjects beedi rollers also showed decrease levels of AMA which was not significant (Table: 14c).

### 5.5.8 Anti Thyro Globulin (ATG)

Anti Thyro Globulin Antibody hormone assay indicate the level of ATG did not showed any predictable change since, they were found to be within the reference value

of 225-325 IU/ml. A comparision of non smokers and smokers enumerated a decrease in all age groups. Beedi rollers exhibited decrease at the age group of 40-50 but it was higher then that of smokers (Table: 14d).

## SUMMARY

The findings of the present study reveal that women beedi workers suffer from poor socio economic status even though this occupation provides a crucial source of employment and income Poverty, debt and lack of worker status forces people into beedi rolling and thus it in a compulsion rather than a choice. The exploration of the data reveal that families are caught in a vicious cycle of poverty, exploitation and helplessness by this highly unorganised home-based industry. Women and children are major working force of the beedi industry and are thus deprived of education and good health. There is a high incidence of musculoskeletal pains, joint pains, back pains, head aches and respiratory diseases. Apart from these occupational illness reported by them, they have physiological disturbances which was evident through haematological analysis of their blood. Routine analysis of the blood revealed that the TEC and TLC levels, Hb content PCV decreased in beedi rollers and smokers compared to non beedi rollers who are non smokers too. SGOT and SGPT increases beedi rollers and smokers when compared with normal humans. Thyroid profile of women beedi rollers show decreased $T_3$ and $T_4$levels when compared with non beedi rollers. But the smokers had high $T_3$ and $T_4$levels. Similarly contrary results were found with regard to TSH in beedi rollers and smokers. $FT_3$ and $FT_4$ of beedi rollers and smokers showed a similar decreasing trend compared to non beedi workers/ smokers. Anti microsomal antibodies and anti thyroglobulin exhibited marginal decrease in beedi rollers whereas smokers showed much decrease. Thus it is evident that the unburnt tobacco dust may have a serious impact on thyroid gland and its products as reported in smokers. Like smokers who inhale tobacco smoke women beedi rollers who are exposed to raw tobacco dust for prolonged hours have the risk of developing hypothyroidism and affect human generation.

**RECOMMENDATION**

- Periodic Health checkup should be made compulsory through agencies.
- Create awareness among the workers regarding causes of how they are being affected physically how could they are able to overcome the problems.
- Masks should be works by the beedi rollers during work to minimize inhalation of tobacco dust.
- Awareness is to be created among the beedi workers regarding the health care facilities available in the government hospitals, which is free of cost.
- To ensure education for workers children and children should be discouraged in taking up beedi rolling.
- The government should initiate suitable action to protect the benefits and provide healthy service conditions for the women beedi workers. The system of middleman should be abolished as it leads to exploitation of the workers. All the companies must be registered and identity cards must be issued to all the workers working both in organized and un-organized sector.
- The health department should initiate measures to start special dispensaries to treat problems arising out of hazards in the industry.
- Ensure the availability of health care centers and proper sanctation facilities by the industries. Portion of profits gained from the sale of

tobacco products should be evenly distributed among these involved in the work education to bring about a lasting impact on the overall health condition of the tobacco workers. Since the magnitude of the health problems is seen more among non passbook holders, efforts should be made to provide passbook to the non passbook holders.

- The labour department should take inititative to provide medical facilities to the most affected beedi rolling sector. Creates and child centers should be established to look after female workers children so that the children are not exposed to the hazards.

- Along with the triple benefit scheme like provident fund, pension and gratuity which is recommended, insurance schemes may be promoted to the beedi companies.

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# QUESTIONNAIRE

1) Age group

a) 20-30 b) 30-40 c) 40-50 d) 50-60

2) Educational qualification

a) Iliterate b) Primary school c) Middle school d) High school

3) Marital status

a) Married b) Single c) Separated d) Widow

4) Type of family

a) Joint family b) Single c) Separated d) Widow

5) Number of family member

a) Below 4 b) 4 to 5 c) 6 to 7 d) Above 7

6) Number of Children

a) Below 4 b) 4 to 5 c) 6 to 7 d) Above 7

7) What is your income per month?

a) Below Rs 1000 b) Rs 1001 to 2000

c) Rs 2001 to300 d) Above Rs 3000.

8) What is the monthly expenditure of the family?

a) Below Rs 1000 b) Rs 2000 c) Rs 2001-3000 d) Above 3000.

9) Where do your save money?

a) S.H.G b) Post office c) Bank d) No Saving

10) Do you have depits?

a) Yes b) No

11) If yes what is the amount of depits you have ?

a) Below Rs 1000 b) Rs 1001-5000

c) Rs 5001-10,000 d) Above 10,000.

12) If yes what is the source of depits you have?

a) S.H.G b) Society c) Bank d) Others.

13) For what reason did you borrow money?

a) Marriage b) Education c) Health d) Others.

14) How did you repay the debts?

a) Daily b) Weekly c) Monthly d) Others.

15) Are you satisfied with your income?

a) Satisfied b) Dissatisfied

16) Are you satisfied with the economic growth of your family?

a) A little satisfied b) Satisfied c) Dissatisfied

17) How many hours do you work per day ?

a) 1-3 hrs b) 4-6 hrs c) 7-9 hrs d) Above 9 hrs.

18) How many days do you get work in a month ?

a) 1-10 days b) 11-20 days c) 21-30 days.

19) What time do you prefer to go for work?

a) Morning b) Evening c) Any time.

20) How many beedis do you roll per day?

a) 500-1000 b) 800-1000 c) 200.

21) Where do you get raw materials?

a) Local b) Outside

22) How many years are experience you have in beedi rolling?

a) Less than 5 years b) 5 to 10 years

c) 11 to 20 years d) Above 20 years.

23) Are your children involved in this work ?

a) Yes b) No.

24) If yes, how are they involved?

a) Part time b) Full time c) During vocation time.

25) If no, why are they not involved?

a) Small children b) Children going to school c) No interest.

26) What type of residence you have?

a) Own b) Rent c) Lease

27) How long you have been staying in this house?

a) Below 5 years b) 5-10 years c) 11-15 years d)Above 15 years.

28) What type of marriage you have?

a) In blood relation b) Others.

29) What was the age when got married?

a) Below 15 b) 15-17 c)18-20 d) Above 20.

30) What is your normal mode of entertainment?

a) T.V b) Radio c) Others.

31) Did your suffer from any following illness?

a) T.B b) Skin disease

c) Asthma d) Other occupational diseases.

32) Did your suffer from any following diseases?

a) Neck pain b) Shoulder pain

c) Finger Numbness d) Gastro intestinal diseases.

33) Where do you go for treatment?

a) PHC b) Private clinic

c) G.H d) Traditional medicine.

34) How often do you go for medicinal checkup?

a) Monthly b) Once in 3 month

c) Once in 6 month d) During the time of sickness.

35) How is your working environment?

a) Good b) Very good c) Bad d) Very bad.

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# Immunostimulant Effect of Vitamin-A in *Channa Punctatus* Challenged with *Aeromonas Hydrophila*: Haematological Evaluation

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**ABSTRACT**

The present study was carried out to evaluate the immunostimulant potential of vitamin A in fish. *Channa punctatus* was chosen for the present study and divided into 3 groups. 'A' group was uninfected, both 'B' & 'C' groups were infected with *A.hydrophila* and only 'C' group was injected with 0.025ml of vitamin A. Haematological parameters were analysed on 1, 2, 3, 4, 7, 14, 21 & 26$^{th}$ day. The total erythrocyte counts and total leucocyte counts exhibited marked increase in vitamin A administered ('C' group) fishes when compared to 'A' & 'B' groups. In differential leucocyte counts the lymphocytes and neutrophils exhibited an increasing trend in both 'B' & 'C' groups but it was significantly higher in vitamin A administered fishes. Further monocyte counts were significantly higher in 'C' group fishes and a gradual declining trend was observed. When the serum of "C' group fishes were titrated with the pathogen in 96 well microtitre plate exhibited agglutination which further supports the increase in lymphocyte counts as a specific immune response. Thus vitamin A can be administered to improve the general resistance in fishes.

## INTRODUCTION

Fish in intensive culture are continuously affected by environmental fluctuations and management practices such as handling, crowding, transporting, drug treatments, under nourishment, fluctuating temperatures and poor water quality. The high susceptibility of fish to stress and the rapid spread of diseases in water have forced aqua-culturists to concentrate their efforts on maintaining their fish in good health in order to achieve sustainable economic performances. Among different practices, administration of immunostimulants is one, which along with good management practices will ensure high survival rates, improve the health status and enhance growth in intensive farming systems. Immunostimulants are a group of biological or synthetic compounds that enhance the humoral and cellular immune response both by specific and non-specific way thereby reducing the risk of diseases (Tewary and Patra 2004). Immunostimulants includes many antioxidants, vitamins, carotenoids and other feed additives.

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Immunostimulants also stimulate the natural killer cells, complement, lysozyme and antibody responses of fish (Sakai 1999, Tewary and Patra 2004). Vitamin A is fat soluble and essential in maintaining epithelial cells, preventing atrophy and keratinization of epithelial cells and also promotes growth of new cells and aids in maintaining resistance to infection.

*Aeromonas hydrophila* is a ubiquitous gram negative rod shaped opportunistic pathogen causing 'Haemorrhagic Septicemia' in fish during stress, overcrowding, transportation, poor level of nutrition and poor water quality. Blood forms an integrated and inevitable part in all immune system and the changes in these parameters can be correlated to the response of the organism to the changing environmental condition and therefore can be used to screen the health status of the fish submitted to the exposed toxicant (Pandey and Pandey 2001). Administration of vitamins as immunostimulants to improve the general health of fish was reported by many workers. Administration of immunostimulant through fish feed will be easier but it has its own drawbacks, for example absorption of the required amount depends on the amount of immunostimulant incorporated in feed, feed consumed and absorbed.

Thus in the present study the immune-stimulant potential of vitamin A was assessed by injecting vitamin A to *Channa punctatus* infected with *Aeromonas hydrophila*.

## MATERIALS AND METHODS

Laboratory acclimated *Channa punctatus* (length 8.28±1.6cm, weight 4.87±0.04g) were recruited for the present study. The fishes were divided into three groups. 'A' group fishes were considered as 'Control' and they were not infected and not injected with vitamin A, 'B' group fishes was infected with a dose of $10^3$ dilution of *A.hydrophila* and not injected with vitamin A. and 'C' group fishes was also infected with a dose of $10^3$ dilution of *A.hydrophila* and also injected with a dose of 0.025ml. The experiment was run in triplicate. During the experimental period water in the containers were changed on alternate days without disturbing the test organisms and fed regularly with the artificial diet. The blood samples were collected on 1, 2, 3, 4, 7, 14, 21, 26 days of exposure to the pathogen. Haematological parameters were performed on pooled blood samples. Total Erythrocyte Counts (TEC), Total Leucocyte Counts (TLC) were counted using Haemocytometer with improved Neubaurer ruling chamber (Weber & sons, England), differential leucocyte counts (DLC) were performed on blood smears stained with May-Grunewald's Giemsa's stain and antibody tire using agglutination principles was analysed in the serum in the 96- well titre plate. The data were analyzed statistically and students't' test was used to test their significance.

## RESULTS & DISCUSSION

### Total Erythrocyte Counts

In group 'A' fishes no significant change was observed during the entire experimental period (Table-1). In both 'B' & 'C' group (Infected) fishes the values remained higher throughout the experimental period. The increase was significantly higher in Vit.A administered group ('C' group). It was also observed that in both infected group ('B' &'C') the values decreased gradually with increase in experimental period. Significant increase in the TEC levels in group 'C' fishes maybe attributed to Vitamin A administration.

Sivagurunathan and Xavier Innocent (2012) observed similar increase in *Channa punctatus* exposed to *Aeromonas hydrophila* and injected with Vit.C. The results of the present study were in accordance with a number of previous observations, which have reported a hike in TEC during unhealthy state of fish (Kumar and Patri 2000; Rahukhan *et al* 1995). Accordingly high counts were associated with the abnormal conditions of a fish. Hence an increase in the TEC might have been accomplished by a rapid mobilization of RBC from the haemopoietic tissue, which may transport higher amounts of oxygen particularly to withstand stress factor caused by *A.hydrophila* (Innocent *et al.,* 2004).

### Total Leucocyte Counts

No significant change was observed in counts in uninfected fishes (group 'A'), however in both 'B' &'C' group fishes the TLCs has increased significantly than control 'A' group and it was significantly higher in group 'C' fishes (Table-2). It was also observe that the counts increased with increase in experimental period. Though increase in the TLC levels can be contributed to pathogen induced defence response, the significant and sustained increase may also be due to early detection of pathogen by the alert immune system which is fortified with a dose of 0.025ml of Vitamin A.

Sivagurunathan and Xavier Innocent (2012) observed similar increase in *Channa punctatus* exposed to *Aeromonas hydrophila* and injected with Vit.C, Harikrishnan et al (2010) observed similar increase in TLC in Cirrhinus mrigala infected with *Aphanomyces invadans*. The results of the present study also agree with the works of Sivagurunathan *et al* (2011), Innocent *et al* (2004) and Pandey *et al* (2000).

**Table. 1:** Total Erythrocyte counts (millions/$mm^3$) in relation to A.hydrophila infection post administered with Immunostimulant Vitamin A. (Mean±SD of three values).

| Sample | Duration (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 7 | 14 | 21 | 26 |
| **A** | 0.41±0.08 | 0.43±0.08 | 0.42±0.07 | 0.41±0.08 | 0.42±0.09 | 0.41±0.08 | 0.43±0.07 | 0.43±0.08 |
| **B** | 0.51*±0.08 | 0.53*±0.07 | 0.52*±0.07 | 0.49*±0.04 | 0.47*±0.04 | 0.49*±0.05 | 0.48*±0.08 | 0.47*±0.02 |
| **C** | 0.55*±0.07 | 0.53*±0.08 | 0.54*±0.05 | 0.52*±0.09 | 0.50*±0.04 | 0.51*±0.08 | 0.52*±0.08 | 0.53*±0.01 |

*=significant (**A** = uninfected, **B** = Infected-Vit.A, **C** = Infected+Vit.A).

**Table. 2:** Total Leucocyte counts (thousands/$mm^3$) in relation to A.hydrophila infection post administered with Immunostimulant Vitamin A. (Mean±SD of three values).

| Sample | Duration (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 7 | 14 | 21 | 26 |
| **A** | 2.60±0.8 | 2.55±0.7 | 2.60±0.9 | 2.56±0.8 | 2.58±0.9 | 2.55±0.5 | 2.53±0.7 | 2.63±0.8 |
| **B** | 3.11*±0.9 | 3.23*±0.8 | 3.41*±0.8 | 3.45*±0.7 | 3.61*±0.9 | 3.65*±1.1 | 3.73*±0.9 | 3.75*±1.2 |
| **C** | 3.85*±0.7 | 3.92*±0.8 | 3.98*±0.5 | 4.21*±0.9 | 4.33*±1.1 | 4.31*±0.8 | 4.36*±0.8 | 4.35*±1.3 |

*=significant (**A** = uninfected, **B** = Infected-Vit.A, **C** = Infected+Vit.A).

**Differential Leucocyte Counts**

As there is no significant changes in the uninfected ('A' group) fishes, the average value from the experimental period was calculated and used in the figure for comparison. In group 'B' &'C' the average of three values were calculated and compared graphically. The lymphocyte counts were less in Infected fishes ('B' & 'C'groups) when compared to 'A' group fishes (Fig.-1), further it was also observed that the counts increased gradually with increase in the duration of experimental period. Further, within the infected groups the counts were higher in 'C' group fishes (Vitamin-A administered). Gradual increase in lymphocytes can be understood as pathogen induced specific immune response and higher counts can be correlated to increase in specific resistance.

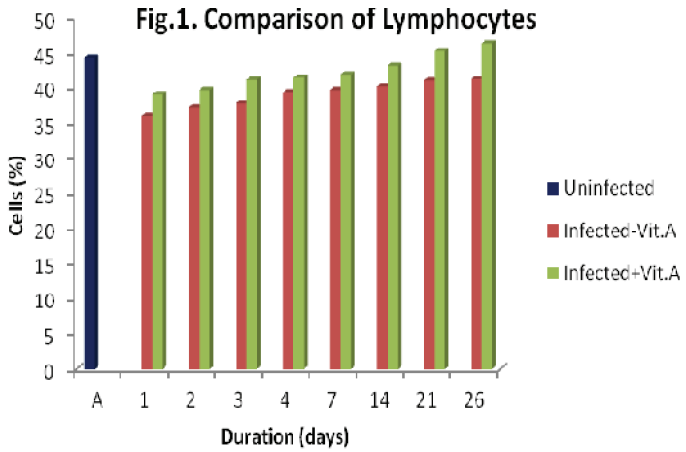


**Fig. 1:** comparison of lymphocytes.

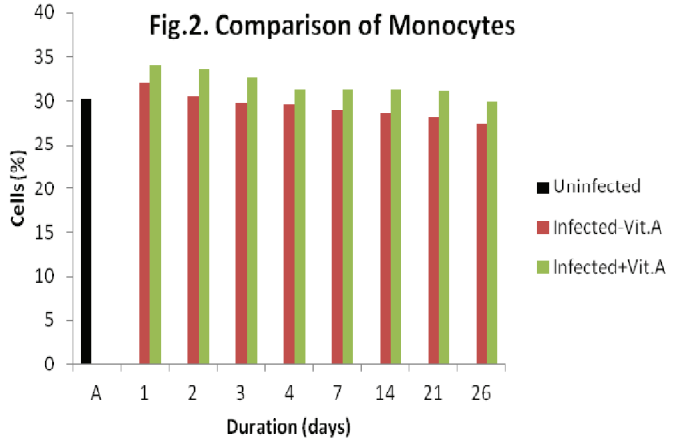


**fig. 2:** comparison of monocytes.

Similarly the neutrophil counts also exhibited an increasing trend from day 1-26 in group 'C' which is a clear indication of improved general resistance. On the other hand the monocyte count was higher only in group 'C' but exhibited a decreasing trend with increasing in the experimental duration. In group 'B' the monocyte counts were less when compared to both 'A' & 'C'. It is well known that the monocyte plays a vital role in both non-specific and specific immunity. They also play an important role in phagocytosis not only to eliminate the pathogen but also to elicit specific immunity. Thus increased levels on monocytes in group 'C' can be attributed to the role of vitamin-A in improving the immunity.

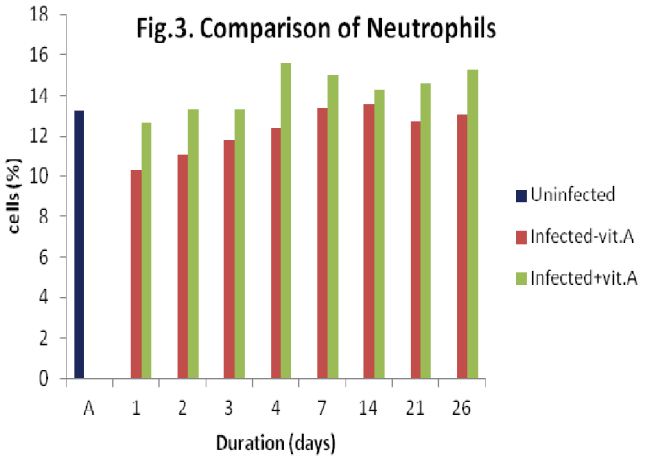


**Fig. 3:** comparison of meutrophils.

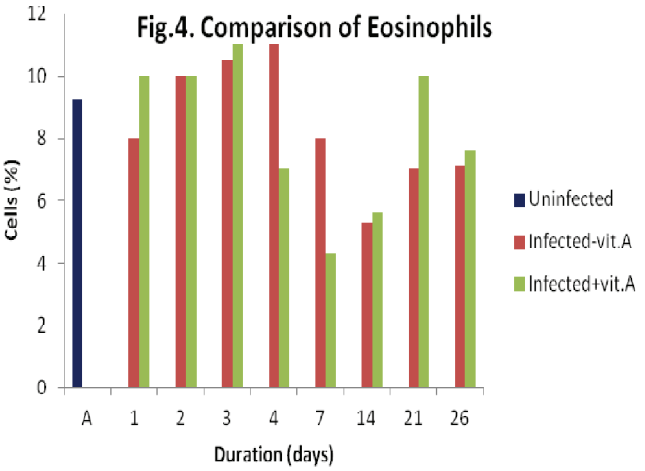


**Fig. 4**: comparison of eosinophils.

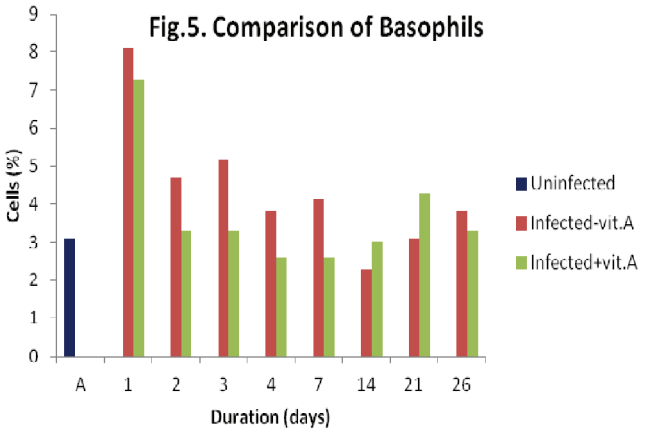


**Fig. 5:** comparison of basophils.

Ainsworth (1992) has suggested that neutrophils and monocytes are responsible for bacterial uptake in studies with Atlantic salmon, shown that neutrophils are capable of ingesting

bacteria, *A.salmonicida*. Similar observations of neutrophil as an inflammatory response were recorded by Innocent *et al* (2004) in *M.montanus* infected by *A.hydrophila*. Sivagurunathan and Xavier Innocent (2012) observed an increase in neutrophil, lymphocyte and monocyte counts in *Channa punctatus* exposed to *Aeromonas hydrophila* and injected with Vit.C, Karuthapandi and Xavier Innocent (2010) observed an increase in lymphocyte counts in Tilapia when infected with Vibrio anguillarum,Garcia *et al*(2011) observed an increase in neutrophil and monocyte counts when *Piaractus mesopotamicus* fed with vit.C supplemented diet and challenged with *A.hydrophila*.

Fluctuating results were observed in both eosinophil and basophil counts in both the infected groups.

Further, Visible results of antigen-antibody reaction in the 96 well microtitre plate in the form of larger aggregates in the $4^{th}$, $5^{th}$,$7^{th}$ well was observed when titrated with the serum obtained from group 'C' fishes which can be correlated with the increasing lymphocyte counts as they are responsible for the antibody production. Montero *et al*(1999) also observed the production of antibody in fishes treated with vitamin A and infected with *V.salmonicia, V.anguillarum, A.salmonicida*.

## CONCLUSION

In the present study fishes infected with *A.hydrophila* and administered with vitamin A exhibited an increase in erythrocyte counts than the fishes not administered with vitamin A further the declining was minimal which is evident that administration of vitamin A supplements the release of more circulating RBC which helps the fish to resist the pathogen induced stress.

On the other hand increase in WBC population in vitamin A administered fishes can be correlated to vitamin induced general resistance against the pathogen. Further, increase in neutrophil counts explains the immediate non-specific immune response and elevated levels of monocytes are an indication of activated immune response as they play a major role in both cell mediated and humoral mediated immunity.

Increase in lymphocyte population explains the activated specific immunity against the particular pathogen wich is further supported by agglutination in the microtitre plate. Thus administration of vitamin A will enhance the immune power of the fish.

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