SRI RAMACHANDRA JOURNAL OF MEDICINE

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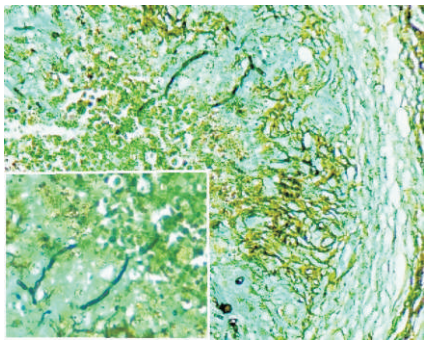

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SRI RAMACHANDRA JOURNAL OF MEDICINE

JAN - JUNE 2009

CONTENT

GUIDELINES FOR AUTHORS

Scope of the Journal

The Sri Ramachandra Journal of Medicine - a scientific journal, entertains communications on all aspects of original biomedical research contributing to the advancement of knowledge in medical sciences. The scope of the journal allows publication of papers on medical education at undergraduate and postgraduate levels in either medical or paramedical courses; innovations in techniques; epidemiologic investigations and case reports. Readers are encouraged to write comments on papers published in the journal in the form of correspondence. Brief communication containing significant findings will be given priority. Review articles are also invited on topics of current interest. The journal is issued thrice in every calender year. All papers are subjected to peer review by the Editorial Board and also experts in the field before acceptance for publication. All papers are accepted subjected to editorial changes.

Articles submitted to the journal should abide by the following manuscript submission guidelines.

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Each manuscript submission should include the following documents.

Part I - Title Page
Part II - Manuscript file
Part III - Acknowledgment, declaration by authors, patient consent and supplemental file.

All contents related to manuscript submission should be in English on a White paper of A4 size (210 x 297) with margins of 25mm (1 inch) wide on all the four sides. Print should be on oneside only with double spacing throughout. Pages should be numbered consecutively, beginning with title page. Lettering should in Times New Roman with a font size of 12. Three copies should be submitted to the editor. A copy of the title page and manuscript file must be emailed (as an attachment) with a covering letter address to the editor.

PART I - Title Page must include : a) Title of the article b) Name of each contributor with the highest degree and institutional affiliation. c) Name, cellphone, e-mail of the corresponding author.

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Declaration by the authors: All the authors should submit a declaration regarding originality of the work, submission to other journals, whether the articles were already published and financial conflicts of interest which might influence the manuscript.

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2. Review articles: These articles addressing an issue / theme of current interest. They should not exceed 4000 words. Should include an unstructured abstract of 400 words with three MesH words. Article may include upto 100 references.

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4. Letter to the Editor: Correspondence to the editor regarding an article published in the journal are invited in this category. The content should be restricted to 300 words with references upto five.

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From the Editor's Desk

The Editorial board is happy to bring out this edition of our journal. It was made possible because of untiring efforts of the members of the editorial board under watchful supervision of the chief editor and Dean of Faculties Prof. K.V. Somasundaram.

Over the period of time the number of articles coming for the editorial board is increasing and in this edition we have 3 original articles, 4 case reports, 2 brief reports, Images in Medicine and a chapter on Recent advances in Pharmacology - New Drug profile.

We thank our Vice-Chancellor Prof. S. Rangaswami for his article on "Sonnet for Hormones"

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The Editorial Board gratefully acknowledges their contribution.

A SONNET FOR HORMONES

A look at "A Dream of Surreal Science" by Sri Aurobindo

S. Rangaswami

Sri Aurobindo is the most celebrated mystic evolutionary philosopher of 20th century India. His prolific writings, completed over a period of half a century, covered the entire gamut of spiritual, philosophical, literary and political thoughts. Interspersed in his colossal literary output are peerless ideas touching all branches of science including cosmology, physics and biology. Particularly striking are his penetrating insights into the origin and evolution of life and the evolution of consciousness from its material foundations to mental and what he described as supramental planes. His intense spiritual *sadhana* in almost total solitude at Pondicherry extending over a period of a quarter of a century allowed him to experience transformative visions of unparalleled clarity and depth. Sri Auribindo's cosmology described man as a 'transient being' on the way to becoming a supramental being. He described integral yoga as the spiritual evolutionary instrument to realize this destination. His ideas on human evolution from the material to supramental planes are described in ***The Life Divine***, his *magnum opus* and ***Savitri***, the spiritual poem of exquisite charm and beauty. ***Savitri*** with its 24,000 verses is recognized as the longest mystical poem written in the English language.

For our discussion, a sonnet written in the period 1930-1950 has been taken. Sonnets are short poems; usually with 14 lines. In "***A Dream of Surreal Science***" Sri Aurobindo's concept on the role of 'glands' and hormones in guiding and controlling the vagaries of human behavior and actions is portrayed. The word 'Hormone' is derived from the Greek *hormao*, a bodily substance that starts, stimulates or irritates. Although the existence of such substances was known ever since Ernest Starling the English Physiologist introduced the term in 1905, it was in the 1930s and 40s that they were seriously incriminated to influence human mind and behavior. Many a capricious human action was squarely blamed on hormones. It is in this context that the sonnet is to be viewed.

A Dream of Surreal Science

Sri Aurobindo

One dreamed and saw a gland write Hamlet, drink
At the Mermaid, capture immortality;
A committee of hormones on the Aegean's brink
Composed the Iliad and the Odyssey.

A thyroid, meditating almost nude
Under the Bo-tree, saw the eternal Light
And, rising from its mighty solitude,
Spoke of the Wheel and eightfold Path all right.

A brain by a disordered stomach driven
Thundered through Europe, conquered, ruled and fell;
From St. Helena went, perhaps, to Heaven.
Thus wagged on the surreal world, until

A scientist played with atoms and blew out
The universe before God had time to shout.

The 'gland' that wrote Hamlet was obviously William Shakespeare and the 'Mermaid Tavern' was his favorite pub in London. The canvas by John Faed (1851) titled 'Shakespeare and His Contemporaries' immortalized the scene at the Mermaid.

It is curious that Sri Aurobindo has given credit for the authorship of Iliad and Odyssey to 'a committee of hormones' on the brink of the Aegean sea rather than to a single author (Homer). There is clear allusion here to the disputes on the authorship of the two epics and support for multiple authorship, a view held by several literary experts.

The thyroid that meditated in 'mighty solitude' under a Bo-tree (Bodhi tree) and spoke of the Wheel and the eightfold Path could be none other than Gautama Budha. But why the thyroid? From whatever sources we have, Gautama Budha is said to have possessed all the 32 major signs of a 'Great Man' in addition to all the 80 secondary characteristics with no indication of any thyroid dysfunction. Whether Sri Aurobindo ascribed the unruffled equanimity of the Budha to an impeccable thyroid is open to speculation. Ecological healing, spiritual meditative practices and mind-body harmonization are identified with optimum thyroid function. Also, in energy medicine, the throat or *visuddha chakra* over the thyroid is recognized as the life energy centre that deals with communication, self expression and creativity.

The hero who went (perhaps!) from St Helena to Heaven must similarly be none other than Napoleon Bonaparte. Didn't the 19th century autocrat thunder through Europe consolidating his ruthless conquests till his final defeat at Waterloo in June 1815 and exile to St.Helena, the small windswept island, 2000 kilometers

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west of Africa? It is quite possible that his skewed mindset and judgment driven as they were by a sickly stomach had a lot to do with his final military debacles. Napoleon is believed to have died of cancer of stomach. (Arsenic poisoning has also been advanced as a cause for his death)

'Thus wagged on the surreal world' gossiping nonchalantly on the whimsical human mind driven by glandular secretions and bodily emissions. The incongruity of such an understanding guided by the dictates of a surreal science is explained by the poet in ***Life Divine:***

"We do not see or know, but it is expounded to us ….that a play of electrons, of atoms, and their resultant molecules, of cells, glands, chemical secretions and physiological processes manages by their activity on the nerves and brain of a Shakespeare or a Plato to produce …. a Hamlet or a Symposium or a Republic…"

The atoms of course, had the last laugh.

What else can one expect when a surreal scientist with an illusory stance and stunted understanding of the fundamentally unintelligible rationale of the universe 'plays with the atoms'? Again, Sri Aurobindo offers the spiritualist's viewpoint in ***Life Divine****:*

"These formulae of Science may be pragmatically correct and infallible, they may govern the ***practical how*** *of Nature's processes, but they do not disclose the* ***intrinsic how*** *or why; rather they have the air of the formulae of a cosmic Magician, precise, irresistible, automatically successful each in its field, but their rationale is fundamentally unintelligible"*

The 'fundamentally unintelligible rationale' of the Universe is the key idiom here; with the consequent implication that before the majesty of enlivening spiritual vision, the formulae of science, their pragmatic infallibility notwithstanding, are at best destined to remain nothing more than dreamy extrapolations.

A POPULATION BASED STUDY OF ACUTE DIARRHOEA AMONG CHILDREN UNDER 5 YEARS IN A RURAL COMMUNITY IN SOUTH INDIA

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ABSTRACT:

Aim of the study

To estimate the prevalence of acute diarrhoea in children under 5 years in a rural population and to study the different modalities of treatment adopted by the mother and family members. The other objective is to find out association between certain risk factors and diarrhoea among children under 5 years.

Methodology

Five hundred and ten under 5 children were selected from Poonamallee block of Thiruvallur District in Tamil Nadu, by Cluster sampling method. Background information, details of acute diarrhoea and treatment modalities were obtained among the respondents of under 5 children. Any child having acute diarrhoea at the time of interview or had acute diarrhoea in the preceding 2 weeks was taken as a case of acute diarrhoea.

Results

The prevalence of Acute diarrhoea was found to be 22.5% (95% CI 17.4% - 27.6%). The prevalence of acute diarrhoea among males and females were 21.4% and 23.8% respectively. The difference in prevalence of acute diarrhoea among male and female children was small and the difference was not statistically significant ($p > 0.05$). Children in the age group 7-12 months had the highest prevalence of diarrhoea to the extent of 40.7% followed by the age group 13-24 months and 0-6 months. The age group 25 months and above had the lowest prevalence. The difference in the prevalence of diarrhoea in different age groups was found to be statistically significant ($P < 0.001$). Oral Rehydration Therapy use rate was found to be 65.2% (95% CI 56.5 – 73.9). The percentage of use of ORT in children who had diarrhoea was found to be higher with increase in the age of the child, though not statistically significant. There was no marked difference in ORT use among male and female children.

Conclusion

The prevalence of acute diarrhoea among under 5 children was found to be 22.5 % and the most vulnerable age group was 7-12 months which corresponds to the time of weaning. The ORT use rate was found to be 65.2% much higher than other studies. The ORT use rate was higher among literate mother.

Key Words

Acute Diarrhoea, Under Five Children, Oral Rehydration Therapy and Population based study.

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INTRODUCTION:

The challenge of the time is to study child health in relation to community, social values and social policy[1]. Child health has been given greatest priority over the years both at National level and at State level[2]. However, acute diarrhoea continues to be one of the main health problems in children. One in four deaths in children under the age of 5 years is estimated to be due to diarrhoea[3]. One out of ten babies born in developing countries fails to reach its fifth birthday falling victim to diarrhoeal diseases[3]. The high mortality and morbidity due to diarrhoeal diseases can be markedly reduced by Oral Rehydration Therapy (ORT) which includes proper home management with Home Available Fluids (HAF) and Oral Rehydration Salt solution (ORS) and by continuing usual feeding. The Oral Rehydration Therapy is rightly considered as one of the important medical advances of the 20th century in terms of simplicity and scope to save lives[4].

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The prevalence of diarrhoea varies from place to place[5]. The community practices relating to ORT and other treatment modalities also vary from place to place[6]. It is important to know the prevalence of diarrhoea in children in different populations and how the mother and other family members respond in treating the child with diarrhoea. This may help in planning appropriate preventive measures for effectively reducing mortality and morbidity due to diarrhoea in children. In view of this a population based study of acute diarrhoea among children under-5 years and ORT was taken up in one of the rural populations in Tamil Nadu.

MATERIAL & METHODS:

This population based cross sectional study was done in Poonamallee block of Thiruvallur district in Tamilnadu. The study population included the under 5 children residing in the Poonamallee block. The Poonamallee block has 160 villages, 34460 house holds and a total of 13790 under 5 children. Cluster sampling method was used for selecting the under-5 children as study subjects from the above study population. Based on the assumption of 20% as prevalence of acute diarrhoea in under 5 children and with an alpha error of 5%, and limit of accuracy of 25% of prevalence and a design effect of 2, the minimum sample size required for the study was found to be 492. Thirty clusters were selected by probability proportionate to size (PPS) method[7]

and 17 under 5 children were selected from each cluster to obtain a total sample of 510 under 5 children for this study.

Selection of Village Clusters and under 5 Children

A list of all the villages and their population in Poonamallee block was first obtained. Adjoining villages with very small populations were clubbed together so that each village cluster had atleast 1000 population. This reduced the 160 villages to 105 village clusters. From the 105 clusters, 30 clusters were selected by PPS sampling method [7]. In each selected village cluster, first house was selected randomly[7]. In the selected house, it was inquired, if there is a child below 5 years. If the family has a child under-5 years relevant data were collected. If not the interviewer moved to the next adjoining house. Similarly adjoining houses were visited in the same direction, till 17 under 5 children were studied. By the same method 17 under five children were studied in each of the 30 clusters for a total 510 under 5 children.

Data Collection

A brief introduction was given to the respondents regarding the purpose of study, if a child under 5years was present in the house. After getting the informed consent orally, relevant information about the under 5 child in the family was obtained from the respondent using a pretested structured questionnaire in the local language. If there were more than one child in the family, one of them was chosen randomly. The environmental conditions of the household were also surveyed. Any of the selected children having acute diarrhoea at the time of interview or had acute diarrhoea in the preceding 2 weeks was taken as a case of acute diarrhoea for this study. The weights of the children were measured and on the basis of IAP Classification, they were classified as undernourished and normal. The duration of breast feeding were also collected from children less than 18 months.

Analysis

Data entry and analysis were done using SPSS-8.0 software. Prevalence of diarrhoea and 95% confidence interval (C.I) were calculated. The 95% C.I was corrected for the design effect of cluster sampling by multiplying the variance by a factor of 2. The association between risk factors and diarrhoea was estimated by odds ratio and 95% CI calculated. Adjusted odds ratios were calculated by using logistic regression. Percentages were calculated for ORT use.

Definition of terms used:

Acute Diarrhoea

Acute Diarrhoea is sudden onset of passage of loose liquid or watery stools which usually lasts 3 - 7 days but may last upto 10 - 14 days.

Literacy

A person is deemed as literate if he or she can read or write with understanding in any language.

RESULTS:

Using cluster sampling method, 510 under 5 children were selected. There was no refusal to take part in the study. Among the selected under 5 children 52.2% were males. The mean age was 25.7 months ranging from 2 months to 59 months. Almost 50% children were in the age group 25 months and above (Table 1). A large proportion of the selected children (80%) belong to nuclear family, the average size of the family was 3.2 ranging from 1 to 4. Based on Gupta's socio economic classification[8], a large proportion of selected children (56.5%) belong to Class IV. Majority of the respondents for the study were mothers (75.5%) followed by grand parents (19.8%) and the rest were brothers, sisters and others.

Table1: Age and Sex Distribution of Children

Age in months	*Male* n (%)	*Female* n (%)	*Total* n (%)
0-6	27 (5.3)	20 (3.8)	47 (9.1)
7-12	32 (6.3)	27 (5.3)	59 (11.6)
13-24	80 (15.7)	82 (16.1)	162 (31.8)
25 & Above	127 (24.9)	115 (22.6)	242 (47.5)
TOTAL	266 (52.2)	244 (47.8)	510 (100.0)

Birth Weight, Feeding and Weaning

Particulars about birth weight could be collected reliably only from 62.6% of respondents. The mean birth weight was 2.6 kg (S.D. 0.4) with a minimum of 1.7 kg to a maximum of 4.1 kg. The proportion of low birth weight (< 2.5 kg.) was found to be 28.2%. The information about breast feeding was obtained for children less than 18 months (n = 203). The mean duration of breast feeding was 10 months ranging from 1 month to 18 months. The details about weaning was obtained for the children belonging to age group 5 – 18 months (n = 180). The mean age of commencement of weaning was 6 months with a minimum of 5 months and a maximum of 9 months.

Status of Immunization, Vitamin–A Supplementation and Malnutrition

Immunization status was obtained from children between 1 - 2 years (n = 162). The children fully immunized were 88.9% and the remaining 11.1% were partially immunized. Information about vitamin A supplementation was obtained from children between 6 - 60 months (n = 463). Among them vitamin A supplementation was given to 79.1% of children within preceding 6 months. Based on IAP classification[9] (weight for age) 23.1% of children were undernourished.

Environmental hygiene

Based on per capita floor area, 58.2% of houses were found to be overcrowded. Sanitary latrine was available only in 25.5% of houses. Most of the houses had public source of water supply which was boiled for consumption constituted to 76.9% and sanitary disposal of garbage was resorted to only in 29.8% of houses. Based on the personal habits of hand washing with soap before preparing food and feeding the child, the personal hygiene was satisfactory in only 18.2% of respondents.

Prevalence of Acute Diarrhoea

Out of the 510 under 5 children, 115 had acute diarrhoea within 2 weeks at the time of interview and the prevalence of acute diarrhoea was found to be 22.5% with 95% confidence interval from 17.4% to 27.6%. Prevalence of diarrhoea among children below 4 years and below 3 years were 23.9% and 26.4% respectively. Among males the prevalence of acute diarrhoea was 21.4% and among females it was 23.8%. The difference in prevalence of acute diarrhoea among male and female children was small and the difference was not statistically significant ($p > 0.05$).

Children in the age group 7-12 months had the highest prevalence of diarrhoea to the extent of 40.7% followed by the age group 13-24 months and 0-6 months. The age group 25 months and above had the lowest prevalence. The difference in the prevalence of diarrhoea in different age groups was found to be statistically significant ($P < 0.001$). The prevalence of diarrhoea in different age group and sex is given in Table 2. The Mean duration of diarrhoea was 3 days with a minimum of 1 day and a maximum of 5 days. The average number of times of diarrhoea per child per day was 5 with a minimum of 3 and a maximum of 10. The percentage of children who did not have any other symptom during diarrhoea was 62.6%. Among those who had symptoms, 26.9% had vomiting, 25.2% had fever, and 6.1% of children had stools mixed with blood and mucus.

Table 2: Prevalence of Diarrhoea by Sex and Age

		Prevalence of Diarrhoea in Percentages
Sex	Males	21.4
	Females	23.8
	$X^2_1 = 0.4, p > 0.05$	
Age group in months	0 - 6	17.0
	7 - 12	40.7
	13 - 24	32.1
	25 & Above	12.8
$X^2_3 = 33.5, p < 0.001$		

Acute Diarrhoea and Risk Factors

Birth weight could be obtained reliably only for 319 under 5 children and it was found that children with birth weight below 2.5 kgs, had 2.1 times greater risk for diarrhoea which was also statistically significant. There were 162 children in the age group 1-2 years. Among them it was found that partially immunized children had 4.6 times higher risk for acute diarrhoea which was also statistically significant. There were 463 children in the age group 6 to 60 months and those who did not take any dose of Vitamin A supplementation had statistically significant higher risk for acute diarrhoea (OR 7.4). Children who were under-nourished had higher risk for acute diarrhoea (OR 14.4). Details given in Table 3.

Where the personal hygiene of the mother or respondent was not satisfactory, the risk of acute diarrhoea was higher (OR 3.2). Similarly under-5 children living in overcrowded houses, living in houses with insanitary garbage disposal, insanitary excreta disposal and with poor water supply had higher risk for acute diarrhoea. All the higher risks found were statistically significant. Details are given in Table 3. Even after adjustment by logistic regression analysis low birth weight, partial immunization, under nourishment and unsatisfactory personal hygiene were found to have statistically significant higher risk for acute diarrhea among under-5 children (Table 4). Since Vitamin A supplementation is a part of nutritional status and personal hygiene is closely related to disposal of excreta and garbage, they were not included in the logistic regression analysis.

ORT Use in Acute Diarrhoea

The number of under 5 year children who received some form of fluid therapy as HAF/ORS during acute diarrhoea along with one or other type of diet was 75 with an ORT use rate of 65.2% (95% CI 56.5 – 73.9). The percentage of use of ORT in children who had diarrhoea was found to be higher with increase in the age of the child, though not statistically significant. There was no marked difference in ORT use among male and female children. The use of ORT was much higher (75.6%) when the mother was literate compared to when the mother was illiterate (43.2%) and the difference was also statistically significant. Details are given in Table 4.

Among currently breast fed children (117), 59 children (50.4%) had diarrhoea and among them breast feeding was continued only in 35 children (59.3%) during diarrhoea. Regarding the diet during diarrhoea, 80% of children received bread, followed by rice kanji (63.9%), milk (57.5%), biscuit (48.8%) and idly (41.25%).

Drugs and Home Remedies Given to Children During Acute Diarrhoea

Out of 115 children who had acute diarrhoea, 74.8% received tablets, 42.6% Suspension, 40% Injections and 7.8% I.V.fluids. The percentage of children who did not receive any drug, but only ORS was 25.2.

Table 3: Association between risk factors and acute diarrhoea in under 5 children

		Diarrhoea		Odds Ratio	95% CI	p value
		Present n	Not Present n			
Birth weight						
N = 319	< 2.5 kgs	38	52	2.1	1.2- 3.6	< 0.05
	> 2.5 kgs	60	169			
Immunization						
1-2 yrs n = 162	Partially Immunised	12	6	4.6	1.5-4.7	< 0.01
	Fully Immunised	44	100			
Vitamin A Supplementation						
6- 60 monthsN = 463	Not Received	54	43	7.4	4.4- 2.6	<0.001
	Received	53	313			
Nutrition						
	Undernourished	74	44	14.4	8.5- 4.4	<0.001
	Normal	41	351			
Personal Hygiene						
	Unsatisfactory	106	311	3.2	1.5 - 7	<0.01
	Satisfactory	9	84			
Overcrowding						
	Yes	88	209	2.9	1.8- 4.8	<0.001
	No	27	186			
Garbage Disposal						
	Insanitary	102	256	4.3	2.2-8.3	<0.001
	Sanitary	13	139			
Source of Water Supply						
	Public Tap& Well	101	291	2.6	1.4- 4.9	<0.001
	House Tap & Well	14	104			
Excreta Disposal						
	Insanitary	103	277	3.7	1.9- 7.3	<0.001
	Sanitary	12	118			

DISCUSSION:

This study has shown that the prevalence of acute diarrhoea among under-5 children in the rural population (Poonamallee block) is high to the extent of 22.5% which once again reinforces the fact that acute diarrhoea in children is an important health priority and that every effort has to be taken to control and prevent acute diarrhoea and its sequale. The 95% confidence interval for the prevalence of acute diarrhoea is quite precise (17.4-27.6) indicating good internal validity for the study. There are few studies done on prevalence of acute diarrhoea in under-5 children in different parts of India and outside India. A study done in Bhopal by S.C. Tiwari et al has reported a prevalence of acute diarrhoea among under 5 children as 27.4% which is little higher than the present study[10]. The study done in Aligarh of Uttar Pradesh by Ansari et al has reported a prevalence of 16%. The study by Ansari et al relates to the patients attending the clinics under Rome scheme which may not be representative of the population[11]. A study done in East Africa by Mtike has reported 18% as prevalence of diarrhoea among children both in rural and urban population[12].

National Family Health Survey – I (NFHS I) was done in the year 1992 and. NFHS–II was done after about 6 years in 1998-99.In both NFHS surveys prevalence of diarrhoea was calculated as percentage of children who had diarrhoea at the time of interview or during the preceding 2 weeks as done in this study. NFHS – 1 has reported prevalence of diarrhoea for children under-4 years and NFHS-II has reported it for children under-3 years. For rural Tamil Nadu the prevalence of diarrhoea for children under 4 years was 12.9%[13] and for children under 3 years it was 14%[14] as per NFHS I and II respectively. The present study in Poonamallee

Table 4 : Association Between Risk Factors And Acute Diarrhoea In Under 5 Children After Adjustment

Background Characteristics	Adjusted Odds ratio	95% CI	p-Value
Birth weight (kg)			
<2.5	3.2	1.2-8.6	**< 0.05**
>2.5+			
Immunization status			
Partial	10.4	1.4-74.8	**< 0.05**
Complete+			
Nutritional status			
Under nourished	10.1	3.9-26.2	**< 0.005**
Normal+			
Sources of water			
Public tap and well			
House tap and well+	2	0.6-6.8	> 0.05
Personal hygiene			
Unsatisfactory	7.0	1.5-33.6	**< 0.05**
Satisfactory+			
Over crowding			
Yes	1.5	0.6-4.0	> 0.05
No+			
'+' Reference category			

block has found much higher prevalence of diarrhoea in children under 4 years (23.9%) and in children under-3 years (26.4%). This may be because the present study was done during peak season for diarrhoea (April to August) or because the study population is more vulnerable and has higher prevalence of diarrhoea compared to the overall prevalence in rural Tamil Nadu.

The present study has shown a very high prevalence of acute diarrhoea (40.7%) in the age group 7 - 12 months, compared to other age groups (Table 2) and the difference is also statistically significant. This may be because at this age, weaning foods are introduced and the child is also exposed more to the environmental condition as it starts crawling and walking. The next vulnerable age group was found to be 13-24 months. Similar trend is reported in NFHS II in which the prevalence of diarrhoea is reported as highest in the age group 7-12 months (17%) followed by 13-24 months (8.6%)[14] though the prevalence reported are much lower.

The prevalence of diarrhoea was found to be only 17% in the age group 0-6 months which reflects probably, the protection offered by breast feeding. Though female children had slightly higher prevalence of acute diarrhoea (23.8%) than males (21.4%), the difference is not statistically significant. A similar pattern is seen in the NFHS I study report, where the females have slightly higher prevalence[13]. However in NHFS II the prevalence of acute diarrhoea in males (14.7%) is reported slightly higher than females (14%)[14].

Table 5: ORT use by Socio Demographic Variables

	ORT given in Percentage	p Value
Age in months		
0 - 6	50.0	>0.05
7 - 12	54.2	
13 - 24	65.4	
25 & Above	77.4	
Sex		
Male	63.2	>0.05
Female	67.2	
Educational Status of Father		
Illiterate	56.7	>0.05
Literate	68.2	
Educational Status of the Mother		
Illiterate	43.2	>0.05
Literate	75.6	

Acute Diarrhoea and Risk Factors

Partially immunised children had higher risk for diarrhoea (OR 4.6) compared to fully immunised children. (Table 3) This is obviously due to the protective effect of immunization especially with reference to measles immunization[15]. The percentage of fully immunised children in the study population was 88.9%. Improving immunization coverage will help to reduce the burden of illnesses due to diarrhoea in children. Those children who did not take any dose of vitamin A supplementation within preceding 6 months had 7.4 times higher risk for acute diarrhoea compared to those who had vitamin A Supplementation. It lays emphasis on the concept that Vitamin A is protective of the intestinal epithelium[16]. The vitamin-A supplementation coverage found in the study population was 79.1%. Improving the vitamin-A supplementation coverage will definitely help in reducing the burden of illnesses due to diarrhoea in children. The under-nourished children had 14.4 times higher risk for acute diarrhoea than normal children. This is in conformity with the statement made by international centre for Diarrhoeal Disease Research in Bangladesh that diarrhoea is common in malnourished children[17]. The prevalence of under nutrition in the study population was 23.1%. It is very important to prevent under nutrition by proper implementation of the various nutritional programmes for reducing the problem of diarrhoea in children.

As expected the study has shown that good personal hygiene has a protective effect against diarrhoea (Table 3). Similar observations have been found in a study done in Yavatmal by Khadse et al who have stated that hand washing with soap and water after defecation and before feeding had a protective value against diarrhoea[18].The risk of diarrhoea was 4.3 times more where insanitary practices of garbage disposal was observed (Table 3) compared to children whose family followed sanitary disposal of garbage. This may be due to increased fly nuisance affecting food hygiene at the family level. Similar observations have been made by this study that children living in over crowded houses and in insanitary condition have higher risk of diarrhoea.

One of the important objectives of diarrhoeal diseases control programme is to increase the use of ORT to prevent death due to diarrhoea as a result of dehydration. The Tamil Nadu state Plan of Action fixed 100% as the target to be achieved for ORT use in Tamil Nadu by 2000 A.D[19]. The ORT use was 27.1% and 23.1% for Tamil Nadu in the year 1991 and 1995 respectively. ORT use increased to 45.4% by the year 1998-1999 showing great improvement in the use of ORT in Tamil Nadu[19]. The present study which was done in the Poonamallee block found ORT use as 65.2% indicating that the trend in increasing use of ORT is probably continuing in Tamil Nadu.

This study has found that ORT use was much higher (75.6%) when the mother was literate compared to (43.2%) when the mother was illiterate and the difference is statistically significant. This shows that improving female literacy will further increase ORT use also. This study found that a major source of ORS as a single entity was private practitioners. Hence it is important to have continuing medical education for them, regarding correct composition and use of ORS through Indian Medical Association and other professional bodies.

Although antibiotics may be useful in reducing the duration and volume of diarrhoea in specific bacterial infection, use of antibiotics and other anti diarrhoeal drugs are not generally recommended for treatment of childhood diarrhoea. However, this study has found that 74.8%, 42.6% and 40% of children who had acute diarrhoea received tablets, Suspensions and injections respectively. NFHS II also found that 41% of children who had diarrhoea received pills or syrup and 28% received injections[14].

The other important unsound practice found in the study population was that 40.7% of mothers who were breast feeding the children did not continue to breast feed them when the children had acute diarrhoea. This may be because of the wrong belief, that intake of milk would further aggravate diarrhoea in children. The above findings indicate poor knowledge about proper treatment of diarrhoea not only among mothers and family members. The results underscore the need for informational programmes for mothers that emphasizes the importance of ORT, increased fluid intake, and continuing feeding.

Key Messages

The prevalence of acute diarrhoea is found to be 22.5% in children under 5 years in a rural population in Tamil Nadu & the most vulnerable age group is 7-12 months. The ORT use rate is found to be 65.2% much higher than found in other studies. ORT use is higher when the mother is literate. Practice of hand washing is a cost effective measure in preventing diarrhoea.

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MOLECULAR GENETIC ANALYSIS OF NEUROPEPTIDE Y (NPY) GENE IN PATIENTS WITH CARDIAC ARRHYTHMIA

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ABSTRACT:

Background: *The heart is powered by an electrical impulse that signals the heart to contract, each at a proper time. Cardiac arrhythmia is any group of conditions in which the electrical activity of the heart is irregular or is faster or slower than normal. In arrhythmia, the heart rate either goes beyond 100 or below 60 beats per minute and this is called tachycardia and bradycardia respectively. NPY is co release with norepinephrine during sympathetic nerve stimulation, and is extensively involved in cardiovascular regulation because it modulates heart rate, cardiac excitability, and ventricular function as well as coronary blood flow.*

Materials and Methods: *In this study, we included 40 arrhythmia and 46 healthy unrelated individuals to examine the NPY gene polymorphisms. All four exons were screened using PCR and sequencing method.*

Results: *Three polymorphisms (Leu7Pro, Ser50Ser and A7735G) and one novel mutation (G172T) were obtained. Association was found only between one marker (Ser50Ser) and the arrhythmia. Weak linkage disequilibrium (LD) was seen between all the pairs of Single Nucleotide Polymorphism (SNPs). The LD between Ser50Ser and A7735G was found to be significant. The distribution of haplotypes in arrhythmia and normal was not statistically significant.*

Conclusion: *NPY gene Leu7Pro polymorphism, which has been reported earlier as a potential cause for many of the cardiac problems, is not associated with arrhythmia in Indian population.*

Keywords: *Neuropeptide Y, Arrhythmia, SNP, Polymorphism.*

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INTRODUCTION:

Cardiac arrhythmia is any group of conditions in which the electrical activity of the heart is irregular or is faster or slower than normal. In a normal resting adult the heart rate should be with in 60 beats to 100 beats per minute. In arrhythmia, the heart rate either goes beyond 100 or below 60 beats per minute and this is called tachycardia and bradycardia respectively. Some of the symptoms of arrhythmia may be palpitations, feeling light headed, and oozing consciousness, shortness of breath and chest pain. Cardiac arrhythmias constitute a major cause of death and disability, with an estimated 300,000 cases of sudden cardiac death annually in the United States alone (1). The heart is powered by an electrical impulse that signals the heart's four chambers to contract, each at a proper time. This can be measured using an Electrocardiogram (ECG). The electrical activity seen is created by sequential and sometimes simultaneous activity of a number of channels in the muscle membrane of the heart tissue which allow certain ions to cross into or out of the cell. These may be sodium, calcium, potassium and some chemicals such as acetylcholine and ATP. Ion channels are pore forming transmembrane proteins that selectively conduct ions and play physiological roles in many cells such as the neurons, skeletal muscle, cardiac muscle and smooth muscle (2). Inherited mutations in genes encoding for ion channels are associated with arrhythmia, also called channelopathies. Mutations in the potassium and sodium channels encoded by SCN5A, KCNQ1, KCNH2, KCNE1, KCNE2 genes are thought to account for 50% to 75% of cases of congenital long QT syndrome (LQTS) and 15% to 30% of Brugada syndrome cases (3).

Neuropeptide Y is 36 amino acid peptide that consists of an alpha-helix folded underneath a proline helix with a tyrosine residue at the carboxy terminus. NPY gene is located on the long arm of human chromosome 7 (7q15.1) (4) and it is divided into and consists of four exons (5). The gene is highly conserved with 92% amino acid sequence identity between the cartilaginous fish Torpedo marmorata and mammals, which are separated by an evolutionary distance of more than 400 million years (6). It is co localized with norepinephrine in both central and peripheral noradrenergic neurons. Studies show that NPY can exert acute effects on post junctional cardiac ion channels (7). NPY has also shown to inhibit contractility in rat heart (8). It is suggested that a T1128C polymorphism in the signal peptide of the NPY gene results in leucine (Leu) 7 to proline (Pro) 7 substitution. Karvonen et al (9) screened the entire coding region of the NPY gene in obese, non-diabetic Finnish and Dutch subjects and found a significant and consistent association between the Leu7Pro polymorphism and high serum total cholesterol and low-density lipoprotein (LDL) levels. This substitution is likely to alter the properties of the signal peptide due to the different physicochemical

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properties of Leu and Pro amino acids. In general, the frequency of the Pro7 allele has been reported to be 8% among Finns and 3% among Dutchmen (9). There was no report of the presence of this allele among Japanese (10, 11,12) and Korean populations (13). Jia et al (14) reported an extremely low frequency of the Pro7 allele in China. Whereas, Bhaskar et al (15) first time documented its presence in Indian populations is with varying frequencies ranging from 0.014 to 0.233.

As NPY is co released with norepinephrine during sympathetic nerve stimulation, and is extensively involved in cardiovascular regulation because it modulates heart rate, cardiac excitability, and ventricular function as well as coronary blood flow (16). Hence the present case–control study aimed to investigate the association of genetic polymorphisms of Neuropeptide Y with arrhythmia.

MATERIALS AND METHODS:

In the present study, we adopted ACC/AHA Guidelines for Ambulatory ECG to evaluate the symptoms of cardiac arrhythmias. Forty arrhythmia individuals who are receiving the treatment and 46 healthy and unrelated individuals were included in the present study. After the informed consent, on each study subject venipuncture was performed and a blood specimen was collected into the EDTA vaccutainer. Genomic DNA was extracted from all participants, using standard procedure (17). All single nucleotide polymorphisms (SNPs) were genotyped by polymerase chain reaction and sequencing. Nucleotide sequence of primers, annealing temperature and amplicon sizes are given in Table 1. The PCRs were performed using 40 ng of genomic DNA, PCR products were checked on 2% agarose gels, and the PCR products were directly sequenced. The protocol was carried out using ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster city, CA) with Big Dye Terminator Cycle Sequencing Ready Reaction Kit. The allelic frequency distribution was tested for Hardy–Weinberg equilibrium by the $\div^2$ test with one degree of freedom using the HWSIM program (18). The distribution of alleles among hypertensive and normotensive individuals was tested using the Fisher's exact test. All individuals were sorted into haplogroups by means of the Arlequin 2.0 computer program (19). SNPs were examined for intermarker LD using the D' measures calculated by the Haploview Ver 4.0 software package.

RESULTS:

The entire genotyping effort of NPY gene yielded 3 reported SNPs and one novel mutation in intron 1 (Fig. 1). These SNPs are located in the coding or untranslated regions of the gene and the names are based on the Karvonen et al.'s notation (9). The genotypic frequencies of all four SNPs are shown in Table 2 and the allele frequency is given in the fig. 2. There was no significant difference between the genotype frequencies of arrhythmia and normal samples except for Ser50Ser. The genotype frequencies for all the SNPs were found to be in Hardy Weinberg equilibrium except for Ser50Ser (HW $\chi^2 = 13.1484$, df = 1, p = 0.0045).

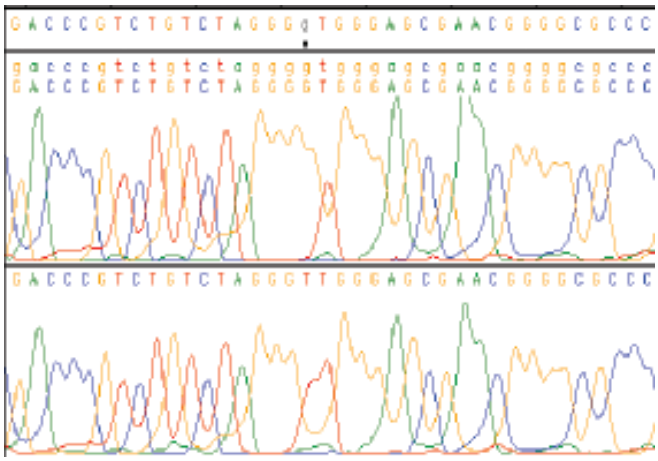

Figure 1: Electropherogram showing the novel base exchange in the Intron 1 of NPY gene.

A novel mutation (G to T transversion) was observed in the first intron of NPY gene only in one arrhythmia sample. Leu7Pro transition which changes the 7th amino acid from leucine to proline (L7P) was observed in both arrhythmia and normal samples. No homozygotes were observed in both the groups. A1258G transition which is a synonymous polymorphism (Ser50Ser) is highly polymorphic in both the groups. A to G transition in 3' UTR (A7735G)

Table 1: Summary of primers used in the present study

Region	Primer sequence	Annealing temperature	Product size
Exon 1	Np1F: 5'-CCCCGCTTCTTCAGGCAGTGC- 3'		
	Np1R: 5'-TGGGGAGTGGAGCGCGATCAT- 3'	59^{0}C	420 bps
Exon 2	NP2F: 5'-CCTGGGTTCTCTCTGCGGGACTG -3'		
	NP2R: 5'-CCCATTTTGTGTAGAGTGTGCCCTGT -3'	60^{0}C	516 bps
Exon 3	NP3F: 5'-TTCCAGATATGGAAAACGAT -3'		
	NP3R: 5'-CTGCCGAAATCTCCCCTAGTCT -3'	55^{0}C	210 bps
Exon 4	NP4F: 5'-CCCGGTCATCTTTCACTTCAG -3'		
	NP4R: 5'-GCGAAACGAACCCTGAATCTG -3'	60^{0}C	417 bps

Table 2: Genotype Frequency, Hardy-Weinberg Frequency in arrhythmia and control.

	Genotype Number (% Frequency)			HW χ^2	Monte carlo simulation
NPY Novel (G172T)					
	GG	TT	GT		
Arrhythmia	39 (0.975)	1 (0.025)	0 (0.00)	40	0.1517
Control	46 (1)	0 (0.00)	0 (0.00)	NA	NA
NPY Leu7Pro					
	TT	CC	TC		
Arrhythmia	38 (0.95)	0 (0.00)	2 (0.05)	0.0263	0.8626
Control	45 (0.978)	0 (0.00)	1 (0.02)	0.0056	0
NPY Ser50Ser					
	AA	GG	AG		
Arrhythmia	21 (0.52)	11 (0.27)	8 (0.2)	13.1484	0.0045
Control	8 (0.173)	7 (0.152)	31 (0.67)	5.5856	0.0058
NPY A7735G					
	AA	GG	AG		
Arrhythmia	37 (0.925)	0 (0.00)	3 (0.075)	0.0607	0.7625
Control	45 (0.978)	0 (0.00)	1 (0.021)	0.0056	1

Table 3: Fisher's p value and odds ratio along with confidence interval of various SNPs observed.

	Fisher's P Value	Odd's Ratio	95% confidence Interval
NPY G172T (Novel)			
Control vs Arrhythmia	0.467	nil	0.029 - infinity
Leu7Pro			
Control vs Arrhythmia	0.447	2.368	0.118 - 142.779
Ser50Ser			
Control vs Arrhythmia	0.001	0.19	0.062 - 0.559
NPY A7735G			
Control vs Arrhythmia	0.257	3.649	0.276 - 195.713

was also seen in both the groups with less frequency. To know the association between the markers and disease we performed Fisher's Exact Test. Except, Ser50Ser none of the other markers have shown significant association with the disease [(novel: p = 0.467), (Leu7Pro: p = 0.447), (Ser50Ser: = 0.001), (A7735G: p = 0.257)] (Table 3). We found a very low frequency of Leu7Pro C-allele carriers in both the arrhythmia and normal samples (Fig. 2). No significant association of the Leu7Pro C-allele and the arrhythmia was found [p = 0.447, df = 1 and odds ratio (95 CI): 2.368 (0.118 - 142.8)]. In this study, we found an elevated frequency of Ser50Ser in both the arrhythmia and normal samples (Fig.2). The results were statistically significant [p = 0.001, df = 1 and odds ratio (95 CI): 0.19 (0.062 - 0.559)]. A7735G was the third polymorphism found in the UTR of the NPY gene. The frequency of this polymorphism was found to be very low in the arrhythmia and the normal samples (Fig. 2). Hence no association was found between A7735G polymorphism and arrhythmia [p = 0.257, df = 1 and odds ratio (95 CI): 3.649 (0.276 - 195.713)]. In this study, a novel G to T heterozygous mutation was found in one of the arrhythmia samples but none of the

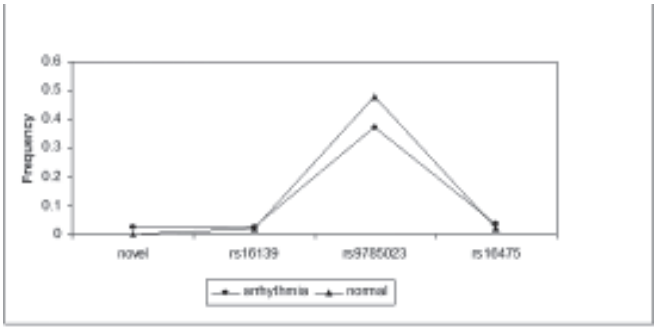

Figure 2: Allele frequency distribution in arrhythmia and control.

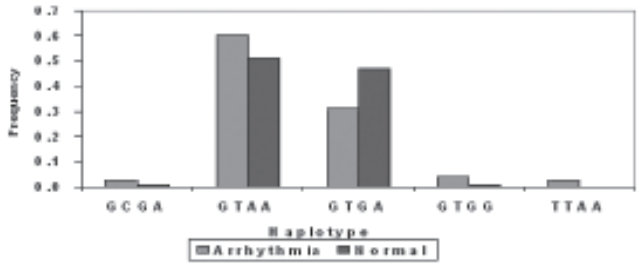

Figure 3: Haplotype frequency distribution in arrhythmia and control.

normal samples. It was also found that this mutation is not associated with arrhythmia [p = 0.467, df = 1 and odds ratio (95 CI): 0.0 (0.029 - infinity)]. Haplotype analysis also indicating no significant differences between the haplotypes of both arrhythmia and normal (χ^2 = 0.035, df = 1 and P = 0.852) (Fig. 3). Linkage disequilibrium (LD) is the non-random association between alleles at two loci (20), and is primarily the result of a physical association. Although LD has been shown not to be uniformly distributed across the genome, limited information is available about the characteristics of LD within candidate genes at large, it is the central concept of genetic association studies. Not much significant LD was observed because of less heterozygosity in the markers of both arrhythmia and normal samples (Fig. 4).

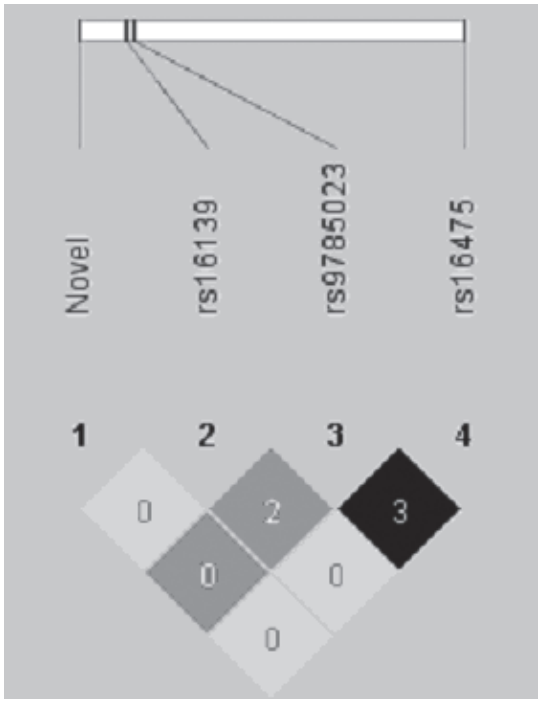

Figure 4: Pattern of linkage disequilibrium between the markers.

DISCUSSION:

Neuropeptide Y (NPY) has been shown to participate in the cardiovascular response mediated by sympathetic system (21). Ullman et al (22) reported elevated levels of Plasma neuropeptide Y-like immunoreactivity (NPY-LI) in patients with acute myocardial ischaemia and congestive heart failure (CHF) owing to increased activity of the sympathetic nervous system. However, although NPY is the most abundant peptide in the mammalian heart (23), and there is evidence of the existence of at leastY1 andY2 NPY receptors in cardiac myocytes (24), the NPY actions in heart are not fully understood (25). NPY exerts long term trophic effects that could be involved in the hypertrophic response of the heart (26). The earlier genetic and epidemiological study indicates that the NPY Leu7Pro allele is a major risk factor for obesity (27). Experimental studies indicate that the Leu7Pro polymorphism influences the heart rate level (28). Presence of the Pro7 variant is a suspected cardiovascular risk factor in Caucasians (9,28,30). There seems to be a variation of frequencies of the Leu7Pro polymorphism between ethnic groups (15). The effects of the Pro7 allele on cardiovascular regulation and diseases are not likely to be relevant in populations other than the Nordic countries because the frequency of Leu7Pro polymorphism shows a strictly geographical distribution, with most if not all positive associations found largely in Nordic countries such as Finland, Sweden and the Netherlands (9,31,32). Hence according to this study, the Leu7Pro polymorphism is not associated with arrhythmia. Contrasting results of Kallio et al (33) and Pettersson-Fernholm et al (34) it is not clear that the Leu7Pro polymorphism translates into higher or lower levels of active NPY; it is difficult to draw conclusions with respect to potential physiological or pathological roles of this polymorphism in cardiovascular control and development of diseases (35). Significant association between Ser50Ser polymorphism and arrhythmia is observed. This synonymous variant Ser50Ser was absent in the 50 obese French white compared with two non-obese controls (36). Whereas this variant is quite common in Mexican Americans (37). At haplotype level also no significant differences were observed in both arrhythmia and normal. Individuals often inherit rather long hunk of DNA from one parent or the other. The hunk is known as a haplotype, and some haplotypes themselves may also be inherited as a group. This is called linkage disequilibrium (LD). LD is the non-random association between alleles at two loci (20), and is primarily the result of a physical association. Although LD has been shown not to be uniformly distributed across the genome, limited information is available about the characteristics of LD within candidate genes at large, it is the central concept of genetic association studies. Not much significant LD was observed because of less heterozygosity in the markers of both arrhythmia and normal samples.

In conclusion, Ser50Ser is significantly associated with arrhythmia. But no association was found between and NPY gene Leu7Pro and arrhythmia. The inconsistencies between association studies may also reflect the complex interactions between multiple population-specific genetic and environmental factors. We accept as true that any association

study obtained even by case-control study should be regarded as provisional and that replication in independent population studies is critical. Relatively smaller sample size of the present study also making it difficult to extrapolate the results to the entire population.

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SIGNIFICANCE OF BLOOD AMMONIA IN UREMIC ENCEPHALOPATHY

Santhi Silambanan[a], Jothimalar[a]

ABSTRACT:

***Aim of the study**: To determine the role of blood ammonia in uremic encephalopathy, and its correlation with other uremic toxins involved in renal failure.*

***Methodology**: The study group included 30 apparently normal individuals and 15 patients with clinically diagnosed uremic encephalopathy in the age group of 30-50 years. Peripheral venous sample was used for the study. Blood ammonia was estimated by Berthelot-Indophenol method.; blood urea by modification of Diacetyl Monoxime method, serum creatinine by modified Jaffe reaction, serum sodium and potassium by flame photometry, serum calcium by modification of cresolphthaline complexon method, and serum phosphorus by modification by Wang et al. Student 't' test was used to find out statistical significance.*

***Results**: The various biochemical parameters were compared using student 't' test with significance of 'p' value at 0.05. Blood ammonia was found to be 70.16 ± 9.11µmol/L in the control subjects. In uremic encephalopathy the level was found to be 78.61 ± 24.42µmol/L.*

***Conclusion**: Blood ammonia was found to be normal in uremic encephalopathy and that it could not be involved in its pathogenesis. Urea and creatinine still remain to be the markers of uremic encephalopathy.*

***Key words:** uremic encephalopathy, uremia, uremic toxins, blood ammonia, hyperammonemia, astrocytosis.*

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INTRODUCTION:

Uremic encephalopathy describes the final stage of progressive renal insufficiency, which culminates in end-stage kidney failure with neurological involvement (1,2,3,4). Under conditions of renal failure where the blood level of urea is high, the amount of urea entering the renal vein and subsequently into the gut is high(5). This is acted upon by urease produced by colonic bacteria converting it to ammonia. Urea acts as a uremic toxin only at extreme levels. At such levels it inhibits argininosuccinate lyase and could exert feedback inhibition of urea production possibly by channeling waste nitrogen into more toxic compounds like ammonia, carbamate or cyanate(6,7,8,9,10). Though ammonia is toxic, systemic blood ammonia levels are normal or only minimally elevated in uremia. Some of the symptoms of uremia especially nausea, vomiting, malaise and possibly bleeding are partly due to its intoxication with urea or a product of urea metabolism which could be ammonia(10,11). The present study was done to find out the significance of blood ammonia in uremic encephalopathy.

Materials and methods

The study was performed on the venous blood of 30 subjects who were apparently normal males and females in equal number. The control group was from the staff of Madras Medical College and Government Hospital belonging to the age group of 30-50 years. The subjects of the diseased group, 15 in number were the patients undergoing treatment in the nephrology department in Madras Medical College. These patients were clinically diagnosed to have uremic encephalopathy. Fasting venous samples were collected after obtaining consent from the patients. The samples were analyzed to estimate ammonia, urea, creatinine, sodium, potassium, calcium and phosphorus.

Estimation of ammonia: Berthelot-Indophenol method (Wako Pure Chemicals) (12,13,14). Berthelot's reagent is an alkaline solution of phenol & hypochlorite. Ammonia reacts with Berthelot's reagent to form a blue product which is estimated.

Reference value: arterial blood: 22-61µmol/L (as per the method). Several recent studies have suggested that it is not necessary to utilize arterial blood when measuring ammonia in blood. Venous blood or a computation of the partial pressure of ammonia in blood sample may suffice. All the older methods have the reference values based on the arterial sample. Only recently venous samples have been used instead of arterial samples because of ease of collection, less trauma and other advantages. Hence arterial blood reference values are given as per the used methodology (16).

Estimation of blood urea by modification of Diacetyl Monoxime method(17,18). Estimation of serum creatinine by modified Jaffe reaction(19). Estimation of serum sodium and potassium by flame photometry (ELICO CL 26D)(20). Estimation of serum calcium by

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modification of cresolphthaline complexon(OCP) method of Moorehead and Briggs(20). Estimation of serum phosphorus by modification by Wang et al of Daly and Ertinghausen method(21).

RESULTS:

The results of the various biochemical parameters of all subjects are tabulated in table I. The various biochemical parameters were compared using student 't' test with significance of 'p' value at 0.05. Table I shows the comparison of biochemical parameters between the control and diseased groups. Blood ammonia was found to be $70.16 \pm 9.11 \mu$mol/L in the control subjects. In uremic encephalopathy the level was found to be $78.61 \pm 24.42 \mu$mol/L. All the parameters except ammonia showed significant difference between the two groups. There was no significant alteration in blood ammonia in uremic encephalopathy patients when compared with that of controls. Level of blood ammonia did not show any correlation with other parameters.

DISCUSSION:

Blood ammonia level in patients with uremic encephalopathy did not show significant change when compared with that of controls. Ammonia being largely contributed by the intestine is taken almost completely by the liver where it is detoxified to urea. In patients with uremic encephalopathy with normal liver functions, most of the ammonia is taken by liver. In the kidney small amount of ammonia is formed by the reaction catalyzed by glutaminase. But this source is insignificant in uremia since the functioning nephrons are much reduced in number. Normally about 40% of the urea filtered by the glomeruli is reabsorbed in the proximal convoluted tubule. This urea through systemic circulation reaches intestine. By the action of urease, urea is hydrolyzed to ammonia. But in patients with uremic encephalopathy urea is converted to guanidine compounds instead of ammonia (22-26). Hence, blood ammonia level does not increase significantly in uremic encephalopathy. This is more in accordance with the author,

Table 1: Comparison of biochemical parameters between control group and uremic encephalopathy group

Groups	n	NH_3 μmol/L	Urea mmol/L	Creatinine μmol/L	Sodium mmol/L	Potassium mmol/L	Calcium mmol/L	Phosphorus mmol/L
Control	30	70.16 ± 9.11	12.93 ± 1.47	82.21 ± 8.84	138.96 ± 2.54	4.10 ± 0.39	2.43 ± 0.81	1.46 ± 0.09
UE	15	78.61 ± 24.42#	55.60 ± 9.30***	881.34 ± 200.67***	137.40 ± 1.67*	4.5 ± 0.51**	2.24 ± 0.17***	1.77 ± 0.20***
Values are mean ± SD ***p < 0.001, ** p < 0.01, *p < 0.05, #p > 0.05								

Nancy A Brunzel, (27) who states that unlike the other non-protein nitrogenous substances; concentration of ammonia in the plasma is not a useful indicator of renal function.

Fasting sample is preferable for the estimation of blood ammonia due to the following reasons; the major source of circulating ammonia is the gastrointestinal tract. Plasma ammonia concentration in the hepatic portal vein is typically five to tenfold higher than that in the systemic circulation. It is derived from the action of bacterial proteases, ureases and amine oxidases on the contents of the colon and from the hydrolysis of glutamine in both the small and large intestine. Hence a protein rich diet causes marked elevation in blood ammonia compared to that of fasting state (28).Proteins in the intestine can be broken down by microflora into ammonia, indoles, phenols, amines etc. Urea and creatinine are elevated as a result of reduced glomerular filtration rate (GFR) and decreased tubular function (29).

Retention of these compounds and of metabolic acids is followed by progressive hyperphosphatemia, hypocalcemia and potentially dangerous hyperkalemia as evidenced by our study. Guanidine compounds, especially methyl guanidine, have been implicated in toxicity of experimental renal failure, but their significance in human uremia remains to be found (30). Generally all uremic toxins exert their effect through enzyme inhibition, irreversible carbamoylation of proteins and derangement in membrane transport (31).

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HANDS ON TONSILLECTOMY BY FIRST YEAR RESIDENTS - A RETROSPECTIVE STUDY

Sanjeev Mohanty[a], Somu L[a] , N S Thirumaran[a], Gopinath[a], Haneesh[a]

ABSTRACT:

Tonsillectomy is the most common surgery performed by an ENT surgeon. So far no study has been conducted to assess the surgical skill of the budding ENT surgeons worldwide. Here is an attempt to compare the surgical skills of first year ENT residents of Sri Ramahandra Medical College and Research Institute. The surgical competencies were compared between two groups – independent group and assisted group. The various parameters analysed included surgical skills such as duration of operation, intra operative bleeding, plane of dissection, preservation of faucial pillars, loss of teeth/injury to lips , usage of diathermy and post operative complications like postoperative pain/oedema, reactionary and secondary hemorrhage and prolonged duration of healing. Study revealed that before performing tonsillectomies first year resident should observe and assist a good number of cases to minimize the morbidity of patients.

Key Words: *Independent tonsillectomy, assisted tonsillectomy, reactionary and secondary bleeding.*

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INTRODUCTION:

Tonsillectomy is the most common operation performed by an otolaryngologist. The first known tonsillectomy was performed by Cornelius Celsus almost 2,000 years ago. He enucleated the tonsil with his fingernails and then suggested that the 'fossae should be washed with a vinegar and painted with a medication to reduce bleeding [1].A study conducted by Bond et al in UK reveals that sore throats cost the NHS an estimated £60 million in GP (General practitioner) consultations alone, result in 90,000 tonsillectomy procedures, approximately half of which are in children, and a loss of more than 35 million school or work days annually. The incidence of tonsillectomy has risen since the early 1990's, although levels are still much lower than in the 1930's, when 100,000 operations were performed in school children[2]

Review of literature revealed information mainly on the indications for tonsillectomy, role of tonsillectomy in recurrent tonsillitis, surgical techniques and complications [3, 4, 5]. However, to the best of our knowledge, there were no studies in the past made to assess the surgical skills of residents newly enrolled in the otolaryngology training programme. This type of study could highlight their deficiency in surgical skills and techniques which needs correction and improvement. Over the years the technique of tonsillectomy has undergone a sea change from the crude methods to the highly acclaimed laser assisted operations. This was a study done to evaluate the competency of a 1st year resident for performing tonsillectomies independently versus surgeries done with assistance.

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MATERIALS AND METHODS:

This was a retrospective study done on 72 tonsillectomies performed by 1st year residents between April 2007 to December 2007.The duration of study has been decided as nine months assuming that by this given time period first year resident will be competent enough to perform tonsillectomies independently. A comparison of the surgical outcome in the first month and the last month of our study in independent group is also been included for further references. Fifty-five cases were performed independently (under supervision) and 17 were assisted.

The surgical competencies were compared between two groups – independent group and assisted group.

SELECTION CRITERIA:

Independent group consisted of supervised tonsillectomies. In this group tonsillectomy was performed by first year resident in ENT (Ear, nose and throat) who was allowed to operate independently under close supervision (immediate surgical assistance available if required), only after observing five cases and assisting five cases.

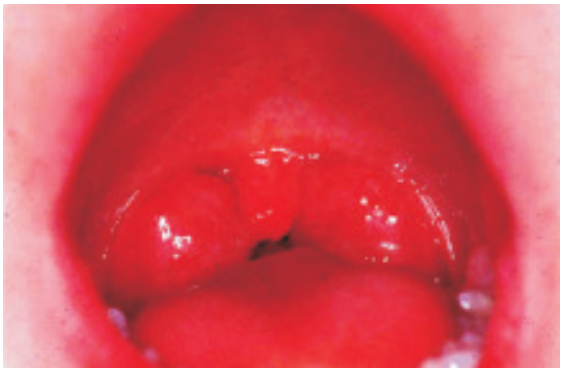
Assisted group consisted of assisted tonsillectomies. In this group with the assistance of a consultant, tonsillectomy was performed by a first year resident after observing 5 cases and assisting another 5 cases. At any point of time if the resident is seeking for hands on assistance in performing surgery, that case also will automatically fall in to assisted group.

Cases for tonsillectomy were selected based on established clinical criteria, counseling and a complete

preoperative work up. The cases included in our study were all performed in service operation theatre of Sri Ramachandra Medical College and Research Institute, done only by first year residents and with no previous experience in performing tonsillectomies.

The various parameters analysed included surgical skills such as duration of operation, intra operative bleeding, plane of dissection, preservation of faucial pillars and loss of teeth/injury to lips. Other parameters assessed included assistance required to complete procedure, usage of diathermy and post operative complications like postoperative pain/oedema, reactionary and secondary hemorrhage and prolonged duration of healing.

ENLARGED PALATINE TONSILS



RESULTS:

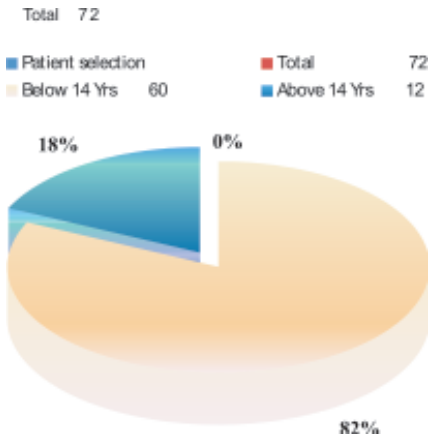
The cases were predominantly in pediatric age group (Graph 1). Results of all the parameters analysed are shown in (Table 1). Duration of operation which was less than an hour in assisted tonsillectomies as compared to 1.2 hours on an average in independent tonsillectomies. Intra operative bleeding was the next parameter which was assessed. Assessment of bleeding was done by measuring the blood collected in the suction apparatus during the procedure. In the assisted group, it was found to be less than 50 ml. In assisted tonsillectomies the level of dissection was always in the right plane (between the capsule and loose areolar tissue). Assessment of pain was done by monitoring how fast the post operative patients returned to their normal swallowing as pain and difficulty in swallowing are constant complaints of post tonsillectomy patients and it is found that patients recover fast in assisted group as compared to independent group. Various intra-operative complications encountered during the surgery were seen mostly in surgeries performed independently (Graph 2). A comparison between the first and last month of independent tonsillectomies are also shown here (table 2) which clearly shows the improvement in surgical skills of first year residents with evolving time.

Table 1: Surgical skills and post-operative complications assessed

Patient selection	Independent group	Assisted group
Duration of procedure (mean)	>1 hour	<1 hour
Intra op bleeding	>50 ml	<50 ml
Plane of dissection in 1st attempt	80%	98%
Preservation of pillars (both)	62%	90%
Incomplete removal	12%	2%
Assistance required	18%	0
Post op pain / oedema	More	Less
Reactionary and secondary bleeding	14%	5%
Prolonged healing >2 weeks	5%	2%
Diathermy usage	7%	1%
Loss of teeth/ injury of lips	3%	1%

The numbers expressed are the % of total number of patients taken for study (tonsillectomy).

Graph 1: Age wise distribution of tonsillectomies performed



Graph 2: Comparison of intra-operative complications between case and control groups

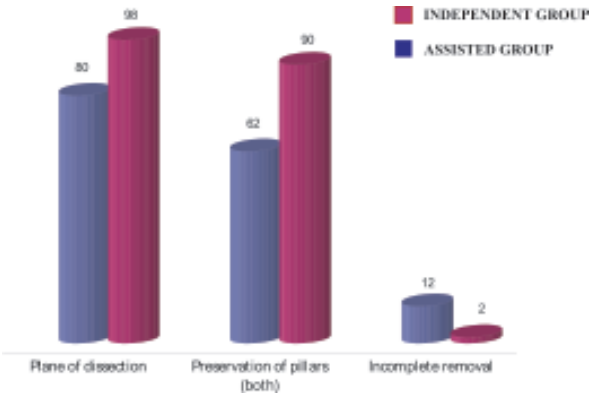


Table 2 : Comparison of tonsillectomies done in independent group in the first and last month of study.

Month	April	December
Total number of tonsillectomies	11	12
Duration of procedure (mean)	1.2 hours	45 minutes
Intra op bleeding	>75 ml	<30 ml
Plane of dissection in 1st attempt	4	11
Preservation of pillars (both)	7	12
Incomplete removal	3	0
Post op pain / oedema	10	7
Reactionary and secondary bleeding	9	3
Prolonged healing >2 weeks	2	0
Diathermy usage	7	1
Loss of teeth/ injury of lips	3	0

DISCUSSION:

Most of the tonsillectomies are done in pediatric age group[6]. Similar age distribution pattern was found in our study as majority (83.3%) of patients were below 14 years of age) as chronic tonsillitis are frequently encountered in pediatric age group. During the initial few months of the study most of tonsillectomies were done under assisted group as we almost always needed assistance. Later, as the confidence level of the resident went up the number of tonsillectomies done under independent group has also come up. By the end of the study there were no assisted tonsillectomies performed which indicates significant improvement in the competency of the first year residents in performing tonsillectomies.

Duration of surgery was less in assisted tonsillectomies as compared to all independent tonsillectomies which needed more than one hour for completion. Reason for prolonged duration of surgery in independent tonsillectomies could be attributed to various reasons such as extra time taken for adjusting head light, positioning of patient and getting synchronized with instruments.

Intra operative complications, tissue damage and incomplete removal of tonsils were found to be much more in independent tonsillectomies. As a result of dissecting in an inappropriate plane, bleeding was more and excessive bleeding which obscured proper visualization of bleeders. This resulted in proceeding blindly which caused more tissue damage. Due to failure to visualize the operating field one may remove anterior pillar or posterior pillar or both along with tonsils. Preservation of faucial pillars needs to be taken care because their removal may lead to difficulty in swallowing and also in delayed healing causing prolonged post-operative pain. Post operative complications such as pain and oedema of uvula were seen much less in assisted tonsillectomies highlighting the need of experienced hands. Some times one might leave behind part of tonsil which will result in incomplete removal, a cause for recurrence[7]. Incidence of reactionary hemorrhage and secondary bleeding was more in independent tonsillectomies as compared to assisted ones. The number of times diathermy was used to achieve complete homeostasis was also very less in assisted tonsillectomies, a skill gained with expierence[8]. Even though injury to the lip and teeth were very rare in it was noticed more in independent tonsillectomies. Healing was much faster in assisted as compared to independent tonsillectomies, this could be due to minimal tissue injury in assisted surgeries.

It is mandatory for a first year resident to observe the various steps of surgery before he starts assisting cases[9]. This is necessary because it is only after few surgeries that one will acquire the correct technique of dissection along with eye and hand coordination and also learn lessons of giving tissue respect[10]. To reduce the overall intra operative and postoperative complications it is advisable that the resident witness more than 10 cases before assisting the surgeon and the number of assisted surgery to be increased from 5 to 10 before performing independent tonsillectomy.

CONCLUSIONS:

To conclude a first year resident of ENT department can perform tonsillectomy independently (under supervision) only after observing and assisting a good number of cases.

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PLASMA FIBRINOGEN LEVELS DURING THE DIFFERENT PHASES OF MENSTRUAL CYCLE – A SCIENTIFIC INSIGHT

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ABSTRACT:

This study was aimed at determining the association between levels of plasma fibrinogen, a marker of blood viscosity and the different phases of the menstrual cycle. Hence this study was conducted on 51 students in the age group of 17 to 19 years. Subjects with regular menstrual cycles were selected and blood samples were collected from them on the 7th and 25th day of the menstrual cycle that fall on the early follicular and late luteal phases respectively. Plasma fibrinogen level was estimated by Microturbidometric method. Plasma fibrinogen was significantly elevated in the early follicular phase when compared to the late luteal phase. ($P < 0.001$) Elevated plasma fibrinogen values in the early follicular phase suggest that ovarian hormonal activity influences haemorheology and blood flow. The clinical significance of these rheological changes remains to be established, but at least theoretically there may be an increased risk of thromboembolism, e.g. at surgery, during days 5-15 of the cycle.

Keywords : *haemorheology, follicular phase, luteal phase microturbidoimetry.*

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INTRODUCTION:

Blood viscosity during the menstrual cycle in healthy women shows that it is the lowest at the start of the bleeding and increases to a peak at day 7. The clinical significance of these rheological changes remains to be established, but at least theoretically there may be an increased risk of thromboembolism, e.g. at surgery, during days 5-15 of the cycle [1].

Therefore, in an attempt to establish possible correlations between blood viscosity with different phases of the menstrual cycle, a study in this regard is conducted where plasma fibrinogen level, a marker of blood viscosity is estimated during the follicular and the luteal phases of menstruation.

Objectives:

1. To assess and compare the plasma fibrinogen level in the early follicular and luteal phases of menstrual cycle in healthy adult population.
2. To associate plasma fibrinogen level variations with the number of days of menstrual bleeding.
3. To study the associations if any between different grades of physical activity and plasma fibrinogen levels.

MATERIALS & METHODS:

The study was conducted on 51 female students of Sri Ramachandra Medical College in the age group of 17 to 19 years after obtaining an ethical committee clearance and volunteer informed consent. Subjects with regular menstrual cycles were included for the study. Blood samples were collected from them on their 7th and 25th day of menstrual cycle that fall on the early follicular and late luteal phases respectively. Subjects with history of any infection, inflammation and those who are under any treatment for any condition were also not included in this study.

The subjects were asked to provide a detailed history regarding their personal, menstrual and physical activity in the proforma handed out. Height and weight were also recorded.

Plasma fibrinogen level, a marker of blood viscosity was estimated by Micro turbid metric method of Parfentjev[2] and Johnson. Once the blood sample was collected, it was centrifuged and the plasma was separated out. The fibrinogen in the plasma was precipitated with ammonium sulphate and the resulting turbidity was estimated as optical density from the amount of light transmitted to the photoelectric cell of a spectrophotometer. A commercial preparation was selected and used as a standard protein solution and various concentrations were analysed. The relationship of increasing optical densities to increasing concentrations of fibrinogen are a straight line in a graph, indicating that the turbidity varies directly with the fibrinogen levels of the protein solutions studied. Now, the plasma fibrinogen concentration was derived from this optical density value using the standard line graph that was plotted to standardise the procedure.

The levels of physical activity are graded as follows.

Grade 0- Inactive; The subject has a sit-down job with no regular physical activity.

Grade 1- Relatively inactive; Three to four hours of walking or standing per day and the subject has no regular organized physical activity during leisure time.

Grade 2- Light physical activity; The subject is sporadically involved in recreational activities such weekend golf or tennis, occasional jogging, swimming or cycling.

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Grade 3- Moderate physical activity; The subject participates regularly in recreational/ fitness activities such as yoga, jogging, swimming or cycling at least three times per week for 30 to 60 minutes each time.

Grade 4- Very vigorous physical activity; the subject participates in extensive physical activity for 60 minutes.

The statistical analysis was conducted using SPSS version 7.0 software. P values less than 0.5 was considered statistically significant.

The levels of physical activity of the subjects were graded from zero to five(0-5) and were correlated with the corresponding plasma fibrinogen levels during both follicular and luteal phase.

RESULTS:

Table 1 : Estimation of plasma fibrinogen in the different phases of menstrual cycle.

Period	Mean plasma fibrinogen level mg/dl	significance
Follicular Phase	364.10 + 65.39	0.000P
Luteal Phase	256.25 + 59.58	<0.001

Table 2 : Correlation between plasma fibrinogen level and the number of days of menstrual bleeding.

Period	No of days of bleeding	Mean plasma fib. Level in mg/dl	SIG.
Follicular phase	< 4 days	366.37+62.57	0.053 P>0.005
	>4 days	308.50+139.30	
Luteal Phase	< 4 days	253.69+54.19	0.003 P<0.01
	> 4 days	319.00+168.29	

Table 3 : Correlation between plasma fibrinogen level and the levels of physical activity.

Follicular phase :

Level of physical activity	Mean plasma fibrinogen level in mg/dl	Sig
0	0	
1	413.4+20.27	0.128P
2	356.19+67.10	>0.05
3	385.11+59.80	
4	332.33+69.82	

Table 4 : Luteal Phase

Level of physical activity	Mean plasma fibrinogen level in mg/dl	Sig
0	0	
1	278.0+76.11	0.512P
2	258.10+58.84	>0.05
3	256.56+78.09	
4	228.17+23.68	

Table 5 : Mean plasma fibrinogen and mean plasma estrogen during the different phases of menstrual cycle

Period	Days in a cycle	Mean estrogen value in pg/ml	Mean plasma fibrinogen level mg/dl
Follicular phase	1-9	57.6	364.10 + 65.39
Luteal phase	24-29	132.8	256.25 + 59.58

DISCUSSION:

Based on the results obtained, I have analysed the plasma fibrinogen level in relation to plasma estrogen, duration of menstrual cycle and the levels of physical activity during the different phases of menstrual cycle and also compared with the results of the other studies.

It is quite evident from the results that the plasma fibrinogen level in the early follicular phase is elevated when compared to the luteal phase and the increase is found to be statistically significant.

Comparison of standard plasma mean estrogen values with that of the mean plasma fibrinogen levels estimated during different phases of menstrual cycle have clearly shown a negative relation between the plasma fibrinogen level and the corresponding estrogen values i.e a decreased estrogen during the folllicular phase and an increased level during the luteal phase.

The above findings are in concurrence with the study done by Solerte SB et al [3] st on blood rheology in healthy women throughout a normal menstrual cycle. Significant increases in fibrinogen, blood and plasma viscosity, was demonstrated during the follicular and ovulatory phase in comparison with mid- and late luteal phase of the menstrual cycle. Associations were found between oestradiol levels and the haemorheological variables suggesting that ovarian hormonal activity influences haemorheology and blood flow.

Larsson H et al[1] studies on blood viscosity during the menstrual cycle in healthy women showed that it was the

lowest at the start of the bleeding and increased to a peak at day 7. The clinical significance of these rheological changes remains to be established, but at least theoretically there may be an increased risk of thromboembolism, e.g. at surgery, during days 5-15 of the cycle.

The levels of physical activity of the subjects were graded from zero to five (0-4) and were correlated with the corresponding plasma fibrinogen levels during both follicular and luteal phase. The results obtained in this regard during both the phases, though the physical activity showed no relation with the plasma fibrinogen levels statistically and there is a difference in the plasma fibrinogen level between grade 0 and 4 indicating that fibrinogen level is moderately high in the physically inactive persons.

Rankinen T, et al studied the relationship between habitual physical activity and plasma level of fibrinogen in a cohort of 180 postmenopausal women, aged 60-69 years[4]. For further analysis, the association between physical activity and fibrinogen level of the subjects were classified into three categories according to their weekly physical activity frequency: i.e 0 to 1, 2 to 3, and 4 or more during the preceding month. The data suggests that in postmenopausal women a low level of physical activity is associated with a high level of plasma fibrinogen. In our study the insignificant relation between plasma fibrinogen and physical activity may be due to the younger age group of the subjects we have selected.

CONCLUSION:

- A statistically significant elevated plasma fibrinogen level in the early follicular phase was observed when compared to the luteal phase.
- Though there was an increased plasma fibrinogen in inactive persons, the physical activity did not corelate significantly with the plasma fibrinogen levels.

Overall this study revealed that blood viscosity during the menstrual cycle in healthy women is the lowest at the start of the bleeding and increases to a peak at day 7. The clinical significance of these rheological changes remains to be established, but at least theoretically there may be an increased risk of thromboembolism, e.g. at surgery, during days 5-15 of the cycle.

There were few limitations encountered in the study. Subjects with irregular menstrual cycles and any history of inflammation, trauma, drug intake and systemic diseases had to be eliminated from the study. Though it was planned to collect four samples from a subject in one cycle initially, on the early and late follicular and also on the early and late luteal phases, most of the subjects were not willing for the same for ethical reasons.

Plasma fibrinogen level can also be estimated from subjects who have undergone natural and surgical menopause. Thereby, the effect of estrogen on plasma fibrinogen level in the above said groups can be studied and the cardiovascular risks involved can be assessed. Also a clinical study looking at incidence of thromboembolic events in female patients with respect to follicular/ luteal phase may be beneficial.

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PLANT KINGDOM CLAIMS FOR INSULIN!!!

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ABSTRACT

Diabetes is a multifactorial disorder affecting a great number of people across the globe. Worldwide prevalence of diabetes was estimated to be 171 million (2.8%) in 2000 and it may rise up to 366 million (4.4%) by the year 2030. Diabetes mellitus is a heterogeneous metabolic disorder characterized by hyperglycemia, glycosuria, and negative nitrogen balance leading to a number of complications like diabetic retinopathy, diabetic nephropathy, diabetic neuropathy, peripheral vascular disease, high cholesterol and high blood pressure, atherosclerosis, and coronary artery disease etc. Of the major three types of diabetes, Type 1 diabetics primarily depends on exogenous insulin for their survival because of absolute deficiency of insulin. ***Insulin*** *is a polypeptide hormone, possessing anabolic property playing a pivotal role in the regulation of carbohydrate metabolism, along with the metabolism of fat and proteins and regulation of certain gene expression. Insulin is required for all animal life. After knowing the importance of insulin and its role in diabetes, higher animals like horses, pigs and cows are exploited for their pancreatic hormone, insulin.Currently human insulin gene cloned in E.coli is considered to be a better method. By the year 2010 global market for insulin is projected to be worth around $11.8bn, and the demand for insulin is predicted to rise from around 5000 kg to 16000 kg. Growing diabetic population and the complications associated with diabetes stimulates the search for new drug targets and more efficient drugs with less adverse effect. Insulin like proteins "glucokinin" is detected in various plants and microbes showing similar functions as that of vertebrate insulin. Isolated glucokinin detected and quantified by ELISA shows its structural/sequence similarity with insulin of animal origin. Peptide sequence of glucokinin isolated from certain plants were similar to that of α and β chain of insulin. Studies on the effect of glucokinin in experimental animals showed its antihyperglycemic property when administered orally/IP with protease inhibitors. Qualitative detection of glucokinin in varied species by ELISA suggests that the insulin mediated carbohydrate pathway have been conserved through evolution. This breakthrough discovery of insulin like protein in plant kingdom showing similar activity as that of animal insulin, lowering blood glucose gives a promising natural source of insulin to meet the global requirement. This article spells out some of the scientific contributions supporting the presence of insulin like hormones/proteins for bioactivity from plants.*

Keywords: *Insulin, glucokinin, diabetes.*

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INTRODUCTION

Worldwide diabetic population is expected to approximately double between the year 2000 and 2030. The greatest increase in the number of people with diabetes will be in India. In the year 2000, diabetic population was 31,705 this number may increase to 79,441 by the year 2030 (1). Diabetes is a chronic metabolic disorder in which the body cannot metabolize carbohydrates, fats, and proteins because of a lack of or ineffective use of the hormone, insulin. Diabetes is classified into three primary types that are different disease entities but share the symptoms and complications of hyperglycemia. Type 1 (Insulin dependent diabetes mellitus [IDDM] or Juvenile onset diabetes) caused by **-**genetic predisposition, - **e**nvironmental exposure to virus, toxin, stress, -autoimmune reaction where beta-cells of pancreas are destroyed by its own immunological system. Type 2 (Non-insulin dependent diabetes mellitus, NIDDM, or adult-onset diabetes) caused by -insulin resistance: unable to utilize insulin produced because of cell-receptor defect, -insufficient production of insulin in response to blood glucose, -excess production of glucose from the liver, -genetic predisposition, and -obesity. The third type of diabetes, is gestational diabetes mellitus (GDM) caused by insulin resistance due to pregnancy**.**

However, diabetes is a manageable disease a large number of classes of drugs are being used for diabetes for the past five decades. Since, a single drug is not effective to control hyperglycemia reasonable combinations of oral agents based on their mechanism of action which includes sulfonylurea and metformin, sulfonylurea and an alpha-glucosidase inhibitor, sulfonylurea and troglitazone, repaglinide and metformin, troglitazone and metformin, insulin and metformin, and insulin and troglitazone are in clinical use now. A schematic representation of the causes of hyperglycemia and the respective drugs blocking the pathway is shown in Fig 1.Diabetes mellitus is known to man since time immemorial and the treatment for this disease from golden days is based on plants and plant derivatives (2). The standard drugs which are widely used for diabetes all over the world were derived from plants. This is in the case of metformin, which is developed from biguanides present in the leaves of the legume plant *Galega*

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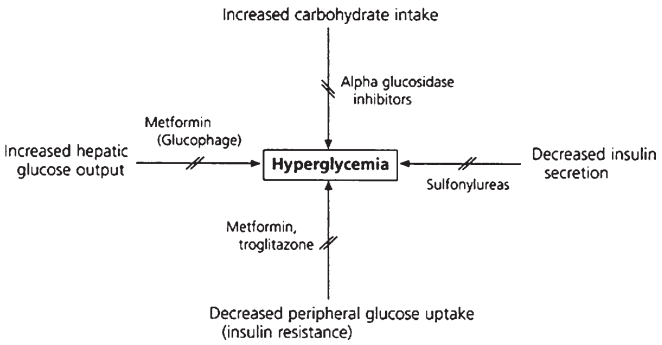

Fig. 1: Showing Pathophysiology of Hyperglycemia and the target of some antihyperglycemic drugs.

officinalis (3), salacinol, an á-glucosidase inhibitor extracted from the roots of *Salacia reticulata* (4), and cryptolepine, an indoloquinolene alkaloid isolated from the leaves of *Cryptolepis sanguinolenta* (5). Over and above a number of herbs such as *Allium cepa, Allium sativum, Aloe vera, Cajanus cajan, Coccinia indica, Caesalpinia bonducella, Ficus bengalenesis, Gymnema sylvestre, Momordica charantia, Ocimum sanctum, Pterocarpus marsupium, Swertia chirayita, Syzigium cumini, Tinospora cordifolia, Trigonella foenum graecum, Mucuna pruriens, Murraya koeingii and Brassica juncea* (6) have been indicated/used for treating Diabetes in various traditional medicines.

Right after the discovery of insulin in young calf serum, J. B. Collip and C. H. Best reported for the presence of insulin-like substances in plant materials like green tops of onions, lettuce leaves, green bean leaves, barley roots, beet roots, etc., (7). The discovery of this hormone in tissues of the higher plants as well as in yeast opened up a new field of research in plant metabolism and afforded another remarkable example of parallelism between certain physiological processes in the plant kingdom with the animal kingdom. This review addresses some of the scientific contributions supporting the presence of insulin like animal hormones/protein capable of communicating with insulin mediated signal pathway in the plant kingdom.

GLUCOKININ OR PLANT INSULIN

Pancreatic insulin's influence on glycogen formation provoked a theoretical concept on the existence of insulin or insulin like protein hormones in organisms rich in glycogen. Collip's efforts on extracts of yeast and onion were successful in altering glucose metabolism. The term glucokinin was proposed by him in order to differentiate insulin of plant origin from that of animals. Following Collip's discovery Charles Best reported on insulin like material in germinating potatoes, rice and even in beetroot (8, 9). In plants, the growth and development process involves metabolisation of large quantity of stored starch into glucose, a process similar to that of liver glycogen mobilization for energy in animals. So a comparative study on glucokinin with insulin isolated from fresh beef pancreas and glucokinin from onion tops revealed that it promotes maize germination (10). No research was done after this pioneering work of the 1920s. And the silence was broken when Khanna and collaborators reported on the presence of insulin in plants and patented a process for its production from the fruits of *Momordica charantia* (bitter gourd) (11). A water soluble fraction of *M.charantia* showed a single band on SDS-PAGE, smaller than recombinant insulin so, it must be less than 6 K Da. A specific fraction of *M. charantia* is about 2.5 K Da with 18 amino acid having an isoelectric point of 8.2 (12, 13). Further work on the possible presence and antihyperglycemic activity of glucokinin in *M. charantia* was done by Ng and co-workers (14).These authors utilized the seeds and employed gel filtration and ionic exchange chromatography which led to the isolation of several pure fractions exhibiting properties similar to animal insulin.

Following these reports, glucokinin was also detected in the prokaryote *Escherichia coli* and in the unicellular eukaryote *Tetrahymena* (15) as well as in fungi (8, 16, and 17) and in *cyanobacterium* (18). Their results suggest that "insulin may have arisen earlier in evolution than had previously been thought" and pointed to the possibility of its presence in plants. The immunoactive material from spinach and *Lemna gibba* was isolated, characterized chromatographically on C-18 hydrophobic liquid chromatography column. The protein nature of the isolated material was observed by its destruction by pronase and unaffected by inactivated enzyme. Isolated glucokinin is also similar to insulin in its net charge characteristic at low pH, since it adsorbed to a CM-Sepharose column and eluted with moderate salt concentration. Furthermore, glucokinin is similar to insulin in its behavior on a C-3 bonded silica reverse phase column. With a relatively step gradient and a short column, glucokinin eluted in the same area as the vertebrate insulin tested i.e. pork, beef, chicken and rat, but differs from guinea pig insulin which is more hydrophilic that the other vertebrate insulin. Glucokinin isolated from spinach and *Lemna gibba* is approximately 6 K Da showing similar properties of insulin immunologically as it reacts with anti-pork insulin antiserum and anti-chicken insulin antiserum, immunodepeleted by anti-insulin antibodies, binds to insulin receptors and stimulates glucose metabolism in rat adipocytes (19).

Reports of Goodman and Davis showed that added exogenous insulin, insulin like growth factors I and II (IGF-I and IGF-II) accelerates the post-germinative development of fat-storing seeds of sunflower, watermelon and cucumber (20). They also measured increased activities of enzymes necessary for the conversion of fat to carbohydrate like fatty acyl CoA dehydrogenase, citrate synthase, malate dehydrogenase, isocitrate lyase, and malate synthase which suggest that plants also have a system that could respond to hormones of higher animals. Recently, a 20 kDa protein, isolated and purified by G-50 Sephadex followed by affinity chromatography through a bovine insulin

antibody-Sepharose column from maize tissue, showed IGF like activity in upregulating maize germination and seedling growth. Maize IGF enhances phosphorylation of S6 ribosomal protein on the 40 S ribosomal subunit, in a similar way as observed when bovine insulin is applied to maize axes during germination. Rapamycin, a specific inhibitor of the insulin-stimulated signal transduction pathway, prevented S6 phosphorylation. Anti-insulin antibody, heat treatment or trypsin digestion attenuated its activity which shows that this protein acts through a pathway similar to that of insulin or IGFs in animal tissues (21). The seed coat of *Canavalia ensiformis* contains protein with a sequence similar to that of bovine insulin (22). With the help of modern techniques such as immunofluorescence microscopy, insulin, insulin receptor and phosphoserine proteins were localized to an internal tissue layer of the seed coat but not in the cotyledonus tissues of *C. ensiformis*. This region was assumed to be important in the transport of the sugars to the embryo. These results proclaimed that insulin, vanadyl sulfate (an insulin mimetic compound), pinitol (a chiro inositol analogue) and glucose are capable of accelerating *C. ensiformis* seed radicle and epicotyl development. On the contrary, tyrphostin (an inhibitor of insulin receptor kinase activity) inhibited these processes (23).

Insulin like antigens was detected in leaves or aerial parts of varied species utilizing a modified ELISA and by western blotting. Plants are found to be positive for insulin like protein belonging to different phyla like Bryophyta (mosses), Psilophyta (wisk ferns), Lycopodophyta (*selaginella* sp), Sphenopsida (horsetails, *Equisetum*), Gymnosperms (Coniferophyta, Cycadophyta, Ginkgophyta) and Angiosperms (flowering plants including monocotyledons and dicotyledons). Alga like *Gracilariopsis* a common red alga, *Spirulina maxima* (Cyanobacterium) and yeast (*Saccharomyces cerevisiae)* also contain insulin like antigen. The protein isolated from *S. maxima by* acidic ethanol extraction showed a similar behavior in reverse phase chromatography as like bovine insulin and the N-terminal amino acid sequence is also shown to have homology with the sequences of proinsulins (18). Wider distribution of insulin in all organisms from unicellular bacteria to multicellular vertebrates suggests that they are conserved through evolution (24).

An ELISA assay using anti-human insulin antibody detected glucokinin in the legume *Vigna unguiculata*. The highest concentration of about 0.5ng/ μg of protein were detected in the seed coats at 16 and 18 days after pollination. It showed similar chromatographic behavior as that of bovine insulin in RP-HPLC. The protein eluted form RP-HPLC was subjected to automated sequencing. Similarity in amino acid sequence of glucokinin with bovine insulin shows another evidence for its existence (25). More recently, isolation of insulin-like molecules from the leaves of *Bauhinia variegate* a plant widely utilized for the management of diabetes showed partial sequence identity to bovine insulin and decreased the concentration of blood glucose when injected in both normal and alloxan induced diabetic mice. Sephadex G-50 column purified fraction which was tested by dot blot analysis showed positive for insulin like protein and was found to have partial sequence identity with that of bovine insulin. When sections of the leaves were examined by immunohistochemical and immunocytochemical microscopical analysis the protein was found to be associated with calcium containing crystals in the vacuoles of chloroplasts. Later, chloroplasts were purified and an insulin-like protein was isolated, purified by gel filtration and reverse phase chromatography. Purified protein cross-reacted with anti-human insulin antibody in an ELISA assay which confirmed the insulin like nature of the protein (26). Association of glucokinin with crystals containing calcium may protect from proteolysis when the decoctions of *Bauhinia variegate* was ingested orally.

ROLE OF GLUCOKININ

Insulin in plant exhibit metabolic functions as those of animal insulin by promoting several metabolic activities through glucose transportation into the cell and by phosphorylating proteins regulating carbohydrate metabolism as evidenced by many studies. Studies on the growth of maize seedlings showed that glucokinin isolated from onion stimulates growth of roots and tops of young maize seedlings as compared with untreated seedling controls. When growth of roots and tops of maize were compared with its endosperm weight as it is reduced much in untreated controls, glucokinin promotes growth by efficient utilization of endosperm i.e. less loss of endosperm by weight (10).

A fraction purified from *M. charantia* about 1ml in PBS (phosphate Buffer saline) given orally showed a significant reduction of blood glucose level at all points i.e. 30, 60, 90, 150 and 180 minutes in a glucose infusion study on SD rats. The same fraction was found to be effective in the prevention and treatment modes of diabetes in SD rats. In the preventive model, animals received orally 1ml (500 μg) of glucokinin from day 1. While in the treatment model, animals received 1ml of purified fraction (500 μg) of glucokinin at the onset of disease on day 5. Serum glucose levels were monitored daily for 15 days. It was found that purified fraction was highly effective in both preventive and treatment modes on day 10 without altering systemic insulin secretion. So, the effect of *M. charantia* on the preventive model of streptozotocin induced diabetes strongly supports its suitability in treating Type I autoimmune diabetes also (11, 12 and 13).

CM purified materials of spinach and *Lemna gibba* were able to compete with labeled insulin for binding to insulin receptors on IM-9 lymphocytes in a dose dependent manner. With increasing concentration of glucokinin there was an increase in the incorporation of ^{14}C from glucose into CO_2 and this stimulation was neutralized by anti-pork insulin

antiserum. Spinach glucokinin enhances lipogenesis as deduced by the conversion of D (^{3}H) glucose into toluene extractable lipid and was neutralized by addition of anti-insulin antiserum (19).

Moreover, plant signaling pathway could respond to exogenous insulin (vertebrate insulin); accelerating seed development, increasing the activity of glyoxysomal enzymes involved in the conversion of stored fat to carbohydrate in fat storing seed species, inducing ribosomal protein synthesis in maize (20). Extracts of *Bauhinia variegate* exhibiting similar properties of bovine insulin were studied for its hypoglycemic activity in swiss albino mice. Intravenous injection of crude protein extract and protein fractions eluted from SDS-PAGE produced a significant decrease in blood glucose levels as similar to that promoted by commercial swine insulin. Total leaf extract did not show any characteristic change in glucose level, possibly due to a lower glucokinin concentration. In the chloroplast, starch is broken down to hexoses (glucose) and transported into cytosol via a glucose translocator. Localization of glucokinin in chloroplast of *Bauhinia variegate* suggest that it might influence carbohydrate metabolism especially through transporting the sugars (26). Despite, many findings with sound techniques for the isolation, characterization and bioactivity assays, there is no direct evidence of glucokinin/vertebrate insulin effects on carbohydrate metabolism in plant. However, isolated glucokinin is found to have hypoglycemic activity in experimental animals and most of the glucokinin of different plant species shows peptide sequence homology with insulin shown in Table 1. Since, glucokinin shares amino acid sequence with human insulin it suggests that it might communicate with insulin mediated signal transduction by binding to insulin receptors.

Table 1 : A comparative tabulation of bovine insulin amino acid sequences with glucokinin isolated from plants. Fractions/subunits of *Canavalia ensiformis* and *Vigna unguiculata* showed high sequence similarity with bovine insulin.

Source	Amino acid sequence	Sequence ID
Bovine insulin á-chain â-chain	 GIVEQCCASVCSLYQLENYCN FVNQHLCGSHLVEALYLVCGERGFFYTPKA	 UniProtKB/Swiss-Prot P01317
C. ensiformis I-SC	GIVEQCCASVCSLYQLENYCN	UniProtKB/TrEMBL Q7M217
C. ensiformis I-LC	FVNQHLCGSHLVEALYLVCGERGFFYTPKA	
V. unguiculata I-SC	GIVEQXXASVXSLYQLENYXN	
V. unguiculata I-LC	FVNQHLXGSHLVEALYLVXGERGFFYTPKA	UniProtKB/Swiss-Prot P83770
B. variegata a *B. variegata b*	GIVEQ FVNQH	 -

INSULIN SIGNALING AND ITS PROTEIN ANALOGUE IN PLANTS

Insulin signal transducing mechanism is a very broad and crucial pathway to understand, where a number of proteins/enzymes participates. So, the insulin pathway is explained in a nutshell in relation to the current topic discussed. Increase in glucose concentration increases its metabolism in the â-cells leading to an elevation in the ATP/ADP ratio. This in turn leads to inhibition of ATP-sensitive K^+ channels causing depolarization of the cell membrane leading to Ca^{2+} influx and insulin secretion. Insulin, in addition to its role in regulating glucose metabolism also stimulates lipogenesis, diminishes lipolysis, increases amino acid transport into cells, stimulates cellular growth and regulates certain gene's expression. Action of insulin is tissue specific promoting glycogen formation in liver and muscle, promotes glucose uptake by increasing the rate of glucose transporter's translocation in muscle and adipocytes, promotes lipogenesis in liver and adipocytes and also activates Na^+ K^+ pump in muscle and adipocytes.

Fig 2 shows a schematic representation of insulin's action in different tissues.

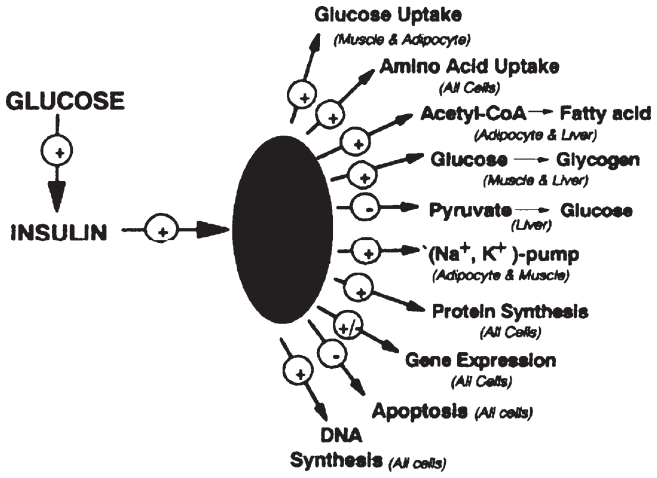

Fig. 2 : Physiological role of insulin in different cells.

Insulin mediates its action by binding to its receptor tyrosine kinases (RTK) which gets autophosphorylated provoking several cascades of signal transduction pathways. Activated RTK promotes serine-threonine phosphorylation of insulin-receptor substrates (IRS1, IRS2, IRS3 and IRS4). Phosphorylated IRS1 and IRS2 leads to binding and activation of phosphatidylinositol 3 kinase (PI3K) forming PI(3,4,5)P3 which then binds to the plasma membrane and associates with phosphoinositol- dependent kinase-1 (PDK-1) activating protein kinase B (PKB or Akt) and PKC zeta by phosphorylation. Finally, the activated Akt is thought to initiate many of the metabolic actions of insulin. Activation of IRS-2/PI3K/Akt/PKCæ is shown to be required for translocation of the glucose transport protein from storage areas to the cell surface and also for the activation of enzymes of carbohydrate metabolism.

The Mitogen-activated protein kinases (MAP kinase) pathway is also activated either through receptor activation of the protein tyrosine phosphatase (Shp-2) or growth factor receptor binding protein-2 (Grb2) which regulates various cellular activities, such as gene expression, mitosis, differentiation, and cell survival/apoptosis. The role of insulin in the stimulation of protein synthesis occurs at the level of translational initiation and elongation and is exerted primarily via a cascade leading to the activation of mammalian target of rapamycin, mTOR. Fig 3 gives a better understanding of insulin mediated signal pathway in relation to the current discussion. A great number of reports exist in literature that suggest the existence of plant proteins with functions, localization and sequences of the corresponding gene or protein, that are similar to proteins which are members of the insulin pathways characteristic of vertebrates. List of such plant protein counterparts in insulin signaling pathway is given in Table 2.

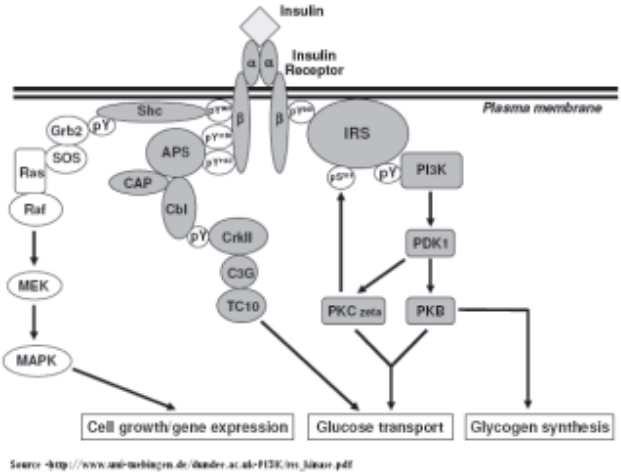

Fig. 3 : A short segment of Insulin signaling pathway showing only proteins playing crucial role in signal transduction

GLUCOKININ TOWARDS CURE

Plant insulin is found to be effective in regulating blood glucose by mimicking insulin signal in eukaryotes as mentioned before. Plant insulin ingested together with protease inhibitors is protected from hydrolysis in the digestive tract, crosses the intestinal barrier and promotes lowering of blood glucose levels (35). Additionally, galactorhamnan a polysaccharide in complex with insulin from the seed coats of Jack bean suggests that the hormone could be protected from hydrolysis in the digestive tract (36, 37). Making use of radioactive compounds, a dose dependent stimulation of glucose metabolism by CM (Carboxy Methyl cellulose) purified insulin-immunoactive material from spinach and *Lema gibba* in rat adipocytes was observed. With increasing concentration, there was an increase in the incorporation of ^{14}C from glucose to CO_2 (19). Most recent and reliable proof was given by

Table 2 : List of proteins (except insulin) found in plants which are associated with insulin mediated signaling pathway.

Proteins of insulin pathway	Its Analogues in plant/microbes
Receptor tyrosine kinases (RTK)	Found in *Arabidopsis thaliana(27)*
Insulin receptor substrates, proteins IRS-1 and IRS-2	*Arabidopsis thaliana*-LSD1 gene shows high sequence homology (28)
Glucose transporter	Sugar beet have high sequence homology (29)
Phosphatidylinositol kinases -PI3K (PI 3-kinase)	CDNA from soyabean shows significant sequence homology (30,31)
Hexokinase	Plant hexokinase involved in sugar sensing processes are regulated by glucose (32)
MAPK pathway	Evolutionarily conserved from unicellular to eukaryote organisms associated with insulin signaling in the promotion of cellular growth in rice (33)
TOR (target of rapamycin)	A potential component of the phosphoinositide 3-kinase (PI3K) pathway in *Drosophila* and was also found in yeast and Arabidopsis (34)
Ribosomal S6 Kinase	A maize insulin-like growth factor signals to a transduction pathway regulating protein synthesis in maize (22)

Azevedo and coworkers that insulin-like protein isolated from *Bauhinia variegata* leaves influenced glucose levels in the serum of diabetic mice. Protein fraction caused a significant decrease in blood glucose levels in diabetic mice similar to that promoted by commercial swine insulin (26). Peptides of *Momordica charantia* (MC6 and MC2) are equally as effective as systemic injection as well as through oral administration. Hypoglycemic effect at a concentration of 500 μg/ml on SD rats showed a significant fall of glucose in glucose tolerance test and also preventive against streptozocin induced diabetes in rats. Serum insulin level is up regulated by this peptide as revealed by radioimmunoassay (12, 13). Research on insulin like proteins in plant has a substantial action on diabetic plaque in animals and has to step into clinical trials.

FUTURE OF INSULIN THERAPY

Current methods of insulin production rely on yeast *Saccharomyces cerevisiae* and *E. coli* genetically engineered to produce synthetic human insulin. These organisms are grown in large steel bioreactors, and the products are subjected to high cost downstream process to yield highly pure protein, devoid of any contaminations. SemBioSys has been working with the transgenic plant for the past five years and its latest results show that plant-derived insulin is analytically and physiologically identical to human insulin. The product was found to be functionally equivalent to Eli Lilly's Humulin. This method achieved a 1.2 per cent accumulation of insulin within the seed protein of the plant, exceeding its commercial target. One acre of safflower would give a yield of over one kilogram of insulin - enough to supply 2,500 patients for an entire year. SemBioSys product will reach the market by as early as 2010.

Pharmaceutical heavy-weights Pfizer, Eli Lilly and Novo Nordisk are now developing new needle-free methods of insulin administration. Pfizer's inhalable insulin product Exubera is a few steps ahead of Eli Lilly's AIR Insulin System, currently undergoing Phase III clinical trials, and Novo Nordisk's AerX product is also in the final stages of clinical studies. There is also competition from US company Mankind, who's inhaled insulin product Technosphere completed Phase III trials with Type 2 diabetes in September last year, Biocon of India is also in the race. On top of this, researchers from the National Tsing Hua University in Taiwan developed insulin pill containing encapsulated insulin in such a way as to protect it from the digestive fluids in the stomach and allows the hormone to reach the bloodstream. Insulin was encased in a polymeric chitosan shell, and administered to diabetic rats. The insulin-loaded nanoparticles were found to lower blood-glucose levels, whereas plain insulin delivered orally resulted in little or no effect. However, all the above described four techniques need some more laboratory work to support their potential bioactivity and safety.

CONCLUSION

Presence of insulin-like protein in plants has been investigated since the discovery of insulin in pancreas of dog. Later contributions of people from different parts of the world with sound modern techniques, evidence the presence of insulin like protein in microbes, as well as in higher plants. All these evidence indicate that plant peptide hormones whose actions are similar to peptide hormones thought to be present only in vertebrates, namely insulin and the insulin-like growth factors, are also present in plants. Some of these have been isolated and characterized, their amino acid sequences determined and shown to share many chemical and biological properties with animal protein. And they are active molecules, effectively communicating with animal cell's signaling mechanism and mediate insulin action. Oral drugs for diabetes lose their effectiveness after an initial period of success, eventually they fail to produce a positive effect on long term treatment. Usage of synthetic drugs at minimal dose was reported to be beneficial, but in the long term they show certain untoward adverse effects such as sulphonylureas are associated with increased cardiovascular mortality, hypoglycemia, allergic skin reactions, headache, fatigue, nausea, vomiting and liver damage. Gastrointestinal intolerance such as flatulence, diarrhoea and mild pain in the abdomen are the major adverse effects of acarbos. Metformin leads to gastro-intestinal obstructions which include nausea, bloating, diarrhoea and abdominal cramping. Moreover, all the drugs are excreted by the kidneys and are complicated in patients with renal disease, liver disease, and cardiac or respiratory insufficiency. The demerits of synthetic drugs and to meet the worldwide growing diabetic population for insulin, attention of researchers, pharmacological companies and other investors in the search of alternative drugs and drug targets to manage diabetes is soaring high. Further exploration of glucokinin in various plants will pave way for a new promising therapy with lesser adverse effects from cheaper sources and also give us a clue of evolutionary existence of the insulin hormone and more. Commercial applicability of glucokinin greatly depends on the level of its expression or production, cost of its down stream processes, extended ligand-receptor interaction and also on biocompatibility.

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NEW DRUG PROFILE: Exenatide

S. Seethalakshmi

INTRODUCTION:

There are various drugs available for the treatment of type-2 diabetes and the main disadvantage with these drugs include hypoglycemia and weight gain (with sulfonylureas, thiazolidinediones), lactic acidosis (with biguanides) and edema (with thiazolidinediones), in addition to restrictions for use in organ dysfunction. It has been shown in diabetes that cell failure is progressive, despite therapy with sulfonylureas and biguanides. So, an ideal anti diabetic agent should delay/arrest, if not reverse the cell decline which can be used synergistically with current therapies with no added adverse drug reaction profile or serious adverse drug reactions, thus reducing long term complications and hence morbidity and mortality. To help these patients further, a new class of agents incretin mimetics has been developed. Exenatide[1] is the first drug in the incretin mimetic class and is indicated for treatment of Type 2 diabetes mellitus.

PROPERTIES OF THE DRUG:

Exenatide is a synthetic 39- amino acid peptide with incretin properties similar to the native glucagon-like peptide. Unlike GLP-1 , it is resistant to in vivo proteolytic degradation by dipeptidase -4 resulting in a significantly longer elimination half -life. Randomized trials have shown exenatide to be efficacious in improving glycemic control when combined with either metformin or Sulfonylureas.

After subcutaneous administration of 10 microgram dose the mean C_{max} is about 193- 220mcg/mL. AUC: 993-1066mcg /mL Median t_{max}: 2.1 hr .Mean apparent volume of distribution: 28.3 L

Exenatide exhibited significant resistance to enzymatic degradation by Dipeptidyl Peptidase IV (DPP- IV) where as GLP(Glucagon like peptide)-was rapidly degraded by this enzyme. Elimination occurs in kidneys via Glomerular filtration followed by proteolytic inactivation, in renal tubules.[2] Mean $t_{½}$: 2.4 hrs

Exenatide is given subcutaneously 60 minutes prior to morning and evening meals. It is initiated as 5 microgram bd and can be increased to 10 microgram bd after 1 month of treatment, seeing the response. It is recommended for patients on metformin or sulfonylurea who have suboptimal glycemic control.

Adverse effects mainly include nausea[3] (44%), vomiting, hypoglycemia, diarrhea, dizziness and headache. Of these nausea is self limiting in 15-30% of patients. Since Exenatide delays gastric emptying, drugs like lovastatin, digoxin and oral contraceptive pills are to be taken 1 hour prior. Careful clinical monitoring is necessary for drugs with narrow therapeutic index.

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MECHANISM OF ACTION

Exenatide is an incretin mimetic drug indicated for treatment of Type 2 diabetes mellitus. Incretin hormones are the hormones produced by the gastrointestinal tract in response to nutrient entry, resulting in stimulation of insulin secretion (insulinotropism). Glucagon like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) are two such examples. Gut insulinotropic agents like GLP-1 are secreted by enteroendocrine L-cells of gastrointestinal tract (duodenum, jejunum, ileum and colon) in response to food.

Therefore decreased incretins levels would result in significant post-prandial hyperglycemia as manifested in impaired glucose tolerance.

In type-2 diabetes there is a decreased GLP-1 responses and decreased insulin secretion as compared to non-diabetics.

Exenatide improves glycemic control by both acute and chronic glucoregulatory mechanisms in patients with Type 2 Diabetes mellitus[4]. It has structural similarity and binds to the receptor for GLP-1 Glucagon like peptide-1 and displays a similar broad range of activities relevant to improving glycemic control. In the beta-cell, exenatide stimulates insulin secretion in a glucose dependent fashion and has been shown to essentially normalize the loss of first-phase insulin secretion in patients with type 2 diabetes. In the alpha-cell, it normalizes the pathologic hyper secretion of glucagon in a glucose-dependent fashion, thereby reducing hepatic glucose production in the postprandial state. The glucose dependency of both of these mechanisms has been well-documented, protecting the patient from hypoglycemia while these delicately counterbalanced hormones are normalized simultaneously.

GLUCOREGULATORY MECHANISM OF EXENATIDE:[4,5]

Acute	Chronic
Enhancement of glucose – dependent insulin secretion	Reduction in food intake.
Restoration biphasic insulin responses.	Reduction in body weight.
Suppression of in appropriate high glucagons secretion	Enhancement of insulin sensitivity
Slowing of gastric emptying	Potentiation of glucose induced pro insulin bio synthesis

Exenatide is comparable to Insulin in reducing baseline fasting blood glucose levels.

In addition, it is, well- tolerated, decreases body weight, has better postprandial glycemic control[6], is rarely associated with hypoglycemia.

Pharmacotherapeutics and Contraindications

Exenatide, a functional analogue of the GLP-1 is a valuable adjunctive therapy option in patients with Type-2 diabetes mellitus who have inadequate glycemic control despite receiving treatment with Metformin and/ or sulfonylurea. In the two randomized, nonblind, insulin –controlled, phase III trial of 26 and 52 weeks duration, patients receiving exenatide 10 microgram twice daily experienced continuous and progressive weight loss and had durable reduction in HbA1c.[7,8]

It is important to remember that it is not indicated in diabetes mellitus type1 or diabetic ketoacidosis and that it is not an insulin substitute. It is not recommended for diabetics with end stage renal disease (creatinine clearance < 30 ml/ min) and severe gastrointestinal disease (like gastroparesis). The FDA has received 30 reports of acute pancreatitis in patients taking exenatide for treatment of type 2 diabetes.

Twenty-seven of the 30 patients had at least one other risk factor for acute pancreatitis such as gallstones, severe hypertriglyceridemia, and alcohol use. It is yet to be studied in pregnant or lactating mothers.

The other DPP-IV inhibitors are vildagliptin, sitagliptin and saxagliptin (currently in development), similar to exenatide rely on GLP-1 actions for the treatment of type 2 diabetes mellitus.

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IMAGES IN MEDICINE
TUMOUR LIKE LESION OF FINGER

Shalinee Rao[a], Aarthi Rajkumar[a], Sandhya Sundaram[a]

In an era of evidence based medicine, histopathology plays a major role in providing a conclusive tissue diagnosis. Surprises in the practice of diagnostic pathology is not uncommon. Quite often, a clinically neoplastic lesion may actually be non tumorous on histopathology. Such tumor like lesions of extremities include developmental anomalies, hamartomas, dermoid cysts and infections.

A 34 year old lady presented with complaints of swelling and mild pain in the left middle finger since two months. She also had a history of injury with a wooden splinter at the same site a few days before the onset of swelling. On examination, a tender nodular swelling over distal phalanx of the middle finger was noted. A clinical diagnosis of neurofibroma was considered and the lesion was completely excised for histopathological examination.

The biopsy showed skin with granulation tissue formation in the subcutis (Figure 1). Confluent histiocytic

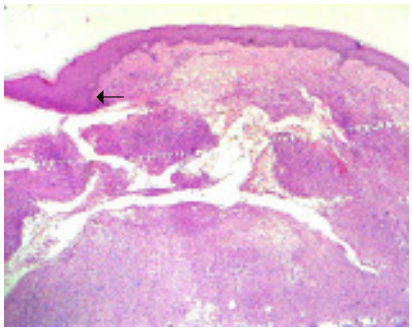

Figure 1 : Section showing inflammatory granulation tissue in the subcutis with focal acanthosis (arrow) of the overlying epidermis (Hematoxylin and eosin x 20).

granulomas were also seen along with inflammatory cells composed predominantly of eosinophils and lymphocytes (Figure 2, 3). The overlying skin was focally hyperplastic. Stain for acid fast bacilli (AFB) was negative. Fungal stains (Periodic acid Schiff and Gomori's Methamine silver) showed short closely septate forms of fungi (Figure 4, 5 and 6). Few of the fungal elements showed branching and constriction at the level of septation. A diagnosis of subcutaneous mycosis possibly phaeohyphomycosis was suggested based on clinical, histopathological and fungal stains findings.

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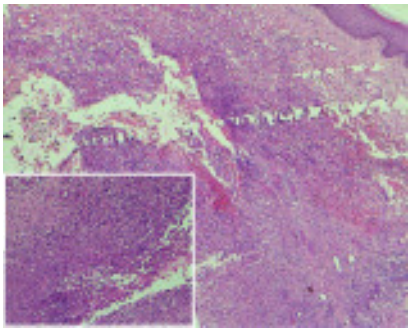

Figure 2 : Coalescing granulomas composed predominantly of histiocytes admixed with eosinophils, plasma cells and lymphocytes (Hematoxylin and eosin x 20). Inset demonstrates higher magnification of granulomas.

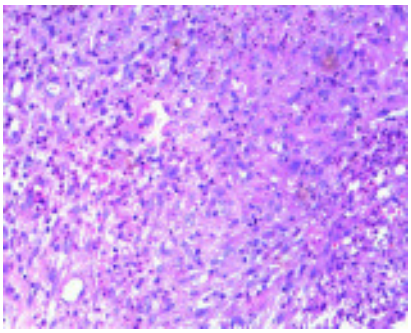

Figure 3 : Granulation tissue formation with mixed inflammatory cell response (Hematoxylin and eosin x 200).

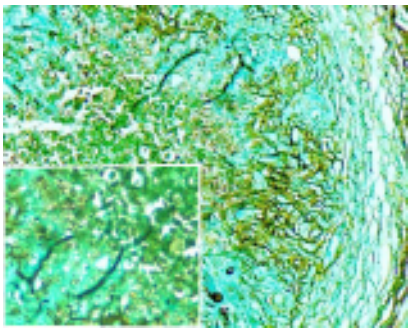

Figure 4 : Broad septate fungi highlighted by silver stain (Gomori's methanamine silver x 200). Inset shows higher magnification of the same.

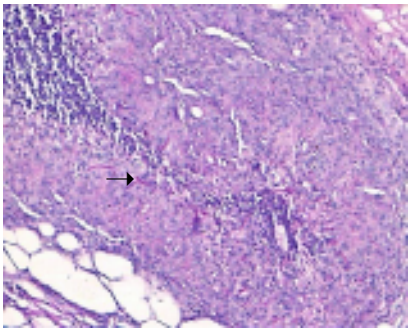

Figure 5 : Fungal elements (arrow) in granulomas staining magenta pink (Periodic acid Schiff x 100).

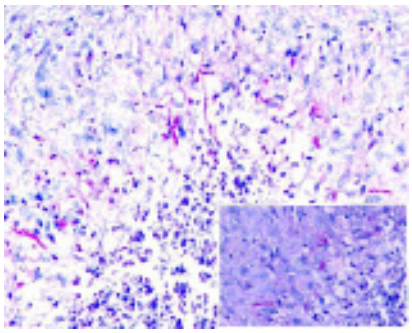

Figure 6: Short hypaeal forms of fungi with branching and nodularity at the level of septations (Periodic acid Schiff x200). Inset show fungi on higher magnification.

Phaeohyphomycosis is a rare infection relatively common in the tropics caused by group of subcutaneous fungi with low virulence and pathogenicty[1,2]. It presents as localized infection in exposed region of the body after penetrating injury by wood splinter or other foreign object, which acts as a portal of infection. Phaeohypomycosis can be caused by variety of polymorphous fungi that are saprophytes of soil and wood, for example Exophiala jeanselmei, Phialophora parasitica and Xylohypha bantiana. Though this infection can occur in healthy individuals immunocompromised are at a greater risk.

Phaeohypomycosis has two main clinical forms: subcutaneous and systemic. Unlike other subcutaneous mycosis these fungi are quite localized and results in an abscess / cyst formation[2]. It does not produce a sinus tract typically seen in mycetoma. Phaeohypomycotic organisms results in a chronic suppurative lesion with tissue response characterized by histiocytic granulomas and granulation tissue formation[3]. It neither induces epidermal hyperplasia nor ulceration characteristic of other subcutaneous fungi like chromoblastomycosis and sporotrichosis. Our case, however, showed mild focal hyperplasia of the overlying skin. Phaeohyphomycotic organisms are occasionally visible on hematoxylin and eosin stained section as brown structures but can more easily be identified and differentiated from other subcutaneous fungi by special stains. Short closely septate hyphae 2-6ì in width and moniliform fungi are usually present in the wall of the abscess. They may be branched and often constricted at the level of septations. Microbiological culture is essential for specific identification of the species. Culture could not be done in our case as the lesion was totally excised and sent in formalin for histopathology.

Treatment of localized subcutaneous fungus is surgical excision. In the present case complete excision of the nodule was done, hence no further treatment was required.

In tropical countries, the possibility of a localized subcutaneous fungal infection should always be borne in mind especially in penetrating injuries of the extremities.

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CAVERNOUS HEMANGIOMA OF THE UVULA – A CASE REPORT

Rebecca Thomas[a], S.B.Jothi Ramalingam[a], John Samuel[a], K.Dinesh Kumar[a]

ABSTRACT

Oral hemangiomas are probably of developmental origin and many of these lesions are present at birth or appear soon after. Often they regress in size and disappear after few years of life. Sometimes they persist and produce symptoms in adulthood. We present two cases of hemangioma involving the uvula, who presented with persistent cough and occasional episodes of bleeding from the oral cavity due to the abnormal length of the uvula. The different types of hemangioma of the head and neck, their incidence and the histopathological features are discussed in this case report.

Keywords: *Cavernous hemangioma, uvula, chronic cough*

INTRODUCTION

Chronic persistent cough hampers a person's quality of life. There are various causes for cough which includes both systemic as well as local conditions. Hemangiomas of the oral cavity are quite rare conditions with a prevalence rate of less than 1% and involvement of the uvula in hemangiomas have been reported only once so far in literature. We report 2 such cases of uvular hemangioma in adults causing chronic persistent cough.

Case 1

A 54yr old lady presented with complaints of persistent cough and foreign body sensation in the throat of 10yrs duration and spitting of blood stained saliva since 2weeks.

On examination, the uvula was elongated, about 4cm long and was lying on the anterior 2/3 of the tongue (Fig. 1). The tip of the uvula was expanded and had a dark bluish discoloration. The ear, nose and throat examination was normal.

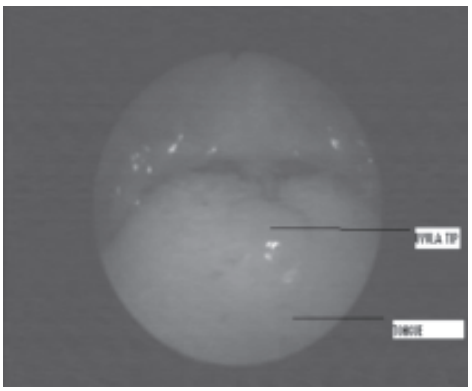

Fig. 1 : Elongated Uvula In Situ

Patient had no other co-morbid conditions and a physician's opinion found no evidence of pulmonary cause for cough. Investigations such as haemogram, ESR, Absolute eosinophil count, Mantoux test, Chest Xray and ECG were done to rule out systemic causes for persistent cough. All investigations were within normal limits. Since the patient was troubled by persistent cough and throat irritation, she was advised excision biopsy of uvula under local anesthesia. The elongated part of the uvula was clamped with an artery forceps and excised (Fig. 2). The mucosal edges were sutured and haemostasis was achieved. Histopathological examination showed features suggestive of cavernous hemangioma (Fig. 3).

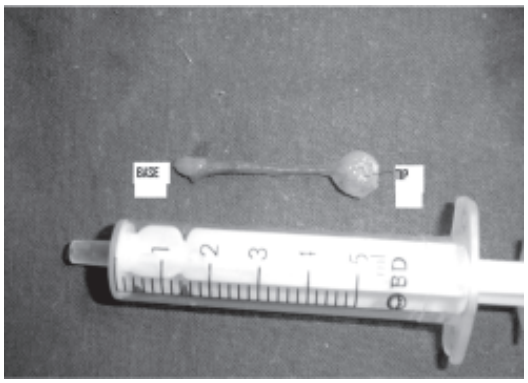

Fig. 2 : Uvula Resected Specimen

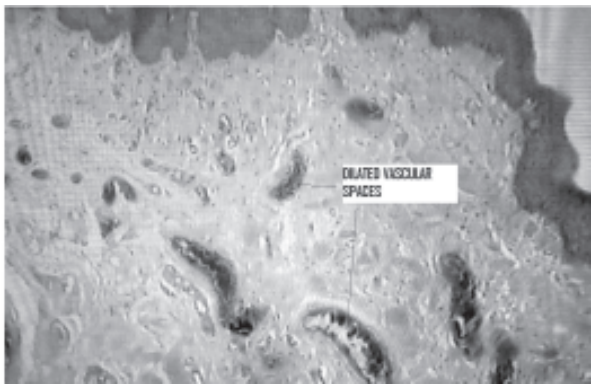

Fig. 3 : Photomicrograph Showing Cavernous Hemangioma

CASE 2

A 24yr old male presented with chronic cough and foreign body sensation in the throat of 1year duration. All the relevant investigations were also done in this case and physician's opinion ruled out systemic causes of chronic cough.

On examination, an elongated uvula about 2.5cms was seen extending up to the vallecula. No other co-morbid

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conditions were noted. The patient underwent excision biopsy of uvula under local anesthesia .The uvula was grasped with forceps and electro-cautery was used to excise the uvula. Histopathological examination of the specimen showed features suggestive of cavernous hemangioma.

On follow up, both these patients are asymptomatic and have been relieved of the nagging dry cough. Excision biopsy under local anesthesia has been curative for both these patients.

DISCUSSION:

Haemangiomas are the most common benign tumors of vascular origin.[1] They are characterized by increased number of normal or abnormal vessels filled with blood. Majority are superficial lesions mostly of head and neck. Common in infancy and childhood, hemangiomas constitute7% of all benign tumors. Prevalence of hemangioma in the oral cavity is 8/1000 in males and 4/1000 in females. Most are present since birth and increases in size along with the growth of the individual. In 90 - 95% of patients the hemangiomas completely resolve by the time they are 9yrs of age. Oral hemangiomas tend to regress less than hemangiomas in other regions. There are several histological variants. They are cavernous, capillary and pyogenic granuloma (lobular capillary hemangioma).

The incidence of cavernous hemangioma of the head and neck is about 5% of the vascular malformations diagnosed by angiography and histologically verified. Commonly seen in 3rd to 5th decade of life, they are also seen in children and elderly patients. Cavernous hemangioma involving the uvula is an extremely rare entity. An extensive literature search was done including the Worldwideweb with Pubmed / Medline search engine which showed that only one such case has been reported and that too in a six month old child[2]. Histologically the mass is sharply defined, not encapsulated and made up of large cavernous spaces partly or completely filled with blood separated by scant connective tissue. Capillary hemangioma is common in skin and subcutaneous tissue and mucous membranes of oral cavity and lips. Histologically they are lobulated aggregates of closely packed thin walled capillaries and lined by flattened epithelium. Pyogenic granuloma is a polypoid form of capillary hemangioma.

Our patients were diagnosed to have cavernous hemangioma of the uvula. The word uvula is derived from the diminutive of *uva*, the Latin word for "grape", due to the uvula's grape-like shape. Histologically the uvula consists of three layers, the mucosa which is made up of non keratinized stratified squamous epithelium, submucosal layer consisting of mucosal glands, blood vessels and nerve endings, few taste buds and lymphoid follicles and deep to the submucosa, few fibres of musculus uvulae Histopathological examination of the two resected specimens showed squamous epithelium beneath which were numerous dilated vascular spaces with back-to-back arrangement and no intervening neural parenchyma suggestive of cavernous hemangioma.

The position of the uvula predisposes it to local trauma and hemorrhage more so when the uvula is longer than usual[3]. Complications of hemangioma in the oral cavity are ulceration and infection. There are various causes for persistent cough and this case report draws attention to a local cause, an elongated uvula, that has caused chronic persistent cough by constantly irritating the posterior pharyngeal wall and posterior 3rd of tongue[4].

CONCLUSION:

Patients with elongated uvula are diagnosed late because the presenting symptoms mimic common ailments and the patients are seen by general practitioners. Such patients need to be thoroughly evaluated to rule out systemic causes of recurrent cough. In the absence of systemic causes, an elongated uvula can be the cause of recurrent cough and excision of the uvula is curative. Histopathological examination of the specimen is a must as it may throw a surprise as in our case .The case is reported for the rarity of hemangioma involving the uvula.

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ULTRASOUND GUIDED COMPRESSION REPAIR, A SUCCESSFUL MODALITY IN THE TREATMENT OF FEMORAL ARTERY PSEUDO ANEURYSM

Ghanshyam Palamaner Subash Shantha [a] , T.R Muralidharan [b], Anita Ashok Kumar [a], Vaibhav Chachra [a], Varun Sundaram [a]

ABSTRACT:

Femoral Artery Pseudoaneurysm (FAP) is a known complication that occurs in 0.1% to 0.2% of diagnostic angiograms and 0.8% to 2.2% following interventional procedures.Surgical repair has been the main modality of treatment of FAP. We report here a 68year old male patient who developed a FAP following a Percutaneous Transluminal Coronary Angioplasty (PTCA), managed conservatively by the noninvasive ultrasound guided compression repair (UGCR). Also this patient was continued on his antiplatelet drugs and heparin during this entire period.

Keywords: *Femoral artery, Pseudoaneurysm, Cardiac catheterization*

INTRODUCTION:

The femoral artery pseudoaneurysm (FAP) is a troublesome groin complication related to the femoral arterial access site used for invasive cardiovascular procedures (1). FAP occurs in 0.1% to 0.2% of diagnostic angiograms and 0.8% to 2.2% following interventional procedures (2). The incidence of FAP has recently increased with the more frequent use of thrombolytics, antiplatelet agents, anticoagulants, and larger-sized cannulas for interventional procedures (1). Iatrogenic pseudoaneurysms (IPA) form when an arterial puncture site fails to seal, allowing arterial blood to jet into the surrounding tissues and form a pulsatile hematoma (3). These lesions lack a fibrous wall and are contained by a surrounding shell of hematoma and the overlying soft tissues. It can present as a new thrill or bruit, pulsatile hematoma, or marked pain or tenderness. Complications of pseudoaneurysms include rupture, distal embolization, local pain, neuropathy and local skin ischemia (3). We report here a 68year old male patient who developed a FAP following a Percutaneous Transluminal Coronary Angioplasty (PTCA), managed conservatively by the noninvasive ultrasound guided compression repair (UGCR).

Case Summary:

A 68 year old male patient, with a background history of coronary artery disease with an old anterior myocardial infarction in July 2006, Coronary Artery Bypass Grafting (CABG) done on 18 th August 2006, was admitted and treated at an outside hospital for acute coronary syndrome in the last week of November 2007. An angiogram done during this time showed a triple vessel disease. Hence, he was referred to our tertiary care center for Percutaneous Transluminal Coronary Angioplasty (PTCA). His admission labs were within normal limits and his coagulation workup was normal (Table 1). He underwent PTCA on 30th November 2007 to Left Main Coronary Artery (LMCA) and distal circumflex coronary arteries. After 3 days of uneventful post procedure period, the patient complained of pain and swelling in the right groin. On examination, there was a warm and pulsatile mass lesion in the right femoral region. An arterial Doppler of the right groin showed a small pseudoaneurysm with a narrow neck at the origin of right superficial femoral artery with right groin hematoma. (Figure 1).

Table 1 : Admission laboratory values as on 29th Nov. 2007

Hemoglobin	13 g/dl
Total count	6900 / mm3
Serum creatinine	0.9 mg/dl
International normalized ratio (INR)	1.12
Platelet count	3.5 lakhs/mm3
Bleeding time	2 min and 45 sec
Clotting time	4 min and 30 sec

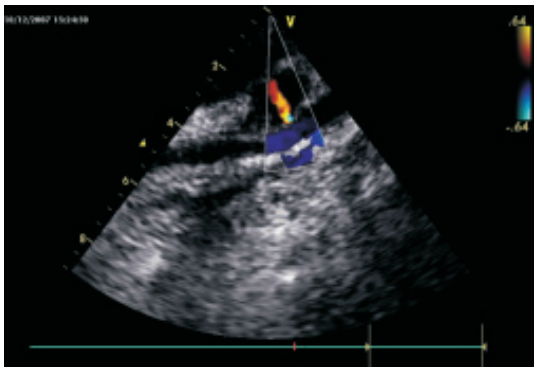

Figure 1 : Arterial Doppler of right groin small pseudoaneurysm with a narrow neck

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The patient was on the antiplatelet agent, low dose aspirin 75mg in addition to low molecular weight heparin (enoxaparin 60mg twice a day subcutaneous injection). These medications were continued and the patient was taken up for Ultrasound guided compression repair (UGCR). This procedure was done in two sittings, a week apart. During each of these procedures a strong compression was applied for nearly 30 minutes at the neck of the pseudoaneurysm and a repeat Doppler was done after each procedure to evaluate the morphology of the pseudoaneurysm following ultrasound guided compression. After the second sitting the patient had good pseudoaneurysm closure and there was absent color flow into the aneurismal cavity. (Figure 2) During this entire period the patient was continued on low dose aspirin, heparin and other cardiac medications. His post procedure period was uneventful and was discharged on the 3 day after the 2nd procedure. He is on regular follow up since then.

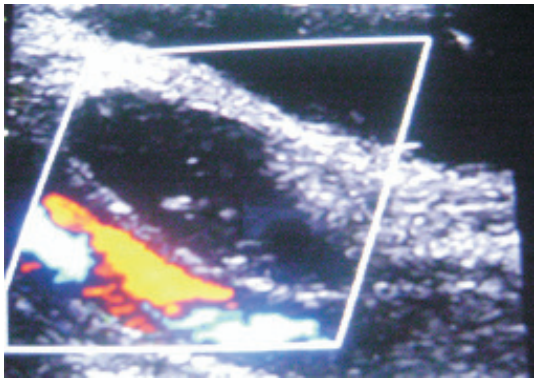

Fig. 2: Repeat arterial Doppler showing absence of blood flow into the aneurysmal sac.

DISCUSSION:

Duplex scanning, along with pulsed and color Doppler flow mapping has been the mainstay in diagnosing FAP. Criteria used to diagnose a pseudoaneurysm include: swirling color flow seen in a mass separate from the affected artery, color flow within a tract leading from the artery to the mass consistent with pseudoaneurysm neck, and a typical "to and fro" Doppler waveform in the pseudoaneurysm neck (3).

Several therapeutic strategies have been developed to treat this complication. They include ultrasound-guided compression repair (UGCR), surgical repair, and minimally invasive percutaneous treatments (thrombin injection, coil embolization and insertion of covered stents)(1).

UGCR has become the preferred line of treatment for pseudoaneurysms at many institutions**.** The introduction of this method in 1991 by Fellmeth et al (4) has significantly reduced the need for surgical repair of FAP. It has been shown to be a safe and cost-effective method for achieving pseudoaneurysm thrombosis(3). However, UGCR has considerable drawbacks including long procedure time, discomfort to patients and a relatively high recurrence rate in patients receiving anticoagulant therapy (as high as 25% to 35%)(3). UGCR has been shown to be less successful in patients with large FAP (i.e., larger than 3 cm to 4 cm in diameter) and those who cannot tolerate the associated discomfort(5). The procedure carries an overall complication rate of 3.6% and risk of rupture of 1%(3). Complications include acute pseudoaneurysmal enlargement, frank rupture, vasovagal reactions, deep vein thrombosis, atrial fibrillation and angina(3). Moreover, UGCR requires the availability of an ultrasound device and the presence of skilled personnel during the procedure. The technique involves applying compression on the pseudoaneurysm neck with the ultrasound transducer until the flow within the neck is obliterated. Pressure is applied for a period of 1 minute, with the procedure repeated 10 times. At the end of each period compression is released briefly to assess pseudoaneurysm patency and to reposition the transducer. Care must be taken to avoid compromising flow within the underlying femoral artery. After successful thrombosis patients should be kept supine for a few hours, with the affected leg in the stretched position. Contraindications to this technique include inaccessible site, limb ischemia, infection, large hematomas with overlying skin ischemia, compartment syndrome and prosthetic grafts (5).

In our patient surgical repair of pseudoaneurysm would have been a high risk procedure considering his cardiac condition. It is unfortunate that most pseudoaneurysms occur in patients least tolerant to general anesthesia, vascular reconstruction and associated blood loss. In the event of surgery the antiplatelet medications should have been stopped which would have increased his risk for another coronary event. Hence, by this UGCR technique the FAP was successfully treated and his antiplatelet medication was continued during the entire course. As this procedure is noninvasive there was no additional cardiovascular risk. The post procedure hospital stay was also very short compared to surgical repair of FAP.

We recommend UGCR as the treatment of choice in CAD patients with FAP as it is a non invasive effective procedure in these patients with high surgical risk.

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MUCINOUS CYSTIC TUMOUR OF APPENDIX INVOLVING CAECUM - A RARE PRESENTATION

Usha Vishwanath [a] , Vishwanath Pai [b], Surendran P [b], Saravanan S [b] , Deepthi Venugopal [a] , Dheeraj Reddy [b]

ABSTRACT:

Mucocele of the appendix is an infrequent event , comprising 0.3-0.7% of appendiceal pathology and 8% appendiceal tumours. It is characterized by a loculated or diffuse distension of appendix with a mucus filled lumen. The main differential diagnosis would include mesenteric cyst, duplication cyst, right ovarian cyst and hydrosalpinx. This case is about a 52year old female with vague, chronic pain abdomen, more on right side for 2 years ; ultrasound was suggestive of a right adnexal mass and explorative laprotomy showed a mucinous cystic tumour of appendix.

KEY WORDS: *Appendix, mucinous tumour, irritable bowel syndrome, cysadenocarcinoma, laparotomy, right hemicolectomy*

INTRODUCTION:

Mucinous cystic tumour of appendix is a very rare entity , with overdistension of the appendix with mucous secondary to luminal obstruction by fecolith or foreign body , carcinoid or endometriosis . Mean age of occurrence is around 55 years of age with four times female preponderance. It is mostly associated with colonic adenocarcinoma or a mucin secreting tumour of ovary. Clinically, 25% of patients are asymptomatic and the rest present with acute or chronic right lower quadrant abdominal pain. Pre-op diagnosis of underlying malignancies in a mucocele is important for the management. However, its identification is difficult on imaging studies.

CASE REPORT:

A 52 year old multiparous female presented to our outpatient department with complaints of occasional right sided pain abdomen for 2 years, which has been aggravated for the past 2 months. There was no history of fever, vomiting, loss of appetite and weight loss. She had no bladder disturbances and there were no features suggesting pelvic inflammatory disease. She was a known case of irritable bowel syndrome for past 9 years on treatment. No other major illness or surgeries in the past except for transabdominal tubecotomy.

Examination of her systems were within normal limits and vaginal examination revealed a fullness in the right fornix which was cystic to firm in consistency, and with normal sized uterus. Basic investigations were done which was within normal limits. Ulrasound-Pelvis was suggestive of a linear right adnexal mass with a possible diagnosis of right sided hydrosalpinx. Patient was taken up for diagnostic laparotomy and to proceed for further management.

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Intraoperatively, uterus, bilateral tubes and ovaries were within normal limits. Appendix was enlarged in size. Hence appendicectomy was performed and the specimen sent for histopathology confirmed a borderline mucinous tumour of the appendix and with base involving the caecum (Fig. 1).

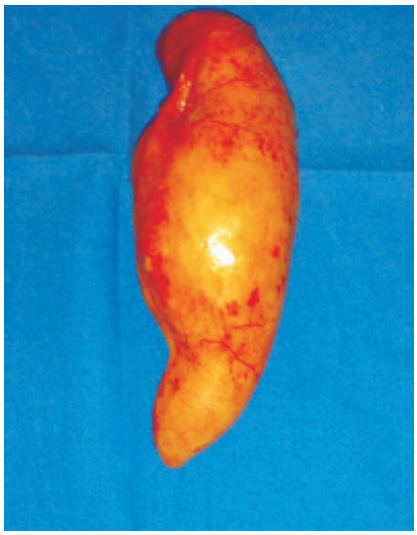

Fig. 1 : Appendiceal Mucocele

There was no evidence of ascites and no signs suggestive of liver metastasis. In consultation with surgeons, a right hemicolectomy, with terminal ileum 15cms upto right one third of transverse colon resection with ileotransversal colostomy anastomosis (Fig. 2). Intra-operative and Post-operative period was uneventful.

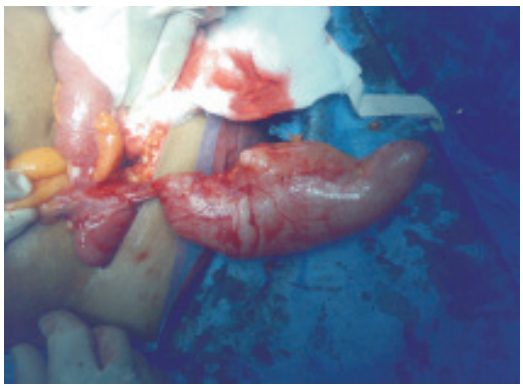

Fig. 2 : Mucocele of the Appendix

DISCUSSION:

The prevalence of appendiceal mucocele is about 0.4% among appendicectomies[1]. It is considered as mucinous distension of the appendiceal lumen regardless of the underlying pathology.

Four causal pathological conditions include [2]:-

(1) Retention cyst

(2) Mucosal hyperplasia

(3) Cystadenoma (of unknown malignant potential)

(4) Cystadenocarcinoma

Clinical manifestations include chronic or acute onset pain abdomen as in our case. It can be associated with palpable abdominal mass, gastrointestinal bleeding, weight loss, nausea, vomiting, changes in bowel habits and anemia. One third are associated with gastrointestinal tumours.

Diagnosis is very difficult. About 60% of cases are diagnosed in the intra-operative period. Pre-operative diagnoses with ultrasound and Computed topography is of utmost importance [3]. Ultrasound (USG) could show an elongated echogenic mass with concentric and echogenic layers within cystic mass (ONION SKIN) appearance [4].

Surgical resection is the method of choice for appendiceal mucocoele. Explorative laparotomy with or without right hemicolectomy is performed as in this case. Laproscopic resection might lead to peritoneal dissemination. The 5 year survival rate according to various other case reports suggests that it was 68% (Right hemicolectomy) versus 20% (appendicectomy alone)[5] .

Inflammatory bowel disease and irritable bowel syndrome (as in our case) is associated with elevated risk of certain gastrointestinal cancers (mucocele of appendix)[6]. Primary adenocarcinoma of appendix is uncommon, it is often an incidental finding and the pre-op diagnosis is very important Characteristic cystic mass in right lower quadrant and with the appearance of "ONION SKIN SIGN" (by USG) clinches the diagnosis (Fig. 3).

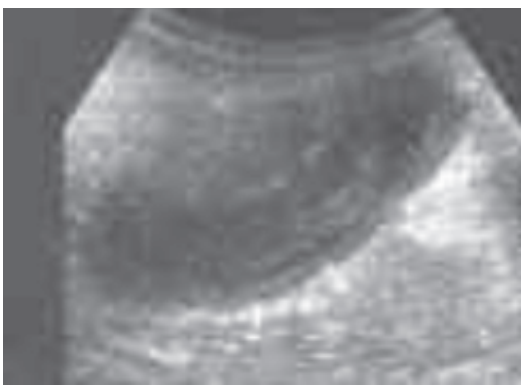

Fig. 3 : Onion Skin Sign in Ultrasound

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THYROID WITH A DIFFERENCE

V Srinivasan[a], C.D.Narayanan[a], Bhaskar Prakash[a], Datson George[a], Nisha Nahar R[a]

ABSTRACT:

It is rare to find carcinoma of thyroid and hyperthyroidism coexisting. While it is common to find occult carcinomas in specimens resected for hyperthyroidism, it is rare to find a metastatic papillary carcinoma thyroid with hyperthyroidism. We present one such case where the patient came to us with enlarged upper deep cervical nodes and thyromegaly, thyroid functions were consistent with hyperfunctioning thyroid and patient also had a meatastatic papillary carcinoma.

Key words: *Papillary carcinoma, hyperthyroidism, hyperfunctioning, thyroid malignancy.*

INTRODUCTION:

The idea that hyperthyroidism is insurance against thyroid cancer has been prevailed for a long time. Hyperthyroidism and malignancy was considered mutually exclusive for long time. We report one such rare association; wherein a patient presented with hyperthyroidism associated with metastatic papillary carcinoma thyroid.

Case Summary

A 25 year old multi parous woman presented with a history of swelling in the lateral aspect of neck of 1 year duration. She complained of anxiety, sweating and palpitations. She had lost weight, however her appetite was normal. Her menstrual cycles were regular and normal .There was no past history of radiation to head and neck .The patient did not receive any other form of treatment. Her mother had suffered from papillary carcinoma and was operated upon, at general hospital 10 years back.

Physical examination revealed an anxious patient with a staring look and fine tremors of the out stretched hands. Her resting pulse rate was 110/min. On examination of the neck, she had a 4x3 cm, firm, and mobile lymph node present in the right posterior triangle. We also found that she had a multinodular goiter, the largest nodule was in the right lobe which was 5x4 cm (Figure 1).

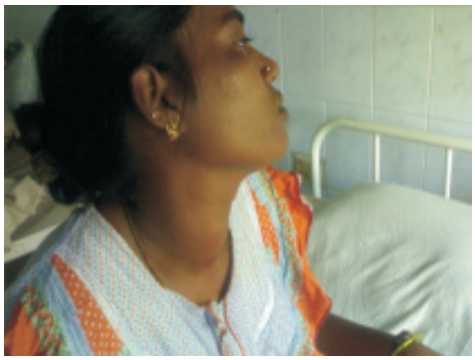

Fig. 1 : 4 x 3 cm upper deep cervical node

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Thyroid function tests confirmed that the patient was in hyperthyroid state, TSH: 0.003micro IU /ML, T3: 10.80pg/dl, T4 :3.59ng/dl. FNAC of the lymph node showed metastatic deposit from papillary carcinoma. Patient was administered antithyroid drugs, T. Neomercazole 30 mg once a day and T.Inderal 20 mg thrice a day for 10 days. After controlling her symptoms she was taken up for surgery. Total thyroidectomy with functional neck node dissection on the right side was done. During surgery the gland was found to be very vascular and the nodes had a characteristic blackish hue of a metastatic deposit of papillary carcinoma. Patient also had pretracheal and suprasternal nodes enlarged which were also removed [Figure2].

Histopathological examination was a 5x3x2 cm right lobe and 3.5x2 x1.5 cm left lobe, cut surface showed multiple tiny whitish foci. Microscopy showed papillary carcinoma pT1, N1 b M0. (T1- tumor size of 1 cm, N1b - suprasternal nodes and pretracheal nodes were positive for malignancy,M0- No distant metastasis).

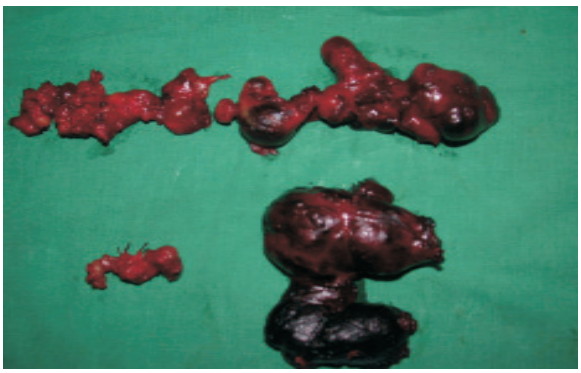

Fig. 2 : Thyroid specimen with right internal jugular nodes and suprasternal node

DISCUSSION

Here is a patient who had concurrent papillary carcinoma thyroid with hyperthyroidism. Risk of malignancy in clinically hyperthyroid patients was considered low until recently. The incidence in various world wide literature ranges from 0.8 t0 4%[1]. In the past five years at our institute there were about 115 cases of papillary carcinomas operated and none of them had hyperthyroidism.

The association can be in two forms. One is an incidental foci of carcinoma in specimens resected for

hyperthyroidism .Second scenario could be carcinoma thyroid presenting as hyperthyroidism which was the case in our patient. The latter association being rare than the former. Such patient presenting with metastatic secondaries is much rare. Most of the carcinomas associated with hyperthyroidism are papillary carcinomas[2].

The basis of this interesting association of malignancy and hyperthyroidism is being investigated. Initially hyperthyroidism was attributed to sheer increased volume of thyroid tissue even in the face of decreased function associated with malignancy[3]. Some workers have raised the role of long acting thyroid stimulator (LATS) and LATS-protector (LATSP) in stimulation of carcinogenesis in Graves' disease.[4] More recently, increasing reports on the possible carcinogenic role of thyroid binding immunoglobulin (TBIg) and other immunoglobulins in Graves' disease are seen in the literature[5].

Activating mutation of thyroid hormone receptor (TSH-r) gene has been demonstrated in a hyper functioning differentiated cancer. This mutation through activation of cAMP signal transduction is believed to cause hyperthyroidism[6].

In an autonomously functioning thyroid follicular carcinoma, a combination of mutations of TSH receptor and K-RAS was found to be responsible for hyper function of the tumor and the carcinogenic process[7].

Hyper functioning thyroid carcinoma should always be considered in the differential diagnosis of thyrotoxicosis / hyperthyroidism. This association of hyperthyroidism and malignancy has considerable therapeutic significance. Functioning thyroid carcinomas require total thyroidectomy whereas incidental carcinomas, because of their small size can be adequately treated with subtotal thyroidectomy.

This case emphasizes the need for thorough evaluation of thyroid to exclude malignancy even in a clinical setting of hyperthyroidism.

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VIDEO-ASSISTED THORACIC SURGERY IN CHILDREN: OUR INSTITUTIONAL EXPERIENCE

Zaffer Saleem Khanday [a], Deepak J [a], R.K. Bagdi [a], Balagopal [a], Prakash Agarwal [a], Madhu [a], Balamourougane [a]

ABSTRACT:

Video-assisted thoracic surgery (VATS) is used commonly for diagnostic and therapeutic procedures in children. Eleven (11) patients which included newborn, infants, children, and adolescents underwent VATS procedures between April 2006 and February 2008. There were 8 boys and 3 girls with an age range of 2 days to 17 years. VATS was performed for lung cyst (n=4) which included hydatid cyst lung (n = 1), encysted traumatic haemopneumothorax (n = 1), inflammatory lung cyst (n=1) infected lung cyst (n=1), decortication of empyema (n=4), congenital diaphragmatic hernia (n = 2), lung biopsy (n=1) and VATS was efficacious for therapeutic purposes in all 11 cases. Overall 1 case of VATS required conversion to open thoracotomy. Average length of thoracostomy tube drainage was 5 days, and average length of stay was 7.1days. Complications included prolonged air leak (>7 days) in 1(hydatid lung).There were no bleeding, complications or deaths related to VATS. VATS is a safe and effective procedure in children resulting in a short length of chest tube drainage and shorter length of hospital stay

***MesH words**: Thoracoscopy, video-assisted thoracic surgery*

INTRODUCTION:

Minimally invasive thoracic surgery has gained increased acceptance over the past decade coincident with the increasing popularity of minimally invasive abdominal surgery. With the advent of smaller endoscopic instruments and improvement in video technology, more VATS procedures are being performed in children. Thoracoscopy or video-assisted thoracic surgery (VATS) involves performance of intrathoracic procedures through several small thoracostomy openings without a thoracotomy. Advantages include less pain, lower postoperative narcotic requirement, shorter hospital stay and smaller incisions with resultant improved cosmesis [1].

CASE REPORT:

Eleven patients which included newborn, infants, children, and adolescents underwent VATS procedures between April 2006 and February 2008. There were 8 boys and 3 girls with an age range of 2 days to 17 years. VATS was performed for hydatid cyst lung (n = 1), encysted traumatic haemopneumothorax (n = 1), infected lung cyst (n = 1), lung biopsy (n = 1) inflammatory lung cyst (n = 1), empyema thoracis (n = 4) and congenital diaphragmatic hernia (n = 2).

TECHNIQUE:

All children received a general anesthesia. Selective ventilation of the right or left bronchus was used in some older children to allow ipsilateral lung collapse. In the operating room, each patient received peri-operative antibiotics. An arterial line and adequate intravenous access was established. The patients were positioned laterally similar to that for thoracotomy.

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A 5-mm or l0-mm skin incision was made and the subcutaneous tissue divided. A clamp was used to spread the chest wall and intercostal muscles and penetrate the parietal pleura. A reusable or disposable trocar was introduced into the thoracic space. Carbon dioxide (<l0 mm Hg) was used selectively as needed to provide lung collapse. Two additional working ports were used generally. All patients were monitored by electrocardiography, pulse oximeter, temperature and end-tidal CO2 monitor.

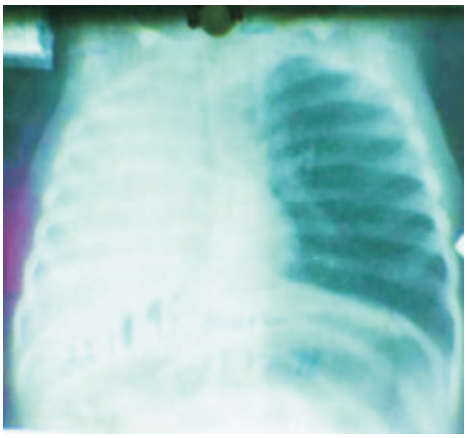

Fig. 1 : Right sided pleural effusion (Empyema thoracis)

In patients with empyema (Fig.1) requiring decortication thin fluid was aspirated with suction cannulas, and more solid fragments were removed with graspers. The fissures were always opened completely, and the lung was mobilized from the mediastinum anteriorly and posteriorly and from the diaphragm. The pleural space was debrided and lung expansion confirmed before closing the chest. Tube thoracostomy was used (Fig. 2).

In CDH (Fig.3) pre and postductal oxygen saturation monitors were placed. Insufflation pressures were initiated at 5 mm Hg. Two additional trocars were inserted under direct visualization in the fifth or sixth intercostal space posteriorly and in the intercostal space below the nipple. Using insufflation and laparoscopic instruments, the herniated intestine, omentum, and spleen were easily

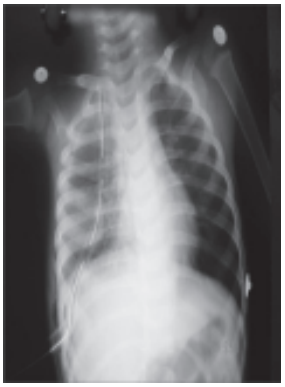

Fig. 2. Postoperative X-ray for empyema thoracis right side with good lung expansion.

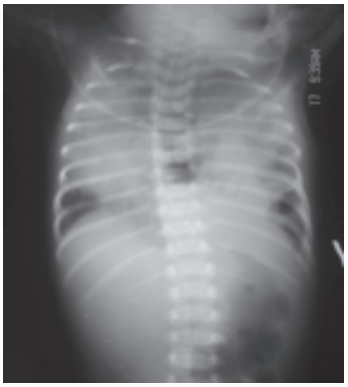

Fig 3 : Left sided congenital diaphragmatic hernia

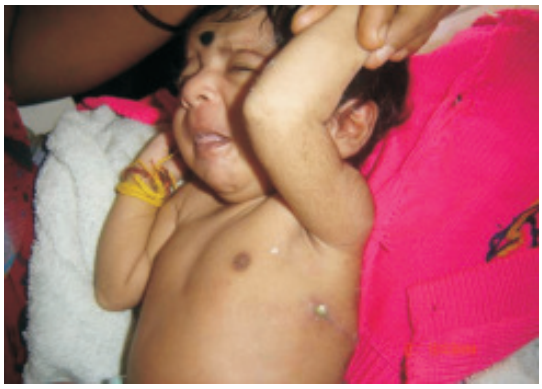

Fig 4: 40 day's old infant with repaired left sided CDH (minithoracotomy scar)

reduced into the abdomen. Brief increase in insufflation pressure to 10 mm Hg often aided the reduction. Elements of bowel malrotation and the arrangement of the intestine within the abdomen after reduction could not be evaluated with this technique. Once the intestine was reduced into the abdomen, the diaphragmatic defect was examined and the diaphragmatic defect was repaired primarily. Due to technical difficulties in suturing the lateral edge of the defect mini thoracotomy (Fig. 4) had to be done for suturing the remaining defect. In the second case of newborn diaphragmatic hernia which had an associated thin sac, the sac was excised and the diaphragmatic defect well defined and closed with interrupted non absorbable mattress sutures. Fig.(5).

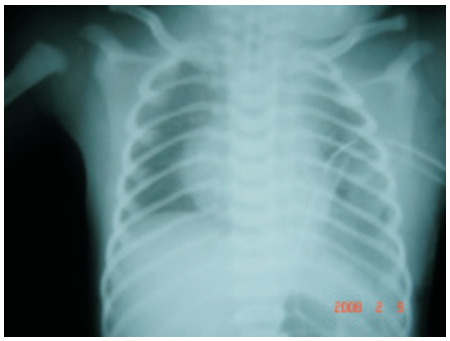

Fig 5 : VATS repair left sided CDH

Hydatid cyst (Fig. 6) was aspirated and exocyst was opened using an electric cautery then the endocyst was excised and extracted using a 10-mm endobag that was introduced through the lower port site. A chest tube was placed through the lower port site. Minimal air leak resolved within 7 days, and the chest tube was removed on the 8th postoperative day. Child was started on oral albendazole preoperatively and was continued for three weeks postoperatively.

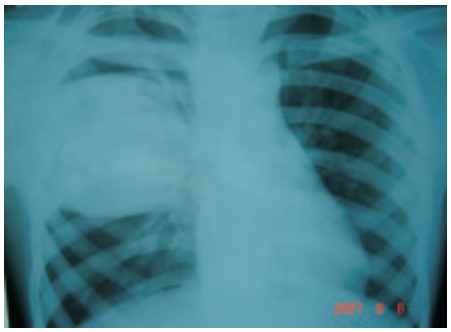

Fig. 6 : Right sided Hydatid lung lesion

Table 1 : Indications and Results for VATS

S.No.	Diagnosis		Number (n = 11)	Age	Chest drainage	Length of stay
1	Empyema thoracis		4	3months-16 years	5days(4-6)	6days(5-7)
2	LUNG CYST(n = 4)	Hydatid cyst lung	1	12 years	7 days	9 days
		Encysted traumatic haemothorax	1	13 years	4 days	5 days
		Infected lung cyst	1	24 days	3 days	14 days
		Inflammatory lung cyst	1	16 years	4 days	5 days
3	CDH		2	2days-40 days	5 days	7 days
4	Lung Biopsy		1	11 years	2 days	4 days
	Average		-	-	5 days	7.1days

In case of loculated haemopneumothorax a 2-cm incision was placed directly over the site of the loculated collection as determined from the CT scan. A suction cannula was inserted into the loculated collection, and as much of the pleural fluid and clotted blood removed. Fluid and blood clots were sent for microbiologic assessment. A 10-mm trocar with the telescope was introduced into the loculated cavity. Another 2-cm incision was placed 8 to 10 cm away from the initial incision, along the same intercostals space, and the suction cannula was introduced through this incision. Further evacuation of the pleural contents was performed under direct vision with the camera. Thus, the procedure was performed from within the loculated collection, gently releasing the adherent lung from the chest wall toward normal lung. Once all the pleural fluid and fibrin was evacuated, adequate lung expansion was observed by ventilating the ipsilateral lung. Chest tube was placed postoperatively into the port site.

Endoloop was prepared with Vicryl and mounted on 5 mm knot pusher, using a 5mm atraumatic grasper, biopsy site of the lung was grasped through the endoloop, endoloop was tightened and the lung biopsy taken after cutting with endoscissors.The specimen was removed through 5mm port. Biopsy site was inspected for bleeding and air leak. ICD was placed and removed after 48 hours.

In a case of infected CCAM involving the the left upper lobe, multiple abscess cavities were drained, using gentle suction, chest tube was kept for 3 days and pus culture reported pseudomonas statzeri.

Lung cyst which was measuring 7 x 7 cms containing pus and altered blood was excised, which was adherent to the parietal pleura, thin septae were broken down using blunt dissection. Instrument commonly used was the tip of the suction cannula. Biopsy was suggestive of inflammatory lung cyst.

All patients were transferred to a high-care unit; a chest radiograph was obtained, blood for arterial blood gas taken, and routine monitoring of vital signs performed. All patients received physiotherapy twice daily.

The only postoperative complication was prolonged air leak in 1 patient. There were no bleeding, complications or deaths related to VATS.

DISCUSSION:

Thoracoscopy has advanced significantly since the 1970s when Rodgers et al[1] introduced the technique for diagnosing intrathoracic pathology in children. The safety and efficacy of the VATS technique were subsequently proven for this indication[2]. Retained hemothorax reportedly occurs in 1% to 20% of patients with chest trauma. Using a protocol based on Vigorous physiotherapy and early withdrawal of tube thoracostomy in 1845 patients, retained hemothorax and empyema rates of 2.7% and 0.5%, respectively, were reported by[3]. The complications of entrapped lung and empyema following inadequately drained pleural blood has traditionally been managed by thoracotomy. Video-assisted thoracoscopic surgery (VATS) has been revitalized with the advent of improved imaging technology and the evolution of endoscopic instrumentation. The current role of VATS in trauma includes evaluation and control of continued chest tube bleeding, early evacuation of retained hemothorax, evacuation and decortication of post traumatic empyema, evaluation and limited treatment of suspected diaphragm injuries, evaluation and treatment of persistent air leaks, and evaluation of mediastinal injuries. The use of VATS in the early evacuation of post traumatic retained hemothorax has been well documented. In a review analyzing the role of thoracoscopy in retained hemothorax, identified eight studies with a total of 99 patients.

Evacuation by VATS was successful in 89 of 99 patients (90%)[4]. Technical failures during VATS evacuation occurred as a result of poor visualization from incomplete lung deflation, dense adhesions or clotted blood. Despite the 10% failure rate, all the studies recommended early VATS evacuation to avoid complications of fibrothorax and empyema. In a series of 24 patients with residual hemothorax, thoracoscopic evacuation was successfully performed in 22 of their patients (92%)[5].

The conventional treatment of hydatid cysts in all organs is surgical. Medical treatment with benzimidazole compound (albendazole) is also effective in properly selected patients. The response of the therapy differs according to age (children and adults), cyst size, cyst structure (presence of daughter cysts inside the mother cysts and thickness of the pericystic capsule allowing penetration of the drugs), and localization of the cyst. In children, small cysts with thin pericystic capsule localized in the brain and lung respond favourably[6]. Percutaneous therapy in the form of puncture, aspiration, injection, and reaspiration is another option to treat hydatid disease. But the need for prolonged hospital stays or repeated visits and development of spillage and abscess formation have limited its widespread use.

In adult, some authors have reported the successful use of thoracoscopic procedures for the treatment of pulmonary hydatid disease. In paediatrics, only 2 similar reports were found: one in the French literature and the other in the English literature. Both have confirmed the feasibility of the thoracoscopic approach in children with pulmonary hydatid cysts. It follows the same principles of the open technique, which include sterilization of the cyst with scolicidal agents (eg. hypertonic saline), complete excision of the endocyst, and closure of bronchial fistula, if present[7].

Thoracoscopy offers the possibility of good visualization and cleansing of the empyema chamber by the use of video techniques and establishes efficient drainage even in patients with advanced stages of the disease. Thoracoscopy enables collection of material not only for bacteriologic, but also

for histopathology examination[8]. This is important to establishing the precise cause of empyema. The described method is minimally invasive, and the complication risk is comparable with that for classical thorax drainage[9].

From an anatomic perspective, we reasoned that the optimal neonatal candidate for a thoracoscopic CDH repair would be one in whom the diaphragm could be repaired primarily.

Given the clinical fragility of patients with CDH, we also sought to use physiologic criteria to select healthier CDH neonate who would have adequate pulmonary reserve to tolerate the operation and expected compromise in postoperative pulmonary function[10]. Neonate in this report had good pre-operative pulmonary function (peak inspiratory pressure (PIP) limit of 24 mm Hg) and no clinical evidence of pulmonary hypertension. Comparing the thoracoscopic approach to the laparoscopic approach for CDH repair, the operation from the chest would appear to be easier. Insufflation helps to reduce the intestine into the abdomen.

The simple use of blunt retractors in a hand-over-hand motion achieves gentle intestinal reduction. We have not found that the chest wall imposes limits on suturing ability if the working ports are placed in appropriate positions[11] which we too appreciated in our study new born. A laparoscopic approach may be hampered by the constant tendency of the bowel to herniate back into the chest. Higher insufflation pressures may also be required, which could be transmitted to the thoracic cavity and adversely affect ventilation. A single disadvantage of the thoracoscopic approach is that one cannot evaluate for intestinal malrotation and arrange the bowel in the abdomen in an orderly fashion from the chest[12].

Neonatal thoracoscopic repair of CDH is feasible and safe in appropriately selected patients. With the refinement in the endoscopic surgery and the introduction of endoscopic stapling instruments, VATS lung biopsy is better alternative to thoracotomy[13]. As the technology improves the indications are further widened which will include lobectomies, complicated cyst excision and Mediastinal tumour excision[14].

CONCLUSION:

We conclude video assisted thoracoscopic surgery is a valuable technique in the management of selective thoracic conditions. It is safe, offers the advantages of less pain, rapid recovery, long-term morbidity and good cosmesis.

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