Effect of Obesity and Associated Disorders like Diabetes, Dyslipidemia & Hypertension on Levels of Serum Complement Component C3 in Indian Ethnic Population

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Abstract

Background: Obesity is a well recognised as a state of chronic low grade inflammation and the main source of complement factors is the adipose tissue which is associated with Insulin Resistance, altered glucose and lipid metabolism, all of which promote the development of metabolic & cardiovascular disorders. Objective: Till date no study has been conducted in Indian ethnic population exploring the relationship of serum complement C3 with obesity and disorders like diabetes, dyslipidemia and hypertension so our objective was to study the effect obesity & associated disorders on serum C3 levels. Material & Methods: The present study included 290 subjects (121 men & 169 women) out of which 203 (70 %) were overweight 61 (21 %) were obese class I and 26 (9 %) were Class II obese according to International Diabetes Federation (IDF) - Modified ATP III criteria. Biochemical parameters like Serum C3, Fasting sugar levels, serum Insulin levels and lipid profile were measured. Homeostasis model of assessment for insulin resistance (HOMA-IR) was calculated. Statistical analysis was done by Medcalc.v11.5.0.0.software. Results: Mean C3 levels in total no. patients were 148.61± 38.82 mg/dl. As BMI increased, there was significant increase in serum levels of C3. When the distribution of variables were studied in both sexes, no statistically significant differences were found for all variables except Age, BMI, blood pressure & C3 levels. Serum C3 also correlated significantly with BMI (r = 0.812, P< 0.0001), insulin resistance (r= 0.262, P <0.001), Triglyceride (r = 0.338, P < 0.001) & LDL (r= 0.431, P < 0.001). As associated disorders with obesity increased, there was significant increase in levels of C3 than only obese patients with no other associated disorders. (ANOVA, P< 0.001). Conclusion: In this study, association of serum C3 with increase in BMI was established & also relationship of C3 with Insulin levels, Insulin resistance and cardiovascular risk factors was found. Our study concluded that obesity associated with dyslipidemia, diabetes & hypertension have a significant effect in increasing the levels of serum C3 concentration.

Keywords: Inflammation, Obesity, Diabetes, Complement component 3, Insulin resistance

Introduction

Obesity is major problem worldwide and prevalence has increased, not only in developed but also in developing countries. Abdominal obesity is a potent risk factor for the development of Type 2 diabetes mellitus, Metabolic syndrome and Cardiovascular disease (CVD). Adipose tissue, particularly white adipose tissue, is an endocrine organ that releases adipocytokines into the blood stream. 1 These cytokines includes Interleukin-6 (IL-6), Tumor necrosis factor-alpha (TNF-a), Leptin, serum complement (C3) and Acylation-stimulating protein (ASP).2 Such cytokines have a role in developing insulin resistance by causing the phosphorylation and proteosomal degradation of insulin receptor substrates or by indirectly interfering with the insulin receptor substrate interaction. Some of them plays an important role in obesity associated insulin resistance and cardiovascular complications.3
The complement system is a complex protein network and initially was considered as part of the innate immune system. Previously, the major source of complement was considered as the liver but in recent years, various non-hepatic sources of complement, like adipose tissue and endothelial cells, have been identified. Complement can be activated by several pathways – the classical, the lectin and the alternative – all of which converge on complement C3, the central component of the complement system. Various Clinical studies shows that elevated plasma C3 levels are associated with Type 2 diabetes, and also correlate with measures of obesity, dyslipidemia and insulin resistance. Functional role for the complement system in the pathogenesis of Type 2 diabetes and insulin resistance is supported by a variety of in vitro and in vivo studies suggesting pleiotropic effects of complement components on adipocytes, endothelial, and inflammatory cell function (3). Increased C3 concentrations is also associated with cardiovascular risk factors, insulin resistance & Obesity, waist circumference and Triacylglycerol concentrations. All these findings suggest that circulating C3 could be a risk factor for the development of Obesity and Type 2 diabetes and also risk indicator for cardiovascular disease. Moreover, C3 concentrations are also useful as biomarkers to identify subjects with metabolic syndrome.

Thus the purpose of the study was to study the effect of different grades of obesity and associated dyslipidemia, diabetes & Hypertension on Serum complement C3 levels and to study its relationship with insulin resistance & different cardiovascular risk factors.

### Material & methods

#### Subjects

The cross-sectional study included 290 subjects attending the daily Outpatient Clinic run by Dept. of Medicine, Dhiraj General Hospital, Gujarat, India during the time period Jan 2014 to Feb 2015. Subjects with BMI more than >23 kg/m² were included in the study. Further these patients are divided in 3 subgroups according to International Diabetes Federation (IDF) - Modified ATP III criteria: 1. Overweight (BMI ≥ 23.00 – 24. 99 kg/m²) 2. Obese class I (BMI ≥ 25.00 – 29.99 kg/m²) 3. Obese Class II (BMI ≥ 30.00 kg/m²). All the subjects detailed history of Diabetes, Hypertension or Cardiovascular disease was taken. Subjects with a systolic blood pressure (SBP) of ≥ 140 mm Hg or diastolic of ≥ 90 mm Hg, or taking any hypotensive agent, were considered hypertensive. Subjects with fasting Glucose Levels ≥126 mg/dl on at least two occasions were considered diabetic. When total cholesterol (TC) or triglycerides (TG) exceeded 200 mg/ dl, or when the subjects were on lipid-lowering drugs, dyslipidemia was diagnosed.

Subjects weight measurement was done on a calibrated weighing machine. Height was measured with bare feet on calibrated fixed scale. The BMI was calculated by dividing the weight in kilograms by the square of the height in metres. Waist Circumference (WC) was measured at the level between the lowest rib margin & iliac crest & Hip Circumference (HC) was measured at the widest points of two trochanters using a measuring tape. Blood pressure was measured by using a mercury sphygmomanometer, maintaining the subject in the seated position, twice consecutively.

#### Blood samples analysis

Fasting blood samples were collected by venipuncture and collected in Fluoride and Plain vacuette. Samples were allowed to clot for 10 min and centrifuged for 15 min at 3000 r.p.m. Serum was separated and analysis of Serum Complement component C3, fasting blood sugar levels, Glycosylated haemoglobin, Insulin levels, Lipid profile was carried out. Serum C3 was analysed on semi-automated analyser ERBA-CHEM 5 plus and Serum Insulin levels were measured on TOSOH AIA-360 system analyser (AIA-IRI pack). The insulin resistance was calculated from the Homeostasis Model Assessment (HOMA). Triglyceride levels (TG) were analysed by GPO-PAP method, Total cholesterol (TC) by CHOD-PAP method and high-density lipoproteins (HDLs) by colorimetric enzymatic assay on fully automated ERBA EM200 analyser. The low-density lipoproteins (LDLs) were calculated using Friedewald’s
Blood Glucose was determined by glucose-oxidase method on fully automated ERBA EM200 analyser.

**Statistical analysis**

Medcalc.v11.5.0.0. software was used for statistical analysis. For comparison between two groups, Student’s t-test was used. To study the differences between more than two groups ANOVA test was done. For Correlation between the groups Spearman’s rank correlation coefficient was used. P < 0.05 was considered significant.

**Results**

The study included total 290 subjects of which 203 (70%) subjects were overweight, 61 subjects (21%) were Class –I obese and 26 (9%) were Class-II obese. Analysis of anthropometric and biochemical parameters is shown in Table 1. It was found that females were more overweight & obese than males. There was significant difference in all the anthropometric and biochemical parameters in Overweight & class-II obese groups except Blood pressure and but no significant differences were found between Overweight & class-I obese except age, BMI, Waist to hip ratio & C3. There was significant increase in levels of Complement C3 in class-I obese & Class-II obese than overweight subjects. (Table-1)

When gender based distribution was studied for anthropometric variables, in total 290 patients (121 men and 169 women) statistically significant differences were found between men and women except age, Blood pressure & BMI. Only significant difference was found between HDL-c levels between men and women among the study subjects. Also no significant difference was found between serum C3 levels in both sexes. There was significant correlation between, serum C3 and all variables, and significant positive correlation found between C3 and BMI (Fig 1), waist to hip ratio, Fasting blood sugar levels (FBS), serum insulin levels and HOMA-IR, Serum Triglyceride levels, LDL and Negative correlation was found between serum HDL levels. (Table-2)

<table>
<thead>
<tr>
<th>Table 1: Anthropometric &amp; biochemical variables in different groups</th>
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<tbody>
<tr>
<td><strong>Overweight</strong></td>
</tr>
<tr>
<td>n (%)</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Gender (M/F)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>Waist-Hip ratio</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
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<tr>
<td>Diastolic blood pressure</td>
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<tr>
<td>FBS</td>
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<tr>
<td>Total Cholesterol</td>
</tr>
<tr>
<td>Triglyceride</td>
</tr>
<tr>
<td>HDL-c</td>
</tr>
<tr>
<td>a.</td>
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<tr>
<td>b.</td>
</tr>
<tr>
<td>a.</td>
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<tr>
<td>b.</td>
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<tr>
<td>a.</td>
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<tr>
<td>b.</td>
</tr>
<tr>
<td>a.</td>
</tr>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>LDL-c</td>
</tr>
<tr>
<td>Insulin</td>
</tr>
<tr>
<td>HOMA-IR</td>
</tr>
<tr>
<td>C3</td>
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</tbody>
</table>

Table 2: Correlation between C3 and anthropometric and biochemical variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>r</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>0.116</td>
<td>0.51</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>0.812</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.262</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic Blood pressure</td>
<td>0.138</td>
<td>0.44</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.109</td>
<td>0.62</td>
</tr>
<tr>
<td>FBS</td>
<td>0.966</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HbA1C</td>
<td>0.943</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S.Insulin</td>
<td>0.909</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.262</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.874</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.338</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-c</td>
<td>-0.281</td>
<td>0.02</td>
</tr>
<tr>
<td>LDL-c</td>
<td>0.431</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>VLDL-c</td>
<td>0.194</td>
<td>=0.02</td>
</tr>
</tbody>
</table>

r = Spearman’s rank correlation coefficient, P<0.05 & P<0.001 considered statistically significant

Fig 1: Correlation between S. C3 and BMI (r = 0.812, P< 0.001)
When serum C3 levels were studied in patients with only obesity and obese patients with one or more associated disorders like Hypertension, Diabetes & Dyslipidemia, it was found that there was significant increase in C3 levels in patients with only obesity and in patients with number of associated disorders (ANOVA P<0.001) (Table 3).

**Table 3: Levels of C3 in obese patients & obese patients with one or more associated disorders like diabetes, hypertension & dyslipidemia**

<table>
<thead>
<tr>
<th></th>
<th>Obese Patients (n= 82)</th>
<th>Obese +1 (n= 147)</th>
<th>Obese + 2 (n= 45)</th>
<th>Obese + 3 (n= 16)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. C3 (mg/dl)</strong></td>
<td>125.83 ± 25.67 (109.76 – 150.23)</td>
<td>129.84 ± 27.12 (124.41 – 125.13)</td>
<td>138.36 ± 24.72 (128.31 – 146.51)</td>
<td>141.36 ± 25.77 (130.25 – 149.67)</td>
<td>P &lt;0.001</td>
</tr>
</tbody>
</table>

Values are Mean ± S.D. and 95 % Confidence Interval. Differences between the groups were analysed by ANOVA (P<0.001).
Discussion

In the present study, it was found that serum C3 levels significantly increased as the BMI increases in overweight & obese population. Serum C3 levels increased significantly in Class-II obese compared to Overweight patients which shows obesity has an effect in increasing serum C3 levels. Moreover, there was significant association Serum C3 levels and serum fasting insulin levels and Insulin resistance. A significant increase in serum C3 levels was found as the associated disorders such as Hypertension, Dyslipidemia and Type-2 diabetes with obese patients increased.

Recently, there has been an increasing consequences of overweight and obesity in adolescents. Adipose tissue secrete a variety of autocrine and paracrine factors, such as TNF-α, IL-6, complement factors & Leptin which are involved in regulation of glucose & body weight homeostasis and is nowadays considered metabolically active. Adipocytes synthesize and secrete C3, Factor B and adipsin. These factors interact and results in the generation of C3a, which is transformed into C3adesarg which is also called acylation stimulating protein (ASP), and is secreted & synthesized by the adipocytes. Also a positive correlation has been found between C3 and ASP.

Previous studies had shown that C3 is significantly increased in patients with conditions such as obesity, Type II diabetes and dyslipidaemia. Fasting C3 concentrations have been significantly raised in Pima Indians, and in familial combined hyperlipidemia (FCHL). There are various evidences where there seems to be an increase in C3 in situations of ischemic cardiopathy and insulin resistance and also C3 were found present on the arteriosclerotic plaque. These findings indicate that C3 could be used as very important factor, which supports the hypothesis that insulin resistance could give rise to atherosclerotic process. Study by Muscari et al. in 1068 subjects (29.8% with BMI > 28 kg/m²), found that there is significant increase in C3 levels in different BMI tertiles. Studies by Halkes et al., Onat et al., Ylitalo et al. shows that anthropometric measurements, such as BMI, waist circumference and waist-to-hip ratio are considered predictors of high C3 concentrations. Koistinen et al., have shown higher C3 expression in obese subjects than lean men.

In the present study, we found that there was significant increase in serum C3 in both men and women as BMI increased and also there was significant relationship between the studied parameters. These results are in accordance with study done by Hernandez-Mijares et al who found increase in C3 in obese subjects but there are no studies in literature regarding C3 levels in Overweight, Class-I obese and class-II obese in Indian subjects. Our study showed a significant correlation between C3 & glycemia, insulin levels & insulin resistance which are in accordance with study done by Bhavita et al. Study done by Muscari et al. found a significant correlation between the glycemic levels (baseline and at 2 h after overload) and insulin resistance. Inflammatory cytokines, like TNF-alpha & interleukin 6 (IL6) inhibit tyrosine phosphorylation of the insulin receptor substrate-1 & causes insulin resistance in majority of obese subjects. TNF-alpha produces 30–40% of the circulating levels of IL6, which is the main source of production of C-reactive protein in the liver. The increase in production of cytokine causes a low-grade chronic inflammation and activates the complement system, which contributes to the metabolic complications observed in obesity.

Also in our study we found positive correlations between C3 concentrations and fasting blood sugar levels, Glycoxylated levels & Cardiovascular risk factors, such as Total Cholesterol, Triglyceride, HDL-c, LDL-c & VLDL-c which is consistent with others studies by Onat et al, Oostrom et al, Hernandez-Mijares et al & Bhavita et al. The relationship between C3 and triacylglycerol can be explained by, ASP which is a hormone produced by adipocytes because of interaction of C3, factor B, and adipsin, stimulates glucose transport through membranes and increase the synthesis of Triacylglycerols in adipocytes. Also in our study we found that there was increase in C3 levels with increase in associated disorders, which is consistent with the study done by Hernandez-Mijares et al & Bhavita et al. Thus in this study we found that this disorders like diabetes, hypertension &
dyslipidemia have effect in increasing serum C3 concentration in addition to obesity which increases cardiovascular risk in patients with obesity.

**Conclusion**

Thus from this study, we conclude that serum concentration of C3 increases with increasing BMI in both men & women and the association between levels of C3 and increased obesity results from synthesis of these proteins by adipose tissue. There is also relationship between Serum C3 levels, Insulin resistance and Cardiovascular risk factors in Indian population. Our study found that disorders such as Dyslipidemia, Diabetes & Hypertension have a role in increasing the levels of serum C3 in Obese patients.

**References**


The Role of Leptin in Obesity-Induced Hypertension

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Abstract

Obesity is linked with the growth of white adipose tissue and associated chronic hyperleptinemia. Leptin is a hormone-like cytokine or adipokine secreted mainly from adipose tissue. High leptin state in obesity rapidly causes selective resistance, focused in the arcuate nucleus of the hypothalamus, and centered round leptin’s role in food intake and satiety. This resistance lowers the body’s reaction to food intake and prevents the anorexigenic effects of leptin. As this resistance builds up, the intake of food increases causing an enhancement in body adiposity and leptin levels. However, some pathways do not build resistance to leptin and continue to exhibit the stimulatory effects, which cause a persistent stimulation of sympathetic nervous system (SNS), particularly in the kidneys and skeletal muscles. The increase in SNS activity in the kidney, along with the endothelial dysfunction and oxidative stress, lead to an increase in blood pressure. Apart from leptin's effects on SNS and renal function, this adipokine influences vascular health and hypertension through several phenomena or mechanisms such as baroreflex sensitivity, release of nitric oxide and cardiac hormones. In this review, an attempt has been made to highlight different aspects of leptin biology, which are relevant to hypertension.

Keywords: obesity, leptin, hypertension, melanocortin, autonomic nervous system

Introduction

Since the early 1990s, leptin has been a source of much interest in the field of obesity and obesity-related comorbidities. Nevertheless, there remains a great deal we do not understand about how leptin works precisely. It has long been established that obesity increases the risk of metabolic syndrome and cardiovascular disease and that this increased risk is due, at least in part, to the increase in blood pressure that tends to follow increased adiposity. However, discovering the cause of hypertension in obesity could revolutionize the way that we treat obesity-related hypertension. Recent studies have implicated leptin in the pathogenesis of hypertension in the obese population due to leptin resistance in the arcuate nucleus and selective activation of a sympathetic nervous response in the kidneys and skeletal muscle.

In this review, an attempt has been made to focus primarily on different biological mechanisms that are linked to blood pressure regulating role of leptin. For this purpose, PubMed system has been used largely to search for relevant literature. The discussion of this paper (i.e., Critical Review section) has been divided into 13 subtopics. In the first part of this review article, topics like leptin's pathophysiological effects and intracellular signaling have been mentioned. Finally, various important issues such as effects on autonomic nervous system, kidney and cardiovascular systems have been discussed in relation to both health and disease conditions.
Physiological effects of leptin

Leptin is one of the many adipokines, which plays an important role in maintaining the balance between food intake and energy expenditure, by way of an anorectic role. Leptin is an adipose-derived hormone and is secreted primarily by adipocytes of white adipose tissue (WAT) (Ray & Cleary 2010). In a physiological state, when energy supply is adequate, leptin is released from adipocytes to alert the hypothalamus, and, in particular, the arcuate nucleus, that enough food has been consumed. Moreover, leptin promotes energy expenditure in the form of thermogenesis and sympathetic nerve activity (SNA) in the kidneys and adrenal glands. When food supply is scarce, leptin levels fall, triggering an adaptive neuroendocrine response. This response leads to decrease in reproductive hormones, thyroid hormone and insulin-like growth factor, and increase in growth hormone to mobilize energy stores (Mantzoros et al. 2011). Leptin also plays a role in wound healing, hematopoiesis, osteogenesis, insulin secretion and sensitivity, and glucose homeostasis (Figure 1).

Pathological effects of leptin in obesity

In the obese individual, the increase in adiposity creates an increase in leptin secretion and a state of chronic hyperleptinemia. In this state, the role of leptin becomes pathological. The arcuate nucleus becomes selectively resistant to leptin’s inhibitory effect on its orexigenic neurons, while continuing to stimulate the neuronal systems in charge of energy expenditure. Leptin continues to act on other hypothalamic targets, which continue to stimulate renal and muscular sympathetic nervous system (SNS) activity (Reed et al. 2010). The SNS stimulation appears to be selective in its targets, generating functional disruption in the autonomic nervous system (ANS). Since the feedback loop between increased leptin levels and satiety has been interrupted, the leptin levels continue to rise, leading to continuous stimulation of sympathetic activity and this imbalance leads to endothelial damage and increased arterial pressure. The mechanism of SNS activation in the state of obesity involves hyperleptinemia, activation of the central nervous system (CNS) melanocortin and renin-angiotensin-aldosterone systems (RAAS), hypoadiponectinemia, hypoghrerinemia, hyperinsulinemia, and baroreflex dysfunction (da Silva et al. 2009; Hall et al. 2015). The primary emphasis of this paper is the impact of the chronic exposure to obesity-induced hyperleptinemia and the cascade of events that ensue.

Leptin and melanocortin system

While chronic high levels of leptin, which is seen in obesity, have been shown to build increasing resistance to its anorexigenic effects, the stimulation of sympathetic nerve continues to rise as leptin levels rise. To understand how some leptin functions are quelled in chronic hyperleptinemic states while others continue to be stimulated is essential to our understanding of the role of leptin in metabolic dysfunction, hypertension and cardiovascular diseases. Biochemically, leptin simultaneously activates the catabolic pathway of proopiomelanocortin (POMC)/cocaine-and-amphetamine related transcript (CART) neuron and inhibits the anabolic neuropeptide Y/agouti-related protein NPY/AgRP pathway in the arcuate nucleus. The POMC is then processed into melanocortins, such as alpha melanocortin stimulating hormone (α-MSH), in second order neurons in a variety of other hypothalamic nuclei. When leptin binds to the lepin receptors (Ob-R, long (Ob-Rb) and short (Ob-Ra) isoforms) on POMC neurons α-MSH is released and binds to melanocortin receptors (MCR) throughout the CNS, mostly MC3R and MC4R type receptors (Simonds & Cowley 2013). Stimulation of MC4R increases
sympathetic nerve activity to the kidneys and in brown adipose tissue. However, when MC4R receptors are blocked only sympathetic nerve activity to the kidneys is affected (Haynes 2005). Interestingly, MC4R deficient mice are hyperphagic, obese, and display signs of metabolic syndrome, but are not hypertensive, despite high levels of leptin. Likewise, deletion of POMC neuron leptin receptors continue to cause mild obesity, without the hypertensive effects of chronic hyperleptinemia (da Silva et al. 2013). In contrast, yellow Agouti-overexpressing mice are resistant to the anorexigenic effects of leptin while renal SNA remains intact, causing hypertension. These mice are very similar to the diet-induced obesity (DIO) mouse model, indicating that leptin resistance in the AgRP neurons of the arcuate nucleus plays a large role in obesity-induced hypertension (Haynes 2005).

A recent study by Purkayastha et al. (2011) was able to uncouple obesity and hypertension by injection of IκB kinase-β (IKK-β) into the mediobasal hypothalamus, near the arcuate nucleus, and further define the role of POMC and melanocortin system in obesity-induced hypertension, while pointing to AgRP role in hyperphagia. Activation of the pro-inflammatory protein nuclear factor κB (NF-κB) and its upstream activator IKK-β in the mediobasal hypothalamus rapidly elevated blood pressure in mice independently of obesity. The study also reported that this form of hypothalamic inflammation-induced hypertension involved the sympathetic upregulation of hemodynamics and POMC neurons that has a crucial role in obesity-related hypertension (Purkayastha et al. 2011). In vivo, the stimulation of the IKK-β/NF-κB pathway has been demonstrated to be activated when leptin binds to Ob-R in the arcuate nucleus (Humphreys 2011). These experiments point to the POMC neurons and the downstream effects of α-MSH on MC4R receptors as the target points for blood pressure regulation by leptin. IKK-β was similarly used in experiments by Zhang et al. (2008) to show the role of NPY/AgRP neurons in the homeostasis of body weight. The role of IKK-β/NF-κB activation in NPY/AgRP neurons has also been implicated in leptin resistance (Figure 2). These studies showed AgRP/IKK-β knockout mice fed a high fat diet were lower in body weight and had preserved leptin signaling, compared to the control group.

**Leptin and intracellular signaling**

Though different studies have been able to draw a strong correlation between various neurons of the hypothalamus and their pathophysiological response to hyperleptinemia, the underlying intracellular signaling pathways are not well understood. Selective leptin resistance occurs due to the different biochemical pathways initiated by leptin, and there are a number of negative feedback loop initiators that have been implicated in this complicated process. Studies have shown that even inhibitors of leptin signaling pathways react differently and are preferentially upregulated in different cell types. The model of leptin signaling includes induction of the Janus kinase (JAK) 2/signal transducer and activator of transcription 3 (STAT3) pathway (Frühbeck 2006). When leptin binds with its receptor on the cell surface of a hypothalamic neuron, it initiates a conformational change that leads to intracellular activation of JAK2 and phosphorylation of the Src homology2 domain of STAT3. The phosphorylated STAT3 then dimerizes, travels to the nucleus and regulates gene expression by binding to promotor regions (Kalil & Haynes 2012). This pathway induces the production of suppressor of cytokine signaling (SOCS) 3 as a negative feedback mechanism. SOCS3 binds to the tyrosine residue (Tyr985), which prevents the phosphorylation of STAT3 (Yang & Barouch 2007). In high leptin states, these leptin-stimulated pathways are increasingly inhibited by SOCS3 (Figure 3).
Transgenic mice bred to overexpress SOCS3 in POMC neurons impaired STAT3 signaling, leading to obesity and leptin resistance. However, when the mice were transgenically modified to overexpress SOCS3 in Ob-Rb neurons no obesity was noted and, in fact, the mice were shown to have a small but significant drop in weight and food intake and in leptin levels, compared to their wild type counterparts. This was an unexpected finding and supports the theory that SOCS3 may influence different Ob-Rb expressing neurons in different ways (Reed et al. 2010). This was further supported by Pedroso et al. (2014) in their experiments with ablation of the Socs3 gene in various mouse models. They found that when SOCS3 was deleted at the neuronal level, the result was improved glucose homeostasis and partial prevention of DIO. However, when SOCS3 was inactivated only in Ob-R–expressing cells there was no change to the effects of DIO, but these mice were protected against diet-induced insulin resistance. These experiments show the down-regulation of the JAK2/STAT3 pathway by SOCS3 overexpression as would be seen in the obese, hyperleptinemic state. Most significantly, however, AgRP neurons build up resistance to leptin through overexpression of SOCS3 at a faster rate than both POMC neurons and other Ob-R-expressing neurons in other hypothalamic nuclei (Olofsson et al. 2012). This ties in to the clinical picture of the yellow Agouti-overexpressing mice which are resistant to leptin’s influence on food intake, but continue to exhibit high levels of SNS activity (Haynes 2005).

Binding of leptin to the Ob-Rb receptor also activates mitogen-activated protein kinase (MAPK) pathway through SHP2 and the phosphoinositol 3 kinase (PI3K) pathway via insulin receptor substrate type 2 (Kalil & Haynes 2012). Though both SHP2 and IRS2 have been implicated in leptin induced intracellular activation, deletion of either of these substances in the entire brain or forebrain, only produce mild hyperphagia and obesity (da Silva et al. 2013). Protein-tyrosine phosphatase-1B (PTP1B) has also been shown to dephosphorylate JAK2 in hyperleptinemia and knockout mice have increased leptin sensitivity and reduced body weight (Mantzoros et al. 2011).

**Leptin and blood brain barrier**

A major component of leptin resistance is possibly an impaired transport of leptin across the blood-brain barrier (BBB). Investigators have suggested the existence of an active transport system via Ob-Ra (Rivest 2002). The impaired transport across the BBB could be due to saturation in the transport of leptin and a subsequent decrease in transport activity. This appears to be supported by the decrease in cerebral spinal fluid (CSF) leptin levels to serum leptin levels in obese individuals. However, it has also been noted that obese individuals still have higher CSF levels of leptin than their lean counterparts (Haynes 2005). In addition, hypertriglyceridemia has been demonstrated to inhibit the transport of leptin across the BBB, thus attenuating the leptin signal across the BBB and providing a mechanism for peripheral leptin resistance (Banks 2008). It has also been noted that different brain regions are saturated at different concentrations. When mice models were centrally injected with leptin equivalent to normal limit concentrations for lean humans, the hypothalamus was shown to reveal a preferentially higher concentration than any other brain region. However, when mice were centrally injected with leptin levels mimicking those of obese humans, the hypothalamus showed the lowest leptin concentration of all brain regions. This study implies that different brain regions may have different leptin level thresholds to be activated and may contribute to the clinical finding of selective leptin resistance (Mantzoros et al. 2011).
Leptin and endothelial function

Leptin has two antithetical effects that result in a balanced action in a normal physiological state. These actions are simultaneous pressor and depressor effects by inducing activation of SNS causing vasoconstriction and also increase production of nitrous oxide (NO) by the endothelium, mediator of vasodilation. Studies were performed to delineate the role of leptin on endothelium-dependent vasodilation as well as endothelium-independent vasodilation (Freeman et al. 2014). Mice treated with leptin depicted no significant change in endothelium-independent vasodilation in response to sodium nitroprusside. Yet, in response to acetylcholine there was a significant reduction in endothelial-dependent vasodilation in leptin-treated mice. On the other hand, leptin deficient obese mice expressed impaired endothelial relaxation in the presence of acetylcholine that was corrected by exogenous leptin treatment. These experiments have demonstrated leptin as a potent vasodilator and indicated the presence of leptin receptors on endothelial cells (Wang et al. 2013).

Endothelial dysfunction is a result of the oxidative damage that is caused by prolonged state of obesity-induced hyperleptinemic effect of increasing reactive oxygen species (ROS). It is of significance to note that short-term exposure of endothelial cells to leptin has been demonstrated to serve as a benefit by stimulating endothelial NO synthase (eNOS). However, long-term exposure of the endothelial cells to leptin actually resulted in decrease NO availability. Among free radicals, superoxide and peroxynitrite in particular have been shown to be increased through the stimulation of endothelial cells by excessive leptin (Korda et al. 2008). Then, ROS have been recorded to inactivate NO or eNOS and act as a potential cause of endothelial damage (Mattu & Randeva 2013). When a superoxide scavenger, membrane-permeable piperidine nitroxide tempol, was administered to mice there was no endothelial dysfunction observed under the influence of leptin (Wang et al. 2013). In DIO mouse models, increased leukocyte-endothelial interactions were seen to correspond with the damage of endothelial cells. The functional vascular impairments are predictive of cardiovascular complications that may occur later.

Nitric oxide release

The role of NO, in a normal physiological state, consists of far more than vasorelaxation. NO has an inhibitory action on oxidation of low-density lipoprotein (LDL), leukocyte migration to the subendothelial space, smooth muscle cell (SMC) proliferation and migration, platelet adhesion and aggregation. NO reduces the expression of adhesion molecules and increases blood flow to hinder coagulation — all function to protect vascular integrity. Studies have shown leptin to participate in phosphorylation of eNOS that leads to NO release. This mechanism was further examined by the administration of leptin to mice that were pretreated with a NOS inhibitor, resulting in an increased blood pressure (Beltowski 2012). Essentially, in non-obese individuals, in the presence of eNOS leptin induces NO release to cause vasodilation, whereas independent of eNOS or in the event of its inhibition, leptin causes vasoconstriction and increase in blood pressure. Hence, it is suggestive that vascular leptin-resistance may exist in obese individuals. In one particular study, leptin was administered in a pulsatile manner to avoid inducing leptin resistance, nonetheless assuring that the same obese levels of leptin were infused. After one week of leptin treatment in this manner, diminished ability of the endothelium relaxation was observed (Wang et al. 2013). Experiments have exhibited impairment of the stimulatory effect of leptin on NO in chronic hyperleptinemia (Beltowski 2012). Therefore, this impairment of leptin induced NO-mediated vasorelaxation probably contributes to leptin-induced hypertension in obesity.
Leptin and SNS activity

There are two pivotal elements that cause an increase in SNS activity: hyperleptinemia and activation of the brain POMC neurons and MC4R. In obese microenvironment there is selective increase in SNS activity to particular organs. Skeletal muscle and kidneys demonstrate elevated SNS activity whereas due to baroreflex inhibitory effect, cardiac sympathetic activity is minimally or not increased. Leptin activates the SNS by local peripheral actions and by the effects on hypothalamus that are centrally mediated. Studies have shown that acutely administered leptin did not affect the blood pressure if it was injected peripherally, whereas central infusion in DIO rats caused resistance to the actions of leptin in peripheral organs such as the kidney (Freeman et al. 2014).

Studies displayed that within a week of exposure to high fat diets in DIO mice, a rise in SNS activity occurred. Furthermore, the same rise was observed in non-obese mice that were subjected to modest weight gain (da Silva et al. 2013). In animals in which SNS was inhibited, leptin was shown to reduce the blood pressure by other factors including NO-mimetic effect on the endothelium (Beltowski 2012). Again, these studies confirm the normal contrasting function of leptin that involves the balanced activation of vasoconstriction by SNS and vasorelaxation by NO, cancelling out any changes in blood pressure. The only way that the depressor response to leptin can be preserved is by sympathectomy. The factors that contribute to increase SNS activity include blunted baroreflex sensitivity, Angiotensin II release, hyperleptinemia, hypoadiponectemia, hyperinsulinemia, and hypogrehelinemia.

Leptin-induced increase in SNS does not cause hypertension in the absence of angiotensin II. Leptin works in synergy by enhancing the presser effect of angiotensin II in acute or chronic treatment. Rats on high sodium diets have chronic vasoconstriction response to angiotensin II, an effect that is eliminated in the event of celiac ganglionectomy. Consequence of the denervation was recovery of the leptin-induced impairment of endothelial-dependent vasodilation in response to acetylcholine; blockage of leptin-induced supplementation of angiotensin II action to increase systolic blood pressure (Wang et al. 2013).

Leptin and renal function

Short-term administration of leptin depicted a natriuretic property of the hormone in rats, increasing excretion of sodium and water, primarily acting at the tubular level. However, in the setting of obesity, leptin has not been shown to increase sodium excretion that might cause an abnormal renal-pressure natriuresis. Impairment of this normal sodium excretion function of leptin in hyperleptinemia participates in causing obesity-related hypertension as a result of sodium retention and a rightward shift in the pressure-natriuresis curve (da Silva et al. 2013). It may therefore be suggested that the environment of hyperleptinemia may cause renal leptin resistance similar to central resistance to leptin impairing the anorectic effect of leptin. When obese rats were placed on calorie-restricted diets, renal regeneration of NO took place that then participated in the restoration of the natriuretic function of leptin (Freeman et al. 2014). Potential causes of renal-leptin resistance in obesity-associated hypertension include oxidative stress as a result of excessive degradation of NO, Ob-R downregulation, post-receptor signaling alterations or antinatriuresis resulting from increased activation of the efferent renal SNS. The latter was supported by a study in which kidney nerve supply was surgically removed resulting in the restoration of leptins natriuretic actions. On the other hand, obstruction of endogenous release of leptin reduced excretion of sodium and water by approximately 20-25% (Freeman et al. 2014).
Leptin and cardiovascular diseases

Obesity is a low-grade pro-inflammatory chronic state in which inflammatory cytokines have multifactorial effects causing pathologies preceding cardiovascular diseases (CVD). The prolonged inflammatory state sustained by abnormal production of adipokines constitutes a central role in the formation and progression of atherosclerosis, which is concomitantly present with endothelial dysfunction (Mattu & Randeva 2013). Leptin also stimulates to express pro-atherogenic and pro-angiogenic factors. Arterial pressure and heart rate are both increased in chronic leptin exposure in mice (Haynes 2005). Along with the heart rate, chronic IV infusion of leptin also increases the mean arterial blood pressure.

Atherosclerosis/Endothelial dysfunction

There is leukocytic and macrophage involvement in the early developmental stage of atherosclerosis in the endothelial wall. In obesity, adipose tissue consists of an increase in macrophage to adipocyte ratio along with alteration in the phenotype of macrophage. This increase in the macrophage recruitment is in association with systemic inflammation and insulin resistance, both of which are determined by which macrophage subtype is expressed in obesity. There are two main types of macrophages, M1 and M2. M1 is expressed in the adipose tissue of obese individuals, causes release of pro-inflammatory cytokines such a tumor necrosis factor alpha (TNF\(\alpha\)) and interleukin-6 (IL-6), and also promotes insulin resistance. These pro-inflammatory cytokines are involved in the pathogenesis of endothelial dysfunction eventually leading to atherosclerosis. M2 is expressed in the adipose tissue of lean individuals, and involved in secretion of anti-inflammatory cytokines and protects against insulin resistance (Nakamura et al. 2014). TNF\(\alpha\) is one of the major pro-inflammatory cytokines, predominantly produced by monocytes or macrophages in chronic inflammatory diseases. TNF\(\alpha\) induces expression of certain adhesion molecules, which participate in coagulation processes, contributing endothelial dysfunction and preceding atherosclerosis. However, decrease in weight in obesity results in decrease of TNF\(\alpha\). Studies have shown that higher levels of adhesion molecules (i.e. E-Selectin, Intercellular adhesion molecule-1 ICAM-1, and P-Selectin) were expressed from the endothelial cells of adipose tissue in obese mice (Nakamura et al. 2014). Interestingly, leptin has been demonstrated to promote the expression of adhesion molecules in CD4+ T cells (like ICAM-1) (Fernandez-Riejos et al. 2010). Evidence of leptin receptors on monocytes and vascular tissue implies that the role of excessive leptin is pivotal in causing this chronic low-grade inflammatory state by the release of pro-inflammatory cytokines (Martin et al. 2008). Leptin was also seen to behave as a monocytic attractant (Fernandez-Riejos et al. 2010). Essentially, the adipocyte hyperplasia in obesity causes increased release of leptin, which stimulates the production of pro-inflammatory cytokines (from monocytes/macrophages), adhesion molecules, and increased oxidative stress — all of which contribute to endothelial damage. Hyperleptinemia-induced pro-inflammatory cytokine release assists in maintaining the inflammatory state that results in the endothelial damage, hypertension, and eventually atherosclerosis.

Baroreflex sensitivity

The baroreflex sensitivity (BRS) counters the effect of SNS induced increase blood pressure, hence BRS is inversely proportional to age, heart rate, and blood pressure. It is important to note that the vagus parasympathetic nerve mediates the function of baroreceptor reflex as a modulator of the heart rate. Additionally, there is inverse relation
between BRS and triglyceride levels and fasting plasma glucose, both of which are relevant to obesity profiles (Skrapari et al. 2007). It was revealed that there was a nearly 50% reduction of BRS in obese women who were otherwise healthy. Though SNS activity is elevated in the skeletal muscles and kidneys among obese humans, the cardiac sympathetic activity is minimally affected due to the role of baroreflex inhibition (da Silva et al. 2009). Therefore, it is of importance to note that the increase in heart rate in obesity is a function of the reduction of the parasympathetic function of the heart. A report has suggested that during aging, endogenously produced leptin contributed to reduce the BRS (Arnold et al. 2014). The chief determining factor for reduced BRS was due to the dominant role of sympathetic activity over parasympathetic. Cardiac vagal dysfunction in obesity has been shown to contribute blunted BRS (Skrapari et al. 2007). Weight loss has a twofold effect in decrease of sympathetic activity and improvement of cardiac vagal activity, as a result enhancing the BRS (Voulgari et al. 2013).

**Leptin and cardiac hormones**

Though there is much room for research, a potential for future treatment methods has been seen in four cardiac hormones: vessel dilator (VD), long-acting natriuretic peptide (LANP), kaliuretic peptide, and atrial natriuretic peptide (ANP)—all of which have blood pressure lowering properties. The heart synthesizes these four hormones, which originate from the same gene. Study of hypertension in obese individuals has shown elevated concentrations of ANP, LANP, and VD. This increase in the cardiac hormones has been observed to revert to normal as the patient loses weight to reduce their high blood pressure. The way by which these cardiac hormones influence blood pressure has been thought to be via decreasing the hypothalamic concentrations of leptin (Lane & Vesely 2013).

**Conclusions**

The means by which leptin resistance occurs and the pathways leptin stimulates to induce hypertension are not yet fully understood. However, there is some exciting progress being made in this field that may soon lead to new pharmacological strategies, which possibly not only reduce the immediate cardiovascular risks associated with obesity-induced hypertension, but could also provide safe and effective weight loss therapeutics for obese individuals. Recent studies regarding the role of pro-inflammatory mediators IKK-β/NF-κB and the effect of this pathway on the arcuate nucleus, obesity, hypertension, and leptin resistance clearly provide an area that warrants further study. Various studies on different gene knockout experimental animals and reports on several anti-inflammatory phenomena have shown significant protection against leptin resistance and obesity, even when fed a high fat diet. These approaches may prove to be promising pharmacological avenues to combat obesity-associated comorbidities.

**References**


[24]. Rivest, S. (2002). Does circulating leptin have the ability to cross the blood-brain barrier and target neurons directly? Endocrinology, 143(9), 3211-3213.


**Figure 1.** Physiological functions of leptin. Leptin maintains the balance between food intake and energy expenditure. Leptin works on many body functions, conserving energy during times of food scarcity and expending energy during times of abundance. SNA: Sympathetic nerve activity.
Figure 2. IKK-β/NF-κB axis is stimulated in both the POMC and NPY/AgRP neurons of the arcuate nucleus. In the POMC neurons, IKK-β/NF-κB promotes the melanocortin system, leading to renal sympathetic nerve stimulation and hypertension. In the NPY/AgRP, IKK-β/NF-κB leads to increased AgRP production, leptin resistance and hyperphagia.

Figure 3. Leptin uses a complex system of intracellular pathways to perform its homeostatic role. Tyrosine 1138 on Ob-Rb is essential for STAT3 phosphorylation and the stimulation of SOCS3. Along with SOCS3, PTP1B and SHP2 are also capable of inhibiting leptin signaling. Leptin binding has different downstream effects, depending on the cell type. This allows for selective leptin resistance in the obese, chronic hyperleptinemic state.

Acknowledgement

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Biofilm: Contributing Factor to Drug Resistance in Staphylococci in Blood Stream Infections

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Abstract

Staphylococci represent the most commonly encountered blood culture isolates from ICU patients. Slime production is considered to be a significant virulence factor for some strains of Staphylococci. The importance of the role played by slime is further increased by its frequent association to reduced antibiotic susceptibility. This study was done to find out relationship of Bio-Film formation on the susceptibility pattern of the isolates. 27.6% of the isolates produced biofilm as detected by Congo Red Agar method; commonest being Staphylococcus epidermidis (53.9%). The percentage of resistance in the group which produces biofilm is always greater than the group which does not produce biofilm. Odds ratio for overall drug resistance was 2.10 (with 95% confidence interval as [1.6 – 2.6]) and with a p-value of 0.006.

Keywords: Biofilm, Staphylococcus, Blood stream infections

Introduction

Management of infections in ICU is one of the most challenging tasks for an intensivist in any hospital across the world; Blood stream infections (BSI) being the most common. It assumes the highest priority in terms of management due to high rates of patient morbidity and mortality; global rates of upto 40% [1] have been estimated. Staphylococcus aureus, Escherichia coli, Coagulase-negative Staphylococcus (CoNS), Klebsiella pneumoniae & Enterococci [2] are the 5 commonly detected bacteria in blood of septicemic patients. Staphylococcus aureus bacteremia is a serious infection associated with high morbidity and mortality because of its ability to cause metastatic lesions and persistent bacteremia. The risk factors for persistence include endovascular sources, cardiovascular prosthesis, metastatic infections, Vancomycin treatment, diabetes and biofilm production. Few organisms like CoNS are characterized by an ability to colonize the surfaces of biomaterials by adhering in biofilm-structured communities of cells encased in a self-produced polymeric matrix, an amorphous slimy material that is loosely bound to Staphylococcal cells. Slime is believed to make the micro-organisms more resistant to administered antibiotics and to host-defense mechanisms. Production of slime is characteristic of many strains of S. epidermis and S. aureus.

It is well known that slime production plays an important role in the pathogenesis of infections caused by different micro organisms especially Staphylococci. But very few studies have been conducted on isolates from human samples to find out the association between drug resistance and biofilm production. This study was conducted to estimate the percentage of Staphylococci producing biofilm and its association with drug resistance.
Methods and materials

It was a prospective study conducted in a 800 bed tertiary care centre with 30 bed ICU. The target population was patients admitted to ICU with a suspicion of Staphylococcal bacteremia. The clinical diagnosis of bacteremia was based on criteria of Bates & Herwaldt [3]. Two blood cultures were collected from each patient. After taking informed consent, 5 ml of venous blood sample was collected under aseptic precautions and inoculated in automated blood culture bottles containing Trypticase soy broth with antibiotic adsorbing beads. Gram stain and subculture were done on the bottles which were flagged positive by the instrument. Staphylococci were identified using standard biochemical tests.

Biofilm production was detected by growing the isolates on 0.8% Congo red agar. A positive result was indicated by black colonies on the surface & Non-slime producing strains developed red colonies after incubation 37°C for 24 h. Susceptibility to antibiotics was determined by the Kirby Bauer disk diffusion method on Mueller-Hinton agar plates. Twelve antibiotics were chosen for the study supplied by Hi Media Laboratories, Mumbai, according to CLSI 2015 guidelines. The antibiotics used were Penicillin (10U), Amoxicillin-Clavulanic acid (20/10µg), Gentamicin (10µg), Cefotaxime (30µg), Cefoxitin (30µg), Linezolid (30µg), Ciprofloxacin (5µg), Tetracycline (30µg), Co-trimoxazole (25µg), Clindamycin (2µg), Erythromycin (15µg), Teicoplanin (30µg).

To check the association between biofilm production and antibiotic resistance, odds ratio was used. The odds ratio is a measure of effect size, describing the strength of association or non-independence between two binary data values.

Results

460 patients satisfied the inclusion criteria from whom 2 to 5 ml of venous blood sample was collected and subjected to further tests. 94 of them yield growth of Gram Positive Cocci in clusters (suggesting Staphylococci) and were considered for further analysis. The following figure (Figure 1) shows the different species isolated

![ Species of Staphylococcus isolated](image)

Figure 1 – Species of staphylococci isolated

Bio-film formation was detected using Congo Red Agar. There were 26 (27.6%) isolates which produced bio-film. The following table (Table 1) gives details on different species of Staphylococci producing biofilms.
Table 1 – Species of staphylococci producing bio-film

<table>
<thead>
<tr>
<th>Species</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>8</td>
<td>30.7</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>14</td>
<td>53.9</td>
</tr>
<tr>
<td><em>Staphylococcus haemolyticus</em></td>
<td>2</td>
<td>7.7</td>
</tr>
<tr>
<td><em>Staphylococcus cohnii</em></td>
<td>2</td>
<td>7.7</td>
</tr>
</tbody>
</table>

The following graph (Figure 2) demonstrates the drug resistance with respect to biofilm production. The percentage of resistance in the group which produces bio-film is greater than the group which does not produce bio-film.

![Relationship of Drug resistance to Bio-film](image)

Figure 2 - Relationship of drug resistance to bio-film

However, significant increase in drug resistance is seen only in Co-trimoxazole, Cefoperazone, Ciprofloxacin and Clindamycin. (p<0.005) Odds ratio for overall drug resistance was 2.10 (with 95% confidence interval as [1.6 – 2.6]) and with a p-value of 0.006. This implies that the organism is 2.1 times more resistant to antibiotics if it produces bio-film. Odds ratio was calculated for individual drugs and the results are as follows (Table 3)

Table 3 – Odds ratio for different antibiotics

<table>
<thead>
<tr>
<th>Drug</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>1.98</td>
</tr>
<tr>
<td>Amoxy Clav</td>
<td>2.63</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2.79</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>2.76</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>0.93</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>3.21</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>4.22</td>
</tr>
</tbody>
</table>
Tetracycline 1.57
Erythromycin 1.80
Clindamycin 3.63

The odds ratio of most of the antibiotics is much higher than 1, indicating that drug resistance is more likely to occur in the bio-film producing group. The exception is Cefoxitin.

**Discussion**

Blood stream infections (BSI) are one of the most common nosocomial infections encountered in hospital especially in ICU patients, less so in ward patients. In the current study, a total of 460 patients were screened, out of which, 94 (20%) positive blood cultures were identified as Staphylococcal isolates by using the standard cultural and identification methods. This is in alignment with the prevalence rate as reported by Aygen and associates [4] and Mathur and colleagues [5].

Slime production plays an important role in the pathogenesis of infections caused by different microorganisms, especially staphylococci. In this study all the species of staphylococci produced bio-film except for *S. lugdunensis, S. capitis* and *S. warneri*. The data reported here indicate an important role of slime production as a virulence marker for clinically significant *S. epidermidis* isolates. The results are similar to those reported by other authors [6], who found that *S. epidermidis* frequently causes nosocomial septicemia, and infects indwelling medical devices like intravascular catheters and prosthetic valves. However, the findings of the present study showed that slime formation was not more prominent in *S. aureus* strains than in CNS strains isolated.

The odds ratio is the ratio of the odds of an event occurring in one group to the odds of it occurring in another group. The measure describes the strength of association or non-independence between two binary data values. In this case the groups are defined by the production and non-production of bio-film. An odds ratio of 1 indicates that the resistance to drug under study is equally likely to occur in the groups, i.e, bio-film producing and not producing. An odds ratio greater than 1 indicates that the resistance to drug more likely to occur in the bio-film producing group. And an odds ratio less than 1 indicates that the resistance to drug is less likely to occur in the bio-film producing group.

In this study it was found that the organism is 2.1 times more resistant to antibiotics if it produces bio-film. The odds ratio ranges from 1.57 (Tetracycline) to a high value of 4.22 (Ciprofloxacin). This indicates that existing drugs may be ineffective in treating isolates producing bio-films involved in bacteremia. This has been found by other studies also which involved prosthetic device related infections[7].

Unlike all other antibiotics, Cefoxitin gives an odds ratio of 0.93 which is less than 1. This indicates that susceptibility to this antibiotic is not affected by production of biofilm. In 2009, CLSI guidelines changed the method of choice to detect MRSA. They recommended Cefoxitin disc diffusion technique. Susceptibility to Cefoxitin not being affected by biofilm may be considered as an advantage to continue the use of this drug to detect MRSA. Kotulova [8] in his paper has discussed that organism producing biofilm is highly resistant to Gentamicin. This study also confirms this finding with an odds ratio of 2.79.
Conclusion

27.6% of the Staphylococcal isolates produced bio-film as detected by Congo Red Agar method. Bio-film production was seen maximum in *S. epidermidis* (53.9%) followed by *S. aureus* (30.7%). The percentage of resistance is always greater in the isolate which produces bio-film than isolate which does not produce bio-film with an odds ratio of 2.10. Significant increase in resistance is seen only to Cotrimoxazole, Cefoperazone, Ciprofloxacin and Clindamycin.

References

A Survey on Ethnomedicinal Plants of Pechipparai Hills, Southern Western Ghats of Tamilnadu, Kanyakumari District

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Abstract

The present ethno-medicinal study was carried out at 3 localities of Pechipparai hills of Southern Western Ghats of Tamilnadu, Kanyakumari district. Frequent field visits were made throughout the study period from 2012-2013. Data presented here are based on personal observations and interviews with informants like medicine men and village head men. In this context the Kani people has rich and abundant terminology. It is common that most of the medicinal plants have their own secret, for many safeguard their specialized knowledge. During the present course of investigation, a total 50 medicinal plant species were distributed across eighteen families and fifty genera. The result of the present study provide evidence that medicinal plants continue to play an important role in the health care system used by tribal community. Documentation of this knowledge is valuable for the communities and their future generations.

Keywords: Medicinal plant, ethno-medicine, Kani people, Western Ghats.

Introduction

Plant products are used as the main source of medicine throughout the world for treating various diseases. About 50% of the present day medicine in the United Nations of America are derived from natural sources especially from various plants. The uses of traditional medicine in both developing and developed countries is significantly increasing in recent time. There is growing demand for the types of medicines like Ayurveda, Siddha, Unani and Homeopathy. During the last two decades scientists all over the world are playing much more attention to the study of an ever branch of science Ethno-biology especially to tribal or ethno-medicine. Ethno-medical practices are preferred largely because medicinal plants are less expensive, readily available and reliable, and they are considered to have fewer side effects than modern medicines.

The World Health Organization (WHO) estimates that over 80 percent of people in developing countries depend upon traditional medicine for treatment of disease and other maladies in their primary health care. India is one of the leading countries in Asia in terms of the wealth of traditional knowledge systems related to the use of plant species and also known to harbor a rich diversity of higher plant species of which 7500 are known as medicinal plants (Kala, 2005). These tribal communities draw their sustenance largely from forests for food medicinal and other requirements. (Janaki Ammal, E.K et al., 1984).

Our country has one of the largest concentrations of the tribal communities in the world. About 68 million tribe people belonging to 573 tribal communities living in different geographic location in our country. Plants play a vital role for the existence of life on the earth and the uses of plant source of medicine is as old as humanity. Since most of this ethnic community do not have their over scripts and written language, the information about prescriptions, pharmacology, attitude towards diseases of the old age etc (Schultes, R.E, 1962),, The people belonging to modern societies are not aware of this rich knowledge system. Inspite of the fact the studies in tribal medicine have enabled to identify 1600 new drug yielding plants. (Ayyanar.M et al., 2005). Thus it has become imperative to collect information and document the same and study them scientifically.
Tribal are in general the followers. It leads to the belief that diseases and death are caused by certain spirits super natural power. These belief have a great influence on their attitude and Psychology about the ailments. In general the tribal people give name to the plants with known good or bad properties. Thus more than one local name have been attributed to a plant which generally creates confusion to ascertain the actual plant species. (Ngari E.W, et al., 2010)

There are wide range of animistic conceptions associated with vegetation groves and forest worships. The tribals are in belief of supernatural power and have doctrine that the landmark things like big trees and unique vegetation of medicinal plants. (Usha.M, 2012) Useful plants near or inside the tribal villages are protected for respective utility. (Ignacimuthu et al., 1998) In many cases they never take whole plants or all fruit for the use but they leave some reproductive parts for next generation. They avoid harvesting medicinal plants in the evening. Some vegetables are not consumed at the ripening stage of the fruit.

The main objective of the ethno-botany is not only to trap the old traditional folk knowledge but also to testify the knowledge and to find out the new resources of various utilization. The present study is focused on the survey of ethno-medicinal plants in Pechipparai hills, Southern Western Ghats of Tamilnadu.

Materials and methods study area

The present study was carried at 3 localities (Valayamthuki, Alamparai, Andipothai) in Pechipparai hills of Southern Western Ghats of Tamilnadu, Kanyakumari district. (8°03’ – 8°35’ N and 77°05’ – 77°36’ E), which is located in the lap of western Ghats. This district covers an area of about 1684 sq km, surrounded by three seas (Gulf of Mannar, Indian Ocean and Arabian Sea), Southern Western Ghats and plains of Kerala. The annual rainfall varies from 89 – 254 cm, and maximum and minimum temperature were 24°C – 28°C in winter and 28˚ C – 32˚C in summer respectively.

The present study of ethno-medicinal plants was carried out at 3 localities of Pechipparai hills of Southern Western Ghats of Tamilnadu, Kanyakumari district. Frequent field visits were made throughout the study period from 2012 -2013. Data presented here are based on personal observations and interviews with informants like medicine men and village head men. Plants were identified botanically using the Flora of Presidency of Madras (Gamble, 1915) and the Flora of Tamilnadu Carnatic (Mathew, 1983).

Results and discussion

Habit wise analysis of these medicinal plants indicates that majority of the plants belongs to shrub category. The highest proportion of Shrubs (62 %) is followed by herbs (14 %), climber (14 %) and trees (10 %). It was interesting to note that 50 plants are distributed among 28 families of these 47 plants belongs to dicots and remaining 3 plants constitutes the monocots (Table 1).

<table>
<thead>
<tr>
<th>Habit</th>
<th>No of Plants</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shrubs</td>
<td>31</td>
<td>62</td>
</tr>
<tr>
<td>Herbs</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Climbers</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Trees</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

Based on the present study, it has been found that the tribal community of Pechipparai hill is rich in ethno-biological knowledge and this knowledge is being transmitted from one generation to another in the verbal form. Traditional medicines are the primary health care resources for the tribes to protect their health.
During the present course of investigation, a total 50 medicinal plant species were distributed across eighteen families and fifty genera. The plants are arranged alphabetically with binomial name followed by habit, family, parts used and its uses were documented. The dominant families with more number of medicinal plants in the present study are Euphorbiaceae (6 species) followed by Fabaceae, Verbenaceae and Lamiaceae (4 species each), Malvaceae, Oxalidaceae, Rutaceae, Asclepiadaceae, Solanaceae, Acanthaceae, Aamaranthaceae and Liliaceae (3 species each), Tiliaceae and Vitaceae (2 species each). Four families were represented with only one species.

<table>
<thead>
<tr>
<th>Table 2: Plant part wise representation of ethnomedicinal plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plants Used</td>
</tr>
<tr>
<td>Leaves</td>
</tr>
<tr>
<td>Whole Plant</td>
</tr>
<tr>
<td>Roots</td>
</tr>
<tr>
<td>Seeds</td>
</tr>
<tr>
<td>Bark</td>
</tr>
<tr>
<td>Flowers</td>
</tr>
<tr>
<td>Fruit</td>
</tr>
<tr>
<td>Latex</td>
</tr>
<tr>
<td>Stem</td>
</tr>
<tr>
<td>Flowers</td>
</tr>
</tbody>
</table>

Most of the plants listed above are used in external application and some plants are used internally. A few plants are used in both ways. External applications is either in the form of poultice, oil paste or extract. For this preparation although root tuber, bark, stem, leaves and whole plants are used. The plants are used to make different preparations like Suranam, Kashayam, Thailam, Kulisai, Powder form etc. All these plants are available in tribal community and are used by the local people as home remedies. They have acquired this art from their ancestors. The plant part wise representation of the species indicates that highest part used is leaves followed by whole plant and other parts of the species (Table 2).

Our present study showed that the few ethno-medicinal plants belongs to the highest proportion of shrubs followed by herbs, climbers and trees. Though the plants are introduced from different parts of the hills, they influence the culture and the traditional system of the tribals. The local people utilize the medicinal plants for the treatment of several diseases.

References

Survey and Comparison of Floristic Diversity and Ethnic culture in Punikkolkavu and Chirakkakavu Sacred Groves of Thalassery, Kannur District, North Kerala, India

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Abstract

Sacred groves exist in various parts of the country and are unique examples of ecological understanding and management. These are locked information sites. The Sacred grove concept is one of the strategies developed by many human societies to conserve biological resources using a traditional approach. In the present study deals with the floristic comparison and ethnobotanical practices of the two sacred groves, Punikkolkavu and Chirakkakavu, Kannur District, Kerala. Punikkolkavu is rich in plant diversity when compared to Chirakkakavu. A total of 70 plant species belonging to 36 families were located in Punikkolkavu and 41 plant species belonging to 22 families were located in Chirakkakavu were recorded. The mode of mythological and therapeutical uses and conservation practices of these plants by the local people have been recorded.

Keywords: Sacred groves, Biological resources, Ethnobotany, Punikkolkavu and Chirakkakavu

Introduction

Biodiversity is the most valuable natural resource without which the overall development of man is not possible. Conservation and Management of Biodiversity is one of the foremost needs as vast expanses of vegetation continue to be under the threat of denudation and degradation all over the world. The western Ghats is one of the three biodiversity centers of India, which in turn is one of the 10 mega biodiversity centers of the world (Nayar, 1996), in which Kerala contributes a major part.

The Sacred grove concept is one of the strategies developed by many human societies to conserve biological resources using a traditional approach. Recognizing the importance of sacred groves, both in terms of conservation of biodiversity and cultural diversity, and in view of the threats faced by the groves, the Government of India has launched a Scheme 'Protection and Conservation of Sacred groves' within its programme 'Intensification of Forest Management'. As a part of this Central Government sponsored Scheme, the Department of Forests and Wildlife

Sacred groves are patches of natural vegetation dedicated to local deities and protected by religious tenets and cultural traditions; they may also be anthropogenic tree stands raised in honour of heroes and warriors and maintained by the local community with religious favours (Ramanujan and Cyril, 2003). Trees are a form of nature which represent life and the sacred continuity of spiritual, cosmic and physical worlds and are the first temple of gods (Frese et al., 1998). Trees may be ‘holy’, ‘blessed’ or ‘sacred’, depending upon the religious attitude of people towards them. A ‘holy’ tree is a species of which all parts are worshipped, e.g. Ficus religiosa Lev. (peepal), Ficus benghalensis L. (bargad).

Sacred groves also help in soil and water conservation, besides preserving biodiversity. The ponds and streams adjoining the groves are perennial water sources, and are often the only source for many of the animals and birds that make them their habitat, especially during summer. Sacred groves are extremely rich in floral and faunal elements. The origin of the groves is likely to have followed the introduction of agriculture. It is often believed that during shifting
cultivation a part of the forest is left undisturbed. Here all the species found in the area are protected. These areas might have developed as sacred groves. Sacred Groves often protect watersheds and water sources. Groves are the result of the reasoned assertion rather than the instinctive behavior of the communities. The taboos, rituals and religious beliefs associated with the groves, supported by mystic folklore, have been the prime motivating factors for preserving the sacred groves.

North Kerala especially the district of Kannur is the pace of temples and folk arts. It is endowed with a number of temples with different worshipers and folk arts like theyyam. According to one version the name Kannur might have assumed its name from one of the deities of the Hindu pantheon, a compound of two words, Kannan(Lord Krishna) and Ur (place) making it the place of Lord Krishna.

Kannur district is known as the land of looms and lores, because of the number of looms functioning in the district and festivals held in temples. The district is the major centre of theyyam, a ritual dance of northern Kerala, and small shrines known as kaavu’s associated with the theyyam dot the district. Many plant parts, flowers, plant extracts are used for theyyam. Every ritual performed in the temple is conducted very traditionally, for example many plants are not eaten before and during religious rites, some plants are used for achieving some specific rituals.

Many of the groves in kannur are bestowed with a rich variety of flora and vegetation. All activities of life in this land centre on trees and flowers. All forms of vegetation in these groves including shrubs and climbers are supposed to be under the protection of reigning deity of that temple and the removal of even a small twig is considered as taboo. The study of conservational practices according to their beliefs is an interesting area. This paper is the first record on the floristic composition and ethno botanical practices on the unreported sacred groves of Thalassery, kanuur district, Kerala.

The present study was undertaken to find the plant diversity in the selected groove and identify the plant species that are used for various religious purposes in the groves. No such study has been undertaken in these groves so far. The specific objectives of the present study are, Preparation of an inventory of plant species conserved in and around the groves, Identification of various plants/plant parts used for religious purpose like Pujas as offerings etc., to study the religious, ritual practices and role of plants and study the mythology behind these practices.

**Materials and methods**

Extensive field visits were carried out to document the floristic diversity. The plants were collected and identified with the aid of regional floras, checklists and herbaria. Personal cross-interview with the local people and authentic literature reference were performed to ascertain the economic importance of the plants. Information of the historical background and social composition were collected from the revenue authorities and traditional leaders. The traditional beliefs, taboos, restrictions and folklores pertaining to the Grove are collected from villagers. Workers of Hindu religious department and devotees, inside the temple are crosschecked during worships and festivals were observed closely for corroboration and the field visits were timed accordingly.

The plant specimens were collected either in flowering or fruiting stage and photographs were taken from the study area. Further, specimens were processed as per routine herbarium techniques and deposited in department of botany, Nirmala college for women, Coimbatore. The specimens were identified with help of different floras, monographs, Gamble, revisions and other available literatures. Most of the information was collected from the elderly people, village head, and headman of the groves and also people with the diverse use of plants.
**Study area**

In Kerala, based on management systems, sacred groves can be categorized into three types. They are, sacred groves managed by individual families, by groups of families and by the statutory agencies for Temple management (Devaswom Board). Area selected for the present study is North Malabar region of Kerala, located to the North east of Kerala within the geographical limit 11° 18' to 12° 48' N latitude and 74° 52' to 76° 07' E longitude, in Kannur district. Topographically the area consists of a narrow coastal belt, undulating midland and mountainous high range. The climate is typical warm-humid tropical type with mean temperature range of 22°C–37°C and relative humidity between 70% and 90%. Studies were undertaken during November 2014 to March 2015 in Punikkol Kaavu and Chirakkakaavu sacred groves, both are located in Thalassery, Kannur District, Kerala (Figure-1).
Results and discussion

During the present study, sacred groves were visited frequently and plant species were documented. These culturally valued species are often ecologically important keystone species, which by their key role in ecosystem functioning contribute to support much biodiversity associated with it. Enumeration provides the list of plant species with scientific name of plants are represented in table 1 and table 2. The primary motive behind the constitution of sacred groves is basically spiritual. However, these groves which doubled as biodiversity conservation areas have almost disappeared. Although the demolition of the groves started over 20 years ago, our results reveal that a greater percentage of them were demolished within the last decade.

The name of grove was given on the name of deity worshipped in the groves. The fruits of many species were used as food and also for performing various religious functions. The villagers also disclosed the fact that the soil in the sacred grove site remained more fertile than the adjacent sites of the village. This was possible due to high bio-mass and accumulation of high organic contents in such sites and further decomposition and nutrients release in such ecosystem.

Habitat fragmentation is a pervasive threat to forest ecosystems throughout the world, eventually leading to a decline in biological diversity and impairment of ecological processes. A number of studies in the last couple of decades have addressed the ecological, demographic and genetic consequences of small fragmented populations. These studies highlight the importance of a set of small groves in harboring the variability among them in an endemic and endangered species of both flora & fauna. The present study is to compare the vegetation in two groves and survey the ethnic culture.

Punikkolkaavu

The presiding deity in this punikkolkaavuis Saasthappan, represents Lord Shiva. The co-deities are Gulikan, Chaamundi and Rakthachaamundi. The grove has more than 100 years old. There is a place for Nagam in the Grove. Noorumpaalum is the main worship for Nagam and Pooyavaykkal is the main worship for Saasthappan.

The Grove has large vegetation around an area of one acre. These plants are protected because of some believes. 70 plant species belonging to 36 families were located from the Grove and these are tabulated in table 1. Photos of some plants also included (plate1-4). The plant families dominated are Fabaceae with 6 species, Lamiaceae with 5 species, Asteraceae with 4 species etc. The plant diversity includes Climbers, Herbs, Shrubs, and Trees etc. These are plotted in a graph (Figure 1). Herbaceous plants were dominated over other life forms representing 22 species followed by trees, 15 species shrubs 14 species etc. Only one fern can be located. The population is largely concentrated in Mimosa pudica, Caryotaurens, Cocosnucifera etc. Some plants like Musa, coconut areca etc are cultivated.

All plant species except one or two are economically important and almost90% plants are used as medicine by indigenous people. (Figure2) shows the percentage of plant parts used by people for various purposes, mostly entire plant is used. Several Sacred plants are there in the Grove. They are Ocimum, Champaka, and Strychnosetc. Different plants are used for worshipping the God (Plate 8 a.) Tender leaves of coconut are used to prepare Thirumudi of Theyyam and also ornaments during the festival (Plate 8 b.).

Chirakkakavu

The presiding deity of this temple is the goddess Kali, who is worshipped in three forms, or Thrigunaathmika. The temple was built by the king of Kolathiri- Chirakkkal Raja after the swayambhoo of the goddess in Koduvally River, and so became renowned as Sree Chirakkakavu. Guntur Kottavaanavar, Ilankarumakan and Poothadi are in a single shrine near the
temple. Outside the Nalambalam there is a Sarppakkavu (Snake Shrine) in the north-west corner of the temple which contains Nagaraja, Nagakanyaka and Chithrkooodam.

In this Grove the plants are conserved more than one acre area. It is tabulated (Table2).42 plant species belonging to 22 families were located. The plant families dominated are Fabaceae and Asteraceae. Here trees are dominant over other life forms. Figure 3 shows the graph of species diversity. Most common tree is Ficus (Plate 9 a.). These are protected in a large area. The area is very cool also. Some rare plants are located here. One of the rare plants that can be seen in the Grove is Rudraksha. It has several medicinal uses. Also this plant is considered as Sacred. Most of the plants are economically important. They are used as medicine to cure various diseases. Percentage of plant parts used was represented by pie diagram (Figure4).

When compared with Punikkol Kavu, the number of plants is lesser in Chirakkakavu. That is plant diversity is higher than Chirakkakavu. But the number of species is higher there. For instance large numbers of Ficus plants are protected in a large area. Rare plants were located in both Groves. The plant diversity includes climbers, herbs, shrubs, trees etc. In diversity trees are dominant in Chirakkakavu, where as herbs are dominant in Punikkolkavu. Different plants and their used were tabulated. A rare tree seen in Chirakkakavu is Rudraksha. Its seed is considered as sacred. Some plants are common in two Groves. These include Cocosnucifera, Mangiferaindica, Mimosa pudica, Pothossrandens, Tectonagrandis, Strychnosnux-vomica, Heliotropiumindicum etc. Most of the plants located from the Groves are economically useful. Among them most have medicinal importance. Of these entire plant is used more as medicines. Both the Groves are associated with pond.

Festivals are celebrated in two Groves. The difference is that the main Deity in Punikkolkavu is Sasthappan and that in Chirakkakavu is Devi. The other Deities are also different in these two Groves. These Groves plays a very important role in maintaining the unity of the village people and also the traditional culture. Some plants in both the Groves are considered as Sacred (Table 3).These plants are protected on the basis of some believes.

The present findings are comparable with other studies in Sacred Groves of Kerala. The floral diversity in fresh water and salt water Sacred Groves was compared by Deep mol and Khaleel in Kannur district. Variation in species was reported by them. Ethno botanical study about medicinal plant was done by Harsh et al., reported a variety of plants and it was represented by Graph and Pie diagram. Similar studies are following. Chandrashekara and Sankar (1998) recorded 73 species from 3 Sacred Groves, Subrahmany Prasad and Raveendran (2013) documented 187 vascular plants from Niliarkottam Sacred Grove in Kannur district. Divya and Manonmani reported 50 plant species from Sacred Groves of Nemmara in Palakkad District. Similar studies were done in other States also.

Ethnic culture

In both the Groves festival is celebrated every year. Different plant parts were used for worshipping God and also for making thirumudi and ornaments of God during festival. Mainly tender leaves of coconut were used.

Punikkolkavu

Each year festival is celebrated during December. During festival Theyyam will be there. Theyyam is a corrupt form of Daivam or God. People of this district consider Theyyam itself as a God and they seek blessings from this Theyyam.
Gulikan

Gulikan Theyyam is worshipped as the Lord Shiva. In every Kaliyattam, the performance of Gulikan Theyyam is inevitable. The 'Kanhiram' and ‘Chempaka’ are important for Gulikan. The story behind Gulikan is following.

Once there was a saint, who was a great devotee of God Shiva. He has no children. As a result of continuous prayer, God blessed him with a child, who was very intelligent and smart. But God remind him that his son will live only up to 18 years old. Thus the child born and he was named Markandeya. He grew up as a great devotee of Shiva. When he attains 18 years the God of death (Yama) came to kill him. But he ran and tightly hold the Shivalinga. But the God of death tried again to take the boy along with Him. God Shiva gets angry and He killed the God of death by the power of His third eye. After this there is no death occur in the earth. So the Goddess of earth (Bhoomi Devi) could not bear the weight and She went to Shiva for a solution. Thus He creates another God of death named Gulikan.

Vishnumoorthi (Chaamundi)

An inevitable constituent in a majority of the Kaliyattams is the performance of the Vishnumoorthi Theyyam and its performance includes complicated rites and rituals. The peculiar drum-beats can be heard up to a distance of 2 km from where the performance of the Vishnumoorthi Theyyam takes place. The enactment involving the Narasimha Avathara of Lord Vishnu by the Koladhari especially thrills the devotees and the spectators as a result of the body movements involved in it. More than a Myth describes the origin, features, rites, centres of worship etc of the Vishnumoorthi Theyyam.

Kuttichathan (Sasthappan)

Kuttichathan’s mythological story is very impressive and a hyperbole. The ‘Lord Shiva’ and his wife ‘Devi Parvathi’ were staying in a remote hill area along with ‘Valluvar’ communities. The ‘Lord’ had two children with ‘Valluvathi’, ie, ‘Karuval’ and ‘Kuttichathan’. The later born in a peculiar manner with flower on his forehead, a third divine eye and a black body with long white stripes. There lived a ‘Kalakadu Namboothiri’; his wife did not conceive a child. As a result of his prolonged fasting the ‘Lord’ decided to donate second child of ‘Valluvathi’ to the Namboothiri family. The child had his early education and was showing certain mischievous acts during his childhood. He even beheaded the cows and drinks its blood to quench his thirst. They felt his actions were beyond their endurance and they killed him.

But even after his death they could hear the unbodied sound of him reverberating inside their ‘Illam’ (House). To get rid of his disturbances they conducted ceremonial fire ‘Kuttichathans’ were is emerging. It was ‘Chalaperumalayan’ alloted certain land for the construction of ‘Sthanas’ for ‘Kuttichathan’ and they started to perform the theyyakolam.

Chirakkakaavu

The temple festival is celebrated every year on days 9-12 of the month of Medaom which usually falls on April 22 to 25th. On Medom 9th the than trikpoojas and Uthsavabali is observed. In the evening the Uthsalvakolam (Thidambu) is taken out of the temple as Ezhunnallathu by the temple priest. This occasion is the only time where the goddess comes out of the temple in full alankaras in UlthasavaThidambu. Bhagavathi, Puthiya Bhagavathi and Cheriya Thampuratty, are the daughters of the mother goddess and Valiya Thampuratty. Guntoor Kottavanavan, Ilankarumakan, Poothadi are the other male Theyyams here. On Medom 10th the Nattathira is celebrated here. On 11th MedomAriyalavu is observed. This is a practice of giving rice.
Theyyam

This is the next three-day festival. The four manifestations of the goddess, Chorakalathil pulses and coconut oils to the all concerned communities to the kavu. This custom recalls the riches of bygone times. In the olden days, the Temple owned land from Vamla to Kali. In the morning of 11th medom Valaiya Thampuratty visits all devotees in the village (hosuses comes under Anuchandey Parambu) and blesses the devotees.

Kalasams come from various parts of the villages to make offerings to the Devi. MothaKalasam and Vaikalasam have the right to enter to the temple first. By the early hours of 12th Medom. The Theyyams start to come to the Thirumuttam in this order: Guntoor Kotta Vanavar, Chorakalathil Bhagavathi and Puthiyabhagavathi. It is considered a blessing when a few drops of rain sweep through as Chorakalathilbhagavthitheyyam appears on the courtyard of the temple. A Mulla Mala (Jasmine garland) is the traditional offering to ChorakkalathilBhagavathi. Thirdly comes the Theyaam of PuthiyaBhagavathi (Theethira), the Theyaam comes with the fire as ornaments and blesses the devotees. By the morning of 12th Medom the theyyam of Ilankarumankan and Poothadi appears on the courtyard of the temple. The theyyam is of divine war between the two. This theyyam makes us to recollect the thought of Bali–Sugreeva war.

Then Thampuratty comes out into the courtyard. The Aattam of Thampuratty with her divine sword is a very rare sight which gives her devotees a life’s blessings. Then the Cheriya Thampuratty appears on the courtyard of the temple with beautiful white hair and other decorations. Then there Thampuratty with Thirumudy and her daughter, Cheriya Thampuratty travel around the temple, followed by the kalasalams and devotees in procession

Conclusion

Sacred Groves are one of the examples of traditional conservation practices of plants. These are patches of natural, near-climax vegetation managed as a part of local cultural tradition and dedicated to certain Deities. Any form of cutting or removal of trees or their parts in the Grove is prohibited, lest it should invite calamities.

The present study is an attempt to compare the floristic diversity in two Sacred Groves and also survey about the ethnic culture. The study reveals that Sacred Groves are rich sources of medicinal plants with many rare species. Sacred Groves are the seat of rare and endangered species of plants. These are being preserved because they encompass village Gods within the Grove, which are worshipped as religious beliefs and taboos of the people weaken, the pressure of these forest increases. The study establishes the role of Sacred Groves in conserving the biodiversity.

Two groves are compared and found most of the plants are common in both the Groves. The species diversity in two Groves are tabulated. Punikkolkavu is rich in diversity when compared to Chirakkakavu.70 plant species belonging to 36 families were located from Punikkolkavu and 41 plant species belonging to 22 families were located from Chirakkakavu. Different habits of plants were plotted by using graph. Percentage of plant parts used is also represented by using pie diagram. In both the Groves plants were seen in which mostly entire plant is used. Also some plants are peculiar in each Grove. In punikkolkavu, herbs are occurred in larger number and in Chirakkakavu trees are larger number. Most of the plants have economic importance, especially medicinal values. Some plant parts like tender leaves of coconut are used during festivals for making Thirumudi and ornaments of God. Some plants are considered as Sacred. These plants are protected and worshipped.

Festivals were celebrated every year. This will helpful for maintaining unity between the people in the village. These Groves also plays an important role in controlling pollution,
conserving medicinal plants, maintaining unity of the society etc. Thus Sacred Groves are an inseparable unit of our culture.

References

FIGURE 4: Species diversity in chirakkakavu

FIGURE 5: Percentage of plant parts used
PLATE 1     Punikkol Grove

Gycosmis maurihna L

Diplocyclos palmatus Arundinella purpurea
(L.)Jeffrey Hochst.exSteud.

Xenostegia tridentate
(L.)Aushin&Staples

Anamirta cocculus
(L.)Wight & Arn

Wattakaka volubilis Stapf.

Cyclea peltata
(Lam.)Hookf&Thomson

Macaranga peltata
Roxb.Mueller

Ocimum tenuiflorum L.
PLATE 2

Grewia nervosa Lour.  Curcuma aromatica Salisb  Lanthana camara Linn.

Glycosmis pentaphylla (Retl.)DC.  Synedrella nudiflora (L.)Gertn.  Musa paradisiaca L.

Drynaria quercifolia (L.)DC.ex Wight  Cosmostigma recemosum(Roxb.)Wight.  Urena lobata L.
PLATE 3

*Caesalpinia mimosoides* Lam

*Triemfetta rhomboidea* Lam

*Strychnos nux-vomica* Linn

*Hyptis suaveolens* (L.) Poit.

*Mallotus philippensis* (Lam.) Mull-Arg

*Sida alnifolia* L.

*Leucas lavandulifolia* L.

*Ziziphus oenopolia* (L.) Mill.

*Caryota urens* L.
PLATE 4

Grewia hirsute Vah.

Ichnocarpus frutescens (L.) R.Br.

Heliotropium indicum L.

Pothos scandens L.

Centrosoma molle Benth.

Acmella paniculata (Kunth.) Cass.

Biophytum sensitivum (L.) DC.

Glirisedia sepium (Jacq.) Kunth ex Walp.

Plumeria rubra Poir.
a) Front view of the Grove

b) Devasthanam

c) Pond behind the Grove
PLATE 6

a) Plants are protected

b) Data collection

c) Preparing thirumudi

d) Front view of (the temple in) Punikkol kaavu
PLATE 7

a) Grove during festival  
b) Preparing thirumudi of Gulikan  
c) Saasthappan theyyam  
d) Rakthachamundi
a) Use of different flowers for worshipping God (Pooya vaykkal)

b) Use of tender leaves of coconut for making the ornaments of God.
CHIRAKKAKAVU

a. Overview of the Grove  b. 

c) Ficus trees are protected
PLATE 10

a) Plants in front of the Grove

b) Grove from one side

c) Sarppakavu
PLATE 11: PLANTS SEEN IN CHIRAKKAKAVU

*Alstonia scolaris* R.Br.

*Naregamia alata* W & A.

*Chromolaena odorata* (L.)King & H.E.Robins.

*Charsalia curviflora* (Wll.ex Kurz.)Thvaiten

*Emilia sonchifolia* (L.)DC.EX Wight.

*Tridax procumbance* L.

*Bauhinia acuminata* L.

*Ixora coccinia* L.
PLATE 12: Plants in Chirakkakvu

*Anisochilus carnosus* (L.f.)Wall.ex Benth.  
*Ruellia tuberosa* L.  
*Vanda sp.*

*Sida acuta* Burm.f.  
*Hibiscus rosacinensis* L.  
*Areca catechu* L.

*Mimosa pudica* L.  
*Setaria parviflora* L.  
*Adenanthera pavonina* L.
PLATE 13: SOME TREES SEEN IN CHIRAKKAKAVU

Caryota urens L.

Anacardium occidentale L.

Artocarpus heterophyllus Lam.

Syzygium cumini (L.) Skeels.

Careya arborea Roxb.

Tectona grandis L.f.
PLATE 14

a) Ilamkarumakan and Poothadi

b) Thamburaatti

c) Kalasham

d) Thee Thira
<table>
<thead>
<tr>
<th>NAME OF THE PLANT</th>
<th>FAMILY</th>
<th>HABIT</th>
<th>PARTS USED</th>
<th>USES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Acmella ciliate</em> (Kunth) Cass.</td>
<td>Asteraceae</td>
<td>Herb</td>
<td>Flower</td>
<td>It is an ornamental plant. Used as an insecticide and as a herbal remedy for toothache and oral infections.</td>
</tr>
<tr>
<td>5. <em>Areca catechu</em> L.</td>
<td>Aracaceae</td>
<td>Tree</td>
<td>Entire plant</td>
<td>Extract have anti depressant properties.</td>
</tr>
<tr>
<td>6. <em>Arundinella purpurea</em> Hochst.ex Steud.</td>
<td>Poaceae</td>
<td>Herb</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7. <em>Arundinella setosa</em> Trin.</td>
<td>Poaceae</td>
<td>Herb</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8. <em>Biophytum sensitivum</em> (L.)DC</td>
<td>Oxalidaceae</td>
<td>Herb</td>
<td>Entire plant</td>
<td>Extracts are antibacterial, anti Inflammatory, antioxidant, antitumor, radio protective, ant metastatic, ant angiogenesis, wound healing, immunomodulation, anti diabetic and cardio protective in nature.</td>
</tr>
<tr>
<td>9. <em>Caesalpinia mimosidis</em> Lam.</td>
<td>Fabaceae</td>
<td>Woody climber</td>
<td>Roots, stem</td>
<td>Gallic acid is extracted from the plant.</td>
</tr>
<tr>
<td>No.</td>
<td>Scientific Name</td>
<td>Family</td>
<td>Type</td>
<td>Parts Used</td>
</tr>
<tr>
<td>-----</td>
<td>----------------------------------</td>
<td>--------------</td>
<td>---------</td>
<td>---------------------</td>
</tr>
<tr>
<td>10</td>
<td>Carica papaya L.</td>
<td>Caricaceae</td>
<td>Tree</td>
<td>Stem, bark, fruit, leaves</td>
</tr>
<tr>
<td>11</td>
<td>Caryota urens L.</td>
<td>Arecales</td>
<td>Palm</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Cassia fistula L.</td>
<td>Fabaceae</td>
<td>Tree</td>
<td>Fruit, leaves</td>
</tr>
<tr>
<td>13</td>
<td>Centrosema molle Benth.</td>
<td>Fabaceae</td>
<td>Climber</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>Charsalia curviflora (Wall.ex Kurz)Thvaiten</td>
<td>Rubiaceae</td>
<td>Shrub</td>
<td>Leaves, root</td>
</tr>
<tr>
<td>15</td>
<td>Chromolaena odorata King</td>
<td>Asteraceae</td>
<td>Shrub</td>
<td>Entire plant</td>
</tr>
<tr>
<td>16</td>
<td>Cissus latifolia Lam.</td>
<td>Vitaceae</td>
<td>Climber</td>
<td>Root</td>
</tr>
<tr>
<td>17</td>
<td>Cleome viscose L.</td>
<td>Capparaceae</td>
<td>Herb</td>
<td>Leaves</td>
</tr>
<tr>
<td>18</td>
<td>Cocos nucifera L.</td>
<td>Araceae</td>
<td>Tree</td>
<td>Entire plant</td>
</tr>
<tr>
<td>19</td>
<td>Cosmostigma racemosum (Roxb.)Wight.</td>
<td>Asclepiaideae</td>
<td>Twining shrub</td>
<td>Leaves</td>
</tr>
<tr>
<td>21</td>
<td>Cyclea peltata (Lam.) Hookf.&amp;Thomson</td>
<td>Menispermaceae</td>
<td>Climber</td>
<td>Root</td>
</tr>
<tr>
<td>22</td>
<td>Cynodon dactylon (L.)Pers.</td>
<td>Araceae</td>
<td>Herb</td>
<td>Entire plant</td>
</tr>
<tr>
<td>No.</td>
<td>Plant Name</td>
<td>Family</td>
<td>Type</td>
<td>Part Used</td>
</tr>
<tr>
<td>-----</td>
<td>-----------------------------------------------</td>
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</tr>
<tr>
<td>24</td>
<td><em>Drynaria quercifolia</em> (Linn.) J.Smith.</td>
<td>Polypodiaceae</td>
<td>Fern</td>
<td>Rhizome</td>
</tr>
<tr>
<td>25</td>
<td><em>Elephantopus scaber</em> L.</td>
<td>Asteraceae</td>
<td>Herb</td>
<td>Entire plant</td>
</tr>
<tr>
<td>26</td>
<td><em>Emilia sonchifolia</em> (L.) DC. ex Wight</td>
<td>Asteraceae</td>
<td>Herb</td>
<td>Entire plant</td>
</tr>
<tr>
<td>27</td>
<td><em>Ficus religiosa</em> (L.)</td>
<td>Moraceae</td>
<td>Tree</td>
<td>Bark, leaves, fruits.</td>
</tr>
<tr>
<td>28</td>
<td><em>Gliricidia sepium</em> (Jacq.) Kunth ex Walp.</td>
<td>Fabaceae</td>
<td>Tree</td>
<td>Entire plant</td>
</tr>
<tr>
<td>29</td>
<td><em>Glycosmis mauritiana</em> Tanaka.</td>
<td>Rutaceae</td>
<td>Shrub</td>
<td>Entire plant</td>
</tr>
<tr>
<td>30</td>
<td><em>Glycosmis pentaphylla</em> (Retl.) DC.</td>
<td>Rutaceae</td>
<td>Small tree</td>
<td>Fruits</td>
</tr>
<tr>
<td>31</td>
<td><em>Grewia hirsute</em> Vah.</td>
<td>Tiliaceae</td>
<td>Herb</td>
<td>Root, leaves</td>
</tr>
<tr>
<td>32</td>
<td><em>Grewia nervosa</em> (Lour.)</td>
<td>Tiliaceae</td>
<td>Shrub</td>
<td>Leaves, bark</td>
</tr>
<tr>
<td>33</td>
<td><em>Heliotropium indicum</em> L.</td>
<td>Boraginaceae</td>
<td>Herb</td>
<td>Leaves</td>
</tr>
<tr>
<td>34</td>
<td><em>Hyptis suaveolens</em> (L.) Poit.</td>
<td>Lamiaceae</td>
<td>Herb</td>
<td>Entire plant</td>
</tr>
<tr>
<td>35</td>
<td><em>Ichnocarpus frutescence</em> (L.) R.Br.</td>
<td>Apocynaceae</td>
<td>Shrub</td>
<td>Root, bark</td>
</tr>
<tr>
<td>36</td>
<td><em>Ixora coccinia</em> L.</td>
<td>Rubiaceae</td>
<td>shrub</td>
<td>Entire plant</td>
</tr>
<tr>
<td>No.</td>
<td>Species</td>
<td>Family</td>
<td>Type</td>
<td>Part Used</td>
</tr>
<tr>
<td>-----</td>
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</tr>
<tr>
<td>37</td>
<td><em>Lantana camara</em> Linn.</td>
<td>Verbenaceae</td>
<td>Shrub</td>
<td>Entire plant</td>
</tr>
<tr>
<td>38</td>
<td><em>Legerstromia microcarpa</em> Wight.</td>
<td>Lythraceae</td>
<td>Tree</td>
<td>Wood</td>
</tr>
<tr>
<td>39</td>
<td><em>Leucas lavandulifolia</em> L.</td>
<td>Lamiaceae</td>
<td>Herb</td>
<td>Leaves</td>
</tr>
<tr>
<td>40</td>
<td><em>Loranthus ferrugineus</em> Roxb.</td>
<td>Loranthaceae</td>
<td>Climber</td>
<td>Leaves, fruits, flowers.</td>
</tr>
<tr>
<td>41</td>
<td><em>Macaranga peltata</em> Roxb. Mueller</td>
<td>Euphorbiaceae</td>
<td>Tree</td>
<td>Leaves, wood</td>
</tr>
<tr>
<td>42</td>
<td><em>Mallotus philippensis</em> (Lam.) Mull-Arg.</td>
<td>Euphorbiaceae</td>
<td>Tree</td>
<td>Entire plant</td>
</tr>
<tr>
<td>43</td>
<td><em>Mangifera indica</em> L.</td>
<td>Anacardiaceae</td>
<td>Tree</td>
<td>Fruits, seeds</td>
</tr>
<tr>
<td>44</td>
<td><em>Mimosa pudica</em> L.</td>
<td>Leguminosae</td>
<td>Herb</td>
<td>Entire plant</td>
</tr>
<tr>
<td>45</td>
<td><em>Mimusops elengi</em> Linn.</td>
<td>Sapotaceae</td>
<td>Tree</td>
<td>Bark, flowers seeds, fruits</td>
</tr>
<tr>
<td>46</td>
<td><em>Mucuna pruriens</em> (L.)DC.</td>
<td>Fabaceae</td>
<td>Climbing shrub</td>
<td>Entire plant</td>
</tr>
<tr>
<td>47</td>
<td><em>Musa paradisiacal</em> L.</td>
<td>Musaceae</td>
<td>Herb</td>
<td>Entire plant</td>
</tr>
<tr>
<td>48</td>
<td><em>Mussaenda frondosa</em> L.</td>
<td>Rubiaceae</td>
<td>Shrub</td>
<td>Entire plant</td>
</tr>
<tr>
<td>No.</td>
<td>Species</td>
<td>Family</td>
<td>Type</td>
<td>Parts Used</td>
</tr>
<tr>
<td>-----</td>
<td>-------------------------------</td>
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<td>------------</td>
</tr>
<tr>
<td>49</td>
<td><em>Ocimum sanctum</em> L.</td>
<td>Lamiaceae</td>
<td>Shrub</td>
<td>Leaves, seeds</td>
</tr>
<tr>
<td>50</td>
<td><em>Ocimum tenuiflorum</em> L.</td>
<td>Lamiaceae</td>
<td>Shrub</td>
<td>Leaves, seed</td>
</tr>
<tr>
<td>51</td>
<td><em>Pajanelia longifolia</em> (Wild.) K.Schum</td>
<td>Bignoniaceae</td>
<td>Tree</td>
<td>Leaves</td>
</tr>
<tr>
<td>52</td>
<td><em>Phyllanthus emblica</em> L.</td>
<td>Euphorbiaceae</td>
<td>Tree</td>
<td>Entire plant</td>
</tr>
<tr>
<td>53</td>
<td><em>Phyllanthus niruri</em> Linn.</td>
<td>Euphorbiaceae</td>
<td>Herb</td>
<td>Entire plant</td>
</tr>
<tr>
<td>54</td>
<td><em>Piper betle</em> L.</td>
<td>Piperaceae</td>
<td>Climber</td>
<td>Leaves</td>
</tr>
<tr>
<td>55</td>
<td><em>Piper nigram</em> L.</td>
<td>Piperaceae</td>
<td>Climber</td>
<td>Dried unripe fruits</td>
</tr>
<tr>
<td>56</td>
<td><em>Plumeria rubra</em> acutifolia Poir.</td>
<td>Mangoliaceae</td>
<td>Small tree</td>
<td>Root, leaves, bark</td>
</tr>
<tr>
<td>57</td>
<td><em>Pothos scandens</em> L.</td>
<td>Araceae</td>
<td>Climber</td>
<td>Root, leaves</td>
</tr>
<tr>
<td>58</td>
<td><em>Rourea minor</em> Leenh.</td>
<td>Connaraceae</td>
<td>Climber</td>
<td>Fruit, bark, root, seed, leaves.</td>
</tr>
<tr>
<td>59</td>
<td><em>Sida acuta</em> Burm.f.</td>
<td>Malvaceae</td>
<td>Herb</td>
<td>Roots, leaves</td>
</tr>
<tr>
<td></td>
<td>Species</td>
<td>Family</td>
<td>Type</td>
<td>Parts Used</td>
</tr>
<tr>
<td>---</td>
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<td>---------------------------------</td>
</tr>
<tr>
<td>60</td>
<td><em>Sida alnifolia</em> L.</td>
<td>Malvaceae</td>
<td>Herb</td>
<td>Leaves, roots</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>61</td>
<td><em>Strychnos nux-vomica</em> Linn.</td>
<td>Loganiaceae</td>
<td>Tree</td>
<td>Entire plant</td>
</tr>
<tr>
<td>62</td>
<td><em>Synedrella nudiflora</em> (L.) Gaertn.</td>
<td>Asteraceae</td>
<td>Herb</td>
<td>Entire plant</td>
</tr>
<tr>
<td>63</td>
<td><em>Tectona grandis</em> L.f.</td>
<td>Lamiaceae</td>
<td>Tree</td>
<td>Wood, bark</td>
</tr>
<tr>
<td>64</td>
<td><em>Terminalia catapa</em> L.</td>
<td>Combretaceae</td>
<td>Tree</td>
<td>Leaves, fruits</td>
</tr>
<tr>
<td>65</td>
<td><em>Triemfetta rhomboidea</em> Lam.</td>
<td>Tiliaceae</td>
<td>Herb</td>
<td>Fruits, flowers, leaves</td>
</tr>
<tr>
<td>66</td>
<td><em>Urena lobata</em> L.</td>
<td>Malvaceae</td>
<td>Undershrub</td>
<td>Roots, leaves</td>
</tr>
<tr>
<td>67</td>
<td><em>Vetiveria zizanioides</em> L.</td>
<td>Poaceae</td>
<td>Herb</td>
<td>Root</td>
</tr>
<tr>
<td>69</td>
<td><em>Xenostegia tridentate</em> (L.) Aushin&amp;Staples.</td>
<td>Convolvulacea e</td>
<td>Creeper</td>
<td>Entire plant</td>
</tr>
<tr>
<td>70</td>
<td><em>Ziziphus oenopolia</em> (L.) Mill</td>
<td>Rhamnaceae</td>
<td>Shrub</td>
<td>Fruit, bark</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
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<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
**TABLE 2: Different plants seen in chirakkakavu**

<table>
<thead>
<tr>
<th>NAME OF THE PLANT</th>
<th>FAMILY</th>
<th>HABIT</th>
<th>PARTS USED</th>
<th>USES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Adenanthera pavonina L.</td>
<td>Fabaceae</td>
<td>Tree</td>
<td>Leaves, seed, bark</td>
<td>It is useful for nitrogen fixation, Leaves can be eaten, Decoction of young leaves and bark are used to treat diarrhea.</td>
</tr>
<tr>
<td>2. <em>Alstonia scolaris</em> R.Br.</td>
<td>Apocynaceae</td>
<td>Tree</td>
<td>Leaves, bark</td>
<td>Purify blood and treatment of respiratory disorders, and to stop cancerous growth.</td>
</tr>
<tr>
<td>3. <em>Anacardium occidentale</em> L.</td>
<td>Anacardiaceae</td>
<td>Tree</td>
<td>Fruit, seeds, timber, bark</td>
<td>Fruits and seeds are edible. Timber is used in furniture making. Bark is used in tanning. Used in the treatment of cancerous ulcers, diarrhea, malaria etc.</td>
</tr>
<tr>
<td>5. <em>Areca catechu</em> L.</td>
<td>Aracaceae</td>
<td>Tree</td>
<td>Entire plant</td>
<td>Extract have anti depressant properties in rodents. It is commercially important seed crop.</td>
</tr>
<tr>
<td>6. <em>Artocarpus heterophyllus</em> Lam.</td>
<td>Moraceae</td>
<td>Tree</td>
<td>Fruit, leaves, seeds</td>
<td>Fruit is edible. Curing fever, boils, skin diseases, skin diseases, diarrhea etc.</td>
</tr>
<tr>
<td>7. <em>Bambusa bambos</em> (L.)Voss</td>
<td>Poaceae</td>
<td>Perennial</td>
<td>Leaves, stem</td>
<td>Manufacturing different household products. Used to treat various inflammatory conditions. Also used in the treatment of kidney troubles.</td>
</tr>
<tr>
<td>8. <em>Bauhinia acuminate</em> L.</td>
<td>Fabaceae</td>
<td>Shrub</td>
<td>Bark, flower, root</td>
<td>Used in skin diseases. It is grown as an ornamental plant.</td>
</tr>
<tr>
<td>No.</td>
<td>Species</td>
<td>Family</td>
<td>Type</td>
<td>Part Used</td>
</tr>
<tr>
<td>-----</td>
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<td>--------</td>
<td>------</td>
<td>-----------</td>
</tr>
<tr>
<td>10.</td>
<td>Carica papaya L.</td>
<td>Caricaceae</td>
<td>Tree</td>
<td>Stem, Bark, fruit, leaves</td>
</tr>
<tr>
<td>11.</td>
<td>Caryota urens L.</td>
<td>Arecaeae</td>
<td>Palm</td>
<td>-</td>
</tr>
<tr>
<td>13.</td>
<td>Cocos nucifera L.</td>
<td>Aracaceae</td>
<td>Tree</td>
<td>Entire plant</td>
</tr>
<tr>
<td>14.</td>
<td>Desmodium gangaticum (L.)DC.</td>
<td>Fabaceae</td>
<td>Undershrub</td>
<td>Root, bark, Leaves.</td>
</tr>
<tr>
<td>15.</td>
<td>Elaeocarpus sphaerius (Gaerth) K.schum</td>
<td>Elaeocarpaceae</td>
<td>Tree</td>
<td>Fruit, leaves</td>
</tr>
<tr>
<td>16.</td>
<td>Elephantopus scaber L.</td>
<td>Asteraceae</td>
<td>Herb</td>
<td>Entire plant</td>
</tr>
<tr>
<td>17.</td>
<td>Ficas religiosa (L.)</td>
<td>Moraceae</td>
<td>Tree</td>
<td>Bark, leaves, fruits.</td>
</tr>
<tr>
<td>18.</td>
<td>Ficus benghalensis L.</td>
<td>Moraceae</td>
<td>Tree</td>
<td>Entire plant</td>
</tr>
<tr>
<td>19.</td>
<td>Heliotropium indicum L.</td>
<td>Boraginaceae</td>
<td>Herb</td>
<td>Leaves</td>
</tr>
<tr>
<td>20.</td>
<td>Hibiscus rosasinensis L.</td>
<td>Rubiaceae</td>
<td>Shrub</td>
<td>Leaves, flowers</td>
</tr>
<tr>
<td>22.</td>
<td>Ixora coccinia L.</td>
<td>Rubiaceae</td>
<td>shrub</td>
<td>Entire plant</td>
</tr>
<tr>
<td>23.</td>
<td>Macaranga peltata Roxb. Mueller</td>
<td>Euphorbiaceae</td>
<td>Tree</td>
<td>Leaves, wood</td>
</tr>
<tr>
<td>No.</td>
<td>Scientific Name</td>
<td>Family</td>
<td>Type</td>
<td>Part(s) Used</td>
</tr>
<tr>
<td>-----</td>
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</tr>
<tr>
<td>24.</td>
<td><em>Mangifera indica</em> L.</td>
<td>Anacardiaceae</td>
<td>Tree</td>
<td>Fruit</td>
</tr>
<tr>
<td>25.</td>
<td><em>Microcos paniculata</em> L.</td>
<td>Tiliaceae</td>
<td>Shrub</td>
<td>Leaves</td>
</tr>
<tr>
<td>27.</td>
<td><em>Mimusops elengi</em> Linn.</td>
<td>Sapotaceae</td>
<td>Tree</td>
<td>Bark, flowers, seeds, fruits</td>
</tr>
<tr>
<td>29.</td>
<td><em>Pothos scandens</em> L.</td>
<td>Araceae</td>
<td>Climber</td>
<td>Root, leaves</td>
</tr>
<tr>
<td>30.</td>
<td><em>Ruellia tuberosa</em> L.</td>
<td>Acanthaceae</td>
<td>Herb</td>
<td>-</td>
</tr>
<tr>
<td>32.</td>
<td><em>Setaria parviflora</em> L.</td>
<td>Poaceae</td>
<td>Herb</td>
<td>-</td>
</tr>
<tr>
<td>33.</td>
<td><em>Sida acuta</em> Burm.f.</td>
<td>Malvaceae</td>
<td>Herb</td>
<td>Roots, Leaves</td>
</tr>
<tr>
<td>34.</td>
<td><em>Sida rhombifolia</em> L.</td>
<td>Malvaceae</td>
<td>Herb</td>
<td>Leaves, roots</td>
</tr>
<tr>
<td></td>
<td>Species</td>
<td>Family</td>
<td>Type</td>
<td>Part(s) Used</td>
</tr>
<tr>
<td>---</td>
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<td>----------------------------------------</td>
</tr>
<tr>
<td>36</td>
<td><em>Syzygium cumini</em> (L.) Skeels.</td>
<td>Myrtaceae</td>
<td>Tree</td>
<td>Fruit, seeds</td>
</tr>
<tr>
<td>37</td>
<td><em>Tectona grandis</em> L.f.</td>
<td>Lamiaceae</td>
<td>Tree</td>
<td>Wood, bark</td>
</tr>
<tr>
<td>38</td>
<td><em>Tridax procumbance</em> L.</td>
<td>Asteraceae</td>
<td>Herb</td>
<td>Entire plant</td>
</tr>
<tr>
<td>39</td>
<td><em>Vanda sp.</em></td>
<td>Orchidaceae</td>
<td>Epiphyte</td>
<td>-</td>
</tr>
<tr>
<td>41</td>
<td><em>Zizipus oenopolia</em> (L.) Mill</td>
<td>Rhamnaceae</td>
<td>Shrub</td>
<td>Fruit, bark</td>
</tr>
</tbody>
</table>
### TABLE 3: Plants in groves which are used for worshipping god

<table>
<thead>
<tr>
<th>S.NO</th>
<th>NAME OF THE PLANT</th>
<th>USES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Bauhinia acuminate</em> L.</td>
<td>The flowers are white in colour and are used for pooja purposes in Kerala.</td>
</tr>
<tr>
<td>2.</td>
<td><em>Cassia fistula</em> L.</td>
<td>This is a golden yellow flower, which is a vital part of God Vishnu. It is used for celebration related to Lord Vishnu.</td>
</tr>
<tr>
<td>3.</td>
<td><em>Elaeocarpus sphaerius</em> L.</td>
<td>Rudraksha names both a Sacred seed and the tree bears it. The seeds are considered as the tears shed by Lord Shiva for the benefit of humanity. There are many stories about this. One among them is following.”Rudra wept when He witnessed the towering metropolies, Tripura or triple city, created by man’s superbly ambitious technology. In its arrogance, this magnificent human creation has undermined the balance between the earth, atmosphere and the sky. Then, according to Mahabharata, having shed the implacable tear which turned in to a Rudraksha bead. The Lord of the Universe drew His bow and unleashed His arrows at the triple city, burning its demons and hurling them into the Western ocean for the welfare of creation. Wearing the Rudraksha, devotees remind themselves of God’s compassion for the human predicament, His watchful love for us.</td>
</tr>
<tr>
<td>4.</td>
<td><em>Ficus benghalensis</em> L.</td>
<td>Hindu mythology says that Brahma was transformed in to a Vat tree and it is viewed as the male to the peepal. It is considered as a sin to destroy both the trees especially the male.</td>
</tr>
<tr>
<td>5.</td>
<td><em>Ficus religiosa</em> (L.)</td>
<td>The tree is considered Sacred by the followers of Hinduism, Jainism and Buddhism. Sadhus still meditate beneath Sacred fig trees and Hindus do Pradakshina around the fig tree as a mark of worship. Also this tree is closely related to Lord Krishna. The peepal is believed to be inhabited by the Sacred triad-Brahma, Vishnu and Maheshwara.</td>
</tr>
<tr>
<td>6.</td>
<td><em>Hibiscus rosasinensis</em> L.</td>
<td>Used for the worship of Devi. Red flowers are dominant. These are used or the worshipping of other Gods also.</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td>---</td>
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<td>---</td>
</tr>
<tr>
<td><strong>7.</strong></td>
<td><em>Ixora coccinia</em> L.</td>
<td>The plant is of a red shade and is thought of being Sacred. Therefore it is offered as prayers in many temples in Kerala.</td>
</tr>
<tr>
<td><strong>8.</strong></td>
<td><em>Ocimum sanctum</em> L.</td>
<td>Holly basil is cultivated for medical and religious purposes and for its essential oil. In particular, it has been used for Thousands of years in Ayurvedic medicine for various types of healing. <em>O. sanctum</em> is considered an adaptogen, balancing the process of the body and allowing it to adapt to stressful situations. It is regarded as an elixir of life and is believed to promote longevity. The seeds are sometimes worn on the body in order to bring balance and longevity.</td>
</tr>
<tr>
<td><strong>9.</strong></td>
<td><em>Plumeria rubra</em> Poir.</td>
<td>It is one of the important plants for God Gulikan. It is used for worshipping God in Groves.</td>
</tr>
<tr>
<td><strong>10.</strong></td>
<td><em>Strychnos-nux vomica</em> L.</td>
<td>This is seen in almost all Sacred Groves. The tree is an important for the Lord Gulikan. Leaves is used for worshipping God Sasthappan (pooya vaykkal).</td>
</tr>
</tbody>
</table>
Clinical Pattern and Outcome of Intrauterine Growth Retardation (IUGR) Babies admitted in the Sick Neonatal Nursery (SNN) of a Tertiary Care Centre in South Tamilnadu, India

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Abstract

Introduction: Intra-Uterine Growth Retardation (IUGR) is failure to attain optimal intrauterine growth. Next to preterm birth, IUGR is the second leading cause of perinatal mortality. As many as 53% of preterm stillbirths and 26% of term stillbirths are growth restricted. Given the immediate and long-term implications of IUGR and its high prevalence in India, a focus on IUGR is both rational and strategic.

Objectives: 1) To study the Clinical pattern and outcome of IUGR babies and their Outcome during hospital stay. 2) To find out the Factors associated with Morbidity and Mortality of IUGR babies.

Methodology: This Cross sectional Descriptive Study was carried out in the Department of Pediatrics, Sick Neonatal Nursery (SNN) ward, Department of Pediatrics, Tirunelveli Medical College Hospital. 120 babies were selected by systematic random sampling. The socio-demographic and antenatal characteristics were collected by interviewing the mother using a structured proforma. The outcome measures like morbidity pattern and condition at discharge were quantified.

Results: Of 120 IUGR babies 22 (18.3%) are Preterm babies and 98 (81.7%) are Term babies. Hypoglycemia (63.3%) and Perinatal Asphyxia (45.0%), Sepsis (33.3%), Hypocalcaemia (30.0%), Hypothermia (28.3%) and Thrombocytopenia (25.0%) are the common complications. 22 (18.3%) have died at hospital and 98 (81.7%) have been discharged. Perinatal asphyxia and Meconium Aspiration are significantly associated with abnormal neurological examination at Discharge. Lower gestational age, Normal delivery and Lower weight of the baby are the statistically significant risk factors associated with mortality.

Conclusion: Hypoglycemia and Perinatal Asphyxia are the commonest complications of IUGR. Perinatal asphyxia, Meconium Aspiration, Gestational age, Delivery category and Weight of the baby are the significant risk factors associated with morbidity and mortality.

Keywords: Intrauterine Growth Retardation, IUGR Babies, Morbidity, Complications of IUGR, Risk factors.

Introduction

Intrauterine Growth restriction (IUGR) is a common complication of pregnancy that carries significant short and long-term sequelae that reaches out to adulthood (Henry, 2008). Next to preterm birth, IUGR is the second leading cause of perinatal mortality (Pallotto, 2006). Intra-Uterine Growth Retardation (IUGR) is failure to attain optimal intrauterine growth which is
defined as either birth weight less than the 10th percentile for gestational age or as birth weight less than 2 standard deviations below the mean value for gestational age. When compared with normally grown fetuses after exclusion of aneuploidic and anomalous fetuses, mortality rates are increased 10-fold with perinatal mortality rates as high as 120 per 1000 for all cases of IUGR. As many as 53% of preterm stillbirths and 26% of term stillbirths are growth restricted. Up to 50% of survivors will experience intrapartum asphyxia, which adds to the already increased risk of end-organ injury (Neelam Kle, 2009).

However, the IUGR condition provides numerous challenges to both researchers of the condition and the clinician caring for the patient and includes the following: varied etiologies and definitions, altered fetal behavioral and vascular responses to IUGR, severely limited treatment options and uncertainty regarding the timing of delivery.

Intrauterine growth-retarded babies face problems not of immaturity, but of in-utero hypoxia, poor nutrition and the resultant stress (Chard T, 1993). There is a substantial overlap between their problems and those that premature babies face. The effects of this disadvantageous start, however, tend to persist. IUGR babies exhibit poor catch-up growth and impaired cognitive and neurobehavioral development (Erich Cosmi, 2011). In addition, emerging evidence suggests that they are also more likely than normal weight babies to suffer from degenerative diseases like hypertension, diabetes and cardiovascular diseases in adulthood (Barker, 1998).

Given the immediate and long-term implications of IUGR and its high prevalence in India, a focus on IUGR is both rational and strategic from a public health perspective. A 20% approximate prevalence of IUGR in India implies that it is a significant public health problem (De Onis, 1998). IUGR is strategic from the point of view of neonatal and infant mortality and adulthood morbidity. So this study was attempted to find the mortality and morbidity pattern and associated the risk factors in IUGR babies admitted in a tertiary care hospital.

Objectives

The Objectives of the study are:
1) To study the Clinical pattern and outcome of IUGR babies admitted in the Sick Neonatal Nursery of Tirunelveli Medical College Hospital and their Outcome during hospital stay.
2) To find out the Factors associated with Morbidity and Mortality of IUGR babies.

Methodology

Study design and setting

This is a Cross Sectional study. This study was carried out in the Department of Pediatrics, Neonatal Unit, Tirunelveli Medical College Hospital between November 2008 and October 2010. According to the following sample size calculation formula, 120 IUGR babies were included in the study: Sample size (n) = 4pq/d2. [p = prevalence; q = 1-p; d = error allowed (25% of p)]. Based on the prevalence of IUGR as 35%, total of 120 babies were included in this study.

The following Inclusion and exclusion criteria were used to select the samples.

Inclusion Criteria:
- Newborns with IUGR defined by Birth weight less than 10th percentile (Annexure: 2) and Ponderal Index admitted in the SNN ward of Tirunelveli Medical College Hospital.

Exclusion Criteria:
- Newborns with chromosomal abnormalities.
- Newborns with congenital anomalies.
Method of data collection

Among all the IUGR babies admitted in SNN during the above study period, the 120 babies who satisfied the Inclusion criteria were selected by systematic random sampling. Informed consent of their parents was taken after explaining in detail about the method and procedures involved in the study in their vernacular language. The socio demographic profile and relevant information of individual babies and their respective mothers were collected by interviewing the mother using a structured proforma. The clinical details, complications and outcome at discharge were noted down.

The following investigations were carried out for all the babies under study:
1) CBC – Hb, PCV, TC, DC, Platelet Count, ESR
2) Blood Sugar, Urea and Serum Creatinine
3) Serum calcium
4) Chest X Ray
5) Total and Direct Bilirubin (Only for Icteric babies)
6) Sepsis Screening (Only for the suspected sepsis babies) - Peripheral smear for band forms and Toxic granules, Blood culture and sensitivity, CSF Analysis with culture and sensitivity.

The following criteria were kept in mind while analyzing the data:

a) Hb: Hemoglobin less than 13 mg/dl was considered as Anemia.
b) PCV: The value of PCV more than 65% was taken as Polycythemia.
c) Total WBC count: A total count of < 5,000/cu.mm or >20,000/cu.mm was taken as abnormal and considered as suspicious of sepsis.
d) Differential count: Differential count was considered mainly to find out Neutropenia which is indicative of sepsis. Absolute Neutrophil count less than 2000 was considered as Neutropenia.
e) Platelet Count: Platelet count less than 1,00,000 was considered as Thrombocytopenia.
f) ESR: Micro ESR value more than 15mm at one hour was considered as sepsis.
g) Blood Sugar: Blood sugar value less than 45 mg/dl in one or more occasions were considered as hypoglycemia.
h) Blood Urea and Serum Creatinine: Value of Blood urea more than 40mg/dl associated with the value of Serum Creatinine more than 1mg/dl was considered as acute renal failure.
i) Serum calcium: Serum Calcium less than 7mg/dl was considered as hypocalcaemia.
j) Peripheral smear for band forms and Toxic granules: Presence of band forms and toxic granules in peripheral smear study was considered as abnormal and considered as sepsis.

The outcome measures like Immediate Complications, Condition at discharge and risk factors associated with IUGR were identified.

Statistical analysis

Data were entered in Excel spreadsheet and analysed using SPSS version 13.0. The results were analysed using the statistical test like simple proportions, Risk ratio and Chi-square test. The p-value < 0.05 was considered as statistically significant.

Results

<table>
<thead>
<tr>
<th>Character</th>
<th>Category</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>65</td>
<td>54.2</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>55</td>
<td>45.8</td>
</tr>
<tr>
<td>Gravida</td>
<td>Primi</td>
<td>82</td>
<td>68.3</td>
</tr>
</tbody>
</table>
**Among the total study population of 120 IUGR babies, 65 (54.2%) are males and 55 (45.8%) are females. As per the gestational age of the respective mothers of the study population, 82 (68.3%) mothers are primi and 28 (23.3%) are 2nd gravida mothers. The 3rd gravida mothers are 8 (6.7%) and only 2 (1.7%) are the 4th gravida mothers. 22 (18.3%) are Preterm babies and 98 (81.7%) are Term babies. 72 (60.0%) have been delivered by normal vaginal delivery and 10 (8.3%) have been delivered by Assisted delivery. 38 (31.7%) have been delivered by Caesarian section. Of the 120 babies 66 (55.0%) are in the birth weight category of 2.0 - 2.5 kg and 38 (31.7%) are in the birth weight category of 1.5 – 2.0 kg. 10 (8.3%) babies are in the category of 1.0 – 1.5 kg and 6 (5.0%) babies are in less than 1.0 kg category. Among the 120 study population 82 (68.3%) babies were classified as asymmetrical IUGR and 38 (31.7%) babies were classified as symmetrical IUGR as per Ponderal Index.**

**Table 2: Morbidity pattern of IUGR babies**

<table>
<thead>
<tr>
<th>Complications</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Metabolic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>76</td>
<td>63.3</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>10</td>
<td>8.3</td>
</tr>
<tr>
<td>Hypocalcaemia</td>
<td>36</td>
<td>30.0</td>
</tr>
<tr>
<td><strong>Hematological</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutropenia</td>
<td>6</td>
<td>5.0</td>
</tr>
<tr>
<td>Polycythemia</td>
<td>6</td>
<td>5.0</td>
</tr>
<tr>
<td>Anemia</td>
<td>24</td>
<td>20.0</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>30</td>
<td>25.0</td>
</tr>
<tr>
<td><strong>Organ Dysfunction</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute Renal Failure</td>
<td>22</td>
<td>18.3</td>
</tr>
<tr>
<td>Perinatal Asphyxia</td>
<td>54</td>
<td>45.0</td>
</tr>
</tbody>
</table>
The above table 2 shows that hypoglycemia (63.3%) and Perinatal Asphyxia (45.0%) are the commonest complications. Other commoner are Sepsis (33.3%), hypocalcaemia (30.0%), hypothermia (28.3%) and thrombocytopenia (25.0%).

Table 3: Mortality in IUGR babies

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dead</td>
<td>22</td>
<td>18.3</td>
</tr>
<tr>
<td>Discharged</td>
<td>98</td>
<td>81.7</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100</td>
</tr>
</tbody>
</table>

Of the 120 IUGR babies 22 (18.3%) have died at hospital and 98 (81.7%) have been discharged.

Table 4: Outcome of IUGR

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dead</td>
<td>22</td>
<td>18.3</td>
</tr>
<tr>
<td>Abnormal Neurological Exam. at Discharge</td>
<td>19</td>
<td>15.3</td>
</tr>
<tr>
<td>Good condition at Discharge</td>
<td>79</td>
<td>66.3</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100</td>
</tr>
</tbody>
</table>

Of the 120 babies 22 (18.3%) have died in the hospital. 19 (15.5%) have been discharged with abnormal neurological examination and 79 (66.3%) have been discharged with Good condition at Discharge condition.

Table 5: Morbidity pattern and outcome

<table>
<thead>
<tr>
<th>Complications</th>
<th>Good condition at Discharge (N=79)</th>
<th>Abnormal N E at Discharge (N=19)</th>
<th>p – Value</th>
<th>Dead (N=22)</th>
<th>p – Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>52 (65.8%)</td>
<td>8 (42.1%)</td>
<td>0.057</td>
<td>16 (72.7%)</td>
<td>0.541</td>
</tr>
</tbody>
</table>
The above table 5 shows the distribution of various morbidity conditions of IUGR babies with their Outcome. Perinatal asphyxia and Meconium Aspiration are significantly associated with abnormal neurological examination at Discharge. Perinatal asphyxia, Pulmonary hemorrhage and Persistent pulmonary hypertension are significantly associated with Death during the hospital stay.

**Table 6:** Fetal risk factors and mortality

<table>
<thead>
<tr>
<th>Fetal Factors</th>
<th>Death N= 22</th>
<th>No Death N= 98</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>Chi-2 Value</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex- Male</td>
<td>13 (59.1%)</td>
<td>52 (53.1%)</td>
<td>1.28</td>
<td>0.50 – 3.26</td>
<td>0.263</td>
<td>0.608</td>
</tr>
<tr>
<td>Weight ≤ 2 kg</td>
<td>15 (68.9%)</td>
<td>39 (39.8%)</td>
<td>3.24</td>
<td>1.11 – 9.76</td>
<td>5.850</td>
<td>0.015</td>
</tr>
<tr>
<td>GA - Preterm</td>
<td>8 (36.4%)</td>
<td>14 (14.3%)</td>
<td>3.43</td>
<td>1.22 – 9.67</td>
<td>5.849</td>
<td>0.016</td>
</tr>
<tr>
<td>Symmetrical IUGR</td>
<td>10 (45.5%)</td>
<td>28 (28.6%)</td>
<td>2.08</td>
<td>0.81 – 5.37</td>
<td>2.367</td>
<td>0.124</td>
</tr>
</tbody>
</table>
Normal Delivery | 18 (81.8%) | 54 (55.1%) | 3.67 | 1.16 – 11.63 | 5.343 | **0.021**

The above table 6 shows that Weight ≤ 2 kg, Preterm and Normal delivery have the statistically significant association with mortality (p-value < 0.05).

**Discussion**

Regarding the complications, Hypoglycemia (63.3%) and Perinatal Asphyxia (45.0%) are the commonest of the complications observed in this study. Carbohydrate metabolism is seriously disturbed and these infants are highly susceptible to hypoglycemia as the consequence of diminished glycogen reserves and decreased capacity to gluconeogenesis (Kliegman, 1989). IUGR infants frequently do not tolerate labor and vaginal delivery, and signs of fetal distress are common.

Other commoner are Sepsis (33.3%), hypocalcaemia (30.0%), hypothermia (28.3%) and thrombocytopenia (25.0%). Other complications are Hyperglycemia (10 - 8.3%), Neutropenia (6 - 5.0%), Polycythemia (6 - 5.0%), Anemia (24 - 20.0%), Acute Renal Failure (22 - 18.3%), Meconium Aspiration (20 - 16.7%), Pulmonary Hemorrhage (6 - 5.0%), Persistent Pulmonary Hypertension (4 - 3.3%) and Meningitis (6 - 5.0%).

There are totally 22 preterm babies. Among them only 7 (31.8%) babies have Respiratory Distress Syndrome. This is due to as McIntire DD et al explained in their study that in IUGR babies, accelerated fetal pulmonary maturation occurs secondary to chronic intrauterine stress (McIntire, 1999).

Perinatal asphyxia and Meconium Aspiration are significantly associated with abnormal neurological examination at Discharge. Perinatal asphyxia, Pulmonary hemorrhage and Persistent pulmonary hypertension are significantly associated with Death during the hospital stay.

Weight ≤ 2 kg, Preterm and Normal delivery have the statistically significant association with mortality (p-value < 0.05) (Table 4.3). Hack M, Fanaroff AA in their study on outcome of extremely low birth weight and gestational age IUGR babies found that lower birth weight and lower gestational age are significantly associated with morbidity and mortality (Hack, 2000). It is consistent with study done by McIntire DD et al (McIntire, 1999). Table 4.3.1 details how the birth weight is associated with mortality during hospital.

Table 6 depicts how lower gestational age is significantly associated with higher chance of Death during hospital stay as compared to higher gestational ages. Garite TJ et al found that preterm infants have higher incidence of abnormalities than the general population because they are subjected to the risk of prematurity in addition to the risks of IUGR. IUGR infants delivered before 28 – 30 weeks had worse outcomes (Garite, 2004). In this study also, of the 8 Infants born before 32 weeks, 7 have died. This is statistically significant (p-value < 0.01).

Morbidity in IUGR is significantly associated with Normal delivery than other mode of deliveries (p-value < 0.01) in our study. IUGR babies frequently have birth asphyxia as they tolerate the stress of labour poorly. This is consistent with other studies by Hawdon JM et al (Hawdon, 1993) and Pérez-Escamilla R et al (Perez-Escamilla, 1992). This may be due to labour is stressful for IUGR fetuses. Skilled resuscitation should be available because perinatal depression is common. The availability of pediatrician for the skillful resuscitation also may contribute for the favorable outcome of IUGR in assisted delivery / caesarian section.
Conclusions

The following are the observations and conclusions of the study.

1. In this study, of the 120 IUGR babies 65 (54.2%) are male and 55 (45.8%) are female.
2. According to Ponderal Index 82 (68.3%) babies are asymmetrical IUGR and 38 (31.7%) are symmetrical IUGR. Ratio Asymmetrical: symmetrical = 2.15:1. Symmetrical IUGR is 2.08 times more risk of having mortality as compared to asymmetrical IUGR.
3. Hypoglycemia (63.3%) and Perinatal Asphyxia (45.0%) are the commonest complications of IUGR. Other commoner are Sepsis (33.3%), hypocalcaemia (30.0%), hypothermia (28.3%) and thrombocytopenia (25.0%).
4. Of the 120 babies, 22 (18.3%) died at hospital and 98 (81.7%) have been discharged. Of these 98 babies, 79 (66.3%) have been discharged in good condition and 19 (15.5%) with abnormal neurological examination.
5. Perinatal asphyxia and Meconium Aspiration are significantly associated with abnormal neurological examination at Discharge. Perinatal asphyxia, Pulmonary hemorrhage and Persistent pulmonary hypertension are significantly associated with Death during the hospital stay.
6. Lower gestational age, Normal delivery and Lower weight of the baby are the statistically significant fetal risk factors associated with mortality.

Recommendations

From the conclusions arrived at this study, the following recommendations can be made to prevent the incidence and the complications in IUGR.

1. Hypoglycemia, hypocalcaemia and hypothermia are the common treatable complications in this study which can be easily identified and treated. So in IUGR babies it is important to anticipate these conditions and treat appropriately to prevent further morbidity and mortality.
2. Perinatal asphyxia is the second most common complication observed in this study which is significantly associated with morbidity and mortality. Hence anticipating perinatal asphyxia, effective neonatal resuscitation measures should be made available for every IUGR delivery.

References

Bionic Eye – A Review
The path traversed and to be traversed

Article by Usha Nandini M
Email:- m.ushanandini94@gmail.com

Abstract
The darkness of the night is broken by the brightness of the sun and people worship
sun for this. Similarly, providing even a flicker of light to a person who has lost
his/her sight is one of the greatest miracles a doctor can perform. Bionic Eye- visual
prosthetic devices serve this purpose and helps to restore some kind of visual
perception in patients with retinal pathologies like retinitis pigmentosa and age
related macular degeneration. The inception of this idea dates back to the 18th
century but the recent advances in electronics, robotics and other technologies has
helped in materializing the idea. The basic function of the device is to receive the
images using a camera, convert it to electric signals and eventually stimulate the
left-over healthier parts of the visual pathway. There are various kinds of devices
based on the position of implants. Each of them have varied advantages.
Understanding the existing systems would help in improvising them or in finding
better systems to serve the same purpose.

Introduction
Bionic Eye is a perfect blend of physics and physiology, a boon to the blind. The
word Bionic Eye is used to refer to an electronic device enabling the re-establishment
of lost vision due to problems in the visual pathway i.e., it is a mixture of physics
(electronics in specific) and physiology of vision in right proportions to form a
miracle. As fascinating as it is, the fact that this is chosen among many other more
significant topics in ophthalmology or even medicine might kindle curiosity. A
research to feed that curiosity would reveal (among other things) that this year 2015
witnessed a breakthrough in this field which was the successful implantation of
Bionic Eye in a patient with Age- related macular degeneration and thus giving a
solution to manage the most common cause of loss of vision in developed countries. 1
This article will be focused on explaining the technical aspects of Bionic Eye in
Simple terms while discussing the path traversed so far and the path to be traversed in
future.

Bionic eye- lexicography
‘Bionics’ is a word derived from ‘Bion’ and ‘ic’ which together means life like. It
can also be simply explained as a blending of the words Biology and Electronics.
Literally, it is the understanding of Biology and application of its principles in
engineering and technology. For example, studying the surface of lotus helped in the
production of dirt and water repellant paint; understanding the echolocation of bats
formed the basis for Sonar, radar and ultrasonography. In medicine, Bionics includes
replacing or substituting the organs and its functions by mechanical versions. The
living systems are studied, devices are developed using its functioning principle and
the device is itself used to serve as a proxy for the system when it is impaired. Ideal
examples would be cochlear implants, artificial hearts or even the proposed
nanodevice called as respirocyte which may serve as bionic red cell. 2 ‘Bionic Eye’ is
also a significant member in this series.
‘Bionic Eye’ is a fancy word used for visual prosthetic devices. It is used to assist
vision for visually challenged people. When defective vision is due to some problem
with refraction, glasses or lens or Lasik is indicated. When there is pathology in the
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cornea, corneal transplant is indicated. All these serve well because in these conditions, the basic units of vision i.e., the rods and cones remain intact. When these are affected, there comes the role of the visual prosthetic devices called “Bionic Eye”.

Critical review

History of evolution of bionic eye- a timeline

The inception of concepts relating to bionic eye dates way back into the 18th century. History is not always fair in offering positions for firsts. Though Luigi Galvani is considered as the first to describe bioelectricity by discovering that when the muscles of a dead frog are struck by electricity, they twitch; about three decades before his discovery in 1780, Benjamin Franklin had predicted an artificial vision system which is also based on the same principle where the retinal cells are stimulated rather than muscles in 1751. And in 1755, Charles Leroy even performed an experiment in this aspect by discharging static electricity from a precursor capacitor of those days - a Leyden jar into a patient who was blind as a result of high fever for three months using two wires- one above the eyes around the head and the other around the leg, the patient experienced a flame like thing passing downwards in before his eyes. That was the first time when a rudimentary prosthesis successfully restored a flicker of visual perception in a visually deprived individual. Since then till date, there are various waxing and waning in the development of bionic eye. The past two decades continued to show rapid progression in this field. The following timeline would help us to better understand the key aspects in the history of evolution of Bionic Eye.

1751
- Benjamin Franklin
  - Mentioned Artificial Vision System in the report of his Kite and Key experiment

1755
- Charles Leroy
  - First use of an electronic device as a rudimentary prosthesis producing a flicker of sensation in a blind eye

1780
- Tiberius Cavallo
  - Wrote an essay on theory and practice of medical electricity

1929
- Foerster observed that electrically stimulating occipital pole produced perception of a small spot of light called as Phosphene

1931
- Krause and Schum
  - Visual cortex retains function despite long time deprivation of visual input

1956
- Graham Tassicker
  - Obtained Patent for Photosensitive selenium cell producing phospene

1960-70
- Brindley and Dobelle
  - Artificial vision by cortical stimulation was capable of producing consistent phosphenes

1983
- João Lobo Antunes
  - First implanted a Bionic Eye in a patient born blind

1989
- Massachusetts Eye and Ear Infirmary along with MIT identified the feasibility of retinal prosthesis

1990
- Early 1990s, Mark Humayun, Eugene Dejuan, Howard D Phillips, Wentai Liu, Robert Greenberg invents the Argus Retinal Prosthesis
  - Late 1990s, Second Sight company was formed by Greenberg with Alfred E. Mann
  - Suprachoroidal implant (silicone carrier with 33 platinum disc shaped electrodes invented in Osaka university in Japan and Nanobioelectronics and Systems Research Centre of Seoul National University of South Korea

1995
- Eberhart Zrenner in South German university Hospital in Tubingen identified subretinal prosthesis which utilizes microphotodiode arrays

1996
- Electrically evoked cortical potentials were identified by stimulation of
retina with a microfabricated electrode array\textsuperscript{13}

1998  
- Beginning of Epiretinal stimulation trials in Harvard and MIT\textsuperscript{11}

2000  
- Experiments were performed on isolated retina such as multi-electrode stimulation and multisite stimulation\textsuperscript{14,15}

2002  
- Microsystem based visual prosthesis was developed by Claude Veraart at University of Louvain\textsuperscript{16-20}
- Artificial Silicon Retina (ASR device) was invented\textsuperscript{21}
- Argus I with 16 electrodes were implanted and studied in 6 subjects (2002-2004)\textsuperscript{22,23}

2003  
- Experiments were done for thresholds required for extracellular retinal stimulation, pattern recognition and visual resolution with retinal prosthetic devices\textsuperscript{24}

2004  
- Suprachoroidal Electrode was tried for transretinal stimulation
- Electronic retinal prosthesis was proposed for the treatment and rehabilitation of visually challenged

2007  
- Trials of Argus-II with 60 electrodes commenced\textsuperscript{25}
- Dobelle Eye-similar to MIT device, but the stimulator sits in visual cortex\textsuperscript{4}

2010  
- Bionic Vision Australia was formed by Kevin Rudd with 42 million dollars fund from the government to develop a retinal prosthetic device
- Retinal implant with 1500 electrodes developed in Germany

2011  
- Argus -II approved for commercial use in Europe

2012  
- First UK implant by Oxford University and King's College Hospital
- Photovoltaic Retinal Prosthesis with high pixel intensity was developed

2013  
- Argus -II obtained FDA approval and was recorded to be the First FDA approved Bionic Eye\textsuperscript{26}

2014  
- Argus-II was implanted in a Retinitis Pigmentosa Patient and continued to be used for the same

2015  
- Argus -II was successfully implanted in a patient with age-related macular degeneration\textsuperscript{1}

Basic units of bionic eye- deciphering the technical jargon

“If you can’t explain it simply, you don’t understand it well enough”- Albert Einstein

The basic components in Bionic Eye are image sensors, processors, transmitters, receivers, retinal implant, and cortical implants. Image sensor is a device similar to a camera and captures the images in front of the eye. Processors process the image obtained and convert them into electric impulses or signals. Transmitters transmit the signal and receivers receive it and transfer it to the electrodes implanted in the retina-the retinal implant. Cortical implants are small electrodes implanted directly into the visual cortex of the brain. The topography of the implant varies in different types of devices. It may be either:

1. In the Eye
   a. Epiretinal Implants
   b. Subretinal Implants
   c. Suprachoroidal Implants
   d. Intrascleral Implants

2. Extraocular Retinal Prosthesis
3. Optic Nerve Prosthesis
4. Into the Brain
   a. Visual Cortical Implants
   b. Lateral Geniculate Nucleus Prosthesis
Epiretinal implants

Epiretinal implants generally consist of a camera that captures images, a component that transforms the captured image into electric stimulation that forms the visual signal and the last component that receives these electric stimulations and then stimulates the remaining cells in the retina by lying on the inner surface of retina. There are various epiretinal implants like Argus II epiretinal prosthesis, Learning retinal implant, EPI-RET3 implant, Epiretinal implant from Bionic Vision Australia, artificial retinal implants using liquid crystal polymers. Each of these devices will be described separately.

Argus II epiretinal implant, a product of the Second Sight Medical Products Inc, is actually a well-developed version of the product of the original inventors of active epiretinal prosthesis Mark Humayun, Eugene Dejuan, Howard D. Phillips, Wentai Liu, and Robert Greenberg in 1990s. Later, the Second Sight Company was formed by Robert Greenberg along with Alfred E. Mann. It consisted of an intraocular epiretinal multielectrode array with 6 platinum electrodes. The array is placed temporal to the fovea and positioned using a single spring-tensioned retinal tack inserted into it. The extraocular component was an external spectacle mounted camera which served as a visual processing unit. This information along with the power was transmitted to magnetic coils implanted in the skull using inductive link telemetry system. The electric signal then reaches the epiretinal multielectrode array via the trans-scleral cables. The power required was supplied by a battery pack that was externally worn. This First generation implant called Argus I with 16 electrodes was implanted into six subjects in the Clinical Trial conducted by Dr. Humayun in 2002 at the Doheny Retina Institute. The subjects had Retinitis Pigmentosa with vision reduced to bare light perception. On testing them, improvements were observed in object detection, object counting, object discrimination and direction of movement with the implant turned on compared to off. Sight restoration was identified to be a learning process based on the fact that the subject’s performance in tasks improved with increased usage. The next generation prosthesis Argus II was approved for clinical study in 2007. The trial was conducted in 11 centres worldwide. The Argus II implant consisted of 60 independently controllable electrodes making it better than its predecessor. It received commercial approval in Europe in March 2011. In February 2013, it became the first of its kind to be approved by the US Food and Drug Administration – First Bionic Eye approved by FDA. It was thereafter used in restoring vision to those affected by retinitis pigmentosa. On 21st July 2015, the Bionic eye- Argus II epiretinal implant was implanted into a patient with dry age related macular degeneration by the surgeons of Manchester. This formed a breakthrough because ARMD was the most common cause of loss of sight in the developed world.

The Learning Retina Implant System is a product developed by Intelligent Medical Implants AG. It consists of an extraocular and intraocular portion. A retinal encoder placed on the frame of a pair of glasses formed the extraocular portion. The intraocular portion is a retina stimulator that rests on the inner surface of retina and positioned using retinal tacks. The processing capability of the Retinal Encoder is comparable to that of the functions of the retinal ganglion cell by its receptive field properties and filtering operations. It improves the visual processing capabilities of the retina by means of 100 to 1000 individually tunable spatiotemporal filters. This implant can be used in assisting the patient with the adjustment of the stimulation parameters individually. Its trials were started in 2003 where 19 out of the 20 patients with Retinitis Pigmentosa who underwent electrical stimulation for 45 minutes described sensation of phosphenes. That result helped in starting the chronic studies that commenced in 2005. Results showed identification of simple patterns and location of light sources. A Second chronic study was done to identify the use of full...
unit with its wearable camera and a pocket sized processing unit. A multicentric clinical trial was registered in Europe and its current status is unknown.

The Aachen University Clinic and the Fraunhofer Institute for Microelectronic circuits in Germany form the EpiRET GmbH group. They have developed a device similar to the Argus epiretinal prosthesis. It also has an extraocular and intraocular part. The image is captured by a metal-oxide semiconductor camera in the frame of the glasses. This is transferred wirelessly to a receiver which is placed in the anterior vitreous similar to an intraocular lens. This eventually stimulates the epiretinal implant through a micro-cable that connects it to the array of 25 electrodes apposed to the ganglion cells. The greatest advantage of the EPI-RET3 implant is that it has all the ocular devices within the eyeball- no wire passing by means of a sclerostomy. It underwent human trials in six patients for 4 weeks in 2007. It was tolerable with mild inflammatory changes and remained stable in its position until removal.\textsuperscript{30} A second generation of the same is being developed with more electrodes and better signal processing capabilities.\textsuperscript{30}

Boston Retinal Implant Project developed an epiretinal prosthesis which underwent acute clinical trials. Since they could not get consistent results, they have abandoned the epiretinal implant and are now developing a subretinal implant instead.\textsuperscript{31,32} It is discussed under subretinal implants.

Bionic Vision Australia is working on two specific devices; one of which is a High acuity device consisting of an epiretinal microchip and implant with 1024 electrodes aimed to provide functional central vision and to assist with face recognition and large print.

The Seoul National University College of Medicine in South Korea has developed a liquid crystal polymer based long term implantable retinal stimulation microelectrode array and it was demonstrated that they were safe, compatible and mechanically stable to be a part of a chronic retinal implant system.\textsuperscript{35}

The advantage of an epiretinal implant is that the surgical technique to access the vitreous cavity and hence the inner surface of retina is a well-known procedure.\textsuperscript{36}

Subretinal implants

Subretinal prostheses involve implantation of the implant in the space between the retina and the Retinal Pigment Epithelium. Surgically, subretinal space can be accessed either externally through scleral incision or internally through the vitreous cavity and retina. Artificial Silicon Retina, Retina Implant AG and Boston Retinal Implant, Photovoltaic Retinal Prosthesis.

The Artificial Silicon Retina was the earliest retinal prosthesis which was developed by Alan Chow and Vincent Chow in Optobionics Corporation.\textsuperscript{21} It was an optobionic device, the energy required by retinal prosthetic devices was derived from incident light, and was composed of approximately 5000 independently functioning electrode tipped microphotodiodes. The Artificial Silicon Retina produced electric charges which altered the membrane potential of contacting retinal neurons and formed images in a way that the retina functions normally. It was found to be safe in animal models and was implanted in six patients with Retinitis Pigmentosa following the FDA approval for clinical trial in 1999.\textsuperscript{37} The Artificial Silicon Retina was well tolerated by all six patients. Visual function improvements occurred in all patients and also included some unexpected vision improvements in retinal areas distant from the implant. This suggested some kind of neurotrophic effect on the retina. They hypothesized that chronic low level electrical stimulation induces an up regulation of protective neurotrophic survival factors that improves the function of remaining photoreceptors. But it was demonstrated that the energy from an optobionic equipment would be insufficient to activate the remaining retina.\textsuperscript{38} After Phase II trials, an involuntary petition to liquidate under Chapter 7 was filed in 2007 and
approved against Optobionics Corporation\textsuperscript{36} halting the further research using Artificial Silicon Retina.

Retina Implant AG was the temporary culmination of the upgradation of an optobionic implant consisting of microphotodiode array with 7000 microelectrodes in a checker-board pattern configuration which was founded in 2003 in Tübingen, Germany.\textsuperscript{12} When used in animal models, they discovered that the energy generated from the microphotodiode array was insufficient and additional source of power would be needed.\textsuperscript{39-41} A compound visual prosthesis device consisting of subretinal, extraocular and subdermal components was developed.\textsuperscript{42} The subretinal part consists of an array of titaniumnitride electrodes and a microphotodiode array with 1550 photodiodes and electrodes. The extraocular portion is a foil strip carrying 22 golden connection lanes to the external connection and the reference electrode. The subdermal portion consists of a silicone cable that leads subperiosteally to the retro-auricular space where it penetrates the skin transmitter and ends in a plug. When implanted for 4 weeks in 12 subjects with Retinitis Pigmentosa without complications, it showed better object localization and differentiation of individual letters in some subjects.\textsuperscript{42-45} A multicentre clinical trial has been registered at http://clinicaltrials.gov/ct2/results?term=NCT01024803 and the results are not yet published. The device is now undergoing further upgradation.

Boston Retinal Implant Project’s subretinal prosthesis consists of a small hermetically encased, wireless device. This array is implanted in the subretinal space using a specially designed surgical technique by an external scleral incision. It affixes the bulk of the prosthesis to the scleral surface. The implanted device includes a hermetic titanium case containing a 15-channel stimulator chip and power supply components. Feedthroughs from the case connect to secondary power and data receiving coils.\textsuperscript{46,47} The device is undergoing animal studies.

Photovoltaic Retinal Prosthesis was developed in Stanford University. It consists of a subretinal photodiode array and an infrared image projection system mounted on video goggles. The light from image is captured by the video camera which is processed by a pocket device and displayed on pulse near Infrared video goggles, infrared image is projected into the retina and stimulates photodiodes which ultimately stimulates the retina.\textsuperscript{48,49}

The Subretinal implants have the advantage of better stimulation of the retinal ganglion cells than their epiretinal counterparts but they are constrained by limited space and difficulty in accessibility to the space. Large implants cannot be used subretinally.\textsuperscript{36}

**Suprachoroidal implants**

One of the devices of Bionic Vision Australia is a wide view device with a microchip containing 98 stimulating electrodes placed in the suprachoroidal space aimed to provide increased mobility to the blind patients. Suprachoroidal transretinal stimulation device is a silicon carrier with 33 platinum disc shaped electrodes. The advantage is that it could provide a wider visual field and a simpler surgical technique.\textsuperscript{33,34}

**Intrascleral implants**

Osaka University in Japan is now trying the development of an intrascleral prosthetic device which could ensure safer surgery while still accessible enough to stimulate the retina.

**Extraocular retinal prosthesis**

A research done at the University of New South Wales, Australia demonstrated the stimulation of retina with a prototype extra ocular retinal prosthesis placed over
sclera. This might lead to the development of a low resolution visual prosthesis. The advantage of this type of prosthesis is that it requires a minimally invasive surgery compared to other types of visual prosthesis. The research was only an animal experiment, this has a long way to go to be successfully available for implantation.

**Optic nerve prosthesis**

Microsystem based visual prosthesis was developed at the university of Louvain by Claude Veraart. The basic functioning of this implant is as follows: The light rays are received by an externally worn camera that sends signals to a spiral cuff of electrode wound around the optic nerve and connected to a stimulator implanted in the skull. The signals received are translated into electric signals that stimulates the optic nerve directly. Similar stimulation of the optic nerve can be achieved by inserting multiple penetrating electrodes through the optic nerve and optic disc. This has to undergo refining to be used as an alternative for retinal prosthesis.

**Cortical implant**

Research to provide vision for patients whose optic nerve is not intact or has not developed at all is aimed at cortical visual prosthesis. This is done in various institutes like Illinos Institute of Technology, University of Utah, the Ecole Polytechnique de Montreal in Canada, Miguel Hernandez University in Spain etc. The Utah Electrode Array developed by University of Utah has obtained FDA approval for short term implantation in human subjects. Though these are the recent works, Dobelle’s Eye developed by Brindley and Dobelle in 1960s and 1970s were the first of its kind. But it was unsuccessful because the milliampere range current needed to evoke phosphenes by cortical stimulation resulted in poor spatial resolution, discomfort and epileptic activity. This shortcoming was overcome by Schmidt’s intracortical electrodes which produced average thresholds of below 25 micro-ampere with better two point resolution.

**Lateral geniculate nucleus prostheses**

Lateral Geniculate Nucleus is a significant part of the visual pathway. Since the location of the nucleus is adjacent to areas that are targeted for deep brain stimulation therapy for movement disorders, it makes it an ideal location for a visual prostheses implantation as the surgical techniques need only slight modifications. But this is at a very premature stage of development in which animal studies are performed.

**Conclusion**

The Bionic Eye seems to be a promising solution for the individuals who were thought to have total irreparable sight loss a while ago. But for the visually challenged individuals of the medium and low income group and especially of the developing nations, it seems like a far outreach by an unfair play of fortune. Even the ultrasonic devices which are now used quite commonly by visually challenged individuals was a distant dream during its days of inception. Bionic Eye is in its initial stages of implementation in the field of ophthalmology, hence it needs to be at a price that can help in further development of the product enough to make it at an affordable price and greater quality. The extensive research on the topic bionic eye has hiked my astonishment and wonder of Human body and Science. I also felt guilty that I had to know these only now while this field has been progressing rapidly with its origin somewhere in the 18th Century itself. I’m sad for the fact that I was not able to mention about the research in India anywhere in this field. I hope events like this kindles the minds of young researchers in India and in future the timeline bears the name of India also. While researching on this topic, I found an app developed by Bionic Vision Australia for educational purposes. It helps us to know what phosphene
vision is and what the bionic eye actually sees. This is an example of such an image of a paper weight:

Only the outline can be deciphered, that too when the object lies in a contrast background. Though this makes up for no vision, this has to be significantly improved. David Hubel and his companion Torsten N. Weisel had long back deduced that: the higher the neuron is located in the Brain’s Visual Pathway- the more complex the stimulus it responds to. So, while researchers work on improving the resolution by increasing the number of electrodes and even electrically stimulating the visual cortex, why can’t they think about sensitizing higher neurons to respond to even in a smaller number of electrical signals? Why always physical stimulation, why not think a little bit of chemistry?

References

How Research Technology and Innovation helps improve primary health care through tele medicine

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Abstract

This article will look into how Research, Technology and Innovation have improved primary health care around the world through telemedicine. Telemedicine and tele health have revolutionised medical care across the globe.

Objective: The paper is an original manuscript which will go into the entire gamut of primary health care and how innovation and technological improvements have revolutionised the concept of telemedicine and how patients across the world have immensely benefitted from this.

Design: This article will be a narrative on the research and technological advances of telemedicine over the years. This will refer to several studies and research works undertaken by several luminaries in this field. The references will be duly documented at the end of this article.

Procedure: The procedure to be followed will be a chronological sequencing of telemedicine as it evolved over the years and how innovation and technology have improved the patient care at the primary health care setting.

Results: A review of the scholarly articles and other studies in this field have pointed to one palpable truth – Research Technology and Innovation have revolutionised way telemedicine and primary healthcare have evolved over the years. With technology driven telemedicine, primary health care has become easy, quick and very affordable.

Conclusion: The bedrock for global development is indeed Research Technology and Innovation. As a doctor, my focus on telemedicine reveals how, technology has touched the lives of millions of people worldwide and how telemedicine has revolutionised this niche market of healthcare.

Keywords: Research, Technology, Innovation, telemedicine, primary healthcare

Introduction

Primary healthcare is a facet of health which touches the life of every person born in this world. Be it the most advanced cash rich country or a country struggling with healthcare facilities, every person reaches out to primary healthcare system at some point or the other in their lives. Right from the time we are born till death, primary healthcare service providers are constantly reaching out to us and enriching our lives. This article will look into how primary healthcare has evolved over the years and how primary healthcare providers have used innovation and technology to reach out to millions of patients.
Over years the focus of primary healthcare has changed. And with this came unimaginable benefits. Nowadays the primary healthcare provider at your neighbourhood can do wonders in liaison with a super specialist sitting hundreds of miles away. This has been facilitated and improved by technological advances. The giant strides which technology has made over these years has made primary healthcare more efficient and patient friendly. The purpose of this article is to look into how innovation and technological advances have benefitted telemedicine at the primary healthcare setting.

**Methodology**

Several studies have been conducted over many years in different clinical settings in many geographically diverse environs spread out in different countries. These studies have been documented in this article and the references section at the end of this article lists these studies clearly.

A topic as vast as this which covers the entire realm of primary healthcare and how telemedicine has changed the game needs to be substantiated with scientific studies. Several medical luminaries have studied the efficacy of telemedicine. Initially the conservative world of doctors met telemedicine with some scepticism and doubt. But with time, the immense benefits of telemedicine and how doctors could visit the home of patients in remote areas started making people realise telemedicine was an easier and patient friendly option. In time, technology made things easier and more affordable and quicker.

**What is telemedicine?**

**Telemedicine** is the use of technology in the field of medicine in order to provide clinical health care at a distance. By doing this, crucial time delays and distance barriers are eliminated and healthcare is improved in remote areas. By using technology, medical information can be transmitted from remote primary healthcare location to a super specialist sitting in a sprawling super speciality tertiary healthcare centre in the city. A person can wonder what the big deal is. But by doing this, the patient sitting in a village gets to avail latest state of the art medical care which will normally not be available at a remote healthcare centre.

Telemedicine involves a growing field of medicine which involves using technology driven applications and services like video conferencing, emails, smartphones, iPad, wireless tools and several other tools using technological advances to connect a patient at a remote location with a doctor at an advanced care giving centre. An example would be a lady in a remote village in India suffering from skin rashes and itchy skin patches for years without realising what she’s suffering from and what she needs to do to treat herself. Telemedicine can connect her with a dermatologist sitting several thousands of miles away in a super speciality hospital who can see the patient using an iPad and do a spot detection of her skin condition and initiate treatment for her.

Telemedicine started over 40 years ago with hospitals extending care to patients in remote areas. Over the years, the scope and reach of telemedicine or telehealth has exploded and now several hospitals have a dedicated department of telemedicine or telehealth dealing with home based healthcare.
Technically speaking telemedicine is not a separate medical specialty. It is a mere extension of hospital facilities to a remote site. This could be tele cardiology, tele surgery, tele medicine, tele dermatology and so on. Telemedicine and telehealth are used interchangeably. Conducting patient consultations using video conferencing, using e Health Medical portals, escalating patient lab results to remote locations, continuing medical education to doctors sitting in different countries, transmission of images and videos of patients to doctors in other cities or countries to facilitate an expert opinion, remote monitoring of vital signs of a patient – including pulse, blood pressure, blood tests, BMI etc., call centres for patient health care and nursing care, home based health care providing nursing and doctor facilities, post discharge monitoring of patients at their homes – all this is part of telemedicine.

**What can telemedicine offer?**

Telemedicine can offer the following variations:

- Primary healthcare
- Specialist healthcare by referral to a specialist in a distant location
- Monitoring of patient in a remote location
- Dissemination and escalation of consumer medical information from one location to a specialist (using safe and medically confidential fool proof methods)
- Medical education, seminars and conferences for medical practitioners – this e conference is an ideal example – being a doctor, this article is written here and would be sent to another country, thousands of miles away and would be read and assimilated by end users sitting in various locations. This is one such example – how technology and innovation has aided telemedicine.

Now let us look at each of these in detail.
Primary healthcare

A primary healthcare set up involves our friendly and affable neighbourhood General Physician (GP) and the patient. This GP model has been working for years on end and has helped thousands of people in every country in every town in every village. However, the GP model has a big disadvantage – critical cases, emergency patients and patients presenting with advanced disease conditions might place the GP in a position where the doctor might be unprepared to deal with the demands of the case. This is where telehealth steps in and unfolds its magic.

Using telemedicine, a specialist could refer the same patient to another super specialist without any travel involved. Without inconveniencing the patient, using technology, a referral could be done to another more qualified specialist who would be able to manage the case better.

With telemedicine, even a critical case or a case which needs expert evaluation can be assessed in a remote location by an expert sitting miles away and through technology driven tools, the GP can assist the patient and deliver a high level of medical care. Video conferencing can be used to connect a super specialist and the patient and this video can be saved for a later date during a follow up visit.

Remote monitoring

Highly advanced investigations like blood tests, cardiac scan or ECG, patient evaluation or monitoring of patient vitals can be done remote diagnostic testing. Nurses and doctors could be dispatched to a patient’s home to provide home based healthcare at the comfy confines of a patient’s home.

Consumer medical information

Using technology, patient’s health information could be disseminated to other specialists to provide online consult. This would enable a fresh relook into the management of critical cases.

Distance medical education

Continuing medical education is an important facet for the development of any doctor or healthcare provider. Telemedicine is a crucial link in this journey. Latest breakthroughs in medical science and advances made in niche specialities are easily cascaded to the team of doctors across the globe. This helps doctors in equipping themselves better and in improving their services.

Though telemedicine brings in information technology and healthcare under one umbrella, the critical aspect here is to ensure the availability of technology for the end user. Without the availability of technology, remote healthcare will not be successful. The most common medium is interactive video.

Technology has grown consistently over the past 40 years but the main challenge is
availability of niche cutting edge technological services for the end user. There is lack of consistency in availability of technology at the grassroots level. Though there are still several critics of telemedicine, it has been widely proved that telemedicine is indeed effective with good technological backup.

Telemedicine includes various specialities like tele radiology, tele pharmacy, tele dentistry, tele surgery, tele rehabilitative therapies, and tele public health. Costs have dramatically declined with advances in technology. With improvements in telecommunication, technology has advanced and this has resulted in declining costs over the past decade, there has been a steady growth in telemedicine. Telemedicine is not only of clinical benefit but cost effective too.

Wherever there is an element of distance between patient and doctor, it is called telemedicine. This is an umbrella term which includes primarily a patient doctor interaction that involves an element of distance. This is similar to using a radio by a ship captain to take advice from the shore or using technology by pilots to take advice from air traffic control.

Online health, e health, telemedicine and telehealth are all interchangeable names. Cost effectiveness and scientific evidence is very crucial for the success of telemedicine.

Findings

Recent advances

• A randomised control trial of home tele nursing was done which showed evidence of cost effectiveness.
• Outpatients are efficiently handled by electronic referrals
• In several locations, GP teleconsulting might turn out cheaper than traditional consulting.
• In several cases, where nurses were handling minor injuries, tele consultation with specialists turned out to be cost effective and quicker.

Teleradiology

A speciality of telemedicine where digital x-ray films, ultrasound scans, CT scans and MRI films are digitally transmitted from the primary healthcare centre to the radiologist sitting in a tertiary healthcare centre.

As is the case with any other form of telemedicine, some technology driven equipment are needed – a modern x-ray unit which can produce digital x-rays is needed. In certain set up, ultrasound scan machine, CT scan and MRI scan machines
are also needed.

The receiving hospital also needs to have technologically compatible set up which can receive and escalate the radiology films to the specialist and there should be a method of returning the reports to the primary healthcare set up.

The advantages of having a tele radiology unit lies in the fact that there no need to maintain a radiologist or a radio diagnosis specialist in certain locations where the numbers of patients do not justify it. By using this tele radiology unit with technologically advanced support, patients living in remote locations can also avail specialist services.

The alternative would be to maintain a radiologist on location which might be an expensive proposition. On top of the money factor, getting specialist radiologists might not be feasible at all locations. Other options involve part time radiologists (visiting radio diagnosis specialist to visit patients at remote locations, once a week), the other option is to refer patients for radio diagnosis at a larger secondary or tertiary medical centre.

The cost effectiveness of tele radiology depends on the number of patients availing the services and costs involved in setting up the tele radiology unit and the revenue generated by the patients paying for the tele radiology services.

A Medline search of over 969 articles was conducted on telemedicine and this included cost effectiveness of tele medicine. Peer reviewed publications on telemedicine were studied. The keywords included “telemedicine”, “telehealth”, “online health” and “e health”. The editorial board of Journal of Telemedicine and Telecare was also consulted.

Over the past several years, there has been a sudden spurt in the interest shown by the medical fraternity in implementing telemedicine for home based healthcare for chronically and terminally ill patients.

The advantages of this set up is that the patient is given care at the familiar home setting and patient compliance is very good. However, this involves a lot of expensive startup expenses involved in purchasing very expensive telecommunication equipment. This makes the concept quite expensive and almost comparable to hospital based expenses.
One very significant study in home based healthcare using telemedicine and telenursing was conducted by Kaiser Permanente. The first randomised controlled trial of home based nursing care for newly diagnosed patients with several diseases like cancer, diabetes, Chronic Obstructive Pulmonary Disease (COPD), Congestive Cardiac Failure (CCF), Anxiety and Cerebro Vascular Accident (CVA) were all selected and studied during this program. Patients were divided into two groups – the control group and the intervention group.

Digital stethoscope, Digital BP Apparatus and home videophones were given to the intervention group. Over 18 months the patients in both the groups were studied and patients in the telemedicine group received 17% less home visits by the nursing staff when compared to the control group.

Though the level of quality of care was the same in both groups, the satisfaction levels in telemedicine group was higher and they had less home visits and more telephonic contact with the care givers.

The best part was the average cost was 27% less than that of the care in the control group. (Outcomes of the Kaiser Permanente Tele-Home Health Research Project, Johnston B, Wheeler L, Deuser J, Sousa KH.) http://www.ncbi.nlm.nih.gov/pubmed/10664641

**Telenursing**

Telenursing is a very important link in the success of telehealth projects. One of the most critical aspects of telenursing is the availability of important tech devices for the nurses, which enable them to monitor the patients and simultaneously keep the data flowing to the specialist sitting miles away. In the Kaiser Permanente Trial, the equipment used were a digital BP apparatus, a videophone and an electronic stethoscope. The availability of these equipment might be a challenge at all locations.

To procure the equipment is a challenge and this is critical for the success of any telenursing project. Which is why it is easier to make such projects succeed at a community nursing home than in a private home because the cost of the equipment could pose a challenge to the public.

Some studies conducted in Hong Kong suggest that the trials of telemedicine maybe cost effective. (The role of telenursing in the provision of geriatric outreach services to residential homes in Hong Kong, Chan WM, Woo J, Hui E, Hjelm NM)

**Referrals to hospitals for specialist care using telemedicine**

A study conducted in Helsinki, Finland at the Peijas Hospital has shown a startling 52% referrals over a 20 month study period were much more cost effective than traditional out patient referrals.

This reduced expenses for the patient, decreased patient visits to hospitals and saved time, money and energy for the ailing patient. A teleconsultation through a video link was all that was needed by the hospital staff to facilitate this. Two groups of patients with similar ailments were studied and it was found that costs were seven times cheaper in the telemedicine group.

Similarly in Bangladesh, a study was conducted wherein a panel of specialists would give email support to the GPs and the general practitioners would then triage and treat the patients coming to them and the ease with which the patients were referred to higher medical centres was also determined by the tele email consultation. This scheme was cost effective and less time consuming.

**GP specialist nexus with telemedicine**

One of the biggest boons of telemedicine is the ease with which an inexperienced, new doctor sitting in a remote location is able to effortlessly and seamlessly consult with a specialist sitting miles away in a specialist tertiary medical centre.
Traditionally, the GP sends the patient to a tertiary medical centre (usually a journey of several miles) and the handover is down through a handover form or a document. In telemedicine, the GP continues to manage the patient at the remote facility but the tertiary medical centre based Specialist is able to closely monitor the case through telemedicine. This avoids travel of the patient to a specialist hospital miles away. Three trials in New Zealand, Norway and UK have shown that teledermatology can be considered cost effective. The trials used real time video and they clearly demonstrated that significant travel time was reduced by telemedicine. This reduced a considerable burden for the patients. In rural areas of Scotland teledermatology is considered feasible, though the same might not be the case in a sprawling city like London with many dermatologists available round the corner.

Injury management and telemedicine

All this said and done, one of the most promising and useful implications of telemedicine is to use real-time video by in the management of minor injuries. This has been studied in Scotland and in more than 20 injury units across UK. This has significantly demonstrated that nursing practitioners avoided unnecessary patient transfer and saved lots of money in managing minor injuries at their remote locations using real time video. A follow-up study by Central Middlesex Hospital has shown telemedicine is clinically safe and effective.

Telephone call centres and telemedicine

Many call centres which provide medical information and health information for public such as NHS Direct, try to triage callers into those who need emergency treatment, those who need primary healthcare referral and those who can safely treat themselves at home. This has shown to be effective and safe. Telephonic conversation usually is the first step of a triage process. Triage in medical parlance means – sorting out a patient based on the need for treatment. Based on the answers to a few questions, the callers are categorised into different groups. Based on this sorting or triaging, patients are managed accordingly.

Online information sources

For health professionals

http://tie.telemed.org Telemedicine Information Exchange database
www.rsm.ac.uk/pub/jtt.htm Journal of Telemedicine and Telecare
www.coh.uq.edu.au Centre for Online Health
Conclusion

Future of telemedicine

The future of conventional healthcare is intertwined with telemedicine. Access to healthcare is improved significantly in remote areas and there has been steep reduction in travel time and costs incurred by patients in remote locations. Geographical barriers are a major factor which help the proliferation of telemedicine, with several patients in lonely remote areas getting to meet a super specialist at the click of a computer mouse. Critics say telemedicine is too expensive and the equipment needed to start off telemedicine consultation are just little more than toys for the boys. Some others critique the equipment and telecommunications companies only provide a technical solution and do not understand the medical aspects. Another challenge in telemedicine is not just the availability of technology but the organisational challenge of how to take advantage of technology and how to train the usually conservative medical experts to become tech savvy and more technology driven.

Telemedicine has now entered the public realm and is now freely being accepted by many as a viable alternative. There are many who have saved a long trip to a referral hospital by employing telemedicine as a viable alternative and several studies have indeed shown in many settings, telemedicine is cost-effective. The long term challenge is using the latest cutting edge technology, sensitising the public and medical experts to the possibility of technology being a life saver and to walk a tight rope between reducing hospitalisation costs and the exorbitant costs of technological gadgets necessary for a telemedicine consult.

Technology and Innovation have revolutionised our lives in several ways and medical science is seeing a great sea change in which patients are treated and healed. Contrary to the conventional practice of a patient going to a doctor and waiting for several days and at times, several weeks to get a reasonable closure, telemedicine has saved time and money for the patient and it has brought the patient, the primary healthcare doctor and the specialist closer than ever before. With technological advances, patient satisfaction has increased. Telemedicine has evolved as a niche branch of Medicine and with newer research and technological breakthroughs, telemedicine is exploding and bringing smiles to the faces of patients and doctors. This article is an attempt to bring the latest advances and benefits of telemedicine to the discerning reader. It is hoped that the reader enjoys this as much as the author enjoyed writing this article. Let’s hope with passing time, medical science evolves further and telemedicine becomes more affordable and easily accessible to the common man. With this, crucial time and money can be saved bringing in rich benefits for the patients.

Conflict of interest

Nothing to declare
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‘A Case Scenario cutting across different HIV Prevention issues’

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Abstract

Background Most new HIV infections are acquired from stable, long-term partners. HIV serodiscordant relationships are among the most vulnerable to acquiring HIV. It is not uncommon getting exposed to body fluids of an HIV infected partner in a serodiscordant (magnetic couples) set ups. This is an interesting case scenario cutting across many issues about the transmissibility of HIV infection. There may be queries from apprehensive people – seeking advice regarding HIV’s probability of acquisition under some very uncommon conditions which may be helpful for training/teaching purposes.

Objectives The scenario (A non-HIV Patient getting exposed to semen of a HIV positive man with an undetectable viral load –VL at the hand having an open cut) is an interesting, educative and unique case scenario as it involves almost all issues related to HIV prevention i.e. scanty information with holding his/her name and gender possibly due to stigma/discrimination issues forwarded by the questioner and issues involving responses to various situations in serodiscordant couples ('magnetic couples ') exposure in an unique non-occupational yet non-sexual mode, counseling& testing issues, initiating nPEP (Non-occupational Post Exposure Prophylaxis) and PrEP (Pre-Exposure Prophylaxis), TasP (Treatment as Prevention) and HTPN 052 Study. The importance of answering this kind of scenarios lies in the fact that it would help many—including the questioner itself.

Methods Descriptive study discussing the different aspects of HIV Prevention.

Results The questioner’s HIV-acquisition risks are extremely low (theoretically) & unwarranted still we will try to put his/her fears to rest by examining the issues involved in this case critically and coming up with scientifically based explanations and accordingly advice him/her taking into the considerations of HIV Testing and Treatment policies and Guidelines of the land available.

Conclusions This scenario is a very interesting one, requiring many basic concepts of prevention of HIV/AIDS for answering and may be used for teaching/training even medical professionals. The questioner’s HIV-acquisition risks are extremely low (theoretically) & unwarranted and we will try to put his/her fears to rest by examining the issues involved in this case critically and coming up with scientifically based explanations and accordingly advice him/her taking into the considerations of HIV Testing and Treatment Policies and Guidelines of the land available. The importance of answering this kind of scenarios lies in the fact that it would help other health professionals and questioners alike and may be useful in teaching/training settings.

Keywords: nPEP, PrEP, Discordant couple TasP

CASE SCENARIO IN QUESTION (this question was asked to me in a site for reply)

'I am in a relationship with a positive undetectable man. I am neg. We refrain from oral and anal sex. Last night while masturbating some of his semen got on my hand and I had an open cut. I have not been able to sleep. I do have a question. Since we are in a monogamous relationship and he is undetectable???, should I get tested on same frequency. Going for testing freaks me out. I have not been tested since 3 months after his diagnosis which was three years ago’
**Introduction**

It is a case of providing inadequate information (about his/her gender, the HIV infected partner being on ART or not, age of cut in his/her hand, which part of the hand—palm or dorsum and has asked question about his/her going for testing of HIV at the frequency specified as per country Guidelines. With whatever little information we have from this person’s history, it is to be advised to him/he that though probability of transmission is extremely low, but due to paucity of information provided and just to be very sure, about non-transmission of the virus, notwithstanding the negligibility of spread he/she should go for testing as per the country guidelines Considering all issues involved, It is suggested that HIV-antibody tests are to be done for the questioner as per the country’s testing guidelines. This will enable the questioner not only to gain psychological peace of mind but also by knowing the status—whether negative or positive help him/her to lead healthy life. Though we expect the result to be negative, considering low risk, but, it is to be remembered that decreased risk is not no risk. Effective cART that drives the viral load to undetectable levels significantly decreases any chance of HIV transmission; however, it does not eliminate the risk completely, so one should always adopt ‘safest sexual practices’. That's the critical point in a discordant scenario. **Strategies Based on Action by Uninfected Individual to Prevent Infection are:** Education/behavior change, Condoms. Male circumcision, Microbicides, PrEP(Pre-Exposure Prophylaxis). Vaccines and possibly ‘PEP(Post Exposure Prophylaxis)

**Strategies to Block Transmission Based on Action by Infected Individual to Reduce Infectiousness /Prevent Virus Release are:** Prevention of mother-to-child transmission, Treatment of positive partner in discordant couples, Treatment as prevention (TasP)—TasP for all, TasP for higher VL, TasP for higher CD4, More rapid clinical linkage to ART Out of the above mentioned, there could be various options available in this scenario for prevention of further transmission (TasP,PrEP, nPEP—Non-Occupational Post-Exposure Prophylaxis). We presume, the positive partner is on ART, as that person is undetectable, so his (positive partner) taking ART, will be acting as Treatment as Prevention(TasP) in this scenario. If this questioner decides to take ART for his/her benefit, to reduce the chances of transmission, subject to fulfilling certain conditions, then this will be called ‘PrEP (Pre Exposure Prophylaxis). Of course in both the set ups (PrEP, nPEP), the questioner has to go for ‘Tests at base line and subsequent times, more so when the questioner decides to start ART as PrEP, because the moment he/she becomes positive after initiating PrEP, then he/she has to stop two drug regimen used as PrEP and further initiate ART as per the Guidelines for HIV infected persons. This is a question involving many issues about the transmissibility of HIV infection under serodiscordant scenarios. The questioner’s HIV-acquisition risks are extremely low (theoretically) & unwarranted and we will try to put his/her fears to rest by examining the issues involved in this case critically and coming up with scientifically based explanations

**Results and discussion**

**Issues involved**

We will critically examine the issues involved in this scenario as follows:

**The first issue: Basic science tells us that HIV/AIDS is not readily contracted and hence the word ‘Acquired’ we find in the acronym ‘AIDS’! One has to put in extra labor to ‘acquire’ it.**

In order for infection to occur, three things must happen:

- One must be exposed to pre-cum semen, vaginal secretions, blood or breast milk, AND
The virus must get directly into one’s bloodstream through some fresh cut, open sore, abrasion etc., AND
Transmission must occur, directly from one person to the other, very quickly (the virus does not survive more than a few minutes outside the body).

No matter what the circumstances are, if one thinks about these three criteria for transmission, he/she should be able to determine whether he/she is at risk for HIV or not.

The second issue: The questioner has not mentioned that ‘how old the cut was and where (palmer/dorsal side). It may be presumed, the cut was in the process of healing when the questioner asked this question, which means that even though the cut was still visible, there was probably not direct access to the blood stream. There would have to be "open, active sores and then "enough fluids entering for there even to be a remote chance. As we know that chances of transmission in intact skin is zero but through mucous membrane is not zero. So the palmer side do has more risk than dorsal side even it is intact. Blood contains the highest concentration of the virus, followed by semen, followed by vaginal fluids. Breast milk can also contain a high concentration of the virus, but transmissibility depends on ‘Who’ and How’?

It is not enough to be in contact with an infected fluid to become infected instead will require prolonged and sustained contact. Healthy, unbroken skin does not allow HIV to get into the body. HIV can only enter through an open cut or sore, or through contact with the mucous membranes in the anus and rectum, the genitals, the mouth, and the eyes. The vulnerability of the mucous membrane can be increased by inflammation, rough sex, the location and thickness of the mucous membrane and STIs. It's important to note though, unless there is something unusual and undeniable as an open sore on the skin, there is no risk from semen on the skin. There is no risk from casual contact either. Also, Mutual masturbation and frottage are not considered HIV transmission risks (a no risk activity –not a very low risk activity !) as long as there are no active bleeding cuts on one’s person. We can have look at the individual risks and calculate the probability

Risk of HIV transmission following an exposure from a known HIV-positive individual

<table>
<thead>
<tr>
<th>Type of exposure</th>
<th>Estimated risk of HIV transmission per exposure (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood transfusion (one unit)</td>
<td>90–100</td>
</tr>
<tr>
<td>Receptive anal intercourse</td>
<td>0.1–3.0</td>
</tr>
<tr>
<td>Receptive vaginal intercourse</td>
<td>0.1–0.2</td>
</tr>
<tr>
<td>Insertive vaginal intercourse</td>
<td>0.03–0.09</td>
</tr>
<tr>
<td>Insertive anal intercourse</td>
<td>0.06</td>
</tr>
<tr>
<td>Receptive oral sex (fellatio)</td>
<td>0–0.04</td>
</tr>
<tr>
<td>Needle stick injury</td>
<td>0.3 (95% CI 0.2–0.5)</td>
</tr>
<tr>
<td>Sharing injecting equipment</td>
<td>0.67</td>
</tr>
<tr>
<td>Mucous membrane exposure</td>
<td>0.09 (95% CI 0.006–0.5)</td>
</tr>
</tbody>
</table>

(The CI, confidence interval)

The third issue: The gender of the questioner has not been mentioned, and the questioner is in relationship with a positively charged male (positive), making them a ‘magnetic couple’ (sero-discordant) which certainly puts one at additional risk of transmission (howsoever small-if the questioner is a female and having ‘vaginal sex’ with HIV + male without protection)

Here are certain facts about —Sexual transmission of HIV in discordant situations:

- Stage of Illness the HIV --the pt is in to (HIV levels in blood are almost 10-times higher during the acute phase of infection, as many as 50% of new infections may occur through sex or sharing needles with someone who has just recently been infected.)
During inflammation, immune cells are brought to the area to fight infection. These immune cells include dendritic cells, which may transport HIV to the lymph nodes, and CD4+ cells (the cells that HIV infects).

In an HIV-positive person, inflammation of the genital tract or rectum increases the viral load in the genital or anal fluids, even though it does not increase the blood viral load. This is because inflammation at a site usually brings more infected immune cells to the area. When these cells become active to fight the infection they unwittingly make more copies of HIV.

If an HIV-negative person has inflammation, a larger number of immune cells will arrive at the site to fight off the cause of the inflammation. This means there is a greater chance that HIV, (if in this period he/she has sex with an infected partner) which will come into contact with these cells and infect them.

Fidelity (faithfulness)/ monogamous in relationship/marriage also affects the transmission as one could never be able to figure out the person’s HIV status, one is going to have sex with as HIV (and not AIDS ! there is difference between HIV & AIDS) is not visible and recognizable by one’s physical appearance

These things may not apply in this case as it is a kind of a non-sexual exposure in non-occupational setup in serodiscordant couple.

The fourth issue: The probability of HIV Transmission with nearly any type of exposure is directly correlated with viral load. Recently there have been studies released that show that in magnetic partners (one being HIV positive and the other negative) if the positive partner is on antiretroviral medications has an undetectable viral load and no other STI's (Sexually Transmitted Infections) present, then the likelihood of HIV transmission is small. (the famous Swiss Federal Commission Report & HTPN 052 Trial) HTPN 052 Trial -- A GAME CHANGER, showed 96% reduction in HIV transmission between serodiscordant partners when HIV infected partner began ART immediately. Even CDC in its campaign for ‘Act against AIDS Campaign ‘ says that at VL 50,000 or more copies/ml, it is projected 26 new infections related to sexual intercourse. In contrast at VL, 3500 copies/ml, the projected no of new infections drops to 2.

Shedding of virus in the male genital tract is not uncommon, even in men with consistently undetectable plasma HIV RNA., and the timing and frequency of this shedding appear to be unpredictable. Furthermore, previous studies have shown no clear association between the presumed penetration of specific antiretroviral into the genital tract and the likelihood of detectable virus in semen.

Three studies all with small number of subjects, have demonstrated that effective ART can reduce VL in serum, to undetectable level, however reductions in HIV in serum do not always lead to reductions of HIV in genital secretion to undetectable levels.

Politch et al in 2012 reported in their research among men having sex with men (MSM) that appropriate treatment of STDs can further reduce their seminal HIV shedding for men on ART. This research confirms that lower VL (Plasma) are also associated with lower genital viral loads. Transmission risks are less but exists.

The threshold level of genital-tract HIV necessary for transmission is not known, HIV transmission can be “very likely”, between magnetic couples, even when the positively charged person is on effective combination antiretroviral therapy (cART-combined ART) that has driven his HIV plasma to viral load undetectable levels., are overestimating the risk considerably.

The fifth issue: While we are on the topic of "undetectable" viral loads (VL), Here some basic things need to be discussed to clear common misunderstandings about this
term’.

- ‘Undetectable’ means the HIV plasma viral load is below the lower limit of detection for the particular test assay that is being used. Early viral load tests could only test down to 10,000 copies. Newer tests were able to test down to 500 copies of the virus per milliliter of plasma. The even newer ultrasensitive viral load assays can test all the way down to 25 or 50 copies/ml. We now have ultra-ultrasensitive assays available in some research laboratories that can test down to a single copy per ml! However, even in HIV-positive patients with HIV plasma viral loads below 1 copy/ml, this does not mean they have zero virus in their body. HIV still exists inside cells in the blood, lymph nodes and other body compartments.

- ‘Undetectable’ does not mean cured! There is no cure for HIV/AIDS, though this has become a chronic treatable and manageable disease just like High Blood Pressure (Hypertension) and High sugar level (Diabetes), you have to gulp’ medicines for life.

- ‘Undetectable’ does not mean noninfectious (that you cannot transmit the virus to others)! We have cases documenting HIV transmission from a man with an undetectable viral load to his HIV-negative wife via unprotected vaginal sex. There are also cases of mother-to-child transmission, despite the mother having an undetectable viral load, though undetectability certainly decreases the risk of transmission.

- ‘Undetectable’ does not mean the virus cannot be detected anywhere in the body. Despite having an undetectable viral load in the blood (plasma), the virus would still be readily detectable in other tissues and body compartments, but the ability to transmit decreases considerably from a HIV pt who is undetectable.

- ‘Effective combination antiretroviral therapy does not kill the virus!’ Rather it merely suppresses viral replication. Consequently, if someone with an undetectable viral load on combination antiretroviral therapy stops taking his drugs, the virus will soon start reproducing again and the viral load will skyrocket to levels near to where the viral load was before treatment was begun. That’s why the compliance/adherence to ART regimen must be at least 95% if not total (100%)!

**The sixth issue:** Testing is an important tool for prevention and treatment as well. One American model tells us that nearly 1.2 millions PLHAs (people living with HIV/AIDS) in US, 20% do not know they are infected. Of an estimated 9,42000 aware of their HIV infection about 77% were linked to care only 51% remained in care Among those retained in care 89% were prescribed ART of whom 77% achieved viral suppression. Following our progress on each step, we can see that only 28% of all HIV infected person in US have a suppressed Viral load, and we know that suppressed VL is essential for non-transfer of the infection.

We see that knowing about one status is very important as 20 unaware out of 100 PLHAs are a big number which is going to have a big impact on any Prevention program as unaware PLHA can further aggravate the pool of infected persons. Unaware persons are unable to realize any of the health and prevention benefits of ART (Anti-retroviral therapy) Increased testing and engagement in care is also at the heart of National HIV/AIDS Strategy in US that has been released by White House in 2010.

Newer research have shown that Anti-retrovirals (ARVs) can be used by uninfected persons to prevent HIV before an exposure (called PrEP) and after a non occupational exposure (called ‘nPEP’). In this set up (sero-discordant), one must be aware be aware about the availability of the options—I believe the positive partner is on ART, as he is undetectable, then this ART is acting as ‘Treatment as Prevention
(TasP/T4P)). If it is decided that the questioner should take ART after this exposure for fixed number of days (as this exposure comes within the time period of 72 hours) then this becomes ‘nPEP’, that is ‘Non Occupational Post Exposure Prophylaxis’.

It has been found through research that fewer MSM population in US are aware of these modalities. If this questioner decides to take ART for his/her benefit, to reduce the chances of transmission, subject to fulfilling certain conditions then this will be called ‘PrEP (Pre Exposure Prophylaxis). Of course in both the set ups (PrEP, nPEP), the questioner has to go for ‘Testing’ at base line and subsequent times as per the protocol of the country, more so when the questioner decides to start ART as PrEP, because the moment he/she becomes positive after initiating PrEP, then he/she has to stop two drug regimen used as PrEP and further initiate ART as per the Guidelines for HIV infected persons.

The seventh issue: Treatment as prevention” (TasP) is the use of combination antiretroviral therapy (ART) in HIV-positive individuals to preserve their health and reduce the risk of transmitting the virus. Anti-retroviral therapy (ART) initiation has been shown to dramatically reduce HIV transmission in discordant heterosexual couples prompting revisions to treatment eligibility criteria. Responding to this, new guidelines recommend starting ART either at HIV diagnosis, or at CD4 counts of ≤500 cells/mm3. In June 2013, the World Health Organization updated its ARV guidelines to reflect treatment and prevention benefits—and suggests that countries offer ART to all HIV-positive individuals with CD4 cell counts of 500 or below, and to specific groups (pregnant or breastfeeding women, HIV-positive people in serodiscordant couples) regardless of CD4 cell count.

A large proportion of HIV infected adults not qualifying for immediate ART at the CD4 count threshold of 350 cells/mm3 may have high viral loads. Sarishen Govender et al reported that of the ART-naïve first time testers whose CD4 count was above the CD4 threshold for ART initiation as per South African guidelines (<350), 34% had a VL > 10,000 copies/ml suggesting that CD4 count at the time of HIV diagnosis may be a poor proxy for HIV transmission risk. Consideration should be given to replacing CD4 count threshold with viral load threshold for ART initiation when planning treatment as prevention (TasP) interventions.

We presume here that, the positive partner is on ART, as he is undetectable, so his (positive partner himself) taking ART, is acting as Treatment as Prevention(TasP) in this scenario.

The eight issue: Stigma and discrimination This issue do have psych-social reasons. The patient has not come up physically to seek help (chosen to seek advice through mail) and has provided inadequate/insufficient information possibly due fear of rejection/stigmatization.

HIV stigma and related discrimination remain key barriers to dealing effectively with the HIV. HIV stigma can deter people at risk from being tested for HIV and deter HIV-positive people from accessing appropriate treatment and care. It also remains the key obstacle for HIV-positive people disclosing their status to friends and family, employers and work colleagues, health care providers, insurance companies, landlords, and sexual partners for fear of being treated less favorably, or being out-rightly rejected or abused. Stigmatization/discrimination often done on moral grounds culminating into to rejection, which prevents them to seek advice openly to as HIV/AIDS is often linked to be at-risk behavior resulting in people labeled as immoral, more so when there are homosexual activities involved (as we presume in this case.)

Conclusions
This is an interesting case scenario involving multiple modalities of HIV.
prevention and generates many though provoking Q & A making them useful in training/teaching setups too. This review yields following learning points and take home messages which forms the base of core knowledge and skills to develop strategies to fight and prevent HIV.

Learning points /take home messages

- Most new HIV infections are acquired from stable, long-term partners.
- No one is immune from HIV.
- HIV is still stigmatized and discriminated in societal terms
- HIV serodiscordant relationships are among the most vulnerable to acquiring
- Discordancy is at the core of HIV sexual transmission, and diagnosing it is key to HIV prevention
- HIV negative partners in discordant couples are at very high risk of infection.
- Acquiring It is not uncommon getting exposed to body fluids of an HIV infected partner in a serodiscordant (magnetic couples/ serodivergent /mixed status couples’) set ups.
- HIV discordance is not a sure sign of infidelity.
- HIV is not transmitted on every exposure.
- Viral load(VL) is important in transmission, but it changes over time
- VL is proportional to acquisition of HIV through any route.
- Undetectable VL reduces the risk of transmission of HIV. But undetectability does not mean no risk or no infection.
- Effective risk reduction options exist(PrEP, nPEP)
- HIV transmission within discordant couples can be prevented.
- A large proportion of HIV infected adults not qualifying for immediate ART at the CD4 count threshold of 350 cells/mm³ have high viral loads.
- It is possible for couples to stay HIV serodiscordant indefinitely if they consistently practice safer sex using condoms.
- Couples’ testing and counseling is as cost-effective than other interventions, such as ART
- Disclosure is important for HIV prevention.
- Treatment for the HIV-positive partner also is highly effective in reducing the risk of transmission to the HIV-negative partner (called Treatment as Prevention—TasPor T4P)
- Combined, treatment and consistent condom use are likely to offer greater protection than either one alone.

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Anticancer and Apoptosis Induction Properties of Apium Graveolens Seeds

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Abstract

Based on traditional medicinal use Apium graveolens seed was selected to evaluate its anti-cancer property with special reference to apoptosis induction, if any. When the water, alcohol and hexane extracts of the dried powder of the plant seeds were tested for cytotoxicity to Dalton’s Lymphoma Acitic (DLA) cells in vitro using Tryphan blue method, only the n-hexane extract showed significant activity at 500 µg/ml level. The extract induced apoptosis as evidenced by morphological changes. The cytotoxicity of this extract was found to be more to cancer cells (DLA) compared to normal cells (thymocytes and macrophages). An active chloroform fraction was separated from this extract. The active fraction at a dose of 200 mg/kg protected 75% of the mice challenged with 1 X 10⁶ DLA cells whereas all untreated control mice died of cancer. Thin Layer Chromatography (TLC) on silica gel further separated this anticancer fraction into 2 cytotoxic components. One of them was identified as a steroid positive component whereas the other was an alkaloid positive component. The steroid component induced apoptotic cell death while the other component induced necrotic cell death. These components are attractive materials for further studies leading to possible anti-cancer drug development.

Keywords: Apoptosis, Apium graveolens, Anticancer, Dalton's lymphoma ascetic

Introduction

Traditional medicinal plants are a rich source of pharmacologically active compounds. These plants are used by various cultural groups from ancient times onwards to treat various diseases including the dreaded disease cancer. These plants were identified for medicinal uses through human experiences, empirical knowledge and beliefs that existed in various periods of human evolution. Many of the herbal drugs are used improperly. Scientific evaluation of these medicinal plants in light of recent advances in medical sciences and therapy as well as modern methods of drug development is sure to revolutionize our health care and socio-economic conditions (1).

Cancer even today escaped the ingenuity of human, to a large extent, and poses a serious threat to modern civilization. Although there are many drugs in use to treat cancer patients, none of them is satisfactory to cure full grown cancers (2). Many plant based drugs discovered and developed through ethno-medical leads such as taxol, vincristine, vinblastine and camptothecin are currently used as chemotherapeutic agents and many more drugs are in various stages of studies (3,4,5,6,7,8,9,10,11,12,13,14). There are many traditional anti-cancer plants, which remain to be studied in light of modern sciences (1,15).

In this context, the discovery of apoptosis, one of the body’s own mechanisms to remove unwanted cells including cancer cells, gives hope to use as a tool to screen medicinal plants for their possible anti-cancer activity (16, 17,18,19). If induction of apoptosis by phyto-chemicals turns out to be specific to cancer cells or specific type of cancer cells such compounds could likely to be invaluable anti-cancer agents (19).

There are many plant extracts and phytochemicals known to induce apoptosis (19). Search for cancer cell specific apoptosis inducing compounds, among anti-cancer
plants is an interesting area to pursue. Based on compelling ethnomedical leads (20), *Apium graveolens* Linn. Apiaceae [Tamil: Oman; Malayalam: Ayamodakam; Sanskrit: Ajmoda; Hindi: Ajmud; English: Celery] was selected for screening for possible apoptosis inducing properties vis-à-vis developing better chemotherapy for cancer.

This plant is used in different parts of the world in traditional medicine to treat as well as prevent diverse types of cancer (20). Generally all parts of the plant, especially seed, leaves and flowers, are in use. The juice of the plant in the form of liquid or syrup or pills is given to treat/control various types of tumors (20). Crude seed extract of this plant was shown to be active against mosquitoes (21). It is currently used in traditional medicine, culinary, drugs, flavouring, food and perfumery (22).

**Material and methods**

**Plant materials**

*Apium graveolens* seeds were purchased from herbal drug market at Nanniyode, Thiruvananthapuram District. These materials were checked for authenticity by taxonomists at TBGRI.

**Chemicals and reagents**

Tryphan blue and Dimethyl sulfoxide (DMSO) were from Sigma Chemicals Co. St. Louis MO. All other chemicals and reagents used were analytical grade and purchased from E.Merk India Ltd. Mumbai and SRL, India

**Preparation of water extract**

The dried seeds were powdered and extracted with distilled water (5 g/100ml) with constant stirring for 4 hrs and then filtered through a filter paper. Residue was again extracted as above with water. The combined filtrate was freeze dried in a lyophiliser. The yield of the water extract was determined (10). (Since the heat sensitivity of the extract with reference to bio-activity is not known, the extraction was carried out at low temperature without using rigorous extraction procedures).

**Alcohol extract**

The alcohol extract of the plant powder was prepared similarly using ethyl alcohol instead of distilled water. However, in this case the combined extract was evaporated to dryness in a rotary evaporator under reduced pressure at 40 °C as described elsewhere (10). The yield of the alcohol extract was determined.

**Hexane extract**

The hexane extracts of the powder was prepared as above using n-hexane instead of alcohol. However, to ensure complete extraction 2g powder was extracted with 100 ml hexane and the process was repeated 3 times. The filtrates from the extractions were combined and dried in a rotary evaporator under reduced pressure at 40 °C. The yield of the hexane extract was determined.

**Animals**

Inbred Swiss albino mice (6-7 week old), reared in Tropical Botanic garden and Research Institute (TBGRI) animal house, were used. Animals were caged in uniform hygienic conditions and fed with standard pellet diet (Lipton, India Laboratories, Bangalore) and water *ad libitum* as per the guide lines of Institute Animal Ethics Committee.

**Cell Lines**

Dalton’s Lymphoma Ascitic (DLA) cells, originally obtained from Amala Cancer
Research Centre, Thrissur, India, were propagated as transplantable tumors in the peritoneal cavity of mice.

Thymocyte preparation

Thymus glands were removed from the mice carefully and trimmed off from the adjoining lymph nodes. Single cell suspensions were prepared in cold RPMI-1640 medium and viability assessed by Tryphan Blue exclusion method (23).

Collection of macrophages

Peritoneal exudates cells (PEC) were collected by injecting 5 ml chilled RPMI-1640 medium in peritoneal cavity of mice. The glass adherent cell population (macrophages) was separated by adhering PEC over glass petri-dishes at 37 °C for 2 hrs in a CO2 incubator having 5% CO2 in air. The viable cell count was taken using Tryphan Blue in a Neubauer counting chamber.

In vitro cytotoxicity assay

Short-term cytotoxicity of extracts was assessed by incubating 1x 10^6 DLA cells, thymocytes or macrophages in 1 ml PBS containing different concentrations of extract. The cell viability was assessed by Trypan Blue exclusion method (23).

Isolation of an active fraction from *Apium graveolens*

The hexane extract from the plant seeds was suspended in water and extracted with chloroform and the chloroform fraction was tested for cytotoxicity. The active chloroform fraction was subjected to chemical analysis to determine the classes of compounds present in it (24). This active fraction was subjected to silica gel Thin Layer Chromatography (TLC) using hexane: chloroform: methanol (5: 4: 1) as a solvent system. The chromatograms were sprayed with different reagents to determine the quality of different components separated (24). Each spot in preparative TLC was identified (with the help of a reagent sprayed plate run simultaneously) based on relative mobility, scrapped off and eluted with chloroform; and tested for cytotoxicity and apoptotic cell death induction in DLA cells.

In vivo anti-cancer assay

*In vivo* anti-cancer activity was evaluated as described elsewhere (14) using DLA growth in the peritoneal cavity of mice as a model. Briefly, mice were divided into 6 groups with 8 animals in each group and were challenged with intraperitoneal injection of 1x 10^6 DLA cells. One group was kept as control without drug treatment. Group 2, 3 and 4 were administered the chloroform fraction of hexane extract of the seeds at 50, 100 and 200 mg/kg respectively, daily, orally, starting from the day of tumour challenge. Group 5 and 6 received the standard drug vincristine at 0.5 and 1.0 mg/kg respectively. The drugs were suspended in 5% Tween 80 and administered. Control mice received the vehicle (0.5 ml 5% Tween 80/mouse).

Results

The yields of water, alcohol and hexane extracts of *A. graveolens* (seed) were 20.7, 17.5 and 15 % of the dry powder respectively.

The cytotoxicity data of the extracts to DLA cells are given in Table 1 and 2. The water extract did not show any toxicity to these cells up to 500 μg/ml studied. In contrast, the hexane extract showed 48% cell death at 50 μg/ml whereas alcohol extract showed almost the same level of toxicity at 500 μg/ml. The cell death was associated with nuclear condensation, membrane blebbing (Fig.1) and formation of apoptotic bodies---characteristics of apoptotic cell death. The cyto-toxicity of the hexane extract to different cell types is shown in Table 2. The DLA cells (tumor cells)
were more sensitive to this extract compared to macrophages (normal cells). Normal thymocytes were resistant to this extract up to 500 μg/ml studied.

An active chloroform fraction was separated from the hexane extract of *A. graveolens*. This fraction showed *in vivo* anti-cancer activity. At a dose of 200 mg/kg, it protected 75 % of the mice challenged with 1 X 10⁶ DLA cells whereas all untreated control mice died of cancer.

The chloroform fraction was separated on TLC into 3 components. Out of the 3 components, 2 of them were found to have cytotoxicity (Table 3). One of the active spots, (Rf 0.60) showed positive to steroid (Liebermann Buchard spray). This spot showed fluorescence under UV light. Interestingly, this steroid positive component showed apoptosis-inducing property, while the other active spot (Rf 0.47) induced necrotic type of cell death. This showed positive to Dragendorff test for alkaloids.

Table 1. Cytotoxicity of different extracts of *Apium graveolens* on DLA cells incubated at 37 °C in PBS.

<table>
<thead>
<tr>
<th>Test material</th>
<th>Quantity of extract (μg/ml)</th>
<th>% cell death</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1% DMSO (Control)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Water extract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>125</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>250</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>500</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Alcohol extract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>125</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>250</td>
<td>11 ± 1</td>
<td>0</td>
</tr>
<tr>
<td>500</td>
<td>51 ± 3</td>
<td>0</td>
</tr>
<tr>
<td>Hexane extract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>48 ± 3</td>
<td>0</td>
</tr>
<tr>
<td>125</td>
<td>75 ± 5</td>
<td>0</td>
</tr>
<tr>
<td>250</td>
<td>100 ± 0</td>
<td>0</td>
</tr>
<tr>
<td>500</td>
<td>100 ± 0</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are mean ± S.D of 3 separate determinations (Values are corrected to whole number). Cytotoxicity was determined by Trypan Blue exclusion method.

Table 2. *In vitro* cytotoxicity of n-hexane extract of *Apium graveolens* to different cells incubated for 3 hours in PBS.

<table>
<thead>
<tr>
<th>Apium graveolens Hexane extract (μg/ml)</th>
<th>% cell death</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLA</td>
<td>Macrophages</td>
</tr>
<tr>
<td>0.1% DMSO (Control)</td>
<td>0</td>
</tr>
<tr>
<td>250</td>
<td>100</td>
</tr>
<tr>
<td>500</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are mean ± S.D of 3 separate determinations. Cytotoxicity was determined by Trypan Blue exclusion method.

Table 3. *In vitro* cytotoxicity to DLA cells of the chemical isolates from the active chloroform fraction from *Apium graveolens*.

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>% cell death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chemical Isolate 1 (Rf 0.60)</td>
</tr>
<tr>
<td>0.1% DMSO (Control)</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>1 (30)</td>
</tr>
<tr>
<td>25</td>
<td>2 (45)</td>
</tr>
</tbody>
</table>
Values are mean of 3 separate determinations. Values are corrected to nearest whole number. % of cells showing membrane blebbing is shown in brackets. Cytotoxicity was determined by Trypan Blue exclusion method. Since apoptotic cell death leads to disintegration of the cells into apoptotic bodies, in this method, cells dying by apoptosis will not be seen as intact stained cells.

Table 4. Anti-tumor activities of chloroform fraction of hexane extract from *Apium graveolens* seeds

<table>
<thead>
<tr>
<th>Days (after tumor challenge)</th>
<th>Number of surviving animals</th>
<th>Chloroform fraction of hexane extract (mg/kg, b.w.)</th>
<th>Vincristine (mg/kg, b.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Mice were challenged with 1x10⁶ cells (i.p.). The fraction was administered daily from the day of tumor challenge for 15 days (the time period required for the death of all the mice in the control group was 14 days)

**Discussion**

The present study shows for the first time the anti-cancer and apoptosis inducing property of the hexane extract of *A. graveolens*. The *in vivo* anti-cancer activity of chloroform fraction (AF) from hexane extract was found to be very promising. Since this fraction contained both apoptosis inducing and necrosis causing components, further studies are required to determine the efficacy of these components separately. Further the apoptosis inducing principle has been tentatively identified as a steroid positive component. This is an attractive material for further studies leading to the possible development of a useful chemotherapeutic agent.

Although it induced apoptosis, the apoptotic mechanism remains to be studied. Detailed studies in various experimental cancer models are required to establish efficacy. It is of interest to note that the cancer cells are more susceptible compared to normal cells. However, there is a need to test the sensitivity of many other types of cancer cells as well as normal cells to determine the likely, beneficial effects of observed cell specific efficacy in the treatment of cancer. Besides acute and subacute toxicity evaluation has to be carried out to determine the likely safety of the extract and or active component. At any rate, the present study has opened up a new vista for further studies.
Fig. 1. Induction of apoptosis in dalton’s lymphoma ascites (DLA) cells by an active steroid positive component (fraction) from A. graveolens seed

A. Control (stained with acridine orange and ethidium bromide)
B. The steroid positive component (fraction) treated cells showing membrane blebbling and formation of apoptotic bodies

References

Medicine: Prevalence and Types of Injuries among Patients treated in Teaching Hospital, Batticaloa

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Email:- mi.hazeem@gmail.com

Abstract

Background: Injuries are the leading causes of death, hospitalizations and disabilities. Traumatic injuries, poisoning and burns are the major types of injuries reported in Sri Lanka, according to the National Health Statistics. Life style changes such as using alcohol and increasing of vehicle usages are believed to be having relation with the prevalence of injuries. Gender and age group also have effects on types of injuries.

Objective: To identify the prevalence of various types of acute injuries in Batticaloa, Teaching Hospital.

Method: It is a cross sectional descriptive study involving simple random sample of one hundred and ninety one (206) injured patients who were admitted at Teaching Hospital, Batticaloa. Data was collected by using a pre designed structured interviewer administered questionnaire.

Results: Altogether, 206 patients with an age range of 01-80 years were studied. Males accounted for 62.8 % of the study sample. Majority of causes are dog bite (33.5 %), and RTA (24.2 %), fall injury (16 %), poisoning (6.3 %) and Cat bite (8.7 %). Among that, 50 % injuries are occur due to the animals in whole injuries. There was a significant relationship between age groups and causes of injuries and there was no relationship between habit of alcohol use and accidental injuries. Among body parts, upper limb (35 %) and lower limb (53 %) are most often affected by injuries. 11.165 % of injured people get disabilities due to their injuries.

Conclusion: According to this study, majority of injuries are caused by dog bites, road traffic accidents (RTA), fall injuries, poisoning and cat bites respectively. Considering the affected body parts, injuries in upper extremities and lower extremities are more common. Around 11. 2 % of patients get disabilities as the consequences of their injuries.

Keywords: Prevalence, Dislocation, Laceration, Upper limb, Lower limb, Skull, Amputation, Linear, Exponential, Strain, Domestic, Stray, Fatal injuries.

Abbreviation: n-number of patients, RTA- Road Traffic Accidents.

Introduction

Background

Injuries are the leading causes of death, hospitalizations and disabilities throughout the world accounting for 9% of all deaths and 16% of the burden of disability annually (1). Injuries account for approximately 11% of all hospital admissions in Sri Lanka (2).

They are also a major public health problem in the South East Asia Region (SEAR) including Sri Lanka. Injuries ranked 5th among all causes of death in the region and was more prominent in the 15-44 years age group (3).

Current situation in sri lanka

Traumatic injuries, poisoning and burns are the major types of injuries reported in the National Health Statistics. Traumatic injuries continue to be the leading cause of
hospitalization since 1995. In 2007, there were 669,052 admissions (proportionate morbidity 16.1%) and 1389 deaths (proportionate mortality 4.0%) in the government hospitals due to traumatic injuries. In the same year, there were 62,721 admissions due to poisoning. This includes 17,723 (28.3 %) due to pesticides and 44,998 (71.7%) due to substances such as drugs, medicaments and biological substances and non-medicinal substances. Poisoning leads to 4.5% (1561) of deaths reported in the government hospitals. Furthermore, there was a total of 13,409 hospital admissions and 292 deaths due to burns and corosions (4). In 2005, injuries (both intentional and unintentional) accounted for 19.1% of all registered deaths in Sri Lanka (3).

The purpose of this study is to assess the prevalence level of acute injuries and to design the guidelines to reduce the high incidence rate of injury.

Objectives

General objective: To identify the prevalence of various types of acute injuries in Batticaloa, Teaching Hospital.

Specific objectives

a. To identify the causes of injuries.
b. To classify the types of injuries and affected body parts associated with injuries.
c. To describe the disability.

Literature review

A study was carried out on Population-based estimates of injuries in Sri Lanka. In this study individuals of all ages were selected from 2000 households in a population-based cross-sectional survey using a stratified cluster sampling technique. The results describe that most of injuries are mechanical injuries, followed by road traffic injuries. The annual injury mortality rate and disability rate were 177 and 290 per 100,000 population, respectively (5).

A study was conducted on Incidence of Physical Injuries in a Rural Community in Sri Lanka. A rural community consisting of 225 families with 1029 inhabitants was studied. Data on major injuries for a period of one year were collected retrospectively. Animal bites being the most common cause of injury was noted in 2.3% of the population followed by falls in 1.6%, contact with objects in 1.5%, cut injuries in 1% and road trauma in 1% (2).

The data were obtained on estimating the incidence of road traffic fatalities and injuries in Sri Lanka using multiple data sources. They used data from multiple sources to estimate the incidence of fatal and non-fatal road traffic injuries in Sri Lanka. They validated the accuracy of the data from the national traffic police by comparing with estimates based on national death registration. For estimating the incidence and patterns of non-fatal injuries, used a nationally represented health survey (6), and data on hospital admissions from a rural setting (Galle district). Estimate that in the year 2005, approximately 2300 people died in Sri Lanka due to road traffic crashes, approximately 300,000 were injured in non-fatal crashes and approximately 140,000 received care for their injuries at hospitals. While the road traffic death rate in Sri Lanka is low compared with other low-income countries, it has been steadily rising for several years. Although young adults are at high risk in non-fatal crashes, the elderly have the highest death rate (7).

A Descriptive cross-sectional study was carried out on incidence and predictors of onboard injuries among Sri Lankan flight attendants. All flight attendants undergoing their annual health and first aid training were invited to participate. Flight attendants who flew continuously for a six-month period prior to data collection were included in the study sample. Recall history of injuries for a period of six months was recorded.
The study sample consisted of 98 (30.4%) male and 224 (69.6%) female flight attendants. The leading causes of injury was pulling, pushing or lifting (60.2%). The commonest type of injuries were strains and sprains (52.3%). Turbulence related injuries were reported by 38 (29.7%) flight attendants. The upper limbs (44.5%) and the back (32%) were the commonest sites affected. After controlling for other factors, female flight attendants had 2.9 times higher risk of sustaining and injury than males (8).

A research work was conducted on injury patterns in rural and urban Uganda Community health workers interviewed adult respondents in households selected by multistage sampling, using a standardized questionnaire. In the rural setting, 1673 households, with 7427 persons, were surveyed. The study reveal that, total incidence of fatal, disabling, and recovered injuries was 116/1000/year. Leading causes of death were drowning in the rural setting, and road traffic in the city (9).

The data was estimated from a research study on injury, incidence and injury patterns in professional football in Sweden. The first team squads of 23 teams selected by the Union of European Football Associations as belonging to the 50 best European teams. The injury incidence during matches was higher than in training. The single most common injury subtype was thigh strain, representing 17% of all injuries. Re-injuries constituted 12% of all injuries, and they caused longer absences than non-re-injuries. The incidence of match injuries showed an increasing injury tendency over time in both the first and second halves. Traumatic injuries and hamstring strains were more frequent during the competitive season, while overuse injuries were common during the preseason. Training and match injury incidences were stable over the period with no significant differences between seasons (10).

A Cross-Sectional study was carried out on incidence and nature of all injuries sustained by elite Western Australian junior Rugby Union players during the 26 weeks up to and including the 1997 National Championship campaign. Injury data were analyzed by phase of play, position, severity and if occurred at games or training. The incidence of injury was significantly associated with the position played and the phase of play in which the injury occurred. Tackling was the most dangerous phase of play (52% of injuries) and the most common site of injury was the lower limb (37%). Most injuries occurred during games (56%) and the flanker was the position most at risk of injury (12%) (11).

A research work was conducted on population based estimates of non-fatal injuries in the capital of Iran. The estimated household was selected for this study. 2,450 households residing in Tehran during 2007-8. The annual incidence of all injuries was 188.7, significant injuries needing any medical care was 68.8, fractures was 19.3, and injuries resulted in hospitalization was 16.7 per 1000 population (12).

**Methodology**

**Study design**

Cross sectional descriptive study.

**Study area**

Medical, Surgical, Orthopedic wards, and Emergency Treatment Unit (ETU) at Teaching Hospital, Batticaloa

**Study population**

The patient who are admitted with acute injuries in the above wards and special unit at Teaching Hospital, Batticaloa
Sampling method, research method & sample size

Simple Random sampling method was used to obtain the 206 samples for our study.

Inclusion criteria Patients who admitted with acute injuries at the Teaching Hospital, Batticaloa will be included.

Exclusion criteria The patients who are unable to speak due to altered conscious level, oral injuries or other reasons will be excluded from this study.

Study period

June 2013 to November 2013.

Study instrument

Data was collected by using a pre designed structured interviewer administered questionnaire.

Data collection

Self-introduction was done and the purpose of this study was explained to the subjects. Each patient was studied individually to avoid the influence by the others and the interviewer administrated questionnaire was filled by the investigator. All the details in the interviewer administrated questionnaire were collected on the basis of their oral statement at the particular time. Researchers did not interfere with their answers. The same procedure was done during our entire study period.

Data analysis

Questionnaire was coded and statistical analysis was done using SPSS (version 16) analytical package.

Ethical consideration

The proposal was submitted to the Ethical Review Committee, Faculty of Health Care Sciences to obtain the ethical clearance. Permission was obtained from Director, Teaching Hospital, and Batticaloa. After explaining the purpose of the study, informed consent was obtained from each participant before data collection. The questionnaires were coded and subjects was identified by a number but not by their names. Privacy, confidentiality and anonymity of the subjects was ensured during interviews and afterwards.

Results

Table 1. Description of study sample

<table>
<thead>
<tr>
<th>Variables</th>
<th>Response</th>
<th>Number (n=206)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>131</td>
<td>62.8</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>75</td>
<td>37.2</td>
</tr>
<tr>
<td>Age group</td>
<td>01-20 years</td>
<td>30</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td>21 - 40 years</td>
<td>86</td>
<td>41.7</td>
</tr>
<tr>
<td></td>
<td>41-60 years</td>
<td>57</td>
<td>27.7</td>
</tr>
<tr>
<td></td>
<td>61 - 80 years</td>
<td>33</td>
<td>16.0</td>
</tr>
<tr>
<td>Alcohol use</td>
<td>Yes</td>
<td>64</td>
<td>31.0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>142</td>
<td>69.0</td>
</tr>
<tr>
<td>Smoking</td>
<td>Yes</td>
<td>79</td>
<td>38.3</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>127</td>
<td>61.7</td>
</tr>
<tr>
<td>Occupation</td>
<td>Farmer</td>
<td>8</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>Business man</td>
<td>6</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>Officers</td>
<td>11</td>
<td>5.3</td>
</tr>
</tbody>
</table>
The Table 1 indicates the demographic data which was gained among the participants at teaching Hospital, Batticaloa. That demographical factor described as below. Among the 206 patients 62.82 % of the patients are male and 37.2 % of the students are female. Age group 21-40 and 41-60 is more prone to effect by injuries than group 01-20 and 61-80. Among the clients, 31. % of the patients are alcohol user and 39% are smoker. Nearly half of the patient have no any occupation.

Table 2. Causes of Injuries

<table>
<thead>
<tr>
<th>Causes</th>
<th>Number</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assault</td>
<td>5</td>
<td>2.4</td>
</tr>
<tr>
<td>Snakebite</td>
<td>6</td>
<td>2.9</td>
</tr>
<tr>
<td>RTA</td>
<td>50</td>
<td>24.3</td>
</tr>
<tr>
<td>Fall Injury</td>
<td>33</td>
<td>16</td>
</tr>
<tr>
<td>Dog bite</td>
<td>69</td>
<td>33.5</td>
</tr>
<tr>
<td>Cat bite</td>
<td>18</td>
<td>8.7</td>
</tr>
<tr>
<td>Cut-Injury</td>
<td>3</td>
<td>1.5</td>
</tr>
<tr>
<td>Rate bite</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Wasp bite</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Stab injury</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>Burn</td>
<td>5</td>
<td>2.4</td>
</tr>
<tr>
<td>Poisoning</td>
<td>13</td>
<td>6.3</td>
</tr>
<tr>
<td>Total</td>
<td>206</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 2 illustrates the classification, the prevalence and the percentages of the various causes for injuries among the patients admitted with the history of injury, at teaching Hospital, Batticaloa. In that results, majority of causes are dog bite (33.5 %), RTA (24.25 %), fall injury (16 %), Poisoning (6.3 %) and Cat bite (8.7 %), 50 % injuries are occur due to the animal in whole injuries.

Table 3. Association between age groups and causes of injuries

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>Major causes of injury</th>
<th>RTA</th>
<th>Fall Injury</th>
<th>Dog bite</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-20</td>
<td>8 (29.6)</td>
<td>12</td>
<td>(44.4)</td>
<td>7 (25.9)</td>
<td>0.46</td>
</tr>
<tr>
<td>21-40</td>
<td>22 (36.6)</td>
<td>5</td>
<td>(8.3)</td>
<td>33 (54.7)</td>
<td>0.005</td>
</tr>
<tr>
<td>41-60</td>
<td>14 (34.1)</td>
<td>7</td>
<td>(17.07)</td>
<td>20 (48.7)</td>
<td>0.045</td>
</tr>
<tr>
<td>61-80</td>
<td>6 (25)</td>
<td>9</td>
<td>(37.4)</td>
<td>9 (37.4)</td>
<td>0.69</td>
</tr>
</tbody>
</table>

RTA –Road Traffic Accident

The Table 3 results revealed that there was no a significant relationship between the Ages group 1-20, 61-80 and Causes of injuries (P > 0.05) and according the above results, there was a significant relationship between Age group 21-40, 41-60 and causes of injuries (P <0.05). So, Age group 21-40 and 41-60 are more prone to effected by RTA and Dog bite than fall injuries.

Table 4. Association between alcohol use and accidental injuries.

<table>
<thead>
<tr>
<th>Accidental Injury</th>
<th>RTA</th>
<th>Fall Injury</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>16</td>
<td>(66.6)</td>
<td>8 (33.3)</td>
</tr>
<tr>
<td>No</td>
<td>34</td>
<td>(57.6)</td>
<td>25 (42.3)</td>
</tr>
</tbody>
</table>
Table 4 results indicate that, weather alcohol user or non-user, there was no a significant relationship between patient and accidental injuries (P > 0.05).

Table 5. Types of injuries

<table>
<thead>
<tr>
<th>Type of Injury</th>
<th>Number</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dislocation</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Strain</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Fracture</td>
<td>38</td>
<td>18.5</td>
</tr>
<tr>
<td>Trauma/Wound</td>
<td>54</td>
<td>26.2</td>
</tr>
<tr>
<td>Puncture/Bite injury</td>
<td>71</td>
<td>34.6</td>
</tr>
<tr>
<td>Scratching</td>
<td>22</td>
<td>10.1</td>
</tr>
<tr>
<td>Abdominal Injury</td>
<td>3</td>
<td>1.5</td>
</tr>
<tr>
<td>Head Injury</td>
<td>8</td>
<td>3.9</td>
</tr>
<tr>
<td>Laceration</td>
<td>3</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Total 206 100

Table 5 describes the prevalence of type of injuries. Nine type of injuries are most frequently occur in Batticaloa. Among that fracture (18.5%), trauma/wound (26.2 %), puncture/bite injury (34.6 %) and scratching (10.1 %) are major types.

Table 6. Affected body parts associated with injuries.

<table>
<thead>
<tr>
<th>Affected Body Part</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head</td>
<td>8</td>
<td>3.8</td>
</tr>
<tr>
<td>Face</td>
<td>7</td>
<td>3.3</td>
</tr>
<tr>
<td>Chest</td>
<td>4</td>
<td>1.9</td>
</tr>
<tr>
<td>Upper limb</td>
<td>72</td>
<td>35</td>
</tr>
<tr>
<td>Rib</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Abdomen</td>
<td>3</td>
<td>1.4</td>
</tr>
<tr>
<td>Lower limb</td>
<td>110</td>
<td>53</td>
</tr>
</tbody>
</table>

Total 206 100

Table 6 results are accounted the frequencies and percentage of affected body parts. In that, upper limb (35 %) and lower limb(53 %) are more prone to affected by the injuries.

Figure 1. Type of disabilities.
Figure 1 illustrate that which types of the disabilities are occur due to the injuries. In the disabilities, lower limb disability and loss of muscle function have high prevalence rate. Lower limb disability are most often occurred by the fracture.

Discussion

Traumatic injuries, poisoning and burns are the major types of injuries reported in the National Health Statistics (3). But, present study in Batticaloa reported that, majority of causes are dog bite (33.5 %), RTA (24.2 %), fall injury (16 %), Poisoning (6.3 %) and Cat bite (8.7 %). In Batticaloa 50 % injuries are occur due to the animal in whole injuries.

In the present study, the results revealed that there was no a significant relationship between the Ages group 1-20, 61-80 and Causes of injuries (P > 0.05) and according the results, there was a significant relationship between Age group 21-40, 41-60 and causes of injuries (P <0.05). So, Age group 21-40 and 41-60 are more prone to effected by RTA and Dog bite than fall injuries. A Study conducted on Epidemiological Study of Road Traffic Accident Cases describe that, the highest number of RTA victims (31%) were found between the age group of 20 and 29 years (13). Another study on knowledge, attitude and practice following dog bite, reported that, majority of the study subjects were in the age group of 15-44 followed by 5-14. A large percentage of the subjects (31.1%) was, in fact, in the age group of 30-44 followed by those in the 15-29 age group (28.8%) (14).

Another Study carried on falls incidence underestimates the risk of fall-related injuries in older age groups show that, the risk of fall-related injuries, calculated on the basis of the incidence of fall-related injuries, showed a linear relationship with age, whereas the risk calculated on the basis of fall-related injuries corrected for exposure (falls risk by exposure, FARE) showed an exponential relationship. Calculations on the basis of the incidence of fall-related injuries underestimated the risk of fall-related injuries in people aged 70 years and older, and especially in women (15).

Alcohol is one of the most important risk factors for serious and fatal injuries, contributing to approximately one third of all deaths from accidents. One study showed that the speed of the vehicle at the time of collision is high when blood concentration of alcohol is high. One Norwegian study showed that 11.5%of the drivers of accident were under the influence of alcohol. In another study group 32% of the victims were with a history of alcohol use (16). In the present study, the results indicate that, weather alcohol user or non-user, there was no a significant relationship between patient and accidental injuries (P > 0.05). So, in the Batticaloa, alcohol use or non-use not associated with accidental injuries.

Most frequently, nine (9) type of injuries are occur in Batticaloa. Among that Fracture (18.5 %), Trauma/Wound (26.29 %), Puncture/Bite injury (34.6%) and Cat bite (10.1 %) are major types and considering the affected body parts, injuries in upper limb (35 %) and lower limb (53 %) are most common. Among that, 11.2 % of patients get disabilities as the consequences of their injuries.

Limitations

The finding of this study cannot be generalized to all injured patients due to small sample size (n=206).

ETU, Surgical Wards and ICU were very rush and most of the time, there was no comfortable situation to interview patient. The interviews conducted to administer the questionnaire were done inside a clinic environment while waiting to consult the doctor. There were some disturbances during the interview which was unavoidable.
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Conclusion
A study was conducted on Prevalence and Types of Injuries among Patients treated in Teaching Hospital, Batticaloa. The study results describes majority of causes for injuries in Batticaloa region. The leading causes are dog bite, RTA, fall injury, Poisoning and Cat bite respectively in descending order. Among then injured individuals, 50 % of injuries are due to the animal bites. The people belong to the age groups 21-40 and 41-60 are more prone to be affected by the RTA and Dog bite. When we consider the affected region of the body, upper limb (35 %) and lower limb (53 %) are most often affected by injuries.

Recommendation

Recommendation for patients who are involve in dog bite

  a  Do not approach an unfamiliar dog.
  b  Remain motionless when approached by an unfamiliar dog.
  c  Avoid direct eye contact with a dog.

Recommendation for older to prevent fall injury
Exercise regularly. It is important that the exercises focus on increasing leg strength and improving balance, and that they get more challenging over time.

Recommendation for road user and driving license provider
There is clearly a need for road safety education and it should be directed towards road users, who are frequently involved and injured in RTAs

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References
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