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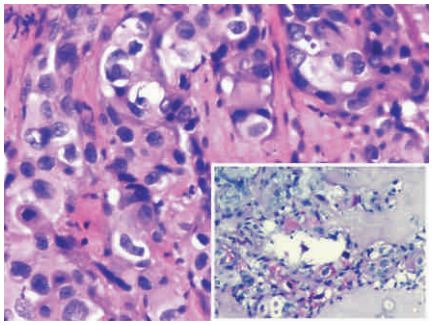

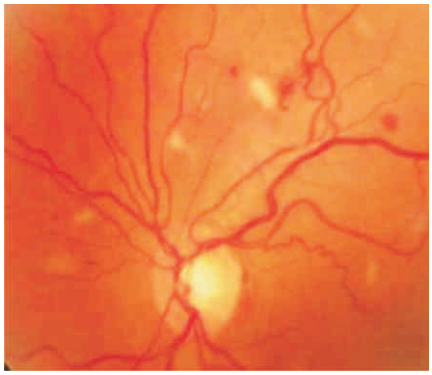

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**JAN - JUNE 2011**


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I am extremely glad to bring out the January – June 2011 issue of SRJM. I take this opportunity to thank the Editorial Board members, reviewers, contributors and the secretarial staff in bringing out this Edition.

This issue has six (6) original articles, one (1) review article and four (4) case reports along with Images in Medicine. The highlight is the contribution of the faculty members other than Sri Ramachandra University, for the first time since the Journal has been published.

One original article on "A cross-sectional study of HIV/AIDS awareness among college students and influence of lifestyle" and another one on "Prevalence of Chronic energy deficiency, overweight and obesity among the geriatric population in a rural area" have been contributed by the faculty of Saveetha Medical College, Chennai and Vinayaka Missions Medical College, Salem respectively.

We also have more articles from clinical departments.

Looking forward for much more contributions,

**P.V. VIJAYARAGHAVAN**
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The Editorial Board gratefully acknowledges their contribution.

# OCULAR MANIFESTATIONS IN HEMATOLOGICAL DISORDERS

**Kalpana Suresh[a], Ramya Sampath[a], Tanvi[a]**

***ABSTRACT:***

***Background & Objectives:*** *To analyse the incidence of ocular involvement in systemic haematological disorders.*

***Methods:*** *This was a prospective non-interventional study done on 40 patients with blood disorders diagnosed from June 2009 to August 2010. Patients with diabetes, hypertension or dense cataracts were excluded. A complete haematological work up and ocular examination was done.*

***Results:*** *Spectrum of diseases identified included anaemia, paraproteinemia, acute lymphocytic leukaemia, acute myeloid leukaemia, chronic lymphoid leukaemia, Hodgkin's lymphoma, non-Hodgkins lymphoma. Bilateral retinopathy in the form of retinal haemorrhages was noted in 62.5% of patients, anterior segment changes and venous changes were found in 7.5% and 56.25% respectively.*

***Conclusion:*** *Flame shaped haemorrhages was the commonest presentation(100%). Reduction in platelet count with associated endothelial hypoxic damage due to anaemia increases the presence of retinal haemorrhages.*

***Key words :*** *anemic retinopathy, haematological disorders, ocular manifestations.*

SRJM 2011;4:1-4

## INTRODUCTION:

Haematological diseases encompass a wide spectrum of disorders that can present with ocular manifestations. Indeed, ocular manifestations may be the initial indication of an underlying haematological disorder. Blood disorders are one of the major health problems presenting with variable clinical manifestations. Nutritional anemia remains the common haematological abnormality especially in third world nations. The retinal metabolism is unable to tolerate this deprivation of its essential supplies with impunity for too long in anemia, falling prey to hypoxic damage in the end. Anaemic changes can thus be an indicator for the retinal damage manifesting either as haemorrhage or pallor.[1,2] Leukaemia while less prevalent, shows ocular manifestations such as roths spots, arteriolar pallor, proptosis 9-90% of the time due to direct infiltration of ocular tissue or due to an opportunistic infection or as an associated haematological abnormality.[3,4,5] Sub conjunctival and retinal haemorrhages may occur with thrombocytopenia irrespective of any aetiology. Previous reports indicate that their indeed exists a link between haematological abnormality and ocular manifestations.[6,7,8] This study was designed to evaluate the ocular abnormalities in anaemia, platelet disorders and haematological malignancies.

## MATERIALS & METHODS

This prospective non-interventional descriptive study was conducted from June 2009 to August 2010 in the Department of Ophthalmology, Sri Ramachandra University (SRU). 40 patients suffering from haematological disorders were evaluated in the ophthalmology department. Exclusion criteria were patients having diabetes, hypertension, dense cataractous changes and other media opacities which prevented posterior segment examination. A proforma was devised to include patients demographical data, a brief medical history, ophthalmic history, anterior and posterior segment examinations with a haematological profile. All patients underwent detailed examination of the anterior and posterior segment which included best corrected visual acuity, slit lamp evaluation of anterior segment, intraocular pressure measurement, dilated retinal examination using direct, indirect ophthalmoscope and slit lamp biomicroscopy using volk 78 D lens. Fundus photography documentation was done in cases with positive findings. Complete haematological profile including haemoglobin levels, total leucocyte count, differential count, erythrocyte sedimentation rate, platelet count, peripheral blood smear, bone marrow study (for leukaemia and paraproteinemias) and lymph node biopsy (in case of lymphoma) was obtained and recorded.

## RESULTS

Among the forty patients enrolled, the spectrum of diseases included - Leukaemia (40 %) Acute lymphocytic leukaemia (ALL) - 7 (43.75%), Acute myeloid leukaemia (AML) -7 (43.75%), Chronic lymphoid leukaemia (CLL) - 2 (12.5%), Anaemia (20 %), lymphoma (17.5%) (Hodgkins - 2, Non-Hodgkins lymphoma (NHL) - 5), paraproteinemias (12.5 %) and Idiopathic thrombocytopenic purpura(ITP)(10%). (Fig.1).

Blood disorders were more prevalent in the age group of 21-40 years (37.5%) followed by 41-60 years (32.5%).

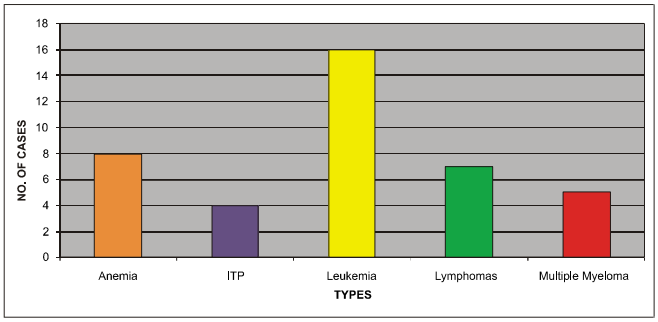


Fig. 1: Spectrum of diseases of study subjects

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Majority were males 62.5% as shown in fig. 2. Females with ocular manifestations predominated in the age group of 61-80 years.

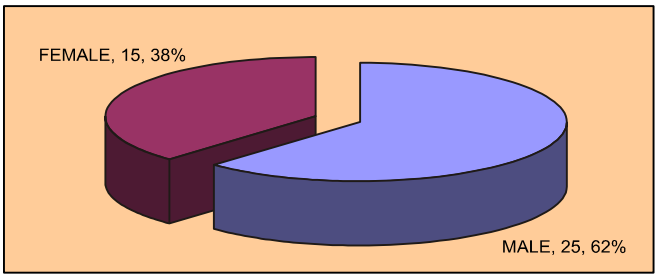


Fig. 2 – Gender related distribution of study subjects

Among the forty patients, 25 (62.5%) had retinopathy, which was mostly bilateral. Of these 58.82 % (20/34) had normocytic normochromic anaemia while 41.17% (14/34) had microcytic hypochromic anaemia on peripheral smear. Among the 8 patients with isolated anaemia and no other additional haematological abnormality was noted. Arterial pallor was found in 5 (62.5%) and background pallor in 5 (62.5%) cases. Of these 60% had Hb < 4 gm % and 40% with Hb between 4 –8 gm %. Venous pallor was present in 5 (62.5%) and dilatation of vessels in 4 (50%). Disc pallor was found in 3 cases (37.5%) of which 66.6% had Hb < 4 gm%. We also encountered anterior segment manifestations in (7.5%) of patients.

Patients who had severe anaemia with a Hb level < 4 g % had 100% venous changes, which reduced to over $^{1}/_{3}^{rd}$ when Hb was above 8g% (25%). Seven cases of anaemia (87.5%) showed retinopathic changes. Among these 57.14% had Hb levels < 4g%, 28.57 % had Hb levels between 4-8 g% and 14.28 % had levels > 8g%. Superficial haemorrhages such as flame shaped haemorrhages (5/7) were found to be most common (77.42%) followed by deep haemorrhages in 57.14% (4/7) and white centred haemorrhages in 42.85 %( 3/7).(Fig.3). Among these 28.57% had a Hb level < 4g/dL. Pre-retinal haemorrhage was present in 28.57% (2/7) and sub hyaloid haemorrhage in 14.28 % (1/7) of cases. It was noted that among patients with Hb level < 4g%, all types of haemorrhages were present while deep (28.57%) and flame shaped haemorrhages were more common in cases with Hb range of 4-8 g% and > 8 g % respectively as shown in Table 1.

Variable retinal changes were seen among the 16 patients with leukaemia. Arterial pallor was noted in 56.2% of patients.[8] Among these 75% (6/8) belonged to a Hb range between 4-8g%. Retinal haemorrhages (Fig.4) were present in 50 %. A subset of this showed haemorrhages to be superficial in 100% and deep in 75%. Roth's spots (Fig.4) were found in 50% of cases among which 75% had TLC count of < 4000cells/ $mm^3$. Venous dilatation was found in 56.25%, background pallor in 5 (31.25%). Majority of these cases 3/5 (60%) had Hb range between 4-8g%. Retinal oedema was present in 25%, optic disc pallor in 3 (18.75%) and hard exudates in 12.5%. About 71.42% of ALL and 42.85% of AML cases showed retinal haemorrhages.

**Table 1 : Types of retinal haemorrhage among study subjects**

| Types of Hge | In Leukemia's n = 16 | In Anemia's n = 8 |
|---|---|---|
| Flame | 100% | 71.42% |
| Roth spots | 50% | 42.85% |
| Deep | 75% | 57.14% |
| Pre-retinal | 12.50% | 28.57% |
| Subhyaloid | 12.50% | 14.28% |

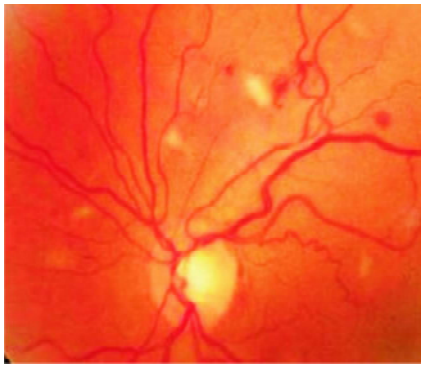

Fig. 3: Retinal haemorrhages and cotton wool spots in anaemia

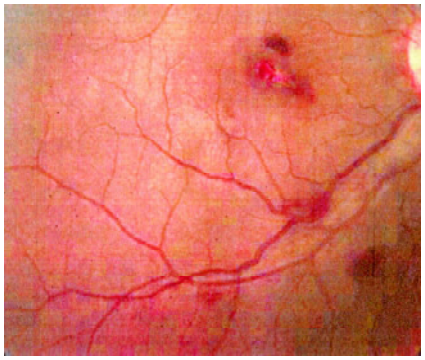

Fig. 4: Flame shaped retinal haemorrhages with Roth spots in leukaemia

Among the 4 cases of ITP background retinal findings predominated with pallor in 50%, hard exudates in 50% and retinal haemorrhage in 50%. A subset of this showed presence of sub-hyaloid haemorrhages in all cases (100%), deep and pre-retinal haemorrhages in 50 % and 75% respectively. Retinal oedema was detected in 25% of cases. Fifty percent of the haemorrhages were associated with a platelet count between 10000-20000 cells/$mm^3$, 25% with a count < 10000 cells/$mm^3$ and > 20000 cells/ $mm^3$ each.

All 5 patients with paraproteinemias were diagnosed with multiple myeloma wherein 40% showed retinal haemorrhages and arterial pallor in 20%.

Among the 7 cases of lymphoma, anterior and posterior segment findings were equally prevalent which included conjunctival congestion (14.28%), anterior chamber reaction (14.28%) and background retinal haemorrhage (14.28%). Patients with non Hodgkins lymphoma had a male predominance with 60 % of the tumors in males.

## DISCUSSION

Hematological disease includes disorders of erythrocytes, leukocytes and platelets as well as disorders of coagulation and plasma proteins. These diseases may affect the eye either as local ocular involvement or as ophthalmic manifestations arising in the disease process. Quite often, ocular manifestations can be the presenting symptom of hematological diseases. Most patients with ocular manifestations are symptomatic requiring an ophthalmic consultation. Hematological disorders can affect any part of the eye and manifestations may vary with disease. Common manifestations include conjunctival pallor and hemorrhages, intraretinal hemorrhages and cotton wool spots. Retinal infiltrates, bleeding manifestations in eyelids, anterior segment, optic nerve, and orbit are relatively uncommon.

Majority of our patients showed a leukemic predominance (40%) followed by anaemia (20%), lymphomas (17.5%), paraproteinemias (12.5%) and ITP (10%). Male showed a greater predisposition (75:25), possibly due to the protective effect of oestrogen in women against retinopathy.

Among 34 diagnosed to have anaemia, 8 had findings of anaemia exclusively without an association with any other haematological disorder. The highest percentage of patients had normocytic, normochromic anaemia (58.82%). This was not consistent with other studies which may probably be due to small sample size. It was observed that it is the decreased amount of Hb in the blood, which leads to the subjective impression of 'pallor'. The critical Hb level was found to be 7g/dl, which was consistent with other studies.[9] The categorisation of anaemia was done as mild, moderate and severe. There was no appreciable difference in patients with disc oedema in severe versus moderate anaemia. The prevalence of pallor was found in all grades of anaemia. Arterial pallor was present in 62.5% of cases.

Flame shape haemorrhages were most frequently documented (100%) followed by deep haemorrhages (75%). Pre retinal haemorrhages were found to be more prevalent in severe anaemia (28.57%). There was no evidence of vitreous haemorrhage in our study. Either the internal limiting membrane halts the forward progress of a pre retinal haemorrhage or the formed posterior vitreous phase prevents the ingress of blood into vitreous in a sub-hyaloid haemorrhage. The prevalence of haemorrhage was found to be 41.17% for microcytic hypochromic anaemia and 58.82% for normochromic, normocytic anaemia.

Venous dilatation was the most common finding (56.25%) in acute leukemias in our study. Literature documents venous dilatation and tortuosity as the initial retinal change in leukaemias.[10] Although it may simply be an evidence of associated anaemia, the entity of anaemia in leukaemia predisposes to haemorrhage than just anaemia due to any other cause.[4,11,12]

Flame shaped haemorrhages (100%) followed by white centred haemorrhages (75%) were prevalent in all patients with anaemia or leukaemia. In most cases of leukaemia , roth spots and superficial flame shaped haemorrhages were seen.[5] However, deep haemorrhages and white centred haemorrhages occur more frequently in anaemia. Males have 3-fold greater chance of developing haemorrhages[5]. The highest prevalence of white centred haemorrhages occurs in patients with total WBC counts $< 4000$ cell/mm$^3$ and upto 25% are associated with counts in between 4000-11000. An increased prevalence is found in the group with platelet counts $< 1.5$ lakh.[13,14] This shows that white centred haemorrhages are linked to the severity of anaemia and lack of platelets. A finding that is inconsistent with previous studies.[11,12]

Ophthalmic manifestations of multiple myeloma can affect equally almost all ocular structures. They may be the first manifestation of the disease. In our study retinal haemorrhages predominated (40%). In Lymphoma, both anterior and posterior segment changes are equally prevalent.[10] Uveitis and extraocular muscle involvement are characteristic ocular features.[15,16] Presence of uveitis in the absence of neurological signs indicates a possibility of intra-ocular lymphoma. Review of literatures reveals non-Hodgkins lymphoma (71.42%) to be found more prevalent than Hodgkins lymphoma.[10] An anterior chamber reaction combined with restriction of extraocular movements are characteristic features to suggest an intraocular lymphoma.

This study was a onetime analysis and patients were referred to us by the concerned experts for their ophthalmic manifestations. Therefore, details of response to therapy could not be documented, a limitation of the present study. The treatment of the underlying haematological disorders was under the direct purview of the primary treating physician and could not be accessed.

## CONCLUSION

This study concludes that males have greater predisposition to ocular haemorrhages as compared to females in setting of haematological abnormalities . Flame shaped haemorrhages is commonest pattern of ocular haemorrhage in hematological disorders. Thrombocytopenia of less than 1,50,000/mm$^3$ increases the propensity for ocular haemorrhages..

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# A CROSS SECTIONAL STUDY OF HIV/AIDS AWARENESS AMONG COLLEGE STUDENTS AND INFLUENCE OF LIFESTYLE

*Shivani Rao [a], G. Palani [b], Ramesh Harihara Iyer [b], B.W.C Sathiyasekaran [b]*

***ABSTRACT:***

***Background:*** *Early phase of youth is a delicate period due to inadequate mental, physical and social maturity. An incomplete psychological development during this period and peer group influence results in adopting risky behaviour making them more vulnerable to HIV/AIDS.*

***Objectives:*** *To assess the awareness regarding HIV/AIDS among college students in South Chennai and to evaluate the association between level of awareness and the influence of certain existing lifestyle issues.*

***Materials and Methods:*** *This was a cross sectional study done on 400 randomly selected undergraduate students of Arts and Science colleges in South Chennai using self-administered questionnaire. Data entry and analysis was done using SPSS 8.0 version and Epi info softwares.*

***Results:*** *Knowledge of awareness regarding, the disease to be viral in etiology, mode of transmission, prevention and treatment was known to 86.3%, 83.8%, 83.8% and 40.5% respectively. Students who did not have risky behaviour had a better knowledge about cause and prevention. Awareness about modes of transmission was better among students indulging in risky lifestyle. Knowledge about treatment was low regardless of their lifestyle. Most common source of information was television and the commonest misconception about HIV transmission was mingling with HIV patients.*

***Conclusion:*** *This study highlights an overall satisfactory level of knowledge on all aspects of HIV except treatment. Students adopting risky lifestyle showed a varied pattern of awareness. Information dissemination should be designed in such a way as to bring about healthy behavioural change targeting the risky lifestyle group. A regular assessment of attitude and level of awareness towards HIV/AIDS is essential to design changes in future educative programmes.*

***Key words:*** *Awareness, college, HIV, lifestyle, students*

SRJM 2011;4:5-10

## INTRODUCTION:

Acquired Immunodeficiency Syndrome (AIDS) caused by Human Immunodeficiency Virus (HIV) is a modern pandemic affecting industrialized and developing countries. Asian AIDS epidemic has risen enormously and about 7.2 million people are presently living with HIV/AIDS in this region.[1] About fifty percent of the new cases occur in individuals below 25 years of age. It is a matter of concern that India holds the second largest absolute number of HIV cases in the world.[1] Though prevalence of HIV has an uneven distribution in India, Southern India and North-eastern states have predominant number of reported cases.[1] To control this epidemic an overall awareness need to be created in general population and especially high risk groups. HIV / AIDS epidemic is worst among the youths as they tend to experiment with practice of risky behavior often with little awareness of the danger. This group is more vulnerable due to incomplete social, emotional and psychological development resulting in risky behavior.[2, 3] Many of them are not prepared to make safe decisions at this age, and without adequate parental monitoring they may be especially susceptible to risky behaviour. Peer group influence paramounts in this period of growth and hence, vulnerability to HIV also increases in individuals with friends who support risky behaviour.

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We need to further concentrate on this younger generation especially the college going students as they represent the country's future. Due to an increase in the incidence of HIV in this younger generation the economy of country would be affected considerably unless further steps are taken to prevent the transmission of this dreadful disease. The young population are the manpower and resource for a economically stable country. Hence, this population being affected means loss of human resources thereby resulting in downfall of economy.[4] Since prevention is the key to AIDS control, empowerment of youth with knowledge about high-risk behaviour and its ominous relation with HIV is one of the most effective tool to control this pandemic.[3]

## AIM

A cross sectional study on awareness and life style issues related to HIV/AIDS, among Arts and Science colleges in South Chennai.

## OBJECTIVES

This study was done to assess the awareness regarding HIV/AIDS among college students in South Chennai and to evaluate the association between the level of awareness with the influence of certain existing lifestyle issues related to HIV.

## MATERIAL AND METHODS

STUDY DESIGN: Cross sectional study comprising of descriptive and analytical components.

STUDY AREA: Arts and Science colleges in South Chennai

STUDY POPULATION: Undergraduate Arts and Science students (Non medical) from randomly selected colleges in South Chennai.

SURVEY PROTOCOL AND SAMPLING: There are 30 Arts

and science colleges in South Chennai. An appeal was made to all the colleges seeking permission to undertake the study. Ten colleges granted permission. A questionnaire was framed relevant to this study population based on various existing issues on awareness of HIV/AIDS from previous studies. Initially a pilot study was carried out among 20 randomly selected students in one Arts and Science college. The inconsistencies were identified during the piloting phase and the questionnaire was modified accordingly to suit the study population. Predominantly these were closed ended questions with few open ended ones. Questionnaire comprised of personal characteristics, their knowledge and awareness about various aspects of HIV/AIDS like aetiology, mode of transmission, diagnosis, treatment and sources of information and lifestyle characteristics of study participants. Confidentiality of the students was ensured. Consent was obtained from students and importance of the study was emphasized before administering the questionnaire.

Sample size was calculated using the formula $n = \frac{Z\alpha^2 pq}{L^2}$

N: Sample size

$Z\alpha$: 1.96

P: Prevalence of awareness of HIV was assumed to be 32% based on literature review as furnished below.

Q: 100-p = 100-32 = 68%

L: Limit of accuracy 5%

Accordingly, $n = \frac{(1.96)^2 X32X68 = 335}{5^2}$

Sample size arrived was 335 + 65 = 400 (We added 65 more to take care of any drop outs or refusal to participate in the study).

List of all students of the 10 colleges were obtained and from each college 40 students were randomly selected using a table of random numbers for a total of 400 of students. Following questionnaire completion, health education was also imparted and their queries were solved.

Data entry and analysis was done using the SPSS 8.0 version and epi info softwares. Frequency was calculated for the variables and $X^2$ test was used wherever necessary to estimate the level of significance.

**RESULTS**

There were 188 Arts and 212 Science students of which 40% were males and 60% were female students in the age group 17 to 25 years with a mean age of 19.46 years and S.D of 1.17years. Seventy two percent of students belonged to nuclear families 72% and 28% were from joint family. As per Prasad's Classification [5] 71% of students belonged to class I, 25.3% to class II, 3.3% to class III and 0.4% belonged class IV. Fathers of 32% students and mothers of 8.25% students were employed in skilled jobs.

Lifestyle issues studied showed higher proportion of males indulged in unfavourable activities as compared to female students. Comparison of risky lifestyle practice adopted with respect to gender is furnished in Table 1.

Table – 1: Life Style Issues among Male and Female students

| Life Style Issue | Male Students | | Female Students | |
|---|---|---|---|---|
| | Number | Percentage | Number | Percentage |
| Alcohol Use | 50 | 12.5 | 15 | 3.8 |
| Drug Abuse | 9 | 2.3 | Nil | — |
| Sexual Exposure | 31 | 7.8 | 9 | 2.3 |
| Late night partying | 20 | 5 | 9 | 2.3 |

Overall level of awareness regarding various aspects of HIV was satisfactory except knowledge regarding treatment (Table 2).

Table – 2: Awareness about various aspects of HIV/AIDS among the college students.

| Aspects of HIV | Number Aware | Percentage | 95% Confidence Interval |
|---|---|---|---|
| Cause | 345 | 86.3 | 82.67 – 89.93 |
| Mode of Transmission | 393 | 98.3 | 97.02 – 99.58 |
| Treatment | 162 | 40.5 | 32.94 – 48.06 |
| Prevention | 311 | 83.8 | 79.70 – 87.90 |

The awareness about cause of HIV was known to 84.2% of males and 87.6 % of females. Awareness about cause of HIV by certain lifestyle issues is shown in Table 3. Awareness about one or more modes of transmission was known to 98.1% of the males and 98.3% of the females. All students who consumed alcohol knew one or more routes of transmission of HIV while awareness about transmission among non alcohol users was 98% (Table 4). All the 9 students who were abusing drugs, had awareness about one or more modes of transmission of HIV while awareness among non drug abusers about transmission was 98.2%.

Table 3: Awareness of cause of HIV with respect to certain lifestyle issues

| Characteristic | Total Number | Number Aware | Percentage |
|---|---|---|---|
| **Alcohol Use** | | | |
| User | 65 | 55 | 84.6 |
| Non-User | 335 | 290 | 86.6 |
| **Drug Abuse** | | | |
| User | 9 | 4 | 44.4 |
| Non-User | 391 | 341 | 87.2 |
| **Sexual Exposure** | | | |
| Yes | 38 | 31 | 81.6 |
| No | 362 | 314 | 86.7 |
| **Late Night Partying** | | | |
| Yes | 29 | 24 | 82.8 |
| No | 371 | 321 | 86.5 |

Table 4: Awareness of Transmission of HIV by certain Life Style Issues characteristics

| Characteristic | Total Number | Number Aware | Percentage |
|---|---|---|---|
| **Tobacco Use** | | | |
| User | 53 | 53 | 100 |
| Non-User | 347 | 340 | 98 |
| **Alcohol Use** | | | |
| User | 65 | 65 | 100 |
| Non-User | 335 | 328 | 97.9 |
| **Drug Abuse** | | | |
| User | 9 | 9 | 100 |
| Non-User | 391 | 384 | 98.2 |
| **Sexual Exposure** | | | |
| Yes | 38 | 38 | 100 |
| No | 362 | 355 | 98.1 |
| **Social Gathering** | | | |
| Yes | 29 | 29 | 100 |
| No | 371 | 364 | 98.1 |

All the 38 students who had sexual exposure knew about some modes of transmission of HIV while awareness about transmission among students who had no sexual exposure was 98.1%. All the 29 students who liked going for social gathering had awareness about some modes of transmission of HIV while the awareness about transmission among those who did not like going for social gathering was 98.1%.

Of the various modes of transmission, sexual route was known to most of the students (89.9%), which was followed by blood transfusion (84.8%), mother to baby (78.3%), using unsterilized needles (70.5%) and sharing razor (25%).

The awareness regarding prevention of HIV was known to 89.9% of males and 94.6% of females. A comparison of level of awareness about prevention of HIV, among students who indulged and did not practice risky behaviour is shown in Table 5.

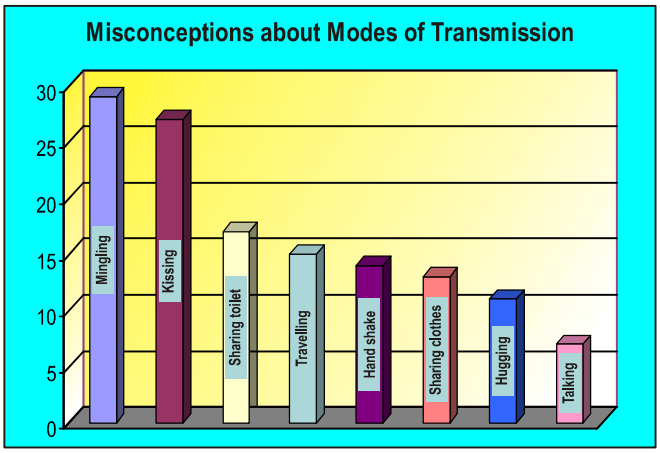
Table 5: Awareness of prevention of HIV with respect to certain lifestyle characteristics

| Characteristic | Total Number | Number Aware | Percentage |
|---|---|---|---|
| **Alcohol Use** | | | |
| User | 65 | 55 | 84.6 |
| Non-User | 335 | 316 | 94.3 |
| **Drug Abuse** | | | |
| User | 9 | 9 | 100 |
| Non-User | 391 | 362 | 92.6 |
| **Sexual Exposure** | | | |
| Yes | 38 | 34 | 89.5 |
| No | 362 | 337 | 93.1 |
| **Late Night Partying** | | | |
| Yes | 29 | 25 | 36.2 |
| No | 371 | 346 | 93.3 |

Condom was the most common method mentioned for prevention of HIV (77.8%). The awareness about modes of prevention of HIV among drug abusers was 100% and among non drug abusers was 92.6%. The awareness about modes of prevention of HIV among students who had sexual exposure was 89.5% and among those who did not have sexual exposure was 93.1%. The awareness about modes of prevention of HIV among students who liked going for social gathering was 89.5% and among those who did not like going for social gathering was 93.1%.

Commonest misconceptions about spread of HIV was mingling with HIV patient mentioned (7.3%), kissing (6.8%), sharing toilet (4.3%), traveling (3.8%), spread by handshake with HIV patient (3.5%), sharing clothes (3.3%), hugging (2.8%) and 1.8% students felt HIV spreads by talking to HIV patient (Fig.1).

Fig. 1 : Misconceptions about Modes of Tranmission




The awareness about treatment of HIV was 43.0% and 38.8% among males and females respectively. Level of awareness regarding treatment of HIV with respect to lifestyle is shown in Table 6.

Table 6: Awareness of treatment of HIV by certain lifestyle issues

| Characteristic | Total Number | Number Aware | Percentage |
|---|---|---|---|
| **Alcohol Use** | | | |
| User | 65 | 37 | 56.9 |
| Non-User | 335 | 125 | 37.3 |
| **Drug Abuse** | | | |
| User | 9 | 5 | 55.6 |
| Non-User | 391 | 157 | 40.2 |
| **Sexual Exposure** | | | |
| Yes | 38 | 18 | 47.4 |
| No | 362 | 144 | 39.8 |
| **Late Night Partying** | | | |
| Yes | 29 | 14 | 48.3 |
| No | 371 | 148 | 39.6 |

Majority of students 83.3% gathered information from television followed by radio 51% (Table 7).

Table 7: Sources of information of HIV

| Information obtained | Number | Percentage |
|---|---|---|
| Television | 333 | 83.3 |
| Radio | 204 | 51 |
| Books | 198 | 49.5 |
| Magazines/Newspapers | 188 | 47 |
| Teachers | 186 | 46.5 |
| Friends | 180 | 45 |
| Posters | 138 | 34.5 |
| Internet | 132 | 33 |
| Health personnel | 127 | 31.8 |
| Hoarding | 73 | 18.3 |

**Statistics derived**

Difference in sex wise awareness about modes of transmission was not statistically significant ($\chi^2_1$ 0.03, p = 0.85). The awareness about modes of transmission of HIV among alcohol users and non users was not statistically significant ($\chi^2_1$ 1.38, p = 0.24). The awareness about modes of transmission of HIV among drug abusers and non drug abusers was not statistically significant ($\chi^2_1$ 0.16, p = 0.69). The awareness about modes of transmission of HIV among students who had sexual exposure was 100% and among those who did not have sexual was not statistically significant ($\chi^2_1$ 0.75, p = 0.39). The difference in awareness about modes of transmission by students attending late night parties and those not attending was not statistically significant ($\chi^2_1$ 0.56, p = 0.46).

The difference in awareness about modes of prevention with respect to gender is not statistically significant ($\chi^2_1$ 3.21, p = 0.07).The risk of lesser awareness about modes of prevention of HIV among males is 1.98 times. The awareness about modes of prevention of HIV among alcohol users was 84.6% and among non alcohol users was 94.3% and this awareness about modes of prevention by alcohol consumption was statistically significant ($\chi^2_1$ 7.64, p = 0.006). Alcohol users have the risk of lesser awareness about modes of prevention to the extent of 3.02 times. Awareness about modes of prevention by students who abused drugs and did not use was not statistically significant ($\chi^2_1$ 0.72, p = 0.40). The awareness about modes of prevention of HIV among students who had sexual exposure and among those who did not have sexual exposure was about modes of prevention by sexual exposure was not statistically significant ($\chi^2_1$ 0.67, p = 0.41). The awareness about modes of prevention of HIV among students who liked going for late night parties and who did not was not statistically significant ($\chi^2_1$ 1.99, p = 0.16).

## DISCUSSION

The alarming rate of spread of HIV, lack of curative therapy and vaccine to prevent it mandates a need for ongoing and consistent health education programme. AIDS prevention largely depends on health education and behavioural changes based on AIDS awareness, particularly among young adults who are prone to high risk behaviour. Before we undertake such kind of awareness programme, we need to know the existing level of awareness of the target population in those areas. An evaluation of HIV awareness is required for two reasons. One to get baseline epidemiological data regarding the existing knowledge and attitude prior to implementing an awareness programme and another reason is to assess the effect or reach of educative campaigns.[3]

The lifestyle characteristics of these students were analysed in order to relate it to level of awareness of HIV. Earlier studies have concentrated on specific groups as high risk groups or a more specific population groups such as nurses, naval officers without any association with lifestyle.[6] In this study, we chose the particular youth as risk groups as listed in the results tables to compare awareness of HIV among them and students with non- risky lifestyle. Substance addiction by youth mainly includes alcohol, tobacco and drugs. Addiction of these substances can result in direct and indirect consequences affecting their health and one substance abuse can also add to another. In a study conducted by Slesnick et al, about 56% of youth indulged in more than one substance-use disorder and 14% in three.[7]

The concept of rave parties started in the '80s in the west where people gathered in weekends around a fire and had enjoyment with disc-jockeys and other electronic dance music. Over time, means of enjoyment in these parties have changed and have been replaced by weird things. In recent years, rave parties and Drug cocktails are becoming the in-thing for college going youth, high salaried executives and young white-collared corporate elite with little knowledge as to how to spend it properly. Now-a-days these parties go on all night with consumption of drugs like cocaine, Marijuana, hash, Ecstasy, acid and speed which results in dreadful consequences.[8]

High risk behaviour was seen in both male and female students. Most common addiction in both genders was alcohol consumption. Though drug addiction was found in 5.7% of males, there were no female students who mentioned that they abused drugs. Considering the nature of subject being explored, the chances of hiding information cannot be ruled out. Probably greater sensitization and confidentiality might be needed for the respondents to come out with more correct responses. Another reason could be that the contact with them was for a brief period without adequate familiarity. Frequent contact and interaction would be beneficial in creating greater confidence and getting a true information regarding these issues.

Majority of respondents (86.3%) were aware of a viral etiology of this disease. Awareness of cause of etiology is quite high in this study as compared to another study done by Bhalwar et al[3] who found less than 50% of students with this knowledge which could be due to the fact that they studied the knowledge of rural students and AIDS

awareness and education programme organized by different agencies are usually concentrated in urban areas.[3] Another reason felt was access to modern facilities (including mass media) which is more in urban areas.[9] A similar finding among drug abusers was noticed by Bhalwar et al.[3] The present study along with other studies mentioned above show a higher level of awareness among girls about cause which could be due to girls being more serious in learning and knowing about this dreadful disease.[10, 11] On analysis of awareness of cause of HIV and certain lifestyle issues, it was found that there was not much of difference between students who consumed alcohol, had sexual exposure, party goers and who did not do these activities. Present study showed that awareness of cause of disease was lower among drug abusers. In a Chinese study, the college going students were even aware of the difference between HIV and AIDS.[12]

Awareness regarding transmission of HIV in this study was high and a better level of awareness about transmission in this study may be due to massive campaigns by Tamilnadu state AIDS control society (TANSAC) and National AIDS control organization (NACO). Sexual route was the single most mode of transmission mentioned by almost 90% of students. Other similar studies have also reported maximum number of respondents being aware of sexual route of HIV transmission.[3, 11]

Only one third of the students were aware that HIV infection can be transmitted by sharing razor in our study. Kore et al found a better response with 85% of males and 64% of female college students who were aware that HIV spreads by sharing razor.[10] This needs to be dealt more seriously since using razor is one of the daily needs of a male gender. Hence, awareness has to be created among the mass about this mode to prevent the infection by this route. The present study showed a good number of students being aware of blood and perinatal transmission of HIV.

There are wrong beliefs that HIV could be transmitted by various means such as mosquito bites, sharing meals, casual contact, and using public swimming pools and toilets.[4] Commonest misconceptions about HIV transmission identified in our study was was mingling with HIV patients. Chatterjee et al found school children having various misconceptions such as hand shaking, kissing, sharing toilets and exchanging clothes.[11] Kissing as a mode of transmission was a major misconception (37%) in a Nigerian study by Gugnani et al done on non medical students as compared to 6.8% in our study.[13] In a Chinese study on college students, on asking if they would like to be involved in the treatment of HIV/AIDS patient, 37.8% answered that they would not discriminate, 31.8% were not sure and 27.1% replied that they would treat an infected person differently.[12] HIV stigmatization can result in mental trauma to those living with HIV causing loss of self-esteem as well as deterioration in social interactions with others.[14] It is the inadequate knowledge that results in such misconceptions, attitude and behavioural differences towards HIV patients. Steps should be taken to remove apprehensions in common man for a better attitude towards affected individuals.

The predominant preventive method mentioned by students was condom. Similar results were obtained by Kore et al and Ganguli et al in their study on college students.[10,15] The reason for such awareness could be attributed to educative programmes.

Our study showed no significant difference in the level of awareness regarding modes of transmission of HIV among groups with respect to lifestyle. The association of awareness of HIV prevention and students who did not consume alcohol was statistically significant in this study ($p < 0.05$). These data suggest a higher level of awareness among students with no addiction. On reviewing the literature such kind of association has not been evaluated by any study in the past. The present study has revealed that high risk group of population are still ignorant about preventive measures in HIV transmission. Low level of awareness regarding HIV prevention seen in late night party goers, reason can be probably due to their engagement in activities such as partying and not spend time in reading educative books and watch educative programmes. This group needs to be identified and implementation of more elaborate programmes focusing this group of population should be done at a place were they frequently visit. Hoardings, posters, music on HIV awareness can be available by concerned authorities near such kind of places as this group needs to be approached in order to educate them as they would never bother to look into such issues themselves.

The level of awareness about treatment was better among males 43% than females 38.8% Although the level of awareness regarding treatment in our study is low, it was still better than the results obtained by Kore et al in a similar study.[10] The knowledge that HIV is not curable, however, progression can be delayed to some extent by giving symptomatic treatment has to be emphasized and spread across in order to educate the mass. Another reason is for them to bring their known ones for symptomatic treatment. In educative programmes, emphasis has to be given that HIV is not curable but preventable by specific means and treatment options are available to treat the symptoms and delay the progress of disease thereby extending their lifespan.

Among various means of communication television was the main source of information of respondents which was followed by radio in this study.[16,17] Similar study done by Lal et al[9] among college students of Kerala, reported newspaper as the major source of information followed by television and radio. In this study knowledge from print media was only 47% which indicates need for motivation of students to read scientific books to update their knowledge. In the present study teachers also played a vital role in imparting knowledge about HIV. At the school level students are much more mouldable in their thoughts and receptive to their teachers so educative lessons on HIV should be introduced in their school curriculum.

In spite of large number of hoardings in Chennai still only 18.3% gathered information from this source. This maybe either due to less number of hoardings in public areas or the presentation was not impressive enough to make

an impact. This study revealed poor performance of health personnel in disseminating HIV related knowledge among college students (31.8%). Doctors remain occupied with clinical work and have less time time to impart health education so it is important to train existing paramedical staff, social workers, anganwadi workers and community health volunteers for creating awareness.[18] This could be overcome by increasing the interaction with health personnel and constant motivation by them.

## CONCLUSION

The present study demonstrates an overall satisfactory level of awareness on routes of transmission and prevention of HIV/AIDS. Analysis on lifestyle revealed that students indulging in risky behaviour had an overall low level of awareness regarding modes of prevention of HIV. We need to target high risk group for dissemination of knowledge on HIV, especially with regards to preventive measures. Newer strategies need to be formulated and executed to draw the attention of this vulnerable younger population in order to educate and also correct their false beliefs to curb this epidemic. A continuous such surveys at regular intervals would further help to assess the level of awareness and attitude toward HIV/AIDS for designing future educative programmes.

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# IN VITRO ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF METHANOL EXTRACT OF *STEREOSPERMUM COLAIS* (BUCH.- HAM. EX.DILLW).

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***ABSTRACT:***

***Background and Objectives:*** *Stereospermum colais (Bignoniaceae) has good ethnopharmacological value in Ayurvedic system of Medicine. However, its antioxidant and anti-inflammatory effects are not explored yet. Therefore, the objective of this study is to evaluate the antioxidant and anti-inflammatory effect of the methanol extract of the leaves of Stereospermum colais by in vitro methods.*

***Methods :*** *The antioxidant activity was studied using 1,1- Diphenyl -2- picrylhydrazyl (DPPH) and nitric oxide radical scavenging activity. In vitro anti-inflammatory activity was evaluated using membrane stabilization assay.*

***Results :*** *The methanol extract of the leaves of S. colais showed the presence of carbohydrates, saponins, flavonoids, glycosides, terpenoids, phenols, anthraquinones, tannins, proteins and aminoacids. In the in vitro antioxidant assays, the free radicals were scavenged by the test compounds in a concentration dependent manner upto the given concentration in both the models which is comparable to that of the standard curcumin. In the in vitro anti inflammatory assay the methanol extract showed significant anti-inflammatory activity at the concentration of 1000µg/ml which is comparable to that of the standard drug Diclofenac sodium.*

***Conclusion :*** *The methanol extract of the leaves of Stereospermum colais showed significant antioxidant and anti-inflammatory effect. The results obtained in the present study indicate that Stereospermum colais is a potential source of natural antioxidant and anti-inflammatory agent.*

***Key words:*** *Stereospermum colais, Antioxidant, Anti-inflammatory.*

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## INTRODUCTION:

In recent years much attention has been devoted to natural antioxidant and their association with health benefits.[1] It is commonly accepted that the reactive oxygen species play an important role related to many chronic and degenerative diseases such as aging[2], cancer, coronary heart disease, Diabetes mellitus, atherosclerosis, neurodegenerative disorders[3] and inflammation.[4]

Inflammation is a complex localized response to foreign substances. In many inflammatory disorders there is excessive activation of phagocytes, production of $O_2$, $OH^{\cdot}$ radicals as well as non-free radical species [$H_2O_2$].[5] These free radicals are the main culprits in lipid peroxidation resulting in membrane destruction followed by production of mediators and chemotactic factors.[6] Hence the agents that can scavenge these reactive oxygen species can be beneficial in the treatment of inflammatory disorders.

Various medicinal plants provide relief from symptoms comparable to that obtained from allopathic medicines.[7] It has been suggested that many anti-inflammatory drugs may exert some of their effects by scavenging oxidants, and decreasing formation of Reactive Oxygen Species (ROS) by activated phagocytes.[8]

*Stereospermum colais* (Buch.- Ham. Ex Dillw) Mabberley (Family: Bignoniaceae) is a large deciduous tree distributed throughout India.[9] It is commonly known as 'Pathiri' in Tamil and 'Parral' in Hindi. In Ayurveda, the leaves of the plant are used in otalgia, odontalgia, rheumatalgia, malarial fever and wounds.[10] So far its antioxidant and anti inflammatory properties have not yet been pharmacologically evaluated. Hence, the present study was undertaken to evaluate the antioxidant and anti-inflammatory activity of *Stereospermum colais* by *in vitro* methods.

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## MATERIALS AND METHODS

### Plant material

The leaves of *Stereospermum colais* was collected from Alagar kovil hills, Madurai, Tamilnadu and authenticated by Prof. P. Jayaraman, PARC, Tambaram, Chennai. The voucher specimen [PARC/2007/80] was deposited at the Department of Pharmacognosy, Madras Medical College, Chennai.

### Preparation of plant extract

The leaves of *Stereospermum colais* was shade dried, powdered and defatted using petroleum ether (60-80$^0$C) and successively extracted with methanol. Extract was filtered through vacuum filter and the filtrate was concentrated in vacuum evaporator.

### Preliminary Phytochemical Analysis

Preliminary phytochemical analysis[11] was carried out for carbohydrates, saponins, flavonoids, glycosides, terpenoids, steroids, tannins, proteins and aminoacids.

**Determination of antioxidant activity**

**DPPH radical scavenging assay**

DPPH radical scavenging activity was done using the method of Yohozowa *et al*.[12] The reaction mixture containing 1ml of DPPH solution (200$\mu$M in ethanol) with different concentrations of the extract was shaken and incubated for 20min at room temperature. The resultant absorbance was recorded at 517nm. The percentage inhibition was calculated using the formula

$$\text{Percentage inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

**Nitric oxide radical scavenging Assay**

The nitric oxide radical scavenging activity was done using the method of Alderson et al.[13] 3ml of reaction mixture containing sodium nitroprusside (10mM in phosphate buffered saline) and various concentrations of the extracts were incubated at 37$^0$C for 4 hours. To the incubation solution, 0.5ml of Griess reagent was added and the absorbance was read at 546nm. The percentage inhibition was calculated using the formula

$$\text{Percentage inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

***In vitro* anti-inflammatory activity**

**Membrane stabilization assay**

The HRBC membrane stabilization method has been used to study the anti-inflammatory activity.[14] Blood was collected from the healthy volunteers and mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000rpm and packed cells were washed with isosaline (0.85%, PH 7.2) and a suspension was made with isosaline (10% v/v).

The assay mixture contained 1ml of phosphate buffer (0.15M, pH 7.4), 2ml of hyposaline (0.36%), 0.5ml of HRBC suspension and 1ml of various concentrations of the extract. Diclofenac sodium was used as reference drug. In the control solution instead of hyposaline, 2ml of distilled water was added. The mixtures were incubated at 37$^0$C for 30min and centrifuged. The absorbance of the supernatant solution was read at 560nm. The percentage haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization was calculated using the formula.

$$\text{Percentage membrane stabilization} = 100 - \frac{\text{O.D of drug treated sample}}{\text{O.D of control}} \times 100$$

**RESULTS**

**Preliminary phytochemical analysis**

The methanol extract of the leaves of *S. colais* showed the presence of carbohydrates, flavonoids, glycosides, terpenoids, phenols, anthraquinones, tannins, proteins and aminoacids are shown in Table.1. Flavonoids, phenols and tannins are compounds basically have been proven with antioxidant activity. So the presence of these compounds may be responsible for the activity.

**Table. 1 – Preliminary phytochemical analysis**

| S.No. | Tests | Methanol extract |
|---|---|---|
| 1. | Carbohydrates | + |
| 2. | Saponins | - |
| 3. | Flavonoids | + |
| 4. | Glycosides | + |
| 5. | Terpenoids | + |
| 6. | Steroids | - |
| 7. | Tannins | + |
| 8. | Phenols | + |
| 9. | Anthraquinones | - |
| 10. | Alkaloids | - |
| 11. | Proteins and aminoacids | + |

Note : + Positive, - Negative

***In vitro* antioxidant activity**

Several concentrations ranging from 62.5$\mu$g/ml to 2000$\mu$g/ml of the methanol extract of *S. colais* were tested

**Table.2 – *In vitro* antioxidant activity of *S. colais***

| S. No. | Concentration ($\mu$g/ml) | Percentage inibition | | | |
|---|---|---|---|---|---|
| | | DPPH Assay | | Nitricoxide scavenging Assay | |
| | | Methanol Extract | Curcumin | Methanol Extract | Curcumin |
| 1. | 62.5 | 23.66 $\pm$ 0.70 | 48.35 $\pm$ 0.80 | 16.43 $\pm$ 0.77 | 30.47 $\pm$ 0.83 |
| 2. | 125 | 40.67 $\pm$ 1.09 | 52.27 $\pm$ 0.89 | 22.25 $\pm$ 0.79 | 45.82 $\pm$ 0.90 |
| 3. | 250 | 59.46 $\pm$ 0.95 | 64.62 $\pm$ 0.86 | 31.63 $\pm$ 0.81 | 59.19 $\pm$ 0.55 |
| 4. | 500 | 65.53 $\pm$ 0.81 | 79.63 $\pm$ 1.17 | 42.48 $\pm$ 0.74 | 67.35 $\pm$ 0.54 |
| 5. | 1000 | 74.68 $\pm$ 1.17 | 89.33 $\pm$ 0.82 | 58.27 $\pm$ 1.06 | 75.20 $\pm$ 0.43 |
| 6. | 2000 | 82.73 $\pm$ 0.92 | 92.47 $\pm$ 1.30 | 70.50 $\pm$ 1.21 | 87.63 $\pm$ 0.54 |

Values are expressed as mean $\pm$ S.D of three experiments

for their antioxidant activity in different *in vitro* models. It was observed that free radicals were scavenged by the test compounds in a concentration dependent manner upto the given concentration in both the models. Table 2 reveals the reductive capability of the methanol extract compared to that of the standard curcumin.

***In vitro* anti-inflammatory activity**

HRBC membrane are similar to lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity. It was observed from the Table.3, that the methanol extract shows significant anti-inflammatory activity at the concentration of 1000$\mu$g/ml which is comparable to that of the standard drug diclofenac sodium (200$\mu$g/ml). The anti inflammatory activity of the extract was concentration dependent.

**Table.3 – *In vitro* anti-inflammatory effect of *S. colais***

| S. No. | Concentration ($\mu$g/ml) | Percentage protection | |
|---|---|---|---|
| | | Methanol extract | Standard (Diclofenac Sodium) |
| 1. | 10 | 08.13 ± 0.27 | - |
| 2. | 50 | 12.43 ± 0.20 | - |
| 3. | 100 | 19.31 ± 0.33 | 49.32 ± 0.18 |
| 4. | 200 | 24.88 ± 0.26 | 78.15 ± 0.35 |
| 5. | 400 | 31.63 ± 0.62 | - |
| 6. | 800 | 53.09 ± 0.13 | - |
| 7. | 1000 | 72.66 ± 0.56 | - |

Values are expressed as mean ± S.D of three experiments

## DISCUSSION

Free radical oxidative stress has been implicated in the pathology of a wide variety of clinical disorders. Antioxidants may offer resistance against the oxidative stress by scavenging free radicals inhibiting lipid peroxidation and by many other mechanisms and thus prevent disease.

DPPH is a relatively stable free radical, the assay is based on the measurement of the scavenging activity of antioxidants towards the stable DPPH. From the present study it may be postulated that *S. colais* reduces the radical to the corresponding hydrazine when it reacts with hydrogen donors in the antioxidant principles.[15]

Nitric oxide (NO) is a very unstable species, under aerobic condition it reacts with $O_2$ to produce stable product nitrate & nitrite through intermediates $NO_2$, $N_2O_4$ & $N_3O_4$. In the present study, the nitrite produced by the reaction mixture was reduced by the methanol extract of *S. colais*. This may be due to the antioxidant principles in the extract which compete with oxygen to react with nitric oxide.[16]

The extract exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane[17] and its stabilization implies that the extract may as well stabilize lysosomal membrane. From the above study it was concluded that the methanol extract of *S. colais* has significant antioxidant and anti inflammatory activity.

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# ENHANCING GENE EXPRESSION IN NON SMALL CELL LUNG CANCER CELL LINE NCI H23 BY 3D AGGREGATE FORMATION AS EVIDENCED BY PROTEIN PROFILING

**H. Madhumitha[a], W. Sai Keerthana[a] and M. Ravi[a]**

***ABSTRACT:***

***Background and Objectives:*** *Cancers are important contributors for high mortality rate globally. Cancer biomarkers are important for research and these uniquely expressed or over-expressed are useful for cancer detection, monitoring and prognosis. Cancer cell lines are invaluable for biomarker discovery. From two-dimensional cultures, cells are now being cultured in three-dimensions to mimic in vivo systems closer. Objective of this study is to optimize simple 3D culture conditions of cancer cell line NCI H23, isolate proteins in a 'three-fraction' model and compare the protein profiles of 2D and 3D cell cultures to ascertain expression changes.*

***Methods:*** *Agarose hydrogels were optimized to obtain healthy 3D aggregates. NCI H23 cells formed floating aggregates with extracellular matrix. Cytoplasmic, membrane and nuclear protein fractions from 2D and 3D cultured NCI H23 cells were extracted and were analyzed both qualitatively (SDS PAGE) and quantitatively.*

***Results:*** *The 3D culture conditions were optimized as 1 ml of 0.5% agarose gel. The 3D aggregates showed pronounced acellular and necrotic cores. Protein profiling by SDS PAGE showed differential expression for 2D and 3D cultures. Quantitative analysis also revealed that the ratio of the protein in the three fractions of 2D and 3D cultures were different.*

***Conclusion:*** *Gene expressions vary in 2D and 3D cultures for the same cell type resulting in a variation of expressed proteins. This reiterates the usefulness of 3D cultures for cancer research including biomarker and drug discovery. Also, these 3D systems will throw more light on the mechanisms of carcinogenesis, metastasis and cancer cell behaviour.*

***Key words:*** *Cancer cell line, 3D cultures, gene expression, protein biomarkers, NCI H23*

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## INTRODUCTION:

Cancer arises when a cell, for a variety of reasons, escapes from the normal constraints placed on its growth and begins to divide in an unregulated fashion.[1] Progressing tumors give rise to distant metastases, which are the cause of 90% of human cancer deaths. They are characterized by changes in Extra Cellular Matrices (ECMs) and their interactions with tumor cells.[2] Recent studies have shown that the molecules mediating adhesion are also capable of signal transduction. These adhesion proteins and motility factors involved could serve as better targets for new treatments for cancers and to prevent metastasis.

Biomarkers which are cancer-specific and capable of early detection are essential for cancer management and therapy of which the genomic and proteomic types being the primarily important ones.[3] Thus, development of new genomic and proteomic approaches towards cancer biomarker discovery can lead to the identification of novel DNA and Protein biomarkers.[4] Biomarkers have a great impact on cancer therapeutics as their role is increasingly promising, suggesting an integrated approach for treatment selection and patient management.[5] *in vitro* cell culture models are good choices for biomarker discovery owing to the limitations of other material available such as patient biopsies.[3]

While traditional monolayer cultures are powerful tools to understand how cells proliferate, grow and respond to stress, they do not recreate the property *in vivo*. Therefore, development of novel *in vitro* model which reflects the actual *in vivo* characteristics is essential.[6] Three-dimensional (3D) scaffolds reflect normal cell morphology & behavior for more realistic cell biology and function, *in vivo*-like morphology, and better intercellular interactions.[7] 3D cultures of tumor cells have been obtained by promoting the aggregation of cells in spheroids via several different methods or by using scaffolds. Extensive studies have shown that growing cells within 3D scaffolds diminishes the gap between cell cultures and physiological tissues. Therefore, a 3D cell culture system may prove to be of tremendous advantage over conventional 2D cell culture system.[8]

The objective of this study is to ascertain if the 3D culture systems can induce a differential expression of proteins when compared to the 2D systems for a same given cell line. A 'Three-Fraction' protein model obtained from both 2D and 3D cultured cells was used for the comparative analysis. Also, the 3D culture characteristics were studied and optimal culture phases as ideally suited for obtaining a particularly localized protein fraction (cytoplasmic, membrane bound and nuclear) were identified.

### Materials and Methods

The cell line NCI H23 obtained from National Center for Cell Sciences, Pune, India was cultured as traditional monolayer attachment cultures in RPMI 1640 medium

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supplemented with 10% Fetal Bovine Serum (FBS). The cell line required 1 to 2 medium changes before attaining confluency in a T-25 culture flask. Passaging, harvesting by trypsinization, cell count along with viability checks were performed in accordance to standard procedures. A freshly thawed stock was cultured for 3 passages and the harvested cells of the third passage were used for 3D cultures. Agarose hydrogels prepared in serum free medium were used as the matrix to obtain 3D aggregates of the cell line. 0.5% of agarose hydrogel was prepared in serum free RPMI 1640 medium by melting at 80ºC. 6 well plates with 1ml of the agarose hydrogels, sterilized by UV exposure inside a laminar air flow cabinet were used. The UV exposure for 45 minutes, apart from ensuring sterility of the matrix also is useful for the polymerization and 'setting' of the low melting agarose used for the gels. As each cell line has a unique optimal 3D culture condition requirements, we have optimized the parameters for the cell line chosen. The various parameters included the concentration of Agarose (0.25%, 0.5% and 0.75%) and the volumes (1ml, 750$\mu$l, 500$\mu$l and 250$\mu$l) as suitable for a 6 well plate. The cells harvested by trypsinization of a monolayer were cultured as 3D aggregates in the optimized conditions. NCI H23 cell line forms floating aggregates in the culture medium above the hydrogels and were harvested by aspirating the culture medium. The floating aggregates harvested were tested for cell counts and viability. Healthy 3D aggregates thus harvested were washed thrice in plain medium and were used for protein fractionation as a 'Three-Fraction' Model. The cytoplasmic, membrane bound and nuclear proteins were extracted as distinct fractions using the Bio-Rad Readyprep Sequential Extraction Kit. Essentially, the kit contains three protein extraction reagents for step wise sequential extraction of cytoplasmic, membrane bound and nuclear proteins from cells and tissues. The Reagent 1 contains 40 mM Tris base, the Reagent 2 contains 10 ml of 8 M urea, 4% (w/v) CHAPS, 40 mM Tris, and 0.2% (w/v) Bio-Lyte 3/10 ampholyte and the Reagent 3 contains 10 ml of 5 M urea, 2 M thiourea, 2% (w/v) CHAPS, 2% (w/v) SB 3-10, 40 mM Tris, and 0.2% (w/v) Bio-Lyte 3/10 ampholyte. 200 mM tributyl phosphine (TBP) in 1-methyl-2-pyrrolidinone (NMP) sealed under nitrogen gas was used as the reducing agent along with reagents 2 and 3.

The cell pellet obtained from the harvested 2D monolayers and the 3D aggregates were re-suspended in 400$\mu$l of Bio-Rad ready Prep Protein Extraction Reagent 1 and centrifuged at 4000 rpm for 10 minutes. The supernatant containing the protein fraction I (cytoplasmic proteins) was transferred to a 1.8 ml Eppendorf tube, labeled as supernatant 1 and stored at -20$^0$C. The pellet obtained after the first fractionation step was re-suspended in 200$\mu$l of Ready Prep Protein Extraction Reagent2, vortexed and was centrifuged at 1000rpm for 10 minutes at 25$^0$C. The supernatant containing the protein fraction II (membrane-bound proteins) of intermediate solubility was collected and stored at -20ºC. The pellet obtained from the extraction 2 was re-suspended in 100$\mu$l of Ready Prep Protein Extraction Reagent 3 and vortexed. The solution containing protein fraction III (nuclear proteins) were also stored at -20$^0$C. The three protein fractions were quantified by using a modified Bradford method. Serially diluted bovine gamaglobulin of concentration 1.5mg/ml was used as the standards. The protein estimation was done in 96 well flat bottom plates in triplicates for each sample; the spectrophotometric absorbance measured at 595 nm ($A_{595}$). The three fractions were also qualitatively analyzed by SDS PAGE. The reagents for the SDS PAGE included 30% Acrylamide mixture, Lower Tris (pH 8.8), Upper Tris (pH 6.8), 10% APS and 10% SDS. The running buffer was composed of 1.875 g of Tris, 9 g of glycine, 0.625 g of SDS dissolved in 300ml of dd. $H_20$. The pH was set to 8.3 and the final volume made upto 500ml. The sample buffer was composed of 2.1 ml of 1.5M Tris HCl (pH.6.8), 1ml of 20% SDS, 0.5 ml 0f 100% glycerol, 0.5 ml of mercaptoethanol. 2.5 mg of bromophenol blue was added to 0.4ml of dd. $H_20$. The Staining solution was prepared from 2 g of CBB added to 500 ml of alcohol to which 70 ml of acetic acid was added. The final volume of the solution was adjusted to 1000 ml with dd. $H_20$. The Destaining solution was 35 ml acetic acid in 200 ml alcohol with the final volume made upto 500 ml with dd. $H_20$. 30 $\mu$l of the samples were loaded to each of the wells along with the standard marker proteins for electrophoresis at 100V till the tracking dye reached the bottom of the gel. The gel was carefully removed from the mold and immersed in staining solution overnight with uniform shaking at 37ºC.

**RESULTS:**

Healthy confluent NCI H23 cells were obtained on Day 3 (72 hours) of seeding a T-25 flask as monolayers. In 3D conditions, small aggregates of cells were observed in the NCI H23 3D culture plates at 24 hours. On day 5 (168 hours) of incubation, a single large aggregate surrounded by small aggregates was observed. The aggregates were not embedded on the agarose and were found to be floating. The presence of extracellular matrix was found more apparent in the floating aggregates at this stage along with acellular regions within the aggregates. Upon harvest, cells in 2D culture formed a solid pellet on centrifugation whereas 3D aggregates formed large, loosely packed cell pellets. Also, the 3D aggregates were visible as floating structures within the centrifuge tubes upon harvest, even before centrifugation. For studies on the shifts in cell culture phase durations the cells in 2D and 3D were harvested every 24 hours. Cell count was performed and the viability was tested using trypan blue dye exclusion for both 2D and 3D cells. The total cell count for 2D and 3D cultures were found to be 132 x $10^4$ and 30 x $10^4$ cells/ml at the end of 144 hours and the cell viability at 72 hours was 97.4% and 88% respectively. A marked shift in the time frames for the cell culture phases was observed for 2D and 3D. (Fig. 1) Of the 0.25%, 0.5% and 0.75% of agarose in volumes of 1ml, 750$\mu$l, 500$\mu$l and 250 $\mu$l, lower concentrations and lower volumes showed a

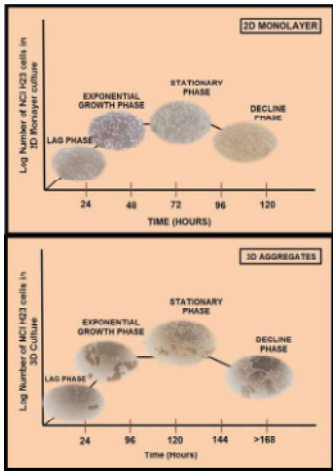


**Figure 1:** A marked shift was observed in the duration of culture phases for NCI H23 cell line in 2D and 3D cultures. Usually, the various cell culture phases, the lag, log (exponential), stationary and the decline phases are determined by the doubling time of a particular cell line/ type and also the nutritional requirements and carrying capacity of a particular culture system. Given the same culture area, medium and supplements, NCI H23 cells showed much longer durations for the progress through each of the culture phases when compared to the monolayers. This is attributed to the fact that the surface area available for monolayer cultures depletes rather rapidly and space available for cell proliferation diminishes faster depending on the doubling time of the cell line. However, in a 3D condition, cells do not depend on a surface as they loose the dependence on attachment to culture surfaces. This induces the aggregate formation where more cells are accommodated in the aggregates. This finally results in a 3D system supporting cell cultures for much longer duration, thus extending each of the cell culture phases in comparison to the 2D systems. When cells from each of the culture phases are harvested and cell counts taken, the 3D system provides more numbers of cell for a given culture phase and also protein expressions differ markedly as the culture phases progress among the 2D and 3D cultures.

varied growth on the hydrogel. The cells were not aggregated and found as single cells among which few were attached like a monolayer. Cells seeded on 0.25% agarose and volume 250 $\mu$l formed typical monolayer. 0.5% and 0.75% of agarose were found to support good aggregate formation. A 1 ml volume of 0.5% gel was found to be optimal for cell aggregate formations. Comparison of culture phases in 2D and 3D as measured by cell counts at 24 hour intervals for 6 days showed lengthy/extended durations in 3D system. (Table 1)

Sequential protein extraction yielded final volumes of 800 $\mu$l, 400 $\mu$l and 200 $\mu$l of CF, MF and NF respectively. Protein estimation of the fractions yielded a concentration of 2.2, 0.9, 2.9 mg/ml of CF, MF, NF in 2D and 0.8, 0.8 and 0.72 mg/ml of CF, MF,NF in 3D. The results of

**Table 1.**The culture feature differences of NCI H23 cell line in 2D and 3D systems showed marked variations apart from the morphology. 3D systems showed a slower proliferation rate with the presence of extracellular matrix surrounding the aggregates and acellular zones as the cultures progressed. Also, the presence of conditioned medium had a positive effect on 3D system but a similar effect was not seen in 2D systems.

| **Features** | **2D** | **3D** |
|---|---|---|
| **Cell proliferation** | Faster | Slower |
| **Necrotic cores** | Absent | Present |
| **Acellular regions** | Absent | Present |
| **Extra cellular matrix** | Absent | Present |
| **FBS requirement** | High | Low |
| **Conditioned medium** | Not Significant | Significant |
| **Cell count upon culture progress** | | |
| 24 hours | 1 x $10^4$/ml | 2 x $10^4$ /ml |
| 48 hours | 2 x $10^4$ /ml | 3 x $10^4$ /ml |
| 72 hours | 6 x $10^4$ /ml | 3.5 x $10^4$ /ml |
| 96 hours | 8 x $10^4$ /ml | 4 x $10^4$ /ml |
| 120 hours | 17 x $10^4$ /ml | 4.5 x $10^4$ /ml |
| 144 hours | 22 x $10^4$ /ml | 5 x $10^4$ /ml |

qualitative analysis by SDS PAGE showed that higher molecular weight proteins in the range of 70-85kDa were expressed in the cytoplasmic fractions of both 2D and 3D whereas lower molecular weight proteins in the range of 34-26kDa were expressed in the 2D and 3D nuclear fractions. In the membrane fractions differential expression was observed in 2D and 3D. (Fig. 2) The lag, log and plateau phases were found best suitable for the identification and

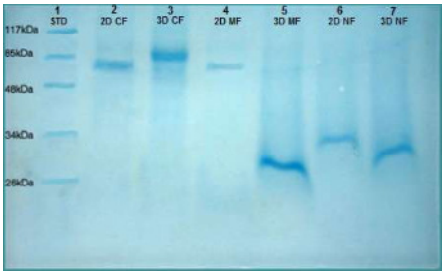


**Figure 2 :** Qualitative analysis of the three protein fractions extracted from 2D and 3D cultures by SDS PAGE showed similar profiles for the Cytoplasmic and Nuclear fractions. However, slight molecular weight differences were noticed with 3D cytoplasmic fractions showing slightly higher molecular weights compared to the 2D fraction. On the other hand, 3D nuclear fractions show lower molecular weight components when compared to the 2D counterparts. A strikingly different profile was observed for the membrane fractions where the 3D fraction showed a much lower molecular weight protein group at around 30 kDa when compared to the 2D fraction which was in the molecular weight rage of 60-65kDa.

characterization of the cytoplasmic, nuclear and membrane proteins respectively.

**DISCUSSION:**

Cancer cells behave differently as monolayers and in a 3D environment. These differences include morphological, metabolic, cell communication, signaling and gene expressions. Thus, cells in a 3D environment are good models as '*near-to-in vivo*' systems and give us useful insights from a variety of ways.[8] They can serve as a cost effective screening platform for drug development & testing and improve the predictive value of cell based assays for safety and risk assessment studies. Cell lysates from lung cancer cell lines and lung cancer animal models can be used for lung cancer biomarker discovery. But 2D culture model do not fully represent the complex circumstances of lung cancers. Therefore, use of cell lysates from well established 3D aggregates can overcome this limitation and facilitate the identification of potential new protein biomarkers. This could be fulfilled by use of natural and synthetic hydrogels which provides essential insights into nearly all aspects of cell behavior, including cell adhesion, migration, and differentiated function. Agarose hydrogels are convenient for obtaining 3D aggregates and optimizing conditions is essential for a given cell type.[9] 3D scaffold used for aggregate formation can be optimized with respect to a variety of parameters. We have shown that decreased gel concentration of 0.25% and decreased volume do not support aggregate formation and results in a monolayer. Agarose concentration of 0.5% and volume of 1 ml was found to be ideal for good aggregate formation. Thus, optimal parameters on standardization, would present us with a simple tool and technique to obtain the required 3D aggregates.

As lung cancer cell lines are very useful for cancer research,[10] it makes them all the more important as 3D aggregate. The 3D structure of cells and interactions with their neighbors significantly influences their ability to grow and function. A marked shift in the time frames for the cell culture phases was observed. An extended lag to log phase duration was observed for the 3D aggregates. Cells in 3D culture generally undergo a slower proliferation. Also 3D cultures stay healthy for a longer period of time (more than 6 days) whereas cells in 2D culture reach decline phase in 72 hours. For the same seeding density, the number of confluent aggregates in 3D culture is lower than the number of cells in 2D culture.

Adding three-dimension to a cell's environment creates significant differences in cellular characteristics and behavior. Therefore, development of bioimetic scaffolds which emulate the natural environment of their native extracellular matrix, ultimately provide a better understanding of lung biology. 3D aggregates of NCI H23 expressed more extracellular matrix around them and also pronounced acellular zones within the aggregates, probably filled with the same extra cellular matrix. The buoyancy of the NCI H23 cell aggregates making them to float might be due to the extracellular matrix around the aggregates and also the core acellular regions. It also showed dark necrotic regions on the surface which reflects the tumor morphology.

Protein estimation was performed using Modified Bio-Rad protein assay using the bovine gamma globulin as standard. The protein concentration of the cytoplasmic, membrane and nuclear protein fraction was much higher for 2D than in 3D. This might be due to the higher number of cells in 2D compared to 3D culture. Also in 2D culture, the ratios of the three fractions followed the order NF > CF > MF whereas 3D cultures showed an order of CF > MF > NF (Fig. 3). These differences in the expression of the three protein fraction ratios indicate that 3D cultures are more suitable towards protein biomarker discovery.

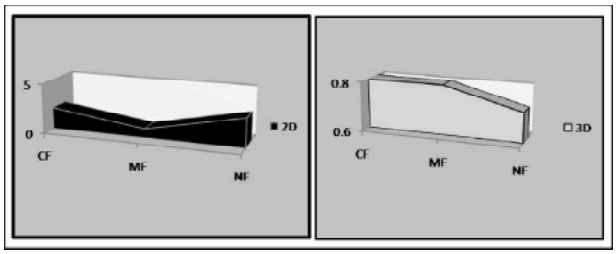


**Figure 3:** The ratios of the three protein fractions (the cytoplasmic, membrane and nuclear) were different in 2D and 3D systems. While the 2D system showed NF > CF > MF order, the 3D fractions showed CF > MF > NF. This indicated differential protein expressions in the 2D and 3D culture systems.

SDS PAGE results have again proven the differential expression of proteins in 2D and 3D culture. In both 2D and 3D cultures, the cytoplasmic protein fractions expressed high molecular weight proteins. In 3D cytoplasmic fractions, certain proteins which were not expressed in 2D were found to be expressed. The representative bands were faint of molecular weight in the range of 50-60 kDa. These proteins might constitute the matrix proteins of the ECM which was a unique characteristic observed in 3D cultures. Membrane protein fractions of 2D and 3D showed a striking difference in the expression pattern. In 2D high molecular weight proteins in the range of 60-65 kDa were expressed whereas in 3D low molecular weight proteins of molecular weight 30 kDa were expressed. The expression pattern of nuclear fractions was almost similar in 2D and 3D. In both the fractions, low molecular weight proteins in the range of 30kDa were abundant indicating active cell proliferation. Because previous studies describe that nuclear proteins responsible for cell proliferation are of low molecular weight. Thus it is very clear that proteins are differentially expressed when cells are provided a three dimensional environment. Therefore more protein profiling studies with 3D cultures can lead to the identification of new potential biomarkers.

Monoclonal antibodies raised against these novel proteins can have potential application in imaging techniques used for cancer diagnosis and also in targeted cancer therapy. Since high molecular weight proteins are highly accumulated in cytoplasmic fractions, raising antibodies through *in vivo* immunizations will be optimal.[11] Nuclear fractions can be a good choice for *in vitro* immunization as they have abundant low molecular weight proteins.[12][13] Membrane fractions which express high molecular weight proteins in 2D culture and low molecular weight proteins in 3D culture will be ideal for *in vivo* immunizations with *in vitro* stimulation which is a new strategy.[14] Further development through better proteomic tools, including more sensitive mass spectrometry analysis, is required for the identification of specific proteins that are differentially expressed in 2D and 3D cultures which has great potentials for the discovery of new biomarkers.

**CONCLUSION:**

Biomarkers have contributed tremendously in cancer research, diagnostics and therapy. Cancer cell lines are contributing important insights into the cancer mechanisms, drug discovery and biomarker identification. 3D cell cultures serve as efficient *in vitro* models for cancer research which include novel biomarker discovery owing to differential gene expressions when compared to 2D culture systems.

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# MARKERS OF OXIDATIVE STRESS IN ANGIOGRAPHICALLY PROVED CORONARY ARTERY DISEASE PATIENTS

**K. Sowmya[a], Jothi Malar[a], Nalini.G[a]**

**ABSTRACT:**

***Background & Objective:*** *Growing evidence indicates that overproduction of reactive oxygen species (ROS) under pathophysiologic conditions is integral in the development of cardiovascular diseases. ROS mediates various signaling pathways that underlie vascular inflammation in atherogenesis - from the initiation of fatty streak development through lesion progression to ultimate plaque rupture. Hence this pilot study was conducted to evaluate the level of oxidative stress markers like malondialdehyde (MDA), superoxide dismutase (SOD) and ascorbic acid in angiographically proved coronary artery disease (CAD ) patient compared with healthy individuals along with routine plasma glucose & lipid parameters*

***Materials & Methods:*** *The study group consisted of 60 subjects, of which 30 were healthy individuals & age matched 30 angiographically proven coronary artery disease patients were taken as cases. Peripheral venous blood was used for analysis. Oxidative stress markers - malondialdehyde, superoxide dismutase & ascorbic acid were analyzed in Hitachi U-2001 spectrophotometer. Lipid profile & glucose values were analyzed in Bayer's Express plus automated system using kits supplied by Accurex India Ltd. The statistical analysis was done using students t- test and p-value of $< 0.05$ was considered significant*

***Result:*** *Dyslipidemia [Increased Total cholesterol, Triglyceride (TGL), Low density lipoprotein-cholesterol (LDL-C) and decreased High density lipoprotein-cholesterol (HDL-C) & increased plasma glucose was seen in CAD patients. Plasma malondialdehyde – a marker of lipid peroxidation was significantly elevated and the levels of ascorbic acid and superoxide dismutase were significantly reduced in CAD patients compared to the healthy group.*

***Interpretation & Conclusion****: There are an increased oxidants and decreased antioxidant levels among the CAD patients when compared to healthy individuals, indicating oxidative stress in CAD patients.*

***Key words:*** *oxidative stress, coronary artery diseases, antioxidants*

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## INTRODUCTION :

Coronary artery disease is the major cause of morbidity & mortality in the entire world.[1] The majority of cardiovascular disease results from complications of atherosclerosis. Progress made in the medical field has attempted to address this insidious disease on all fronts – etiology, pathogenesis, treatment and prevention. Despite all-round efforts, it remains a major challenge to the health managers and scientists. In developing countries, the incidence of CAD is increasing alarmingly.[2] India is on the verge of cardiovascular epidemic. In India, epidemiological studies have revealed that the prevalence of CAD has increased from 4% in 1960 to 11% in 2001. It has been predicted that by the year 2020 CAD will persist as the major and the most common threat to human life.[3,4]

The underlying cause of coronary artery disease is atherosclerosis. An important initiating event for atherosclerosis is impaired endothelial function. The endothelial function is impaired by Reactive Oxygen Species (ROS) and various risk factors.[5] Reactive Oxygen Species reduces the endothelial nitric oxide production. Lack of nitric oxide contributes to impaired vascular relaxation & increased platelet aggregation thereby leading to atherosclerosis. Role of dyslipidemia in causing CAD is well established by various studies.[6,7,8] Moreover oxidized LDL-C plays an important role in the pathogenesis of atherosclerosis[9] and this oxidative modification of LDL-C is brought about by free radicals. Goldstein[10] in his work has proved oxidized LDL-C as an important atherogenic factor. The oxidized LDL-C bypasses the normal feedback control of LDL-C receptor and is avidly endocytosed by the scavenger receptor pathway of macrophages. The scavenger receptor pathway is not regulated by the intracellular cholesterol concentration thus leading to loading of macrophages with cholesterol thus converting them to foam cells and thereby leading to atherosclerosis.

Malondialdehyde is a breakdown product of peroxidation of long chain fatty acids.[11] Increased lipid peroxides lead to endothelial cell damage, uncontrolled lipid uptake, and increased thrombogenecity thus leading to atherosclerosis.[12] Endothelial cells have a comprehensive array of antioxidant defense mechanisms to reduce free radical formation or limit their damaging effects. These include enzymes such as superoxide dismutase and catalase to degrade superoxide and peroxides respectively, and essential free radical scavengers like ascorbic acid. superoxide dismutase is a secretory glycoprotein found in blood vessel walls which represents an important vascular enzymatic antioxidant defense system. It dismutates superoxide ions to $H_2O_2$ and thereby improves endothelial function.[13] Ascorbic acid (vitamin C) is an important antioxidant in plasma, it consumes free radicals and helps to preserve alpha

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tocopherol in lipoproteins.[14] Thus the antioxidants tend to reduce the risk and severity of atherosclerosis by inhibiting lipid peroxidation.

There are very few studies on markers of oxidative stress in angiographically proved CAD patients. Hence this pilot study was undertaken to estimate the level of malondialdehyde, superoxide dismutase and ascorbic acid, in angiographically proved CAD patients and compare it with healthy individuals. Thus the estimation of routine parameters, together with oxidant–antioxidant profile in an individual will help in risk assessment and management of CAD.

## MATERIALS & METHODS

All the tests were performed in the laboratory of Sri Ramachandra Medical College & Research Institute and the procedures followed were in accordance with the ethical standards of the committee on human experimentation of the institution. The written consents were taken from the patients prior to study and the objectives of the study were fully explained. The complete clinical and personal history of the subjects was recorded in the form of Questionnaire. Patients with smoking habits and those suffering from renal disease, chronic obstructive pulmonary disease, hypertension and hepatitis were excluded from the study. Selection of the patients was done after confirmation of CAD by electrocardiogram and angiogram. The control group comprised of 30 apparently healthy individuals in the age group of 40-60 years of both sexes (14 males and 16 females) and age matched 30 angiographically proven cardiac cases of both sexes (18 males and 12 females) from the Department of Cardiology, Sri Ramachandra Medical College & Research Institute, Porur, Chennai were taken as cases. Blood samples were drawn after overnight fasting. Fasting fluoride plasma and serum samples were collected using BD vacutainer tubes for the estimation of glucose and lipid profile respectively. Heparinized plasma samples were used for the analysis of malondialdehyde and ascorbic acid & haemolysate was used for estimation of superoxide dismutase.

Lipid profile & glucose values were analyzed in Bayer's Express plus automated analyser using kits supplied by Accurex India Ltd. Fasting plasma glucose values were measured by glucose oxidase-peroxidase method, serum Total cholesterol was measured by cholesterol oxidase-peroxidase method, TGL was measured by glycerol kinase, glycerol phosphate oxidase, peroxidase method, HDL-C was determined by cholesterol esterase, cholesterol oxidase & catalase method - a direct enzymatic method & LDL-C was calculated using Friedewald's equation.

Plasma ascorbic acid was estimated by the method of Omaye et al[15] and expressed as mg/dl. SOD was estimated by the method of Marklund & Marklund[15,16] in which the degree of inhibition of auto-oxidation of pyrogallol by superoxide dismutase was used as a measure of enzyme activity and expressed as U/ml. Malondialdehyde was estimated as a marker of lipid peroxidation by the method of Yagi et al[17] and expressed as nmol/ml. SOD, malondialdehyde & ascorbic acid were analyzed in Hitachi U-2001 spectrophotometer. Statistical analysis was done using students t- test and p –value of $< 0.05$ was considered significant

## RESULT

The present study was conducted on 30 CAD patients and the results were compared with healthy controls. Mean age in the study group was 46 ± 4.4 years and that in control group was 48 ± 4.8 years. The controls of our study group were not a diabetic, hypertensive nor had the habit of smoking. Among the cases, 36 % (11 out of 30 cases) were diabetic and were on oral hypoglycemic drugs (metformin). Table I and Fig.1 shows the comparison of the Biochemical parameters of Conventional risk factors. The mean values of Plasma glucose, Total cholesterol, LDL-C, and TGL were found to be higher in CAD cases & the mean HDL cholesterol was significantly low in CAD cases as compared to normal group. The difference in mean values among the cases and controls were calculated and was found to be statistically significant for all the parameters. Table II and Fig. 2 shows a statistically significant ($p = 0.001$) increase in malondialdehyde levels and significant decrease in SOD ($p = 0.001$) & vitamin C ($p = 0.001$) levels in CAD cases compared to the normal group

**Table 1: Conventional risk factors in normal individuals & Angiographically proved CAD Patients**

| Parameters (mg/dl) | NORMAL (n = 30) | CAD (n = 30) | p-Value |
|---|---|---|---|
| Plasma Glucose | 89 ± 12 | 140 ± 39 | <0.001 |
| Total Cholesterol | 164.7± 31.06 | 234.4 ± 38.41 | <0.001 |
| TGL | 112.6 ± 30.75 | 165.9 ± 38.20 | <0.001 |
| LDL | 102.9 ± 30.01 | 171.02 ± 38 | <0.001 |
| HDL | 39.26 ± 7.22 | 30.2 ± 4.2 | <0.001 |
| p-value <0.001 is considered significant | | | |

**Fig.1: Graphical representation of conventional risk factors in normal individuals & Angiographically proved CAD Patients**

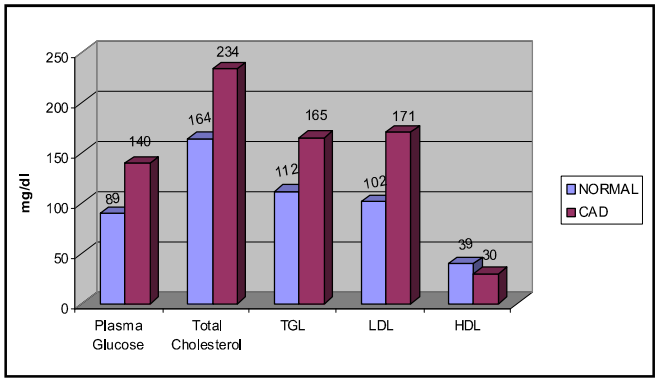
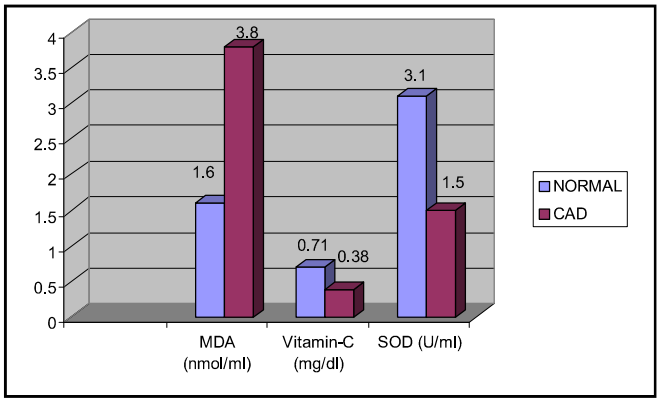


**Table 2: Oxidant & antioxidant levels in healthy individuals and Angiographically proved CAD Patients**

| Parameters | NORMAL (n = 30) | CAD (n = 30) |
|---|---|---|
| MDA (nmol/ml) | 1.6 ± 0.32 | 3.8 ± 0.76*** |
| Vitamin-C (mg/dl) | 0.71 ± .21 | 0.38 ± 0.09*** |
| SOD (U/ml) | 3.1 ± 0.59 | 1.5 ± 0.39*** |
| ***p-value < 0.001 | | |

**Fig.2: Graphical representation of oxidant & antioxidant levels in healthy individuals and Angiographically proved CAD Patients**




## DISCUSSION

Coronary artery disease continues to be a leading cause of death among the Indian population. It is associated with various risk factors. Dyslipidemia is a major risk factor for CAD & the leading cause of death world wide.[7] In this study the levels of risk associated lipids, i.e. Total cholesterol, Triglycerides and LDL-C were high and levels of HDL-C were low in CAD patients compared with the normal individual, thus proving the association of dyslipidemia with CAD. A positive relationship between dyslipidemia and risk of CAD has been well established by many studies[16, 18, 19, 20] thus favouring the study finding. Plasma glucose levels were found to be significantly high among the cases than among the controls and the levels of lipid peroxidation were found to be high among the cases indicating that diabetes is associated with increased generation of reactive Oxygen Species. malondialdehyde is a product of auto oxidation of polyunsaturated fatty acids which is used as an index of oxidative damage.[21] Enhanced lipid peroxidation is the result of increased free radical generation and suppressed scavenging mechanisms.[22] In this study malondialdehyde levels were significantly increased in CAD patients compared to the normal individuals thus confirming increased lipid peroxidation in CAD patients. Mendis [23] & Kostner et al [24] in their study have reported high malondialdehyde levels in CAD patients compared to controls. This study results also akin to the above study finding.

The levels of antioxidants such as ascorbic acid & superoxide dismutase were found to be low in CAD patients compared to healthy controls in this study. Ascorbic acid is a water-soluble vitamin and a powerful antioxidant that acts as the body's primary defense against peroxyl radicals formed during the metabolic process. Gokce[25] and Santillo[14] in their study have proved the beneficial effect of ascorbic acid supplementation in CAD patients and its protective role against the peroxidative damage of lipids, favouring this study finding. Superoxide dismutase is a major enzyme in plasma which removes the superoxide radical. In this study the level of superoxide dismutase was low in CAD patients compared to healthy controls. The decreased levels of superoxide dismutase in CAD patients may be because of reduced production or increased utilization of the enzymes, thereby rendering an individual susceptible to oxidative damage due to decreased clearance of free radicals. Landmesser[26] in his study have proved, reduced superoxide dismutase activity as a major contributor of endothelial dysfunction in patients with CAD. Wang[27] in their study have established the association of decreased superoxide dismutase levels in CAD patients, supporting this study finding. Thus the levels of superoxide dismutase may be envisaged as a potential marker of risk assessment for CAD in addition to glucose & lipid profile.

## CONCLUSION

The study results show an increased lipid peroxidation, decreased antioxidant status and dyslipidemia among the cases, thus indicating increased oxidative stress among coronary artery disease patients. Hence oxidative damage due to lipid peroxidation can be managed by increasing the antioxidant status by supplementation of antioxidants and by taking measures to correct dyslipidemia, thereby we can reduce the events of coronary artery diseases.

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# PREVALENCE OF CHRONIC ENERGY DEFICIENCY, OVERWEIGHT AND OBESITY AMONG THE GERIATRIC POPULATION IN A RURAL AREA IN TAMILNADU

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**ABSTRACT:**

***Background:*** *The Current geriatric population in India is about 8% which is expected to rise by 10% in 2025. Among various health problems in geriatric population, nutritional problem plays a major role. Chronic energy deficiency, overweight and obesity are the commonest nutritional problems among the elderly. Limited studies are available in literature documenting nutritional problems in geriatric population. The present study would highlight the nutritional status of the elderly in a rural community of Tamil Nadu.*

***Objectives:*** *1) To assess the prevalence of Chronic Energy Deficiency, overweight and obesity among the geriatric population in a rural area in Tamil Nadu and 2) To study various factors influencing the nutritional status among the geriatric population.*

***Methodology:*** *A cross sectional study was conducted on 400 early individuals (aged > 60 years) on basis of simple random technique, at Attayampatti village near Salem in Tamil Nadu. The height and weight of all those individuals were measured and the Body Mass Index(BMI) was evsaluated and also various questions were administered to them to assess their nutritional status.*

*Results: The present study revealed that the prevalence of chronic energy deficiency (CED), obesity and overweight was 10%, 13% and 27% respectively. The prevalence of CED among both males and females were almost equal (10%) while males were slightly more as compared to the females(25%) . However, the BMI suggested that the prevalence of obesity in females (17%) was almost twice as that of the males (8%). The factors like age, occupation, income, dependency, calorie and protein intake showed an impact over the nutritional status of the elderly population.*

***Conclusion:*** *Apart from CED, overweight and obesity are emerging as another major nutritional health challenge among gertiatric population in India.*

***Key words:*** *chronic energy deficiency, geriatric, nutritional status, obesity.*

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## INTRODUCTION:

Ageing is a natural process. Discoveries in medical science and improved social condition during past few decades have increased the life span of man. The expectation of life at birth in developed countries is over 70 years. In India, the current geriatric population is about 8% which is expected to go upto 10% by 2025.[1]

Among various health problems in geriatric population, nutritional problem plays a major role. With increasing age, there are metabolic changes and also reduction in physical activity and, as a result, energy requirement in elderly is substantially lower than younger adults. Elderly individuals also face problems in ensuring appropriate dietary intake because of alteration in taste with increasing age and loss of teeth.[2] There is relatively little data on the prevalence of undernutrition among the elderly in the developing world. However, studies of the African elderly showed that up to 36 percentof the men and 27 percent of the women were undernourished.[3] A recent study in the Philippines showed about 30 percent of the elderly were underweight.[4] The tribal population in India is among India's poorest groups, and one study found that more than 60 percent of the tribal men and women over age 60 suffered from a chronic deficiency in needed calories.[5]

Similarly ,obesity and overweight is emerging as an important health problem in India. The Nutrition Foundation of India (NFI) study showed that 32.3% of middle class males and 50% of middle class females in Delhi are obese. The prevalence of obesity has increased and is now an epidemic worldwide.[6]

As such in India, very few studies have been done to assess the nutritional status in geriatric population, when compared to children or adolescent. So the present study was undertaken to highlight various factors affecting the nutritional status among geriatric population in a rural area in Tamilnadu.

## MATERIAL AND METHODS

The objective of the study was

1) To assess the nutritional status-Chronic energy Deficiency, overweight and obesity among the geriatric population in a rural area in Tamilnadu.
2) To study various factors influencing the nutritional status among the geriatric population in a rural area in Tamilnadu.

**Study area:** Attyampatti village

**Study period:** October 2010- December 2010

**Study design:** cross sectional study

**Study population :** population of 400 aged > 60 years, got by using the standard formula for calculation of sample size ($4PQ/e^2$)

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**Sampling :** simple random technique.

**Methodology :** Using a pretested semistructured questionnaire, house to house visit was done on simple random basis. A two page questionnaire in Tamil was made and the CRRI's of our hospital was trained in administering the questions to the study population and in assessing the diet survey. Questions regarding family history, diet history-including their calorie and protein consumption, their personal habits, their dependency status ,socioeconomic and occupation history and history of any chronic morbidity among elderly were taken. Their height and weight were measured and the BMI was calculated. Attayampatti town panchayat has a population of 10,000 and in that the geriatric population is 800( keeping the national average of 8%). Keeping the prevalence of chronic energy deficiency as 50% [5] n the geriatric population and taking the confidence interval as 95% and the maximum allowable error as 5%. Applying these values in the standard formula for calculation of sample size ($4PQ/e^2$), the study population came to 400. Total of 2500 houses are there in the Attayampatti village which constitutes the sampling frame and all the houses were numbered accordingly. Then by using a random number table we selected the houses and in those houses all the geriatric people were interviewed still we obtained the required sample size of 400.

The dietary assessment was done by diet survey questionnaire, using a 24 hr recall period for three consecutive days. The food item taken by the individuals for the past 24 hours listed and the amount of calorie and proteins in those food items was calculated by using a Standard nutritional chart. The average of those three days is calculated as the total calorie and protein consumed by the individual.

The chronic energy deficiency is defined as BMI less than 18.5 and overweight is BMI $>30$ and obesity is BMI $>35$[5]

Relation of nutritional status with respect to occupation, income and dependency was assessed. The occupation was categorized as skilled (tailor, carpenter etc), semiskilled (powerloom worker , agriculturist), unskilled (coolie, peon), semiprofessional (teacher, bank employee). The income of the study population was categorized at the scale of 1000 interval stating from 1000 to 6000. The dependency was categorized based on their economic needs as dependant and not dependant.

Statistical analysis: The data was entered in the SPSS software and the analysis was done. Statistical inference test life "F" test was used in the analysis to see the association between BMI and the other variables used in the study.

## RESULTS

Table-1 shows the prevalence of chronic energy deficiency (CED) is 10%,the prevalence of obesity is 13% and prevalence of overweight is 27%.The prevalence of CED among both male and female are almost equal(10%),and that of the overweight is slightly more in males(29%) as compared to the females(25%),whereas the prevalence of obesity in female(17%) is almost twice as that of the males(8%).

Table 1 also shows the relationship between sex and BMI among different age-groups. It is found that among males, the prevalence of CED and overweight is almost same in the age group of 60-62yrs (37%), and the prevalence of obesity is more in the age group of 69-71yrs(41.7%). It is found that among females, the prevalence of CED is more in the age group of 78-80yrs (29.2%).The prevalence of overweight is more in the age group of 60-62yrs(48.4%) and the prevalence of obesity is more in the age group of 60-62yrs(50.0%).As the age increases in females, the prevalence of CED increases, which is found to be statistically significant by F-test.($p<0.001$)

Table-2 shows that the maximum number of males (57.1%), were engaged in semi-skilled occupation. It was found that , prevalence of CED is more in semiskilled workers (68.8%),as compared to other occupations. The prevalence of overweight and obesity is also more among semiskilled workers 64.4% and 75% respectively.

Similarly among females, maximum number of them 39.3% were unemployed. The prevalence of CED is more in unemployed females 45.8%,as compared to other occupations. The prevalence of overweight is 32.3% equal in both homemakers and unemployed women and the prevalence of obesity is 40.5% among unemployed women. Both the differences among males and females are not statistically significant.

Table 3 shows that maximum number of elderly people 25.8% were getting income in the range between Rs1000-2000. The prevalence of CED (60%), overweight(28%) and obesity(27.8%) found to be maximum in the income group of less than 2000 as compared to other income groups and the difference is not statistically significant.

Table-4 shows that maximum number of people(72.5%),who had CED,were consuming less than 1700 calories per day. Contrast to this ,maximum number of them who were overweight(62.6%) and those who were obese(61%), were consuming 1900-2300 calories per day. Therefore it was found that, the intake of calories was directly proportional to the increase in BMI, which was found to be statistically significant by F- test($p<0.001$)

Table-5 shows that maximum number of people (52.5%), who had CED, were consuming proteins of less than 40 gms per day,while ,maximum number of them who were overweight(75.7%) and those who were obese(61%), were consuming 46-60gms of proteins per day.Therefore it was found that,with increase in the intake of proteins, BMI increased,leading to overweight and obesity,which was found to be statistically significant by F- test($p<0.001$)

Table 6 shows that 236(59%)persons are dependent on the others for their living. The prevalence of CED among dependents are 47.5%,while that of overweight is 61.7% and prevalence of obesity is 70.3%.The prevalence of CED among non dependents are 52.5%, while that of overweight is 38.3% and the prevalence of obesity is 29.6%. CED is

**TABLE-1** : BMI related to age and sex of the study population

| Sex | Age | BMI | | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | | < 18.5 (%) | 18.5-24.99 (%) | 25-29.99 (%) | 30-34.99 (%) | 35-39.99 (%) | > 40 (%) | |
| Male | 60-62 | 6(12.4) | 25(51.02) | 17(34.7) | 1(2.0) | 0(0) | 0(0) | 49 |
| | 63-65 | 2(7.7) | 14(53.8) | 7(26.9) | 3(11.5) | 0(0) | 0(0) | 26 |
| | 66-68 | 4(22.2) | 7(38.9) | 6(33.3) | 1(5.5) | 0(0) | 0(0) | 18 |
| | 69-71 | 1(4.0) | 16(64.0) | 3(12.0) | 5(20.0) | 0(0) | 0(0) | 25 |
| | 72-74 | 1(7.7) | 8(61.5) | 4(30.8) | 0(0) | 0(0) | 0(0) | 13 |
| | 75-77 | 0(0) | 7(53.8) | 5(38.5) | 0(0) | 1(7.7) | 0(0) | 13 |
| | 78-80 | 2(22.2) | 4(44.4) | 2(22.2) | 0(0) | 0(0) | 1(11.1) | 9 |
| | > 80 | 0(0) | 2(66.6) | 1(33.3) | 0(0) | 0(0) | 0(0) | 3 |
| | **Total** | **16** | **83** | **45** | **10** | **1** | **1** | **156** |
| Female | 60-62 | 4(4.5) | 33(37.5) | 30(34.1) | 16(18.2) | 5(5.7) | 0(0) | 88 |
| | 63-65 | 2(3.4) | 34(57.6) | 12(20.3) | 3(5.1) | 7(46.7) | 1(1.7) | 59 |
| | 66-68 | 1(5.6) | 7(38.9) | 7(38.9) | 1(5.6) | 2(11.1) | 0(0) | 18 |
| | 69-71 | 5(13.9) | 20(55.6) | 8(22.2) | 2(5.6) | 1(2.8) | 0(0) | 36 |
| | 72-74 | 2(40.0) | 2(40.0) | 0(0) | 1(20.0) | 0(0) | 0(0) | 5 |
| | 75-77 | 2(20.0) | 4(40.0) | 3(30.0) | 1(10.0) | 0(0) | 0(0) | 10 |
| | 78-80 | 7(36.8) | 9(47.4) | 2(10.5) | 1(5.3) | 0(0) | 0(0) | 19 |
| | > 80 | 1(11.1) | 7(77.8) | 0(0) | 1(11.1) | 0(0) | 0(0) | 9 |
| | **Total** | **24** | **116** | **62** | **26** | **15** | **1** | **244** |

**Table-2** : BMI related to the occupation of the study population

| Sex | Occupation | BMI | | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | | < 18.5 (%) | 18.5-24.99 (%) | 25-29.99 (%) | 30-34.99 (%) | 35-39.99 (%) | > 40 (%) | |
| Male | Unemployed | 1(2.7) | 24(64.9) | 10(27.0) | 1(2.7) | 0(0) | 1(2.7) | 3 |
| | Semiskilled | 11(12.4) | 40(44.9) | 29(32.6) | 8(8.9) | 1(1.1) | 0(0) | 89 |
| | Unskilled | 3(11.5) | 17(65.4) | 5(19.2) | 1(3.8) | 0(0) | 0(0) | 26 |
| | Skilled | 1(33.3) | 2(66.7) | 0(0) | 0(0) | 0(0) | 0(0) | 3 |
| | Semiprofessional | 0(0) | 0(0) | 1(100.0) | 0(0) | 0(0) | 0(0) | 1 |
| | **Total** | **16** | **83** | **45** | **10** | **1** | **1** | **15** |
| Female | Home maker | 6(9.1) | 29(43.9) | 20(30.3) | 5(7.6) | 5(7.6) | 1(1.5) | 66 |
| | Unemployed | 11(11.5) | 48(50.0) | 20(20.8) | 10(10.4) | 7(7.3) | 0(0) | 96 |
| | Semiskilled | 3(7.3) | 18(43.9) | 13(31.7) | 6(14.6) | 1(2.4) | 0(0) | 41 |
| | Unskilled | 4(10.3) | 21(53.8) | 8(20.5) | 5(12.8) | 1(2.6) | 0(0) | 39 |
| | Semiprofessional | 0(0) | 0(0) | 0(0) | 0(0) | 1(100.0) | 0(0) | 1 |
| | Skilled | 0(0) | 0(0) | 1(100.0) | 0(0) | 0(0) | 0(0) | 1 |
| | **Total** | **24** | **116** | **62** | **26** | **15** | **1** | **244** |

**Table-3** : BMI related to the income of the study population

| Income | BMI | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | **<18.5 (%)** | **18.5-24.99 (%)** | **25-29.99 (%)** | **30-34.99 (%)** | **35-39.99 (%)** | **>40 (%)** | |
| **<1000** | 12(12) | 47(47) | 26(26) | 10(10) | 5(5) | 0(0) | 100 |
| **1000-2000** | 12(11.65) | 51(49.51) | 30(29.12) | 6(5.82) | 3(2.91) | 1(0.97) | 103 |
| **2001-3000** | 7(12.06) | 3(5.17) | 14(24.13) | 3(5.17) | 2(3.44) | 1(1.72) | 58 |
| **3001-4000** | 4(8) | 2(4) | 1(2) | 4(8) | 3(6) | 0(0) | 50 |
| **4001-5000** | 4(8) | 25(50) | 14(28) | 7(14) | 0(0) | 0(0) | 50 |
| **5001-6000** | 0(0) | 8(40) | 6(30) | 5(25) | 1(5) | 0(0) | 20 |
| **>6000** | 1(5.26) | 9(47.36) | 6(31.57) | 1(5.26) | 2(10.52) | 0(0) | 19 |
| **Total** | **40** | **199** | **107** | **36** | **16** | **2** | **400** |

**Table-4 BMI related to the calorie intake among the geriatric population**

| BMI | Calorie intake | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | **<1700 (%)** | **1700-1900 (%)** | **1900-2100 (%)** | **2100-2300 (%)** | **2300-2500 (%)** | **2500-2700 (%)** | **>2700 (%)** | |
| **<18.5** | 29(72.5) | 9(22.5) | 2(5) | 0(0) | 0(0) | 0(0) | 0(0) | 40 |
| **18.5-24.99** | 23(11.55) | 76(38.19) | 60(30.15) | 24(12.06) | 14(7.03) | 2(1) | 0(0) | 199 |
| **25-29.99** | 1(0.93) | 16(14.95) | 34(31.77) | 33(30.84) | 13(12.14) | 7(6.54) | 3(2.80) | 107 |
| **30-34.99** | 0(00 | 3(8.33) | 10(27.77) | 11(30.55) | 3(8.33) | 5(13.88) | 4(11.11) | 36 |
| **35-39.99** | 0(0) | 0(0) | 6(37.5) | 5(31.25) | 1(6.25) | 2(12.5) | 2(12.5) | 16 |
| **>40** | 0(0) | 0(0) | 1(50) | 0(0) | 0(0) | 1(50) | 0(0) | 2 |
| **Total** | **53** | **104** | **113** | **73** | **31** | **17** | **9** | **400** |

**Table-5 : BMI related to the protein intake among the geriatric population**

| BMI | Protein Intake | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | **<40 (%)** | **40-45 (%)** | **46-50 (%)** | **51-55 (%)** | **56-60 (%)** | **61-65 (%)** | **66-70 (%)** | |
| **<18.5** | 21(52.5) | 18(45) | 1(2.5) | 0(0) | 0(0) | 0(0) | 0(0) | 40 |
| **18.5-24.99** | 18(9.04) | 87(43.71) | 62(31.15) | 15(7.53) | 13(6.53) | 4(2.01) | 0(0) | 199 |
| **25-29.99** | 0(0) | 8(7.47) | 26(24.29) | 25(23.36) | 30(28.03) | 18(16.82) | 0(0) | 107 |
| **30-34.99** | 0(0) | 2(5.55) | 3(8.33) | 9(25) | 12(33.33) | 9(25) | 1(2.77) | 36 |
| **35-39.99** | 0(0) | 0(0) | 2(12.5) | 3(18.75) | 3(18.75) | 7(43.75) | 1(6.25) | 16 |
| **>40** | 0(0) | 0(0) | 0(0) | 0(0) | 2(100) | 0(0) | 0(0) | 2 |
| **Total** | **39** | **115** | **94** | **52** | **60** | **38** | **2** | **400** |

**Table-6 BMI related to the dependency factor among the geriatric population**

| Dependency | BMI | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | **<18.5 (%)** | **18.5-24.99 (%)** | **25-29.99 (%)** | **30-34.99 (%)** | **35-39.99 (%)** | **>40 (%)** | |
| **Dependant** | 19(8.0) | 113(47.9) | 66(28.0) | 24(10.2) | 13(5.5) | 1(0.4) | 236 |
| **Independent** | 21(12.8) | 86(52.4) | 41(25.0) | 12(7.3) | 3(1.8) | 1(0.6) | 164 |
| **Total** | **40** | **199** | **107** | **36** | **16** | **2** | **400** |

more in people who are not dependant, and the prevalence of overweight and obesity is more among people who are dependant than people who are not dependant.

## DISCUSSION

According to the NNMB reports 2002, the prevalence of chronic energy deficiency (CED) among the geriatric population as assessed by BMI < 18.5 was relatively more among males (53.5%) than in females (49.4%). It also stated that over the two decades there is a reduction in the prevalence of CED both in men and women but the reduction is more in women where as in our study, the prevalence of CED among both male and female are almost equal (10%). The NNMB reports also shows that the prevalence of overnutrition was higher in elderly men as compared to elderly women[5] and similarly in our study the overweight is slightly more among males(29%) as compared to the females(25%) and the prevalence of obesity in female(17%) is almost twice as that of the males(8%).

According to the study done on the older adults of desert areas of western Rajathan, the prevalence of Chronic Energy Deficiency (CED = BMI < 18.5) was e" 40% in desert areas of India, indicating a "very high" public health problem. It was higher among older women (52%) compared with men (42.4%) and higher in those belonging to Scheduled Caste and Scheduled Tribes and in HHs of laborers, artisans, landless individuals, marginal farmers, and below poverty line families[7], whereas the prevalence of CED in our study is only 10% without any sex variation and it is more likely related to their diet, physical activity and their dependency factor.

The study done on the nutritional status of the tribal elderly in India, Chronic Energy Deficiency (CED = BMI < 18.5) was relatively higher (65.4%) in females compared with their male counterparts (61.8%). The prevalence of CED was significantly higher ($p < 0.001$) among the elderly living in kutchaand landless households. The tribal elderly are subsisting on inadequate diets, which are reflected in the poor intakes of all the nutrients and higher prevalence of undernutrition,[8] whereas, in our study there is no sex predominance among the CED prevalence but it is reflected to the poor intake of calories and proteins.

The National Cardiovascular Disease Database of the year 2000 showed a higher prevalence of 54% (criteria: BMI > 22.25) was recorded among elderly populations (age group: > =60) during 2000.[9] An Epidemiological Study of obesity among elderly in Chandigarh showed the prevalence of overweight was 33.14% and obesity was 7.54% of the elderly and that of CED is only 14.36%. Over weight/obesity was higher among females (42.1%) than males (20.9%).[10]

According to the study done on the prevalence of overweight and obesity amongst elderly patients attending a geriatric clinic in a tertiary care hospital in Delhi, the mean age of the study subjects were 68.5 years. It was found that 34% of men and 40.3% of women were overweight and obese, respectively. The prevalence of obesity was higher in females as compared to males, whereas, in our study the prevalence of overweight is slightly more in males(29%) as compared to the females (25%),whereas the prevalence of obesity in female(17%) is almost twice as that of the males(8%). The results of the present study revealed that overweight and obesity highlight an emerging health problem amongst elderly.[11]

A study on prevalence of nutritional disorders and nutrient deficits in elderly people in a rural community in Tamilnadu, India, the mean height and body weight of both male and female were 155cm/48kg and 145cm/43kg respectively. The prevalence of underweight in this rural elderly community (55%) while obesity was uncommon (1,9%). It was found that 93.2% of the elderly people studied were on low calorie intake when compared to RDA (ICMR 1989), the average calorie intake in the study being 929 Kcal. 98 % of the subjects had their protein intake less than ICMR recommended allowance. The average daily intake of protein was 24g,[12] and similar study done on morbidity pattern among the elderly population in the rural area of Tamil Nadu, India. The average height was 1.53m. (SD 0.096), males 1.58m and females 1.48m. The average weight was 45.4 kgs (SD 10.9), males 49.4 kgs and females 42.2 kgs. The mean BMI was 19.02 kg/m2 (SD 9.21), males 19.31 kg/m2 (SD 12.7) and females 18.83 kg/m2 (SD 8.95). CED at the community level was 49 % and 9.7% were overweight[13] where as the mean height and weight of both male and female in our study is 154cms/56kg and 143cms/ 50kg respectively and the average calorie intake in our population is 2000Kcal and protein is 50gms.

## CONCLUSION :

Though many past studies indicates that in geriatric population CED is the Chief nutritional problem whereas the present study reflects that along with CED, overweight and obesity is also becoming a major nutritional problem in India . Since very few studies as such for assessing the nutritional status of the elderly, has been done in India a large multi centric studies should be taken up in different parts of India to throw some light on this dual problem and based on the results obtained, suitable interventions can be planned for them.

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# SIX MINUTE WALK TEST: A LITERARY REVIEW

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**ABSTRACT:**

*Exercise testing forms an important part in assessment of any patient requiring physical training. The methods of such exercise testing have undergone enormous changes Apart from being a basis for training; such testing procedures have helped to delineate patients having high risk from beneficiaries for major surgical procedures. The conventional methods of physical capacity evaluation needed many sophistications and facilities to make them reliable and safe. In developing country like India, the cost of treatment burdens the patient, as most is spent on initial assessment. Moreover periodic evaluation is precluded due to lack of the required setting all times. Thus, the usage of field tests gained importance, as they are noninvasive, easy to administer, patient friendly and cost effective. Initially twelve-minute walk test (TMWT) was developed to assess the exercise capacity of patients with respiratory dysfunction, as they were unable to complete the conventional sub maximal exercise testing. The TMWT also posed limitation to patients with moderate to severe cardio respiratory dysfunction. Hence Six-minute Walk Test (SMWT) was developed and validated in different patient groups. The importance of SMWT was recognized and usage widened from being a basic exercise testing of patient to the level of prediction of patient outcomes, reflection of quality of life, prediction of mortality and morbidity. This review intends to describe and analyse the clinical utility of the most widely used field test. It also outlines the limitations and future applications to be explored.*

***Key words :*** *Six minute walk test, exercise testing, Cardiopulmonary rehabilitation*

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## INTRODUCTION:

Exercise testing is an important component of initial patient assessment in cardiac rehabilitation. Assessment of functional exercise capacity has gained importance in the patient care in various diseased states. Timed walking tests are widely used to evaluate functional exercise performance, as they are likely to measure the ability to undertake the activities of day-to-day life. Functional capacity is an important clinical outcome measure in rehabilitation of any patient and thus necessitating an exercise testing procedure.[1]

Assessment of functional capacity was traditionally done by asking patients about the work capacity like how many times they climb stairs, or how much they walk etc. However these recollection methods and questionnaire methods most often report over estimation or under estimation of the true functional capacity.

### Exercise Testing:

An objective exercise testing procedure was developed by using a treadmill or bicycle ergo meter to determine maximal exercise capacity. Maximal exercise testing has been extensively validated for diagnosis, prognosis and exercise prescription. Maximal exercise testing requires specialized facilities, equipment and personnel and is associated with considerable cost. However, most of the human activities are always associated with sub maximal exercise capacity level. Hence sub maximal exercise testings were considered to be safe and feasible for more debilitated patients or patients with low exercise capacity or high risk population for graded, maximal exercise testing.

### Field tests:

In 1960's Balke developed a simple walk test with defined period of time [2,3] which was later modified to twelve minute walk test.[4] When this 12 minute walk test was performed in patients with respiratory and cardiac problems, it was too exhausting[5] and therefore a six minute walk test was developed which is easy to administer, better tolerated and more reflective of daily activities of living.[6] The six minute walk test was developed by Guyatt *et al*. for exercise testing before exercise prescription.[3]

Lipkin has first introduced the 6MWT as a functional exercise test in 1986. Its results were highly correlated with those of the 12-minute walk test from which it was derived and with those of cycle ergometer or treadmill based exercise tests. The 6MWT was also found to be a valuable instrument to assess progression of functional exercise capacity in different clinical intervention studies.

In a cardiac rehabilitation set up while prescribing exercises, six minute walk test as originally described by Guyatt et el[3] can be used for both initial assessment and document functional outcomes after completion of cardiac rehabilitation program.[7] There are equations in six-minute walk test using which, the distance walked can be converted into a measure of functional capacity [vo2max].[8,9] The distance walked can be used as the marker of disease, severity, and prognosis and as the outcome measure in clinical trail testing in medical and surgical procedural therapy.

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**Validity and Reliability of SMWT:**

Usually age, gender, height, body mass determine the walk performance in adult population.[10,11,12] According to American association of cardiovascular and pulmonary rehabilitation (AACVPR) risk stratification most of the adults having history of sedentary life style and low physical function, determined by SF 36 questionnaire[7], showed corresponding low performance in SMWT. There are several studies that have assessed the correlation between the functional capacity derived out of 6MWT and symptom limited graded exercise testing and found to be highly correlated.[13]

There is maximum correlation in the rehabilitation equivalent value when compared to rate of perceived exertion suggesting that the 6MWT is more a sub maximal exercise test [4,14] and hence can be considered as the exercise testing procedure in cardiac rehabilitation set up.[15]The reliability of the test in healthy elderly persons and patients were high (Intra Class Correlation = 0.93) and it has been established as a valid and reliable test to assess the exercise capacity of various patient groups.[7]

The baseline 6MWT distance in (UAB) University of Alabama at Birmingham cardiac rehabilitation (9) program of ischemic heart disease patients in total number of n = 30 was mean equivalent to 1351 feet $\pm$ 361.(411meter) with minimum value of 120ft (36meters) and maximum value of 2322ft (707meters). A British cardiac rehabilitation program reported an improvement in 6-min walk distance from 1032 $\pm$ 249 to 1238 $\pm$ 258 ft over 6 weeks of training (two exercise sessions per week) but did not indicate the proportion of patients improvement.[7]

**Technical Aspects of the 6mwt:**

A complete description of the test is given by ATS.[12]

**Location**

The 6MWT should be performed indoors, along a long, flat, straight, enclosed corridor with a hard surface that is seldom traveled. If the weather is comfortable, the test may be performed outdoors. The walking course must be 30 m in length. A 100-ft hallway is, therefore, required. The length of the corridor should be marked every 3 m. The turnaround points should be marked with a cone (such as an orange traffic cone). A starting line, which marks the beginning and end of each 60-m lap, should be marked on the floor using brightly colored tape.

**Required Equipment**

1. Countdown timer (or stopwatch)
2. Mechanical lap counter
3. Two small cones to mark the turnaround points
4. A chair that can be easily moved along the walking course
5. Worksheets on a clipboard
6. A source of oxygen
7. Sphygmomanometer
8. Telephone
9. Automated electronic defibrillator

**Patient Preparation**

1. Comfortable clothing should be worn.
2. Appropriate shoes for walking should be worn.
3. Patients should use their usual walking aids during the test (cane, walker, etc.).
4. The patient's usual medical regimen should be continued.
5. A light meal is acceptable before early morning or early afternoon tests.
6. Patients should not have exercised vigorously within 2 hours of beginning the test.

**Measurements**

1. Repeat testing should be performed about the same time of day to minimize intraday variability.
2. A "warm-up" period before the test should not be performed.
3. The patient should sit at rest in a chair, located near the starting position, for at least 10 minutes before the test starts. During this time, check for contraindications, measure

   Pulse and blood pressure, and make sure that clothing and shoes are appropriate.
4. Pulse oximetry is optional. If it is performed, measure and record baseline heart rate and oxygen saturation (SpO 2) and follow manufacturer's instructions to maximize the signal and to minimize motion artifact. Make sure the readings are stable before recording. Note pulse regularity and whether the oximeter signal quality is acceptable.
5. Have the patient stand and rate their baseline dyspnea and overall fatigue using the Borg scale
6. Set the lap counter to zero and the timer to 6 minutes. Assemble all necessary equipment (lap counter, timer, clipboard, Borg Scale, worksheet) and move to the starting point.
7. Instruct the patient as follows:

   "The object of this test is to walk as far as possible for 6 minutes. You will walk back and forth in this hallway. Six minutes is a long time to walk, so you will be exerting yourself.

   You will probably get out of breath or become exhausted. You are permitted to slow down, to stop, and to rest as necessary. You may lean against the wall while resting, but resume walking as soon as you are able. You will be walking back and forth around the cones. You should pivot briskly around the cones and continue back the other way without hesitation. Now I'm going to show you. Please watch the way I turn

without hesitation." Demonstrate by walking one lap yourself. Walk and pivot around a cone briskly. "Are you ready to do that? I am going to use this counter to keep track of the number of laps you complete. I will click it each time you turn around at this starting line.

Remember that the object is to walk as far as possible for 6 minutes, but don't run or jog. Start now or whenever you are ready."

8. Position the patient at the starting line. You should also stand near the starting line during the test. Do not walk with the patient. As soon as the patient starts to walk, start the timer.
9. Do not talk to anyone during the walk. Use an even tone of voice when using the standard phrases of encouragement. Watch the patient. Do not get distracted and lose count of the laps. Each time the participant returns to the starting line, click the lap counter once (or mark the lap on the worksheet). Let the participant see you do it. Exaggerate the click using body language, like using a stopwatch at a race.

   After the first minute, tell the patient the following (in even tones): "You are doing well. You have 5 minutes to go." When the timer shows 4 minutes remaining, tell the patient the following: "Keep up the good work. You have 4 minutes to go." When the timer shows 3 minutes remaining, tell the patient the following: "You are doing well. You are halfway done." When the timer shows 2 minutes remaining, tell the patient the following: "Keep up the good work. You have only 2 minutes left." When the timer shows only 1 minute remaining, tell the patient: "You are doing well. You have only 1 minute to go." Do not use other words of encouragement (or body language to speed up). If the patient stops walking during the test and needs a rest, say this: "You can lean against the wall if you would like; then continue walking whenever you feel able." Do not stop the timer. If the patient stops before the 6 minutes are up and refuses to continue (or you decide that they should not continue), wheel the chair over for the patient to sit on, discontinue the walk, and note on the worksheet the distance, the time stopped, and the reason for stopping prematurely. When the timer is 15 seconds from completion, say this: "In a moment I'm going to tell you to stop. When I do, just stop right where you are and I will come to you." When the timer rings (or buzzes), say this: "Stop!" Walk over to the patient. Consider taking the chair if they look exhausted. Mark the spot where they stopped by placing a beanbag or a piece of tape on the floor.
10. Post-test: Record the post walk Borg dyspnea and fatigue levels and ask this: "What, if anything, kept you from walking farther?"
11. If using a pulse oximeter, measure SpO 2 and pulse rate from the oximeter and then remove the sensor.
12. Record the number of laps from the counter (or tick marks on the worksheet).
13. Record the additional distance covered (the number of meters in the final partial lap) using the markers on the wall as distance guides. Calculate the total distance walked, rounding to the nearest meter, and record it on the worksheet.
14. Congratulate the patient on good effort and offer a drink of water.

**Clinical Application:**

Initially, SMWT was used as field test to monitor the outcomes of therapy specifically in pulmonary conditions; Later was used to assess the exercise tolerance in patients with low work capacity and high risk group for graded, maximal exercise testing. Thus, gradually SMWT was used in cardiac rehab programs with validation with standard testing protocols and self-reported measures.[15, 16, 17] The 6MWT data guides the exercise prescription in cardiac Rehabilitation by making use of American College of Sports Medicine (ACSM) working equation.[13]

**Example of Using 6-Min Walk Data for Exercise Prescription**

Distance walked by the patient: 420 metres in 6 min.Calculate walking speed: Calculate speed in m/min: 420 m/6 min = 70m/min Calculate metabolic value for walking without grade:1 MET = 3.5ml/kg/min; 0.1 is the constant for converting m/min to ml/kg/min Estimated oxygen consumption(V)2) = 3.5ml/kg/min + O2 consumed in 6MWT(in ml/kg/min)Thus, VO2 = 3.5ml/kg/min + 70m/min × 0.1 = 3.5 + 7ml/kg/min = 10.5 ml/kg/min.MET level achieved: 10.5 ml/kg/min/3.5 = 3 METs. Metabolic value can be used for exercise prescription on apparatus other than treadmill MET, metabolic equivalent.

Using the cahalin formula [VO2 MAX = 0.006 X 6MWD (FEET) + 3.38 also can be used Walter villobos *et al.*, have concluded in their study that 6MWT is more reliable in cardiac rehabilitation and have better correlation in term of $Vo_2$ Max.[16, 17] In their study they also concluded patients with a 6MWD of less than 360 have low level exercise capacity and those with 6MWD great than 546 have moderate to high level of exercise capacity. They also use an equation to find the predicated value for 6MWT equation 218 + (5.14 X HT IN CM – 5.32 X AGE) – (1.80 X WT + 51.31). It is observed that in healthy individuals will have a predicted 6MWD of 631 ± 93meter.

**Widening Horizons:**

Six-minute walk test is a universally accepted field test. It is safe, simple and well tolerated by most patients, even by frail elderly. It is reliable and feasible for many patient populations including the renal patients. The six-minute walk test (SMWT) is a simple, safe, and inexpensive test that uses an exercise mode relevant to everyday activities. The SMWT is a good measure of functional exercise ability because it is self-paced and sub maximal

in nature. The SMWT is also well-accepted by patients, easily administered, and easily reproduced.[15-20] Recent studies indicate that Six minute walk distance predicts the mortality in different groups of patients. The SMW distance was found to be correlated with mortality prediction inpatients with colon cancer and lung transplant.[21, 22] It is quite interesting to note that a recent study have found the prediction ability of the SMWT in patients waiting for liver transplant, which was equivalent to predictability as that of MELD (Model for End stage Liver Disease) score which is used for prioritizing patient waiting for liver transplantation.[23] Thus, the applications of SMWT extends beyond Cardiopulmonary testing and monitoring therapy response.

**Limitations:**

The SMWT could an useful method for fragile, debilitated individual but may not be a suitable tool for normal subjects and sports training as it does not show a correlation with maximal exercise testing in this population. Moreover, it has value of correlation rather than exact measure of sub-maximal exercise capacity of an individual.[12, 18] However, psychological factors such as depression and cognitive impairment all have a negative effect on the timed walking distance.[24] The SMWT interpretation should include consideration of vascular, pulmonary, and musculoskeletal exercise limitations, as the mechanisms to limitation in the physical capacity might not have same physiological process and may have confounding variables also.[25] The reliability in prediction of changes in $VO_2$ max among the patients awaiting cardiac transplantation still remains unestablished.[26]

**Future directions:**

The SMWT is well established for safe clinical test for variety of patients. As its role expands to prediction of outcomes of patients requiring surgical interventions and high risk individuals, the test needs to carefully used with due precautions taking into consideration of the limitations. The normative data, effects of medications, nutritional status, and level of racial differences in patient groups needs further testing before generalization. The predictive valve still needs clarification due to less number of published data in various conditions other than cardiac and pulmonary diseases and need to consider the confounding variables as SMWT could not point out the source of deficit in all patients.

**Conclusion:**

Six minute walk test is a simple, valid and reliable field test with high level of patient compatibility and clinically sensitive with predictive of outcomes in different groups of cardiac and pulmonary patients. The normative and comparative data in Indian population for various groups is yet to be established, which could provide more insight on the minimal standards of exercise tolerance and predictive values in those patients. Thus, SMWT could serve as a clinically useful tool facilitating efficient method of estimating an individual's physical capacity with a functional test. The SMWT should be considered as useful complementary information about the functional status of patients with cardiovascular or pulmonary disease, while the utility needs further testing in other groups of patients.

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# AN UNCOMMON CASE OF AMNIOTIC BAND SYNDROME

**R. Preetha[a], Usha Vishwanath[a], Preet Agarwal[a], Parimala[a]**

***ABSTRACT:***

*Amniotic band syndrome is a set of congenital malformations ranging from minor constriction rings to complex multiple congenital anomalies that are attributed to amniotic bands that entangle and disrupt fetal parts. Usual manifestations are constriction rings around the digits, arms and legs, amputation of digits and limbs, club feet, club hands, etc. Here we present a case of amniotic band syndrome with symmetric intrauterine growth retardation (IUGR) with oligohydramnios. The baby had a small cleft adjacent to left big toe without any other obvious deformities. This case of amniotic band syndrome is reported to highlight the importance of how heightened clinical suspicion, appropriate obstetric intervention and neonatal care can improve the outcome of child wellbeing.*

***Key words :*** *amniotic band syndrome, constriction rings, oligohydramnios.*

SRJM 2011;4:35-37

## INTRODUCTION:

Amniotic band syndrome (ABS) has been studied since the time of Hippocrates and Aristotle.[1] As early as 1968 Richard Torpin worked on the pathogenesis of amniotic band syndrome. The prevalence of amniotic band syndrome for live births is 7.7:10,000.[2] Among pregnant mothers with amniotic band syndrome, spontaneous abortions is a common outcome and it can be as high as 178:10,000.[3] Here we report a case of amniotic band syndrome with symmetric IUGR with oligohydramnios. The aim of this paper is to describe how a high clinical suspicion and appropriate obstetric management can help in improving fetal wellbeing in the case of amniotic band syndrome.

## CASE REPORT

A 23 year old lady with 37 weeks of gestation was referred from a peripheral center with a diagnosis of amniotic band syndrome for further management. She had one previous history of abortion following which she conceived spontaneously and was on regular folic acid supplementation. She reported quickening at the fifth month and also received two doses of tetanus toxoid. Her dating scan and anomaly scan were normal. Ultrasonogram examination done at 36 weeks revealed the presence of amniotic bands with symmetric intrauterine growth retardation (IUGR) and oligohydramnios (Fig.1).

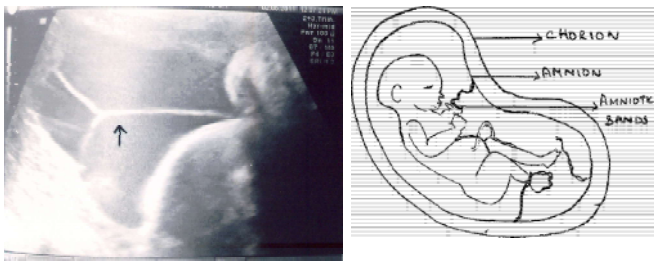


Fig.1 : Ultrasonogram image showing presence of amniotic bands; adjacent picture gives a diagramatic picture of amniotic band

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At 36 weeks, two doses of steroids, injection Betamethasone 12mg were given 24 hours apart, (prophylactically in view of IUGR).

She had no significant past medical or surgical history. Her menstrual cycles were regular. Her first pregnancy was a spontaneous abortion at 8 weeks for which dilatation and curettage was done.

On examination she had no pallor, icterus, cyanosis or clubbing.

Her pulse rate was normal and her blood pressure was 110/70 mmHg. All the systemic examination was normal. Per abdomen: uterus corresponded to 32 weeks, which was relaxed with cephalic presentation. Clinically the liquor was decreased. Fetal heart rate was 140 beats per min. Her laboratory parameters were all within the normal range (Table 1). Her ultrasound scan reports revealed amniotic bands after which a repeat ultrasound was performed that confirmed the presence of amniotic bands, oligohydramnios and symmetric IUGR (Table 2). Her urine routine, serum electrolytes and liver function tests were normal.

Table 1. Laboratory parameters of the patient with Amniotic Band Syndrome.

| INVESTIGATIONS | REPORTS |
|---|---|
| Haemoglobin | 11.3g/dl |
| PCV | 41.1 |
| FBS | 91mg/dl |
| Platelets | 1.61 lakhs/cu mm |
| Blood Group | 'A' Positive |
| HIV, HBSAG | Negative |

All laboratory parameters were in the normal range.

Intraoperatively the placenta showed marginal insertion of the umbilical cord with multiple areas of calcification. Amniotic bands were seen extending from each avascular area.

She delivered a girl baby weighing 1.43kg with an Apgar score of 8/10, 9/10. Baby was immediately assessed by paediatrician. Baby had a small cleft adjacent to left big toe (Fig. 3). No other obvious congenital deformities was

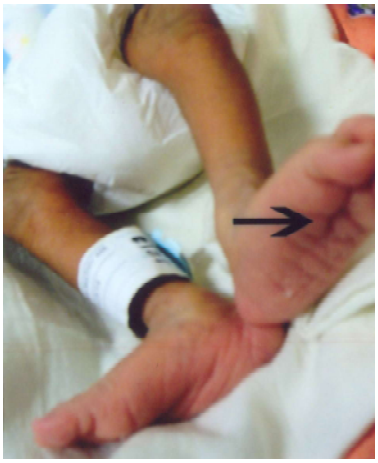

Fig.3 : Presence of Cleft near left big toe.

TABLE 2: OBSTETRIC ULTRASOUND FINDINGS OF THE PATIENT

| | 29.04.2011 | 02.05.2011 |
|---|---|---|
| Gestational Age (Dates) | 36weeks | 36 weeks + 3 days |
| Gestational Age (Scan) [lags by 4 weeks compared to the gestational age according to last menstrual period] | 31-32 weeks | 31-32 weeks |
| Presentation | Cephalic | Cephalic |
| Placenta | Posterior | Posterior |
| Amniotic fluid index | 8.9 cm | 8.5 cm |
| Estimated fetal weight | 1523 +/-152.3 g | 1674 +/-251.17g |
| | Symmetric IUGR with few amniotic bands with oligohydramnios | HC falls at 2nd percentile and AC falls less than first percentile for 36 – 37 weeks suggestive of symmetric IUGR.<br>Multiple amniotic bands seen applied closely to fetal body but not attached to it.<br>Limited assessment of fetal anatomy. No obvious defect in parts assessed. Normal Doppler study. |

noted. Post operatively the baby was monitored in neonatal intensive care unit in view of low birth weight.

Baby was followed up for 2 weeks after delivery. The baby was on direct breast feed. For further management of small cleft seen adjacent to left big toe, the patient was referred to the Department of Orthopaedics and General Surgery. No surgical intervention was suggested.

## DISCUSSION

Amniotic band syndrome is a set of congenital malformations ranging from minor constriction rings to complex multiple congenital anomalies that are attributed to amniotic bands that entangle and disrupt fetal parts.

ABS occurs when the inner membrane of the amniotic sac tears and wraps around the developing baby and causes problems with limbs, clefts in the face and band marks in different areas of the body.[4]

The etiology is unknown. There have been reports associating amniotic band syndrome with maternal trauma, oophorectomy during pregnancy(5), intra uterine contraceptive device, and amniocentesis.[6] There are case reports in families with connective tissue disorders like Ehler Danlos syndrome.[7]

The clinical features) range from minor constriction rings around the digits, arms and legs. Swelling of the extremities distal to the point of constriction (congenital lymphedema). Severe complications like amputation of digits, arms and legs (congenital amputation) can occur.[8] Congenital deformities like clubfoot, clubhands, cleft lip, cleft palate and hemangioma can occur.

Amniotic band theory states that ABS occurs due to a partial rupture of amniotic sac. This rupture involves only the amnion; the chorion remains intact.[9] The bands can constrict fetal parts reducing blood circulation and hence causes congenital abnormalities.

Vascular disruption theory suggests an "intrinsic" defect of blood circulation because the constricting mechanism of the amniotic band theory does not explain the high incidence of cleft palate and other cleft defects occuring in ABS.[10]

Amniotic band syndrome can be diagnosed prenatally by ultrasound which can show amniotic bands, congenital malformations, oligohydramnios.[11] The most important diagnostic criteria are visible amniotic bands, constrictions rings and irregular amputations of toes or fingers. Three dimensional and four dimensional ultrasound and MRI contribute to more sensitive prenatal diagnostics of amniotic band syndrome.

## TREATMENT

The accepted modality of treatment for ABS in utero is by foetoscopic laser surgery before the bands can compress fetal parts.(12)

Plastic and reconstructive surgery to treat any resulting deformity has been performed after birth. Physical and occupational therapy for long term rehabilitation must be considered.

## RECURRENCE

Amniotic Band Syndrome is often sporadic with no recurrence in subsequent pregnancies. However there are some reports of ABS occurring among families with collagen disorders like Ehler-Danlos syndrome.[13]

## CONCLUSION

This case report illustrates how heightened clinical suspicion can help in pre-operative diagnosis of amniotic band syndrome.

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# AN UNUSUAL CONCERN IN PREGNANCY..... IDIOPATHIC THROMBOCYTOPENIC PURPURA – A CASE REPORT

**Pushpalatha [a], Gonnabaktula Naga Vasanthalakshmi [a], Priyanka Mehta [a], S. Asha Devi [a]**

**ABSTRACT:**

*Idiopathic thrombocytopenic purpura accounts for 3% of thrombocytopenia in pregnancy with an overall incidence of 0.1-1/1000 pregnancies. The greatest concern with this disorder is the risk of postpartum hemorrhage and the chances of neonatal thrombocytopenia. A 29yr old G2P1L1 was admitted at SRMC with 38 weeks for safe confinement. She was a known case of ITP since 6 yrs with a significant past history of menorrhagia and purpuric spots. She was advised prednisolone and azothioprine for the same. She was serially monitored with platelet count during her course of pregnancy and had an uneventful intrapartum period. She delivered a female baby vaginally without any postnatal complications. The aim of this case report is to focus on the clinical aspects of ITP and the management guidelines of ITP with pregnancy in labour.*

***Key words :*** *menorrhagia, postpartum hemorrhage, thrombocytopenia*

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## INTRODUCTION:

Platelet count in pregnancy is normal in most women even though as a consequence of hemodilution in pregnancy, there is a downward drift in the platelet count of upto 10%.[1,2,3] The causes of thrombocytopenia in pregnancy includes gestational thrombocytopenia,Idiopathic Thrombocytopenic Purpura(ITP), Thrombotic thrombocytopenic purpura, HELLP syndrome (Hemolysis Elevated liver enzymes Low platelets), viral infections, leukaemia, drugs and pseudo-thrombocytopenia.Diagnosis of ITP is mainly by excluding the above causes. ITP is caused by antibodies against platelet surface glycoproteins which result in immune mediated platelet destruction; these antibodies are capable of crossing the placenta and can cause fetal thrombocytopenia.[4]

## CASE REPORT :

A 29yr old Gravida2 Para1 Living1 was admitted at Sri Ramachandra Medical Centre in 2010 with 38 weeks with ITP for safe confinement.

In 2004, she had her first clinical symptoms in the form of menorrhagia and purpural rashes for 3 months leading to a drop in hemoglobin to 3g%. She was stabilized with 3 units of packed cells as she was severely anemic and thrombocytopenic and was started on continuous combined pills (ethinyl estradiol 35micrograms + levonorgertrel 0.15mg). On evaluation, the patient had a microcytic peripheral smear picture with a platelet count of 17,000; Coomb's test was negative. IdiopathicThrombocytopenic Purpura was diagnosed after excluding the other causes. She was advised prednisolone 60mg for the same with restoration of the platelet count to normal. She had her marriage in 2005 and conceived spontaneously in the same year.

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In her first pregnancy,in 2005, she was advised prednisolone 30mg at 30 weeks in view of serial fall in the platelet count from 1.68 lakhs/cu.mm. to 35 thousand/ cu.mm. The counts gradually improved upto 1.76lakhs at 36weeks; she was induced at 38 weeks in view of declining platelet count (90,000). She had an uncomplicated spontaneous vaginal delivery with episiotomy.

In her second pregnancy in 2010, she was on azothioprine pre-conceptionally . Her platelet count was serially monitored and at 5th month of her gestation, anomaly scan and fetal echo was done which was normal. Even though there is a concern of intracranial hemorrhage with vaginal delivery, Caesarean section is not routinely recommended ;[5] hence the patient was allowed for a spontaneous vaginal delivery with episiotomy with an uneventful third stage. In the postpartum period, she was advised to withhold azothioprine till lactation was established and was changed to prednisolone 30mg.

## DISCUSSION :

Differential diagnosis of thrombocytopenia in pregnancy includes gestational thrombocytopenia, Pre-eclampsia, Acute fatty liver of pregnancy,Hemolytic uraemic syndrome, Systemic Lupus Erythematosus, viral infections, Disseminated Intravascular Coagulation, Congenital hyperslenism, bone marrow dysfunction, viral infections and drug-induced causes.

Most women with ITP have normal findings on physical examination (splenomegaly is absent) and purpura may be present especially in the lower limbs.[6] Although antibodies can be demonstrated in these cases, the tests lack sensitivity and specificity. Majority require no treatment except before delivery. In pregnancy,the first line of management is Prednisolone, starting at 20mg and escalating to 60 mg in 1 week if needed.Second line is Azathioprine / i.v.immunoglobulin/ anti-D immunoglobulin[7] and the third line is platelet transfusion / spleenectomy (in the non pregnant state)

Although there is no universally accepted safe platelet count, the following can be taken as a general guideline for

the intervention as per (Table-1) in pregnancy, so as to have a safe delivery avoiding the risks of post partum hemorrhage.[8]

Table-1

Safe platelet count as per Royal College of Obstetrics & Gynaecologists[9]

| INTERVENTION | PLATELET COUNT (*$10^9$/l) |
|---|---|
| Antenatal, no invasive procedures planned | >20 |
| Vaginal delivery | >40 |
| Operative or instrumental delivery | >50 |
| Epidural anaesthesia | >80 |

Prednisolone has been assigned to pregnancy category C by the FDA. Some animal studies have revealed evidence of fetal harm, although data are conflicting. There are no controlled data in human pregnancy. Prednisone is only recommended for use during pregnancy when there are no alternatives and benefit outweighs risk. Azathioprine in pregnancy falls under category C. Although fetus is at risk for cardiac anomalies (atrial/ventricular septal defect), fetal growth restriction and preterm birth, benefits outweigh the risks and hence are used in the management of ITP.

The incidence of neonatal thrombocytopenia is 14-37%[10] but the neonates are usually without physical findings.[5] There is no recommendation for routine cordocentesis. In labor, to avoid intracranial hemorrhage, measures should be taken to avoid fetal scalp electrodes and instrumental delivery. A cord sample should be taken to assess the platelet count and repeated on days 1 and 4 of birth. Intramuscular vitamin K is to be avoided until the count is known. Babies with severe thrombocytopenia should be treated with platelets and i.v.immunoglobulins.

Neonatal Alloimmune Thrombocytopenia, which occurs as a result of maternal immunization develops in fetal life, with 25-50% of fetal intracranial haemorrhage detected on prenatal ultrasound. Neonatal morbidity is by far more common in Neonatal Alloimmune Thrombocytopenia, with 10% of affected newborns dying and 20% experiencing neurological sequale secondary to intra cranial haemorrhage. Affected infants can have generalized petechiae, haemorrhage of abdominal viscera and excessive bleeing following venepuncture or circumcision.

To conclude, eventhough pregnancy is considered an hyper-coagulable state,there are always exceptions as in this patient who had a thrombocytopenic picture which was of special concern to us.

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# SUBCUTANEOUS NODULES IN ACUTE RHEUMATIC FEVER – A CASE REPORT

**J Dinesh Kumar[a], S Saji James[a], P Venkataraman[a] , P SubbaRao [a] M S Latha[a]**

**ABSTRACT:**

Acute Rheumatic fever commonly presents with features of arthritis, carditis and less commonly with chorea and erythema marginatum. The association of subcutaneous nodules in rheumatic fever with carditis is <1% We present a case study of this rare presentation with review of literature.

***Key Words:*** *Acute Rheumatic fever, Subcutaneous nodules, Rheumatic heart disease, Carditis.*

SRJM 2011;4:40-41

## INTRODUCTION:

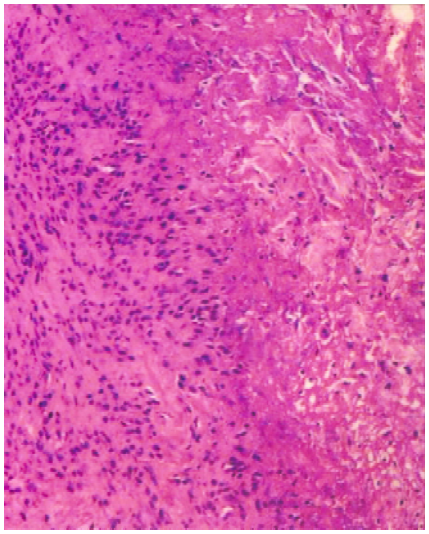
The prevalence of acute rheumatic fever and rheumatic heart disease in Indian population varies from 0.5/1000 to 11/1000 as documented in various studies.[1] Acute rheumatic fever is a non – suppurative complication of group A beta hemolytic streptococcal sore throat. Rheumatic fever usually affects joints, skin, subcutaneous tissues, brain and heart.[2] Except heart, all other effects are reversible, needing only symptomatic relief during the episodes. Significant cardiac complications like valvular heart disease can occur if secondary prophylaxis were not followed.[3]

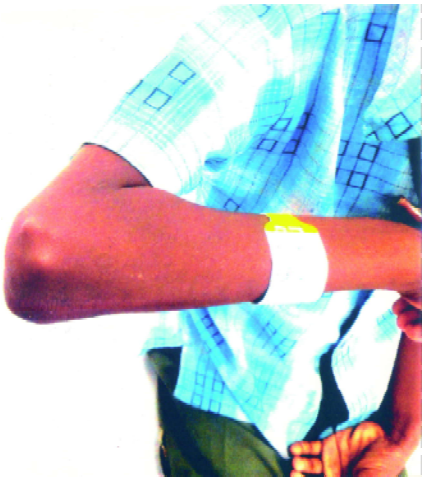
## CASE REPORT :

A 10 year old boy, apparently normal till 1½ months back, first had fever and throat pain lasting for 3-5 days, treated symptomatically. One month later he complained of pain in both knee joints, associated with swelling and redness of the joints, restricting his daily activities, which subsided by 1 week. After a week, he developed fever, pain in both ankle joints, chest pain and nodular lesions over both extensor aspect of elbows.

On examination, 3 to 4 subcutaneous nodules could be seen on the extensor aspect of both elbows which were rounded, firm, mobile and non tender each measuring 0.5 to 1cm in size. He also had hyperdynamic precordium, pericardial rub, apical impulse at 6$^{th}$intercostal space half an inch lateral to mid clavicular line, grade 3/6 pansystolic murmur radiating to axilla and back and loud P2.

Blood investigations revealed ESR of 57mm/hr and raised C- Reactive Protein, Hb 7.3gm/dl, Anti Streptolysin O – 200 IU/L, throat culture positive for streptococcal pyogenes. Echo done revealed severe mitral regurgitation, dilated Left atrium and Left ventricle, thickened mitral valve and minimal pericardial effusion and pulmonary hypertension of 46 mm Hg. Clinical examination of the patient ruled out any other systemic involvement apart from cardiovascular system.





Excision biopsy of the subcutaneous nodule was done to confirm the diagnosis which showed fibrocollageneous tissue with interstitial spindle cells, collagen denegration and associated histiocytic reaction with occasional neutrophil, features suggestive of rheumatic nodules.

He was treated with a stat dose of 0.6 million units of Benzathinepencillin and Steroid 2mg/kg/day which was tapered at a dose of 2.5 mg every 3$^{rd}$ day after 2 weeks, Aspirin was started at a dose of 75 mg/kg during the tapering of steroids, and given for a total period of 10 weeks. He was started on low dose diuretics for 2 weeks and stopped. After two weeks all nodules were slowly disappeared.

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**DISCUSSION:**

According to modified Jones criteria, the diagnosis of Acute Rheumatic Fever (ARF) can be made if it fulfills with 2 major criteria or 1 major and 2 minor criteria with supporting evidence of antecedent streptococcal infection. The major criteria are poly arthritis, carditis, rheumatic chorea, erythema marginatum and subcutaneous nodules. Minor criteria includes fever, arthralgia, raised acute phase reactant (erythrocyte sedimentation rate, C-reactive protein, prolonged PR interval). Supporting evidence of antecedent group A streptococcal infection like positive throat culture or rapid streptococcal antigen, elevated or rising titer of Antistreptolysin O.[4] Subcutaneous nodule (SCN) is one of the major clinical criteria to diagnose Acute Rheumatic fever (ARF). Subcutaneous nodules have been reported in < 1% to 21 % of cases.[5] Subcutaneous nodules were invariably associated with severe carditis. They usually appear several weeks after the onset of the acute episode, persist from days to weeks and rarely last longer than one month. The nodules are small, varying in size from millimeters to 2 centimeters, firm, painless and fully movable under the skin. They are encountered in clusters, on the extensor surface of the joins and overlying bone prominences, mainly in large joints of limbs, knuckles, scalp and along the spine in the paravertebral areas.

A prospective study of 42 cases of ARF with SCN attempts to analyse the accuracy of such statements.[6] The group comprised of 12.5% of 336 consecutive cases of ARF. Other major criteria associated with SCN were carditis in 38 (90.4%), arthritis in 33 (78.5%) and chorea in two (4.7%). No evidence of carditis could be found in 4 (9.5%). When a detailed study of SCN was done the average number of nodules found in the group was 18 (range 4-49). Thirteen (30.9%) had less than 10 nodules and 5 (11.9%) had only 4-5 nodules. With initiation of treatment SCN disappeared within 4 weeks in 29 (69%), within next 5-8 weeks in 8 (19%) and within 9-12 weeks in 3 (7.1%). In two cases (4.7%) multiple nodules were observed 12 weeks later when all other evidences of activity had disappeared. Number of nodules appearing in ARF might be quite small and they could persist for long inspite of treatment.[7] Subcutaneous nodules never present as a sole manifestation of Rheumatic fever and Subcutaneous nodules are not exclusive to Rheumatic fever. They occur in 10% of children with Rheumatoid arthritis.[8]

The most common differential diagnosis is the rheumatoid nodule. Nodules in acute rheumatic fever are smaller and more short lived than the nodules of the Rheumatoid arthritis. Although elbows are most frequently involved in both diseases, Rheumatic fever nodules are more common on olecranon while Rheumatoid nodules are usually found 3-4 cms distally.[9] The other differential diagnosis are Lipoma, Sebaceous cyst, Xanthomas, Erythema Nodosum, Polyarteritis nodosa.

There is no specific treatment for subcutaneous nodules and it will disspapear when the carditis is adequately treated. Prognosis mainly depends upon the presence or absence of carditis at the initial attack of Rheumatic fever.

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# ATYPICAL PRESENTATIONS OF GALL BLADDER CARCINOMA

**T Karthik[a], A V P Sivalingam[a], Ramya Ramakrishnan[a], Shalinee Rao[b]**

***ABSTRACT:***

*Gallbladder carcinoma is a highly malignant and uncommon tumour that affects predominantly women in the 6th and 7th decade of life. Most of the patients are either asymptomatic or have only dyspeptic symptoms and usually present at an advanced stage, when treatment options becomes limited. Some patients may have atypical presentations and unusual associations.*

*In this case report, we document two cases of carcinoma gallbladder who presented with an acute abdomen. Relevant literature about atypical presentations of carcinoma gallbladder is also reviewed.*

***Key Words:*** *acute abdomen, atypical presentation, carcinoma, gallbladder.*

SRJM 2011;4:42-44

## INTRODUCTION:

The incidence of gallbladder carcinoma is 1.2 cases per 100,000/ year and increases with age, particularly after the sixth decade of life.[1] Ethnic background and geographical location are important factors in the incidence of this cancer. It is more common in Central and South America, Japan and North India. Risk factors include cholelithiasis, calcified gallbladder wall, adenomatous gallbladder polyps, choledochal cyst and anomalous pancreatobiliary junction.[2,3,4]

The clinical presentation of gallbladder cancer is identical to the more prevalent symptoms of biliary colic and/or chronic cholecystitis, making it difficult to suspect the diagnosis pre-operatively. Some patients are completely asymptomatic until widespread metastasis occurs.[5] Rarely, patients with gallbladder carcinoma may have atypical presentations and unusual associations. In this case report, we discuss the case details about two of our patients with carcinoma gallbladder one of which presented with features of emphysematous cholecystitis and another with acute pancreatitis.

## CASE DETAILS

**Case 1:** A 45 year old female was brought to the emergency department with complaints of epigastric pain and vomiting for three days. On examination, she had mild tachycardia and normal blood pressure. She was icteric and afebrile. Abdominal examination revealed tenderness in the epigastrium with no features of peritonitis. Blood investigations revealed leucocytosis, mild obstructive jaundice with elevated pancreatic enzymes (Serum amylase- 431/l, Serum lipase- 1102/l). Ultrasonogram of the abdomen showed distended gallbladder filled with sludge and dilatation of the common bile duct. A diagnosis of gallstone pancreatitis was made and the patient was managed conservatively. Subsequently, patient was taken up for open cholecystectomy after symptomatic improvement and reduction of serum pancreatic enzyme levels. Intra-operatively, the gallbladder was thick walled and distended. Cholecystectomy was done by the standard technique. On cutting open, the gallbladder was seen to be studded with polypoidal nodules over the entire mucosa. No gall stones were identified (Fig 1). Histopathology of the excised specimen revealed papillary adenocarcinoma of the gallbladder (Fig 2). Grossly the tumor was seen involving the fundus, body and portion of

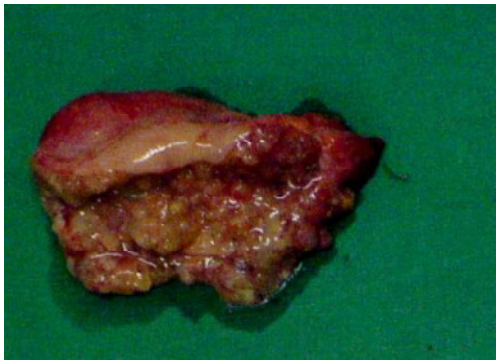

**Fig. 1:** Cut open gall bladder showing mucosa studded with glistening nodules

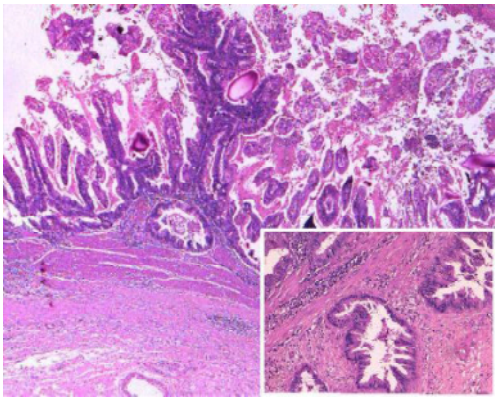

**Fig. 2:** Tumour cells arranged in papillary pattern showing stratification and nuclear atypia (Hematoxylin and eosin X 10 x); Inset show islands of tumour cells arranged in glandular pattern infiltrating the muscle layer (Hematoxylin and eosin X 10)

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the neck. One lymph node was identified in the specimen which was found to be positive. The tumor was moderately differentiated with a pathologic staging of p $T_2$ $N_1$ $cM_0$ . Patient was referred to medical oncology for further management.

**Case 2:** A 41 year old male, a smoker and alcoholic, visited the outpatient department with complaints of dyspepsia for one month and acute abdominal pain for three days. On examination, patient was febrile and had tenderness in the right hypochondrium and a positive Murphy's sign was elicited. Ultrasonogram of the abdomen revealed a distended gallbladder with pericholecystic fluid and specks of air in the wall of the gallbladder. The total leucocyte counts was elevated. His liver function tests, renal parameters and pancreatic enzymes were within normal limits. A diagnosis of emphysematous cholecystitis was made and patient was taken up for laparoscopic cholecystectomy. During surgery, gallbladder was found to be oedematous and the Calot's triangle could not clearly be identified. Hence, it was converted to an open surgery and gallbladder was removed and sent for histopathological examination. Grossly the gall bladder was thickened and mucosa flattened out. Sections showed mucosal ulceration with islands of atypical cells arranged in glandular pattern with surrounding desmoplastic stromal reaction (Fig 3). A histopathological diagnosis of moderately differentiated adenocarcinoma was made. Post-operative period was uneventful. Patient is under follow-up with medical oncology.

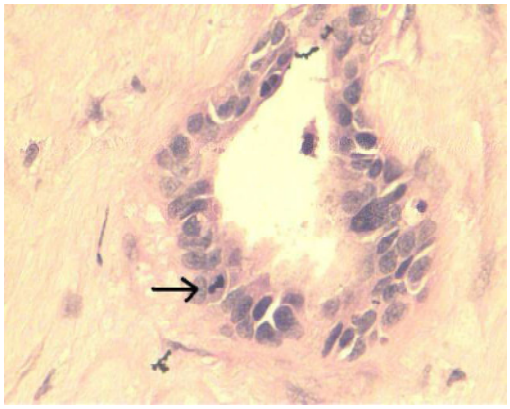

Figure 3: Atypical cells arranged in glandular pattern with surrounding desmoplastic stromal reaction, Note an atypical mitosis (arrow)(Hematoxylin and eosin X 40)

## DISCUSSION

Carcinoma of gall bladder is an uncommon malignancy with a high incidence in certain parts of the world. The incidence of gall bladder cancer in India is very high in the north as compared to the Southern India.[6] It is found incidentally in 1-2% of patients undergoing cholecystectomy for symptomatic cholelithiasis.[7] Early stage tumours(T1) do not require any further surgery. Exceptions to this rule are presence of intra-operative bile spillage and cystic duct margin positivity. Unfortunately, most of the patients are asymptomatic and present in an advanced and unresectable stage requiring only palliative treatment.

The most common factor associated with gall bladder carcinoma in India is the presence of gall stones, while in Japan and Chile, it is the presence of anomalous pancreatic biliary duct junction (APBDJ).[8,9] Pathogenesis of carcinoma arising in APBDJ is different as compared to carcinoma from cholelithiasis. Constant irritation of epithelium of gall bladder from reflux of pancreatic juice and frequent K-ras mutation at codon 12 results in gall bladder carcinoma in APBDJ.[10,11] While the sequence of events in carcinoma occurring due to gall stone starts with inflammation of mucosa with subsequent metaplasia followed by dysplasia with p53 mutation ultimately transforming into a carcinoma.[12,13] Misra et al in a study documented over-expression of p53 protein in 70% of cases with gall bladder carcinoma that were associated with gall stones.[14]

Gall bladder carcinoma is usually diagnosed at an advanced stage. In most series, the 5 year survival rate is 3-5%[15] since they are usually asymptomatic and presents only at an advanced stage. Symptomatic patients have features of dyspepsia or biliary colic. Rarely, they may present with an acute abdomen. Occasionally , patients with gallbladder cancer may have atypical presentations and unusual associations. Haribakthi et al studied a large series of carcinoma gall bladder in a tertiary health care center of North India . Their study included 324 patients of which, 26(8%) had atypical clinical presentations and 34(10%) with unusual associations.[16] The atypical presentations included empyema, acute cholecystitis, post-cholecystectomy biliary stricture, carcinoma head of pancreas, gastric outlet obstruction and liver abscess. In our experience, we encountered two cases of carcinoma gallbladder one presenting as emphysematous cholecystitis and another with acute pancreatitis. In the former case, it would have probably occurred due to tumour blocking the cystic duct with distension and mucosal necrosis with secondary infection. While, in the latter case of acute pancreatitis, the mucoid material produced by the papillary carcinoma would have obstructed the pancreatobiliary junction resulting in pancreatitis. Anomalous pancreatic biliary duct junction is also known to cause pancreatitis by way of reflux of bile and pancreatic juice into the pancreas. This indicates that the patient who presented with acute pancreatitis could have had an anomalous pancreatico biliary junction. Another unusual feature about our patients was their age at presentation. Carcinoma of the gallbladder is unusual among patients less than sixty years of age and both our patients were less than fifty years of age. Rashid et al found a genetic abnormality involving extensive instability in repeated nucleotide sequences(microsatellites).[17] This condition is termed as microsatellite instability, and it occurs most often in patients with gallbladder cancer at a younger age.

To conclude, unusual presentations of gallbladder cancer in certain geographic locations (like India) must be thought of when managing patients with acute abdomen with symptoms related to the hepatobiliary/ pancreatic systems.

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# CARCINOMA EX PLEOMORPHIC ADENOMA: A RARE SIGHT ON CYTOLOGY!

**Shalinee Rao [a], Sandhya Sundaram [a], Prathiba Duvuru [a]**

## INTRODUCTION:

Malignancy in pleomorphic adenoma is an uncommon event and has been categorized as carcinoma ex pleomorhic adenoma; carcinosarcoma (true malignant mixed tumor) and metastasizing pleomorphic adenoma.[1]

Fine needle aspiration cytology (FNAC) has been a widely accepted diagnostic tool for palpable salivary gland lesions. Diagnostic accuracy of FNAC of salivary gland lesions ranges from 80-95% in adequately sampled specimens.[2, 3] One of the entities rarely diagnosed on cytology is carcinoma ex pleomorphic adenoma.[4] We present a case report of this uncommon entity diagnosed on cytology.

A 55 year old male presented with swelling in front of the left ear of 10 years duration. It started initially as a small nodule and increased gradually to the present size. Patient also gave history of a rapid increase in size during the last 3 months associated with pricking type of pain. There was no history of skin discoloration or increase in size while chewing. There was no history of fever, ear discharge or pain. General and systemic examinations were within normal limits.

Local examination showed a 3.5x 3cm swelling in front of the left ear. The swelling was firm in consistency, fairly circumscribed and was lifting the ear lobe. There was no warmth, pain or tenderness over the swelling. A clinical diagnosis of pleomorphic adenoma of left parotid gland was made and sent for FNAC.

Fine needle aspiration cytology of the left parotid swelling was performed using a 22 gauge needle and the material aspirated was smeared on to the glass slides. Smears fixed in 95% ethanol were stained with hematoxylin and eosin stain while the air dried smears were stained with

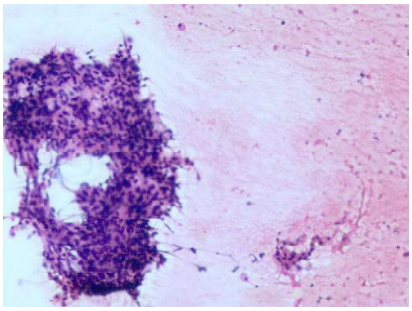

**Fig. 1:** Scanner view showing cellular cell cluster (Hematoxylin and eosin X 20)

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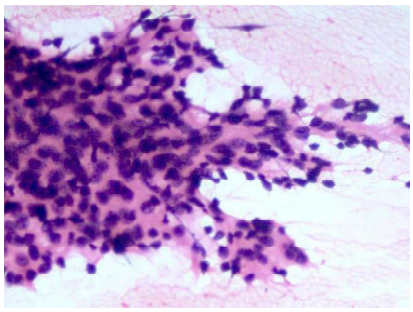

**Fig. 2:** Closely admixed benign cells with bland nuclei and malignant epithelial cells having coarse nuclear chromatin (Hematoxylin and eosin X 100)

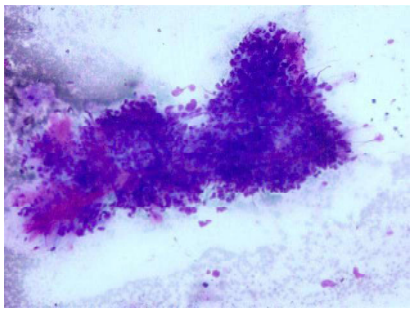

**Fig. 3:** Cluster showing biphasic pattern with epithelial cells and chondromyxoid background seen as by magenta coloured material (May-Grunwald Giemsa X 40)

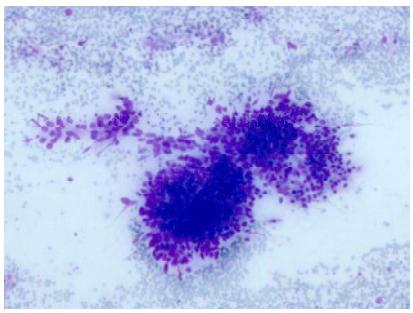

**Fig. 4:** Benign epithelial cells in the clusters appear small oval with round nuclei having a smooth nuclear membrane. Few atypical cells are also seen (May-Grunwald Giemsa X 40)

May-Grunwald Giemsa stain. Examination of the cytological smears revealed a high cell yield (Fig. 1). Smears showed a mixed population of cells composed of benign and malignant elements (Fig. 2). Some clusters showed a metachromatic stroma in the background (Fig. 3). The benign epithelial component appeared as small round to oval cells with bland oval nuclei (Fig. 4). The malignant population consisted of large cells with large pleomorphic nuclei having coarse chromatin and high nucleo-cytoplasmic ratio (Fig. 2, 4 & 5). Malignant change occurring in a background of pleomorphic adenoma was suggested based on the above mentioned cytological features.

A superficial parotidectomy was performed under general anesthesia and the excised specimen was sent for histopathological examination.

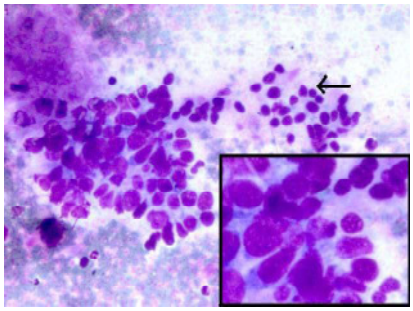

**Fig. 5:** Loose cluster of highly atypical cells with an occasional cluster of benign epithelial cells (arrow) in the adjacent area (May-Grunwald Giemsa X 100); Inset shows large atypical cells with large pleomorphic nuclei, coarse chromatin and irregular nuclear membrane (May-Grunwald Giemsa X 200)

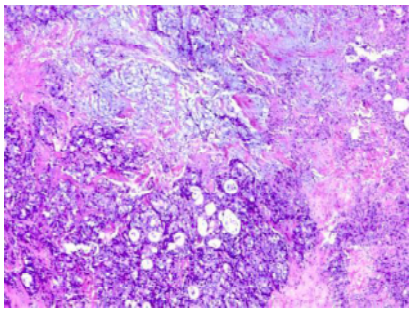

**Fig. 6:** Microscopic section showing mixed tumor with epithelia cells and mesenchymal chondo-myxoid material(Hematoxylin and eosin X 20)

Gross examination showed a soft tissue mass measuring 5x4x3.5cm. External surface appeared irregular. Cut surface showed a fairly well circumscribed grey tan nodular tumour measuring 3cm in diameter having focal myxoid areas compressing the adjacent salivary gland tissue to the periphery (Fig. 6). The margins were uninvolved.

Microscopic sections showed tumor with features of biphasic pattern consisting of epithelial and mesenchymal components. Tumor displayed islands of bland cells arranged in glandular pattern and cords with a chondro-myxoid background.

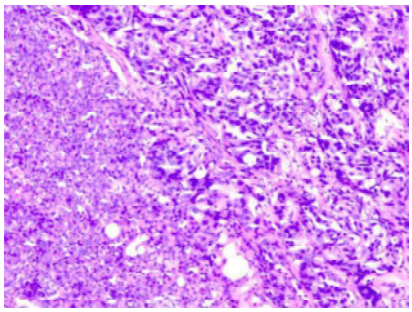

**Fig. 7:** Histology illustrating benign epithelial cells arranged in glandular and trabecular pattern along with a focus of highly atypical cells showing hyperchromatic pleomorphic nuclei(Hematoxylin and eosin X 40)

In addition, one large focus showed groups of highly atypical epithelial cells with pleomorphic nuclei and

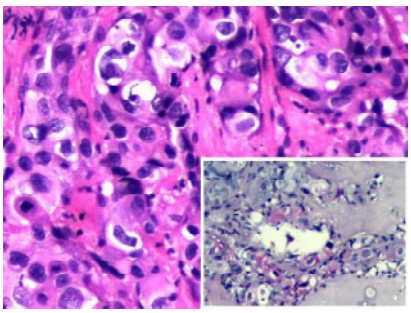

**Fig. 8:** Malignant cells showing cytoplasmic mucin and desmoplastic stroma (Hematoxylin and eosin X 100); inset shows magenta colour in cytoplasm highlighting mucin (Periodic Schiff stain X 100)

increased nucleo-cytoplasmic ratio (Fig. 7 and 8). Pleomorphic cells were arranged in squamoid nests, glandular pattern and sheets. Few of the tumor cells showed presence of cytoplasmic mucin demonstrated by periodic Schiff (PAS) (Fig. 8). Surgical margins were negative for tumour infiltration. A final diagnosis of mucoepidermoid carcinoma occurring in a background of pleomorphic adenoma was made.

## DISCUSSION

Fine needle aspiration cytology is a well accepted tool for the pre-operative diagnosis of salivary gland tumours as they are easily accessible. However, cytology of many of the salivary gland lesions may not always display classical features leading to diagnostic dilemma to the cytopathologist.

The wide spectrum of morphological patterns and frequent overlap of cytomorphological features in a salivary gland lesion leads to difficulty in cytological diagnosis. The other possible reason for inaccurate diagnosis is needle miss in a heterogenous tumor leading to a sampling error.[5] Carcinoma ex pleomorphic adenoma accounts for 3.6% of salivary gland tumours.[6] It is difficult to diagnose this rare entity on cytology.[7] In a series studied by Verma et al on pleomorphic adenoma, all the cases of carcinoma ex pleomorphic adenoma were missed on cytology resulting in a false negative pre-operative cyto-diagnosis.[4]

Quite often the cytologist is satisfied with a single needle pass in FNAC, provided the aspirated material has adequate cellularity for deriving an opinion. In our case, since we made multiple needle passes from different areas of the swelling, we were able to sample the areas of malignant transformation. There are no prescribed criteria for the number of aspiration attempts to be made on salivary gland lesions. However, it has been recommended to make at least 3 passes to completely sample the lesion for a presumptive diagnosis.[8]

The common clinical feature suggestive of a malignant change includes sudden increase in size of a longstanding lesion, pain and facial nerve palsy. In our case, there was a sudden increase in size for the past 3 months in a swelling present for 10 years. The possibility of malignant transformation in a longstanding pleomorphic adenoma

increases from 1.6% in < 5 years to 9.5% in a tumor present for 15 years.[9] The common malignancies occurring in a background of pleomorphic adenoma are adenocarcinoma not otherwise specified (42.4%) and salivary duct carcinoma(32.8%).[8] The other uncommon malignancies that can arise in a setting of pleomorphic adenoma are adenosquamous carcinoma, adenoid cystic carcinoma, undifferentiated carcinoma, myoepithelial carcinoma, epithelial-myoepithelial carcinoma and sarcomatoid carcinoma. Mucoepidermoid carcinoma arising in a pleomorphic is a rare event.[9] On cytology, it may show features of non-specific high grade malignancy as noted in our case. Presence of mucin producing cells in addition can support a diagnosis of mucoepidermoid carcinoma. Although mucin producing cells were appreciated on histopathological sections of our case, it was not evident on cytological smears and hence due to lack of specific findings only a diagnosis of malignancy owing in a background of pleomorphic alenona was offered in cytology. Our inability to provide a precise cyto-diagnosis as mucoepidermoid carcinoma was due to lack of specific cytomorphological features on cytosmears.

A careful scrutiny of all smears helped us in making a cytodiagnosis of malignant change in a background of pleomorphic adenoma. This vital information is necessary for the surgeon to plan out the therapeutic modalities for a better outcome.

Considering the polymorphic nature of some salivary gland tumours, it is advisable to aspirate from more areas to render the correct cytodiagnosis. Aspiration from multiple sites is mandatory in salivary gland lesions clinically suspicious of malignancy.

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