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# nuJHS

# NITTE UNIVERSITY JOURNAL OF HEALTH SCIENCE

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Editorial

# ONE SMALL STEP

Arunachalam Kumar, Editor, NUJHS, Mangalore 575018, India

It is with much pride (and with some trepidation!) that I launch this, the first issue of the Nitte University Journal of Health Science. The nascent university has, through this publication, made a bold foray into the world of publishing. The space and leverage in the scientific research publishing world is limited and already crowded. The sole factor that determines stature, standing and tenure of any technical publication is the relevance, readability and reference-worthiness of articles: the ultimate position NUJHS occupies will depend on the quality and range of articles it carries. Towards reaching our self-set targets, we have set ourselves rigorous benchmarks - a vigorous review process being one among the cardinal.

The need for a readily accessible forum for interaction and exchange of knowledge and scientific information on health and disease, especially in developing and under-developed third world countries, long been felt as desirable, is today become, imperative. With the launch of NUJHS it is our hope that this long-felt vacuum will, at least partially, be now be partially filled.

The domain of science, unfortunately, has remained an exclusive turf for academicians and researchers operating from economically privileged locales. The abysmal lack of finance and poor funding has strapped growth of scientific temper and stymied the third world from participating as equals with their contemporaries and counterparts, blessed to be based in advanced hi-tech set-ups, in richer countries[1].

The odious system of mandating a 'publication fee' from contributors (even afflicting the open access to information and its dissemination) has effectively nullified the third world researcher from being heard or read, no matter how positive the quality of contribution is to the sum knowledge on health sciences[2]. Hopefully, NUJHS will span that divide by providing free space for meaningful scientifically sound submissions from less renowned or more remote research centers.

Rapid processing and quick publication are the ideals we aim for – ingenious ideas, novel innovations or astute scientific observations are as vital to information banks as are scholarly reviews and seminal original treatises. The laboratory is not, nor needs to be the sole sanctum for spewing and spawning papers: even random bedside observations, seemingly trivial diagnostic aids or ridiculously simple therapeutic ideas add to the sum total of information on healthy related issues.

Have your say - if you have something interesting to say and can say it well enough – your voice will find ears and eyes on the pages of NUJHS. To the extent of our limited expertise can, we offer to help anyone, especially young medical or health science students – in polishing your presentation and submission skills, provided that what you submit contains any stimulating, educative or thought-provoking idea[3].

As a postscript, I caution the 'in-a-hurry to-publish' novice author: there are no detours or shortcuts in the dog-eat-dog medical world – so beware and be-warned: the perils of plagiarism and pitfalls of unethical journalistic ambitions are very real.

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Nitte University Journal of Health Science
Original Article

# CERVICO-FACIAL HEMANGIOMAS; THE TREATMENT THAT ELUDES US

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Abstract
Cervicofacial hemangiomas treated from January 2001 to December 2009 was clinically evaluated. This retrospective clinical study consisted of 42 females and 20 males with the age ranged from 20 days to 55 years. The lesions were present with-in first month in 45 patients (72.6%). Two patterns of tumor growth were evident: focal and diffuse. There were 59 focal hemangiomas (80.8%) and 14 diffuse hemangiomas (19.2%). Complications noted at the time of first consultation include residual skin changes in the in 35 patients (56.5%), obstruction of orifices in 14 patients (22.6%), ulceration in 6 patients (9.7%), and infection occurred in 2 patients (3.2%). Overall, there is reduction in size and improvement in color and texture of lesion following intervention in each group. No significant difference in outcome was observed in between groups with respect to change in size and texture. However, improvement in color showed statistically significant difference and combined treatment modality and surgical treatment was found to be better.

Keywords : Hemangioma, cervicofacial, management, challenges in treatment,.

## Introduction

The most common congenital deformity observed in infants and children are the Cervico-facial Vascular anomalies. They frequently involve the head, neck, and oral cavity.[1] In 1982, Mulliken & Glowacki[2] classified childhood vascular lesions as either hemangiomas or malformations. This classification was ground breaking and has served as a cornerstone for the proper identification, investigation, and management of vascular birthmarks, More specifically, hemangiomas were differentiated from vascular malformations by their clinical appearance, histopathologic features, and biologic behaviour.

Hemangioma is the most common tumor in infancy, with a perinatal incidence of 1% to 3%[3,4] and affecting 10% of infants by one year of age[5,6]. They are speculated to affect 4% to 12% of white children.[5] The incidence seems to be lower in Asian infants and is low in children of African decent[7]. Up to 30% of preterm infants with low birth weight (<1000g) may have hemangioma[8]. A predilection for the female sex has been reported, with a ratio of 2:1 to 5:1[9-11].

The hallmark of hemangiomas is rapid growth during the first several months of the child's life. When involution occurs, the process is usually completed by the child's seventh year. Therefore, a strong opinion developed in the mid-20th century that appropriate treatment for hemangioma was no treatment, this became known as benign neglect[12]. However, symptomatic problems such as ulceration, bleeding, infection, and residual skin changes which may be disfiguring require early intervention[13]. Also children begin to develop self awareness at 18 to 24 months of age[14]. Therefore, the psychosocial impact on a child with facial hemangiomas or its resultant scar cannot be underestimated[15]. The past decade has witnessed a revolution in the understanding and treatment of these vascular lesions. Previous complacency in treatment is changing to a more proactive approach to circumvent immanent aesthetic sequelae. Wiliams et al[16] developed a useful approach to the management of hemangiomas based on the stage of the lesion (proliferative or involutive phase), type of lesion (superficial, deep, compound) and the management of residual deformity. Freiden et al[17] stated major goals of management of hemangioma of infancy

are (1) to prevent or reverse any life-threatening complications of hemangiomas; (2) to prevent permanent disfigurement left by residual skin changes following involution; (3) to minimize the psychosocial distress from the presence of hemangiomas for both patient and family; (4) to avoid overly aggressive, potentially scarring procedures or toxic therapies for the treatment of those hemangiomas that are likely to have an excellent prognosis without therapy; and (5) to prevent or adequately treat ulcerated hemangiomas so that scarring, infection, and pain are minimized.

A number of treatment modalities are available for the management of hemangiomas. These include observation, compression[18], corticosteroids - systemic[19-24], intralesional[23-26] and topical therapy[27]; sclerotherapy[28], interferon α-2a[29], laser therapy[30,31], vincristine[32], becaplermin gel[33], and β-blockers[34,35]. Surgical treatment includes intratumoral ligation[36], tobacco-pouch suture technique[37], and excision[38-45]. The question is how to identify those lesions that are most likely to require treatment and also which treatment modality is best suited for a particular individual. Therefore, a study that will characterize various features of hemangiomas, discussing various therapeutic options with emphasis on the timing of intervention, and also focusing on correlation between various managements of hemangiomas remains elusive.

## Material and methods

A retrospective clinical study of patients with confirmed diagnosis of Cervicofacial hemangiomas reported from January 2001 to December 2009 was done in the department of Oral and Maxillao-Facial surgery. The following information was recorded from each patient's medical records, operative notes, investigations and clinical photographs: i) Age, ii) Sex, iii) Onset of hemangiomas, iv) Anatomic location, v) Size of hemangioma, vi) Complications noted at the time of consultation, vii) Type of treatment, and vii) Time period of treatment.

Each patient was then assigned to different treatment groups based on type of treatment done, like: group I, steroid treatment (figure 1); group II, sclerotherapy (figure 2); group III, surgical treatment (figure 3); group IV, combined therapy (figure 4).

Final results for each patient were assessed on available pretreatment data and present post treatment outcome based on: I) Reduction in size of tumor, II) Improvement in texture, and IV) Improvement in color. The results were analyzed by a single observer using the following scales: 1) poor (0 to 25 percent), 2) fair (26 to 50 percent), 3) good (51 to 75 percent), and 4) excellent (76 to 100 percent). Results of each of these parameters were summarized by groups. Finally, comparison of the outcomes between groups was analyzed by means of Chi-square statistical test. $p \leq 0.05$ was considered to be statistically significant.

## Results

Over a period of eight years from January 2001 to December 2009, 62 patients with 73 hemangiomas were reviewed. The age of these patients ranged from 20 days to 55 years with the average being 9 years and 6 months. The gender distribution was 42 females and 20 males with the ratio being 2.1:1. The lesions were present at birth in 7 patients (11.3%), with-in first month in 45 patients (72.6%), and after first month in 10 patients (16.1%). The involved area of hemangiomas in each group was as follows: group I, 0.25 to 120 $cm^2$; group II, 3.0 to 140 $cm^2$; group III, 1.0 to 180 $cm^2$; group IV 3.0 to 225 $cm^2$. The mean and median for each group, respectively, were group I, 6.2 $cm^2$ and 6 $cm^2$; group II, 20.5 $cm^2$ and 24 $cm^2$; group III, 12.3 $cm^2$ and 15.5 $cm^2$; group IV, 21.2 $cm^2$ and 22 $cm^2$.

Two patterns of tumor growth were evident: focal and diffuse. There were 59 focal hemangiomas (80.8%) and

14 diffuse hemangiomas (19.2%). Focal hemangiomas were mapped to 15 sites of occurrence, the most common being lateral upper lip in 15 patients (25.4%), lower lip in 14 patients (23.7%), mid-cheek in 7 patients (11.9%), and nasal tip in 5 patients (10.2%) (Table 1). The most common sites for segmental hemangiomas were maxillary segment in 6 patients (42.9%), mandibular segment in 5 patients (35.7%), and frontonasal segment (21.4%) (Table 2).

Complications noted at the time of first consultation include residual skin changes in the form of fibro-fatty masses of tissue in 35 patients (56.5%). Obstruction of orifices was present in 14 patients (22.6%). They were the eye (3), mouth (5), and nose (6). Ulceration was seen in 6 patients (9.7%). Infection occurred in 2 patients (3.2%) & was secondary to previous ulceration (Table 3).

Management of the hemangiomas and mean age of treatment in each group is summarized in Table 4. The time period of treatment ranged from 1 month to 4 years 2 months with the mean period in each group as follows: group I – 1 year 8 months, group II – 1 year 2 months, group III – 1 year 4 months, and group IV – 2 years 6 months.

The final results of each of the three parameters are also summarized by group (Table 5). Statistically no significant difference was obtained in reduction in size of tumor (p=0.683). With respect to improvement in texture, also no statistically significant difference was observed (p=0.152). However improvement in color resulted in statistically significant difference (p=0.006). Comparison of treatment outcome with respect to improvement in color amongst the groups yielded significant difference in outcome between group III (surgical treatment) and group II (sclerotherapy){p=0.037}, as well as between group IV (combined modality treatment) and group III{p=0.005}. Surgical treatment was found to be better compared to sclerotherapy and combined modality treatment was better when compared to surgical treatment alone.

| Distribution of focal | Frequency | Percentage |
|---|---|---|
| Lateral forehead over eyebrow | 1 | 1.7 |
| Nasal bridge/ glabella | 3 | 5.1 |
| Lateral nasal bridge | 2 | 3.4 |
| Directly beneath eye | 1 | 1.7 |
| Nasolabial fold | 1 | 1.7 |
| Mid cheek | 7 | 11.9 |
| Nasal (alar) | 2 | 3.4 |
| Nasal tip | 5 | 8.5 |
| Columella | 2 | 3.4 |
| Philtrum | 3 | 5.1 |
| Lateral upper lip | 15 | 25.4 |
| Lateral lower lip | 14 | 23.7 |
| Chin | 1 | 1.7 |
| Preauricular area | 1 | 1.7 |
| Ear | 1 | 1.7 |

Table 1: Distribution of focal hemangiomas

| Segmental hemangiomas | Count | Percentage |
|---|---|---|
| Frontonasal | 3 | 21.4 |
| Maxillary | 6 | 42.8 |
| Mandibular | 5 | 35.7 |

Table 2: Distribution of segmental hemangiomas

| | Group I (n= 11) | Group II (n=9) | Group III (n=22) | Group IV (n=20) | Total (n=62) |
|---|---|---|---|---|---|
| Fibro-fatty tissue | 0 | 5 | 16 | 14 | 35 |
| Obstruction | 4 | 2 | 3 | 4 | 13 |
| Ulceration | 3 | 1 | 1 | 1 | 6 |
| Infection | 1 | 0 | 0 | 1 | 2 |
| Number (%) of patients | 8(72.7) | 8(88.9) | 20(91) | 20(100) | 56(90.3) |

Table 3: Complications noted at the time of first consultation

| Group | | Number | Percentage | Minimum | Maximum | Mean age |
|---|---|---|---|---|---|---|
| | | (n=62) | (100%) | age | age | |
| I | Steroid treatment | | | 20 days | 1 year | 5 months |
| | | | | | 4 months | |
| | 1. Oral administration | 3 | 4.8 | | | |
| | 2. Intralesional administration | 8 | 12.9 | | | |
| II | Sclerotherapy | 9 | 14.5 | 2 years | 35 years | 12 years |
| | | | | 1 month | | |
| III | Surgical treatment | 22 | 35.5 | 1 year | 55 years | 8 years |
| | | | | 6 months | | 6 months |
| IV | Combined modality | | | 1 year | 38 years | 14 years |
| | | | | 6 months | | 6 months |
| | 1. Sclerotherapy + surgical treatment | 14 | 22.6 | | | |
| | 2. Steroid treatment + surgical treatment | 6 | 9.7 | | | |

Table 4: Management of hemangiomas of infancy

| Type | Group | Poor | Fair | Good | Excellent |
|---|---|---|---|---|---|
| Volume | I | 2 | 3 | 4 | 2 |
| | II | 1 | 3 | 3 | 2 |
| | III | 2 | 6 | 8 | 6 |
| | IV | 0 | 3 | 8 | 9 |
| Color | I | 1 | 3 | 7 | 0 |
| | II | 2 | 1 | 5 | 1 |
| | III | 0 | 11 | 7 | 4 |
| | IV | 0 | 1 | 14 | 5 |
| Texture | I | 0 | 7 | 3 | 1 |
| | II | 1 | 4 | 4 | 0 |
| | III | 0 | 8 | 12 | 2 |
| | IV | 0 | 4 | 13 | 3 |

Table 5: Final outcome of the number of patients in each group

## Discussion

The management of hemangiomas has been a subject of intense controversy for many decades. They are remarkably heterogeneous in terms of size, location, rate of growth and involution. Despite, the benign and trivial nature of most hemangiomas, a significant minority, cause functional compromise, or permanent disfigurement. They are also notoriously unpredictable early in infancy: some barely grow, while others blossom forth into huge tumors. Therefore, the treatment of vascular lesions has undergone a revolution in clinical practice in the past decades which remains elusive. Earlier intervention and advanced therapeutic modalities have permitted the patient and family, the opportunity to remove the hemangioma earlier and more effectively and, thereby, to mitigate the aesthetic and psychological impact that the hemangiomas may otherwise have.

The aim of this study was to characterize hemangiomas depending upon the onset, gender distribution, anatomic location, and associated complications as well as to evaluate the treatment outcomes following various managements. The ratio of female to male in our group of patients was 2.1: 1, and was similar to the ratio generally reported in the literature of 2:1 to 5:1[2,9,10,11,44].

The age of these patients ranged from 20 days to 55 years with the average being 9 years and 6 months. Achauer et al[44] reported patients from 1 day to 59 years with the average age being 4 years and 7 months. The average age in present group was more because patients reported to our hospital after the complications had developed.

Hemangiomas usually appear soon after birth in 60% to 70% of patients[2]. In this study also, majority of the hemangiomas arise soon after birth within first month in 72.6% of patients. Thus, it can be concluded that infantile hemangioma usually arise after birth in majority.

Waner et al[46] mapped sites of occurrence of facial infantile hemangiomas and analyzed two patterns of tumor growth: focal (177 lesions [76.3%]) and diffuse (55 lesions [23.7%]). Hagstrom et al[47] described four primary segments – frontotemporal, maxillary, mandibular and frontonasal involved in segmental infantile hemangiomas. In present study among 73 hemangiomas, there were 59 focal hemangiomas (80.8%) and 14 diffuse hemangiomas (19.2%). The focal hemangiomas were mapped to 15 sites of occurrence, the most common being lateral upper lip in 15 patients (25.4%), lower lip in 14 patients (23.7%), mid-cheek in 7 patients (11.9%), and nasal tip in 5 patients (10.2%). The segmental hemangiomas are maxillary segment in 6 patients (42.9%), mandibular segment in 5 patients (35.7%) and frontonasal segment (21.4%). Thus it can be concluded that infantile hemangiomas involving face have two distinct patterns of involvement with focal type predominating.

Our study demonstrates non treatment rate of complications at initial consultation was of 90%. This large rate of complications noted at the time of first consultation is definitely due to the fact that these patients seek treatment after complications had developed. Residual skin changes in the form of fibro-fatty masses of tissue in 35 patients (56.5%).

Obstruction of orifices was present in 14 patients (22.6%). They were the eye (3), mouth (5), and nose (6). Ulceration was seen in 6 patients (9.7%). Infection occurred in 2 patients (3.2%) and was secondary to previous ulceration. Thus, it can be concluded that obstruction and ulceration are the most common indication for management of hemangiomas during proliferative phase.[16,17]

Our study showed that reduction in size of tumor in steroid treatment group was excellent in 2, good in 4, fair in 3, and poor in 2 patients. With regards to improvement in color good outcome was observed in 7, fair in 3, and poor in 1 patient. Improvement in texture was excellent in 6, good in 32, fair in 23, and only 1 patient had poor result. Also, the mean age of treatment in this group was 5 months. Hence, steroids resulted in improved outcome if treatment is recommended during proliferative phase.[19-27]

Most of the patients in our study received sclerotherapy, surgical treatment or combined approach, as they reported to us during involuting or involuted phase and with residual skin changes. In 9 patients who received sclerotherapy, reduction in volume was excellent in 2, good in 3, fair in 3, and poor in 1 patient. Improvement in color was excellent in 1, good in 5, fair in 1, and poor in 2 patients and improvement in texture was good in 4, fair in 4, and poor in 1 patient. The mean age of treatment in this group was 12 years. In a study by Kane et al[28], sclerotherapy with sodium tetradecyl sulfate was used solely in 12 patients and resulted in a favorable outcome. Surgery and combined modality approach resulted in improved outcome in majority of patients either in proliferative, involuting or involuted phase. This is similar to studies done by Kane et al[28], Achauer et al[44], Demiri et al[45].

Comparison of treatment between groups yielded no significant difference in outcome with respect to change in size and texture. However, improvement in color showed statistically significant difference and combined treatment modality and surgical treatment was found to be better. This indicates that proper selection of a particular treatment modality during course of hemangioma is important for a favorable outcome.

## Conclusion

The successful treatment of cervicofacial hemangiomas is guided by an understanding of tumour natural history and ultimately judged by an improvement in function and appearance of the patient. Given their inherent heterogeneity, developing a rationale for interventions can be challenging; however, management of these lesions should ultimately be determined on the individual basis. Safe, active intervention is possible during all stages of development of hemangiomas. Therefore, treatment should be started early in the course of hemangioma as it will be more successful in preventing scarring, disfigurement, and life or function threatening sequelae and also in alleviating psychosocial burden of disease carried by the child and the family. It should, however be kept in mind that whatever action is undertaken should in no way result in a worse outcome than that which is seen with natural involution.

Group I: Steroid treatment

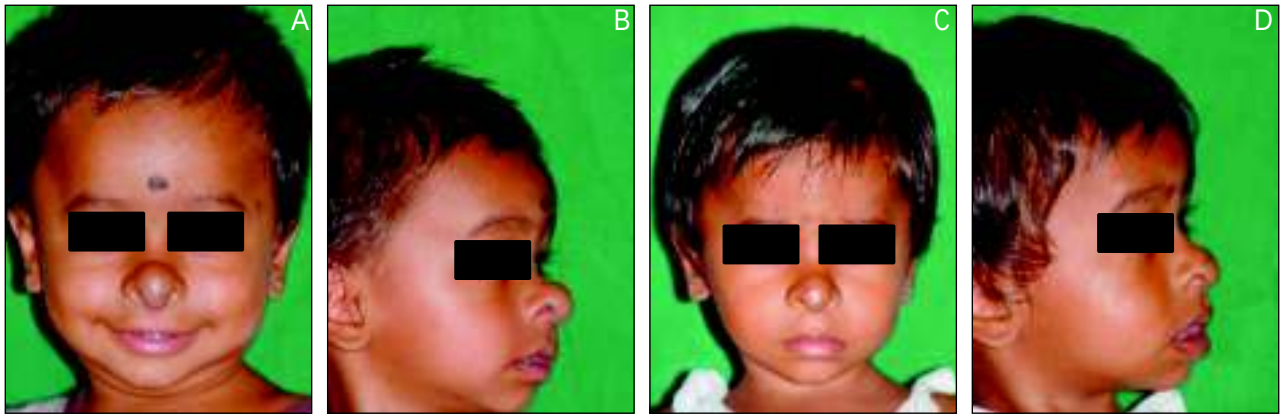


Figure 1: Pretreatment photographs of a child with hemangioma at tip of nose in A) frontal view and B) lateral view. Post steroid treatment follow – up photographs of same patient in C) frontal view and D) lateral view.

Group II: Sclerotherapy

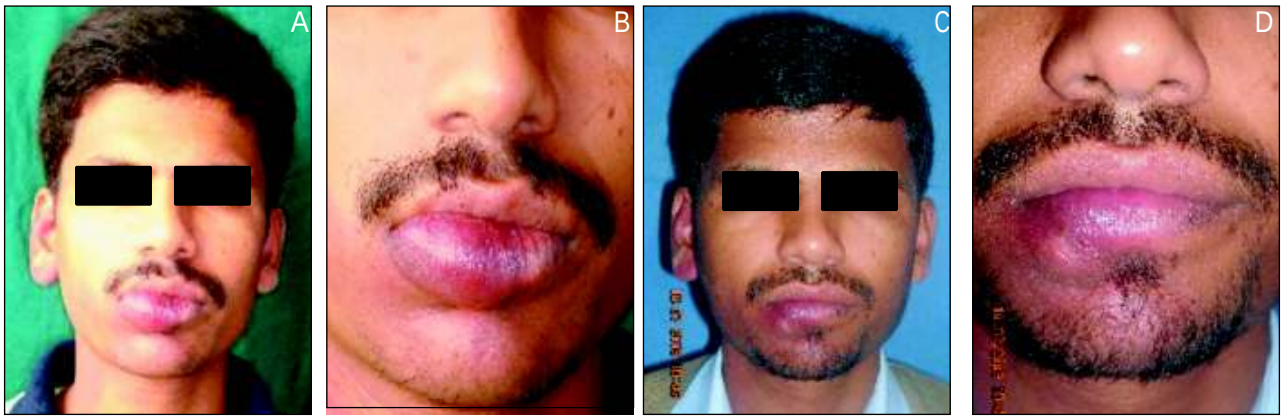


Figure 2: Pretreatment photographs of a patient with hemangioma on lateral portion of lower lip in A) frontal view and B) close-up view. Post sclerotherapy follow – up photographs of same patient in C) frontal view and D) close-up view.

Group III: Surgical treatment

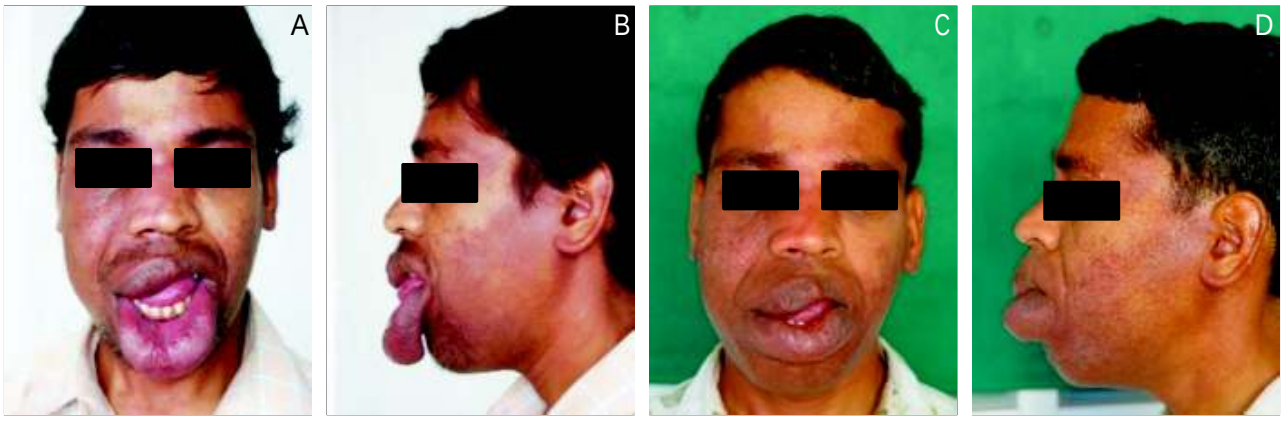


Figure 3: Pretreatment photographs of a patient with massive hemangioma involving lower lip and lateral upper lip in A) frontal view and B) profile view. Follow – up photographs of same patient following surgical treatment in C) frontal view and D) profile view.

Group IV: Combined treatment

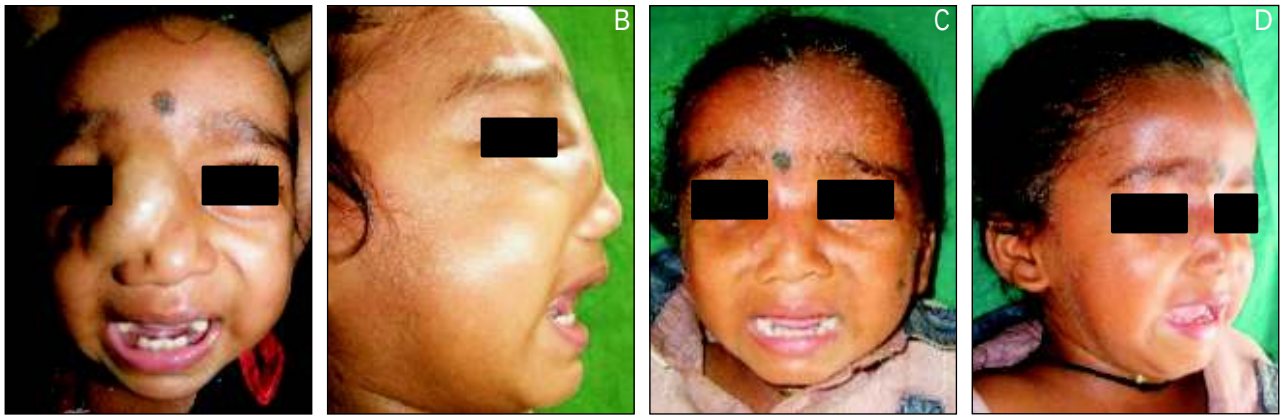


Figure 4: Pretreatment photographs of a child with hemangioma at the base of nose in A) frontal view and B) profile view. Follow – up photographs of same patient following steroid treatment and surgical resection in C) frontal view and D) lateral view.

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Original Article

# EFFECT OF YOGA THERAPY ON BODY MASS INDEX AND OXIDATIVE STATUS

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Abstract:

Background and Objective: Human beings are under threat from many chronic diseases and life style disorders. A major cause of all these diseases was found to be improper lifestyle and stress leading to obesity and excessive lipid peroxidation, indicating increased production of reactive oxygen species. Yoga therapy concentrates on purification of body and mind through its integrated holistic approach one can overcome the different kinds of afflictions in life. Hence the present study was undertaken to find out the effect of intervention of yoga therapy in obese individuals.

Materials and Methods: The study was conducted at MRPL ladies club auditorium in the MRPL Township after an informed and written consent from all the participants. The research protocol was approved by institutional ethics committee. The study consisted of forty obese male and female subjects. The changes in Body weight, Body Mass Index, Blood sugar, MDA level and Total antioxidant status was estimated before and after one month of yoga therapy. The data obtained was analyzed using paired't' test.$p<0.05$ was the level of significance.

Result: There was a significant decline in the Body Weight ($p=0.020$), BMI ($p=0.000$), Fasting Blood Sugar ($p=0.03$) and Post Prandial Blood Sugar ($p=0.000$) MDA ($p=0.000$) and significant increase in Total Antioxidant Level ($p=0.021$) after yoga when compared to that before the yoga therapy.

Conclusion: Yoga therapy is beneficial in maintaining better health by regulating BMI, Oxidative status by improving the biochemical functions of the body and helpful to overcome the complications of obesity.

Keywords : Body Mass Index, Yoga Therapy, Blood sugar, MDA level, Total Antioxidant.

Running title: “Effect of yoga therapy on obesity”.

## Introduction

Obesity is the most hazardous factor found in modern sedentary society and is a complex disorder of the modern world. It is emerged as the most prevalent sedentary lifestyle disorder in urban society. Excessive body weight is associated with various diseases particularly cardiovascular diseases, Type-2 Diabetes Mellitus, Obstructive sleep apnea, certain types of cancer osteoarthritis etc.

Yoga has been shown to be a simple and economical therapeutic modality that may be considered as a beneficial adjuvant for many of the health problems.Yoga therapy is the two fold therapeutic system that prevents and cures various diseases through practice of yoga system. This system concentrates on purification of body and mind, through this integrated holistic approach one can overcome almost all kinds of afflictions in life. It is a kind of low-impact physical exercise, and is used for therapeutic purposes [(1,2,3)]. Yogasanas have been practiced in India from Vedic period and was coordinated and organized in a systematic way, as known today, by Sage Pathanjali. He defined yoga as a systematic practice for purifying one's mind, intellect and body.

In the present scenario, human beings are under threat from many chronic diseases, life style disorders and non communicable diseases etc. A major cause of all these diseases was found to be improper lifestyle and stress. Excessive stress is known to cause hormonal imbalances and chemical imbalances in human body. It disturbs the

metabolic activities and causes improper coordination of the metabolic and bio-chemical functions. One of the major fallouts of stress in the human body is excessive lipid peroxidation, indicating increased production of reactive oxygen species (ROS). Also during an immune response in the body, there is an increase in the production of ROS which will cause an imbalance in the body between ROS production and antioxidant defenses against the pathogen. Abnormally high levels of peroxidation and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and oxidative stress[4]. Hence the natural balance between pro-oxidants and antioxidants will be shifted towards the oxidant side to cause further biological damage. As the oxidative stress increase, cell damage and accumulation of the toxic compounds in the body increases, leading to many pathological conditions.

According to tridosha theory in Ayurveda and Naturopathy literature, namely, Vatha, Pitta and Kapha are the structural and functional factors of the body, which govern the biochemical and physiological activities of the body. These three elements must be in a dynamic equilibrium with each other for the maintenance of health. Any imbalance of their relative equilibrium in the body results in disease. In human body Vatha dosha is responsible for all the physiological activities. Since yoga keeps the balance between ROS production and antioxidant defenses to prevent/ reduce oxidative stress, the present study was undertaken to find out the effect of intervention of yoga therapy in obese individuals.

Materials and Methods:

The present study was conducted to assess the effect of selected yogic practices in obese male and female subjects belong to the age group of 48 ± 13 years. The present study involved 40 obese individuals. All the subjects were comparatively new to yogic practices. The study was conducted in MRPL ladies club auditorium in the MRPL Township after an informed and written consent from all the participants. The research protocol was approved by institutional ethics committee. All the individuals were subjected to yoga practices for one month. The yoga practice was performed six days per week between 6 pm to 7 pm under the direct supervision of a trained yoga expert. This practical session utilized a standard sequence of selected Kriya, Asanas, Pranayamas and Relaxation Techniques, taking appropriate precautions for patients like Hypertension and back pain.

The various a*sanas included in the present study are* Swastikasana, Vajrasana, Suptavajrasana, Tadasana, Trikonasana, Parshwakonasana, Paschimotthanasana, Purvotthanasana, Ardhabadha padmasana, Janusirsasana, Mahamudra, Pavanamuktasana, Bhujangasana, Dhanurasana,Viparitakarani and Uttanapadasana. The different pranayama techniques used were Ujjayi, Anuloma viloma and Bhastrika. The kriyas and relaxation techniques were Yoganidra and Agnisara respectively.

The Body Mass Index (BMI) was estimated in all the participants before and after the experimental procedure using the formula, BMI = Weight in kg/ Height in meter$^2$.

For biochemical estimations, about 5 ml of venous blood was collected from all the participants before and after the yoga practice and the serum was used for the estimation of fasting blood sugar, post prandial blood sugar, total antioxidant capacity and lipid peroxidation using commercially available kits.

Statistical Analysis: The data obtained was analyzed for the statistical significance using a paired "t" test .$p<0.05$ was considered the level of significance.

Results:

The present study involved the assessment of effect of

yoga therapy on obesity and oxidative status in obese individuals. The present study showed that the obese individuals reduced their Body Weight, Lipid Peroxidation, Blood Sugar and Total Antioxidant Level. The significant reduction in Body Weight (p=0.000, Fig-1), BMI (p=0.000, Fig-2), Fasting Blood Sugar (p=0.03, Fig-3) and post prandial blood sugar (p=0.000, Fig-4) after yoga when compared to that before the yoga therapy. This decline was the clear evidence that one month yoga therapy improved the metabolic and biochemical mechanisms in obese individuals. A significant reduction in the level of MDA (p=0.000, Fig-5) was also recorded in the present study. We also observed a significant increase (p=0.021, Fig-6) in the level of Total Antioxidant after yoga when compared to that before the yoga therapy, which further indicates that one month yoga therapy improves the biochemical and metabolic functions in the body.

Discussion:

The present study confirmed the positive effects of yoga therapy as a conventional modality of treatment on biochemical imbalances and oxidative status in obese subjects.

Body Mass Index provides a simple numeric measure of a person's "fatness" or "thinness", allowing health professionals to discuss over- and under-weight problems more objectively with their patients. Excessive body weight is associated with various diseases, particularly cardiovascular diseases, Type-2 Diabetes mellitus, Obstructive sleep apnea, certain types of cancer, and osteoarthritis [5]. As a result, obesity has been found to reduce life expectancy [6]. Obesity is most commonly caused by a combination of excessive food intake, lack of physical activity, and genetic susceptibility, although a few cases are caused primarily by genes, endocrine disorders, medications or psychiatric illness. Obesity is one of the leading preventable causes of death worldwide [4, 2, 7]. On an average, obesity reduces life expectancy by six to seven years [5, 8]; a BMI of 30–35 reduces life expectancy by two to four years [9] while severe obesity, BMI > 40 reduces life expectancy by 10 years [10].

The significant reduction in the Body Weight and BMI as recorded in the present study are in line with the earlier studies, wherein, a 6-day yoga program led to decreased body mass index (BMI), waist and hip circumference, fat-free mass, total cholesterol, high-density lipoprotein and fasting serum leptin levels [11], and 12 weeks of yoga led to an overall reduction in food consumption, in eating speed and in food choices [12]. Eating disorder symptoms have also decreased following yoga [13], and in those diagnosed with an eating disorder, physical activity increased following yoga, and symptoms decreased, as did BMI and hip and waist measurements [14].

According to yoga, increased body mass is the indication of imbalance in the triguna and tridosha. The root causes of the abnormalities are adhi or mental stress. To overcome the stress the person habituates overeating leading to the deposition of fat in the body. To manage overweight or obesity, one has to reduce the mental stress and has to provide the sufficient physical activities. The practice of yoga can regulate all the body functions in a balanced manner and helpful in providing sustainable health. Analysis of our results clearly indicated that the complications of obesity can be reduced by yoga therapy. The reduction in the body weight might be due to reduction in the deposited fat on adipose tissue.

A reduction in the FBS and PPBS level after the practice of one month yoga indicated the improvement in the biochemical functions in obese individuals. Our findings are similar to the findings of Malhotra et al. who showed that yoga asanas significantly decreased FBG concentrations in Type-2 Diabetic patients after forty days [15].

Lipid peroxidation being an important process of

oxidative degradation of the lipids increases the oxidative stress in the body. Oxidative stress has been identified and proven to be the root cause of more than seventy different chronic degenerative diseases such as heart disease, cancer, stroke, diabetes, Alzheimer's dementia, Parkinson's disease, macular degeneration and other serious ailments. The significant reduction in lipid peroxidation as recorded after one month yoga therapy might be due to enhancing the antioxidant system of the body.

Conclusion:

The prevalence of obesity is increasing among all ages, including the elderly. Research has shown that both high BMIs and low BMIs indicate increased morbidity and mortality. Yoga therapy is beneficial in maintaining good health by regulating BMI, Oxidative status by improving the biochemical functions of the body and helpful to overcome the complications of obesity. Hence, from our study, it is concluded that the efficacy of yoga therapy on Body Weight, Fasting and Post Prandial blood glucose, Lipid peroxidation and Total Antioxidant status in obese individuals. This may have direct impact on the use of yoga therapy as a safe therapeutic modality in combating obesity.

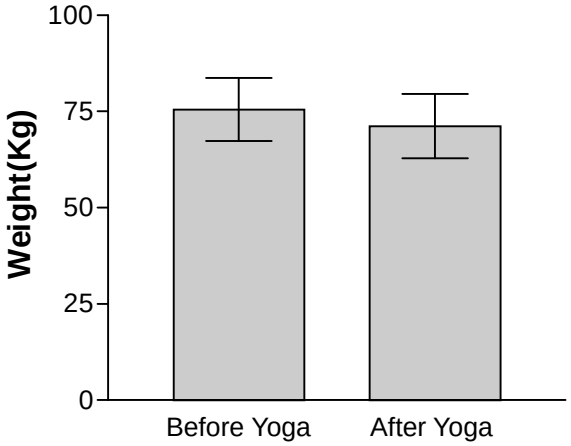


Figure-1: The effect of yoga therapy on body weight. No. of individuals were 40. There was a significant decline (p=0.02) in body weight after yoga when compared to that before the yoga therapy.

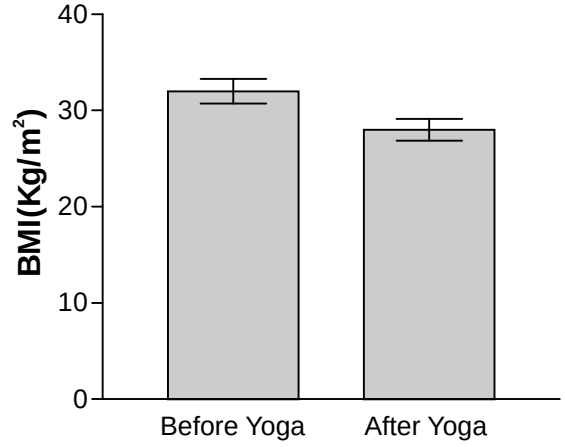


Figure-2: The effect of yoga therapy on Body Mass Index. No. of individuals were 40. There was a significant decline (p=0.000) in BMI after yoga when compared to that before the yoga therapy.

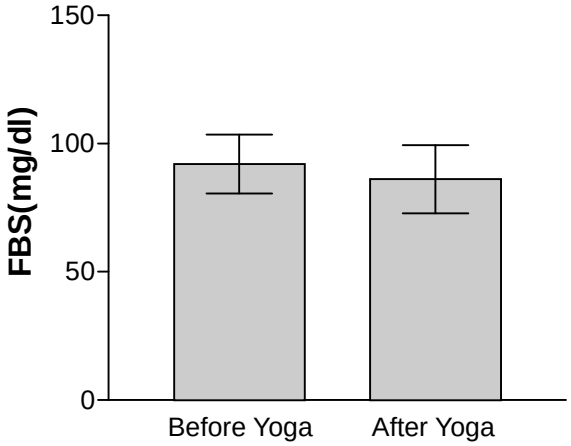


Figure-3: The effect of yoga therapy on Fasting Blood sugar. No. of individuals were 40. There was a significant decline in FBS (p=0.03) after yoga when compared to that before the yoga therapy.

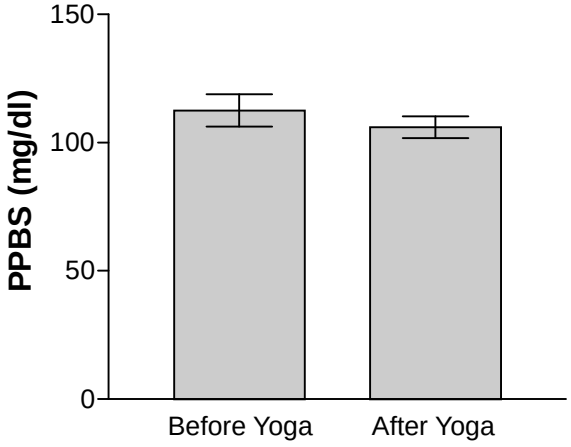


Figure-4: The effect of yoga therapy on Post Prandial Blood sugar. No. of individuals were 40. There was a significant decline in PPBS (p=0.000) after yoga when compared to that before the yoga therapy.

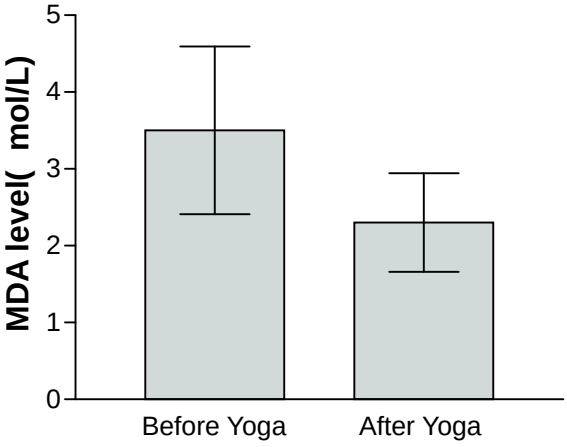


Figure-5: The effect of yoga therapy on Lipid peroxidation (MDA level). No. of individuals were 40. There was a significant decline in MDA level (p=0.000) after yoga when compared to that before the yoga therapy.

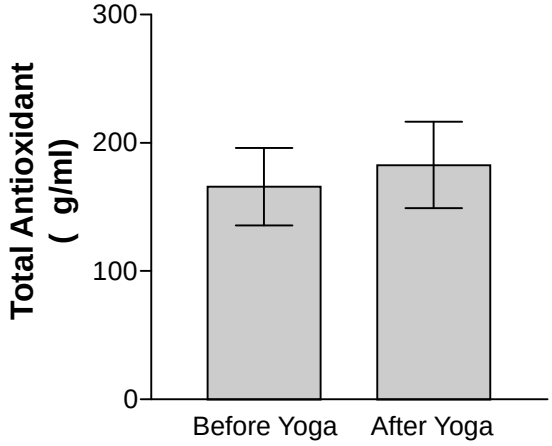


Figure-6: The effect of yoga therapy on total antioxidant status. No. of individuals were 40. There was a significant increase in the total antioxidant (p=0.021) after yoga when compared to that before the yoga therapy.

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# EFFECT OF ELECTRON BEAM RADIATION ON HEMATOPOIETIC CELLS OF SWISS ALBINO MICE

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Abstract:
Ionizing radiation which results in the free radical formation and it leads to damage of biological macromolecules such as DNA, proteins, lipids. 36 male Swiss albino mice were used for survival assay, to find out the lethal dose of Electron Beam Radiation. It was found to be 10Gy was the lethal dose for mice. Different dosages (4Gy, 6Gy and 8Gy) of electron beam radiation were used to study the micronucleus formation in irradiated mice. The results showed micronucleus formation will increase linearly with radiation dosage.
Keywords : Electron beam radiation, $LD_{50}$, Micronucleus

Running title: Micronucleus Induction by Sub Lethal Ionizing Radiation

## Introduction

Radiation therapy has been used in cancer treatment for many decades; it is used to eradicate cancer and as a palliative to relieve pain associated with metastases. In the course of treatment, radiation produces numerous biological perturbations in cells; because normal cell toxicity limits the doses used in effective treatment, approaches are designed to strike a balance between eliminating cancer cells and protecting normal tissues. The primary focus in radiotherapy is to increase DNA damage in tumor cells, as double strand breaks are important in cell death. Another course of action is to alter cellular homeostasis, modifying signal transduction pathways, redox state, and disposition to apoptosis. The cellular changes ideally would enhance the killing of tumor cells while reducing the probability of normal cell death [1, 2, 3].

Ionizing radiation consists of electromagnetic radiation (photons), including X-rays and gamma rays, and particulate radiation, such as electrons, protons, and neutrons. Clinical radiation oncology uses electromagnetic radiation and particulate radiation, mostly electrons and to a lesser extent neutrons and protons [4]. Radiation damages cells by direct ionization of DNA and other cellular targets and by indirect effect through ROS [5].

Radiation induces breaks in the chromosomes and chromatids. This break leads to chromosomal aberrations and micronucleus formation. Micronuclei induced by sub lethal ionizing radiation have been studied in mouse bone marrow cells and peripheral blood erythrocytes. In humans they are scored in the mitogen stimulated peripheral lymphocytes *in vitro* [6].

Electron beam radiation is a form of ionizing energy that is generally characterized by its low penetration and high dosage rates. It is a concentrated, highly charged stream of electrons, generated by particle accelerators which are capable of producing beams that are either pulsed or continuous. This high energy electrons are used for various purposes in the field of biology, it it is also used in the radiation therapy [7].

With this background our aim of the study is to evaluate the lethal dose of electron beam radiation on Swiss albino mice. Also to score the sub lethal whole body electron beam radiation induced micronucleus in bone

marrow cells of mice.

## MATERIALS AND METHODS

### Animal care and handling

Animal care and handling was carried out according to the guidelines set by WHO (World Health Organization; Geneva, Switzerland). The institutional animal ethical committee has approved this study. Swiss albino mice aged 6 -8 weeks and weighing 25±5 g, taken from an inbred colony, was used for this study. The mice were maintained under controlled conditions of temperature and light (light: 10 h; dark: 14 h). Four animals were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment. They were provided standard mouse feed and water ad libitum.

### Survival assay

36 male Swiss albino mice were used for the Survival assay. These animals were divided into 6 groups. Each group contains 6 animals each. These animals were irradiated to 4Gy, 6Gy, 8Gy, 10Gy, 12Gy and 14Gy radiation dosages. The percentage of mice surviving 30 days after exposure against each dose will be used to construct survival dose response curve [8].

### Irradiation

The irradiation work was carried out at Microtron centre, Mangalore University, Mangalore, Karnataka, India. The animals were restrained in well-ventilated perspex boxes and exposed to whole-body electron beam at a distance of 30 cm from the beam exit point of the Microtron accelerator at a dose rate of 72 Gy/min.

### Micronucleus assay

The mouse bone marrow micronucleus test was carried out according to the method described by Schmidt [9] by evaluation of chromosomal damage in experimental animals. The animals exposed to sub lethal dose 4Gy, 6Gy and 8Gy electron beam radiation were scarified on 31st day post irradiation. The bone marrow cells from femur were flushed in the form of a suspension into a centrifuge tube containing 5% BSA. The cells were dispersed by gentle pipetting and collected by centrifuge at 2000 rpm for 5 min at 4°C. The cell pellet was resuspended in a drop of BSA and bone marrow smear were prepared. After air drying the smear were stained with May-Grunwald/Giemsa. Micronucleated polychromatic erythrocytes and Non chromatic erythrocytes were observed under Microscope. The percentage of micronucleated polychromatic erythrocytes (MnPCEs), micronucleated normochromatic erythrocytes (MnNCEs) and ratio of PCE to (PCE + NCE) was calculated.

### Statistical analysis:

All values were expressed as Mean ± SD. Comparison between different groups were performed by analysis of variance (ANOVA) with Bonferroni. In all these test criterion for statistical significance was $P<0.05$.

## Results

The radiation dose was determined by exposing the mice with various doses (4Gy, 6Gy, 8Gy, 10Gy, 12Gy and 14Gy) of electron beam radiation. It was found to be non toxic up to a dose of 6Gy, where no radiation induced mortality was observed. A further increase in the electron beam dose to 8Gy resulted in 33% mortality. An increase in radiation dose to 10Gy caused a 50% reduction in the survival of mice. 100% of the mice died when the electron beam dose was increased to 12Gy and 14 Gy. The $LD_{50}$ of electron beam for acute radiation induced mortality was 10Gy (Graph 1).

The whole body electron beam exposed mice showed the formation of micronucleus in the bone marrow cells. The frequency of micronuclei was increased with increase in radiation dose (Table 1).

## Discussion

A single whole-body exposure of mammals to ionizing radiation results in a complex set of syndromes whose onset, nature and severity are a function of both total radiation dose and radiation quality. At the cellular level, ionizing radiation can induce oxidative stress [10] which results in the damage of biologically important

macromolecules such as DNA, proteins, lipids and carbohydrates in various organs [11, 12]. Irradiation produces different types of lesions in DNA molecule which lead the formation of micronucleus and breaks [6].

The $LD_{50}$ of ionizing radiation such as X ray and gamma rays for mice found to be 6.5-9.5Gy (6). The present study showed 10Gy is the LD50 for mice; this might be due to the low penetrating power of electrons.

The survival assay results revealed that the death of animals in 12 and 14 Gy irradiated mice were observed after 10th day of irradiation. Death between 11th day to 30th day post irradiation is due to haemopoietic damage inflicted by radiation [13]. The result obtained by micronucleus assay justifies the haemopoietic damage.

Ionizing radiation induces micronucleus formation and DNA damage [14]. The micronucleus formation was found to be in dose dependent manner, micronucleus was found to increase linearly with radiation dose ($P<0.05$). These sub lethal dose induced damage to haemopoietic organs of mice will result in leucopenia, thrombocytopenia and haemostatic disorders [6].

## Conclusions

The sub lethal doses of radiation also induce micronucleus formation in mice. The similar kind of mechanism may occur in humans during exposure of therapeutic radiation doses in radiotherapy. This damage is due to the direct radiation effect or by the effect of free radicals on cellular system. This damage can be reduced by antioxidant supplementation prior to the radiation exposure.

GRAPH 1: SURVIVAL ASSAY FOR ELECTRON BEAM RADIATION

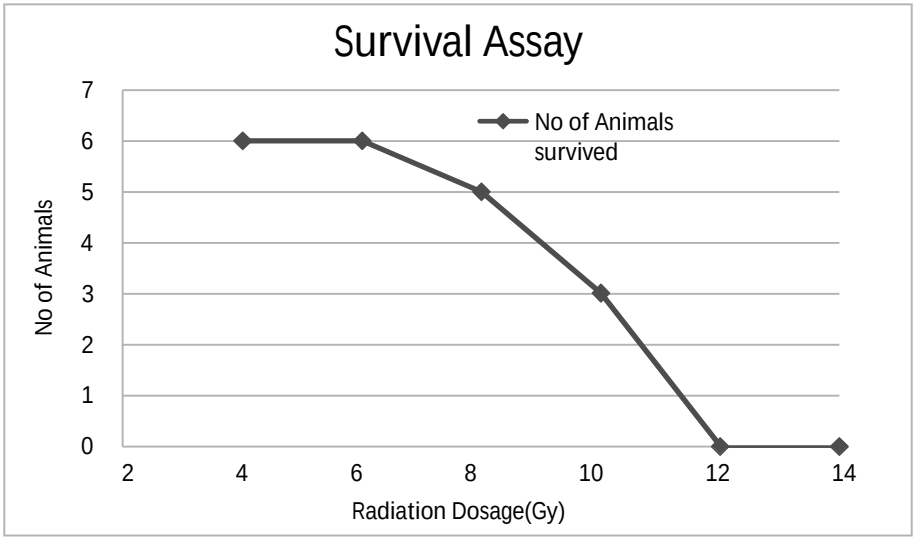


TABLE 1: EFFECT OF ELECTRON BEAM RADIATION ON MICRONUCLEUS FORMATION IN BONE MARROW CELLS OF SWISS ALBINO MICE

| | 4Gy | 6Gy | 8Gy |
|---|---|---|---|
| MnPCE/PCE (%) | 12.07±0.09 | 28.40±4.44 | 32.2±0.98* |
| MnNCE/NCE (%) | 4.08±0.10 | 12.67±7.30 | 18.47±0.44* |
| PCE/PCE+NCE (%) | 33.03±2.35 | 26.50±8.32 | 14.43±3.16* |

*P<0.05

PCE: Polychromatic erythrocytes, NCE: Non chromatic erythrocytes, MnPCE: Micronucleated Polychromatic erythrocytes, MnNCE: Micronucleated Non chromatic erythrocytes.

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# INFLUENCE OF THE TOTAL ANTIOXIDANT CONTENT OF SALIVA ON DENTAL CARIES.

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Abstract:
Objective: The aim of the study was to evaluate the Total Antioxidant level in saliva of caries patients with and without smoking habit.
Methods: A total of 80 individuals were included in the study. Unstimulated saliva was colleced. Samples of smoking and non smoking caries patients showing only Streptococcus mutans growth were analysed for Total Antioxidant level and smokers and nonsmokeres without any caries were considered as control groups. Caries isolates were confirmed as Streptococcus mutans by grams staining and biochemical tests. Total Antioxidant level was determined by spectrophotometric method.
Result: Non smokers with caries showed significantly(P <0.0001) higher TAC level to Smokers with caries. Smokers without caries showed significantly (P=0.0005)lower level of antioxidants than nonsmokers without caries.
Conclusion: This invasive study shows that smoke adversely effects the Total Antioxidant Level in caries patients.
Keywords : Dental caries, Streptococcus mutans, Total antioxidant level. Smokers and nonsmokers

## Introduction

Dental caries is one of the most common, but rarely life threatening disease[1]. It is characterized by a localized, transmissible, pathological infectious processes that ends up in the destruction of hard dental tissue [2]. Among the factors that have been related to grater cariogenic activity are inadequate dental hygiene and care, the existence of active caries or a family history of caries, a high concentration in the mouth of bacteria with acidophilic activity (*Streptococcus mutans* or *lactobacillus*), reduced salivary flow a cariogenic diet or inadequate levels of fluoride in drinking water [3].

*Streptococcus mutans* is considered to be the main cause of dental caries. A study stated that *Mutans Streptococci* participate in the formation of biofilms on tooth surfaces [4]. Antioxidants constitute an important part of our diet and these, together with extra cellular antioxidants and those of the enzymatic systems, can prevent various inflammatory, infectious or tumoural processes [5] .Various authors [5,6] have examined the relationship between the composition of the saliva and cariogenic activity. A study showed that the amount of caries in deciduous teeth is in direct proportion to the observed total antioxidant capacity of saliva[7].

Cigarette or tobacco smoke is involved in the pathogenesis of several diseases including local toxic effects in the oral cavity. The noxious effects of smoke compounds justify the high incidents of periodontal diseases, caries and neoplastic diseases of oral tissues in smokers [8]. Exposure to oxidant chemicals in smoke is associated with depletion of endogenous levels of antioxidants in the systemic compartment. Studies have reported that smoking results in low antioxidant concentrations in plasma [9].

The aim of the present study is to evaluate the status of Total Antioxidant Capacity (TAC) in caries patients with and without smoking habit.

## Materials and Methods

The present study was done at A. B. Shetty Memorial Institute of Dental Sciences after getting the institutional ethical clearance. The study was comprised of 80 participants, belonging to age group 30-40 years, in four groups. A written informed consent was obtained prior to enrollment of participants.

Group I: 20 Smoking individuals with caries.
Group II: 20 Smoking individuals without caries.
Group III: 20 Healthy individuals without any caries or smoking habits.

Group IV: 20 Non-smoking individuals with caries.

### Collection and isolation of Streptococcus mutans

Unstimulated saliva was collected in sterile sample collection bottles from patients with caries and without caries with and without smoking habit [10]. Saliva samples were vortexed and serially dilutedto 10folds in 0.05M phosphate buffer. 100µl of the aliquotes were cultured onto Mutans Sanguis Agar (Himedia, Bombay) plates. The plates were incubated at 37$^0$C for 24-48 hrs. Colonies were identified as *Streptococcus mutans* by doing grams staining and biochemical tests like Inulin and Mannitol fermentation, Esculin hydrolysis in the presence and absence of bile, Urease and resistance to Bacitracin. The *St. mutans* ferments both Inulin and Mannitol.Esculin hydrolysis is negative in presence of bile and positive in absence of bile. Urease is negative and it is resistance to 2U of Bacitracin.

### Estimation of total antioxidant capacity (TAC)

Total antioxidant capacity was estimated by phosphomolybdenum method[11]. This quantitative assay is based on the conversion of Molybdenum (Mo VI) by reducing agents like antioxidants to Molybdenum (Mo V), which further reacts with phosphate under acidic pH resulting in the formation of a green coloured complex. The intensity of which can be read spectrophotometrically at 695 nm.

## Results

The data of TAC was analyzed by one way Annova using Prism software. Table 1 is representing the level of TAC as mean $\pm$ standard deviation in all four groups. The data shows significant decrease in the TAC of smokers with caries than non-smokers with caries ($P < 0.0001$) and it is also significant over smokers without caries ($P = 0.0005$). Nonsmokers with caries showed higher level of TAC ( 162.0 $\pm$ 6.339 µg/ml) than healthy individuals without any smoking habit (136.5 $\pm$ 17.08 µg/ml )(Table 2 and Fig 1)

## Discussion

Dental Caries is a bacterial plaque-dependent disease of the dentition that is characterized by a progressive, intermittent demineralization of enamel, dentin and cement with a characteristic pattern of decay that may lead to total destruction of coronal dental tissues and the formation of pulpal abuses, Oral microorganisms, when organized in voluminous masses as dental plaque on tooth surfaces, hydrolyte starches and metabolize sugars to form acids (mainly lactic acid) that de-mineralize the hard tissue underneath [12].

When antioxidant defenses are weakened, body cells and tissues become more prone to develop dysfunction and /or disease. Total antioxidant capacity evaluation is the first step in the search of diseases in biochemistry, medicine, food and nutritional sciences [13].

Saliva is considered as mirror of the body is readily available and collection process is fairly straight forward [14]. Saliva being the diagnostic tool for detection of dental caries shows lower flow rate, viscosity, pH and buffering capacity[15]. In this study unstimulated saliva was collected for the assessment of *Streptococcus mutans* and total antioxidant level.

In the present study smokers with and without caries (group 1 and 2 ) shows decrease in Total Antioxidant level compared to the nonsmokers with and without caries

(group 3 and 4). This may be due to the presence of high amounts of free radicals in cigaratte smoke that generate an oxidative stress in the smokers body that may cause exhaustion of antioxidants of the body[16].

In our study we found significant increase (P=0.0142) of Total Antioxidant level in nonsmokers with caries (group 4) than healthy individuals (group3) without any habit of smoking. Similar studies done on children reveals the same results [17,18,19]. The increase may due to an increase in the suspension of proteins and of cariogenic activity [6] and it has been suggested that the levels of antioxidants could be altered in response to an infection or disease [20].

There is significant decrease (P=0.0005) in total antioxidant level of Smokers without any caries when compared to nonsmokers without any dental caries. This finding was also reported in few previous studies[7,16].

The Total antioxidant level in smokers with *St.mutans* caries (Group 1) decreased significantly (P<0.001) than in nonsmokers with *St.mutans* caries (Group 4). Cigarette smoking was shown to be associated with the prevalence of caries [21] and free radicals in cigarette smoke [22,23] deplete the antioxidant level.

## Conclusion

In this noninvasive study we considered the patients with *St.mutans* caries and smoking habit. We have found that the Total antioxidant level does not decrease in individuals with *St.mutans caries* without any smoking habit, but it decreases in individuals with smoking habit. So it may be suggesting that smoking may increase the prevalence of dental caries or it may worsen the conditions in dental caries as the free radical produced by the smoke reported to deplete the total antioxidant level.

## Acknowledgement

We acknowledge the financial support given by the Nitte University for this study.

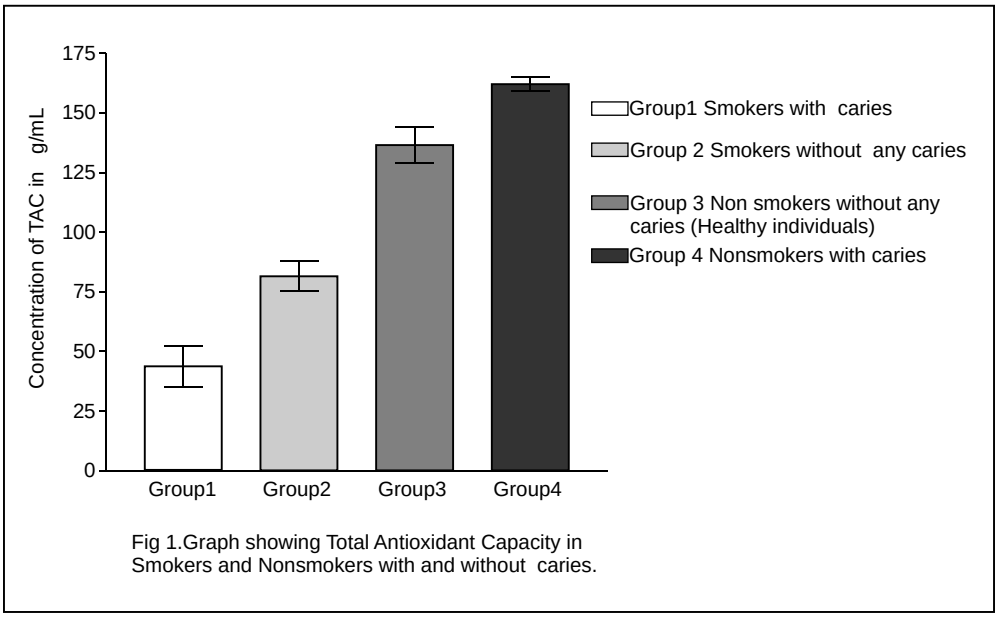


Fig 1.Graph showing Total Antioxidant Capacity in Smokers and Nonsmokers with and without caries.

Tables

| Groups | Group 1 | Group 2 | Group 3 | Group4 |
|---|---|---|---|---|
| Level of TAC in µg/ml | 43.50 ± 19.04 | 81.50 ± 13.85 | 136.5 ± 17.08 | 162.0 ± 6.339 |

Table 1: Showing the levels of Total Antioxidant Capacity in all the four groups. Data are presented as mean ± standard deviation. P <0.0001 and level of significant at the level of 0.05

| Groups | Group 1 | Group 4 | P value |
|---|---|---|---|
| Level of TAC | 43.50 ± 19.04 | 162.0 ± 6.339 | P<0.0001 |
| Groups | Group 2 | Group 3 | P value |
| Level of TAC | 81.50 ± 13.85 | 136.5 ± 17.08 | P=0.0005 |
| Groups | Group 1 | Group 3 | P value |
| Level of TAC | 43.50 ± 19.04 | 136.5 ± 17.08 | P<0.001 |
| Groups | Group 2 | Group 4 | P value |
| Level of TAC | 81.50 ± 13.85 | 162.0 ± 6.339 | P=0.0005 |
| Groups | Group 3 | Group 4 | P value |
| Level of TAC | 136.5 ± 17.08 | 162.0 ± 6.339 | P=0.0142 |

Table 2: Showing significance in the levels of Total antioxidant capacity between Group 1 and 4(P<0.0001), Group 2 and 3(P=0.0005),Group 1 and Group 3 (P<0.001) , Group 2 and Group 3 (P=0.005) and group 3 and Group 4 (P=0.0142). level of significance at P<0.05.

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# EVALUATION OF THE EFFICACY OF CARDAMOM AROMATHERAPY ON AEROBIC FITNESS & AUTONOMIC FUNCTIONS AMONG STUDENTS.

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Abstract:
Introduction: Aromatherapy is categorized as a form of complementary and alternative medicine (CAM), and has been steadily gaining popularity in today's society. Aromatherapy is considered by many to promote comfort, well-being and invigorating. However there appears to be insufficient supporting evidence to validate physiological changes that may reflect invigoration.
Objectives: This study examined the physiological effects of cardamom (Elettaria cardamomum) aromatherapy as indicated by heart rate variability. Aerobic fitness is one of the non invasive and simplest parameters which help in accessing one's fitness. In this present study we have also evaluated the efficacy of aromatherapy on the individual's physical fitness.
Methods: Total thirty healthy college students were selected as a subject who has attended four sessions in random order involving only exercise, aromatherapy and exercise combined. Each intervention lasted 15 minutes. Heart rate data were recorded for all sessions, and heart rate variability was analyzed. Aerobic fitness parameters also evaluated with the help of standard methods.
Results: There was statistically significant difference in LF/HF between the two intervention studies. During this aromatherapy, physiological responses such as oxygen consumption, respiratory exchange ratio and minute ventilation were significantly altered among different groups. Aerobic fitness was observed higher in aromatherapy combined exercise group against the only exercise group.
Conclusion: These results suggest significant physiological effect of cardamom (Elettaria cardamomum) aromatherapy interventions occurs in the autonomic nervous system as indicated by heart rate variability. The present study demonstrates that good estimated aerobic fitness was independently associated with aromatherapy and exercise.
Keywords : Aromatherapy, autonomic function, aerobic fitness, exercise.

## Introduction

Ancient writings provide insight into how religions and cultures of old used the aroma of burning herbs, flowers, tree leaves and other natural sources in their spiritual practices. It is learnt from the Hebrew, Christian, Buddhist and Hindu cultures more about these types of ritualistic observances[1]. All these cultures from around the world had access to various plants from which to develop a recipe for pleasing the nose and mind. They assigned mystical energies to these plants and learned over the ages which ones provided results and which ones failed. They also provided us with instructions for using aromas that were pleasing not only to the senses of the human nose, but also to the senses of the Divine forces in their lives. Incense sticks are part of the 16 essential offerings during a Hindu ritual. According to Saurabh Bhattacharya[2] each of these offerings has symbolic spiritual significance and is offered to the Divine in a particular order.

Physical activity is defined as any bodily movement produced by skeletal muscles that result in energy expenditure[3]. Physical fitness can be defined in multiple ways, such as a set of outcomes or traits that relate to the ability to perform physical activity[3]. Aerobic fitness determines the degree of fatigue that almost everybody experiences in daily life. The higher the aerobic fitness, the less fatigue one experiences. Aerobic fitness is the ability to sustain work for prolonged periods. In order to

enhance aerobic fitness, athletes are known to try a variety of aids to enhance performance and boost their chances of winning. The term ergogenic aids identifies those agents or procedures which if followed before a competition will potentially enhance the athlete's performance[4].

Aromatherapy, another possible ergogenic aid, has been growing in recent years and has received much attention by both traditional and alternative medicine practitioners. However, there is very little evidence which supports or refutes the claims made by merchants, practitioners, and manufacturers[5]. Since smell is the least understood of all our senses[6] it is logical to understand why the number of discrepancies exist within the lay and scientific literature regarding the validity of aromatherapy. Buckle et. al. (1998) argues that aromatherapy is becoming more and more valuable in holistic nursing practice and should be a part of nursing protocols[7]. Martin et. al. (1996) suggested that drawing conclusions regarding aromatherapy is premature since much of the data is qualitative as much of it is based on pure historical content and anecdotal reports from individuals[8]. Researchers have argued against aromatherapy secondary to the poor follow-up of clients after sessions. Additionally many reports are purely subjective in nature and cannot be considered truly scientific[9].

Earlier it was demonstrated that inhaling peppermint is reported to be a stimulant for increased energy which would certainly benefit any athletic or non-athletic individual during an exercise bout[10]. By definition, peppermint could be considered an ergogenic aid. Lavender is marketed as an aroma which promotes relaxation and a calming effect[7]. As with any ergogenic aid, multiple questions arise regarding legal and ethical issues. Also important is the additive being used a substance the athlete would normal consume or use in everyday life[4]. How effective are the aromatherapy and environment conditioning fragrances? It has been said that aromas from lavender, basil, cinnamon and citrus flavor aid relax, whereas cardamom, peppermint, thyme and rosemary invigorate. Ginger, cardamom, licorice and chocolate are supposed to arouse a sense of romance, while rose combat depression[11, 12]. Stimulating or invigorating odors such as cardamom, rosemary and lemongrass affect the locus ceruleus with the resultant release of noradrenalin into the brain and this has the effect of arousal/waking up. Researchers at the Royal Berkshire Hospital NHS Trust recently broke new ground by studying the effects of aromatherapy in the intensive care unit as a means of helping to alleviate anxiety and stress[13].

The purpose of this pilot investigation was to examine the potential benefits that common cardamom (Elettaria cardamomum) aromas might have on basic physiological measures before, during and after a 15-minute exercise bout. There is little evidence in the literature addressing the actual physiological responses after the introduction of an aromatherapy during exercise. It was hypothesized that cardamom (Elettaria cardamomum) aromatherapy can induce significant changes in the autonomic activity. Our main concern is to elucidate the effect of cardamom aromatherapy is synergistic or antagonistic. It was hypothesized that the effect of aromatherapy on heart rate would be a greater alteration in autonomic nervous system activity than the effect of exercise alone.

## MATERIALS METHODS

Subjects: Sixteen male and fourteen female apparently healthy college students volunteered to participate in this investigation. Mean ages were 22.5 ± 0.8 for males and 21.75 ± 0.9 for females. These students considered themselves sedentary. In this investigation, an individual was considered sedentary if he/she did not regularly participate in physical activity more than two days per week at equal to or greater than 60% of their maximal heart rate. All subjects were familiar with exercise

protocols and walking on a motorized treadmill. This study was approved by the departmental review board for institutional ethical committee. Informed consent was obtained from every individual who has participated in this study. Using an in-house designed questionnaire, background information was gathered from the subjects.

Methods:

Anthropometric measurements: All subjects underwent a clinical examination; weight, height, and waist and hip circumferences have been measured. Blood pressure was recorded by auscultatory method with a mercury sphygmomanometer according to the American Heart Association guidelines.

Measurement of Peak Expiratory Flow Rate (PEFR): Peak expiratory flow rate was examined with an Airmed peak-flow meter. The test was performed in standing position holding the peak flow meter horizontally. A tight fitting disposable cardboard mouthpiece was inserted in the inlet nozzle. After proper rest, subject was requested to take a deep breath and followed by exhalation as forcefully as possible in one single blow into the instrument. The procedure was repeated three times and best of the three was recorded.

Measurement of Physical Fitness Index (PFI): PFI was measured by Harvard step test[14]. The standard procedure for the original step test was modified and used with a difference that the stepping height is reduced from 20 inches to 18 inches in the line of originator of the tests who suggested that for evaluating subjects with body surface area below 1.85sq.m, an 18 inches stool should be appropriate. The subjects stepped up and down on a stool at the rate of 30 complete steps per minute keeping time to a metronome for duration of 5 minutes unless one stops from exhaustion. The recovery pulse counts were measured at 1 to 1.5, 2 to 2.5, 3 to 3.5 minutes recovery. Physical fitness is scored as PFI = [Duration of exercise in sec x 100] / [2 x (sum of 1 to 1.5 min., 2 to 2.5 min., 3 to 3.5 minutes recovery)].

Oxygen consumption ($VO_2$), minute ventilation (VE) and respiratory exchange ratio (RER) were obtained via open circuit spirometry using a metabolic analyzer. HR (Heart rate) was recorded using a Polar Heart Rate Monitor. A motorized treadmill was used for the exercise mode. RPE was obtained using the modified Borg scale[15].

Aromatherapy: In this treatment schedule the subject has been categorized and in order to assess each subject's olfactory sensation, they were asked to identify three recognizable aromas: garlic, ginger and a control [water with food coloring made to resemble other aroma mixtures]. As subjects confirmed their olfactory sensation by recognizing each aroma, their suitability for participation was confirmed. After acclimatization to the laboratory, subjects returned once per week for three weeks. Each week cardamom (Elettaria cardamomum) aroma was administered in a double blind fashion. The administration of the cardamom essential oil was delivered through a sealed plastic container with the oil concentrated on a sterile cotton pad. Subjects inhaled the aroma with only nose breathing. The procedure of administration cardamom aromatherapy was modified from the Pournemati P. et.al.[16]. Each subject inhaled the aroma for one minute and began walking on the treadmill at a speed of 90.5 meters/minute for 15 minutes. At every three-minute interval, the subject's HR, $VO_2$, VE and RPE were obtained and recorded. Also at each interval the designated aroma was again administered using the sealed container for a period of 60 seconds. After the 15-minute period was completed, subjects walked for five minutes at a speed of 55.3 meters/minute. Recovery heart rates were obtained until it was confirmed that subjects had returned to within 15-20 beats of their pre-exercise heart rate.

Measurement of HRV parameters: The ECG was recorded using lead II to obtain a QRS complex of

sufficient amplitude and stable base line. ECG signals were conveyed through an A/D converter (Biopac MP 30, Biopac system) at a sampling frequency of 500 Hz to PC and were analyzed

Statistical analysis: The data obtained were analyzed for their statistical significance by one way ANOVA test and paired t-test using SPSS. P<0.05 was considered the level of significance.

RESULTS

The data represented in Table 1. highlights the characteristics features such as age, height, blood pressure and different habits of the subjects. In addition, weight and height was obtained to determine the body mass index of each subject, waist and hip measurements are included to determine waist to hip ratio.

The information gathered from the subjects during the interactive session clearly shows that few of them were also got the addiction to the alcohol, caffeine, and smoking. The collected information about the lifestyle parameters such as food habits, walking habits, health and sports habits and stress related conditions at the college. It was demonstrated that students are having poor attitude towards their lifestyle parameters and highly sensitive to the stressful conditions.

Table 1 – Base line general features of selected students

| Particulars | |
|---|---|
| Age (Years) | 22 ± 0.5 |
| Weight (kg) | 58.7 ± 5.95 |
| Height (cm) | 159.5 ± 6.74 |
| BMI (kg/m$^2$) | 23.2 ± 0.8 |
| WHR (waist-hip-ratio) | 0.69 ± 0.03 |
| Systolic blood pressure (mmHg) | 124 ± 10.68 |
| Diastolic blood pressure (mmHg) | 74 ± 2 |
| Smoking | 2 % |
| Alcoholics | 1 % |
| Excess Caffeine | 2 % |

Subjects (n) in each group, n= 30. The values are expressed as mean ±SD.

Each subject completed three separate exercise bouts of treadmill walking lasting 15 minutes. During each bout subjects inhaled one of three samples in a double blind fashion. Table 1 contains the data obtained from analysis of expired gases Oxygen consumption ($VO_2$) minute ventilation (VE) and Respiratory Exchange Ratio (RER) remained the same among all three trials.

Table 2 - Comparison of selected physiological parameters among exercise and exercise combined aromatherapy.

| Parameters | Excercise group | Aromatherapy combined exercise | P Value |
|---|---|---|---|
| Physical Fitness Index (PFI) | 44.5 ± 2.42 | 49.4 ± 1.41 | P<0.01 |
| $VO_2$max (ml.kg$^{-1}$min) | 29.6 ± 1.24 | 35.5 ± 3.79 | P<0.01 |
| Peak expiratory flow rate (PEFR) (Lmin$^{-1}$) | 376.1±67.67 | 429.9±.42.91 | P<0.01 |
| VE [L/min] | 28.65 ± 2.7 | 32.16 ± 3.2 | P<0.01 |
| RER | 1.55 ± 0.07 | 1.92 ± 0.08 | P<0.01 |

Subjects (n) in each group, n= 30. The values are expressed as mean ±SD.

Table 1 shows the higher aerobic capacity in aromatherapy group as compared to exercise group, expressed by higher (P < 0.01) oxygen uptake and physical fitness index.

Heat rate response can be found in Table 2. During exercise, heart rate increased approximately 30 beats per minute above pre-exercise rate for all three trials. Based on the rate of exercise intensity which subjects performed, this response was considered normal. The exception to this increase was with the cardamom trial which only elicited a 29 beat increase secondary to the higher pre-exercise heart rate of 71 BPM as compared to 77 BPM for the exercise group.

Table 3. Heart rate and rating perceived exertion (RPE) response in exercise and exercise combined aromatherapy group.

| Parameters | Excercise group | Aromatherapy combined exercise | P Value |
|---|---|---|---|
| Pre-exercise heart rate | 77.23±8.5 | 71.64 ± 7.7 | P<0.1 |
| Exercise Heart Rate | 100.23 ± 8.7 | 100.9± 9.3 | P<0.1 |
| Time of running (minute) | 12.45 ± 1.2 | 14.30 ± 0.9 | NS |
| RPE | 2.4 ± 0.8 | 3.7 ± 0.8 | P<0.1 |

Subjects (n) in each group, n= 30. The values are expressed as mean ±SD.

Subjective measures of the modified Borg Scale or Rating of Perceived Exertion [RPE] were obtained and these results demonstrated an increase for the aromatherapy combined exercise group [3.7] compared to a [2.4] for the exercise group. An RPE of 3 equates to a perception of hard work. An RPE of 2 equates to a perception of light work.[17]

The results depicted in table.4, Data are reported as medians and interquartile range. RR = standard normal RR interval; LF, HF = low and high frequency power, respectively; LF/HF = ratio of absolute LF power to HF power values. Frequency ranges: LF: 0.04-0.15 Hz and HF: 0.15-0.4 Hz.

According to the results presented in table. 4 It is apparent that after the aromatherapy treatment blood pressure shows significant changes in both systolic and diastolic. There was also an increase in heart rate, LF and LF/HF ratio. Apparently, treatment of aromatherapy decreases parasympathetic nervous activity.

The differences in the heart rate and other parameters between the two groups at baseline, before and after exercise are shown in Table 4, indicating a significant difference in LF between the exercise and exercise combined aromatherapy groups before and after exercise. The median LF, HF level in exercise combined aromatherapy group was significantly higher than those in the exercise group. The smaller HF and larger LF/HF after exercise may indicate possible elevation of sympathetic activity. All the other HRV measurements, indicates significant difference between the cardamom aromatherapy combined exercise and exercise group.

Table 4. Comparison of heart rate variability parameters in the exercise combined aromatherapy group.

| Parameters | Exercise group | | Aromatherapy combined exercise | |
|---|---|---|---|---|
| | Pre-exercise | Post-exercise | Pre-exercise | Post-exercise |
| Heart Rate (BPM) | 69.32 ± 8.75 | 76.05 ± 6.11 | 78 ± 10.49* | 82.7 ± 15* |
| LF(msec$^2$) | 357.55 ± 159.84 | 305.88 ± 227.74 | 534.64 ± 567.80** | 370.27 ± 257.52*** |
| HF(msec$^2$) | 1191.32 ± 1298.74 | 399.18 ± 419.84 | 787.73 ± 755.52** | 482.36 ± 429.80** |
| LF/HF | 0.299 ± 1.017 | 0.764 ± 1.351 | 0.705 ± 1.671** | 0.968 ± 1.145*** |
| Systolic blood pressure (mmHg) | 112.04 ± 17.29 | 129.52 ± 11.31 | 123.27 ± 11.16** | 139.09 ± 6.49** |
| Diastolic blood pressure (mmHg) | 74.32 ± 8.72 | 83.00 ± 6.89 | 78.82 ± 8.94** | 86.00 ± 6.72*** |

Subjects (n) in each group, n= 30. The values are expressed as mean ±SD.
*P < 0.05; **P < 0.01; ***P < 0.001.

## DISCUSSION

Aromatherapy uses concentrated essential oils extracted from herbs, flowers, trees and other plants. The essential oils are believed to have an effect on both psychological and physiological level. Depending on the mix and blend, this effect may be either to stimulate or relax [18]. Cardamom (Elettaria cardamomum) is also known as cardamon; it is related to several spices, such as ginger (Zingiber officinale), and consequently possesses some similar therapeutic properties and benefits as ginger.

Some important neurotransmitters have been identified in cardamom, aceylcholine and choline, which explain herbal medicine's position that cardamom, may behave as a stimulant to the nervous system, and uses it to help prevent convulsions or spasms. Scientific studies have shown that cardamom has a blood thinning action, due to its high concentration of linoleic acid[18].

We found a significant increase in mean heart rate and increases in analysis of heart rate variability parameters after 15 min of cardamom inhalation. Moreover, HF is

also increased, and all these indices have been used to reflect primarily sympathetic influences. LF has been shown to reflect both sympathetic and parasympathetic influences, making the contributive components of this measurement less clear[19, 20]. However, the change in LF and ratio of LF/HF infer that there is an impact on sympathetic drive to the heart.

In this present study we also investigated that the differences in autonomic cardiovascular control could appear between exercise and exercise combined aromatherapy group during a situation of exercise stress, which is a natural stimulus, leading to sympathetic excitation and vagal withdrawal in the heart. During exercise the initial increase in the heart rate response is mediated by a decrease in vagal activity, followed by an enhanced sympathetic activity that maintains higher values of heart rate during the test.

The finding of increased low to high frequency (LF/HF) ratio in response to cardamom aromatherapy indicates that aromatherapy may in fact cause a sympathetic response, rather than a parasympathetic response. This finding warrants further research. Further, it is not known what mechanisms play a role with aromatherapy and metabolism. At 15 minutes of exercise the average individual is just beginning to achieve a true point when oxygen demands equal oxygen availability or more commonly referred to as steady state of oxygen consumption[17].

The use of aromatherapy is rapidly growing within western society. It is obvious from the literature that there are vast differences in opinion regarding the efficacy, validity and the overall claims towards the role of aromatherapy in all aspects of medicine, particularly preventative and rehabilitative medicine. Exercise does fall into both categories, preventive and rehabilitative; therefore the use of aromatherapy in concert with exercise is quite popular. Although it is claimed that peppermint may accentuate energy by stimulating the adrenal cortex[10] it is not known what dosage and how this increased energy may affect exercise performance.

Cardamom has reputation for having simulative effects on autonomic bodily systems like digestion and circulation. Cardamom may improve blood circulation to the lungs, and is indicated in folk medicines to relieve symptoms of asthma and bronchitis.[11-13] Our study clearly suggests that during dynamic exercise, heart rate increases due to both a parasympathetic withdrawal and an augmented sympathetic activity. The relative role of the two drives spectral depends on the exercise intensity.[20-25]

In the present investigation, cardamom aromatherapy had significant effect on resting or exercises heart rate during 15 minutes of moderate treadmill walking. The result of present study about rating perceived exertion shows a slight increase in aromatherapy groups. Rating perceived exertion is a good index for evaluating individual's effort during an exercise bout [15].

Earlier study mentioned that inhaling the essential oil can affect the performance in both positive and negative ways[20] and the cardamom essences is known as aphrodisiac and invigorating which may be the reason for increase in concentration and improves performance. The results of this study indicate that inhaling a cardamom essence has brought the significant differences when compared to exercise group. The value of respiratory exchange ratio indicates that fat is being consumed for energy generation during exercise. It is possible that cardamom aroma may have more beneficial effects when fat are the major fuel source as opposed to carbohydrate.

The higher aerobic capacity of the athletes was reflected in greater oxygen consumption and physical fitness index in both groups. Also, the resting heart rate was significantly lower in exercise combined aromatherapy group than in exercise group. Changes in the intrinsic mechanisms acting on the sinus node and alterations in

the autonomic nervous system control of the heart have been reported to contribute to this phenomenon.[23, 24] These data are exciting regarding aromatherapy and exercise performance; however, given the above limitations regarding time and introduction of aroma, these results are understandable.

The benefits of aroma on exercise performance is not yet established, the questions regarding their ethical use must be addressed. However, further research on the normal and strenuous exercise in combination with appropriate control needs to be conducted to clarify the efficacy of cardamom aromatherapy in the management of well-being in combating with lethargy, sedentary life style and or other health issues. Heart rate and other cardiac variables should have been measured during exercise to clarify physiological responses more in details. We demonstrate the need of further research to investigate subjects, measurement indicators, and experimental conditions to clarify the relationship between physiological and emotional responses produced by cardamom aromatherapy.

## CONCLUSION

Everyone experiences some degree of pressure at work, and being under pressure can help improve performance and productivity. Excessive levels of pressure, however, can have adverse effects, resulting in stress and intense long-lasting stress can lead to mental and physical ill-health. In such situations, simple techniques that can help to cope up with stress, such as aromatherapy, can be of major benefit. We have demonstrated on young healthy adults the effect of inhaling the aroma from Cardamom (Elettaria cardamomum) essential oil on autonomic balance. The results provided support for the stimulatory effect of aroma, as indicated by a shift of the autonomic balance toward sympathetic predominance. Combining exercise with cardamom aromatherapy is not only enjoyable because of its effects on mood but also promotes physiological excitation, thereby increasing physical activation. Inhalation of cardamom essential oil may provide a relatively simple, safe, well-being, invigorating and effective method of handling the stressful conditions.

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# ROLE OF MATERNAL ERYTHROCYTE ARGINASE ACTIVITY IN PREGNANCY – A PILOT STUDY

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Abstract:
Introduction: Arginase is an urea cycle enzyme which catalyzes the cleavage of L arginine to L- ornithine and urea. It is expressed in liver, erythrocyte, brain, kidney, mammary gland and intestine. The arginase activity detected in nonhepatic tissues that lack a complete set of urea cycle enzymes is thought to provide ornithine, the biosynthetic precursor of proline, an important constituent of collagen, and the polyamines, which are important for cell proliferation.
Aim and objectives: In the current study arginase level in maternal erythrocytes were determined to ascertain any possible role in pregnancy.
Study design: The study group comprised of total 45 subjects including twenty non – pregnant women (mean age 31.0 ± 6.0 years) and twenty five pregnant women (mean age 29.6 ± 6.1 years) of gestational age between 28 – 38 weeks.
Results: We found a significant increase in the level of maternal erythrocyte arginase (p 0.05) in pregnant women when compared to non – pregnant women.
Conclusion: Our study suggests that the increased maternal erythrocyte arginase activity may have a role in fetal growth and development.
Keywords : Arginase, Ornithine, Non-pregnant women, Pregnancy, Erythrocyte, polyamine, proline

## Introduction

Arginase (L- Agginine amidino hydrolase, E,C 3.5.3.1) is an urea cycle enzyme which catalyzes the cleavage of L arginine to L- ornithine and urea. Arginase exists in two isoforms. Arginase-1 is a cytosolic protein, expressed primarily in the liver and to some extent in the erythrocytes. Hepatic arginase-1 activity serves urea synthesis and nitrogen homoeostasis.Arginase-2 is a mitochondrial protein, expressed in many extrahepatic tissues, such as brain, spinal cord, kidney, small intestine and mammary gland, but not in mature erythrocytes [1-4].

Arginase activity detected in non-hepatic tissues that lack a complete set of urea cycle enzymes is thought to provide ornithine, the biosynthetic precursor of proline, an important constituent of collagen and the polyamines, which are important for cell proliferation. Erythrocyte arginase activity is one of the sources for ornithine present in plasma. The availability of ornithine may be important for peripheral tissues such as cartilage and bone, since these tissues have low or no arginase activity [5]. Different studies have revealed that the requirement of rapidly dividing tissues for enhanced polyamine biosynthesis is apparently met by increased arginase activity, for eg, in gastric cancers, breast cancer, colorectal carcinoma [6 – 8]. In lactating mammary gland arginase activity rises to about 25% of that found in liver in order to supply the proline required for milk protein biosynthesis [9]. Experimental evidences suggest that, myometrial arginase activity increases ~ 25-fold during pregnancy to supply the rapidly growing fetus with polyamines to facilitate cell proliferation [10].

In our survey of literature regarding the erythrocyte arginase in pregnancy, no previous data was available .Hence; the current study was taken up to find out the probable role of erythrocyte arginase in pregnancy.

## Materials and Methods

The study group consisted of total 45 subjects which included twenty non – pregnant women (mean age 31.0

± 6.0 years) and twenty five pregnant women (mean age 29.6 ± 6.1 years) of gestational age between 28 – 38 weeks .Subjects with history of coronary heart disease, hypertension, renal disease, diabetes mellitus, gestational diabetes mellitus, pre-eclampsia or any systemic disease were excluded from the study. The study was approved by the institutional ethical committee and informed consent was obtained from all the subjects involved in the study.

Collection of samples: 5 ml of venous blood was collected from the anticubital vein under aseptic conditions from each subject. 2ml of blood was added to the EDTA bottles for the separation of RBCs for the arginase estimation and 3ml to the plain bottles for separation of serum. The samples were then subjected to centrifugation for 3000g for 10 minutes within 2hrs of collection.

Preparation of RBC suspension: From the EDTA added blood the plasma was removed and the RBCs were washed with saline. For this they were mixed with about 10 ml of 0.9% saline and centrifuged. Supernatant was removed and the process was repeated for three times. Then the RBCs were suspended in saline to get 50% of RBC suspension.

Serum separated from the plain tube sample was used for the estimation of urea.

Determination of arginase activity: Arginase activity is measured colorimetrically. L- arginine is cleaved by arginase into urea and ornithine. The amount of urea formed by the action of arginase is measured by Berthlot reaction. One unit enzyme activity is the amount of enzyme required to produce1 µmol urea per minutes at 37° C [11.] Arginase activity is expressed as units per gm hemoglobin.

## Results

Statistical analysis was performed using the statistical package for social sciences (SPSS 11.5). Independent student's't'test was used to compare mean values between the groups. The results are expressed as mean ± standard deviation (SD) in pregnant and non- pregnant women. Probability less than 0.05 was considered statistically significant.

A significant increase is observed in the level of maternal erythrocyte arginase (p 0.05) in pregnant women when compared to non – pregnant women. There is a significant decrease in urea level in pregnant women when compared to non- pregnant women.

## Discussion

In intact erythrocytes, arginase catalyses the hydrolytic cleavage of the guinidino group of arginine, a semi essential amino acid to urea and ornithine. Since ornithine transcarbamoylase is absent in erythrocyte they cannot have functioning urea cycle. Urea formed in the erythrocyte comes out and accounts for one percent of blood urea. Several investigators have suggested that ornithine is an important precursor for proline, a critical constituent of proteins, e.g., collagen, in peripheral tissues and polyamines which are essential for cell proliferation. Peripheral tissues have relatively high levels of ornithine aminotranferase and proline -5-carboxylate reductase, the enzymes that sequentially convert ornithine to proline, but they have relatively low levels of arginase. Hence, an enzymatic complementarity exists between red blood cells and peripheral tissues with regard to the conversion of arginine to proline; i.e., red blood cells convert arginine to ornithine, and release into plasma. Peripheral tissues take up this ornithine and convert it sequentially to pyrroline -5- carboxylate and proline. The arginase activity present in RBC may be important also during healing process, where red blood cells within a fibrin clot can act as a metabolic source of ornithine [2, 6.]

Growing fetus requires polyamines for cell proliferation and differentiation and proline for bone growth (13). Fetal growth and development are dependent on the adequate provision of substrates from the maternal circulation. Red blood cells act as source of amino acids from one site to another and they have a role in

interorgan amino acid transport[13]

Investigators have suggested that there are various sources such as myometrium, placenta and plasma which supply ornithine required for the fetal growth and development. Experimental evidence suggests that, myometrial arginase activity increases ~ 25-fold during pregnancy to supply the rapidly growing fetus with polyamines to facilitate cell proliferation. [10]. Elevated arginase activity observed in the placental villi in the early gestational period may be responsible for proliferation of trophoblasts by increasing polyamines production. These results suggest that the l-arginine-ornithine-polyamine pathways play a role in placental growth and development [14]

A Study conducted in rats suggests that plasma arginase activity is increased in late pregnancy as well as during lactation, but not reflected on the circulating urea level. The arginase activity changes have been related to known changes in feeding and nitrogen handling pattern as well as hormonal variation[15].

In the present we found that erythrocyte arginase activity increased significantly in pregnant women. There is significant decrease in the serum urea level in pregnant women due to hemodilution. This suggests that the increased maternal erythrocyte arginase activity may be an additional source of ornithine required for fetal growth and development. However, there are few limitations. Number of sample used for the study is less. Another limitation is that arginase activity has been studied only in the third trimester

## Conclusion

Erythrocyte arginase activity increases in the third trimester of pregnancy .This suggests that it has a role in providing ornithine for fetal growth and development. To the best of our knowledge, this study is first of its kind. Investigation of erythrocyte arginase activity in all trimesters of pregnancy is required to correlate the increase in activity with progressive fetal growth.

Table 1: Levels of hemoglobin, serum urea and erythrocyte arginase in Non –pregnant women and pregnant women

| Parameters | Non –pregnant women (n= 20) | Pregnant women (n= 25) | Significance ( p value) |
|---|---|---|---|
| Hemoglobin (gm/dl) ( mean± SD) | 12.2 ± 0.56 | 10.56 ± 0.9 | 0.001 |
| Serum urea (mg/dl) ( mean± SD) | 32.16 ± 3.12 | 22.0 ± 0.56 | 0.001 |
| Erythrocyte arginase (µmol/gm Hb) (mean± SD) | 6.13 ± 0.68 | 17.92± 3.2 | 0.0001 |

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# FINGERPRINT ANALYSIS OF ETHNIC FEMALE KUWAITIS

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Abstract:
Fingerprint patterns and palmar dermatoglyphics have, over the decades not only been of significant application in identity establishment in forensics, but also played an adjunct role in diagnosis and confirmation of quite a few medical afflictions and syndromes.
Many data banks on fingerprint profiles covering gender and diverse populations, ethnic groups and occupations have been compiled and are readily available for application and analysis. Observations of oddities, peculiarities or exclusiveness in print patterns across peoples have led to the accumulation of reliable markers that aid diagnostics and in specific assignments based on sex, disease, inheritance (both genotype and phenotype) and in classifications.
This paper presents observations on fingerprint patterns and their variations found in adult Kuwaiti females from the Middle-east. A total of 360 prints were collected and results were collated for analysis. The results show a distinct difference in pattern in this ethnic populace, an increased frequency in ulnar loops. This undertaking, to my knowledge, is the first ever to cover a native Arab, could form the nucleus for more such and larger scale studies.
Keywords : dermatoglyphics, dactylography, physical anthropology, ethnicity

## Introduction

The history fingerprinting and their study (dactylography) has special significance to India as it is the original home to the first ever forensic fingerprint institute to be established in 1897 (Calcutta) the world. Since those early times, the science of recording fingertip pulp prints and their classification into characteristic individualized patterns, according to their digital ridge counts and shapes, has grown in leaps and bounds universally. The near foolproof system of establishing identity through exclusively individual characteristics has formed the backbone and mainstay in forensic investigations and in criminal judicial processes. However, despite the impressive and time proven record of dactylography as a major and reliable tool in physical anthropological sciences, the potential of application of dermatopglyphics as a dependable and adjunct tool n the world of diagnostics and clinical medicine has remained unexploited.

In recent years, analysis of specific loci of the proximal and distal tri-radii and the measurements of the A t D angles through palmar prints has emerged as a significant subject area for research in prognosticating and diagnosing a slew of medical afflictions, congenital or acquired. The study of palmar ridges, creases and flexure lines and their variations led to the consistent observation of a transverse palmar crease (simian crease) as an unfailingly accurate external marker for Down syndrome is proof enough if any needed be provided, on the scope and ambit of dermatoglyphics and dactylography as major areas for clinical investigations and research. This paper however, does not include the observations on palm patterns in its scope. Significantly, in the world of physical and forensic anthropology, the analysis of prints from data banks the world over has resulted in extraction of reliable and conclusive similarities in print patterns distinct to populations, geo-locales, races and ethnic groups (1).

In this paper we present and analyze fingerprint patterns from 36 adult Kuwaiti women and compare the results with that found in other population groups. The middle

eastern population is of ethnic Arab descent and little, if anything, by way of dermatographics is known about these nationals. Though small, the sample population taken up for this study is perhaps the first ever to be researched on.

## MATERIAL & METHODS

Individual digital and palm prints were collected from 36 healthy adult female volunteers from the Faculty of Medicine of Kuwait University. Each volunteer was required to impress transfer inked palm and finger prints onto clean non-absorbent white drawing sheet. Two palm prints and ten finger prints were taken from each individual. Each print was then separately studied using hand-held magnifying lens. The patterns, shapes, ridge counts, variations and oddities (if any) for each finger print was recorded.

The results of the observations were tabulated for statistical analysis and comparison.

## Observations & Results

The results of this study are presented in the Tables I, II, III, IV, V & VI. The most significant of the observations was that the radial loop pattern found in Kuwaitis was 5 to 10 times higher when compared to similar loops in other population groups. Taking only the female gender for comparison showed that this loop was 4 times more in Arab women than in females elsewhere. The ulnar loop too, was found strikingly lower in numbers in the study group when compared to the results from that recorded in other studies.

The percentage and numbers of whorls and arches however were consistent with those recorded in other data compilations. The levels in individual print pattern variations were same as those found in the general population elsewhere.

## Discussion

For long, the hand has been considered as a mirror of health and disease. The size, shape, pallor, elasticity, strength, mobility of the hand have been used as diagnostic factors in general physical examination: more specifically the positions of fingers, their lengths, positions, shapes metacarpal formulas, nail growth rates patterns and rates have often led to clues to particular affliction or disease, be it congenital or acquired (2). Study of the skin of the hand, more importantly on its volar aspect, such flexure lines, creases, finger tip ridge shapes and counts, are covered under the science of palmar dermatoglyphics (3) and dactylography.

Research work involving the creases and patterns, apart from use as base markers by themselves, have also led to newer applications. The position of the distally positioned tri-radii ( A B C & D) and the A t D angle formed by the first and last digital base tri-radius (A & D) with the axial tri-radius (t) has been very effectively and confidently used and applied and are of pathognomic value if congenital genetically inherited conditions such as Marfan's syndrome

It is known that 20% to 30% of hand and fingerprints show bilateral asymmetry in patterns. Whorls and radial loops being more frequent on the right side and ulnar loops being more common on the left. Females generally show a tendency to be lower in presence of whorls and radial loops while simultaneously display higher numbers in arches; World over, men show a more complex print pattern than in women.

In contrast and in contradiction the observed norm, this study, however, women showed a higher frequency of ulnar loops on their right hand than on the left. The significance or import of the observed ethnic variations are still nebulous and may require larger study groups covering bigger populations in the middle-east for interpretation.

## Conclusions

Large scale studies on diverse populations and large

Finger print patterns, ridge counts, palmar crease studies, flexure line analysis, statistical and scalar and angular analysis of tri-radii loci (A t D) could throw up much by way of our understanding and interpretations on the complexities of disease diagnosis and treatment: With wider applications of collated data and further research, the science of dermatotoglyphics and dactylography may yet prove to be a more potent and reliable tools in medical armamentarium than they presently are.

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I wish to thank the Chairman, Department of Anatomy, Faculty of Medicine, Kuwait University for permitting me to conduct this study during my tenures as Visiting Professor.

Table - I
(Fingerprint patterns)

| | RIGHT | | | | | LEFT | | | | | RIGHT | | | LEFT | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | A | L | W | A | L | w |
| 1 | UL | UL | TA | TA | UL | TA | TA | TA | TA | RL | 2 | 3 | | 4 | 1 | |
| 2 | SW | CW | UL | UL | UL | CW | SW | TA | RL | RL | | 3 | 2 | 1 | 2 | 2 |
| 3 | UL | CW | UL | SW | UL | RL | TA | TLW | SW | SW | | 3 | 2 | 1 | 1 | 3 |
| 4 | UL | SW | UL | SW | UL | SW | SW | SW | CPW | RL | | 3 | 2 | | 1 | 4 |
| 5 | SA | SA | UL | CPW | TA | SA | SA | RL | RL | CPW | 3 | 1 | 1 | 2 | 2 | 1 |
| 6 | CW | SW | UL | CPW | CPW | CW | SW | RL | RL | RL | | 1 | 4 | | 3 | 2 |
| 7 | CW | CW | CPW | SW | CPW | CW | RL | CW | CW | UL | | | 5 | | 2 | 3 |
| 8 | UL | TA | UL | UL | UL | CW | SA | RL | RL | SA | 1 | 4 | | 2 | 2 | 1 |
| 9 | UL | UL | UL | TLW | SW | RL | RL | TA | CPW | CW | | 3 | 2 | 1 | 2 | 2 |
| 10 | UL | CW | UL | CPW | UL | RL | RL | RL | RL | RL | | 3 | 2 | | 5 | |
| 11 | UL | TA | UL | SW | CW | RL | TLW | RL | CW | CW | 1 | 2 | 2 | | 2 | 3 |
| 12 | UL | TA | TA | TA | UL | RL | TA | RL | RL | CPW | 3 | 2 | | 1 | 3 | 1 |
| 13 | CW | SW | UL | SW | UL | TLW | CPW | RL | RL | RL | | 2 | 3 | | 3 | 2 |
| 14 | CW | SW | SW | SW | CW | CW | SW | SW | SW | CLW | | | 5 | | | 5 |
| 15 | UL | TA | UL | NC | NC | RL | RL | TA | RL | RL | 1 | 2 | | 1 | 4 | |
| 16 | CW | TLW | SW | CW | CPW | SW | SW | RL | SW | RL | | | 5 | | 2 | 3 |
| 17 | RL | TA | RL | RL | RL | TLW | UL | RL | RL | RL | 1 | 4 | | | 4 | 1 |
| 18 | CW | SW | SW | SW | SW | RL | CW | CW | TLW | RL | | | 5 | | 2 | 3 |
| 19 | SW | TLW | SW | SW | CPW | SW | CPW | CPW | CPW | RL | | | 5 | | 1 | 4 |
| 20 | TLW | UL | UL | SW | SW | CW | RL | RL | TLW | RL | | 2 | 3 | | 3 | 2 |
| 21 | UL | UL | CW | SW | RL | RL | RL | RL | CW | RL | | 3 | 2 | | 4 | 1 |
| 22 | UL | UL | UL | UL | UL | RL | RL | RL | SW | RL | | 5 | | | 4 | 1 |
| 23 | UL | UL | UL | CW | UL | RL | TA | RL | CPW | RL | | 4 | 1 | 1 | 3 | 1 |
| 24 | CW | TLW | UL | UL | UL | TLW | RL | RL | RL | RL | | 3 | 2 | | 4 | 1 |
| 25 | SW | TLW | UL | UL | UL | TLW | SW | RL | RL | RL | | 3 | 2 | | 3 | 2 |
| 26 | UL | UL | UL | UL | UL | RL | RL | RL | RL | RL | | 5 | | | 5 | |
| 27 | UL | UL | UL | UL | UL | RL | UL | UL | RL | RL | | 5 | | | 5 | |

Table - I (Continued)

| | RIGHT | | | | | LEFT | | | | | RIGHT | | | LEFT | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | A | L | W | A | L | w |
| 28 | UL | CPW | SW | SW | UL | RL | SW | SW | CPW | RL | | 2 | 3 | | 2 | 3 |
| 29 | UL | RL | UL | UL | UL | RL | RL | RL | CPW | RL | | 5 | | | 4 | 1 |
| 30 | SW | SW | UL | CPW | UL | SW | CPW | TA | CPW | CPW | | 2 | 3 | 1 | | 4 |
| 31 | UL | CPW | UL | UL | UL | RL | RL | RL | RL | RL | | 4 | 1 | | 5 | |
| 32 | UL | CPW | CPW | CPW | CPW | RL | CPW | CPW | CPW | UL | | 1 | 4 | | 2 | 3 |
| 33 | UL | UL | UL | UL | UL | RL | CW | RL | RL | RL | | 5 | | | 4 | 1 |
| 34 | CPW | CPW | UL | SW | UL | SW | SW | CPW | SW | CPW | | 2 | 3 | | | 5 |
| 35 | CPW | CW | UL | RL | UL | SW | CW | RL | SW | RL | | 3 | 2 | | 2 | 3 |
| 36 | UL | CPW | UL | UL | UL | RL | RL | RL | CPW | RL | | 4 | 1 | | 4 | 1 |
| | | | | | | | | | TOTAL | | 12 | 94 | 72 | 15 | 96 | 69 |

Table – II
(Total Patterns)

| | ARCHES | LOOPS | WHORLS |
|---|---|---|---|
| RIGHT | 12 | 94 | 72 |
| LEFT | 15 | 96 | 69 |
| TOTAL | 27 | 190 | 141 |

Table – III
(Percentage Frequencies)

| | ARCH | | RADIAL LOOP | | ULNAR LOOP | |
|---|---|---|---|---|---|---|
| DIGIT | Right | Left | Right | Left | Right | Left |
| I | 1 | 2 | 1 | 18 | 20 | 0 |
| II | 6 | 6 | 1 | 12 | 9 | 2 |
| III | 2 | 5 | 1 | 21 | 25 | 1 |
| IV | 2 | 1 | 2 | 15 | 11 | 0 |
| V | 1 | 1 | 2 | 25 | 22 | 2 |
| TOTAL | 12 | 15 | 7 | 91 | 87 | 5 |
| MEAN | 2.7 | | 9.8 | | 9.2 | |
| % | 7.5 | | 27.72 | | 25.5 | |

Table – IV
(% Comparative data)

| ORIENTAL | WHORL Mean | UL Mean | RL Mean | ARCHES Mean |
|---|---|---|---|---|
| 1. Caucasians | 35.4 | 55.5 | 4.3 | 4.3 |
| 2. Negroes | 27.1 | 61.4 | 2.6 | 8.8 |
| 3. Asian Ind. | 42.6 | 33.59 | 2.2 | 3.4 |
| 4. Kuwaiti | 39.16 | 25.5 | 27.72 | 7.5 |

Table – V
Percentage data on female population

| FEMALES | ARCH | RADIAL LOOP | ULNAR LOOP | WHORLS |
|---|---|---|---|---|
| World | 9.2 | 3.8 | 63.0 | 24.0 |
| Kuwaiti | 7.5 | 27.72 | 25.5 | 39.16 |

Table – VI
(Digital Pattern Types)

A. Caucasians

| | | | | | |
|---|---|---|---|---|---|
| Whorl | IV | I | II | III | V |
| Loop | V | III | I | IV | II |
| Arch | II | III | I | IV | V |

B. Negroes

| | | | | | |
|---|---|---|---|---|---|
| Whorl | I | IV | II | III | V |
| Loop | V | III | IV | II | I |
| Arch | II | I | III | IV | V |

C. Asian Ind.

| | | | | | |
|---|---|---|---|---|---|
| Whorl | I | IV | II | V | III |
| Loop | V | III | II | I | IV |
| Arch | II | III | I | IV | V |

D. Kuwaiti

| | | | | | |
|---|---|---|---|---|---|
| Whorl | IV | II | I | V | III |
| Loop | V | III | I | IV | II |
| Arch | II | III | I | IV | V |

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# DEVELOPMENT OF GASTRO RETENTIVE FLOATING MATRIX TABLETS OF DILTIAZEM HYDROCHLORIDE

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Abstract:

The objective of the present investigation was to formulate and evaluate hydrodynamically balanced floating matrix controlled drug delivery system of diltiazem hydrochloride. Floating matrix tablets are associated with advantages of increased bioavailability and minimizing the dosing frequency. Diltiazem hydrochloride is a calcium channel blocker, an anti-hypertensive and anti-anginal drug, which undergoes extensive firstpass metabolism and display poor bioavailability. It has an elimination half-life of 3 to 4.5 h and an absorption zone from the upper intestinal tract. Gastric floating of diltiazem hydrochloride tablets results from effervescence produced by the reaction between sodium bicarbonate and hydrochloric acid in stomach. Seven formulations of floating tablets were prepared using direct compression technique with low viscosity polymer such as HPMC K100LV, high viscosity polymers such as HPMC K4M, K15M, and carbopol in different ratios. The evaluation results revealed that all formulations comply with the specification of official pharmacopoeias and/or standard reference with respect to general appearance, content uniformity, hardness, friability and buoyancy. Accelerated stability studies carried out at different temperatures, 27 ± 2 °C, 40 ± 2 °C and 7 ± 2 °C did show no changes in physicochemical properties at the end of 8 weeks indicating all the formulations are stable. Out of all the formulation developed, formulation $F_6$ containing equal ratio of HPMC K4M and K100LV showed optimum floating time and *in vitro* drug release of 82.19% at the end of 8 h. Thus it is summarized; high viscosity grade polymer HPMC K4M, low viscosity grade polymer HPMC K100LV and carbopol can be successfully used in formulation of sustained release gastro retentive floating drug delivery system.

Keywords : Gastro retentive floating matrix tablets, controlled drug release, diltiazem hydrochloride, low density polymers, high density polymers

## Introduction

The oral drug delivery is by far the most preferable route of drug delivery system, due to ease of administration, patient compliance, and flexibility in formulation etc. It is evident from the recent scientific and patent literature that an increased interest in novel dosage form that are retained in stomach for a prolonged period of time exists today in academic and industrial research groups[1]. One of the most feasible approaches for achieving a prolonged and predictable drug delivery in the GI tract is to control the gastric residence time, i.e. gastro retentive dosage form. Effective oral drug delivery may depend upon the factor such as gastric emptying process, gastro intestinal transit time of dosage form, drug release from the dosage form and site of absorption of drug. Most of the oral dosage forms possess several physiological limitations such as variable gastrointestinal transit, because of variable gastric emptying leading to non-uniform absorption profiles, incomplete drug release and shorter residence time of the dosage form in the stomach. The gastric emptying of dosage forms in humans is affected by several factors because of which wide inter- and intra-subject variations are observed. Since many drugs are well absorbed in the upper part of the gastrointestinal tract, such high variability may lead to non-uniform absorption and makes the bioavailability

unpredictable[1,2,3]. Among the various gastro retentive systems, gastric floating drug delivery systems (GFDDS) offer numerous advantages over the gastric retentive systems. These systems have a density lower than the gastric fluids and thus remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. While the system is floating on the gastric contents, the drug is released slowly at a desired rate from the stomach[4,5,6]. Cardiovascular diseases are one of the life threatening diseases of the world. Angina pectoris, hypertension and cardiac failure are the commonest diseases and require constant monitoring[7,8]. Calcium channel blockers are emerging as very important group of the management of angina pectoris and hypertension. Diltiazem hydrochloride is a calcium channel blocker. It is widely prescribed for the treatment of hypertension and angina. Diltiazem hydrochloride undergoes an extensive biotransformation results in bioavailability of 30% to 40% only. It has an elimination half-life of 3 to 4.5 h and has an absorption zone from the upper intestinal tract. Efficacy of the administered dose may get reduced due to incomplete drug release from the device above the absorption zone. The dosage is 30 mg, 4 times a day and increased as necessary up to 360 mg/day in divided doses[9,10]. Due to short half-life diltiazem hydrochloride require frequent administration. These disadvantages can overcome by developing a floating dosage form to be remained buoyant in the stomach. The gastroretentive drug delivery systems can be retained in the stomach and assist in improving the oral sustained delivery of drugs that have an absorption window in a particular region of the gastrointestinal tract. These systems help in continuously releasing the drug before it reaches the absorption window and thus ensuring optimal bioavailability.

## Materials and Methods

Diltiazem Hydrochloride was obtained as a gift by Cadila Zydus, Ahmedabad and HPMC K4M, HPMC K15M, HPMC KIOOLV and Carbopol 934P was gifted by Wockhardt, Aurangabad.

### Formulation of floating matrix tablets of Diltiazem Hydrochloride by direct Compression

The powder mixture containing drug, polymers and other excipients were weighed as per required quantity and thoroughly blended in a mortar and pestle and then passed through sieve No. 100. An Appropriate amount of the mixture was weighed and fed into the die of Minipress II using 8 mm punch to get tablets of average weight of 250 mg[11,12,13,14]. The formula for the different batches is given in the Table 1.

### Evaluation of Floating Tablets of Diltiazem Hydrochloride

#### I. Pre-Compression Evaluation Parameters

(a) Angle of Repose

The angle of repose of powder blend was determined by the funnel method. Accurately weighed powder blend was taken in the funnel. The height of the funnel was adjusted in such a way the tip of the funnel just touched the apex of the powder blend. The powder blend was allowed to flow through the funnel freely on to the surface. The diameter of the powder cone was measured and angle of repose was calculated using following formula[15,16].

$$\text{Tan } \theta = h/r$$

$$\theta = \tan^{-1} h/r$$

Where, $\theta$ = angle of repose, h = height, r = radius.

(b) Bulk Density

The bulk density of a powder is dependent on particle packing and changes as the powder consolidates. A consolidated powder is likely to have a greater arch strength than a less consolidated one and therefore more resistant to powder flow. The ease with which a

powder consolidates can be used as an indirect method of quantifying powder. Apparent bulk density (g/ml) was determined by pouring preserved bulk powder into a graduated cylinder via a large funnel and measuring the volume and weight[15,16]. Bulk density can then be calculated by the following formula.

$$\text{Bulk density} = W/Vo$$

Where, W = wt. of powder, $V_0$ = initial volume.

(c) Tapped Density

A quantity of 2 gm of powder blend from each formula, previously shaken to break any agglomerates formed, was introduced in to 10 ml measuring cylinder. After that the initial volume was noted and the cylinder was allowed to fall under its own weight on to a hard surface from the height of 2.5 cm at second intervals. Tapping was continued until no further change in volume was noted[15,16]. Tapered density was calculated using the following equations.

$$\text{Tapped density} = W/V_f$$

Where, W = wt. of powder, $V_f$ = final volume.

(d) Compressibility Index (Carr's Consolidation Index)

The Compressibility index is measure of the propensity of a powder to be compressed. As such, they are measures of the relative importance of interparticulate interactions. In a free-flowing powder, such interactions are generally less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater interparticle interactions, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the Compressibility Index[15,16]. The compressibility index is calculated using measured values for bulk density ($D_b$) and tapped density ($D_t$) as follows:

$$\text{Compressibility index} = Dt\text{-}D_b/D_t \text{ X } 100$$

Where $D_b$= Bulk density, $D_t$= Tapped density

II. Post- Compression Parameters

(a) Tablet Dimensions

Thickness and diameter of five tablets randomly selected were measured using vernier calipers. The Pharmacopoeia states that the extent of deviation in a batch of tablet should not exceed the limit of ± 5% of their determined standard values[16,17].

(b) Hardness Test

The crushing strength kg/cm$^2$ of prepared tablets was determined for tablets of each batch by Monsanto tablet hardness tester. Hardness indicates the ability of a tablet to withstand mechanical shocks while handling[16,17].

(c) Friability Test

The friability of tablets was determined using Roche friabilator. It is expressed in percentage (%). Ten tablets randomly selected were initially weighed ($W_0$ initial) and transferred into friabilator. The friabilator was operated at 25 rpm for 4 minutes or run up to 100 revolutions. The tablets were weighed again (W final)[16,17]. The percentage friability (%F) was then calculated by

$$\%F = (1 - W/W_0) \times 100$$

Where, $W_0$ = weight of tablet before test,

W = weight of tablet after test.

(d) Weight Variation Test

Twenty tablets were selected randomly from each batch and weighed individually using electronic balance (Ohause) to check for weight variation[16,17]. Pharmacopoeial parameters are displayed in Table 2.

(e) Drug Content Estimation

Ten tablets were randomly selected and powdered. A quantity of powder equivalent to 60 mg of diltiazem hydrochloride was accurately weighed and transferred into a 100 ml volumetric flask and dissolved in 0.1 N HCl and the volume was made with 0.1 N HCl (pH 1.2). The flask was shaken on a flask shaker for 24 h and was kept for 12 h for the sedimentation of undissolved materials.

The solution was filtered through Whattman filter paper. 1 ml of the above solution was transferred to a 100 ml volumetric flask and diluted to 100 ml with 0.1 N HCl and the absorbance was measured at 236 nm using UV / visible spectrophotometer (Shimadzu UV – 1600/1700). The percentage of diltiazem hydrochloride was determined using calibration curve[9,10].

(f) In Vitro Buoyancy Test

The prepared tablets were subjected to in vitro buoyancy test by placing them in 250 ml beaker containing 200 ml 0.1 N HCl (pH 1.2, temp. 37±0.5 °C). The time between introduction of the dosage form and its buoyancy in the medium and the floating durations of tablets was calculated for the determination of lag time and total buoyancy time by visual observation. The Time taken for dosage form to emerge on surface of medium called Floating Lag Time (FLT) or Buoyancy Lag Time (BLT) and total duration of time by which dosage form remain buoyant is called Total Floating Time (TFT)[9,10,11,13].

(g) In Vitro Dissolution Studies

The dissolution study was carried out using USP II (paddle method) apparatus in 900 ml of 0.1 N HCl (pH 1.2) for 12 h. The temperature of the dissolution medium was kept at 37± 0.5 ºC and the paddle was set at 100 rpm. 10 ml of sample solution was withdrawn at specified interval of time and filtered through Whattman filter paper. The sample was replaced with fresh dissolution medium. The sample diluted to a suitable concentration with 0.1 N HCL. The absorbance of the withdrawn samples was measured at $\lambda_{max}$ 236 nm using a Shimadzu UV-1600/1700 series spectrophotometer[11,13,20].

(h) Stability Studies

The stability studies of all the formulations were studied at different temperatures using the reported standard procedure. The tablets were wrapped in aluminum foil and placed in Petri dishes. These containers were stored at ambient humid conditions, at room temperature (27 ± 2 °C), oven temperature (40 ± 2 °C) and in refrigeration temperature (7 ± 2 °C) for a period of 8 weeks. The samples were analyzed for physical changes such as color, texture, in vitro floating time[21].

Results and Discussion

I. Pre-Compression Evaluation Parameters

The angle of repose for all the powders lies below 25°, indicating excellent flow properties. The Compressibility Index (Carr's Consolidation Index) is determined from the values of bulk density and tapered density. The values from the Table 3 reveals that HPMC K4M, K15M and K100LV are having good flow properties as their values are in range of 12 to 16, where as the values between 18 to 21 of carbopol 934P and diltiazem hydrochloride indicates fair flow properties. The overall values obtained from the study of Pre-Compression evaluation parameters indicates that the powder blend have the required flow property to undergo tablet formulation by direct compression technique.

II. Post-Compression Parameters

Tablet Dimensions

Thickness and diameter of the seven formulations did not exceed the limit of ± 5% of the determined standard value; hence all the formulations comply with the proposed limits of pharmacopoeia. The values are shown in the tablets 4.

Hardness Test

The measured hardness of tablets of each batch ranged between 6.5 to 7.3 kg/cm$^2$ as reported in Table 4. This ensures good handling characteristics of all batches.

Friability Test

The % friability was found to be in the range 0.52% to 0.80% ensuring that the tablets are mechanically stable. The values of friability test are tabulated in Table 4.

Weight Variation Test

All the formulated tablets comply with the weight variation limits of the Indian Pharmacopoeia as the values are in the range of 1.5% to 3% as displayed in Table 4.

Drug Content Estimation

The percentage of drug content for $F_1$ to $F_7$ was found to be in the range of 97.82% to 99.41%, as tabulated in Table 4, of diltiazem hydrochloride, it complies with official specifications.

In Vitro Buoyancy Test

In Vitro buoyancy test was performed on all the batches ($F_1$ to $F_7$). On immersion in 0.1N HCL solution pH (1.2) at 37 °C, the tablets floated, and remained buoyant without disintegration. Table 5 shows the results of in vitro buoyancy study from the results it can be concluded that the batch containing high viscosity grade HPMC K4M or HPMC K15M polymer and carbopol i.e. formulations $F_1$, $F_4$ and $F_6$ showed good buoyancy, total floating time of more than 8 h, whereas formulation $F_3$ containing low viscosity grade polymer HPMC K100LV displayed floating time of less than 5 h and the formulations $F_2$, $F_5$ and $F_7$ floated till 7 h.

In Vitro Dissolution Studies

In vitro dissolution studies showed highest drug release of 82.19% from the formulation $F_6$ followed by $F_5$, $F_2$, $F_7$, $F_3$, $F_4$ and $F_1$. $F_6$ formulation contains equal ratio of HPMC $K_4$M and K100LV. Fig 1 shows the drug release pattern of all the formulations.

Discussion

The overall values obtained from the study of Pre-Compression evaluation parameters indicates that the powder characteristics of diltiazem hydrochloride, HPMC K100LV, K4M, K15M, and carbopol 934P, with the selected excipients has the optimum flow properties necessary to be blended together and undergo tablet formulation by direct compression technique.

The Post-Compression evaluation parameters of 7 formulation, reveals that, all the prepared formulation comply with the official standards mentioned in pharmacopoeia for tablet dimension, hardness test, friability test, weight variation test and drug content estimation. The in vitro buoyancy studies showed optimum floating time of more than 8 h for the formulation $F_1$, $F_4$ and $F_6$ when compared to other formulations. The results reviles that the formulation $F_6$ containing equal ratio of HPMC K4M and K100LV is the best formulations as the extent of in vitro drug release was found to be 82.19% in 8 h and followed zero order drug release kinetics. The drug release profile of $F_6$ formulation appear to be biphasic; as the first phase is characterized by initial burst effect followed by controlled slow release in second phase. This peculiar behavior may be explained as, when the tablet comes in contact with dissolution medium, the low viscosity grade polymer HPMC K100LV result in faster and greater amount of gel formation initially resulting in immediate drug release from the pores formed during gelling. The drug release is then controlled by the high viscosity grade polymer HPMC K4M which also contributes to higher floating time i.e. more than 8 h. when high molecular weight carbopol 934P, a cross linking polymer, comes in contact with water it swells and hold water inside the microgel network. This property helps in partially retarding the drug release which further enhances the control release property of the formulated gastro retentive floating drug delivery system. The tablets remained intact during the dissolution period. The accelerated stability studies performed at room temperature (27 ± 2 °C), oven temperature (40 ± 2 °C) and in refrigeration temperature (7 ± 2 °C) did show no changes in physicochemical properties at the end of 8 weeks indicating all the formulations are stable.

Conclusion

The pre-compression evaluation parameters concludes

that it is feasible to formulate diltiazem hydrochloride gastro retentive floating matrix tablet by direct compression technique, using low viscosity polymer such as HPMC K100LV, high viscosity polymers such as HPMC K4M, K15M, and carbopol 934P in different ratios.

The post-compression evaluation parameters reveals prepared Floating matrix tablets of diltiazem hydrochloride possessed optimum tensile strength, hardness and friability. This indicates good mechanical strength for handling and transportation of the prepared tablet formulation. It was observed that the floating matrix tablet formulated with equal ratio of high viscosity grade polymer HPMC K4M and low viscosity grade polymer HPMC K100LV with carbopol 934P resulted not only in higher in vitro buoyancy time but also showed optimum controlled in vitro drug release profile that followed zero order kinetics. The fact this tablet formulation also remained intact during the dissolution period, is one of the encouraging observations. The Accelerated stability studies carried out at different temperatures did show no changes in physicochemical properties at the end of 8 weeks concluding all the formulations are stable.

The specific objective of formulation and evaluation of the gastro retentive floating matrix tablets of diltiazem hydrochloride for reducing the dose frequency and control release of the diltiazem hydrochloride is optimally achieved. There is a need to extend the formulations reported in this research article for commercial exploitation.

Table 1. Formulation composition of floating matrix tablets of diltiazem hydrochloride

| Ingredients | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | $F_6$ | $F_7$ |
|---|---|---|---|---|---|---|---|
| | (mg) | (mg) | (mg) | (mg) | (mg) | (mg) | (mg) |
| Diltiazem Hydrochloride | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| HPMC K4M | 75 | - | - | 50 | - | 50 | 37.5 |
| HPMC K15M | - | 75 | - | 50 | 50 | - | 37.5 |
| HPMC KLOOLV | - | - | 75 | - | 50 | 50 | 37.5 |
| Carbopol 934P | 75 | 75 | 75 | 50 | 50 | 50 | 50 |
| Sodium bicarbonate | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| Lactose | 29.6 | 29.6 | 29.6 | 29.6 | 29.6 | 29.6 | 29.6 |
| Magnesium stearate | 5.2 | 5.2 | 5.2 | 5.2 | 5.2 | 5.2 | 5.2 |
| Talc | 5.2 | 5.2 | 5.2 | 5.2 | 5.2 | 5.2 | 5.2 |

Each tablet contains uniform weight of 250 mg.

Table 2. IP standards of percentage of weight variation

| Percentage deviation allowed under weight variation test. | |
|---|---|
| Average weight of tablet | Percentage deviation |
| 80 mg or less 10 | 10 |
| More than 60 mg but less than 250 mg 7.5 | 7.5 |
| 250 mg or more 5 | 5 |

Since, the average weight of the tablets is 250 mg; the percentage deviation is taken as ± 5

Table 3. Pre-Compression evaluation parameters

| Pre-Compression evaluation parameters | HPMC K4M | HPMC K15M | HPMC K100LV | Carbopol 934P | Diltiazem hydrochloride |
|---|---|---|---|---|---|
| Angle of Repose | 17.28 | 18.26 | 18.12 | 19.28 | 20.67 |
| Bulk Density | 0.472 | 0.471 | 0.475 | 0.485 | 0.446 |
| Tapped Density | 0.561 | 0.568 | 0.555 | 0.598 | 0.568 |
| Compressibility/ Carr's Index | 15.86 | 17.077 | 14.414 | 18.896 | 21.478 |

Table 4. Post-Compression parameters

| Formulation Code | Tablet thickness ± SD (mm) | Diameter (mm) | Hardness ± SD kg/cm$^2$ | Friability (%) | Weight variation | Drug content (%) test (%) |
|---|---|---|---|---|---|---|
| $F_1$ | 2.7 ± 0.04 | 8 | 6.5 | 0.25 | 2.29 | 99.41 |
| $F_2$ | 2.6 ± 0.02 | 8 | 7.2 | 0.64 | 1.84 | 99.00 |
| $F_3$ | 2.9 ± 0.01 | 8 | 6.9 | 0.80 | 2.37 | 97.82 |
| $F_4$ | 2.7 ± 0.03 | 8 | 6.7 | 0.55 | 2.19 | 98.28 |
| $F_5$ | 2.6 ± 0.02 | 8 | 6.8 | 0.72 | 2.71 | 99.25 |
| $F_6$ | 2.9 ± 0.01 | 8 | 7.3 | 0.55 | 2.04 | 98.51 |
| $F_7$ | 2.5 ± 0.03 | 8 | 7.1 | 0.60 | 2.90 | 98.82 |

SD = Standard Devation

Table 5. In Vitro Buoyancy studies

| Formulation Code | Buoyancy Lag Time (sec) | Total Floatation Time (h) |
|---|---|---|
| F1 | 100 | >8 |
| F2 | 120 | ~7 |
| F3 | 150 | ?5 |
| F4 | 120 | >8 |
| F5 | 125 | ~7 |
| F6 | 132 | >8 |
| F7 | 140 | ~7 |

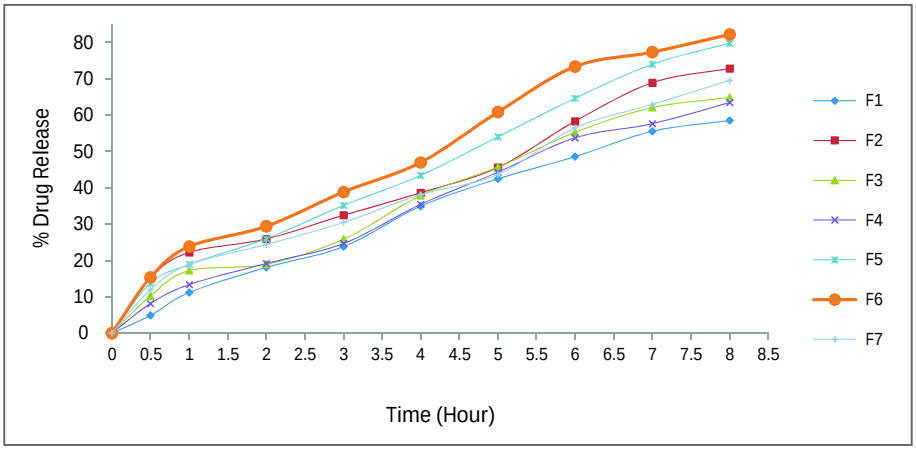


Fig 1. In-vitro dissolution profile of formulations $F_1$ to $F_7$

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Short Communications

# MATRIX BUTTON PROVISIONALS

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Abstract:
Fabrication of provisional restorations has been explained using different materials and techniques in the past. Formation of an external matrix before preparation of the abutment, which is later used in combination with a resin material, is one of the techniques. Here, a new material has been used as matrix in direct, indirect and indirect-direct methods to produce esthetic and functional provisional restorations. There is neither wasting of chair side time nor a second visit for the patient.

Keywords : Provisional Restorations, External Surface Form, Thermoplastic, Matrix

MATRIX BUTTON PROVISIONALS

## Introduction

Provisional Restorations are an integral part of fixed prosthodontic treatment. Malone et al[1] named them as "treatment restorations" or "Healing Matrix" and explained various metallic and non-metallic materials for their fabrication. For non-metallic materials there are a variety of techniques available to suit the individual needs of the clinician and the situation, whether it is an inlay, single crown, bridge, complete arch restoration or even implant.[1-5]

Malone et al[1], Rosenstiel et al[6] and Dykema et al[7] suggest a method to form a mold cavity or an External Surface Form, into which a plastic material is poured and the internal or the tissue surface form is fabricated. For creating external surface form, an alginate impression, base plate wax impression, silicone putty impression, polycarbonate preformed shell, or custom preformed Fixed Partial Denture (FPD) shells can be used. They can also be custom fabricated using a thermoplastic sheet, which is heated and adapted to a stone cast with vacuum or air pressure while the material is still pliable. This requires an electric heating element and a vacuum source.

The purpose of this article is to present a new thermoplastic material that does not require elaborate equipment for its manipulation. It is a circular disc of thickness about 2 millimeters and diameter 22 millimeters.[8] The softening temperature is around 65 - 70$^{C}$ and the softening time is approximately 45 seconds. There is a change in color from white to clear when the disc is completely soft and ready to be kneaded. (Figure 01) Working time is around 5 minutes.

Provisional restorations can be fabricated using any one of the following techniques:

Techniques:

I INDIRECT

1. Diagnostic casts of the entire mouth are required for this method. On the casts, make all the modifications as per the case. For the edentulous area, an artificial tooth may be arranged in wax, corresponding to the patient's occlusion. (Figure 02)
2. The matrix is formed on the cast. More than one disc can be softened and kneaded according to the requirement. Place discs in hot water bath at 65 - 70$^{C}$ in a clean rubber bowl or ceramic cup. In approximately 45 seconds discs change their color from white to clear. (See Figure 01)
3. Pick up the soft discs using tweezers and knead them to form an oblong mass.
4. Press it down on the occlusal surfaces of the teeth on the cast and mold it buccally, lingually and into the

undercuts. (Figure 03)

5. Mark the buccal side with explorer for reference while reseating later. Cool the matrix with air syringe for 30 seconds and watch the color revert back to white! Remove the matrix and examine for completeness. (Figure 04)
6. After the abutments are prepared, an impression is made with irreversible hydrocolloid. Cast is poured with quick setting stone.
7. Cast is retrieved from the impression. Then the matrix is filled with the selected provisional material (Figure 05-a) and it is placed over the cast with prepared abutments. A rubber band can be used to hold the matrix and the cast together while the material is polymerizing. (Figure 05-b)
8. Once the material is set remove the matrix from the preparation and peel the matrix away. (Figure 06) Trim the excess material (Figure 07), polish and lute the restoration with suitable cement.
9. There are several materials that can be used with this matrix for the fabrication of provisionals. Ethylacrylates, auto mix bisacryls or light cure resins may be used and the matrix is seated on the prepared tooth. When using acrylics, it is advisable to coat the internal surface of the matrix lightly with separating medium. Remove the crown in rubbery phase or wait till final set, lifting it on and off the preparation.

II DIRECT (for small restorations)

1. Anesthetize the tooth concerned. (Not required if non-vital). While the anesthesia is taking up, soften the disc and form the matrix directly in the mouth.
2. Complete the abutment preparation and try the matrix on to check for accurate seating.
3. Fill the matrix with required material, apply petrolatum on the tooth and soft tissue around. Place the loaded matrix on the prepared tooth. Remove the matrix when the material reaches rubbery phase, lift it on and off the preparation.
4. Once it is set, remove the matrix from the preparation and peel the matrix away. Trim the excess material, polish and lute the crown with suitable cement.

III INDIRECT-DIRECT

1. In this method at least the diagnostic cast of the arch concerned is required. If the cast of the opposing arch available, the provisional restoration would be made to respect the occlusion.
2. The pontic area/s are replaced with artificial tooth/teeth fixed firmly in wax.
3. The matrix is formed on the cast as explained earlier utilizing one or more discs.
4. After the abutments are prepared, fill the matrix with the material of choice and place it on the abutments. Allow the material to completely set. Trim, adjust the occlusion if required, polish and cement the restoration.

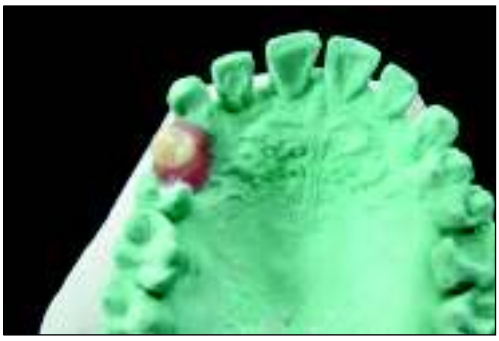
Summary

A new matrix material used for the fabrication of provisional restoration has been explained. The advantages of the technique include, accurate reproduction of the contours of the tooth satisfying esthetics and occlusal demands of the treatment. Density of the plastic drives the crown material into the margins extremely well.

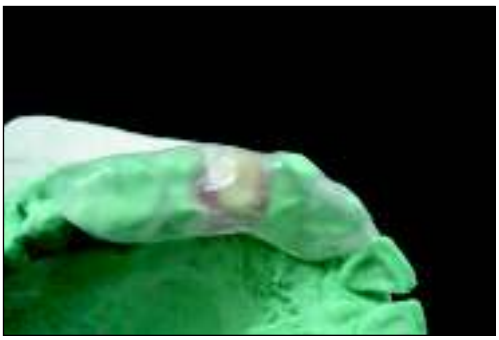
The thermoplastic material is easy to handle, requires no extra armamentarium other than what is available in any dental office, quick to manipulate when soft and mold on to the tooth. It has sufficient working time for kneading and adaptation on the teeth or the cast. In a busy practice, it saves valuable chair side time for the dentist.

The crown material can be completely light cured through them. The matrix can be saved along with other information in the event patients lose or break their provisionals. Several discs can be softened together and molded to form a larger mass for multiple unit restorations.

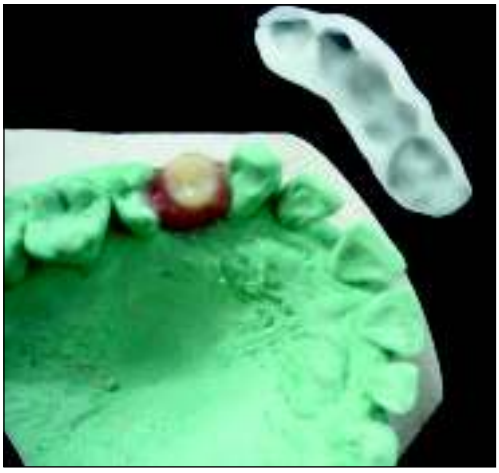
Matrix button, before and after softening



Modified Diagnostic cast



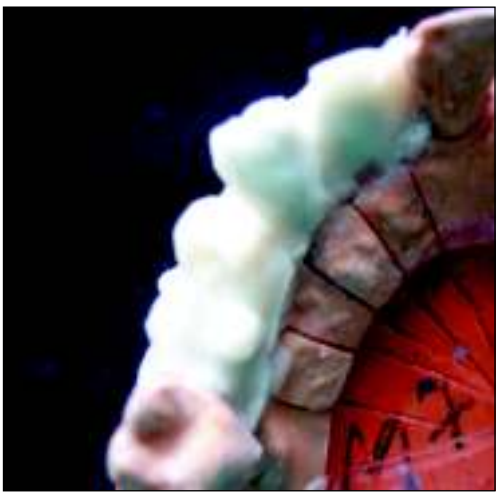
Molded Matrix buttons



Matrix-completed

Matrix loaded with provisional material

Loaded matrix on the prepared abutments



Provisional restoration – before trimming

Provisional restoration – after trimming(before polishing)

It can also be used in a fractured tooth after it is modified with rope wax. If more bulk is required around the cervical area of the restoration, all that has to be done is to spring open the matrix buccally and lingually.

Extended advantage of this material is that it can also be used to form provisional laminates for anterior teeth with light cure acrylics.

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Short Communications

# TEMPERO-MANDIBULAR JOINT: THE KINETICS OF ELEVATION & DEPRESSION

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Abstract:
The temperomandibular joint (TMJ) is a multiaxial ginlymoarthrodial synovial joint endowed with a capacity to perform a wide range of movements , primarily through its bicondylar mandibule freely articulating with a stationary (fixed) fossa of the squamous temporal. This short paper elaborates on an odd, yet possible, movement that can be performed by the joint: An illusory and paradoxical 'depression' that can result by a reversal in the roles of the two articulating bones, the mandible and the temporal. Whereas in classical depression, the TMJ utilizes two axises, the same joint during performance of the reversed articulation uses just a single axis to achieve an identical final outcome, separation of the alveolar ridges of two bones.
This paper here discusses the kinetics and articular dynamics that coordinate the plethora of performed or possible movements with special reference and a closer observation of the depression – elevation sequence.
Keywords : Temperomandibular joint, hinge, bicondylar, illusory depression, axis

## Introduction

One of the most active and complex joints, is thetemperomandibular. The TMJ is one among the only two synovial joints endowed with an articular disc (the other being the sternoclavicular. Classical descriptions of the movements possible at the joint, usually describe it as a multiaxial, synovial and gynglimoartrodial. Orthodontist and prosthodontists, however have debated off and on, often disagreeing on the type the joint is classified as: Is it a condylar or a hinge, or both?

The mandible, for one, is one of the few bones to ossify in membrane (covering the cartilage of the 1st. arch) and for another, it is characteristic in that it possesses features that lineate it under in three distinct and different classifications in arthrology – the two halves of the mandible unite through a symphisial joint (secondary cartilaginous), its two rami, along with the temporal ,form bilateral condylar synovial joints and its alveolar process houses a tandem of tooth-socket gomphosial (fibrous joints). The joint exhibits a wide range of movements, the bicondylar parts of the mandibular rami articulating with the temporal fossae: the oddity of the intervening cartilagenous disc, divides the joint cavity in an upper and lower compartment, each half performing its role in enabling complex movements of the mobile mandible as mandated in mastication.

This brief communication takes a fresh approach to understanding the kinetics (1) and presents yet another view of the dynamics of the elevation-depression movement at the TMJ

## Discussion

Classical teaching and texts on TMJ functional anatomy and movements, describe the involved processes in definitive and isolated terms: elevation - depression, protraction - retraction and rotation. The upward, downward, forward, backward and side-to-side excursions of the mandible require a wide range of action between the condylar head of the mandibular ramus and the temporal fossa. The spin, rotation, roll, pivot and

glide of the mobile condylar element using both, the upper and lower units of the articular disc divided joint cavity produce all known movements of the bone. While the antero- posterior (horizontal) axial movement produces

Figure 1: Norma Lateralis

Figure 2: Complete depression of mandible involving two levels of transverse axises (a) across the condyle and (b) across the ramus through the mandibular foramen

Figure 3: Paradoxical 'depression' of mandible caused by elevation of maxilla involving only a single transverse axis passing across the condyle

protraction and retraction is a glide / slide (Figure), side-to-side (chewing) movements are produced by alternating asynchronous movements of each half of the mandible. One dominant half of the mandible pivoting anticlockwise or clockwise through its head, the axis being vertical passing through the temporal fossa and condyle (rotator) and the other half essaying a similar, but passive sortie (orbiter) – the vertical axis, marginally shifting to the left or right or vice versa in the rotator half and their translation in the non-dominant orbital half.

Mastication, a dynamic and continuous process using all axises , is primarily and exclusively performed by the mandible's alveolar process abutting, grinding, gliding or sliding on, against and across the alveolar ridge of a static maxilla.

Mandibular depression and elevation though, are however not as simple as appear – for these hinge (2) movements the transverse axis passes through the mandibular foramens and not the condylar heads as is often assumed. While for a transient initial phase of

depression, the head rotates as a hinge with the transverse axis passing through the two condyles (3), a greater part of depression is produced by an imaginary hinge located at the ramus of the mandible, with a fresh transverse axis passing through the level of the mandibular foramen (Fig: 1, 2 & 3). In essence the head, first rotates and then glides forwards and downwards (4). The transverse axises for depression passing along hinges (one conventional and the other a fulcrum) at two locales and levels (5) (Fig 3 A).

Kinetics and dynamics of motion permit yet another hitherto unexplored movement in the temporomandibular joint. Though seemingly impossible and logic defying, the TMJ can be activated to move the base skull and maxilla upward and downward on a fixed and stationary mandible. To elaborate, despite the loss of mechanical advantage (as postulated under the 'principles of levers' in physics), extension at the cervical joints (neck extension) if generated powerfully enough through extreme action (contraction) of the muscles of the neck, extensors of the suboccipetal triangle and extensors such as erector spinae (iliocostalis cervicis, longissimus cervicis, spinalis cervicis, splenius capitis, semispinalis capitis, sternocleidomastoid and trapezius and the extensor spinalis, can and will elevate the maxilla along with the entire skull around a transverse access, to duplicate a reversed depression of the mandible – the difference being, instead of the mandible being depressed by its own muscles (lateral pterygoids and geniohyoids, for example), the maxilla is elevated by action of the cervical (neck) extensors. The role of the digastrics is worth a second look into. While the anterior belly aids mandible depression, the posterior belly helps in neck extension, creating a illusory depression through maxillary elevation. This 'paradoxical' opening of the mouth (by elevating the maxilla instead of depressing the mandible), by definition, coverts the temperomandibular joint to an illusory but functional 'mandibulotemporal 'joint. (Fig: 4, 5 & 6)

Though paradoxical action of muscles is known to mycologists (6), the biomechanics and physics of 'reverse' articulations is an unexplored commodity. Though the human anatomy permits, and all of us do perform many reverse movements without being conscious of them (to example -one can flex the thigh and raise the knee while standing, by using femoral flexors origination from the hip bone complex through acetabulo-femoral (hip) joint, but one can also mimic femoral flexion by flexing one's back (as in bending forwards to pick up something from the floor from a standing position). In the latter movement, even though the femoral flexors are inactive, the recti of the anterior abdominal wall bring the axial skeleton to forwards and downwards – producing in effect a femoral flexion in which no hip or femoral muscle involves. The oddity of the reversed articular mechanics however has an overriding dictum: whether performed, as designed to, by skeletal biomechanisms or 'engineered' to perform through remote leverages and non-antagonistic muscle groups – the final results are similar in either and by using the very same axises of movement through the reference parent joint.

The TMJ is perhaps one of the few, if not the only cranial joint that can be maneuvered to reverse movement by a functional inversion in the active -passive roles of the condyle of the mandible and the temporal fossa. Moreover, far more peculiarly, the temperomandibular joint when reversing to the mandibulotemporal , solely utilizes the transverse axis passing through the mandible's condylar head , moving clockwise to raise the skull base and maxilla and totally exempting the transverse axis passing through the two rami (the axis used in normal depression – elevation). Though the final results, vis-à-vis the positions of the mandible and maxilla, whether one is depressed or the other is elevated, are seemingly similar – the locales of the axises

used are dissimilar: whereas in mandibular depression, the axis passes transversely through the mandibular foramen of the ramus and the bone rotates around it; in elevation of the maxilla the transverse axis passes through the condlylar head. In effect, despite the juxta-positioned status of the two articular surfaces being identical, in the first case it is achieved by a forward and downward glide of the head brought about by rotation along the transverse axis through the ramus and in the second it is a backward rotation of the temporal bone brought about by a rotation of entire skull along a transverse axis, passing through the two mandibular condyles. The forward glide of the condyle within the fossa, a characteristic component of mandible depression, is totally absent during maxillary elevation.

Figure 5: Depression of mandible brought about by rotation along two levels of transverse axises

Figure 4: Norma frontalis

Figure 6: Paradoxical depression of mandible through elevation of the maxilla involving a single transverse axis

There is no other joint in the skeleton, save the temperomanibular, which can use two different axises, one, as a classical hinge and the other as a modified fulcrum to engineer one final result - enhancement of the gap between the alveolar ridges of the upper and lower jaws

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Case Report

# TRANSPOSITIONAL AUTOKERATOPLASTY

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Abstract:
Rejection is the most common cause for corneal graft failure[1, 2]. Therefore, for obvious reason, ideal material for corneal graft is the host's own cornea, either from the same eye or the fellow eye, which is totally blind from the cause that leaves the cornea healthy and intact or possibly, from an identical twin, that has a cornea to spare for the same reason. Herewith, we report a case of Transpositional Autokeratoplasty.
Keywords : Autokeratoplasty, Transpositional, Rotational, graft rejection

A 70 year old lady presented to the cornea services of the Ophthalmology Department of Justice K.S. Hegde Charitable Hospital, Mangalore with complaints of bilateral loss of vision since about 20 years following cataract surgery in both eyes. She had recurrent episodes of pain, redness and watering in the right eye, since the time of initial surgery. She had no previous records of the details regarding the surgical procedure. She was neither a Diabetic, nor a Hypertensive, and was not on any ocular or systemic medications at the time of presentation.

On examination, her visual acuity was light perception with accurate projection of rays in all quadrants in the right eye whereas there was no light perception in the left eye. Slit lamp examination showed mild conjunctival congestion, with epithelial bullae and diffuse stromal oedema in the right eye, whereas left eye showed a clear cornea. She was aphakic in both eyes with no evidence of vitreous in the anterior chamber. The status of the posterior capsule was not visible in the right eye, whereas it was found to be intact in the left eye. The intra-ocular pressure was 17.3 mm of Hg and 14.6 mm of Hg with 5.5 gm. by Schiotz Tonometry in the right eye and the left eye respectively. Dilated fundus examination revealed a chalky white disc with a normal retina in the left eye, whereas that in the right eye could not be visualized.

B-scan of the right eye showed normal posterior segment. A-scan biometry in aphakic mode was also done for the right eye and posterior chamber intraocular lens power calculated by SRK II formula, using the keratometry values of the left eye, was +22.0D. Specular microscopy or pachymetry was not performed.

Figure 1(a): (RE) Aphakic Bullous Keratopathy, (LE) Clear cornea

Figure 1(b): B-Scan (RE) within normal limits

Surgery was performed under peribulbar anaesthesia to both the eyes[3]. First, an 8.2mm corneal button was trephined from the left eye, which was then carefully placed in a sterile bowl and covered with viscoelastic. Donor tissue from our Eye Bank was sutured in place and the eye was patched.

Subsequently, in the right eye, an8.0 mm cornea was trephined out, posterior capsule was found to be intact, and hence, a +22.0D posterior



Figure 2(a)& 2(b): Preparation of Donor button from Left Eye

Figure 2(c): Preparation of Recipient Bed

chamber intraocular lens was placed in the sulcus. The corneal button trephined earlier from the left eye was now sutured using 16 interrupted 10-0 nylon sutures. Peripheral button-hole iridectomy was also done and residual viscoelastic was washed from the anterior

On the first post-operative day, the right eye revealed a mild stromal oedema in the graft with few Descemet membrane folds. The graft-host junction was well apposed, anterior chamber well-formed and the intraocular lens was in place. The vision was counting

fingers 3 metres. She was asked to use Moxifloxacin-Prednisolone Acetate eye drops 6 times in a day, tab. Acetazolamide (250 mg) b.d. for 3 days and ocular lubricants.

At the end of one week, the right eye showed a clear graft with an uncorrected vision of 6/60 improving to 6/18 with pin-hole. The steroids were now tapered off and lubricants were continued. At 4 weeks, the visual acuity of 6/60 was maintained, and the graft was clear.

Figure 3(a): Post-Op Day 1

Figure 3(b): Post-Op 4 weeks

Figure 3(c): Post-Op 6 months

Discussion:

Autokeratoplasty is a surgical procedure, wherein the patient's own cornea is used for visual rehabilitation, the greatest advantage being negligible chances of immune rejection. Traditionally, 2 types of autokeratoplasty have been described – transpositional and rotational. Transpositional grafts are those which involve exchange of corneal buttons between the two eyes of the same person, whereinthe eye with clear cornea has no visual potential, while the recipient eye has opaque cornea, but good visual potential[5].

Review of literature shows many case reports of rotational grafts, but very few reports of transpositional graft. To the best of our knowledge, there have been 11 reported cases of Transpositional grafts in the literature so far.[6, 7, 8]. Therefore, we would like to report this particular surgical procedure as an alternative to penetrating keratoplasty.

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Case Report

# IMMUNOCOMPROMISED STATUS A CAUSE OF OPPORTUNISTIC INTESTINAL INFECTION LEADING TO GRAM NEGATIVE SEPSIS

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Abstract:
Gram negative sepsis and intestinal opportunistic infections including Cryptosporidium, Isospora, Cyclospora, Microsporidia, S.stercoralis and Candida albicans are increasingly becoming prevalent in acquired immunodeficiency syndrome (AIDS) patients. These infections are clinically important primarily because many of these have the potential for serious and even lethal complications in immunosuppressed patients. Here, we are reporting a patient with Gram-negative sepsis, who was found to be co-infected with three of these opportunistic pathogens. She was also found to be positive for human immunodeficiency virus (HIV) antibodies. To our knowledge, this has been reported once previously, and serves as a reminder to actively exclude S.stercoralis infection in immunocompromised individuals presenting with bacteraemia.

Introduction Strongyloides stercoralis is an intestinal nematode of humans. It has a worldwide geographical distribution but is endemic in the tropics and the subtropical regions of the world. It is estimated that tens of millions of persons are infected worldwide, although no precise estimate is available. Strongyloidiasis in an immunocompetent host is an indolent disease, but in an immunocompromised host the overwhelming accelerated autoinfection cycle can potentially lead to life threatening illness with multi organ failure due to massive larval invasion known as hyper infective syndrome [1]. The hyperinfective state is associated with massive invasion of the gastrointestinal and respiratory systems and may result in widespread dissemination into other body organs or invasive strongyloidiasis.

Case report

A thirty five year old lady with unknown HIV status, was admitted in the medicine department of K.S.Hegde Medical Academy, Mangalore, with complaints of loose stools of 4-5 episodes per day, weight loss and low grade fever on and off for more than three months duration. She gave history of recent aggravation of symptoms, with high fever and chills for 2 days. Blood and stool samples of the patient received by the microbiology department were subjected to standard diagnostic microbiological examination.

Two of the three blood cultures revealed the presence of E. coli which was identified by standard biochemical reactions. The stool sample was concentrated by formol-ether concentration technique. A wet mount preparation revealed numerous larvae of Strongyloides stercoralis and moderate number of yeast like budding cells. Gram smear revealed moderate number of gram positive yeast like budding cells with pseudohyphae. Z-N stained smear did not reveal any acid fast bacilli. A smear stained by modified Z-N staining method revealed a large number of round pink cysts of *Cryptosporidium parvum* measuring 4-6 µm in diameter. However, gastric aspirate and sputum were found negative for larvae of S. stercoralis.

The stool sample was also inoculated on the plates of MacConkey agar, nutrient agar, and XLD. No pathogenic bacteria were isolated. Thus, the role of bacteria which may cause chronic diarrhea, were ruled out. However, culture on SDA revealed colonies of Candida species which was subjected to confirmatory tests and identified as Candida albicans. She was also found to be positive for human immunodeficiency virus (HIV) antibodies.

## Discussion

Although most infected individuals are asymptomatic, Strongyloides stercoralis is capable of transforming into a fulminant fatal illness under certain conditions that are associated with an immunocompromised host such as patients on steroid therapy, those infected with human T cell lymphotropic virus-1 (HTLV-1) and Human immunodeficiency virus (HIV).[2] The isolation of Cryptosporidium in stools of HIV-infected patients from different developing countries yielded prevalence rates of 6% to 94% and that of C. albicans was 7.6% to 39.1%. [3] Strongyloides stercoralis causes heavy infection in AIDS patients with prevalence of about 2.5%. Co-infections with Cryptosporidium and S. stercoralis have been reported from AIDS patients. [4] Strongyloides stercoralis is an important human parasitic infection primarily because of its potential for serious and even lethal disease in immunosuppressed patients. In the present case, Strongyloidiasis might be responsible for gram negative sepsis as intestinal flora attached to the larvae, during invasion, may also migrate throughout the body [5]. To our knowledge, gram negative bacterial sepsis in an immunocompromised patient associated with gastrointestinal Strongyloides stercoralis, has been reported once previously and serves as a reminder to actively exclude S. stercoralis infection in immunocompromised individuals presenting with bacteraemia [2].

Intestinal coccidial infection with Cryptosporidium, Cyclospora, Isospora and Microsporidia are increasingly becoming prevalent in AIDS. Intestinal infection with Cryptosporidium is self limiting in immunocompetent individuals but leads to persistent diarrhea in advanced stage of AIDS. Yeast in stool specimen is due to transient or commensal growth in GIT. However in immunocompromised individuals it may lead to invasive disease thus proving to be a fatal opportunistic pathogen. [3,4]

To conclude, we wish to emphasize that, patients with multiple opportunistic infections, especially Strongyloidiasis, are at a high risk of developing fatal gram negative sepsis- particularly when the patient is immunocompromised. Hence, early diagnosis and treatment of parasitic infections will help in improving the quality of life in AIDS patients.

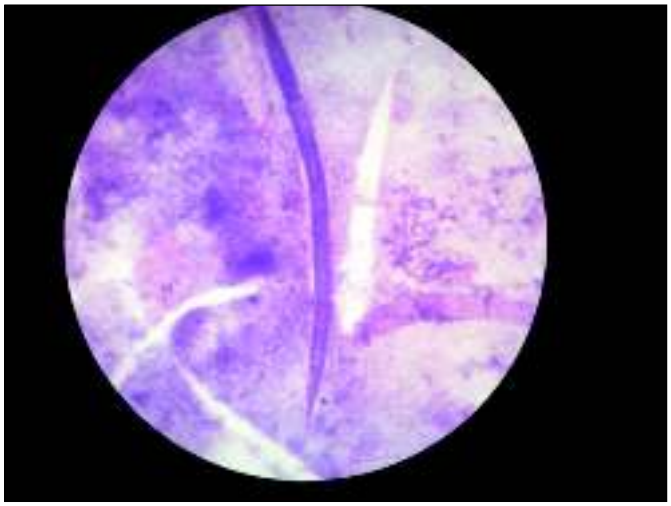

Fig 1: Larvae of Strongyloides stercoralis

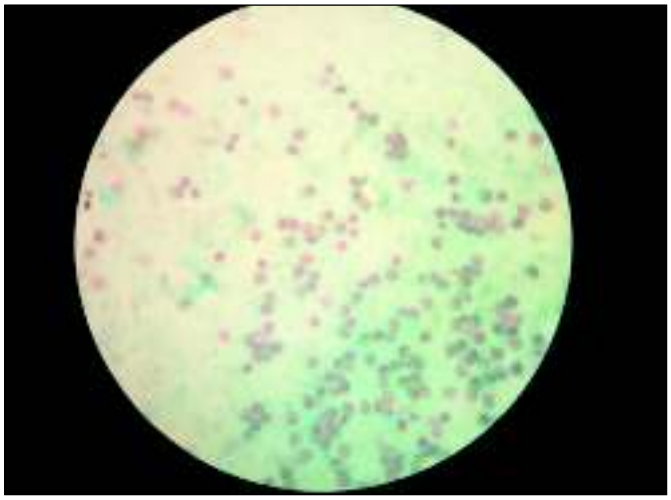

Fig 2: Cysts of *Cryptosporidium parvum*

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Case Report

# HUMAN DIROFILARIASIS: AN UNCOMMON CASE OF SUB CUTANEOUS INFECTION WITH DIROFILARIA REPENS WITH A BRIEF REVIEW OF LITERATURE

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Abstract:
Dirofilaria are a group of arthropod borne filarial nematodes that cause infection in wide range of domestic and wild animals. Dirofilaria repens is a common zoonotic infection in countries like Sri Lanka. Human infection with Dirofilaria repens is not widely recognized in India. Most of the documented cases of human dirofilariasis recorded in India presented with ocular infections, affecting the eyelid, periorbital region and occasionally the sub conjunctivae. Here we report a rare case of sub cutaneous dirofilariasis, which presented as a swelling at an uncommon site.

Keywords : Dirofilariasis, Dirofilaria repens, Dirofilaria tenuis

## Introduction

Dirofilaria are a group of mosquito borne filarial nematodes that cause infection in wide range of domestic and wild animals. The widely recognized parasites among them are Dirofilaria immitis (D.immitis), the agent of cardiovascular Dirofilariasis, and Dirofilaria repens causing sub cutaneous infection. D. repens is a mosquito borne filarial parasite of the sub cutaneous tissue of domestic and carnivores such as dogs, cats and foxes. Dirofilaria species may be divided into two groups: subgenus Dirofilaria represented by Dirofilaria immitis, which is characterized by smooth cuticle and normally found in the right heart and pulmonary vessels of dogs, the natural host ; and subgenus Nochtiella, which parasitize the sub cutaneous tissue. Species of this group have longitudinal ridges on the cuticle. Representative species are Dirofilaria (Nochtiella) repens, found in dogs and cats, and Dirofilaria tenuis, found in raccoons[1] .The mature D. repens live in the tissues and organs of vertebrates, while the immature stages or the microfilaria prefer the blood and the lymph vessels[2].

D. repens is a common zoonotic infection in countries like Sri Lanka. Human infection with D. repens is not widely recognized in India. However, there is probably a focus of human infection with D. repens in Kerala, from where few cases are being reported[3].

## Case report

An 18 year old female patient, hailing from Allepey district of Kerala, presented with swelling on the right side of the neck of six months duration. The swelling increased in size in the past one week and was associated with fever and pain.

On examination a 4cm x 3cm swelling was noted in the right supraclavicular region (Figure1). The surface of the swelling was smooth and the borders ill defined. It was not fixed to the underlying muscle or deeper tissue. The swelling was tender on palpation and redness was noted over the swelling. No similar swelling was noted elsewhere. Clinical diagnosis of cervical lymphadenopathy was made. Needle aspiration cytology was advised. On FNAC a thin thread like white worm was

removed in toto.

The worm was thin, thread like, cylindrical measuring 9cms long and 497um wide with rounded anterior end and tapering posterior end. On glycerin wet mount the worm revealed thick cuticle with longitudinal ridges. Muscles were separated into dorsal and ventral bands. The thick cuticle had prominent longitudinal ridges with fine transverse striations (Figure 2). The body cavity showed elongated esophagus and uterus with small round structures within the uterus, which were the immature eggs. The worm was unfertilized or immature. The posterior end was tapering and unremarkable. Based on size, cuticular and internal morphology, the worm was identified as D. repens. The identity of the worm was confirmed as immature female D. repens at the Veterinary Sciences College, Hebbal, Bangalore.

Routine hematological and biochemical test results were normal and no microfilaria was seen in peripheral smear

Discussion

Dirofilariasis is often reported from European countries surrounding the Mediterranean particularly from Italy. The first D.repens case is said to have been reported by Angelo Pace in Palermo in 1867[1]. In India, sub cutaneous dirofilariasis is rare, only few cases having been reported from Kerala[4]and most of the documented cases of human dirofilariasis recorded in India presented with ocular infections, affecting the eyelid, periorbital region and occasionally the sub conjunctivae.[3].

Dirofilaria repens infection, rarely seen in humans, is a zoonotic illness. Humans get infected through blood sucking arthropods such as mosquitoes, fleas and ticks. For D. repens, the human body is not an appropriate host, and therefore, no mature stages of the parasite are found in man[2]. With changing host, agent and environmental factors, an increasing number of human infections are being reported. Though man is not a suitable host, there are reports of the infective larvae developing into adult worm and in exceptional cases even producing blood circulating microfilaria[5].

Identification of D.repens is made by studying the morphology of the worm. An adult male worm is 5-7cm long and 370-450µm wide with 2-6 pre anal papillae on the right side and 4-5 on the left. The spicules are unequal. The left spicule is 460-590µm and right ones are 180-210µm. The female are 10-17cm long and 460-650µm wide with a vulva 1.15-1.62 cm from the anterior end. The microfilaria is unsheathed and occurs in the sub cutaneous lymph spaces and in the blood of natural host[2].

The other species of Dirofilaria reported from India are D.immitis and D.tenuis[6, 7]. However, D.immitis can be differentiated from D.repens by the absence of longitudinal ridges and transverse striations[3]. Many parasitologists believe that D. tenuis is restricted to USA and consider that D. tenuis is synonymous with D.repens[5]. In the natural hosts, like dogs, studies have reported parasitemia varying from 12-37%[4].

Simple extraction of the worm or surgical excision of the lesion is the treatment of choice for human dirofilariasis. There is seldom a need for chemotherapy as Microfilaraemia is extremely rare. In a small number of cases, ivermectin and/or diethylcarbamazine has been tried with good results. The symbiosis of filarial nematodes and intracellular bacteria, Wolbachia, has been recently exploited as a target for antibiotic therapy of filariasis. Antibiotic treatment of filarial nematode results in sterility and inhibits larval development. In the first trial on human onchocerciasis, depletion of bacteria following treatment with doxycycline resulted in a complete and long term block of embryogenesis[8].

## Conclusion

Human cases of dirofilariasis are most probably under reported because many of them remain undiagnosed or unpublished. The current case was diagnosed as subcutaneous Dirofilariasis based on parasitological observation. Definitive diagnosis can be made by molecular techniques like multilocus analysis of gene enzyme system and PCR. However these tests are not available for routine diagnostic purpose. Diagnostic constraints and lack of awareness often result in under reporting of cases. Awareness and high degree of suspicion is the key to diagnosing human Dirofilariasis.

Figure 1- Clinical presentation of swelling

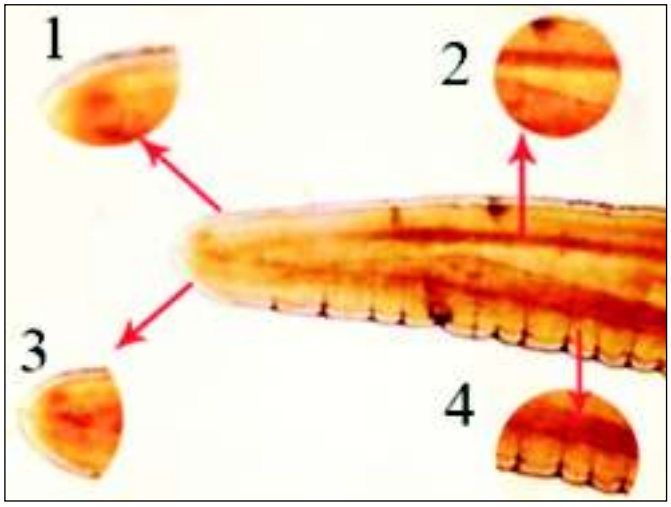


Figure 2- Immature female worm of Dirofilaria repens in wet mount showing (1) Thick cuticle (2) Intestine (3) Posterior end (4) Thick muscular coat with longitudinal ridges and transverse striations.

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Case Report

# LIPOSARCOMA OF SPERMATIC CORD PRESENTING AS INDIRECT INGUINAL HERNIA- A RARE CASE REPORT

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Abstract:
Liposarcoma in the inguinal region though rare are clinically significant lesions. Preoperative diagnosis is difficult since the clinical findings are very similar to that of inguinal hernia. We report a rare case of Liposarcoma of the spermatic cord in 85 year old male, clinically diagnosed as left sided indirect inguinal hernia. Surgical excision specimen showed multiple globular lipomatous masses which were yellowish and grey tan with areas of myxoid degeneration and necrosis seen. Microscopic examination showed adipocytes arranged in lobules with numerous blood vessels, lipoblasts and myxoid stroma confirming the diagnosis of myxoid liposarcoma. He is on regular follow up since two years without any recurrence or metastasis. Our case report highlights the importance of sampling and examination of fatty masses in the inguinal region to rule out the possibility of liposarcoma as they are mistaken for lipoma at surgery.

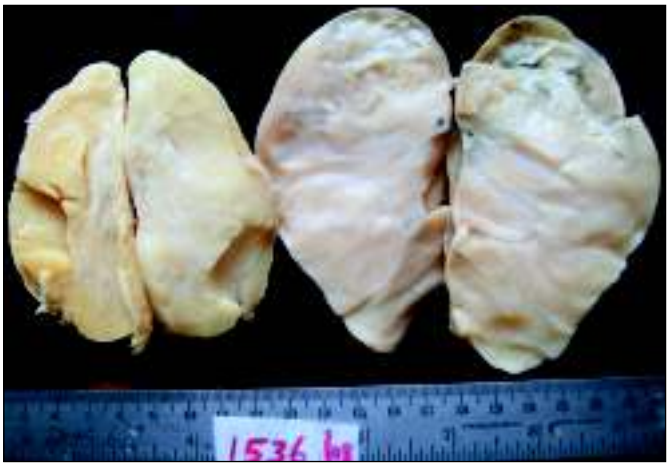
Keywords : Liposarcoma, spermatic cord, indirect inguinal hernia.

## Introduction

Liposarcoma is a malignant mesenchymal tumor of the adipose tissue. [1] Its incidence in the paratesticular region is very low, varies from 10-12 percent of all soft tissue sarcoma between fifth and seventh decades. The two major sites of liposarcoma are the extremities, particularly the thigh and the retroperitoneum which account for about two-third of all cases. [2] Its clinical presentation characterized by symptom which is usually late and nonspecific. [3] They presumably have a good prognosis. Well differentiated Liposarcoma have no metastasis with high five year survival rates. [4] Since the inguinal region communicates with the retroperitoneum, liposarcoma in both the regions may be detected during hernia repair operations. [5] We report a rare case of liposarcoma of spermatic cord presented clinically with indirect inguinal hernia.

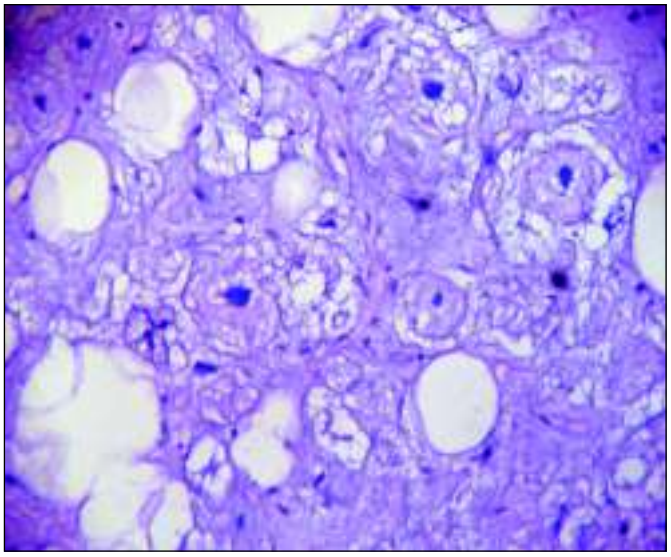
## Case report

A- 85- yr old male presented with swelling and pain in the scrotal and left inguinal region since 1 year with sudden increase in the last two months. He was a known asthmatic and hypertensive on treatment. Local examination showed swelling in the left scrotal region measuring 15x15 cms. The swelling was not reducible on lying down. It was tender and soft in consistency. Routine haematological, biochemical and serological investigations were within normal limits. Scrotal ultrasonography showed bilateral hydrocele with left inguinoscrotal hernia. The clinical diagnosis of bilateral hydrocele with left inguinal hernia was made and treated surgically for hydrocele and hernia. At operation, multiple diffuse lipomatous swellings were seen along the spermatic cord which were excised and sent for



Gross picture showing globular yellowish and grey tan lipomatous masses .

histopathological examination. Gross examination showed multiple globular lipomatous masses, larger measuring 12x7x6 cms. Cut section of the lipomatous masses was yellowish and grey tan with areas of myxoid degeneration and necrosis seen. (Figure 1) Microscopic examination showed adipocytes arranged in lobules with numerous blood vessels and lipoblasts seen. (Figure 2)



Microscopy picture showing adipocytes arranged in lobules with numerous blood vessels and lipoblasts. (Hematoxylin and Eosin X 100)

The lipoblasts are large cell having cytoplasmic vacuolations indenting the pleomorphic nucleus. In the stroma there were scattered multinucleated floret like giant cells and atypical lipocytes. Foci of necrosis haemorrhage and myxoid change was also seen. Final diagnosis of myxoid Liposarcoma was considered. The postoperative course was uneventful. He was on regular follow up since two years without any recurrence or metastasis.

## Discussion

Liposarcoma in the paratesticular region is rare representing 7-10% of intrascrotal masses. [6] Sarcomas account for 90% of malignant lesions of the spermatic cord of which liposarcoma form approximately 3-7%. [6] They are most likely to involve the spermatic cord than the testicular tunis; occasionally there may be extension from retroperitoneal sarcomas. [4] Diagnosis of liposarcoma in the inguinal region preoperatively is not easy because unlike other sarcomas they have a long clinical history and on examination is diagnosed as inguinal hernia or lipoma at surgery [7]. Histologically the differential diagnosis of liposarcoma is benign fatty tumors, fibromatosis, fibrosarcoma and malignant peripheral nerve sheath tumor (MPNST). The presence of atypical cells with large hyperchromatic nuclei within the fibrous septa or in the fat, variation in size of adipocytes and presence of lipoblasts favours the diagnosis of liposarcoma.[4] our case showed similar histological features. Low grade fibrosarcoma and malignant peripheral nerve sheath tumors are more cellular with a fascicular architecture. MPNST are at least focally S-100 positive. [4] The treatment of choice is radical orchidectomy with high ligation of the cord [8], while some recommend wide excision of the mass [7]. Though some authors recommend radiation and chemotherapy for both primary and metastasis [9], the use of these treatment modalities is still controversial. [10] More than half of the cases of paratesticular well differentiated Liposarcoma showed recurrence with progression to low or high grade dedifferentiation in some of the recurrence. [4] Our patient was treated by surgical excision of the masses .On regular follow up since two years there was no recurrence or metastasis in our patient.

We conclude that awareness and histopathological confirmation of fatty masses in the inguinal region is necessary as Liposarcoma have prolonged clinical course with late recurrences.

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Case Report

# PRIMARY ANORECTAL MELANOMA- A RARE CASE REPORT WITH REVIEW OF LITERATURE

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Abstract:

Malignant melanoma is very rare accounting for 1% of all anorectal malignancies. Long term survival is rare, as most patients die of disseminated disease. A 64-year old female presented with the history of bleeding per rectum and constipation since 2 months with recto-sigmoidoscopy showing an exophytic growth measuring 4x3 cms obstructing the lumen of the rectum. Biopsy showed malignant spindle cell tumor, with possibilities of spindle cell variant of squamous cell carcinoma, leiomyosarcoma and malignant melanoma. The tumor cells were immunoreactive for S-100 and HMB-45, confirming malignant melanoma. MRI showed a tumor just behind the anal verge, without evidence of invasion in the sphincter or enlarged lymph nodes. An abdominal perineal resection was performed with colostomy. On four years of follow up, the patient is doing well without recurrence or metastasis. We report this case because of its rarity, diagnostic dilemmas, early diagnosis and treatment of this aggressive malignancy for better outcome and long term survival.

Keywords : primary, anorectal melanoma, HMB-45.

## Introduction

Primary malignant melanoma of the anus and rectum is rare and highly lethal neoplasm constituting less than 1% of all melanomas and 4% of anorectal tumors other than adenocarcinoma. [1, 2] The delayed clinical manifestations and lack of clinical suspicion contributes to delayed diagnosis. [2] Owing to its rarity and histologic variability, misdiagnosis as carcinoma, sarcoma and lymphoma is common. [1] Sometimes this can be mistaken for benign conditions like hemorrhoids or rectal polyp as they present with rectal bleeding. The melanocytes as demonstrated by HMB 45 are usually located in the anal squamous zone, but are also seen in transitional and colorectal zone. Thus the demonstration of melanocytes in all the three zones of the anal canal substantially supports the observation that malignant melanoma of the anal canal may originate not only below but also above the dentate line. [3] We report a case of anorectal melanoma in a 64 year old woman who underwent surgery with complete recovery without any recurrence or metastasis after 4 years of follow up. We highlight the diagnostic dilemmas, importance of early diagnosis and treatment of this aggressive malignancy for better outcome with long term survival.

## Case Report

A 64-year old female presented with the history of bleeding per rectum and constipation since 2 months with recto-sigmoidoscopy showing an exophytic growth measuring 4x3 cms obstructing the lumen of the rectum. Biopsy from the growth showed clusters of tumor cells arranged in sheets, clusters and fascicles. The cells were round to oval to spindle shaped consisted of vesicular nucleus with prominent eosinophilic nucleoli. Many abnormal mitotic figures were seen. The possibilities of spindle cell variant of squamous cell carcinoma, leiomyosarcoma and malignant melanoma were

considered. The tumor cells were immunoreactive for S-100 and HMB-45, confirming malignant melanoma. MRI showed a tumor just behind the anal verge, without evidence of invasion in the sphincter or enlarged lymph nodes. Abdominal ultrasonography, CT-thorax showed no evidence of distant metastasis. An abdomino-perineal resection was performed with permanent colostomy. The postoperative period was uneventful. Histopathology of the resected specimen confirmed the diagnosis of malignant melanoma with tumor limiting only to the submucosa and at places showing melanin pigmentation. The resected margins and lymph nodes were free from tumor. The patient is on regular follow up since 4 years without any evidence of recurrence or metastasis.

## Discussion

Primary anorectal melanoma is a rare disorder accounting for 4% of anorectal malignancies other than adenocarcinoma. [4] It usually affects women in the fifth or sixth decade and presents with rectal bleeding or altered bowel habits. [5] Our patient was a 64 year female presented with bleeding per rectum and constipation. Patients presenting with rectal bleeding and sensation of a mass is usually attributed to hemorrhoids or polyp. The epithelial lining of the anal canal is glandular in the upper part and squamous in the lower part. The middle zone also known as anal transitional zone is characterized by an epithelium which bears resemblance to that of anal glands, but show little mucous secretion.[3] The absence of early clinical manifestations and the lack of clinical suspicion contributes for delayed diagnosis. Up to 60% have metastasis at the time of diagnosis. [7].In our case the disease was limited to rectum and anal canal without evidence of metastasis. The factors for poor prognosis include, advanced disease at the time of diagnosis and rich vascularity which increases the risk of hematogenous metastasis. [8] Abdomino-perineal resection (APR) is the treatment of choice for patients with <2 mm wide lesion.[9] Radiotherapy is palliative in locally extensive tumors while combined with chemotherapy is used for metastasis. APR appears to have some effect in controlling symptoms caused by local and regional disease but has minimal impact on prognosis. [10] However in our case only APR with permanent colostomy was done without chemo-radiotherapy. Extended follow up for 4 years showed no recurrence or metastasis.

## Conclusion

Although anorectal melanomas are rare, this should be considered as one of the differential diagnosis in malignancies other than adenocarcinoma, so that early diagnosis with histological features confirmed with immunohistochemistry will help in appropriate management and long term survival of the patient.

Figure 1

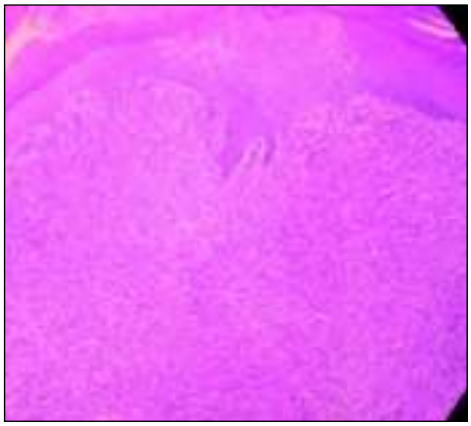

Figure 2

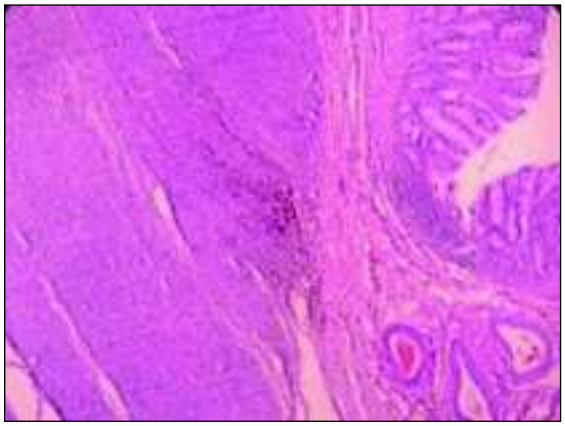

Figure 3

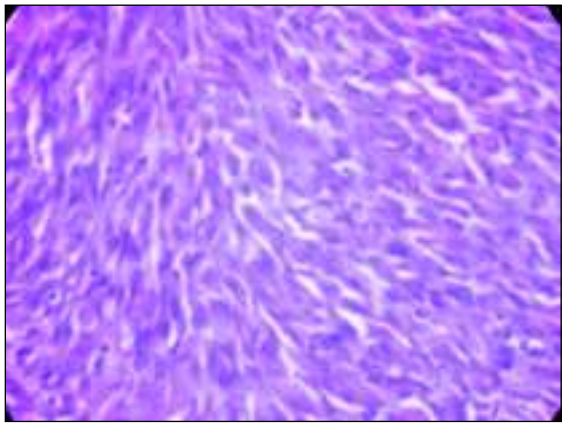

Figure 4

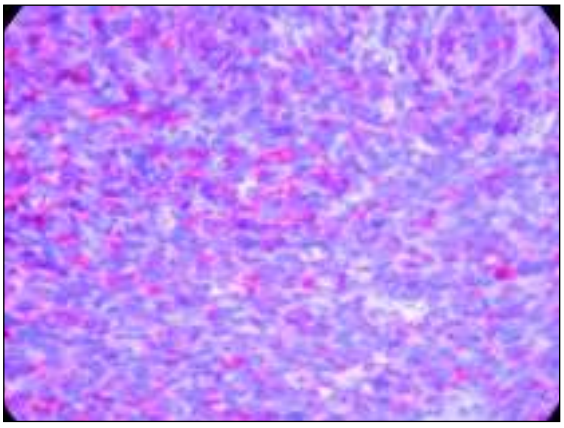

Figure 5

LEGENDS

Figure 1 -Gross photo showing nodulo proliferative growth with blackish pigmentation in the anorectal region.
Figure 2 - Microscopy showing tumor beneath the stratified squamous epithelium (Anal canal). (H&E X 100)
Figure 3 -Showing tumor with melanin pigment in the rectum. (H&E X100)
Figure 4 -Showing spindle shaped tumor cells having vesicular nucleus with prominent eosinophilic nucleoli (H&EX400)
Figure 5 -Showing tumor cells immunoreactive for HMB-45.

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Case Report

# RARE DISTINCT SUBTYPE OF CLEAR CELL RENAL CELL CARCINOMA - MULTILOCULAR CYSTIC RENAL CELL CARCINOMA

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Abstract:
Multilocular cystic renal cell carcinoma (MCRCC) is an uncommon low- grade tumor with unique morphologic features and excellent prognosis. Currently it is classified as subtype of clear cell renal cell carcinoma. We report a case of multilocular cystic renal cell carcinoma presented with right renal mass. Computed tomography showed heterogeneously enhancing partly cystic mass lesion in right kidney. Patient underwent right nephrectomy. Microscopic features were suggestive of multilocular cystic renal cell carcinoma. Patient is doing well with no evidence of recurrence.

Keywords : cystic renal cell carcinoma, renal cell carcinoma

## Introduction

Multilocular cystic renal cell carcinoma is a rare variant of clear cell carcinoma comprising of approximately 1 to 2% of all renal tumors with good prognosis.[1,2] Various cystic diseases of the kidney such as multilocular cystic renal cell carcinoma, cystic nephroma, mixed epithelial and stromal tumor of the kidney may present with similar findings on imaging and are difficult to distinguish.[3]Clinically, MCRCC behaves as a low grade variant of renal cell carcinoma in 83-88% of cases and have a good prognosis.[2]

## Case report

We report a case of a 40 year old man presented with the complaints of mass in the right loin and frequency of mictuirition since two months. Computed tomography abdomen showed a heterogenously enhancing partly cystic mass in the lower pole of the right kidney measuring 5 X 4cm.(figure 3) Nephrectomy specimen showed tumor measuring 6.8 X 5cm that occupied lower pole. The cut section showed multiloculation with cysts ranging from 2mm to 2cm in diameter filled with mucoid fluid.(Figure 1)Microscopy showed a multicystic tumor with tumor cells having clear cytoplasm with hyperchromatic nuclei with anisonucleosis and no visible nucleoli (Fuhrman nuclear grade I).These tumor cells were separated by delicate fibrovascular septa. (Figure 2) Renal capsule, vessels and perinephric fat were free of tumor. A diagnosis of Multilocular cystic renal cell carcinoma, Fuhrman nuclear grade I(stage 1) was made. Patient is well with no evidence of recurrence after three years of follow up

## Discussion

MCRCC is a subtype of clear cell renal cell carcinoma with distinct morphology.[4] Main pathological features of MCRCC, according to the WHO classification of kidney tumors are on gross are multilocular cystic appearance , encapsulated, yellowish solid component limited to small areas, no expansile nodules, and tumor necrosis is absent with microscopy showing cyst lined by cuboidal clear cells or flattened epithelium, septa containing aggregates of epithelial cells with clear cytoplasm and low Fuhrman grade.[6]

The term multilocular cystic renal cell carcinoma should be used exclusively to identify cystic renal cell carcinoma

be used exclusively to identify cystic renal cell carcinoma with a small volume (25% or less) of neoplastic clear cells in the cyst wall.[2, 3, 5] The differential diagnosis of multilocular cystic mass includes multilocular cystic nephroma and mixed epithelial and stromal tumor of the kidney. Multilocular cystic nephroma is a benign entity, characterized by communicating cysts of variable size separated by fibrous septa. Cystic nephroma in adults is considered to belong to the spectrum of cystic renal lesions, ranging from typical cystic nephroma to mixed epithelial and stromal tumor of the kidney.[2]On US and CT images, MCRCC appears as a well defined , multilocular cystic mass with serous , proteinaceous or hemorrhagic fluid, with no expansile solid nodules in the thin septa and sometimes with small, slightly solid areas.[7] When the radiological examination demonstrate a cystic renal mass of this kind in adult males, MCRCC should be included in the differential diagnosis.[3]

Shams H et al study observed chromosome 3p deletion in MCRCC which provides support for the hypothesis that it is a subtype of clear cell renal cell carcinoma.[4]

Multilocular cystic renal cell carcinoma has a better outcome than non cystic conventional renal cell carcinoma.[6] The tumor has extremely high cure rate, ranging from 92 – 100% following surgical resection.[2, 4] Hence, few authors have suggested renaming it as multilocular cystic renal cell carcinoma of low malignant potential.[2,3]

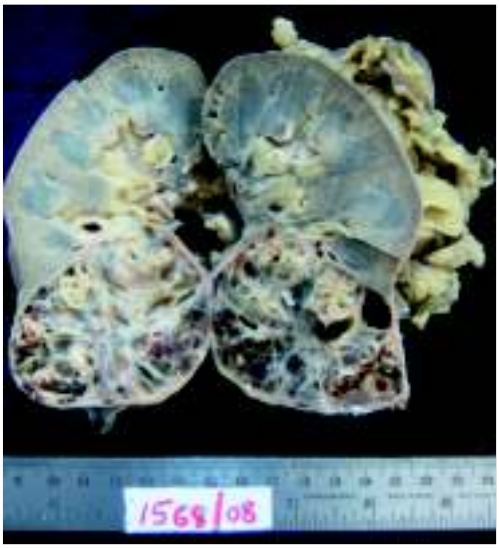


Figure 1 -Right nephrectomy specimen showing cystic tumor in the lower pole of the kidney.

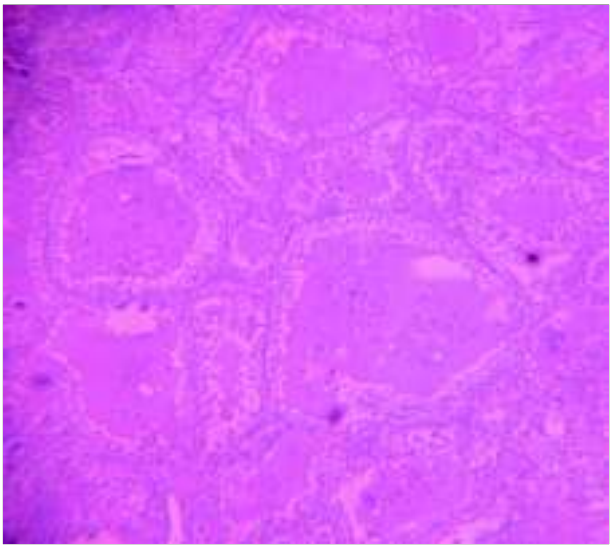

Figure 2 -Multicystic tumor with tumor cells having clear cytoplasm.

Figure 3 -C T image showing heterogeneously enhancing cystic mass in the lower pole of right kidney.

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Case Report

# NECROBIOTIC XANTHOGRANULOMA PRESENTING AS A CLINICAL VARIANT WITHOUT PARAPROTEINEMIA

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Abstract:
Necrobiotic xanthogranuloma is a rare granulomatous disease involving skin and subcutaneous tissues. A 61 year old woman presented with a 5 year history of enlarging plaques around both eyelids.it was associated with diminished vision in both eyes. The lesions are often bilateral,symmetrical and ophthalmologic complications are observed. A histopathological examination showed features of necrobiotic xanthogranuloma. Necrobiotic xanthogranuloma is usually associated with paraproteinemia which was not seen in our case.The pathogenesis remains unclear. Although there is no first line of treatment, reports have shown variable benefits with chemotherapeutic agent and steroids.
Keywords : necrobiotic xanthogranuloma, necrobiosis.

## Introduction :

Necrobiotic xanthogranuloma(NXG) is characterized by multiple, sharply demarcated nodules and plaques which have a violaceous to red to orange-yellow appearance. There may be central atrophy, ulceration and telangiectasia. These lesions occur around the eyes and face but may occur in the trunk and limbs. These lesions may be chronic and may be progressive. It is usually associated with paraproteinemia, bone marrow plasmacytosis, hypocomplementemia and hyperlipidemia.

## Case Report :

A 61 year old female came with raised non tender lesions around both eyes since 5 years . Initially the lesion started as a small papule around both eyelids.it was painless and gradually to the present state. Local examination of the lesion showed multiple non tender diffuse swellings in the periorbital region, which was soft to firm in consistancy. Laboratory investigations showed a mildly elevated lipid profile. The hemoglobin level was 7.7gms/dl.,the packed cell volume was26.Aperipheral smear report showed dimorphic anaemia with eosinophilia. The erythrocyte sedimentation rate was 45mm/hr.protein electrophoresis was done twice and there was no increase in paraproteins. The lesion was excised and sent for histopathological examination. The lesion was grossly yellow. histopathologic examination shows granulomatous infilterate comprising of foamy histiocytes, in focal aggregates and intersecting bands.plenty of giant cellsare seen of both the foreign body type and touton type. Places show dense aggregates of lymphocytes forming germinal centres. There are areas of necrobiosis. The diagnosis of necrobiotic xanthogranuloma was made.

## Discussion :

Necrobiotic xanthogranuloma(NXG) is a rare progressive disease showing destructive cutaneous lesions and usually a close association with paraproteinemia.

NXG is a disease of adults usually seen in the sixth decade of life. The lesions have a characteristic yellow color(1,2,3). The most common involvement is the faceparticularly the periorbital region(85% of cases) . the trunk and proximal extremities are other affected sites. Ophthalmologic complications are seen in 50% of cases include orbital mass, conjunctival involvement keratitis, scleritis and uveitis(1)

NXG can involve other sites like lungs, kidneys ,liver, spleen, intestines, skeletal muscle and central nervous system(1,4,5). Paraproteinemia is closely associated with nxg, with approximately 80% of patients demonstrating a monoclonal gammapathy on serum protein electrophoresis. A case report discusses a case of nxg without paraproteinemia as seen in our case. there was no signs of paraproteinemia, malignancy or new skin lesionsin our case ,in the year to follow(12).

Histopathologic examination shows a xanthogranuloma with areas of necrobiosis, comprising of degenerated collagen that infiltrates into the mid dermis and subcutaneous fat. Multiple foamy histiocytes, foreign body giant cell, touton giant cells, cholesterol clefts and lymphoid follicles are also seen(1,2).

Treatment of NXG - improvement was seen in skin lesions with chlorambucil, melphalan, localized radiotherapy, systemic glucocorticoids, inteferons2a and plasmapheresis (1,6,7,8,9,10,11). In our case improvement was seen with steroids.

To conclude – with the characteristic histopathological picture, the diagnosis of necrobiotic xantho granuloma should be considered even in the absence of paraproteinemia.

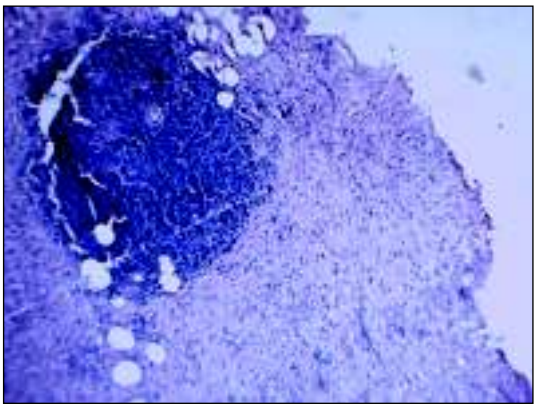

Fig 1-a lymphoid follicle is seen surrounded by foamy histiocytes(H&EX100)

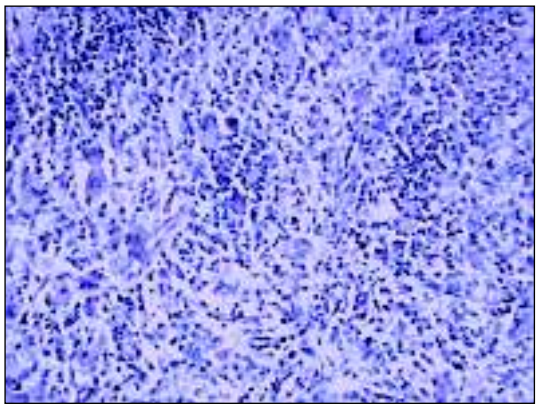

Fig 2-foreign body giant cells and touton giant cells seen amidst lymphocytes and histiocytes(H&EX400)

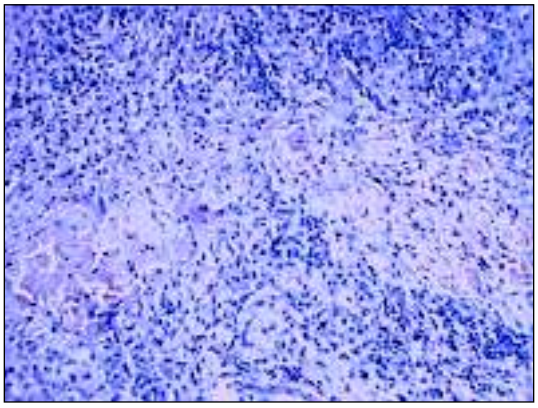

Fig3-area of necrobiosis seen in the centre surrounded by lymphocytes and histiocytes.(H&EX400)

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Review Article

# LARYNGECTOMY: FROM STRIDOR TO SURVIVAL- OUR EXPERIENCE

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Abstract:
Laryngeal cancer is among the commonest cancers in the body. It constitutes one fifth of all head and neck cancers. India accounts for 17% of the world burden. Even though it is curable in early stages, majority of them in India present in the later stages due to ignorance and poverty. The mainstay of treatment in advanced cases is total laryngectomy, with or without post operative radiotherapy which results in loss of voice. However, by speech rehabilitation and by modification of the technique in the form of near total laryngectomy, these patients can be assured of good quality disease free survival.
Keywords: Laryngeal cancer; Total Laryngectomy; Near total laryngectomy; voice rehabilitation

## Introduction:

The larynx is an integral part of upper aerodigestive tract and has vital functions like protection of the lower airways, phonation and fixation of thoracic cage. Any lesion involving the larynx or any procedure that ablates the larynx, adversely affects these functions.

Larynx cancer accounts for 3.4% of all cancers and one-fifth of head and neck cancers. Larynx cancer accounts for 152,000 cases globally, and India accounts for 17% of the world burden. (1) In India the population based registry estimates , 25000 new cases of laryngeal cancers and 2500 cases of hypopharyngeal cancers are added every year, that means Indian every year 50000 people are at risk of compromised voice.

The larynx is divided into sub-sites: Supraglottis, Glottis and Subglottis and malignancies involving these areas behave differently as far as the clinical presentation, spread and management are concerned(2). In the western world, glottic carcinomas constitute 55% to 75% of laryngeal malignancies. These patients present early with hoarseness as the symptom and hence the diagnosis is made in the early stages.(3,4) However in India, the patients with glottis malignancy often present in the later stages because they tend to neglect the initial symptom of the disease.

Hypopharynx is closely related to larynx as the pyriform fossa, which is a part of hypopharynx shares its medial wall with the larynx. Because of the close proximity of the hypopharynx to the larynx, in the surgical management of the hypopharyngeal malignancies, larynx needs to be sacrificed as an innocent bystander.

The early laryngeal and hypopharyngeal cancers are treated by modalities like radiotherapy, partial laryngectomy procedures and endolaryngeal laser surgery whereas advanced laryngeal and hypopharyngeal tumours are best treated by laryngectomy or laryngopharyngectomy resulting in loss of natural voice.

Total laryngectomy was first performed by Theodore Billroth in 1873 (5). In this procedure the entire larynx is removed and the cut end of the trachea is brought out as a permanent stoma for respiration. Creation of a

neopharynx allows oral feeding. However removal of larynx results in loss of natural voice which is perceived as disability in the community and also a psychosocial handicap. These patients hence require rehabilitation in the form of Oesophageal voice, Tracheo esophageal prosthesis and electrolarynx to attain alternative mode of speech.

Early laryngeal cancers are considered as curable cancers either with radiotherapy or surgery with cure rates of 80% to 95% with both forms of treatment.

Even in patients with advanced T3, T4 laryngeal cancers Total laryngectomy with post operative radiotherapy has a 5 year survival rate of 80%, which overweighs the disability produced by the surgery. The treatment results of T3 and T4 tumors demonstrate better cure after surgery as compared with irradiation. Vermund found surgical cure in 59% (41 of 69) T3N0 and T4N0 tumors as compared with 25% (9 of 36) by irradiation.(5)

With the introduction of near total laryngectomy by Bruce Pearson in the 1980s, there is a ray of hope of voice conservation in advanced laryngeal and hypopharyngeal malignancis. Lesions which are suitable for near total laryngectomy are those which are well lateralized and have sufficient uninvolved mucosa for reconstruction of a neo laryngeal pathway. The survival rate after near total laryngectomy is 70 to 75 % and speech acquisition is seen in 83-85% of the patients. Local recurrence of malignancy is seen in 8%.(7)

In the recent years Chemoradiation is gaining larger importance even in surgically resectable advanced laryngeal and hypopharyngeal malignancies in an attempt to preserve voice. Although some tumors respond to primary chemoradiation therapy, there are many cases in which the tumor does not respond and require salvage surgical resection, which results in high surgical complications. Laryngectomy, when performed meticulously in selected indicated patients, and when these patients are rehabilitated thoroughly, can offer good quality disease free survival.

In this study, we conducted a retrospective analysis of ninety patients who underwent laryngectomy for laryngeal and hypopharyngeal cancers. These patients were studied with respect to age and sex, habits, clinical features, stage and site of the disease, type of laryngectomy done, complications of surgery and survival.

Materials and methods:

Ninety patients who underwent Laryngectomy for malignancy of the larynx and hypopharynx in the department of ENT, K.S.Hegde Hospital from the year 2000 to 2010 were identified and their records reviewed. Age and sex distribution, Clinical features, type of presentation, and history of smoking and alcohol consumption, type, site and stage of malignancy, type of laryngectomy done were recorded in a proforma. Post operatively, swallowing, speech acquisition, and complication of the procedure, disease free survival were analysed. Quality of life after surgery was recorded.

Those patients who underwent laryngectomy as a primary modality of treatment and those patients who presented with recurrent or residual lesion after chemotherapy or radiotherapy were included in the study.

Results:

In our study, of the ninety patients who underwent laryngectomy, 87 (96%) were males and 3 (4%) were females. Most of the patients were in the age group of 55-65 years. The youngest patient in our study group was aged 34 years. Among the male patients 83 (92%) patients had history of smoking. Sixty patients (66%) also had history of alcohol consumption. Three female patients did not have any habits. Most of the patients presented with hoarseness (58%). Stridor, Foreign body sensation and Dysphagia were the other presenting

complaints (Table 1). Eight patients with stridor required tracheostomy during the first visit.

The most common malignancy in the study group was Transglottic malignancy (n=42) followed by supraglottis (n=24) and pyriform fossa (n=18), and T2 Glottis Malignancy (n=6).

Majority of the patients in our study had no lymph nodes on presentation (n=57) followed by N1 (n=12) and N2a (n=12), closely followed by N2b (n=9). Majority of patients with pyriform fossa malignancy in the study group had lymph node metastasis (n=12). All the tumors were squamous cell carcinoma on histopathology.

Of the ninety patients, 60% underwent total laryngectomy 27% underwent near total laryngectomy and salvage surgery following radiotherapy or chemotherapy was done in 10%

Average hospital stay was 12 days after surgery, in patients without complications. The hospital stay was prolonged in those patients developing complications, average stay being 22 days. Nasogastric tube feeding was started 24 hours after surgery. Oral feeding was started on 10$^{th}$ postoperative day, initially with liquids, followed by semisolids and solids.

The most common complication that followed total laryngectomy was hypothyroidism (n=45) (table 2). Stomal stenosis was seen in 12 patients, most of them were those who underwent Near Total Laryngectomy. None of them developed severe breathing difficulty. Eight patients developed salivary leak which was healed after conservative management in all patients after average three weeks.

Speech rehabilitation was offered to all the patients. All the patients (27%) who underwent NTL acquired laryngeal speech. 40% percent of the patients developed oesophageal speech after speech training. Three percent required tracheoesophageal puncture for phonation. 7% failed to acquire speech of any form, 8% were lost to follow up and 6 % died of disease. Table 3.

The average three year survival in the study group was 90%. And average five year survival was 80%. Majority of them continued to do their original work ( 80%), where as others who could not perform hard manual work, changed their occupation.

Discussion:

Laryngeal and hypopharyngeal malignancy accounts for one fifth of all head and neck malignancies (1). The treatment option for laryngeal malignancy revolve around various factors like the Stage of tumour, site of the tumour, lymph node metastasis, general condition of the patient and associated comorbidities (3). For advanced T3 and T4 stage laryngeal and hypopharyngeal tumours, mainstay of treatment is Laryngectomy with post operative radiotherapy.

The treatment protocols have evolved in three notable stages; the first was focused on curing patients using radical surgical procedures, mainly by total laryngectomy. The second era developed with the goal of voice preservation while using sound oncological principals. The final and current era is of organ sparing protocols utilizing a combination of radiation and chemotherapy. (4) However, chemotherapy with radiotherapy is not an option for every patient. The toxic effects, like severe nausea, fatigue, mucositis, xerostomia, nephrotoxicity and suppression of the hematopoetic and immune system may cause significant morbidity in some patients. Also, mere preservation of larynx does not ensure its function since exposure to chemotherapeutic agents and radiation results in pain, dysphagia, and continued speech disturbances due to post treatment edema and fibrosis. Even though these patients get rid of the cancer, they continue to have original symptoms as sequelae of treatment.

Total laryngectomy is perceived as a disabling surgery, having detrimental effect on the quality of life. The disadvantages of this surgery are loss of voice, permanent stoma (fig 1), inability to perform heavy manual work and social disruption. In this study, we studied 90 patients, who underwent laryngectomy, regarding the swallowing, speech, quality of life and survival.

Among the 90 patients, 87 were males and only three were females. This male preponderance in laryngeal and hypopharyngeal cancers can be attributable to the high incidence of smoking in males.

All patients had normal swallowing after surgery.

After laryngectomy, restoration of voice remains the major challenge. After total laryngectomy, voice rehabilitation offered includes oesophageal speech, tracheoesophageal puncture (fig 4 ) and electrolarynx.

Oesophageal speech is a type of non lung powered speech, which involves swallowing of air and controlled expression of air across pharyngoesophageal segment. The vibration of this segment results in voice production.

Tracheoesophageal puncture involves shunting of air across the tracheoesophaeal fistula for phonation (8).

Electrolarynx is an electronic battery operated hand held device which produces a tone when applied in close approximation to the skin of the neck. The tone produced is then articulated and speech is produced.

Near total laryngectomy is a voice conservation surgery. It involves preservation of uninvolved vocal cord and ipsilateral functioning recurrent laryngeal nerve. Air is shunted across the myomucosal shunt (fig 3)that is created from the tracheostoma to the neopharynx, to produce lung powered by vibrating the preserved vocal cord. This surgery is oncologically sound and the quality of speech produced is good. Since it is not dependent on prosthesis it is maintenance free.

Majority of the patients who underwent total laryngectomy acquired alaryngeal speech in the form of oesophageal speech, Tracheoesophageal puncture or electrolarynx, except 8% who failed to acquire speech. All patients who underwent near total laryngectomy acquired laryngeal speech.

Conclusion:

Improving survival is the main goal in treating patients with cancer of the larynx and hypopharynx. Total laryngectomy, which is a radical procedure, has disabling outcomes like loss of speech. So, voice conservation surgeries and chemoradiation have emerged as alternative to radical surgery. However, advanced laryngeal and hypopharyngeal malignancies, still require total laryngectomy for better survival rate. When properly rehabilitated, these patients can acquire alaryngeal speech and can lead a good quality life. Various options for acquisition of speech reduce the impact of the loss.

Near total laryngectomy is an oncologically sound procedure in selected advanced cases which preserves laryngeal speech. These two modalities offer rehabilitation at no extra cost of prosthesis and recurring expenses of its maintenance

Therefore the option laryngectomy should not be deferred for patients with advanced laryngeal and hypophayngeal malignancy for the fear of loss of voice, as the disease free survival and the quality of life outperforms all the other modalities of treatment.

| Table 1: Presentation of patients with laryngeal and hypopharyngeal malignancy. | |
|---|---|
| Symptoms | Number of patients |
| Hoarseness of voice | 52 |
| Stridor | 21 |
| FBS | 27 |
| Dysphagia | 27 |
| Referred otalgia | 10 |

| Table 2: Complications following laryngectomy. | |
|---|---|
| Complications | No. of patients |
| Hypocalcemia | 9 |
| Pharyngocutaneous fistula | 8 |
| Nodal Recurrence | 6 |
| Chylous leak | 1 |
| Second Primary | 3 |
| Stomal Stenosis | 12 |
| Hypothyroidism | 45 |
| Death due to disesae | 1 |

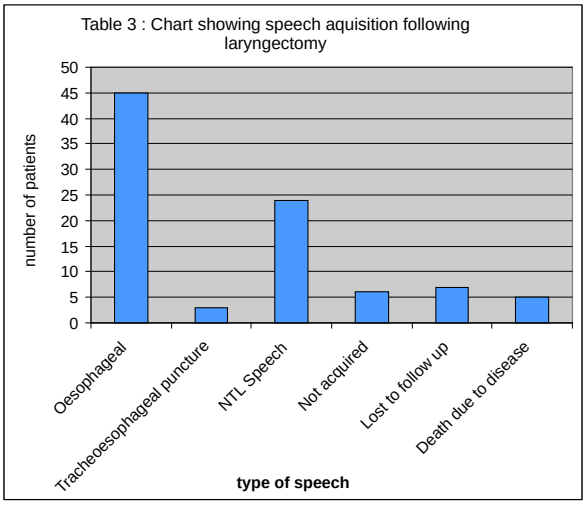


Fig 1 : Well healed stoma.

Fig 2: Laryngectomy specimen.

Fig 3 : Neopharyngeal stoma being constructed.

Fig 4: Tracheoesophageal prosthesis. (Nagpur prosthesis)

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