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Evaluation of Lumber Lordotic Angle in Patients with Inter Vertebral Disc Prolapse using Cobb's Method

By Caroline Edward Ayad, Doaa Mohammed Abd-Alsaid Wahby, Elsafi Ahmed Abdalla & Samih Awad Kajoak

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Abstract - This study was done to evaluate the lumbar lordotic angle (LLA) in patients with inter vertebral disc prolapse examined by magnetic resonance imaging (MRI) using Cobb's method.

This study was conducted at Antalya Medical center and Elnilin Medical Center and extended from November 2012 up to March 2013.

Total sample of 62 Sudanese subjects were included in the study, with ages ranging between (24-80 years), 50 of the total sample were 25 males and 25 females underwent MR lumbar scan and were diagnosed to have inter vertebral disc prolapse at different vertebral levels, the remaining 12 were diagnosed as normal lumbar spine and they were considered as control group.

Measurement of (LLA) was done from the mid-sagittal slice of T2 MRI lumbar spine using Cobb's method; by drawing a perpendicular line to a line drawn across the superior endplate of first lumbar vertebra and the superior endplate of first sacral vertebra; the angle formed by the intersection of the two perpendicular lines is the Cobb angle or lumbar lordotic angle.

Keywords : *cobb, inter vertebral disc, mri.*

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Evaluation of Lumber Lordotic Angle in Patients with Inter Vertebral Disc Prolapse using Cobb's Method

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The Cobb angle and inter vertebral disc prolapse level were then correlated with Gender, age, weight, height, body mass index (BMI) and jobs to demonstrate if there is any degree of association.

The study concluded that Cobb angle and Disc prolapse levels have no significant relation with job, height, weight, age and BMI, no significant difference was detected between Cobb angle of the normal subjects and patients with prolapsed disc and the results did not differ among male and female patients.

Using MRI in the detection of vertebral morphological changes and end plates degeneration is recommended since it involves no ionizing radiation and has excellent demarcation of disc prolapse. The dependence upon the Cobb angle in diagnoses of disc prolapse is of no significant value.

Keywords: *cobb, inter vertebral disc, MRI.*

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I. Introduction

The spine is an elastic rod structure, loading of the spine leads to its deformity, strengthening its physiological S-shaped lordosis and kyphosis [1–3].During loading; the disk becomes dehydrated causing the accompanying ligaments to become loosened, the disk-height is reduced and the spine loses its homogenous elasticity. In turn, localized overloading of the disk and subchondral spinal endplates may take place.

In the last few decades, MRI has become the standard imaging method; it allows direct visualization of the disk and because of its high water content, the nucleus pulposus is bright on T2-weighted images. With aging and degeneration, the size, character and height of the disk decline continuously [4, 5]

MRI can diagnose degenerative changes within the bony endplates. This border region is damaged during overloading. This results probably in pain and activation of fibrovascular tissue ending in neovascularization of the disk, particularly at the anterior and posterior part [1, 6] producing degeneration. The endplate fails before the injured annulus fails. Endplate failure seems to be the precursor to disk degeneration, which means they are correlated to each others.

The first signs of degeneration may be localized malalignments with or without rotation of the vertebral body. The evaluation of lumber attitude is commonly assessed to help guide diagnosis and plan treatment [7,8]During an examination of spinal posture; lumbar lordosis should be assessed. It has been suggested that its deviation may increase a person's risk of developing low back pain [9, 10, 11]

Lumbar lordosis is defined as the curvature assumed by the intact lumbar spine to compensate for the inclination of the sacrum, restore an upward spinal orientation, and consequently avoid a forward inclination. Its measure, is influenced by various parameters, including age, gender, pelvic bend, and thoracic curvature, among others [12, 13].

Value of sagittal curves measurements on spine; present great variability in normal individuals with a wide variation range for those, within normality limits.

That great measurements variation must be considered as physiological, indicative, but not normative [14]

Several different methods are used to measure lumber lordosis including Centroid, Cobb, Trall, and Harrison posterior tangent method. Cobb's method is commonly used for curvature analysis on lateral lumber radiograph, whereas the Centroid, Trall, and Harrison posterior tangent method are not widely used [15]

Normal lordosis may range from 31 to 50 according to Cobb's method. The Cobb technique based on measurement of vertebral endplates is the method most frequently adopted for clinical diagnosis. However, because of the variation in the vertebral endplate architecture, the vertebral surface angle is difficult to identify. In this method, the angle of interception sustained by the most tilted upper and lower vertebrae of the lumber curvature is measured [16]

To our knowledge, no reliable measurements were done to the lumber lordotic curve for Sudanese patients in the open literature which may aid in the early diagnosis and management of spine conditions before irreversible neurologic change ensues.

So this study is to evaluate lumbar lordotic angle by magnetic resonance imaging using Cobb's method in patients with inter vertebral disc prolapse. To determine the effect of inter vertebral disc prolapse on the lumber lordotic curve as well as to investigate whether the angle changes according to age, weight, height, BMI and job for Sudanese.

II. Materials and Methods

The study was carried out during the period from November 2012 up to March 2013 in Antalya Medical Center and Elnilin Medical Center.

a) Study population

Total sample of 62 subjects were included in the study, the average age ranging between (24-80 years), 50 of the total sample were 25 males and 25 females underwent MR lumbar scan and diagnosed with inter vertebral disc prolapse, the remaining 12 were diagnosed as normal lumbar spine MRI and they are the control group.

b) Machines used

General Electric. Signa. HD. 1.5 tesla MRI machine in Antalya medical center, Semiens. Magnetom. CI. 0.35tesla. Open MRI machine in Elnilin Medical Center.

III. Lumbar mri Technique

Axial and sagittal slices of lumbar spine obtained with T2 weighted images while the patients in supine position with their knees elevated over a foam bad , the patient positioned so that the longitudinal alignment light lies in the midline, and the horizontal alignment light passes just below the lower costal margin.

a) Image interpretation

Measurement of lumbar lordotic angle (LLA) was done from the mid-sagittal slice of lumbar spine MRI using Cobb's method. By drawing a perpendicular line to a line drawn across the superior endplate of (L1) and the superior endplate of (S1); the angle formed by the intersection of the two perpendicular lines is the Cobb angle.

IV. The study Variables

The mean of the angles was correlated with variables which are: age, height, weight, body mass index (BMI), job, and the level of inter vertebral disc prolapsed. The data were analyzed through the statistical method (SPSS programme) version 16.0 and included frequency tables, percentages, correlations, cross tabulation. P-Value is significant at 0.05.

V. Results

The Following tables and figures presented the data obtained from 25 males and 25 females came to MRI department for lumbar spine examination as they all were complaining of Lower backache, the Cobb angle was measured to study the relations regarding the Cobb angle variations.

Table 1 : The age classes and frequency of patients affected with disc prolapse

Age Classes Male	Frequency And percentage	Age Classes Female	Frequency And percentage	Age Classes Total Sample	Frequency And percentage
24-34	4(16%)	27-35	3(12%)	24-34	7(14%)
35-45	9(36%)	36-44	6(24%)	35-45	17(34%)
46-56	7(28%)	45-53	5(20%)	46-56	12(24%)
57-67	3(12%)	54-62	8(32%)	57-67	10(20%)
68-78	1(4%)	>62	3(12%)	68-78	3(6%)
>78	1(4%)	-	-	>78	1(2%)
Total	25(100%)	-	25(100%)	-	50(100%)

Table 2 : The mean and standard deviation of the Variables according to gender

Variables	Age	Weight	Height	BMI	Cobb Angle*
Male	46.96±12.8	74.88±12.2	171.32±8.4	25.50±3.79	36.9±10.84
Female	48.71±7.5	73.44±16.87	160±6.7	28.53±5.1	40.8±8.80
Total	48.4±12.4 *Min:24.0* *Max:82.0*	74.12±5.4 *Min:55.0* *Max:127.0*	165.64±9.5 *Min:145.0* *Max:198.0*	27.02±4.7 *Min:19.7* *Max:41.5*	38.8±9.96 *Min:20.0* *Max:60.0*

BMI = Body Mass Index, *Min*=Minimum, *Max*=Maximum.* Cobb's angle in the cases of disc prolapsed and Gender (P-value = 0.172)

Table 3 : Association between Diagnosis /vertebral disc prolapse level & Gender (P-value =0.614)

Diagnosis/ *intervertebral disc prolapse level*	Gender		Total
	Male	Female	
L2,L3	1.0(4.0%)	0.0(0.0%)	1.0(2.0%)
L3,L4	1.0(4.0%)	2.0(8.0%)	3.0(6.0%)
L4,L5	7.0(28.0%)	9.0(36.0%)	16.0(32.0%)
L5,S1	4.0(16.0%)	5.0(20.0%)	9.0(18.0%)
L1,L2,L3	1.0(4.0%)	0.0(0.0%)	1.0(2.0%)
L3,L4,L5	1.0(4.0%)	1.0(4.0%)	2.0(4.0%)
L4,L5,S1	9.0(36.0%)	6.0(24.0%)	15.0(30.0%)
L2,L3,L4,L5	0.0(0.0%)	2.0(8.0%)	2.0(4.0%)
L3,L4,L5,S1	1.0(4.0%)	0.0(0.0%)	1.0(2.0%)
Total	25.0(100.0%)	25.0(100.0%)	50.0(100.0%)

Diagnosis stands for all cases examined by MRI and diagnosed to have intervertebral disc prolapse at different levels by the expertise Radiologist.

Table 4 : Association between Diagnosis /intervertebral disc prolapse level and patients demographic data.

Diagnosis/ *intervertebral disc prolapse level*	Age	Weight	Height	BMI	Cobb's angle
L2,L3	43.0 ± 0.0	66.0 ± 0.0	167.0 ± 0.0	23.7 ± 0.0	38.0 ± 0.0
L3,L4	50.7 ± 17.5	61.7 ± 11.5	159.7 ± 16.8	24.2 ± 1.8	30.0 ± 8.0
L4,L5	52.9 ± 11.0	76.3 ± 18.3	167.8 ± 12.8	27.1 ± 5.8	41.6 ± 10.0
L5,S1	40.4 ± 11.5	83.0 ± 15.8	166.2 ± 8.8	30.0 ± 5.0	38.9 ± 9.4
L1,L2,L3	37.0 ± 0.0	73.0 ± 0.0	164.0 ± 0.0	27.1 ± 0.0	31.0 ± 0.0
L3,L4,L5	59.5 ± 0.7	62.5 ± 10.6	165.5 ± 6.4	22.7 ± 2.1	37.5 ± 6.4
L4,L5,S1	48.0 ± 13.4	69.6 ± 8.1	164.6 ± 5.7	25.8 ± 3.4	38.0 ± 10.8
L2,L3,L4,L5	47.0 ± 12.7	81.0 ± 1.4	160.0 ± 0.0	31.7 ± 0.5	48.5 ± 0.7
L3,L4,L5,S1	42.0 ± 0.0	85.0 ± 0.0	171.0 ± 0.0	29.0 ± 0.0	23.5 ± 0.0
P-value	.360	.272	.930	.270	.385

Values are express as Mean ± SD

Table 5 : Correlation between Cobb's angle & Variables

Cobb's angle	Age	Weight	Height	BMI
Correlation Coefficient	.147	.121	-.076	.182
P-value	.309	.402	.599	.206

Table 6 : Cobb's angle & Occupation (P-value = 0.439)

Occupation	Mean ± SD
Employee	37.0 ± 12.8
Worker	37.6 ± 9.2
Unemployed	41.0 ± 8.3

Table 7 : Association between Diagnosis/ vertebral disc prolapse Level & Occupation (P-value =0.244)

Diagnosis/ *intervertebral disc prolapse level*	Occupation			Total
	Employee	Worker	Unemployed	
L2,L3	0.0(0.0%)	1.0(6.3%)	0.0(0.0%)	1.0(2.0%)
L3,L4	0.0(0.0%)	1.0(6.3%)	2.0(10.0%)	3.0(6.0%)
L4,L5	5.0(35.7%)	3.0(18.8%)	8.0(40.0%)	16.0(32.0%)
L5,S1	1.0(7.1%)	3.0(18.8%)	5.0(25.0%)	9.0(18.0%)
L1,L2,L3	1.0(7.1%)	0.0(0.0%)	0.0(0.0%)	1.0(2.0%)
L3,L4,L5	1.0(7.1%)	0.0(0.0%)	1.0(5.0%)	2.0(4.0%)
L4,L5,S1	6.0(42.9%)	7.0(43.8%)	2.0(10.0%)	15.0(30.0%)
L2,L3,L4,L5	0.0(0.0%)	0.0(0.0%)	2.0(10.0%)	2.0(4.0%)
L3,L4,L5,S1	0.0(0.0%)	1.0(6.3%)	0.0(0.0%)	1.0(2.0%)
Total	**14.0(100.0%)**	**16.0(100.0%)**	**20.0(100.0%)**	**50.0(100.0%)**

Table 8 : Correlation between Cobb angle in cases with inter vertebral disc prolapsed and Control Group.

Correlations			
		Cobb angle in cases with inter vertebral disc prolapse	*Cobb angle in the Control Group*
Cobb angle in cases with inter vertebral disc prolapse	Pearson Correlation	1	-.132-
	Sig. (2-tailed)		.683
	N	50	12
Cobb angle in Control Group	Pearson Correlation	-.132-	1
	Sig. (2-tailed)	.683	
	N	12	12

VI. Discussion

50 patients were examined by MRI, (25 males and 25 females), their ages ranged between 24-80 years old as seen in table [1], all were complaining of Lower backache and were diagnosed to have intervertebral disc prolapsed at different levels. The males and the females mean age, weight, height; BMI and Cobb angle were presented in table [2]

The mean Cobb angle was measured from the superior end plate of L1 to the superior end plate of S1, The level where the disc prolapse was taken place had been evaluated, and the mean Cobb angle was found to be 38.8±9.96. For the female patients the mean Cobb angle was 40.8±8.80, where the mean Cobb angle for male patients was 36.9±10.84. It is higher in female than male but the difference is not significant, similar findings was found by [17] The disc prolapse may affect one or more inter vertebral disc, the study showed that the most affected level was between L4 and L5 in both gender as presented in table [3]

The largest Cobb angle was found when the level of disc prolapse affected more than three vertebral disc at the level of (L2 L3, L4, L5) where the higher mean age of the patients affected with disc prolapse was found at the level of (L3, L4, L5) and it was found to be greater than the other above or below levels. But the Cobb angle was neither correlated significantly with the patient age nor the level of prolapse (p-value=90.385, 0.360) respectively [table4], reverse results were found

by Ghassan [17] who had mentioned that the age can be predictors of the level of lumbar disc herniation.

The association between the levels of inter vertebral disc prolapse with weight, height, BMI was found to be insignificant at P-value, 0.272, 0.930, 0.270 correspondingly as presented in table [4]

Cobb angle in cases with intervertebral disc prolapse was found to have insignificant relation with the Sudanese patients characters including age, weight, height and BMI at P value (0.309, 0.402, 0.599, 0.206), in respectively as seen in table [5] but different findings were found by Khodadad et al who found that obesity, gender, body mass index have significant effects on low back pain and lumbar total and segmental lordosis[18]

According to the job classification, the largest Cobb angle was found in the unemployed patients followed by the workers then the employee as presented in table [6]. Our study showed that Lumbar lordosis Cobb angle has insignificant correlation (P-value=0.439)with the job as Sudanese may do different work load related physical activity in their residence, It is postulated that lifestyle might cause lower back pain and may affected the lumbar lordosis angle [19]

From table [7] there is no association (P-value =0.244) with the different level of inter vertebral disc prolapse and the patients jobs.

Different results were found in the Cobb angle difference related gender and age; Amonoo-Kuofi [20], Guigui et al. [21], Gellb et al [22] and Damasceno et al[14]

By testing the correlation between Cobb angles in cases with inter vertebral disc prolapse and the control group as presented in table [8]; the study showed that there was no significant difference between Cobb angle measured in patients with inter vertebral disc prolapse and the control group.

MRI is a valuable tool to demonstrate the vertebral body end plate borders which have value in applying the Cobb method as well as to diagnose inter vertebral disc prolapse at the same level of measurement. The study concluded that Cobb angle has no significant relation with height, weight, age and BMI. Disc prolapse levels have no association with, work intensity, age, weight, height, BMI and Cobb angle.

The study recommend to use MRI in detecting and monitoring vertebral morphological changes and end plates degeneration since it involve no ionizing radiation and has excellent demarcation of disc prolapse. More studies are needed in this area with bigger sample to determine the normal range of lumbar lordotic angle in normal Sudanese individuals.

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Trichrome Stain for Diagnosis of Amoebae in Parasitology Laboratory

By Daissy J Vargas Sepúlveda
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Abstract - For many years the trichrome staining technique (TricrómicWheatley) has been considered as the most important technique for the identification ofthe most common intestinal protozoa and popularin parasitology (1).

Currently the mostsensiblidad procedure for detecting and identifying protozoa trophozoites stool sample as it helps to easily highlight the morphology of amoebic cysts and trop-hozoites however, the procedure is complicated and tediousto perform and require at seven different reagents which is probably the most critical especially in laboratories with limited staff, this makes it complicated the routine use of this technique in most of the clinical laboratory, using koplic additionally facilitates reagent contamination by repeated use.(4,5)

Keywords : *tricrómicwheatley, diagnosis of amoebae.*

GJMR-K Classification : *NLMC Code: WF 141*

Strictly as per the compliance and regulations of:

Trichrome Stain for Diagnosis of Amoebae in Parasitology Laboratory

Coloración Tricromica Para El Diagnostico De Amebas En El Laboratorio De Parasitologiaclinica

DaissyJ Vargas Sepúlveda

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Resumen- Por muchos años la técnica de coloración tricromía (TricrómicWheatley) ha sido considerada como la técnica más importante para la identificación de protozoos intestinales la más común y popular en parasitología (1).

Actualmente es el procedimiento con mayor sensiblidad para detectar e identificar trofozoitos de protozoarios en muestra de materia fecal ya que ayuda a evidenciar fácilmente la morfología de quistes y trofozoitos de amebas sin embargo, el procedimiento es complicado y tedioso de realizar y requiere siete reactivos diferentes lo cual es probablemente lo mas critico especialmente en laboratorios con personal limitado, esto hace que sea complicado el uso rutinario de esta técnica en la mayor parte de los laboratorio clínicos, adicionalmente el uso de koplic facilita la cont- aminación de los reactivos por el uso repetido. (4, 5)

Palabras clave: coloración tricromica, diagnostico de amebas.

I. Introduction

The main purpose of this study was to evaluatea new method for obtaining atrichromic staining faster and effective for it used the samedyesand proce-ededim plementing two different technical koplicone with and one with direct drops of reagent in the lamina.

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There were 20 positive smearsall parasites and made several technical modifications in order to simplify and expedite the procedure equally maintaining the excellent staining qualities, the nimplemented the steps mentioned in the original technique and then the technique modified.

Original Technical Steps

Wheatley's Modification of the Gomori Trichrome stain

1. Schaudinn 30 minutes
2. 70% Ethanol 5 minutes.
3. Place slide in70% ethanol iodado al 1 minute.
4. Place slide in 70% ethanol for 5 minutes
5. Place slidein 70% ethanol for 3 minutes in other Koplic.
6. Trichrome stain 10 minutes
7. Destain in 90% ethanol y acétic acid por 1 a 3 seconds
8. Rinse several times in 100% ethanol
9. Place in two changes of 100% ethanol for 3 minutes each
10. Place in two changes of xylene or xylene substitute for 10 minutes
11. Mount with coverslip using mounting medium (e.g., permount).
12. Examine the smear microscopically utilizing the 100× objective. Examine at least 200 to 300 oil immersion fields

Step in the technique modified

1. Perform aslidesheetth in extended Let dryat room temperature
2. Add saturated mercury chloride for 20 minutes
3. Add Trichrome stain + alcohol 96% for 10 minutes
4. Wash and add 96% ethanol 4 minutes
5. Examine the smear microscopically utilizing the 100×objective.

Recommendations for modified technique

1. The most important step for aisgood results is the fixation of the sample, because if not set the samplemay shrink orprotozoamaytakean abnormal color identification difficult.

2. The smear should not be too thick to facilitate identification of cysts and trophozoites.
3. You must completely remove mercury or Schaudin in the first step of coloring because if left too much, it tends to form crystalsor granules that can prevent the identification of any organism.
4. After adding trichrome bleaching should be performed in a short timeasmay appear washed staining is likely due to excessive discolouration.
5. May. And the final stage of dehydration with 100% ethanol should be as free of water as possible to avoid both the reactive evaporation of moisture absorption as that can preventeasy identification of the parasite.(2)

Note: formalin fixed Fecal samples are suitable for this dyeing process

a) Important considerations

The fund continues to see green and cytoplasm of protozoa is stained a blue green and purple. There dnuclei with inclusions purple and intracellular structures are easy to distinguish as glycogen vacuoles are the Iodamoeba butschlii. (6)

Experimental development

Validation of the art Stian Modified Trichrome in Cmd Siplas

Table 1 : Compared observer 1 and observer 2 with the modified technique

NUMBER SAMPLE	RESULT OBSERVER 1	RESULT OBSERVER 2	COMMENTS
250529	Cysts *Endolimax nana* ++	Cysts *Endolimax nana* ++	Agreement in identifying parasitic forms
252022	Cysts *Entamoeba coli* Cysts *Blastocystis Hominis*	Cysts *Entamoeba coli* Cysts *Blastocystis Hominis*	Agreement in identifying parasitic forms
252029	Yeasts ++	Yeasts ++	Agreement in identifying parasitic forms
253555	Cysts *Entamoeba coli* +	Cysts *Entamoeba coli* +	Agreement in identifying parasitic forms
253912	Cysts *Blastocystis Hominis*+	Cysts *Blastocystis Hominis*+	Agreement in identifying parasitic forms
255717	Cysts *Blastocystis Hominis*+	Cysts *Blastocystis Hominis*	Agreement in identifying parasitic forms
256920	Cysts *Endolimax nana*	Cysts *Endolimax nana*	Agreement in identifying parasitic forms
257583	Cysts *Endolimax nana* escasos	Cysts *Endolimax nana* +	Agreement in identifying parasitic forms
259110	Cysts *Blastocystis Hominis* +	Cysts *Blastocystis Hominis* +	Agreement in identifying parasitic forms
259209	Cysts *Blastocystis Hominis*++	Cysts *Blastocystis Hominis*++	Agreement in identifying parasitic forms
261161	Cysts *Blastocystis Hominis*++	Cysts *Blastocystis Hominis*+	Agreement in identifying parasitic forms
254021	Cysts Entamoeba coli ++	Cysts Entamoeba coli ++	Agreement in identifying parasitic forms
266114	Cysts Iodamoeba Butschlii+	Cysts *Iodamoeba Butschlii*+	Agreement in identifying parasitic forms
264223	Cysts *Endolimax nana* +	Cysts *Endolimax nana* + Cysts de *Blastocystis Hominis* escasos	Agreement in identifying parasitic forms
264532	Parasitic structuresare not observedin the sample	Parasitic structuresare not observed in the sample	Agreement in identifying parasitic forms

269688	Cysts Entamoeba hysto-litica/dispar ++	Cysts Entamoeba hysto-litica/dispar ++	Agreement in identifying parasitic forms
264746	Cysts *Endolimax nana* ++	Cysts *Endolimax nana* +	Agreement in identifying parasitic forms
264939	Leukocytes++	Leukocytes ++	Agreement in identifying parasitic forms
p- 03	Cysts Giardiaspp +	Cysts Giardiaspp +	Agreement in identifying parasitic forms

Observer 1: DAISSY VARGASS, Bacteriologyst CMDSIPLAS.
Observer 2: YENY BALLESTEROS,Bacteriologyst CMDSIPLAS.

II. Analysis of Results

a) Parasitic kappa index identification forms

		OBSERVER 1	
	-	concordance in identifying parasitic forms	negative
OBSERVADOR 2	concordance in identifying parasitic forms	16	0
	negative	0	1
	17	16	1
	%Sensitivity	100,0	
	%Specificity	100,0	

TABLE DE 2*2		
	Reference Reagent	No reference Reagent
Reagent to validate	Vp	Fp
No Reagent to validate	FN	VN
	Vp+FN	VN+Fp
Sensitivity	Vp/(Vp+FN)=True positives	
Specificity	VN/(VN+Fp)=True Negatives	

		Acceptability
ÍNDICE KAPPA	1,00	DIAGNOSTICACCURACYIS NOTED
VPP (%)	100,0	
VNP (%)	100,0	
Total positives	16	
Total Negatives	1	

ÍNDICE KAPPA	
Pe	0,886
Po	1,00

b) Kappa index leukocytes

	OBSERVER 1		
	-	concordance in identification	negative
OBSERVADOR 2	concordance in identifying parasitic forms	1	0
	negative	0	16
	17	1	16
	%Sensitivity	100,0	
	%Specificity	100,0	

TABLA DE 2*2		
	Reference Reagent	No reference Reagent
Reagent to validate	Vp	Fp
No Reagent to validate	FN	VN
	Vp+FN	VN+Fp
Sensitivity	Vp/(Vp+FN)=True positives	
Specificity	VN/(VN+Fp)=True Negatives	

	Aceptability
ÍNDICE KAPPA	1,00
VPP (%)	100,0
VNP (%)	100,0
Total positives	1
Total Negatives	16

ÍNDICE KAPPA	
Pe	0,003
Po	1,00

c) Kappa index yeast

	OBSERVER 1		
	-	concordance in identification in yeast	negative
OBSERVADOR 2	concordance in identifying parasitic forms	1	0
	negative	0	16
	17	1	16
	%Sensitivity	100,0	
	%Specificity	100,0	

TABLA DE 2*2		
	Reference Reagent	No reference Reagent
Reagent to validate	Vp	Fp
No Reagent to validate	FN	VN
	Vp+FN	VN+Fp
Sensitivity	Vp/(Vp+FN)=True positives	
Specificity	VN/(VN+Fp)=True Negatives	

	CRITERIO DE ACEPTABILIDAD	CRITERIO DE ACEPTABILIDAD
ÍNDICE KAPPA	**1,00**	DIAGNOSTICACCURACYIS NOTED
VPP (%)	100,0	
VNP (%)	100,0	
Total Positives	1	
Total Negatives	16	

ÍNDICE KAPPA	
Pe	0,003
Po	1,00

Kappa: the agreement between observers for the identification of parasitic forms, leukocytes, yeasts, and negative for themis 1.0, which shows diagnostic accuracy and level of agreement between observers for the samples with the latest changes made by SIPLAS medical laboratory, concluding that the changes mentioned here allow adequate identification of both parasite forms leukocytes, yeast and other fungal forms structures that allow the definition diagnosed patients, ensuring diagnostic accuracy versus the clinical definition

kappa	Degree of agreement
< 0	without agreement
0 – 0.2	insignificant
0.2 – 0.4	low
0.4 – 0.6	moderate
0.6 – 0.8	good
0.8 – 1	very good

III. Sensitivity and Specificity

The sensitivity and specificity of the samples analyzed for fungal structures, yeast and parasiticl-eucoidesis 100%, which shows that the stain can classify patients according to the irpositive or negative real state against it sclinical definition

a) Parasitic forms identification with modified technique

Micrographs of amoebae obtained modified tech-nique implemented

Figure 1 : Cysts Blastocystis hominis

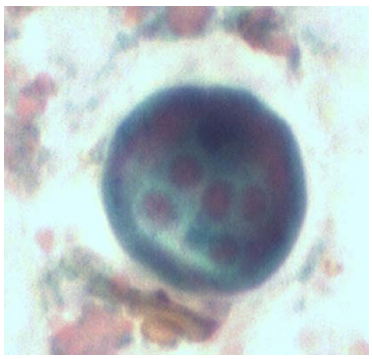

Figure 2 : Cysts Entamoeba coli

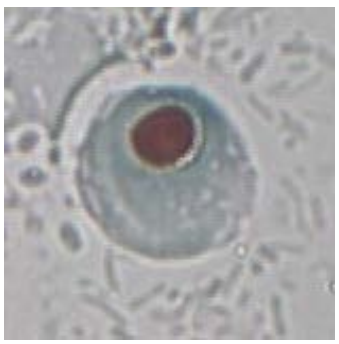

Figure 3 : Cysts Iodamoeba butschlii

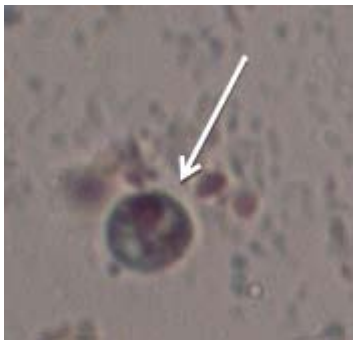

Figure 4 : Cysts Endolimax nana

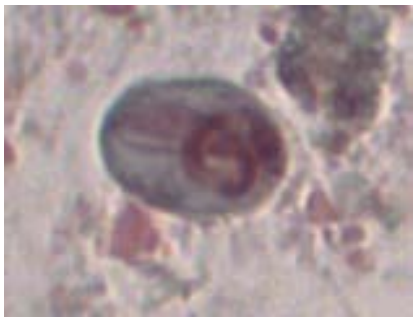

Figure 5 : Cysts Giardia Duodenalis

Note: The photomicrographs were taken by the bacteriologys Daissy Vargas Sepulveda in CMD SIPLAS

IV. Conclusions

- The quick method is effective and accurate.
- It requires less processing time and therefore the patient must wait less time to get your results
- Iteliminates contamination of the reagents considering that it is not necessary to use Koplic
- It saves time and money by having as only three reagent required to implement this technique

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Determining Injury Severity Amongst Victims of Motorcycle Accidents in Sokoto, North West Nigeria

By M Oboirien, O Adegbala, P S Agbo & B K Adedeji
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Abstract - *Background:* Injuries from motorcycle accidents are a major contributor to mortality and morbidity in Nigeria. Injuries are varied amongst victim from bruises to severe head injuries. We therefore sought to determine the degree of severity of injuries sustained by motorcycle accident victims in our environment.

Methodology: A prospective study of motorcycle accident victims including riders, passengers, and pedestrians was undertaken over a one year period from January 2012 to December 2012 at the trauma Centre of a tertiary hospital in Sokoto, North-West Nigeria. Information obtained from the trauma register included the age and sex of victims, use of protective helmet, and nature of collision, injury severity, determined by the injury severity scores and outcome.

Keywords : *injury; severity; score; motorcycle; victims.*

GJMR-K Classification : *NLMC Code: WA 250*

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Determining Injury Severity Amongst Victims of Motorcycle Accidents in Sokoto, North West Nigeria

M Oboirien [α], O Adegbala [σ], P S Agbo [ρ] & B K Adedeji [ω]

Abstract- Background: Injuries from motorcycle accidents are a major contributor to mortality and morbidity in Nigeria. Injuries are varied amongst victim from bruises to severe head injuries. We therefore sought to determine the degree of severity of injuries sustained by motorcycle accident victims in our environment.

Methodology: A prospective study of motorcycle accident victims including riders, passengers, and pedestrians was undertaken over a one year period from January 2012 to December 2012 at the trauma Centre of a tertiary hospital in Sokoto, North-West Nigeria. Information obtained from the trauma register included the age and sex of victims, use of protective helmet, and nature of collision, injury severity, determined by the injury severity scores and outcome.

Results: A total of 803 victims of motorcycle accidents were seen over the 12 months period with majority of victims in the age range of 21-30 years representing 44.3%. Most of the accidents occurred in the months of October 13.8%, May 13.2%, June 11.7% and April 11.0 %. Fifty-five per cent of victims were the riders while passengers constituted 34.8%. The nature of accident was such that 43.6% of victims were as a result of motorcycle-motorcycle collision while 24.2% of victims were of lone motorcycle accidents. Victims from motorcycle-car collision were 21.9 %. Eighty-one per cent of victims had injury severity score (ISS) of 4 and below while the highest ISS of 29 was seen in one victim. Eighty four per cent of victims were treated and discharge while nine victims died. Twelve per cent of victims were admitted for further management while 3% signed and left against medical advice.

Conclusion: The severity of injuries in motorcycle accidents victims though low, measures are however needed to reduce and regulate the use of motorcycle. This can be done through licensing, education and enforcement of use of helmet.

Keywords: injury; severity; score; motorcycle; victims.

I. Introduction

The motorcycle has evolved over the years as a means of transportation. In developing countries it serves as a mode of commercial transportation ferrying passengers from place to place. Their operators are mainly youths who are untrained and sometimes drive under the influence of alcohol. Safety and security concerns have been raised by stakeholders and this has led to the total ban of motorcycles as mode of transportations in some major Nigerian cities like Lagos and Calabar. The incidence of motorcycle accident i cities like Lagos and calabar is as high as 27% 1,2. In Sokoto metropolis, motorcycle accidents constituted 40% of road traffic accidents in 2009 3.

In the United States available statistics indicates that motorcyclist are 35 times more likely to experience a deadly accident on the road than those in passenger car and 11% of all road accidents involves motorcycles 4. The vulnerability of users of motorcycle as means of commercial transportation makes them a target audience in injury prevention strategies 5, 6, 7.

The pattern of injuries resulting from motorcycle accidents is varied with musculoskeletal and head injuries being the most common. Sokoto is a cosmopolitan city in Northwestern Nigeria and has a population of 427,760 as of the 2006 census. Sokoto lacks a public transport system and it is easier to commute by the aid of a motorcycle as is the case in some Nigerian cities. Patronage of commercial motorcyclist cuts across social economic status as some do use them to navigate the poor road network and sometimes to beat traffic. Stakeholders in road safety have raised concerns about the safety of this mode of transportation. Efforts at enforcing safety measures through the use of crash helmets have been largely unsuccessful. It is against this background we sought to determine the injury severity in victims of motorcycle accidents in Sokoto, North Western Nigeria.

II. Methodology

This was a prospective descriptive study of victims of motorcycle accidents carried at the trauma Centre of a tertiary hospital in Sokoto, North-West, Nigeria over a period of one year from January 2012 to December 2012. The trauma Centre caters for patient from Sokoto metropolis, neighboring cities and states. Trauma records of victims of motorcycle accidents (MCA) were recorded in a proforma that included the age and sex, class of victim in terms of rider, passenger or pedestrians. Other information gathered was the nature of collision motorcycle-motorcycle collision (MMC), motorcycle-car collision, lone motorcycle accidents (LMA) and motorcycle- pedestrians' collision. The use of helmets amongst rider and passenger, the injury severity score and outcome were also recorded. The injury severity score (ISS) system is a process by which complex and variable patients' data was reduced

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to a single number. It is an anatomical scoring system and can be determine by the attending casualty doctor. Each injury was assigned an abbreviated injury scale (AIS) in the six body region of Head and Neck, Face, Chest, Abdomen, Extremity and External. AIS range from 1 to 5 depending on the severity of injury. Minor injuries were assign 1, moderate 2 and severe 5. The 3 most severely injured body regions have their scores squared and added together to produce the ISS. The least ISS is 1 and the highest is 75. Score reflective of injury severity include 1 to 9 as Minor, 10 to 15 as Moderate, 16 to 24 as Moderate to Severe and 25 an above as Severe to critical.

Statistical analysis was done with SPSS 17 and results presented as graphs.

III. Results

A total of 803 victims of motorcycle accidents were seen over the 12 months period with majority of victims in the age range of 21-30 years representing 44.3% follow by those within the age range of 11-20 and 31-40 with 21% and 15% respectively as shown in figure 1. Time of arrival in hospital from the accident site showed that 79.8% of victims arrived at the hospital in less than 2 hours while 20.2% arrived between 2 to 6 hours. The male to female ratio is 7:1. Most of the accidents occurred in the months of October 13.8%, May 13.2%, June 11.7% and April 11.0 % as shown in figure 2. Fifty-five per cent of victims were the riders while passengers and pedestrian constituted 34.8% and 9.8%. The nature of accident was such that 43.6% were victims of motorcycle versus motorcycle collision while 24.2% were victims of lone motorcycle accidents. Lone motorcycle collisions are crashes with stationary objects or where there is loss of control. Victims from motorcycle and car collision were 21.9 %. Motorcycle–pedestrian collision resulted in 10.3% of victims. None of the riders and passenger used protective helmet. Eighty-one per cent of victims had injury severity score ISS of 4 and below while the highest ISS of 29 was seen in one victim as shown in figure 3. Eighty four per cent of victims were treated and discharge while nine victims died. Twelve per cent of victims were admitted for further management while 3% signed and left against medical advice.

IV. Discussion

Majority of our patients were below 40 years with a peak at 21-30 years and this was similar to findings in North Central Nigeria 8. These are the productive age group in the society: and with increasing unemployment the business of commercial motor transportation becomes a ready alternative. There was a preponderance of male victims as is in some other studies 1, 5, 8.

The female victims are either passengers or pedestrian as none was a rider. The practice of commercial motorcycle transportation is usually the exclusive preserve of the men. Sociocultural and religious considerations are major determinants of economic adventures in this part of the world.

Majority of the victims arrived at the Centre within 2 hours and delays usually results from the absence of ambulance service and lack of a pre-hospital emergency preparedness plan 9. It is also not unusual to have delays from arguments among riders and drivers of commercial vehicles trying to establish who is at fault rather than see to the rescue of affected victims to hospital.

Our study noted that the highest numbers of patients were seen at the beginning and in the peak of the dry season. Rain and poor weather conditions affect outdoor activities and for motor cycle transport business, this may be far reaching. This could explain the increased numbers seen during the dry season.

Majority of the victims were the riders while most victims were from a motorcycle to motorcycle collision. This goes to show the level of training and recklessness of some of these riders. The business of commercial motorcycle transportation is an all comers affair. Riders are not licensed and they do not have regards for road signs. In a study in Ilorin, North central, Nigeria, motorcycle- vehicle collision produced the largest amount of victims 10. The number of victims was almost twice as high in motorcycle-motorcycle collision as in motorcycle- vehicle collision in our study. This may be an indication that the numbers of motorcycle plying the roads are high. This may also be an indication of the level of impatience associated with some of these riders. Reasons adduced for the high number of motorcycles on our roads include cheaper cost of acquisition compared to cars, increasing fuel cost and sometimes these motorcycles are given out to youths as gratifications by politicians. Other reasons are that most of our cities lack good road network and public transport system and for the inpatient individuals the motorcycles serves as a means of navigating traffic 10, 11, 12.

Another worrisome trend is the number of victims from lone motorcycle accidents. From the mechanism described earlier it shows that some of these riders are either under the influence of drugs or they are too reckless or they are not trained. Studies in Zaria, a city about 450 km South-east from our study city indicated a high prevalence (59.5%) of use of psychoactive drugs amongst motorcycle riders involved road traffic accidents13.

The use of helmet has been a difficult safety measure to enforce in most Nigerian cities especially in the northern part where cultural and religious reasons have been adduced for their non-compliance. While the

use of helmet has found acceptance in western nations as a result of legislation, that cannot be said of developing countries. A study in Thailand showed a high number of helmet use in riders of about 60% while only 28% of passengers reported they always wore helmet 14. Reports from some Nigerian cities show the near absence of helmet use either by the riders or passengers 10, 15.

Majority of the victims had injury severity that was termed minor and this includes bruises and lacerations. About half of the victims were from a motorcycle-motorcycle collision and this could explain the severity of the injuries sustained. MMC results in low energy collision while injuries sustained from a motorcycle-car collision or from a lone crash or motorcycle-pedestrian collision are likely to be more severe because of the seemly high energy involved. In all the events, the rider and passenger are vulnerable in the crash because of their lack of protection. It should be noted cases of fatality from motorcycle accidents occurring at the scene of the accident were not included in the study, hence the injury severity that appears to be low only tells of those that were rescued to hospital.

Traumatic brain injuries (TBI) and thoracic trauma as well as abdominal injuries are common fatal injuries sustained in motorcycle accidents12, 15, 16. While these major traumatic events can be handled in developed countries with established pre-hospital care, thus reducing mortality to the least, the same cannot be said of poor and developing countries with absent pre-hospital care. Deaths from secondary brain injury and hemorrhage occur from delays in accessing health care and inappropriate triage 11, 16.

The injury severity score system was used to assess the degree of injury sustained, the limitation of this anatomic based system is that it does not reflect the seriousness of the injuries. It was however chosen because of the simplicity of scoring as various officers were involved.

Mortality was quite low and this may be a reflection of the degree of severity and the fact that a dedicated Centre is involved in the care of traumatic surgical emergencies.

V. Conclusion

The study found out that injury severity amongst victim of motorcycle accident can be categorize as minor, the large number of victims however shows that commercial motorcycle transportation is inevitable in developing society like ours. Regulation of motorcycle riders in terms of licensing, numbers, education and enforcement of use of helmet can reduce the number of motorcycle accidents.

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Figures

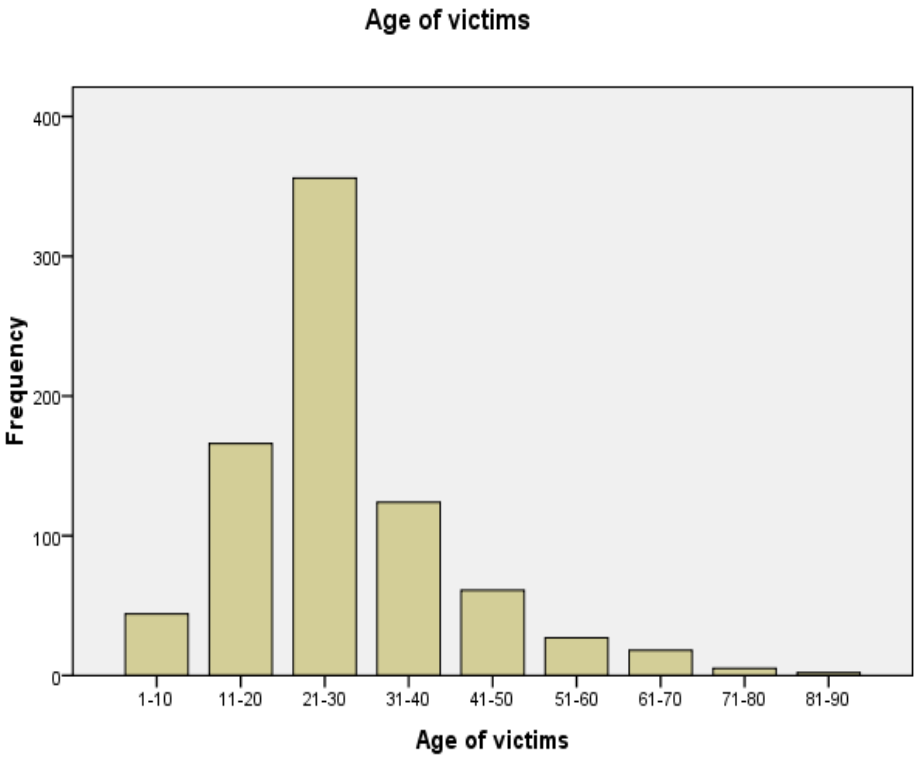


Figure 1 : Age of Victims

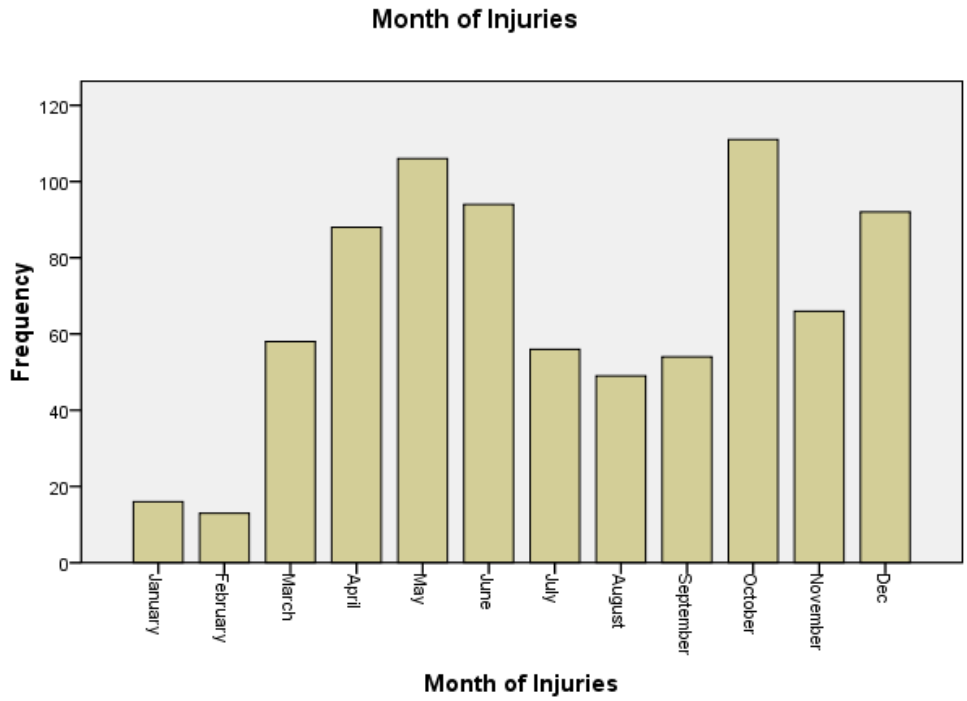


Figure 2 : Monthly Distributions of Accidents

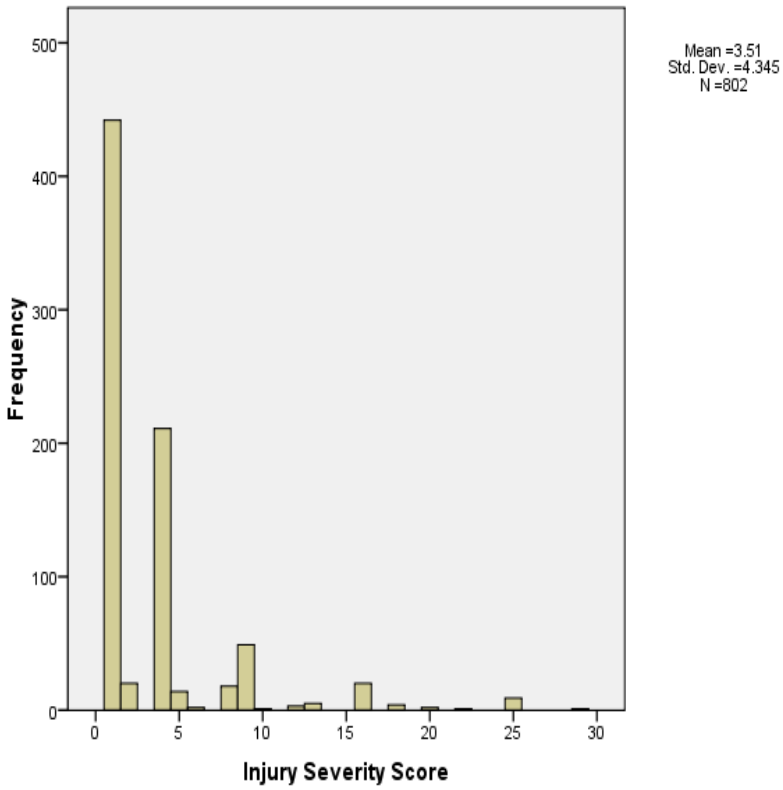
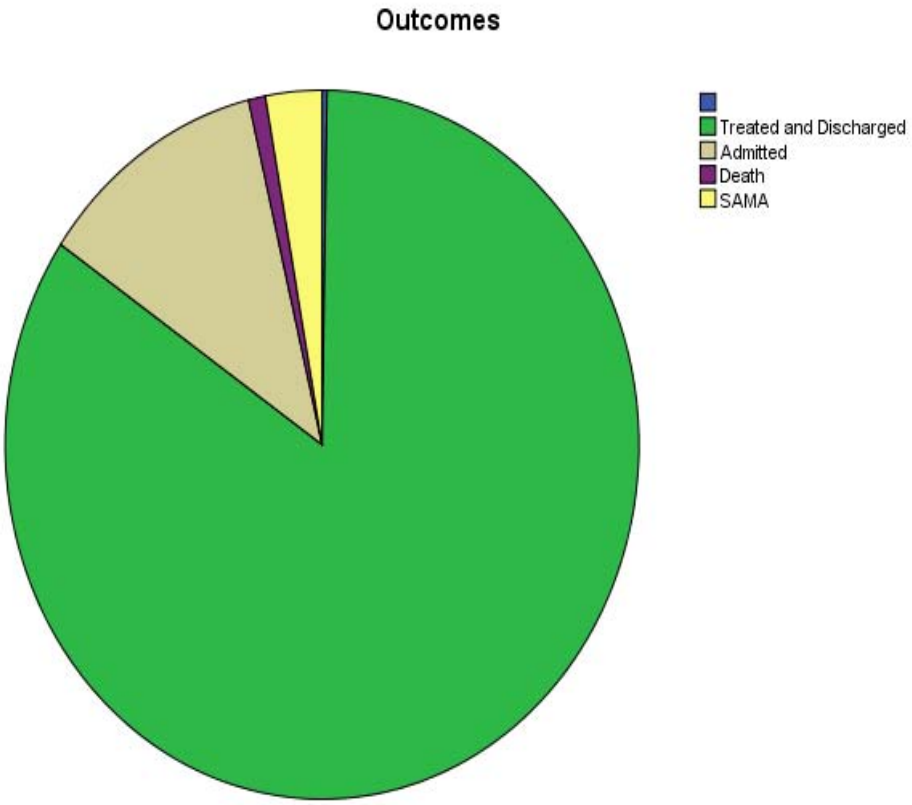


Figure 3 : Injury Severity Score of Victims of Motorcycle Accidents



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The Heating Value of a Different Location of Human Body Lipids

By Kuat Pernekulovich Oshakbayev, Kenneth Alibek, Igor Olegovich Ponomarev, Bibazhar Abayevna Dukenbayeva, Nurlybek Nurlanovich Uderbayev, Pernekul Oshakbayev & Halit Mustafin

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Abstract - *Background:* Distribution character of fats can influence to emergence of different severe life-threatens diseases.Body lipids morphology is enough well investigated, but there is little data on the calorific properties of various lipids, including atherosclerotic plaque (AP).Aim of the study was to investigate the calorific properties of a human body lipids of various anatomical sites.

Methods : Trial design is a prospective randomized pilot physical experimental trial.

Adipose tissue in the amount of 252 samples from 36 individuals (17 female sex) at autopsy. The subjects were dying from various injuries and were between 36-54 years old.

Interventions: Differential scanning calorimetry («Mettler Toledo», USA) was used with an increments temperature of 10.37 °C per minute. In an experimental set up specimens were heated up from 26.0 °C to 700.0 °C for 70.0 minutes.

Keywords : *atherosclerotic plaques,lipids, calorific value, specific heat capacity, different localization.*

GJMR-K Classification : *NLMC Code: QU 85*

Strictly as per the compliance and regulations of:

The Heating Value of a Different Location of Human Body Lipids

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Results: The heat capacity of the studied lipids decreases from AP (dense) to AP (loose), VF (omentum fat), SF (umbilical area), SF (shoulder area), SF (buttock area) and VF (pararenal fat). The dense AP (-3, 97±0,16ºC) has higher a heat capacity (p=0.02) than the loose AP (-3, 44±0,15ºC). The lowest thermal capacity has a pararenal fat of VF (-1, 25±0,21ºC) in compare with SF (buttock area) (p=0.027).

ConclusionsIn: Conclusion, the fats of a human body have different calorific properties depending on a location. Atherosclerotic plaques carry the highest energy potential in comparison to the other body lipids. The lowest thermal capacity has pararenal fats. The results of the study suggest that an atherosclerotic plaque is not an accidental phenomenon in the body, but it is a logical pathophysiological process in result of fats compaction.

Keywords: atherosclerotic plaques,lipids, calorific value, specific heat capacity, different localization.

I. Introduction

According to numerous studies an atherosclerotic plaque (AP) is the main cause of an atherosclerotic \ diseases, and it is a non-homogeneous structural formations [1, 2]. AP shave layered structure, but they always have atheromatous masses [2, 3]. It is well-known fact that the body lipids are an origin of energy andthey have a different diversity and structure both by location and function [3, 4]. The body fats are distributed in the body throughout, for instance, in subcutaneous area, in submucosa, inside and around of parenchymatous and hollow organs. They are represented in different forms, such as saturated, non-saturated, atheromatous, etc. [5, 6]. Distribution character of fats can influence to emergence of different severe life-threatens diseases [6, 7]. Therefore, it is a scientific interest to study a heat capacity (caloric value) of the different body fat depend on a place of location. Also, there are little data on studies of the calorific properties of various lipids of the body [8, 9].The aim of the study was to investigate the calorific properties of human body lipids of various anatomical sites.

II. Study Design

A prospective randomized pilot physical experimental trial in vitro.

a) Participants

Adipose tissue in the amount of 252 samples was obtained from 36 individuals (19 males, 17 females) at autopsy. The subjects had died from various injuries and were between 36-54 years old. The autopsy material (lipids) was taken for research purposes after forensic medical examinations had been carried out. The criteria used for inclusion of material in the research were:

1. sampling was performed within 2 hours after death(interval of time between death and collection);
2. tissue donors had no chronic somatic diseases (such as cardiovascular, endocrine, cancer pathologies, etc.) prior to death,and cause of death of their was road accident;
3. every Monday (after weekend) the four tissue donors were included in the study during nine weeks ofa summer season of an year (a total of 36 tissue donors).

Removal of autopsy material was performed at the Centre for Forensic Medical Examination of the city of Almaty. Tissue was collected from 7 various locations:

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visceral fat (VF), from the omentum and paranephric regions; subcutaneous fat (SF) from the buttock area, the abdomen (umbilical region), and shoulder area; APs from the descending aorta: homogeneous AP, at the stage of smooth/dense plaque (hereafter referred to as dense), and heterogeneous AP at the stage of destruction (loose plaque).

b) Research methods

Differential scanning calorimeter («Mettler Toledo», USA) was used with an increments temperature of 10.37 °C per minute. In an experimental set up specimens were heated up from 26.0 °C to 700.0 °C for 70.0 minutes. The calorific value of lipids was determined according to the heat capacities of lipids. The greater the temperature difference between the sample (sample) and a standard (reference), the more of the heat generated, thus the higher is heating value [4,8].Heating value was determined indirectly, by measuring heat capacities of organic substances.

The more a temperature difference between the sample (sample) and the standard (reference) the more a substance releases heat [9, 10].

Statistical analysis. Student's two-t-test (with Bonferroni correction,α/2) with confidence interval (CI) were used. The study data are presented in tables as mean standard error of the mean (M± SEM), and P values of <0.025 were considered significant. Statistical analysis was performed using SPSS for Windows version 17.0 (SPSS: An IBM Company, Armunk, NY) and Microsoft Excel-2010.

III. Results

The results of the study are shown in a Table 1.

Table 1 shows that lipids from various sites have different abilities to store a heat. The heat capacity of the studied different lipids decreases in a rowfrom AP (dense) to AP (loose), VF (omentum fat), SF (umbilical area), SF (shoulder area), SF (buttock area) and VF (pararenal fat). APs have the highest heat capacity among the lipids, at once the dense AP (-3, 97±0,16°C) has higher a heat capacity (p=0.02) than theloose AP (-3, 44±0,15°C). The lowest thermal capacity has a pararenal fatof VF (-1, 25±0,21°C) in compare with SF (buttock area) (p=0.027).

For a more in depth analysis of the properties of these lipids, theheat capacity values are presented in correlation with temperature dynamics. Figure 1 shows how the properties of the heat capacity of the analyzed lipids change during of the combustion process. Thecombustion process indicates the difference between the sample t°C and the reference t°C.Figure 1 clearly shows that the atherosclerotic plaques, both dense and loose are almost below zero in the scale of t °C difference between the sample and the standard. Thisunderlies an intense absorption of the heat in the calorimeter. Thatcan indicate that the APs have a relatively higher heat capacityin comparison to other lipids. For example, in contrast to APs other lipids have relatively similar combustion characteristics: they absorbs the heat actively at approximately 200 °C, and they actively releasesthe heat starting from 300 °C to 500 °C and completely burns after 600°C.

It is interesting to note that the lipids from the omentum areahave an intermediate position between the atherosclerotic plaques and the rest of the lipids from other locations. This can suggest that the omentumfat, at least according to physical parameters of the heat capacity, are close to atherosclerotic fats, and they have a high thermal capacity as the APs.

According to the Table 1 and the Figure 1 we can conclude firstly, that all lipids have the ability to store a heat. Secondly, the lipids of various locations of the body have different abilities to store a heat. Third, atherosclerotic plaques carry ahigher energy potential in compare with the rest of the body lipids. So,thedense and loose APs have the highest heat capacity. It is known that a heat capacity of substances depends on its chemical composition, structure, and biological nature [11, 12].

IV. Discussion

The fact, that different body fats have different biophysical and biochemical properties, has also been confirmed by others [13]. The study of the mRNA expression of proteins secreted by adipocytes in the subcutaneous and visceral adipose tissue in humans have shown that visceral and subcutaneous adipocytes have different properties with regard to the synthesis of bioactive molecules [14].

Fats are energy accumulators, but not all fats are the same between themselves [15]. Triglycerides containing saturated fatty acids are main energy source in the body. The harder the fat, the greater is the content of saturated fatty acids [16, 17].

Heating value of lipids according to the chemistry rules depends on the content of saturated and branched hydrocarbon chains [18].

Appearance of APs in the body precedes transient, sometimes permanent hyperlipidemia [19]. Because of a reserve capacity of the body accumulation of APs takes some years [20, 21]. Could we guessthat genesis of APsis the result of the transformation of body fats which were not used? Despite the small volume APs intrinsically possess a high heating value. Therefore, over time a certain amount of excess fat within the body is transformed into a more compact, but energy consuming lipid. Perhaps this process of increasing density of fats is adeliberate and intentional process which is required for saving body space without loss of energy resources?

The research result revealed that the lipids of a human body have different heating capacities

depending on their location where APs have had the greatest heating capacityamong of the studied lipids. Our findings can allow to look at the nature of anatherosclerosis occurrence and development is not just from the standpoint of pathology, but itis possible to tell from the position of "physiological" changes of body fats. The results of the study suggest that an atherosclerotic plaque is not an accidental phenomenon in the body, but it is a logical pathophysiological process in result of fats compaction.This point may allow to develop new treatment methods of atherosclerotic diseasesin the future.

V. Conclusion

The fats of a human body have different calorific properties depending on a location.

The lipidsof various locations of the body (dense AP, loose AP, VF from omentum fat, VF from pararenal fat, SF from umbilical area, SF from shoulder area, SF from buttock area) have different abilities to store a heat. Atherosclerotic plaques carry the highest energy potential in comparison to the other body lipids, especially the dense APs havethe highest a heat capacity. The lipids from omentum area have an intermediate position between lipids of atherosclerotic plaque and the rest lipids from other locations.The lowest thermal capacity has pararenal fats.

Competing interests: Conflicts of interest werenotdeclared by any author.

VI. Endnotes

Study limitation. Several limitations of the study deserve comment. First, the design of the present study was experimental-based, which is susceptible to selection bias. Second, the sample size was small, limiting its ability to detect significant results. Third, the physical investigations indicated only some of organic substances, and calorific value was estimated by specific heat capacity. Fourth, the heterogeneous content of organic substances in the human fats was not analyzed in the present study. Finally, it is important to mention that our study was performed on Kazakhstan citizens, and our findings may not be relevant to people of other countries.

Trial national registration: State registration # 0109RK000079, code O.0475 at the National Center for Scientific and Technical Information, the Republic of Kazakhstan.

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VII. Abbreviations

atherosclerotic plaque (AP)
visceral fat (VF)
subcutaneous fat (SF)
confidence interval (CI)
mean□standard error of the mean (M± SEM)

Table 1 : Values of overall heating capacities of lipids from various sites (the temperature difference between the sample and thereference)

	AP (dense)	AP (loose)	VF (omentum fat)	SF (in umbilical area)	SF (in shoulder area)	SF(in buttock area)	VF (pararenalfat)
Difference between sample and reference* (t °C)	-3,97 ±0,16	-3,44 ±0,15	-3,35 ±0,23	-2,87 ±0,44	-1,97 ±0,23	-1,81 ±0,19	-1,25 ±0,21
CI max	-3,65	-3,15	-2,91	-2,00	-1,23	-0,99	-0,49
CI min	-4,282	-3,73	-3,79	-3,73	-2,69	-2,63	-2,01

Abbreviations:
*,mean±standard error of the mean
AP, atherosclerotic plaque
VF, visceral fat
SF, subcutaneous fat
CI, confidence interval
toC, temperature measure in Celsius

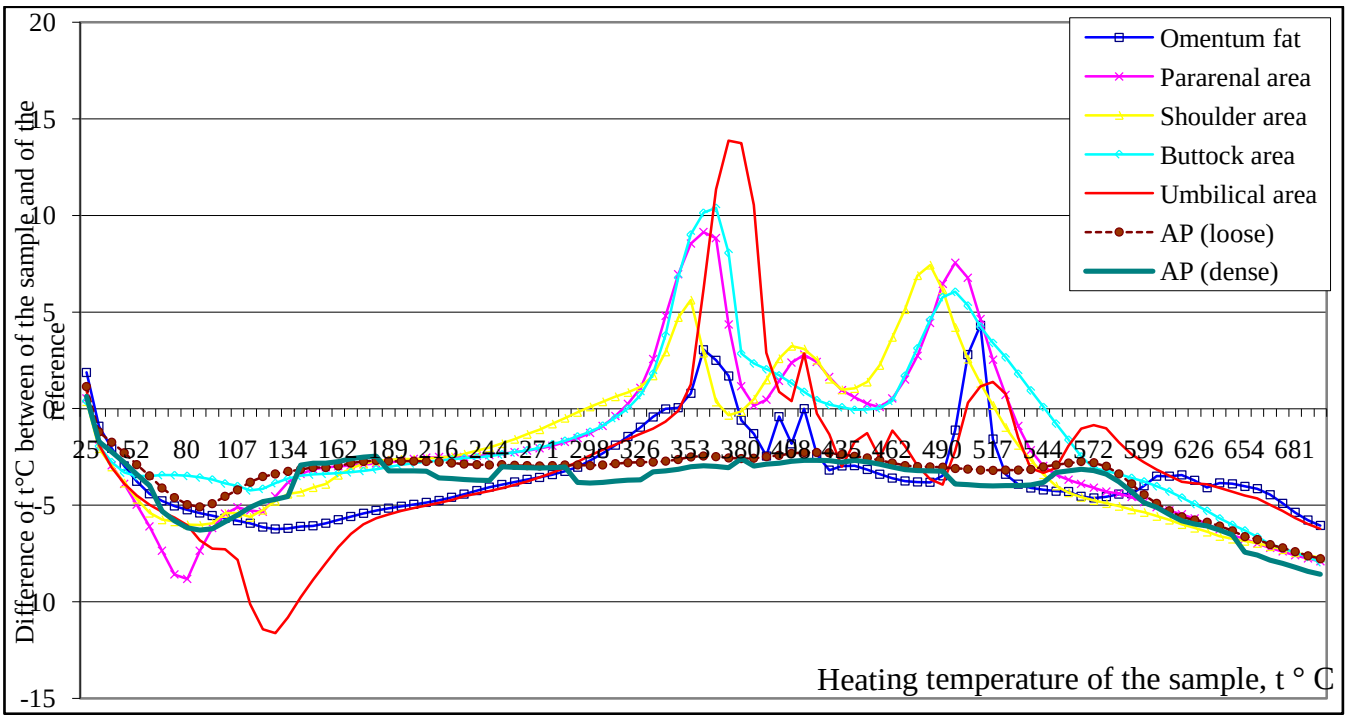

Figure 1 : Comparative values of heating capacities of the lipids with temperature dynamics between of the sample and of the reference

Abbreviations:
AP, atherosclerotic plaque
toC, temperature measure in Celsius

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DNA Normality Following in Vitro Sperm Preparation with Pentoxifylline and L-Carnitine for Asthenozoospermic Infertile Men

By Saad S. Al-Dujaily, Yahya K. Al-Sultani & Nawras N. Shams Alddin
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Abstract - Background: Sperm cells from infertile patients have poor motility and defected DNA. Pentoxifylline (PX) and L-carnitine (LC) are added in the medium for in vitro activation to increase sperm motility and improve the pregnancy outcome. However, there are few studies about the effect of PX with LC medium on sperm DNA normality.

Objective: The goal of this study is to found out the optimum medium can increase the active motility percentage and reduce sperm DNA damage for asthenozoospermic men using the motility stimulant substances, PX and LC for this purpose.

Methods: Semen was collected from 100 infertile men involved in the current study. Each semen sample was divided into four portions. One part was considered as a control group and in vitro activated by using culture medium only. The other portions were considered as treated groups and in vitro activated by adding PX (1mg) and/or LC (0.5mg) to the culture medium. Certain sperm function parameters were examined before and following in vitro activation using layering technique. Sperms DNA damage was detected by using acridine orange (AO) test.

Keywords : pentoxifylline, l-carnitine, in vitro activation, layering preparation technique, male infertility.

GJMR-K Classification : NLMC Code: QU 58.5, WJ 780

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Results: The results revealed a highly significant ($P<0.01$) increment in the mean of sperm concentration (m/ml) percentage of progressive sperm motility grade (A), grade (B) and grade (A+B) and the percentage of morphologically normal sperm (MNS) after using PX and/or LC medium in comparison with control medium .As well as, a highly significant ($P<0.001$) increase in the percentage of sperm normal DNA by using AO test.

Conclusion: It was concluded from the results of the present study that using layering technique with adding PX and/or LC to the culture medium resulting in an improvement in certain sperm function parameters and in the percentage of sperm normal DNA of asthenozoospermic men.

Keywords: pentoxifylline, l-carnitine, in vitro activation, layering preparation technique, male infertility.

I. Introduction

Infertility is the inability of a sexually active non-contraception couple to achieve pregnancy in one year (WHO ,2010) . A male factor is solely responsible in about 20% of infertile couples and contributory in another 30- 40%, if a male infertility factor is present, it is almost always defined by the finding of an abnormal semen analysis for the assessment of male fertility (Agarwal et al.,2003). Problems with the production and maturation of sperm are the most common causes of male infertility. Sperm may be immature, abnormally shaped, or unable to move properly. But, normal sperm may be produced in abnormally low numbers (oligozoospermia) or seemingly not at all (azoospermia) (Diemer et al., 2000).

Asthenozoospermia is one of the major causes of infertility or reduced fertility in men (4). Motility is the prime functional parameter that determines the fertilizing ability of spermatozoa, the cause underlying loss of sperm motility may be either hormonal, biochemical, immunological or infection (Henkel and Schill, 2003; Twigg et al., 1998).

Semen preparation techniques for assisted reproduction were developed to concentrate progressively motile, functional and morphologically normal spermatozoa, and to remove defective and non vital sperms as well as cells e.g. spermatogenic cells and leukocytes. Leucocytes, bacteria and dead spermatozoa produce oxygen radicals that negatively influence the ability to fertilize the egg (Aviad and Dettelbache, 1984). Layering technique is used for normospermic and asthenospermic semen, which allow self-selection of motile sperm.Two factors protect the sperm DNA from oxidative insult: the characteristic tight packaging of the DNA; and the antioxidants present in seminal plasma (Stanic et al, 2002).

On the other hand, pentoxifylline is dimethylexanthine derivative designated chemically as 1-(5-oxohexyl)-3, 7-dimethylexanthine (Okada,1997).The PX prevents cAMP breakdown by inhibiting the activity of the cAMP phosphodiesterase and presumably, stimulates sperm motion (Steiber et al.,2004). Moreover, PX has a protective effect on sperm membranes (i.e.it would preserve functional membrane integrity of sperm tail) as it scavenges ROS and then reduces lipid peroxidation (Muller et al 2002).

Moreover, L-carnitine is a quaternary ammonium compound biosynthesize from the amino acids lysine and methionine (Claudette and Lawrence, 1996). In human body, the primary L-carnitine function is to carry fatty acids into the mitochondria where they

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can be broken down with the ultimate production of energy (Oosterhuis et al., 2000). In the epididymis, the sperm use fatty acids as a source of metabolic energy and scientists believe that one of the functions of LC in sperm is to carry fatty acids into the sperm mitochondria, thereby assisting in the production of energy. Secondly, the conversion of some of the LC to ALC in the mature sperm facilitates the continuation of energy production within the sperm and the newly formed ALC serves as a readily available source of acetyl groups, i.e. energy, for the sperm (Sills, 2004).

A number of studies have investigated the relationship between human sperm DNA damage and semen parameters, such as concentration, motility and morphology (Gil-Guzman et al, 2001; Sikka, 2001). Oxidative stress (OS) may develop as a result of an imbalance between ROS generation and antioxidant scavenging activities (Cocuzza et al., 2007). Sperm preparation techniques can be used to decrease ROS damage production to enhance and maintain sperm quality after ejaculation (WHO, 1999). Therefore the aim of the present study was to use PX and LC for stimulate certain sperm functions and protect the male germ cell from the influence of free radicals.

II. Material and Methods

Semen samples were obtained from 100 infertile men during their attendance to the IVF High Institute, Al-Nahrain University through the period from November 2011 to October 2012. The samples of seminal fluid were collected after 3 to 5 days of abstinence directly into a clean, dry and sterile disposable Petri-dishes by masturbation in a room near the laboratory. After liquefaction time, macroscopic and microscopic analysis of semen samples was don using standardization of WHO (1999) to determine certain sperm function parameters namely; sperm concentration (million/ml), percentage of sperm motility and morphologically normal sperm (MNS) percentage. The DNA denaturation was examined by using acridine orange test according to Tejada, et al. (1984).

a) Preparation of Pentoxifylline Stock Solution

This solution was prepared by dissolving 10 mg from PX powder (sigma, USA) in 10 ml of PBS (0.1%) then stirring until dissolve. These concentrations prepared daily under sterile condition using UV light and Millipore filter (0.45μM).

b) Preparation of L-Carnitine Stock Solution

This solution was prepared by adding 0.5mg of LC powder (Natrol,USA) to 10 ml of phosphate buffer solution in plastic test tube . Then it was filtered by using Millipore 0.45 μM and have been fixed at pH 7.4- 7.8 at 25˚C.

c) In vitro activation technique

After liquefaction of human semen, layering activation technique was used according to Hall, et al. (1995), each semen sample was divided into four portions, one portion was considered as a control group by using Hams F-12 medium (Sigma, Germany) and the other three portions of semen is considered as treated groups by adding the following substances: Pentoxifylline (PX) 1mg/ml, L-Carnitine (LC) 0.5 mg/ml and both equally added PX + LC. Certain sperm function parameters were examined following in vitro activation according to WHO (1999) too.

Statistical analysis: Data from treated and control media groups were expressed as mean ± SEM and statistically analyzed using analysis of variance (ANOVA) to compare the differences between the four prepared media. When F values reach the significant level at 5%, least significant difference (LSD) test was used (Sorlie, 1995).

III. Results

Tables 1,2,3 and 4 shows that no significant ($P< 0.05$) difference in the mean of sperm concentration between control and treated media groups after activation in most infertile patients (asthenozoospermic men,table-1,oligoasthenozoospermic men, table-2, astheno- teratozoospermic men ,table-3 and oligoasth-enoteratozoospermic men ,table-4). The activation of human sperm in vitro with both control (Hams F-12) and treated (PX and/or LC) media caused a significant ($P<0.05$) and a highly significant ($P<0.001$) increase in the percentage of progressive sperm motility grade (A,B,A+B) compared to before activation by layering activation technique in all treated infertile groups(Tables 1,2,3,4). There was a highly significant ($P<0.001$) increment in the mean of sperm concentration, percentage of progressive sperm motility grade (A), grade (B) and grade (A+B) with the percentage MNS after using mixing PX+LC medium in comparison with using PX and LC alone and with control medium in the asthenozoospermic patients (Table-1) and other mild male factors infertility (Tables 2, 3, 4). Activation of human sperm caused a highly significant ($P<0.001$) improvement in the MNS in both control and treated group when compared to before activation and between treated semen samples when compared to control semen samples in asthenoteratozoospermic patients(Table-1) and oligoasthenoteratozoospermic (Table-4) patients following layering technique. There was a significant ($P<0.05$) and a highly significant ($P<0.001$) decrease in the round cells in both control and treated groups when compared to before activation of all infertile men. Also the results show a highly significant ($P<0.001$) improvement in the percentage of normal DNA sperms after activation with PX and/or LC by layering technique in most infertile patients.

Table 1 : Effect of in vitro activation with Hams F-12, Pentoxifylline , L-carnitine on certain sperm function parameters of asthenozoospermic patients using layering technique

Certain sperm function parameters		Before activation	hams F12	hams F12 +PX	LC	PX + LC
Sperm Concentration (Million/ml)		53.96±3.77^{a}	50.52±3.23^{a}	56.29±5.34^{a}	58.13±5.49^{a}	59.78±4.62^{a}
Active sperm motility (%)	Grade A	4.62±0.89^{A}	19.13±2.21^{B}	30.86±4.62^{B}	29.13±4.2^{B}	32.83±3.3^{B}
	Grade B	34.35±1.17^{A}	39.43±1.85^{A}	42.86±3.34^{b}	42.4±2.4^{b}	44.52±2.5^{B}
	Grade A+B	38.96±1^{A}	58.57±3.39^{B}	73.71±5.98^{Ba}	71.53±4.88^{Ba}	77.35±4.13^{B}
Morphologically Normal sperm (%)		35.92±0.81^{A}	39.13±1.3^{a}	45.57±2.31^{b}	46.2±2.42^{B}	51.35±2.34^{B}
Round cells (cell/HPF)		7.31±1.2^{a}	5±0.94^{ab}	3.21±1.28^{b}	3.67±1.2^{b}	3.96±0.99^{b}
Green sperm %		50.14±6.51^{a}	68.87±6.06^{b}	77.74±7.1^{b}	66.47±6.99^{ab}	73.12±6.46^{b}
Orange sperm %		49.86±6.51^{a}	31.12±6.06^{b}	22.25±7.09^{b}	33.77±6.92^{ab}	26.87±6.46^{b}

Values are expressed as Mean± SEM.
Different small letters mean significant difference at P<0.05.
Different capital letters mean significant difference at P<0.001.

Table 2 : Effect of in vitro activation with Hams F-12, Pentoxifylline, L-carnitine on certain sperm function parameters of oligoasthenozoospermic patients using layering technique

Certain sperm function parameters		Before activation	hams F12	hams F12 +PX	LC	PX + LC
Sperm Concentration (Million/ml)		10.81±1.22^{a}	16.92±3.83^{a}	22.70±2.30^{Ab}	26.15±2.96^{Ab}	32.94±4.16^{B}
Active sperm motility (%)	Grade A	0.94±0.50^{A}	6.42±2.23^{A}	28.90±5.54^{B}	22.31±3.64^{Ab}	29.94±3.04^{B}
	Grade B	25.63±1.91^{A}	23.67±1.96^{A}	35.80±2.10^{b}	38.00±3.30^{B}	42.19±2.28^{B}
	Grade A+B	26.56±1.85^{A}	30.08±3.63^{A}	64.70±7.20^{B}	60.31±6.55^{B}	72.13±4.79^{B}
Morphologically Normal sperm (%)		33.44±0.94^{a}	37.92±1.82^{a}	44.90±1.79^{b}	30.00±2.89^{a}	45.63±2.77^{B}
Round cells		4.63±0.61^{a}	3.33±0.85^{a}	2.40±0.58^{b}	1.85±0.60^{b}	1.88±0.66^{b}
Green sperm %		7.39±1.19^{A}	35.75±6.85^{B}	81.03±4.56^{B}	77.42±3.54^{B}	84.69±4.52^{B}
Orange sperm %		92.60±1.19^{A}	64.24±6.85^{B}	18.96±4.56^{B}	22.57±3.54^{B}	15.30±4.52^{B}

Values are expressed as Mean±SEM.
Different small letters mean significant difference at P<0.05.
Different capital letters mean significant difference at P<0.001.

Table 3 : Effect of in vitro activation with Hams F-12, Pentoxifylline, L-carnitine on certain sperm function parameters of asthenoteratozoospermic patients using layering technique

Certain sperm function parameters		Before activation	hams F12	hams F12 +PX	LC	PX + LC
Sperm Concentration (Million/ml)		31.15±2.85^{a}	40.96±3.89^{a}	36.50±5.82^{a}	37.33±5.52^{a}	59.87±4.59^{B}
Active sperm motility (%)	Grade A	6.58±1.33^{A}	18.39±2.10^{B}	21.71±3.06^{B}	21.47±3.03^{B}	32.78±2.92^{B}
	Grade B	24.31±1.98^{A}	37.61±2.63^{B}	36.14±4.62^{Ba}	36.60±3.13^{Ba}	46.65±1.94^{B}
	Grade A+B	30.88±2.48^{A}	56.00±4.20^{B}	57.86±7.04^{B}	58.07±5.66^{Ba}	79.43±3.49^{B}
Morphologically Normal sperm (%)		19.96±1.29^{Aa}	31.26±2.23^{b}	33.36±4.06^{B}	36.87±2.97^{B}	52.22±2.56^{B}
Round cells(cell/HPF)		7.46±1.44^{a}	5.74±1.30^{a}	3.07±1.03^{b}	6.07±1.76^{a}	4.52±1.09^{a}
Green sperm (%)		62.92±6.44^{a}	63.77±6.42^{a}	80.88±6.51^{b}	76.73±6.72^{a}	71.22±6.72^{ab}
Orange sperm (%)		37.12±6.43^{a}	36.24±6.42^{a}	19.12±6.51^{b}	25.26±6.72^{a}	28.77±6.72^{ab}

Values are expressed as Mean±SEM .
Different small letters mean significant difference at P<0.05.
Different capital letters mean significant difference at P<0.001.

Table 4 : Effect of in vitro activation with Hams F-12, Pentoxifylline , L-carnitine on certain sperm function parameters of oligoasthenoteratozoospermic patients using layering technique.

Certain sperm function parameters		Before activation	hams F12	hams F12 +PX	LC	PX + LC
Sperm Concentration (Million/ml)		12.75±0.76^{a}	18.24±2.59^{ab}	20.38±2.33^{bB}	21.86±1.78^{b}	28.44±3.10^{b}
Active sperm motility (%)	Grade A	2.60±0.97^{A}	15.59±1.55^{B}	23.50±2.63^{B}	14.93±1.78^{b}	27.89±3.09^{B}
	Grade B	19.55±1.46^{A}	35.18±2.13^{B}	37.31±2.80^{B}	31.57±2.20^{Bb}	43.33±2.26^{B}
	Grade A+B	22.15±1.94^{A}	50.76±2.99^{B}	60.81±5.03^{B}	46.50±3.37^{B}	71.22±4.77^{B}
Morphologically Normal sperm (%)		21.90±1.17^{A}	33.47±1.15^{B}	34.38±2.11^{B}	33.79±2.18^{B}	41.78±2.74^{Ba}
Round cells(cell/HPF)		6.65±0.66^{A}	2.41±0.49^{B}	2.38±0.93^{B}	2.21±0.70^{B}	2.94±0.92^{B}
Green sperm (%)		21.02±4.65^{A}	51.82±6.52^{B}	62.43±6.56^{B}	68.91±4.27^{B}	74.71±5.55^{B}
Orange sperm (%)		78.97±4.65^{A}	48.17±6.52^{B}	37.56±6.56^{Ba}	31.08±4.27^{B}	25.29±5.55^{B}

Values are expressed as Mean±SEM.
Different small letters mean significant difference at P<0.05.
Different capital letters mean significant difference at P<0.001.

IV. Discussion

In this study, there was a highly significant increase in the sperm motility grade (A) and grade (A+B), while there was a significant increase in sperm motility grade (B) in treated group. This finding is in agreement with studies that revealed a significant improvement in grade (A), hyperactivation and the acrosome reaction following activation by PX (Abid, 2005 and Al-Dujaily et al., 2007). PX has been demonstrated to increase testicular sperm motility when it added to culture media (Sato and Ishikawa, 2004). It inhibits the breakdown of cAMP and it is known that intracellular cAMP concentration plays a central role in cell energy which in turn sustain sperm motility. The increase of cAMP lead to increase progressive sperm motility. The cAMP plays an important role in the glycolytic path way of the sperm and, through its effect on glycolysis. It can influence the energy generation required for sperm motion (Steiber et al., 2004). The highly significant increase in most sperm parameters after adding LC to sperm activated medium which showed a highly elevation in active sperm motility was in agreement with other studies that reported a significant increase in the sperm motility when LC was added to ejaculated human spermatozoa (Al-Dujaily et al.,2012). The other positive effect of LC addition to the medium in current study may be its function to carry fatty acids into the sperm mitochondria to assisting the production of energy. (Agarwal and Said 2004).

This study believed that the medium contains both LC and PX gave excellent improvement in progressive sperm motility grade (A) and grade (A+B). This is in agreement with Aliabadi, et al. (2013) who

stated that in vitro administration of LC and PX to extracted testicular sperm samples led to increased sperm motility. Whereas giving a highly improvement results in the percent of MNS may be resulting from the important effect of both PX and/or LC that works as antioxidant ROS scavengers to reduce sperm DNA damage after activation (Menezo et al.,2007).

Further significant improvement in the percentage of MNS was recorded after activation. This finding may be related to the fast movement of normal spermatozoa from seminal plasma into upper layer of culture medium, and consequently elicited from impact of some seminal plasma components like leukocytes, round cell and others leading to kept the sperm out of stress factor and ROS production that responsible for DNA damage (Sharma et al., 2004). Thus, layering activation technique remove the immotile and dead cells from the sample. The same observation was noticed by other studies using culture for separation and activation of sperm in vitro (Al-Dujaily and Malik, 2013 and Al-Dujaily et al., 2006).

The results of the present study has found a highly significant reduction in abnormal (DNA damaged) sperms in the infertile semen after activation by all activation media compared with results before activation.This may be caused by the affection of antioxidants (including LC and PX every one alone or both of them)which added to the medium (HamsF12), PX has been oxygen-free radical scavenging capacities by reducing the superoxide release from human spermatozoa (McKinney et al.,1996).The PX has been shown to decrease ROS production. Moreover, the anti-oxidant property of LC may also have an influence on sperm motility. L-carnitine, as anti-oxidant (Solarska et al., 2010) may protect sperm plasma membrane with high level of unsaturated fatty acid content (Aitken and Clarkson, 1987). Free radicals can also decrease mitochondrial energy availability and impaired sperm motility (De-Lamirande and Gagnon, 1992). It has been emphasized that acridine orange staining of semen smears improve the information obtained by semen analysis with respect to sperm fertilizing capacity (Kosower et al.,1992). The cause of infertility in the infertile men with normal semen parameters could be related to abnormal sperm DNA (Menezo et al., 2007). Therefore, the present work depends on the evaluation of sperm DNA integrity to improve the positive effects of the activation technique and the motility stimulants and to add further information on the quality of spermatozoa that will be used in future on reproductive outcomes (Schulte et al.,2010).

It was concluded that LC and PX can be added for the medium as activator substances to stimulate certain sperm function parameters in vitro of asthenozoospermic patients with or without other male infertility factors to reduce DNA damage in sperms. This results can be utilized for in vitro activation medium used in the ART centers.

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Evolution of Life Expectancy and Health Equity in Canadian Health Regions from 1986 to 2007

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Abstract - *Background:* Life expectancy (LE) analysis was conducted in this study to explore time trends for Canadian mortality rates from 1986 to 2007 by age and sexand compare these trends to socio-demographic characteristics.

Objective: To measure Canadian's LE by age, sex, provinces/territories and health regions, detect lowerLE in Canada due to variant lifestyle and socioeconomic status, compare the difference of Canadian LE among these areas over time.

Results: Canadians' LE at birth has increased 3.2 years in 1986-2007 in women, and 5.2 years in men. LE in British Columbia and Ontario were 81.2 and 81.0, higher than the average in Canada (80.7) in 2007.Regions with the highest LE were Richmond Health Service Delivery Area (BC), York Regional Health Unit (ON), and Peel Regional Health Unit (ON) in compared to the three regions with lowest LE:Région du Nunavik (QC), Burntwood/Churchill (MB), and Nunavut Territory in both sexes.

Keywords : *life expectancy, age, sex, mortality, health status, risk factors, biostatistics, canada.*

GJMR-K Classification : *NLMC Code: WX 150*

Strictly as per the compliance and regulations of:

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Conclusion: LE is lower for males than females, and shorter in the North than in the South consistent with limited primary health care, higher rates of smoking, heavy drinking and obesity, higher levels of long-term unemployment, fewer high school and university graduates, a relatively larger Aboriginal population and being generally rural and remote.

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I. Introduction

Life expectancy (LE) is frequently used as an indicator of the health of a population. Identifying gaps in life expectancy between different groups in different provinces and territories helps draw attention to particularly vulnerable populations in Canada. However, life expectancy measures the length rather than the quality of life, and does not necessarily represent the number of years spent in good health [1].

In the last five decades, life expectancy in Canada has been ranked in the top 10 among the 34 countries now in the Organization for Economic Co-operation and Development (OECD) [2-3]. In 2007, the most recent period for which data are available for all OECD countries, Canada ranked ninth, with a life expectancy at birth of 80.7 years for both sexes combined. This was 1.9 years lower than the first-ranked country, Japan. Canadian men were in eighth place, 1.2 years below top-ranked Switzerland, and Canadian women were tied for ninth with Sweden, 3.0 years below Japan [4].

The gap in life expectancy between males and females differs by country. In Canada, life expectancy at birth is 4.6 years longer for women than men. Among the top 10 OECD countries, the gap in life expectancy is largest in France (7.0 years) and smallest in Iceland (3.5 years) [4].

The objective of this study is to identify where Canada has the highest overall life expectancy, and to relate the findings to associated health behaviours and socio-demographic characteristics.

II. Data and Methods

We used the Canadian Vital Statistics Death Database for the period of 1986 to 2007. All death records are based on information abstracted and compiled from death certificates and are provided to Statistics Canada by the vital statistics registrars in each province or territory. The mortality data in this analysis are coded using both the 9th and 10th Revision of the *International Classification of Diseases* (ICD-9 and ICD-10). Annual population estimates were taken from Statistics Canada's annual demographic statistics (Statistics Canada. Annual Demographic Estimates: Canada, Provinces and Territories 2011). For each province or territory (Northwest Territory, Yukon, and Nunavut) and health regions, overall average and sex-specific mortality rates were calculated for the study period.The annual population was considered as a weight to produce a weighted average of annual mortality rate and life expectancy for provinces/territories and health regions.

Life expectancy was then related to the health regions by age and sex. These variables were examined together, with socio-demographic characteristics. A peer group was created based on similarities in the populations of health regions along a number of sociological categories, including: income inequality; percentage of the population aged 65+; unemployment rate; property ownership; and average years of schooling [5].We compared the significant differences among respective peer groups, provinces/territories and the Canadian national level group.

When the result is "0", this means there is no statistical difference; "+1" means there is a positive

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statistical difference; and "-1" means there is a negative statistical difference between compared groups.

LE analysis was conducted to explore time trend for Canadians mortality rate from 1986 to 2007 by all causes, age, and sex. All analyses were conducted by using SAS, version 9.1, statistical software.

III. Results

Life expectancy in Canada improved significantly from 1986 to 2007. Women's life expectancy at birth increased from 79.8 years in 1986 to 83.0 years in 2007, while men's increased from 73.2 to 78.4 years in the same period—increases of 3.2 years for women and 5.2 for men (Figure 1).

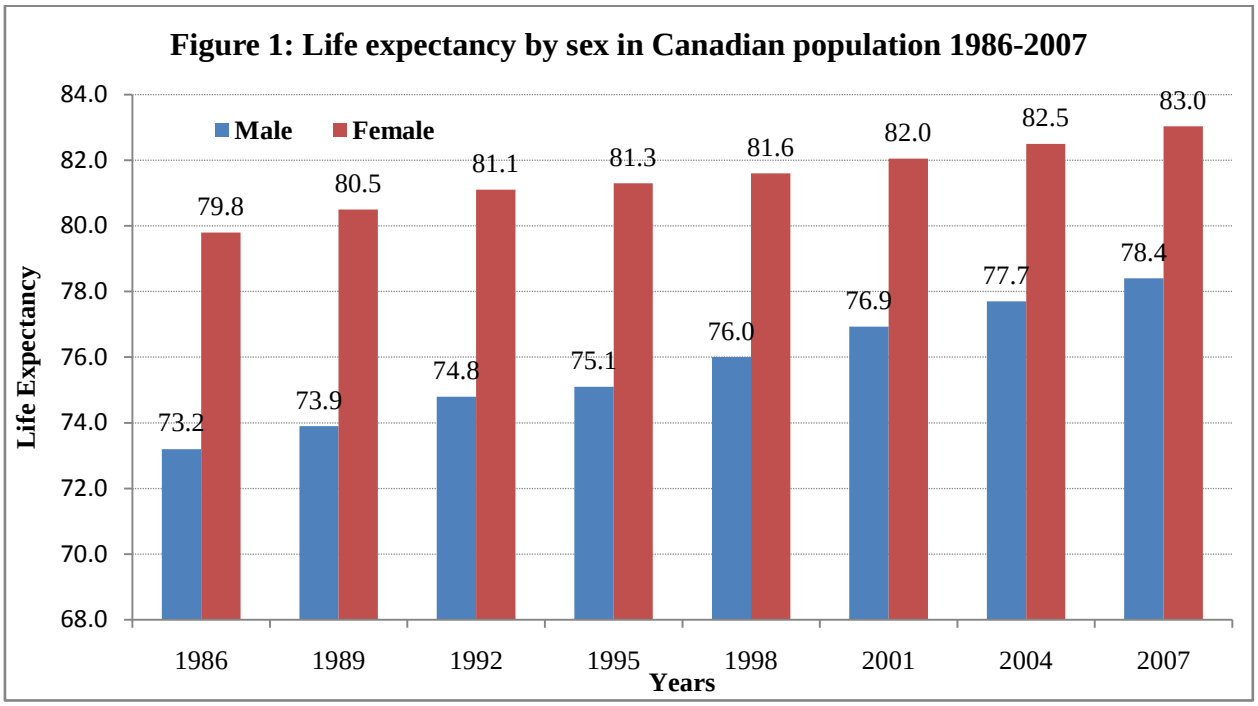

Figure 1 : Life expectancy by sex in Canadian population 1986-2007

Our study noted only modest variability in LE by province, with the only noteworthy exception being the much lower female LE in Newfoundland and Labrador. Figure 2 shows how life expectancy at birth varies across Canada. Among the provinces in 2007, British Columbia (BC) had the longest life expectancy, 81.2 years, following by Ontario (81.0), Quebec (80.7), Alberta (80.5), PEI (80.2), and New Brunswick (80.0). Newfoundland and Labrador had the lowest, 78.3 years. However, when we compare the differences between 1986 and 2007 period, we found that the highest increase in LE was for PEI, there are 4.3 years difference, higher than average (4.2) in the general population. Ontario was 4.2 years, the same as in the average LE in Canada. LE in other provinces and territories were lower than general population (Figure 2).

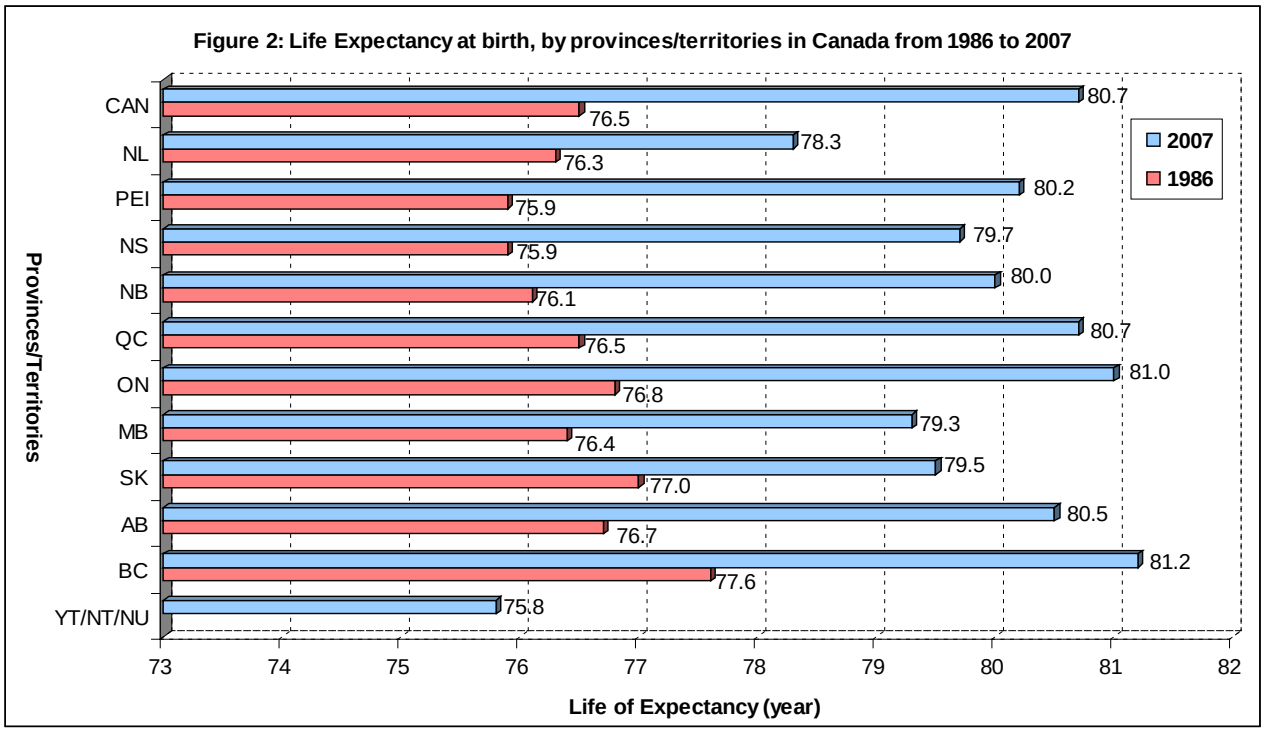

Figure 2 : Life Expectancy at Birth, by Provinces/Territories in Canada from 1986 to 2007

Notes: CAN-Canada; NL-Newfoundland and Labrador; PEI-Prince Edwards Island; NS-Nova Scotia; NB-New Brunswick; QC-Quebec; ON-Ontario; MB-Manitoba; SK-Saskatchewan; AB-Alberta; BC-British Columbia; YKT/NWT/NUT- Yukon Territory/North West Territory/Nunavut Territory

LE in the different health regions of different provinces and territories varied in health regions and sex. The three regions with the highest LE were Richmond Health Service Delivery Area (BC), York Regional Health Unit (ON), and Peel Regional Health Unit (ON) in both sexes (84.6, 83.8, 83.2), males (82.6, 82.0, 81.3), and females (86.2, 85.4, 84.9) respectively. In contrast, the three regions with the lowest LE were Région du Nunavik (QC), Burntwood/Churchill (MB), and Nunavut Territory in both sexes (71.3, 71.3, 72.0), males (69.3, 68.4, 68.9), and females (72.5, 74.6, 76.0). Results showed that the three highest LE have positive statistical difference in comparing with the previous reference period (+1), the Canadian national level (+1), the peer group rate (+1) and the provincial level (+1). However, the three regions with the lowest LE were negative statistical lower than the Canadian national level (-1), the peer group rate (-1) and the provincial level (-1). (Table 1,Map 1, Map 2).

Table 1 : Significant difference from Life expectancy (LE) at birth by health region compared to peer groups, provincial/territorial level and Canadian national level group in Canada 2005-2007*

Table 1--- Significant difference from Life expectancy (LE) at birth by health region compared to peer groups, provincial/territorial level and Canadian national level group in Canada 2005-2007*

		Life expectancy by sex and 95% confidential interval								Compared to previous period		Compared to national level		Compared to peer group		Compared to provincial level	
	Three top highest LE in the health regions																
	Code	Both	95%C.I.		Male	95%C.I.		Femae	95%C.I.	Male	Female	Male	Female	Male	Female	Male	Female
Richmond Health Service Delivery Area	5931	**84.6**	84.2	84.9	**82.6**	82.1	83.2	**86.2**	85.7	86.7	1	1	1	1	1	1	1	1
Peel Regional Health Unit	3553	**83.2**	83.1	83.4	**81.3**	81.0	81.5	**84.9**	84.7	85.2	1	1	1	1	1	1	1	1
York Regional Health Unit	3570	**83.8**	83.6	83.9	**82.0**	81.7	82.3	**85.4**	85.1	85.6	1	1	1	1	1	1	1	1
	Three lowest LE in the health regions																	
Nunavut	6201	**72.0**	70.5	73.4	**68.9**	67.2	70.6	**76.0**	73.3	78.6	0	0	-1	-1	0	0	0	0
Région du Nunavik	2417	**71.3**	68.9	73.8	**69.3**	65.8	72.7	**72.5**	69.3	75.7	0	0	-1	-1	0	-1	-1	1 -
Burntwood/Churchill	4685	**71.3**	70.3	72.2	**68.4**	67.1	69.7	**74.6**	73.1	76.0	0	0	-1	-1	-1	-1	-1	1 -

Note: 0---No statistically significant difference between compared groups
1---Statistically significant difference above compared group
-1---Statistically significant difference below compared groups

***** Data source: Statistics Canada, CANSIM table 102–4307

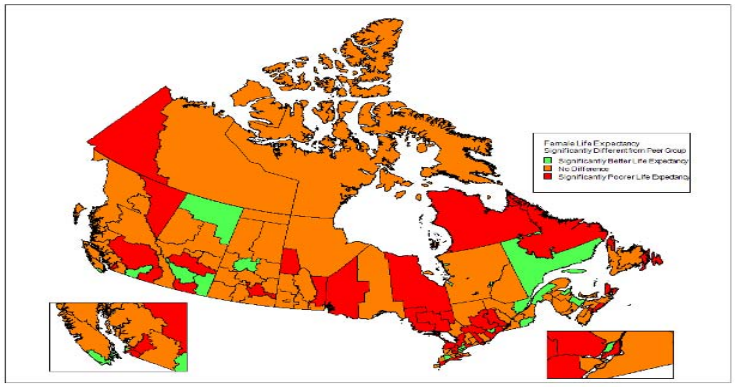

Figure 3 : Female life expectancy at birth by health region, significantly different from peer groups in Canada 2005–2007

Source:Statistics Canada. Vital Statistics.Life expectancy.

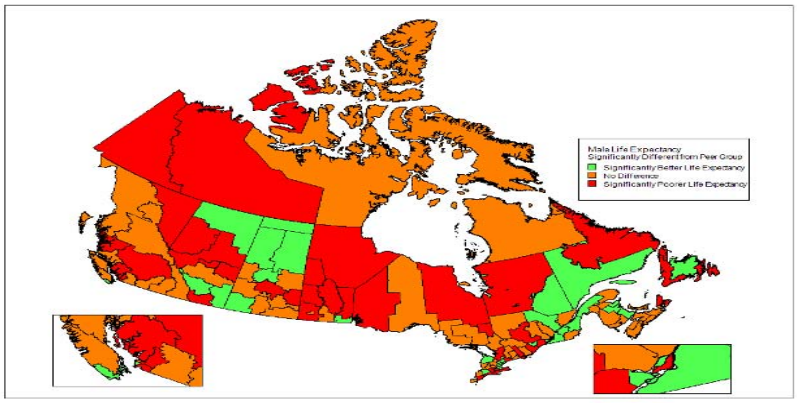

Figure 4 : Male life expectancy at birth by health region, significantly different from peer groups in Canada 2005–2007

Source:Statistics Canada. Vital Statistics.Life expectancy.

IV. Discussion

Life Expectancy (LE) is a commonly used component of the Human Development Index (HDI), along with adult literacy, education, and standard of living [6].

Although our study noted only modest differences in LE by province, we observed large variations in LE by health regions. This highlights the importance of conducting surveillance with sufficient granularity to adequately inform public health action. Health regions with low LE in tended to be clustered in the north, while those with high LE in tended to be in regions in the south which were most urbanized and had experienced the highest levels of immigration. The most disadvantaged health units had life expectancies comparable to those experienced in Canada as a whole 40 years ago [7].

Life expectancy is lower for males than for females: this gap has been present to varying degrees for nearly a century. This gap between the sexes narrowed over the study period [8-10].

The reasons for this may be that life expectancy tends to be lower in regions with poor living conditions, a lack of primary health care, higher accident rates, and the rates ofsmoking, heavy drinking and obesity are relatively higher than other regions in Canada [11-16]. These regions also have higher rates of extended unemployment, fewer high school and university graduates, a relatively larger Aboriginal population and are generally tended to be more rural and remote [17].

Our results are comparable to those in some European countries and in Japan. The difference in life expectancy at birth between the best and worst European countries in this respect is more than 10 years for both sexes. Life expectancy at birth in the European Union-27 countries (new members after 2004) was 75.1 years (men) and 81.3 years (women). The difference between the 10th and 90th percentile of 272 regions was 8.0 (men) and 5.6 years (women). Men lived 6.1 years and women 3.9 years shorter in the new member states (NMS, new members since 2004) than in the European Union-15 countries.

The main causes explaining differences in life expectancy are ischemic and other heart disease, stroke, alcohol related mortality, lung cancer and injuries [18-21]. The contributions of medical care and pollution are likely to be modest; health behaviour, diet, and alcohol consumption seem to be more important; smoking seems to have the largest impact [11-13, 22-24]. In contrast, people in health regions with life expectancy higher than the Canadian average practice good health behaviours, have high average years of schooling, high

levels of property ownership, and there is a high proportion of immigrants, and low unemployment.

V. Conclusion

Life expectancy in Canada has increased substantially over the last 25 years, and Canada ranks consistently among the top countries in the world. At the same time there are large regional differences. Contributing reasons likely include poor living conditions, access to limited primary health care, and higher rates of smoking, heavy drinking and obesity. These regions also have higher rates of long -term unemployment, fewer high school and university graduates, a relatively larger Aboriginal population and rural and remote.

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Calculating the Carbon Cost in Critical Care: A Global View from an Intensive Care Window

By P Ramesh Menon

Abstract - The "Global Climate Change and Children's Health" is a technical report and policy statement (1), that outlines the specific ways global climate change impacts child health, and calls on health practitioners to understand the threats, anticipate the impact on health, and advocate for strategies that will lessen the effects.

A carbon footprint is defined as: The total amount of greenhouse gases produced to directly and indirectly support human activities, usually expressed in equivalent tons of carbon dioxide (CO2). It is often understood as in the following examples:

Other greenhouse gases which might be emitted as a result of human activities are methane and ozone. These greenhouse gases are normally also taken into account for the carbon footprint. They are converted into the amount of CO2 that would cause the same effects on global warming. This is called equivalent CO2 amount. Carbon footprint may also be expressed in kg carbon rather than kg carbon dioxide (By multiplying with a factor 0.27 i.e.1'000 kg CO2 equals 270 kg carbon).

Keywords : *carbon footprint, ventilation, health.*

GJMR-K Classification : *NLMC Code: WA 320*

Strictly as per the compliance and regulations of:

Calculating the Carbon Cost in Critical Care: A Global View from an Intensive Care Window

P Ramesh Menon

I. Introduction

The "Global Climate Change and Children's Health" is a technical report and policy statement (1), that outlines the specific ways global climate change impacts child health, and calls on health practitioners to understand the threats, anticipate the impact on health, and advocate for strategies that will lessen the effects.

A carbon footprint is defined as: The total amount of greenhouse gases produced to directly and indirectly support human activities, usually expressed in equivalent tons of carbon dioxide (CO2). It is often understood as in the following examples:

Each of the following activities adds 1 kg of CO2 to carbon footprint:-

- Travel by public transportation (train or bus) a distance of 10 to 12 km
- Drive by car a distance of 6 km (assuming 7.3 litres petrol per 100 km)
- Fly with a plane a distance of 2.2 km
- Operate a computer for 32 hours (60 Watt consumption assumed)
- Production of 5 plastic bags/ 2 plastic bottles/ one third of an American cheeseburger (the production of each cheeseburger emits 3.1 kg of CO2!)

Other greenhouse gases which might be emitted as a result of human activities are methane and ozone. These greenhouse gases are normally also taken into account for the carbon footprint. They are converted into the amount of CO2 that would cause the same effects on global warming. This is called equivalent CO2 amount. Carbon footprint may also be expressed in kg carbon rather than kg carbon dioxide (By multiplying with a factor 0.27 i.e.1'000 kg CO2 equals 270 kg carbon).

The carbon footprint is a very powerful tool to understand the impact of personal behaviour (including healthcare) on global warming. Individual activities like, e.g. travelling by car, train, bus or air plane, fuel consumptions, electricity bills are called "carbon stamps" (individual contributions). In the medium- and long term, the carbon footprint must be reduced to less than 2'000 kg CO2 per year and per person. (2)

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Keywords: *carbon footprint, ventilation, health.*

Carbon footprint in the ICU: In the medical parlance, Carbon dioxide (CO2) is produced by cell metabolism in the mitochondria. It depends on the rate of metabolism and the relative amounts of carbohydrate, fat and protein metabolized. The amount is about 200ml.min-1 when at rest and eating a mixed diet; this utilizes 80% of the oxygen consumed, giving a respiratory quotient of 0.8 (RQ = rate of CO2 production / rate of O2 consumption). A carbohydrate diet, in a healthy human in a resting state, gives a quotient of 1 and a fat diet 0.7. (3) In a sick child as the metabolic processes are in a compensatory overdrive / decompensated mode, the CO2 production increases and the homeostatic processes go into overdrive with increasing Work of breathing (WoB) to eliminate the excess CO2 generated and to get more O2 available for gas exchange. (4) It is in this context of increased WoB that the intensivist manages the patient with Mechanical ventilation and sedation/analgesia and nutrition (enteral or parenteral) and supportive care (temperature / fluid etc).

Considerable changes in healthcare delivery practices have been suggested and adopted to improve efficient utilisation of energy.(5,6) However, no formal assessment of actual health care/delivery services in terms of their carbon footprint/ stamps have been done to date.(7) This would enable improvisations of the services which consume the energy rather than the technology available to the end user.(2) While the Hospital sector and health care organizations have made great efforts to improve hospital sustainability practices, the transition may be guided by Life Cycle Assessment (LCA),(8) a method being increasingly used to determine the entire "cradle to grave" economic and climatic effects (carbon cost) of processes and products. (9) Advanced computing methods have been used to look at the amount of CO2 produced for energy consumed per unit of medical equipment (e.g.: cannulae, ventilator, operation theatre equipment, anesthetic gases etc) and other healthcare accessories including hospital buildings, biological waste disposal etc.(5,6,10,11). However, computing carbon cost of actual processes of health care interventions (like cannulation, mechanical ventilation, parenteral nutrition) have not been considered, yet. It is only recently that an

approach at estimating the carbon cost of "processes" in service of the end user have begun to be looked at.(6) Sustainable health care practices can be maintained into future by conserving an ecological balance. Its relative importance may be assessed by audits that found over 8% of US total carbon cost originating from health care system. (12)

Here, we intend to look at the concept of carbon cost of the process of mechanical ventilation of a sick child, in the intensive care setting, to understand the perspective of accounting for the individual practices of health care intervention in terms of their ecological impact. Hypothesis: In a ventilated child, other supportive care remaining the same (e.g. sedation / analgesia / nutrition / temperature/ inotropic support), the PaCO2 levels across time (x axis: time; y axis: PCO2) would directly yield the carbon footprint of the process of mechanical ventilation (in their respective modes of ventilation) for the duration of ventilation.

Approximately 75% of carbon dioxide is transported in the red blood cell and 25% in the plasma. There is a difference between the percentage of the total carbon dioxide carried in different forms in blood and the percentage exhaled from them. For example, 5% of the total is in solution (in plasma) but 10% of exhaled carbon dioxide comes from this source; 10% is protein bound, particularly with Hb, but this supplies 30% of the exhaled amount. This corresponds to 0.5ml.kPa-1 carbon dioxide in 100 ml blood at 37oC. The partial pressure of carbon dioxide is 5.3pKa in arterial blood and 6.1kPa in mixed venous blood; therefore, arterial blood will contain about 2.5ml per 100ml of dissolved carbon dioxide and venous blood 3ml per 100ml. A cardiac output of 5l.min-1 will carry 150ml of dissolved carbon dioxide to the lung, of which 25ml will be exhaled. Because of this high solubility and diffusion capacity, the partial pressure of carbon dioxide in alveolar and pulmonary end-capillary blood are virtually the same.(3) Hence PA CO2 (= PaCO2) is a direct correlate of CO2 produced in metabolically active tissues of the body and brought to the lungs for exhalation, via blood. PaCO2 is the only blood gas measurement that provides information on VA. Furthermore, PaCO2 states directly, with one number, the relationship of VA to carbon dioxide production, at least at the time the sample is taken. (4) The production of CO2 (and thereby PaCO2) in a steady state is dependent on the metabolic state of the tissues and the work done by the mechanical ventilator (which offloads the work of breathing in a sick child). After the initial stabilization of a sick child, the metabolic state of the tissues attains a new equilibrium, including the work shouldered by the mechanical ventilator. (4) Other factors and interventions like sedatives/ analgesics / inotropes/ parenteral nutrition etc are significant factors in the attainment of the equilibrium. Once attained, the metabolic equilibrium is a function of the underlying disease process and the compensatory work demand performed by the mechanical ventilator. Given that the body's homeostatic mechanisms in overdrive / distress become less efficient (generating more CO2 in disease / decompensated state), mechanical ventilation may actually have benefits in terms of saving the carbon cost due to the disease or death. There has to be a method of quantifying the carbon cost of the process of health care intervention.

This concept of saving carbon cost with health care intervention (like heart surgery) (13, 14) finds an echo in the similar concept of carbon cost saved in the process of E-news. (15) Technical improvements in any specific sector (e.g. communication, transportation) may not generate per capita reductions in energy use or GHG emissions as large as reductions possible through changing the means by which people achieve the ends currently provided in those sectors (e.g. E-news, social interaction). However, reductions are constrained by how well the alternative (e.g., e-readers, vehicle sharing) substitutes for the existing means of providing the service. (15)

Carbon cost and health care (a Global view): This reminds people in health care that we're not a trivial part of the issue. The primary focus is on issues surrounding patient safety, health care quality, and cost containment at this current point in time. The health care sector, in general, may be a bit slower than other sectors to put this [emissions] on their radar screen. But given the focus on health care policy and environmental policy, it might be interestingif not wise to start accounting for environmental externalities in health care.

According to a WHO report (16)

- Climate change affects the fundamentals for health – clean air, safe drinking water, sufficient food and secure shelter.
- Many of the major killers such as diarrhoeal diseases, malnutrition, malaria and dengue are highly climate-sensitive and worsen as the climate changes.
- Areas with weak health infrastructure – mostly in developing countries (12). – will be the least able to cope without assistance to prepare and respond.

Treating climate-related ills will require preparation, and early-warning systems. Climate change can contribute to such diseases as diarrhea, malaria and infectious illnesses in a number of ways. (17,18) In warmer temperatures, for example, the parasite that spreads malaria via mosquitoes develops more quickly.(17) A 2000 study conducted in Peru(19) found that when the periodic El Nino phenomenon boosted temperatures there, hospital admissions of children with diarrhea increased exponentially.

Pediatric perspective: For children, this can mean post-traumatic stress, loss of caregivers,

disrupted education and displacement. Increased climate-sensitive infectious diseases, air pollution-related illness, and heat-related illness and fatalities also are expected. (20) Disruptions in the availability of food and water and the displacement of coastal populations can cause malnutrition, vitamin deficiencies and waterborne illness. Direct health impacts from global warming include injury and death from more frequent extreme weather events, such as hurricanes and tornados. India, like most other developing countries with high growth indices can grow differently, because "it is in an early stage of development". In other words, it can leapfrog to a low carbon economy, using high-end and emerging technologies and by being different. As doctors, our field of care must broaden to ensure that today's children who would inherit the burden of our actions today and bequeath it to tomorrow's children, are well prepared. The new paradigm of an abruptly changing climate system has been well established by research over the last decade, but this new thinking is little known and scarcely appreciated by the wider community of natural and social scientists and policy makers– National Academy of Sciences/National Research Council 2002. (9)

II. Hospital Perspective

1) Use point of contact for health care delivery to discuss with parents regarding potential impact on health of climate change. Antenatal clinics, Immunisation clinics, well baby clinics; School health services .Doctors can effectively create awareness about "future" effects of climate change; make sure that patients understand the air quality index, pollen counts and UV measures used in most metropolitan areas. These may be opportunities to introduce the broader issue of climate change and the importance of reducing CO2 emissions.

5) Emulate other hospital sectors and organisations that have made great efforts to improve hospital sustainability practices e.g.: using less volatile anesthetics like desiflurane. (9) Some measures that hospitals can use to improve energy efficiency include creating recycling programs and purchasing goods and services from environmentally friendly suppliers. At the University of Chicago Medical Centre, the Sustainability program has implemented a plastic recycling program that diverts more than 500 pounds of waste each day from landfills to recycling plants and ensured that 90 percent of cleaning supplies used by the hospital have Green Seal certification. Such efforts have reduced waste costs from $55,000 per month to $35,000 per month, suggesting that reducing environmental impact can go hand in hand with reducing costs in a hospital setting. An audit of Hospital care, scientific research and the production and distribution of pharmaceutical drugs, found that they produce 3-8%t of the total carbon dioxide output. (5, 6) The audit used 2007 health care spending and a model of environmental impact, called the environmental input-output life cycle assessment (EIOLCA) model, developed by the Green Design Institute at Carnegie-Mellon University. The analysis found that hospitals were by far the largest contributor of carbon emissions in the health care sector, and were attributable to the high energy demands needed for temperature control, ventilation and lighting in large hospital buildings. The second largest health care contributor to the overall carbon footprint was the pharmaceutical industry, where carbon emissions were attributable to manufacturing combined with transportation costs associated with distribution.

5) Policy advocacy: To advocate and support policies that strengthens public transportation, expand green spaces and reward energy efficiency. It's also crucial that children are given specific attention in emergency and disaster response planning.

According to the Intergovernmental Panel on Climate Change (21,22) global mean temperatures could increase by 1.5 to 5.8 °C by the end of the next century in response to this additional radiative forcing. While this may appear to be a minor warming when compared to diurnal or seasonal amplitudes of the temperature cycle, it should be emphasised that this is a warming unprecedented in the last 10000 years. It is time for a motivated implementation of on-the ground adaptation strategies and policy initiatives immediately.

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A Self-Rating Ayurveda Scale to Measure the Manasika Prakrti of the Children

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Abstract - *Background:* Self-rating inventories to assess tridoshas (Vata,Pitta and Kapha) have been developed and validated for adults. The objective of this study was to develop and standardize self-rating scale to assess the M**ā**nasika prakrti(Sattva,Rajas and Tamas) of the children according to Ayurveda .

Method: The 54-item Sushruta Child Personality Inventory(SCPI) was developed on the basis of translation of Sanskrit verses describing Sattva (A), Rajas(B) and Tamas prakrti (C) characteristics, described in Ayurveda texts and by taking the opinions of 6 Ayurveda experts and two psychologists. The scale was administered on children of the age group 8-12 years in New Generation National Public school.

Results: This inventory was named Sushruta Child personality inventory (SCPI) and showed high internal consistency. The Cronbach's alpha for A, B and C scales were 0.60, 0.64 and 0.61 respectively. And the Split-Half reliability for A,B and C scales were 0.62, 0.68 and 0.54 respectively. Factor validity coefficient scores on each items was above 0.3 on Sattva , Rajas and Tamas scale.

Keywords : *triguna, prakriti, sattva, rajas, tamas, ayurveda.*

GJMR-K Classification : *NLMC Code: WB 55.A9*

Strictly as per the compliance and regulations of:

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The result of SCPI was compared with parent rating scale Ayurveda Guna Inventory for Children (AGIC). Subscales of SCPI correlated highly positively (above 0.9) with subscales of AGIC, which was applied to check validation of SCPI with respect to AGIC.

Conclusions: The Manasika prakrti (Sattva, Rajas and Tamas) of the children can be measured reliably utilizing Sushruta Child Personality Inventory (SCPI). Correlation with parent rating scale suggested criterion –related validity.

Keywords: triguna, prakriti, sattva, rajas, tamas, ayurveda.

I. Introduction

According to western psychology, traits determine individual's behavior. While Indian philosophy and Indian psychology proclaim individual's character based on predominance of any of the Trigunas (Sattva, Rajas, Tamas). Sattva refers to balance, tranquility, and purity. Rajas refers to action, passion. Tamas refers to laziness, delusion. [1]

Āyurveda considers Rajas and Tamas as mānasika doshas, which are responsible for the manifestation of neurosis and psychosis. Classical texts of Āyurveda describes 16 types of mental personalities (seven types of Sattva, six types of Rajas, three types of Tamas) determined by pre dominance of each gunas. [2-7]. Imbalance of Rajas and Tamas can be counter-acted by increasing Sattva (Satvavajaya) by Yoga as also diet regime.

Shilpa et al. [8] have discussed the importance of analyzing trigunas in individuals. Dube KC. [9] has reviewed the concept of prakrti according to Āyurveda perceptive and showed the similarities between Āyurveda concept and modern gestalt theory, he mentioned the correspondence of 16 types of personalities with 16 types of psychological disorders. David Wolf. [10] has developed and standardized Vedic personality inventory, and has found psychometric properties of shortened version of the inventory.

Stempel HS, [11] has correlated Vedic personality inventory with Daily Spiritual Experiences Scale and the Brief Symptom Inventory. Gīta personality inventory.[12] was developed based on Bhagavad-Gīta concept. A study, [13] Quoting the existing, paper-pencil tests to measure spiritual and transpersonal construct is available. One of the earliest available inventories was developed by Paramswaran. [14] And Uma, Lakshmi and Parameswaran. [15] named as 'Guna Inventory" to assess the three Gunas. This inventory is based on the descriptions of the characteristics of the three Gunas as outlined in the Sāmkhya Karika and The Bhagavad-Gita.

Mohan and Sandhu, [16] have developed a Triguna personality inventory based on the Gita typology of personality (TGPI) to measure the three Guans as separate dimensions with one being predominant. They found that Sattva was distinct from Rajas and Tamas. Kapur et al. [17] attempted to provide a theoretical model of infant temperament based on ancient Indian thought with special focus on the resilient or competent child. Most of the items of the checklist are drawn from the items of the inventory developed by Marutham. [18] For adult population, along with some items from the standard checklist used in studies on temperament in the west.

Marutham et al. [19] consider the three factors as independent of each other. The inventory is constructed on views depicted in Sāmkhya Karika and Bhagavad-Gita. Ayurveda Guna Inventory for Children (AGIC), [20] a parent rating scale has been developed and standardized. This was developed on the basis of Samskrita verses explained in 5 classical texts of Ayurveda and content validity of the 10 Ayurveda experts and 3 psychologists. Which consisted 32, 20, 18 items respectively for Sattva, rajas and tamas scales.

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Which was administered on 70 parents of the children in Maxwell public school, and was associated with good reliability, which refers to the consistency of the scale and the items in the scale (Cronbach's alpha and split half reliability which describes the analysis of the internal consistency, homogeneity of the items in the particular scale was above 0.5) and construct validity (factor loading) for each items was above 0.5.

Effect of Integrated yoga module and yoga nidra on Trigunas has been discussed. [20-22]

A simple, self-rating scale to assess manasika prakrti (constituiton) of the children according to Ayurveda concepts is not available.

The aims and Objective of the present study were to develop a self – rating scale Sushruta Child Personality Inventory(SCPI) to measure trigunas in children and to compare with parent rating scale AGIC (Ayurveda Guna Inventory for Children) for the purpose of establishing ,Criterion related validity (which describes that the particular scale measures what it supposes to measure), which refers to the usefulness of a test in closely relating to other measures, of the scale, to assert, construct validity, which refers to whether a scale measures or correlates with the theorized psychological construct, using factor analysis. And to determine d discriminant validity, which refers to whether measurements that are supposed to be unrelated are, in fact, unrelated, by assessing correlation between subscales.

Foot note: Prakrti corresponds to constitution or Personality. According to Āyurveda which is based on Sāmkhya Philosophy which emphasizes on the point that universe is governed by the prakrti and Purusha which are the causal factors for the creation. prakrti is the unconscious principle and Soul is conscious principle. And, is made up of Sattva,Rajas and Tamas gunas. Which are responsible for creation, maintenance and destruction of the universe? And as well, forms the personality of the individual.

II. Methods

The Sushruta Child Personality Inventory (SCPI) was developed based on one hundred three Sanskrit characteristics from the five authoritative ancient Ayurveda texts (Table-1) describing characteristics typical of 7 Sattvikaa, 6 Rajasika and 3 Tamasika prakṛti. Item reduction by researcher with the help of Ayurveda expert ,was carried out by deleting the repeated items (described similarly in all texts and alike for different types of Sattvika, Rajasika and Tamasika prakrti for example Sattvika prakrti person will be free from anger, jealousy ,hatred is described in all texts and different types) , ambiguous items (Which are impossible to educe for example Person with predominance of Rajas and Tamas will have different tastes for food), and by selecting those items specifically suitable for children (For example the Rajasika and Tamasika prakrti persons will be having wealth and very much interested in sex and engaged in sex) (See Table-1).

Table-1 Texts and number of items

Table-1 gives the number of initial items (Samskrita) collected from five Ayurveda texts with a: Initial number of items, b: Repeated (retained) number if items, c: Ambiguous items (removed) and d: Items not concerned with children (removed)

84 items, translated into English utilizing Sanskrit dictionary, were presented to ten Āyurveda experts, for content validity. They were asked to judge the correctness of each statement and to check (1) whether any of the items were repeated or should be added?. (2) Whether the features of Sattva, Rajas,and Tamas prakṛti .selected for the scale are correct and (3) if the items constructed represented acceptable translation of the Sanskrit in the original texts. Of these, 80 items which were agreed by all the experts were retained, out of which, some of the items were changed and refined.

Based on the final Sanskrit statements 54 questions were framed by the researcher. The scale was again presented to five Āyurveda experts and one psychologist, who reviewed the format of this scale and recommended a two point scoring (0 and 1), which was adopted in the final SCPI. Suggestions in the phrasing of questions were incorporated. After obtaining consensual validity on 54 questions, by all Ayurveda experts and psychologist, the scale was finalized.

Table-2 Content validity by experts (items agreed by experts)

Table 2 gives the opinion of 5 Ayurveda experts and a psychologist.

The SCPI has 20 items for Sattva scale (A-scale) 18 items for Rajas scale (B-scale) and 16 items for Tamas scale (C-scale) subscales.

Table-3 Demographic data

Table-3 gives mean and standard deviation of demographics. Out of 200 children 104 were boys, 96 were girls, aged around 8-12 years. Mean age being 10.27. Students of 3rd standard to 7th standard, mean education being 4.65.

Data collection and analysis

For testing up reliability and validity of the scale was administered on parents of the children who were the students of New Generation National Public School in Bangalore, of both sexes with an age range of 8 to 12 years, of class of 3rd standard to 7th standard. The 54 items SCPI was answered by 200 children. The Criterion Ayurveda Guna Inventory for Children AGIC) was administered on parents of 30 children of the age group of 8-12 years,for the purpose of cross-validation.

The statistical package for social sciences (SPSS-16.0) was used for data analysis

The item difficulty level was first assessed. The data was next analyzed for reliability. The split-half and Cronbach's alpha tests were applied for reliability-internal consistency analysis. Discriminant validity was analyzed by Pearson's correlation analysis. This was done to check the degree of association between Sattva, Rajas and Tamas scale scores. Criterion related validity was assessed by Pearson's correlation between subscales of SCPI and parent rating scale AGIC.

III. Results

a) Content validity

Amongst, 6 experts who judged the items, content of all 54 questions were agreed by four to five experts.

b) Item difficulty level

This is defined as the presence of a said symptom expressed as the percentage of children who score positive to that item 20-22. The results obtained from the administration of SCPI on 60 children showed 70 items that had less coefficient than 0.9 (answered yes by the most) and more than 0.3 (answered yes by the less subject) were retained.

c) Internal consistency

Refers to, the homogeneity of the items in the particular scale. An analysis of the data collected from 200 children showed the Cronbach's alpha (which is the particular formula based on variance to assess the internal consistency) for S, R and T scales were 0.60, 0.64 and 0.61 respectively. The Split-Half reliability (which refers to the correlation between first half and second half of the scale) for S, R and T scale were 0.62, 0.68 and 0.54 respectively. This shows that the three scales have good internal consistency. [24, 25, 26]

d) Correlations

The Sattva scale correlated negatively with Rajas and Tamas scales. While Rajas and Tamas scales correlated positively.

Table-4: Correlation among Sattva, Rajas and Tamas

Table-4 gives (**) r-Pearson correlation values and significance of correlation between subscales which is at 99% confidence level. Sattva highly negatively correlated with Rajas and Tamas, while Rajas correlated significantly positively with Tamas.

Table: 5 Correlation of the subscales of self-rating scale (Sushruta Child Personality Inventory) with parent rating scale (Ayurveda Guna Inventory for Children)

*Table -5 gives Pearson correlation of each subscales of SCPI with subscalesof AGIC (** p< 0.01).Subscales of SCPI (Sattva,Rajas,Tamas) correlated highly positively with subscales (Sattva, Rajas Tamas)of AGIC(parent rating scale)*

Table-6 Mean score differences between Boys and Girls in trigunas

Table -6 presents the mean scores of Boys and Girls in each subscales. Showing high scores on Tamas in girls (98, for boys it is 7.6), high scores on Sattva and Rajas in Boys (10.5 and 7.4, for girls it is 9.9 and 6.3) . Changes were significant p<0.05 (One sample t-test)

IV. Factor Analysis

Factor analytic co-efficient obtained for each items in; S-scale, R-scale, also T-scale were more than 0.3. (Appendix-1)

Kaiser-Meyer-Olkin Measure of Sampling Adequacy for subscale item analysis was above 0.5, showing good sampling adequacy.

V. Discussion

This present study, has been carried out to develop and standardize a 54 item, self- rating, the Sushruta Child Personality Inventory (SCPI), as an instrument to assess the mental personality (prakrti) of the children. It was developed based on Sanskrit statements from five authoritative texts of Ayurveda. Though the scales are standardized, they doesn't consider the comprehensive outlook of Ayurveda. [11-19] An SCPI has the wide-ranging approach of analyzing trigunas according to the concepts of Ayurveda.

The reliability which refers to the consistency of the scale, was supported by Cronbach's Alpha co-efficient and Split-half analysis (analyzed through SPSS and it has particular formula based on variance of scores on items). Similarly, validity (which refers to utility of the scale) was supported by Factor–analysis which was done to check the association of the items with respective subscales was as good as parent rating scale. [20] Cronbach's Alpha ranged from 0.60 to 0.64. This provided the evidence of homogeneity of items. While for parent rating scale, Ayurveda Guna Inventory for Children (AGIC) it ranged between 0.55 and 0.80. However, Split-half analysis was high as parent rating scale which ranged from 0.56 to 0.79, which is not been addressed other earlier studies.[10-18] Factor loadings for each items in the subscales , ranged from 0.37 to 0.74, 0.43 to 0.75,0.39 to 0.79 0.53 to 0.85, while for parent rating scale. [21] It ranged from 0.50 to 0.80 and 0.40 (only one item) to 0.80 respectively for Sattva,Rajas and Tamas subscales. While for Vedic Personality Inventory subscales it was 0.62 to 0.87, 0.57 to 0.80, and 0.55 to 0.76 respectively. This proved the validity of the items in the subscales. (See Appendix-Table-7)

Co-relation of Sattva with Rajas and Tamas was highly negative, suggesting discriminant validity (See Table-4). Comparing to parent rating scale.20 correlation of Sattva with Rajas and Rajas with Tamas has been improved (-0.77 to -0.85, 0.37 to 0.41) other studies have not shown high correlation (10, 11, 12). While association of Rajas with Tamas was positive this was shown by earlier study. [12].

The subscales of SCPI correlated highly ('r' above 0.9) positively with subscales of parent rating scale AGIC (Ayurveda Guna Inventory for Children). [20] suggesting concurrent validity(SeeTable-5).

The divergence in results (Cronbach's alpha,factor loadings) of self rating and parent rating scales, may be because of discrepancy in prakrti of the children and religion, as parent rating scale (to check reliability) and SCPI were administered in different schools. Ayurveda texts assert persons of one religion, one place will have one prakrti.

The strength of the study is that it is the first attempt to develop and standardize a self–rating scale to measure the manasika prakrti of the children, which is important aspect of maintaining one's health [2-5]. Ayurveda emphasizes on maintenance of the health of a healthy person. Early measurement of Rajas and Tamas can reduce the vulnerability to psychological disorders. By following diet regime and by particular Yoga module one can move towards perfect health. Though published scales are available to assess the trigunas of an individual.[8-9] They have been standardized for adult ale group. However, children require different mode of questions. Hence, SCPI can be potentially used to identify the predominant manasika doshas in children, thus helps to plan suitable régime, yoga at an early age to maintain the health.

Limitations of the study

Although, SCPI (Sushrura Child Personality Inventory) is a reliable, valid instrument, it has not addressed test-retest reliabiltiy. Future studies could establish the norms by the study on more number of samples.

VI. Conclusions

An SCPI is reliable and valid instrument. Researchers can adopt this instrument to assess the effect of Yoga, personality development programme, treatment for children about the age of 8 to 12 years.

VII. Acknowledgment

We thank, Dr. Kishore, Dr.Uma. Dr.Aarati Jagannathan, Āyurveda experts in Bangalore Āyurveda medical College, for their support and participation in the study.

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Table 1 : Texts and number of items

Text	*Sattva prakrti*				*Rajas prakrti*				*Tama prakrti*			
	a	b	c	d	a	b	c	d	a	b	c	d
Caraka Samhita	31	0(31)	2	2	22	0(22)	2	2	11	0(11)	2	1
Sushruta samhita	15	3(12)	2	0	15	3(12)	2	0	9	0(9)	0	0
Astanga samgraha	5	5(0)	0	0	4	4(0)	0	0	4	4(0)	0	0
Astanga hridaya	5	5(0)	0	0	4	4(0)	0	0	4	4(0)	0	0
Kashyapa Samhita	35	35(0)	0	0	25	25(0)	0	0	15	15(0)	0	0
Total	91	48(43)	4	2	70	36(34)	2	2	43	23(20)	2	1

Table 2 : Content validity by experts(items agreed by experts)

Experts	Comment
1 (RM)	Agreed all questions 5^{th} ,10^{th} questions
2 (RA)	Agreed all questions except 5^{th} .12^{th} questions.
3 (SUM)	Agreed all questions except 2^{nd} ,4^{th} questions
4 (AHA)	Agreed for all items except 7^{th} ,10^{th} questions
5 (SHK)	Agreed for all items 6^{th}, 7^{th}, 8^{th} ,12^{th} ,13^{th} questions.
6 (AAJ)	Agreed for all items, suggested changes in the format of questions.

Table 3 : Demographic data

Sample	Boys	Girls	Total
Gender (Boys)	104	96	200
Age range	8-12 years	8-12 years	8-12 years
Mean± SD	10.13± 1.23	10.0±1.18	10.27±1.28

Table 4 : Correlation among Sattva, Rajas and Tamas

Sattva vs *Rajas*	-	0.85**	P < 0.01
Sattva vs *Tamas*	-	0.77**	P < 0.01
Rajas vs *Tamas*		0.41**	P < 0.01

Table 5 : Correlation of the subscales of self-rating scale (Sushruta Child Personality Inventory) with parent rating scale (Ayurveda Guna Inventory for Children)

Sc vs Sp	r = 0.97 **
Rc vs Rp	r = 0.91**
Tc vs Tp	r= 0.93**

Table 6 : Mean score differences between Boys and Girls in trigunas

Sample	*Sattva*	*Rajas*	*Tamas*
Boys	10. 5	7. 4	7.6
Girls	9.9	6.3	9.8

Appendix-1

Table 7 : Factor loadings for each items

Sattva	Loadings
s1	.427
s2	.430
s3	.674
s4	.576
s5	.529
s6	.567
s7	.745
s8	.625
s9	.437
s10	.388
s11	.589
s12	.559
s13	.409
s14	.658
s15	.547
s16	.711
s17	.548
s18	.511
s19	.374
s20	.626

Rajas	Loadings
r1	.753
r2	.650
r3	.581
r4	.788
r5	.592
r6	.558
r7	.748
r8	.546
r9	.485
r10	.684
r11	.439
r12	.575
r13	.566
r14	.676
r15	.687
r16	.629
r17	.565
r18	.652

Tamas	Loadings
t1	.793
t2	.707
t3	.665
t4	.560
t5	.450
t6	.418
t7	.486
t8	.407
t9	.601
t10	.382
t11	.527
t12	.620
t13	.568
t14	.611
t15	.391
t16	.541

Legend-*Table-7 gives factor loadings (correlation of each item with respective subscales)*

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Status, Symptomatology and Partial Characterization of Virus Causing Ring Spot Disease in Bell Pepper (Capsicum Annum L.) in Himachal Pradesh

By Nisar Ahmad Dar, Anil Kumar Handa, M Iqbal Jeelani, Aflaq Hamid & Mudasir Ahmad Bhat

SKUAST-K, India

Abstract - Surveys were conducted to determine the occurrence and distribution of tospovirus affecting bell pepper in Solan district of Himachal Pradesh during 2009 and 2010.Bell pepper crop was more affected especially in Kandaghat (65%), Pandah (65%), Naganji (60%), and Kumarhatti (35%).Infected bell pepper samples were collected from different localities in Solan district of Himachal Pradesh.The symptoms observed under field conditions include plant stunting, rosetted leaves and ringspots. On leaves, concentric rings were observed. The bell pepper fruits produced on infected plants were misshapen with browning and showing nail head symptoms.Based on the reaction on certain indicator plants belonging to familiesAmaranthaceae, Asteraceae, Chenopodiaceae, Cucurbitaceae, Compositae, Malvaceae and Solanaceae as well as serological relationship, isolates from Pandha and Naganji vegetable farm were identified to be a tospovirus. Attempts were made to characterize the virus at biological and serological levels so as to identify whether the tospovirusis a distinct virus species or a strain of TSWV serogroup I, II, III.

Keywords : *serology, ringspots, tospovirusand capsicum annuuml.*

GJMR-K Classification : *NLMC Code: QW 180, QW 570*

Strictly as per the compliance and regulations of:

Status, Symptomatology and Partial Characterization of Virus Causing Ring Spot Disease in Bell Pepper (Capsicum Annum L.) in Himachal Pradesh

Nisar Ahmad Dar [α], Anil Kumar Handa [σ], M Iqbal Jeelani [ρ], Aflaq Hamid [ω] & Mudasir Ahmad Bhat [¥]

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I. Introduction

Capsicum (Capsicum annum L.) popularly known as 'Shimla Mirch'is among the world's most popular vegetables belonging to family Solanaceaeafter potato and tomato. Peppers are extensively cultivated throughout tropical Asia and equatorial America for their edible fruits. It is widely distributed throughout the tropical and subtropical areas of the world particularly Malaysia, India, Pakistan, Thailand, Indonesia, Philippines, tropical Africa, North Africa, South America as major capsicum producing countries (Tindal, 1983).

In world, capsicum (including hot peppers) is grown in an area of 17, 03,486 hectare with a production of 2,60,56,900 tonnes and productivity of 15.03t/ha. China is the largest capsicum producing country (1, 40, 33,000 tonnes) in the world followed by Mexico (16, 90,000 tonnes) (FAO, 2009). In India Bellpepper is grown over an area of 5,761 hectare with the production of 53,198 tonnes and productivity of 9.23 t/ha (FAO, 2009). The major bell pepper growing areas of India includes Himachal Pradesh, Jammu and Kashmir, Arunachal Pradesh and Hills of U P and Darjeeling district of West Bengal during summer months and as autumn crop in Maharashtra, Karnataka, Tamil Nadu and Bihar (Singh et al., 1993). In Himachal Pradesh, capsicum covers an area of 2,503 ha with a production of 33,923 tonnes including hot pepper. The major belts of capsicum cultivation in HP include districts of Solan, Kullu, Shimla, Mandi, Sirmour, Chamba and Kangra (Anonymous, 2009).

Bell pepper occupies an important place among the commercial vegetable crops of Himachal Pradesh. It ranks third after pea and tomato as far as remuneration is concerned, since it is exported to the plains during June to September. But several abiotic and biotic stresses affect the productivity of capsicum crop' worldwide. Among biotic factors besides fungal, bacterial and nematodes, viral diseases attract considerable attention because they impose significant production constraints affecting both yield and quality and are difficult to control (Nono-womdim, 2001). Viruses have become the most devastating diseasecausing agents of capsicum, causing serious losses, thus putting the farmer to the great loss every year (Kang et al, 1973; Lockhart and Fischer, 1974). The major viruses infecting capsicum are Cucumber mosaic virus (CMV), Pepper mottle virus (PeMV), Potato virus Y (PVY), Tobacco mosaic virus (TMV) Alfalfa mosaic virus (AMV) and Tomato spotted wilt virus (TSWV). Among them Tomato spotted wilt virus is one of the most important virus worldwide.

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II. Materials and Methods

An extensive survey of different bell pepper (Capsicum annuum L.) growing localities in Solan district of Himachal Pradesh was conducted during 2009-2010 cropping seasons to determine the distribution and incidence of virus diseases in the state.Incidence counts were made mostly at flowering to fruiting stage of the crop on at least 100 plants by choosing 4-5 locations in the field at random and observations on the number of healthy and diseased plants were recorded.

The per cent disease incidence was calculated by using following formula:

$$\text{Per cent disease Incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \times 100$$

The isolates collected from different bell pepper growing localities of Solan district on the basis of symptoms were maintained on their natural hosts under insect proof glasshouse conditions. Young symptomatic leaves form infected bell pepper plants of each location were used for preparing the inoculum of respective isolates. Commercially available immunoreagents [Tospovirus (serogroup I, II, III), BIOREBA-AG Switzerland] by following protocols of suppliers of ELISA Kits with little modification, if any, were followed for the detection of the virus isolates.

The isolateswere mechanically transmitted to different plant species belonging to families Amaranthaceae, Asteraceae, Chenopodiaceae, Cucurbitaceae, Compositae, Malvaceae and Solanaceae. Both localized and systemic infections were observed. These plants species could serve as potential reservoirs of virus under natural conditions.The isolates infecting bell pepper ware easily transmissible to Nicotianadeneyii, N. tabaccumvar White Burley and N. tabaccumvar Samsun hosts and have been found to be good diagnostic hosts because of the production of distinct localized and systemic symptoms.

Healthy test plants of the same age and uniform size were raised by sowing seeds for mechanical transmission studies. Test plants of bell pepper and other indicator plants were inoculated at 4-5 leaf stage by leaf rub method. The standard extract was applied by rubbing the sap with fore finger or by cotton swab method. Inoculated leaves were washed thoroughly with distilled water immediately after inoculation to eliminate the excess of inoculum and abrasive form leaf surface. During the mechanical transmission test, every possible care was taken to avoid lethal injury to leaves by abrasive or through hand pressure.

For the aphid transmission tests, virus free colonics of aphid species viz., MyzuspersiceaSulz., Aphis craccivoraKoch., A. fabae and Brevicoryn-ebrassicae Linn. Most commonly encountered in and around bell pepper fields were examined for their possibility to act as vectors. Few adults of these species were collected from their healthy host plants and maintained on Capsicum annuumL. and Nicotianatob-acumvar. White Burley in isolation chambers of 3'x3'x3' size covered with nylon net of 80 mesh.

Apterous form of each aphid species were removed from their colonies with gentle tapping and by moist camel hair brush in separate Petri dishes. These were then given one hour pre acquisition access. Sections of leaf tissue infested with 6-10 aphids were placed on the leaf of the test plants. For each isolate, ten plants were inoculated and kept in separate insect proof cage. After 24 hours of inoculation access, the plants were sprayed with 0.1 per cent Malathion to kill theaphids. These plants were observed for 3-4 weeks for symptoms development under glasshouse conditions.

To check the possibility of seed borne nature of isolates, one hundred seeds of variety Capsicum annuum var. California Wonder from fruits of infected plants were collected during 2009. These seeds were sown during the growing season of 2010 in pots having sterilized potting mixture. The plants thus germinated were allowed to grow under insect proof conditions and were observed for symptom expression up to 40 days. Plants raised form seeds collected form healthy plants were kept as control.

III. Results and Discussion

a) Survey and Incidence

Surveys were conducted to determine the occurrence and distribution of tospovirus affecting bell pepper in Solan district of Himachal Pradesh during 2009 and 2010. Typical symptoms were observed on bell pepper. The symptoms included stunting of plants, rossetting of leaves and formation of ringspots (Plate I). Leaves developed concentric rings of different sizes were observed. The bell pepper fruits produced on infected plants were misshapen and developed irregularly shaped brown spots.

During surveys, incidence of ringspot disease was recorded at different vegetable growing areas of Solan district (Table 1). The data presented in table indicates that during year 2009, bell pepper crop was more affected especially in Kandaghat, Kumarhatti, Naganji and Pandah. Though ringspotting symptoms repoted form Pandah, Naganji farm, Kandaghat during year 2010, the incidence of the disease was relatively low than the year 2009.

Table 1 : Incidence of virus disease in major bell pepper growing areas of solan district

SOLAN	Per cent disease incidence (%)	
	2009	2010
Rangah	60	20
Kalaghat	40	20
Kandaghat	90	40
Kumarhatti	50	20
Naganji Farm (UHF, campus)	80	40
Pandah	90	40

b) Collection, Maintenance and Immunoassay of Virus Isolates

Present investigations were based on the ringspot disease in bell pepper. Infected bell pepper samples were collected form Naganji Farm (UHF, Nauni) and Pandah designated as isolates CC-1 and CC-2, respectively (Plate I). The association of tospovirus with infected bell pepper plants was confirmed by biological and immuno-assays. After confirmation, the virus isolates were maintained under glass house conditions on Nicotiana. The virus isolate collected form Pandah was selected and redesignated for further detailed investigations such as symptom-matology, transmission, and serology.These results go in line with the findings of Verhoevenet al (1995) who have also reported Nicotiana sp. to be the best indicator for the maintainance of Tospovirus.

Out of the three polyclonal antisera viz. Pepper veinal mottle virus, Cucumber mosaic virus, Tospovirus (serogroup I, II, III) directed against nucleocapsid (N) protein of different viruses, only tospovirus antisera showedpositive reaction with bell pepperisolate in direct antigen coated (DAC) form of ELISA. The data on optical density (O.D) i.e. absorbance value at 405 nm is presented in table 2. It is evident from the data set out in the table that tospovirus (serogroup I, II, III) antiserum reacted strongly with the virus isolate under study. No positive reaction was observed with other antisera used for detection (Plate II).

Table 2 : Serological reaction of bell pepper virusisolate (pandah isolate) with different antisera in direct antigen-coated enzyme-linked immuno-sorbent assay (DAC-ELISA)

Antiserum	Reaction	Absorption values at 405 nm	
		CC-1	CC-2
Tospovirusserogroup I,II,III	++	0.753	0.943
Pepper veinal mottle virus	-	0.019	0.141
Cucumber mosaic virus	-	0.026	0.207
Positive control	+++	1.021	
Negative control	-	0.114	

(-) = No reaction; (++) = Strong positive reaction; (+++) = Very strong positive reaction

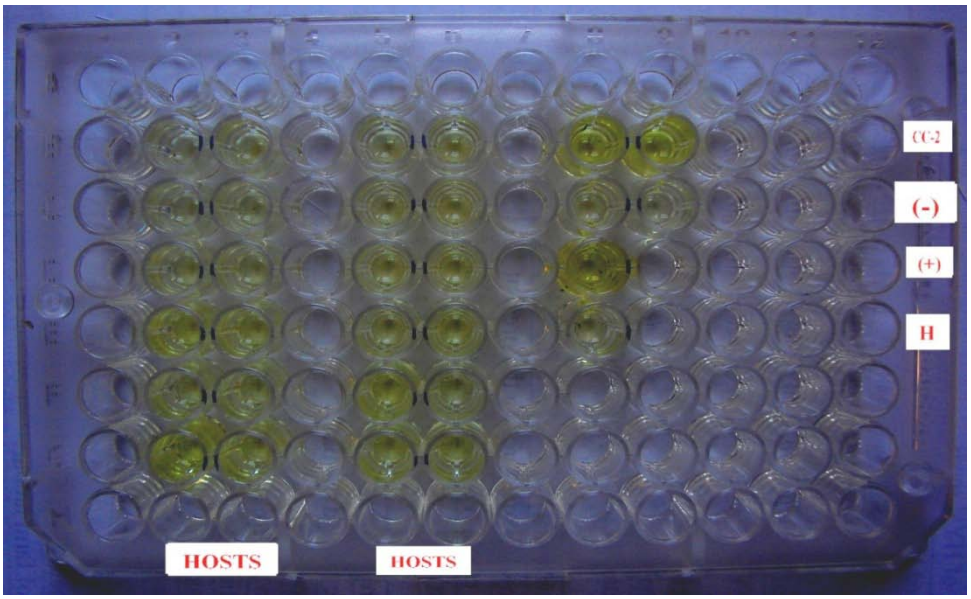
DAS-ELISA tests conducted on both the isolates revealed that the isolates reacted positively and strongly with the antiserum against tospovirus (sero group I, II, III). Since isolate CC-2 had higher O.D. value of 1.012 at A405nm, this isolate was used for further studies (Table 3). On the basis of nucleocapsid (N) protein serology, tospoviruses have been classified into TSWV and WSMV serogroupsand a group containing serologically unrelated viruses (Moyer, 2000). Similar observations were also recorded under present investigations wherein direct DAS and indirect DAC-ELISA were both found to be highly efficient for the detection of the isolates infecting bell pepper.

Table 3 : Serological reaction of different virusisolates in DAS – ELISA

Isolate	Host	Place of Collection	Mean O.D value (A405nm) / serological reaction
CC-1	Bell pepper (C.annuum L.)	Naganji farm	0.945 (++)
CC-2	Bell pepper (C.annuum L.)	Pandah	1.012 (+++)
Positive controle	-	-	2.012 (+++)
Negative Controle	-	-	0.315 (-)

(-) = No reaction; (++) = Strong positive reaction; (+++) = Very strong positive reaction

Serological Detection of Virus Isolate in Different Hosts (DAS-ELISA)



CC-2 = Pandah Isolate; (+) = Positive Control; (-) = Negative Control; B.C. = Buffer Control

c) Symptomatology

The first manifestation of the disease on the inoculated plants was observed after 14-21 days of inoculation. Symptoms of tospovirus vary depending largely upon the age of the plant at the time of infection. Initially, infected plants showed vein clearing, curling, necrotic spots and rings on the leaves. The plants were small and stunted as compared to the healthy plants. In bell pepper, chlorotic and necrotic lesions, vein chlorosis and rugosity followed by leaf chlorosis, severe growth reduction and stem necrosis were the most striking symptoms observed (Plate I). Fruits produced on infected bell pepper plants were misshapen with nail head symptoms (Plate I). Tomato plants showed bronzing, curling, necrotic streaks and spots on the leaves. The ripe fruit shows pale red or yellow areas on the skin often appearing as ringspots of alternate colors. Symptoms resulting from mechanical inoculation were similar to those observed on naturally infected plants (Plate I).Tospovirus infections are generally characterized by a variety of symptoms like necrotic and chlorotic lesions, stunting, systemic necrosis, systemic wilt, spots, mottling, leaf distortion, vein yellowing and ringspot (Peters and Goldbach, 1995; Moyer, 2000). Under the present investigations bell pepper plants showed also similar type of symptoms (plant stunting, rosetted leaves, ringspots, and misshaped fruits with browning and nail head symptoms) during surveys conducted at different localities of Solan district of Himachal Pradesh.

Plate I : Symptoms of Isolates under Field Conditions Collected From Different Localities of Solan District

d) Transmission

i. Transmission Through Sap

The standard extract of the plant virus prepared form infected leaves of bell pepper showing prominent symptoms were inoculated on healthy test plants. The inoculated plants were kept under observations for six weeks for the development of symptoms. The results of the mechanical sap inoculation experiment revealed that the isolate was easily sap transmissible with incubationperiod of 18 to 20 days. Using mechanical inoculation, plant species representing seven families were virus infected. Infected plants showed chlorotic and/or necrotic spots and rings on inoculated leaves, followed by systemic veinal mottle or necrosis. The virus infected systemically many solanaceous species, including D. stramonium, N. rustica, N. glutinosa, Chenopdium album and N. tabacum var. Samsun. These species reacted with local lesions or rings on inoculated leaves followed by mosaic or systemic necrosis (Plate II).

Plate II : Symptoms on Leaves of Different Hosts under Glasshouse Condition

ii. Transmission Through Insect Vectors

In order to test the transmission of tospovirus by Aphids vectors, namely four aphid species Myzuspersicae Sulz, A. craccivora Koch, A. fabae and Brevicoryne brassicae Linn were tested. The results of the experiment have been presented in the table 4 and it is evident from the table that none of the aphid species tested transmitted the virus isolate form infected plants to healthy plants.It is well documented that the tospoviruses are easily transmissible by mechanical sap inoculation and there are no reports that support the transmission of tospoviruses through aphid vectors and seeds (Pappuet al., 1999a).

Table 4 : Transmission of virus isolate by aphids

Aphids	Reaction
Myzuspersicae Sulz.	(-)
A. craccivora Koch.	(-)
A. fabae	(-)
Brevicorynebrassicae Linn.	(-)

(-) = No reaction

IV. Conclusion

During an extensive survey of Solan district, the incidence and distribution of ringspot disease was recorded. Incidence of ringspot disease on bell pepper ranged between 20 to 90 %. Suspected tospovirus infected bell pepper samples from Solan district, showed positive reaction with tospovirus (serogroup I, II, III) antisera in direct antigen-coated enzyme-linked immunosorbent assay. On the basis of serology, the virus isolate under study has been found to be a tospovirusas the antigen reacted positively with tospovirusantiserum (serogroup I, II, III) in DAS- ELISA.

The virus was found to be transmissible through sap but not through aphid vectors and seeds of bell pepper. Bell pepper tospovirusisolatewas mechanically transmitted to different plant species belonging to families Amaranthaceae, Asteraceae, Chenopodiaceae, Cucurbitaceae, Compositae, Malvaceae and Solanaceae. Both localized and systemic infections were observed. These plants species could serve as potential reservoirs of virus under natural conditions. Tospovirus isolate infecting bell pepper was easily transmissible to Nicotianadeneyii, N. tabaccumvar White Burley and N. tabaccumvar Samsun hosts and have been found to be good diagnostic hosts because of the production of distinct localized and systemic symptoms.

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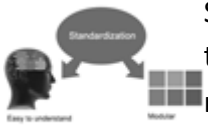

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- Paper Title should be of Font Size 24 with one Column section.
- Author Name in Font Size of 11 with one column as of Title.
- Abstract Font size of 9 Bold, "Abstract" word in Italic Bold.
- Main Text: Font size 10 with justified two columns section
- Two Column with Equal Column with of 3.38 and Gaping of .2
- First Character must be three lines Drop capped.
- Paragraph before Spacing of 1 pt and After of 0 pt.
- Line Spacing of 1 pt
- Large Images must be in One Column
- Numbering of First Main Headings (Heading 1) must be in Roman Letters, Capital Letter, and Font Size of 10.
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You can use your own standard format also.
Author Guidelines:

1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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Research letters: The letters are small and concise comments on previously published matters.

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(b) A brief Summary, "Abstract" (less than 150 words) containing the major results and conclusions.

(c) Up to ten keywords, that precisely identifies the paper's subject, purpose, and focus.

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(e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition; sources of information must be given and numerical methods must be specified by reference, unless non-standard.

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(h) Brief Acknowledgements.

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References

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30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

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Key points to remember:

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- Please note the criterion for grading the final paper by peer-reviewers.

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- To the point depiction of the research
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- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
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- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

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	A-B	**C-D**	**E-F**
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Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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