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Contents

RJM

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Proceedings of Annual Academic Session-2013, Faculty of Medicine, University of Ruhuna

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Cover picture

Hospital Street, Galle Fort, Sri Lanka

In Galle the Portuguese introduced the Misericorda, a house of mercy established by the Franciscan friars to serve the needy and the sick. There were 262 Portuguese families living in the fort. The Misericorda was located in the present court house square. The Portuguese term "espirital" for hospital incorporated into Sinhalese colloquial vocabulary indicates the impact thier few hospitals made upon the people.

Source: *Galle as quiet as asleep* by Norah Roberts
Cover page designed by Dr. Channa Yahathugoda

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Forward

Inaugural Annual Academic Sessions of the Faculty of Medicine, University of Ruhuna

I am very pleased to witness the beginning of Annual academic Sessions of the Faculty of Medicine, University of Ruhuna and the launching of official journal of the Faculty. This is a significant milestone of the academic front of the Faculty which now nearly 33 years old. I hope that this activity will continue in future and grow to bigger dimensions.

In order to gain international recognition, Faculty needs to uplift its academic profile and activities of this nature allow academics to showcase their research findings. This year we have selected six completed PhD/Mphil projects and three abstracts to fill the program. This is of course what the organizing committee considered best, given that it is the first event of this nature in the Faculty and we welcome the comments of academic members to improve the content and quality of the future sessions.

While thanking those involved in organizing this event I wish well for those contributing to the program. Also hope the audience will enjoy the activities of the day.

Professor Sarath Lekamwasam
Chairperson

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Message from the Vice Chancellor

It indeed gives me a great pleasure to send this message for the proceedings of the 1st Annual Academic Sessions of the Faculty of Medicine, University of Ruhuna. It is inspiring to note that the Academic Sessions have now become an annual event in almost all faculties of the University of Ruhuna, fostering and nurturing a research culture among the academics. This is a very good opportunity for the academic staff of the Faculty of Medicine to present their research endeavors.

As the Vice Chancellor of a University committed to academic excellence and high quality research, I am proud to witness a remarkable improvement in quantity and quality as well as relevance of research conducted by our staff. Academic Sessions will facilitate our academics in developing and sharpening their research skills and capability. Therefore I believe that the publication of research findings presented at the Academic Sessions will be an incentive to our academics for future evaluations in their areas of expertise. I wish the 1st Annual Academic Sessions every success.

Professor Gamini Senanayake,
Vice Chancellor, University of Ruhuna, Matara.

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Message from the Dean

Dr. Sampath Gunawardena,
Dean, Faculty of Medicine, University of Ruhuna, Galle.

Review

Upper gastrointestinal tract abnormalities in patients referred for gastroscopy and the prevalence of *Helicobacter pylori* infection among them: a hospital based study in Sri Lanka

Waidyarathne EI
Department of Anatomy, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.

Abstract

Upper gastrointestinal endoscopy is a common procedure in routine clinical practice. This study examines the types of upper gastrointestinal abnormalities, their correlation with histological changes and the prevalence of *Helicobacter pylori* (*H pylori*) infection among a group 251 Sri Lankan patients referred for upper gastrointestinal endoscopy.

Nine gastric mucosal biopsies were obtained from each patient for three diagnostic tests i.e. histology, rapid urease test and culture and 5 ml of peripheral venous blood was obtained for the detection of anti-*H.pylori* IgA and IgG. Three case control studies were performed by comparing patients with peptic ulcer disease, gastro-oesophageal reflux disease and gastritis with age and sex matched, hospital based control groups to asses the risk and protective factors for each disease entity. The quality of life of patient group was compared with a control group using the validated Sinhala translation of WHO Quality of Life BREF (WHO QOL BREF) questionnaire.

Approximately 86% of patients had histological evidence of chronic gastritis. There was a poor correlation between endoscopic and histological gastritis with more than 60% of patients with histological gastritis failing detection endoscopically. The prevalence of *H.pylori* determined by histology was 49.4%. When compared with histology which was taken as the gold standard (either Hematoxyline & Eosin or modified Giemsa positive) for the detection of *H.pylori*, all other diagnostic tests had low sensitivity and specificity. In the three case control studies, the middle socioeconomic group had lesser tendency to develop upper gastrointestinal diseases. While smoking had no effect, alcohol consumption, frequent use of certain groups of drugs and bad food habits significantly increased the risk of upper gastrointestinal diseases. Patients with upper gastrointestinal symptoms showed significantly low scores in physical and psychological domains of WHO QOL BREF.

A majority of patients referred for upper gastrointestinal endoscopy had a clinically relevant abnormality. There was relatively a low prevalence of *H.pylori* infection. Histology using H & E or modified Giemsa stain appeared to be the most reliable technique to detect *H.pylori*. Bad food practices, commonly used drugs and alcohol consumption were significant risk factors for upper gastrointestinal diseases and the symptoms significantly affect the quality of life of patients.

Corresponding author: Waidyarathne EI

<eiwaidya@yahoo.co.uk>

This study was performed in University of Ruhuna, Sri Lanka and the results were included in a thesis with two published papers for a PhD degree with the University of Ruhuna, Sri Lanka and defended the thesis on 11th of June 2008.

Background

Upper gastrointestinal complaints are very common among the general population. Every day, a large number of patients seek medical advice and undergo investigations at government and private sector hospitals for upper gastrointestinal complaints. Upper gastrointestinal endoscopy units in almost every tertiary care hospital in Sri Lanka are overcrowded with patients.

The patients present with a wide spectrum of complaints ranging from regurgitation to haematamesis. Endoscopic findings are either confined to oesophagus, stomach and duodenum or a combination of abnormalities in two or all three sites to a varying degree.

Helicobacter pylori (H.pylori) is a bacterium that inhabits the gastric mucosa and areas of gastric metaplasia in the duodenum. This organism is mostly responsible for chronic gastritis and it is a key etiological factor in the pathogenesis of peptic ulcer disease. Two Australian pathologists, Robin Warren and Barry Marshall isolated the particular organism from gastric tissue for the first time in 1982 (1).

The infection is acquired during childhood, persisting as chronic gastritis if the organism is not eradicated. The exact mode of transmission of the *H.pylori* infection is unclear, but intraepithelial clustering suggests person-to-person spread, either oral-oral or faeco-oral. Due to the progression of gastritis over the years, gastric mucosa undergoes a sequence of changes that may lead to glandular atrophy, intestinal metaplasia, increased risk of gastric dysplasia and carcinoma. The organism is also responsible for gastric mucosa associated B cell lymphoma.

The prevalence of *H.pylori* is high in developing countries ranging from 80%-90% of the population (2-4). More affluent countries show lower prevalence figures around 25-50% (5-7). Reported prevalence data from Sri Lanka showed a wide variation (8-11).

The prevalence of *H.pylori* infection was studied in 251 patients referred for upper gastrointestinal endoscopy at the Teaching Hospital, Karapitiya from November 2005 to June 2007. Age of the subjects ranged from 15-84 years and 134 of them were males.

Patients whose upper gastrointestinal endoscopy is contraindicated due to bleeding disorders, decompensated cirrhosis and those who were on anticoagulant therapy were excluded from the study. In addition to that patients who have taken specific *H.pylori* eradication therapy during the previous 6 months and who have consumed antibiotics active against *H.pylori* during the 4 weeks preceding the endoscopy were also excluded.

During the procedure, nine gastric biopsy specimens (antrum-5, corpus-4) were obtained for investigations. The ethical approval for the study was obtained from the Ethics Review Committee, Faculty of Medicine, Galle.

The association between endoscopic and histological abnormalities

A complete upper gastrointestinal endoscopy was performed in each patient. Of the 251 patients, 175 (69.7%) had abnormal findings in the stomach. The most frequently detected endoscopic abnormality was gastritis. Of the patients who had gastritis, 49 (67.1%), 22 (30.1%) and 2 (2.7%) had pan, antral and corporal gastritis respectively. Ninety (35.9%) patients had abnormalities in the esophagus while only 13 (5.2%) patients had endoscopically detected abnormalities in the duodenum. In one previous Sri Lankan study, gastritis with or without duodenitis was the most frequent abnormality (one third of cases) detected among patients who had non-specific upper abdominal symptoms (12). Duodenal abnormalities were less frequent among the sample population studied. Similarly, in another Sri Lankan study, duodenitis and duodenal ulcer contributed to 8% and 5% cases of upper gastrointestinal bleeding respectively (13).

Five gastric biopsies were obtained for histology. Three were collected from the gastric antrum 2-3 cm away from the pylorus from the distal parts of greater and lesser curves. Two biopsies were obtained from the corpus in a similar manner. Formalin fixed biopsy specimens were processed and stained with both Haematoxylin & Eosin (H & E) and modified Giemsa stains. The histological findings of gastric mucosa were interpreted according to the updated Sydney system (14).

Histological abnormalities were detected in 222 (88.4%) patients. Altogether 217(86.5%) patients had chronic gastritis. Among them 182(83.9%), 29 (13.7%) and 6 (2.8%) had pan, antral and corporal gastritis respectively. Of the 217 patients with histological chronic gastritis, 71 (32.7%) were correctly detected endoscopically while 146 (67.3%) were not detected endoscopically (sensitivity = 0.33, specificity = 0.94) (kappa=0.096).Two patients having gastritis endoscopically had normal gastric mucosa histologically.

Analysis of our study showed that endoscopy had only 32.7% sensititivy in detecting histologically manifested gastritis. However it showed 94% specificity. Poor to moderate sensitivity of endoscopy in detecting gastritis was seen irrespective of its topographic distribution. This observation shows that endoscopic gastritis does not always coincide with histological gastritis. Various gastroscopic features may be interpreted as signs of gastritis, but the significance of such features in relation to histomorphology is less clear in many cases. The macroscopic features of gastritis recorded include erythema, inflammation and erosions of the gastric mucosa, reduced height and increased distance between rugae in the gastric corpus, and presence of visible vessels. Results showed that morphological features recorded were insensitive to detect gastritis in a majority of cases and if gastritis is suspected those patients should be subjected to biopsy and histological

Table 1 Sensitivity and specificity of different *H.pylori* diagnostic techniques when histology (H & E or modified Giemsa) was taken as the gold standard

Test	True Positives	True Negatives	False Positives	False Negatives	Sensitivity	Specificity
Urease test (n=210)	36	67	31	76	32%	68%
Serology (n=125)	3	63	2	57	5%	97%
Culture (n=62)	2	32	1	27	7%	97%
H&E† alone (n=251)	91	127	0	33	73%	100%
Giemsa alone (n=251)	101	127	0	23	81%	100%

†Hematoxylin and Eosin

Table 2 Sensitivity and specificity of *H.pylori* positivity in antrum and corpus when compared with the "gold standard" in which two staining methods are taken together

Test	True Positives	True Negatives	False Positives	False Negatives	Sensitivity	Specificity
H.pylori in antrum (H&E† alone)	88	127	0	36	71%	100%
H.pylori in corpus (H&E† alone)	35	127	0	89	28%	100%
H.pylori in antrum (Giemsa alone)	98	127	0	26	79%	100%
H.pylori in corpus (Giemsa alone)	91	127	0	33	73%	100%

†Hematoxylin and Eosin

Table 3 The association between drug usage and upper gastrointestinal diseases among patients and controls

Peptic ulcer disease		Controls	Cases	P*	OR(95% CI)	P**
Antirheumatic drugs	**never/infrequent use**	110(98.2%)	32(76.2%)		1	
	used in the recent past	2 (1.8%)	10(23.8%)	<0.001	17.18 (3.58 -82.48)	<0.001
Analgesics	**never/infrequent use**	109(97.3%)	28(66.7%)		1	
	used in the recent past	3 (2.7%)	14(33.3%)	<0.001	18.17(4.88 - 67.62)	<0.001
Antibiotics	**never/infrequent use**	112 (100%)	34(81.0%)		1	
	used in the recent past	0	8 (19.0%)	<0.001	46.6(9.48-229.3)	<0.001
Gastro-oesophageal reflux						
Antirheumatic drugs	**never/infrequent use**	266(98.9%)	107(83.6%)		1	
	used in the recent past	3(1.1%)	21(16.4%)	<0.001	17.4(5.08 - 59.5)	<0.001
Analgesics	**never/infrequent use**	264(98.1%)	105(82.0%)		1	
	used in the recent past	5(1.9 %)	23 (18.0 %)	<0.001	11.6(4.28-31.2)	<0.001
Antibiotics	**never/infrequent use**	266(98.9%)	116(90.6%)		1	
	used in the recent past	3 (1.1%)	12 (9.4%)	<0.001	9.17 (2.54-33.12)	<0.001
Gastritis						
Antirheumatic drugs	**never/infrequent use**	282(98.6%)	140(82.4%)		1	
	used in the recent past	4 (1.4%)	30 (17.6%)	<0.001	15.1(5.2 - 43.6)	<0.001
Analgesics	**never/infrequent use**	280(97.9%)	131(77.1%)		1	
	used in the recent past	6 (2.1%)	39 (22.9%)	<0.001	13.8 (5.7 - 33.6)	<0.001
Antibiotics	**never/infrequent use**	283(99.0%)	145(85.3%)		1	
	used in the recent past	3 (1.0%)	25 (14.7%)	<0.001	16.21 (4.82 -54.50)	<0.001

P*= contrasts proportions in two groups and calculated using Chi-square test.
P**= P for odds ratios

Table 4 The association between social habits and upper gastrointestinal diseases among patients and controls

Peptic ulcer disease		**Controls**	**Cases**	**P***	**OR(95% CI)**	**P****
Smoking	never	82(73.2%)	30(73.2%)		1	
	ever	30(26.8%)	11(26.8%)	1	1.00(0.45 -2.24)	0.98
Alcohol consumption	never	84(75.0%)	25(61.0%)		1	
	ever	28(25.0%)	16(39.0%)	0.09	1.92(0.89 - 4.10)	0.11
Gastro-oesophageal reflux						
Smoking	never	209 (77.7%)	92 (72.4%)		1	
	ever	60 (22.3 %)	35 (27.6 %)	0.26	1.33(0.82 - 2.15)	0.26
Alcohol consumption	never	203 (75.5%)	82 (64.6%)		1	
	ever	66 (24.5%)	45 (35.4%)	0.031	1.69 (1.07 - 2.67)	0.03
Gastritis						
Smoking	never	217(75.9%)	118(69.4%)		1	
	ever	69 (24.1%)	52 (30.6%)	0.081	1.39 (0.91 - 2.12)	0.15
Alcohol consumption	never	211(73.8%)	102(60.0%)		1	
	ever	75 (26.2%)	68 (40.0%)	0.002	1.88 (1.25 - 2.81)	0.002

P*= contrasts proportions in two groups and calculated using Chi-square test.
P**= P for odds ratios

examination even though endoscopic findings are negative.

Prevalence of *H.pylori* infection

The prevalence of *H. pylori* infection was determined by histology. A particular patient was identified as positive when either H & E or Giemsa stained histology demonstrated morphological evidence of *H. pylori* in at least one biopsy specimen.

Overall prevalence of *H. pylori* in the study sample was 49.4%. This contradicts the popular belief that *H.pylori* infection is very high among patients in developing countries. Fernando *et al* (15) have shown a similar prevalence of *H.pylori* (46%) among 100 consecutive Sri Lankan patients with upper gastrointestinal disease. Other studies from Sri Lanka have reported similar low prevalence figures of 30-40% (16-18). In contrast to above studies, one Sri Lankan study using PCR reported a higher prevalence of 75.4% of infection among 57 dyspeptic individuals (9). Histologically, 52.5% of patients with chronic gastritis and 41.4% patients with gastric ulcers had *H.pylori*. According to the distribution of gastritis, the prevalence of *H. pylori* in pangastritis and antral gastritis was 53.3% and 48.3%, respectively. The number of duodenal abnormalities was too small to determine the prevalence of infection in different duodenal diseases. Even though *H.pylori* is the key etiological factor in chronic gastritis, only 52.5% of our patients with chronic gastritis had *H.pylori*. These figures are relatively low when compared with other Asian countries (2-4). *H.pylori* infection is known to be associated with low socioeconomic status, overcrowding, unhygienic practices and poor sanitation. Sri Lanka has better health care indices compared to other South Asian countries and that may explain the low prevalence observed. Furthermore, some culinary and medicinal plants used in Sri Lankan cooking (e.g. tumeric, cumin, ginger etc) have shown bactericidal and anti-adhesive properties against *H.pylori* (19).

The patients with histological evidence of gastritis (52.5%) had a higher prevalence of *H.pylori* infection when compared to patients with other diseases (P=0.012). Furthermore, patients with histological evidence of pangastritis (53.3%) had higher prevalence of *H.pylori* when compared to patients with antral gastritis (48.3%), but the difference was not statistically significant (P=0.61).

Hence, it seems that *H.pylori* infection in Sri Lankan patients is associated with histologically manifested gastritis but not with its topographical distribution. Small sample size may have limited the significance of our analysis. The well known association between antral gastritis and *H.pylori* infection observed in other studies published in Asian and non-Asian countries was not apparent in this study (20, 21).

Sensitivity and specificity of diagnostic tests to detect *H.pylori* infection.

Several tests are available for the diagnosis of *H.pylori* infection. Depending on the need of endoscopy they are categorized into two groups; endoscopic dependent and endoscopic independent tests. Endoscopic dependent tests include histology, rapid urease test and culture. Endoscopic independent tests include urea breath test, serology and *H.pylori* stool antigen tests. Molecular diagnostic tests can either be dependent or independent of endoscopy depending on the type of material used for the diagnostic purposes.

Four diagnostic tests were used to detect the infection in this study i.e histology, rapid urease test, culture and serology.

Histology was performed in all patients. Formalin fixed biopsy specimens were processed and stained with both H & E and modified Giemsa stains. The histological findings of gastric mucosa were interpreted according to the updated Sydney system (14).

Urease test was performed in 210 patients. For this purpose two biopsy specimens each from antrum and corpus were immersed in homemade urea solution (22). The solution contained a mixture of urea, sodium chloride, potassium dihydrogen phosphate and phenol red as the indicator. Urease produced by *H.pylori*, converted urea to ammonia giving a change in colour in the solution. A positive test was indicated by yellow to pink colour change observed within 6-12 hours after the immersion of the biopsy specimens.

Culture was performed in 62 patients. Two biopsy specimens each from antrum and corpus were collected into 0.9% sterile saline stored at 4^0C. Soon after the collection the specimens were inoculated into Columbia agar base (CM331) supplemented by laked horse blood (SR 48) and *H.pylori* selective supplement (SR 147). The culture plates were incubated for 3 days at 37^0C under microaerophilic conditions (5%O_2, 10% CO_2, 85% N_2) using special gas kits (CampyGen CN0025A). On the 4^{th} day, positive cultures were identified by colony morphology, Gram stain and biochemical testing (positive uraese, catalase and oxidase activity). Positive colonies appeared discrete, translucent and non coalescent and Gram stain revealed Gram negative curved bacilli. Subcultures were performed as required.

Out of the 251 patients, a randomly selected subgroup of 125 underwent Enzyme –Linked Immuno-Sorbent Assay (ELISA) for the detection of anti-*H.pylori* IgA and IgG antibodies in plasma. For this purpose, 5 ml of peripheral venous blood was collected to EDTA bottles. The blood samples were centrifuged, plasma was separated and anti *H.pylori* IgA and IgG were measured using ELISA test kits (Human Gesellschaft fur Biochemica und Diagnostica GmbH, Germany).

Histologically, the prevalence of *H.pylori* in this referred sample was 49.4%. Culture isolated *H.pylori* from three patients. Positive urease activity was demonstrated in 67 patients either or both in antrum and corpus. Four patients were positive for IgG only while one patient was positive for both IgG and IgA.

The sensitivity and specificity of each diagnostic test was calculated taking histology (either H & E or Giemsa positive) as the gold standard. According to the gold standard the sensitivity and specificity of diagnostic methods are summarized in table 1.

Histology was the most sensitive method for the detection of *H.pylori* among these patients. Giemsa staining showed a marginally better sensitivity when compared to H & E. Urease test showed a moderate sensitivity and specificity while culture and serology had lower sensitivity. As histology was taken as the reference, both staining methods showed 100% specificity. Serology and culture also showed a very high specificity while urease test had the lowest specificity.

There is no gold standard for the detection of *H.pylori* in clinical practice. We considered histology as the gold standard in our study. Modified Giemsa stain was used as a special stain to improve the sensitivity and specificity of diagnosing *H.pylori* as recommended by the updated Sydney system (14). As the chosen gold standard was based on these two techniques, both stains, as expected, showed 100% specificity. Our findings support the fact that modified Giemsa stain is reliable, less technically demanding and easily reproducible amongst many available staining techniques for *H.pylori* (23).

According to our data, histology from multiple biopsies representing different areas of the stomach increases the detection of *H.pylori*. If endoscopist has a practical limitation in obtaining multiple biopsies, a single biopsy obtained from antrum and stained with modified Giemsa gives an acceptable sensitivity to detect the organism (Table 2).

The rapid urease test when compared to histology, showed relatively low specificity and sensitivity, 32% and 68% respectively. This is not in line with many other studies where the rapid urease test has reported high specificity and sensitivity (24,25).

Table 5 The association between food habits and upper gastrointestinal diseases among patients and controls

		Controls	Cases	P*	OR(95% CI)	P**
Peptic ulcer disease						
Consumption of tea	None/occasional	11 (9.8%)	13(31.0%)		1	
	≥ 2 cups /day	101(90.2%)	29(69.0%)	0.002	0.24 (0.10 -0.59)	0.002
Consumption of acidic/spicy foods	infrequent	51(46.4%)	19(46.3%)		1	
	frequent	59 (53.6%)	22(53.7%)	1	1.00(0.49 - 2.05)	0.98
Missing/delaying meals	no	71(64.5%)	13(31.7%)		1	
	yes	39(35.5%)	28(68.3%)	<0.001	3.92(1.82 - 8.43)	<0.001
Frequent intake of fruits/vegetables	no	81(73.6%)	22(53.7%)		1	
	yes	29(26.4%)	19(46.3%)	0.019	2.41(1.14 -5.09)	0.03
Frequent intake of starchy foods	no	63(57.3%)	26(63.4%)		1	
	yes	47(42.7%)	15(36.6%)	0.495	0.77 (0.37 -1.62)	0.58
Satisfactory intake of dairy foods	no	92(82.9%)	33(80.5%)		1	
	yes	19 (17.1%)	8 (19.5%)	0.732	1.17(0.47 -2.94)	0.81
Gastro-oesophageal reflux						
Consumption of tea	None/occasional	22 (8.2%)	33(25.%)		1	
	≥ 2 cups /day	247(91.8%)	95(74.2%)	<0.001	0.25(0.14-0.46)	<0.001
Consumption of acidic/spicy foods	infrequent	123(46.1%)	44(34.6%)		1	
	frequent	144(53.9%)	83(65.4%)	0.038	1.61(1.04 -2.49)	0.038
Missing/delaying meals	no	164(61.4%)	32(25.2%)		1	
	yes	103(38.6%)	95(74.8%)	<0.001	4.72 (2.95 -7.57)	<0.001
Frequent intake of friuts/vegetables	no	184(68.9%)	76(59.8%)		1	
	yes	83(31.1%)	51(40.2%)	0.088	1.48(0.96-2.31)	0.089
Frequent intake of starchy foods	no	159(59.6%)	61(48.0%)		1	
	yes	108(40.4%)	66(52.0%)	0.039	1.59(1.04 - 2.44)	0.043
Satisfactory intake of dairy foods	no	212 (9.1%)	103(81.1%)		1	
	yes	56 (20.9%)	24(18.9%)	0.69	0.88(0.52 -1.50)	0.68
Gastritis						
Consumption of tea	None/occasional	24 (8.4%)	43 (25.3%)		1	
	≥ 2 cups /day	262(91.6%)	127(74.7%)	<0.001	0.27(0.15 - 0.47)	<0.001
Consumption of acidic/spicy foods	infrequent	128(45.1%)	52 (31.1%)		1	
	frequent	156(54.9%)	115(68.9%)	<0.002	1.82 (1.21 -2.71)	0.004
Missing/delaying meals	no	171(60.2%)	43 (25.7%)		1	
	yes	113(39.8%)	124(74.3%)	<0.001	4.36 (2.87 - 6.64)	<0.001
Frequent intake of friuts/vegetables	no	192(67.6%)	104(62.3%)		1	
	yes	92 (32.4%)	63 (37.7%)	0.15	1.26 (0.85 - 1.88)	0.25
Frequent intake of starchy foods	no	171(60.2%)	81 (48.5%)		1	
	yes	113(39.8%)	86 (51.5%)	0.01	1.61 (1.09 - 2.36)	0.016
Satisfactory intake of dairy foods	no	223(78.2%)	133(79.6%)		1	
	yes	62 (21.8%)	34 (20.4%)	0.41	0.91(0.57 - 1.47)	0.81

P*= contrasts proportions in two groups and calculated using Chi-square test.
P**= P for odds ratios

Table 6 Comparison of the quality of life of patients with upper gastrointestinal symptoms and the control group on the dimensions of the WHOQOL-BREF

Domain			Mean	SD	95% Confidence Interval for Mean		F	P value
					Lower	Upper		
Physical	Patient	(n=126)	54.4	17.7	51.3	57.5	45.0	0.000
	Control	(n=200)	67.1	16.0	64.9	69.4		
Psychological	Patient	(n=123)	62.1	17.6	58.9	65.2	15.7	0.000
	Control	(n=179)	69.4	14.6	67.3	71.6		
Social relationship	Patient	(n=121)	60.6	21.7	56.7	64.5	1.2	0.283
	Control	(n=169)	63.3	21.0	60.1	66.5		
Environment	Patient	(n=124)	63.0	15.0	60.3	65.6	1.5	0.221
	Control	(n=176)	65.0	13.0	63.0	66.9		

Table 7 Effect of the *H.pylori* density on the quality of life among the symptomatic patients

Domain			Mean	SD	95% Confidence Interval for Mean		F	P value
					Lower	Upper		
Physical	None	n=68	56.6	17.2	52.5	60.8	1.8	0.166
	Mild	n=24	53.0	18.1	45.4	60.7		
	Moderate	n=25	48.9	18.3	41.3	56.5		
Psychological	None	n=67	65.1	16.5	61.1	69.1	3.2	0.044
	Mild	n=23	59.4	19.3	51.1	67.8		
	Moderate	n=24	55.2	17.3	47.9	62.5		
Social relationship	None	n=65	68.0	18.7	63.4	72.6	10.1	0.000
	Mild	n=23	55.8	21.8	46.4	65.2		
	Moderate	n=25	48.7	19.0	40.8	56.5		
Environment	None	n=67	66.2	14.5	62.6	69.7	5.2	0.007
	Mild	n=23	60.1	17.2	52.7	67.5		
	Moderate	n=25	55.7	11.5	50.9	60.4		

Since the urease solution was sterile and sterility was maintained during the biopsy procedure, contamination is unlikely for false positive urease test. Studies have shown that hypochlorhydric patients could harbor many urease-positive bacteria in gastric mucosa other than *H. pylori* (26). The strong urease activity they possess could be the reason for false positive results.

Even though many studies have shown the sensitivity of urease test to be around 80-90%, the sensitivity observed is lower. The sensitivity can vary with the site chosen for biopsy due to patchy distribution of the infection. In one study the gastric angle site was positive in 100%, while the prepyloric and corpus sites were positive in 87% and 84.4%, respectively (27). Hence a false negative test could occur due to sampling error. Satarasinghe, *et al* (16) have postulated that Sri Lankan *H.pylori* strains are different from strains found elsewhere that they have shorter survival and they produce inconspicuous rapid urease results.

Infection with *H. pylori* induces several antibodies; anti *H.pylori* IgG, IgA and less frequently IgM. Anti *H.pylori* IgM can be detected shortly after infection is acquired and IgG and IgA antibodies indicate a chronic infection. In most studies the prevalence of infection has been determined by IgG-type antibodies. Detection of serum IgA or IgM is known to have poor

discriminatory value when compared to serum IgG. Some investigators have reported a subset of patients who are positive for IgA but negative for IgG antibodies for *H.pylori* (28).

In serology, the two cases with false positive results observed may have had a previous infection with *H.pylori* which resulted in persistently elevated antibody levels. Due to the patchy distribution of *H.pylori* a sampling error could also have been the cause for the false positive results. A similar picture was observed in elderly people where the progression of atrophic gastritis has spontaneously eliminated the organism while a detectable level of antibody was observed in serum (29).The false negative results could be due to many reasons. It was reported that certain test kits were not successful to detect the infection in certain populations (30). In this study plasma instead of serum was used. Assurance was given by the manufacturer that both plasma and serum would give the same result when the test kit was used (31).

Culture of bacterium which is urease, oxidase and catalase positive from gastric biopsy specimens is a definitive proof of *H pylori* infection. However, the ability to isolate the organism from infected subjects varies widely among laboratories. That makes culture the most technically demanding *H pylori* diagnostic test. Biopsy specimens must be rapidly transferred to the laboratory in chilled transport medium. Upon the receipt, the sample is ground or minced to produce a homogenate which is inoculated on to freshly prepared media. When these fastidious requirements are met, culture yields positive results.

Even after fulfilling all these requirements, the sensitivity of culture varies among laboratories. Moayyedi, and Dixon (32) have performed *H.pylori* culture with the sensitivity and specificity of 90.5% and 99.2% respectively. In one study that compared eight different methods for detection of *H.pylori*, culture revealed 55.9% sensitivity with 100 % specificity (33).

Administration of drugs such as antibiotics, omeprazole or Bismuth– containing drugs, three months prior to the culture are likely to provide negative results (34, 35). Recent users of proton pump inhibitors were not excluded in our study and this may have contributed to negative cultures. Furthermore, commonly used non-steroidal anti-inflammatory drugs (NSAIDs) can also inhibit the growth of *H.pylori* in vitro (36).

Cost of *H.pylori* diagnostic tests

Of the four tests performed, histology was the most expensive test. It costs around 4 - 5 US Dollars (USD) per test to purchase consumables. Approximately 2 hours was required to complete the test for one patient. The estimated cost for man power for one test was 2 USD and therefore the total cost of the test was between 6 -7 USD.

Many western countries, where the prevalence of infection is low, do not use histology in their routine clinical practice. However, in Sri Lanka endoscopy and histology are performed free of charge in most of the government owned tertiary care hospitals around the country. Hence, the cost factor has not limited the diagnostic utility of this investigation.

Serology was the second most expensive test. On average the consumable cost for one serology test was 3.8 USD. Out of the total time required to perform the test, approximately one hour was utilized for the direct involvement of man power. The estimated cost for man power per one serology test was approximately 1 USD and the total cost was 4.8 USD.

Culture was third in place when the total cost was compared. But it was more time consuming than serology. It required around 2.9 USD for consumables to perform the culture for one patient. On average, it required about 1.5 hours to perform the culture. The approximate cost for man power to perform one culture test was 1.6 USD. The total cost was 4.5 USD.

Urease test was the cheapest of all tests. Since the home-made urease solution was used, it required less than 0.2 USD to prepare 100 ml of the solution and the time spent was approximately one hour. One milliliter of this solution was required per test, therefore the approximate cost of man power to perform one urease test was 1 USD. Hence the total cost for urease test was 1.2 USD.

There were certain limitations in direct and indirect cost calculations. For histology, when calculating the reagent volumes required for staining techniques, total calculated volume was more than the true volume that was required. The reason is that once a staining procedure was performed, reagent tanks were refilled without waiting for the tanks to be emptied. So the estimate tends to be more than the actual value. Furthermore, in all four diagnostic tests there were varying time periods with minimal personnel involvement e.g. tissue processing time in histology, incubation periods in serology and culture. This was not taken into consideration when calculating indirect costs. So the estimated time was less than the actual number of hours taken to perform the test.

The risk factors of peptic ulcer disease, gastro-oesophageal reflux disease and gastritis - three case control studies

Upper gastrointestinal diseases are known to be associated with many risk factors. At the same time there are many protective factors as well. However, Sri Lankan studies are scarce on this aspect. There are

various beliefs and practices in the society based on personal experiences of individuals but no documented data are available. The impact of several possible risk and protective factors in three upper gastrointestinal diseases; peptic ulcer disease, gastro-esophageal reflux and gastritis was studied by comparing the patient group with a control group.

A hospital based control group (n=350) was selected from attendees to the out patients department of the same hospital seeking treatment for brief illnesses. Those who sought medical advice, those who have taken medication for, and those who have experienced any upper gastrointestinal symptom during the previous year were excluded from the study. Two controls were selected for one case approximately after stratifying for age (within ten years) and sex.

Patients from both test group and control group were interviewed and basic information such as age, sex, occupation, level of education were recorded with presenting complaint, past medical history, drug history, social habits, food habits, sanitary practices etc. At the end of the interview, a brief clinical examination was performed. The height and weight were measured and blood pressure and pulse rate were recorded while resting.

Risk and protective factors for peptic ulcer disease

The impact of risk factors on the development of peptic ulcer disease has been shown to vary among different populations. Factors such as *H. pylori*, smoking, alcohol use, and NSAIDs use are well documented risk factors for peptic ulcer disease (37, 38).

Cases for this part of the study were selected from the study sample. There were 56 patients with peptic ulcer disease (male/female: 31/25) and they were compared with 112 controls (male/female: 62/50).

Cases and controls had significant differences with regards to socio economic status, type of drinking water and physical activity. Smoking and alcohol consumption were not different between the two groups. When compared with the higher socio economic group, people in the middle group had lesser tendency to develop peptic ulcers. People who did not drink boiled cooled water regularly had lesser chance of having ulcers when compared with people who regularly drank boiled cooled water. Similarly less physical activity was seen as a risk factor for ulcer disease. Anti-rheumatic and analgesic use increased the risk of peptic ulcer disease. Apart from frequent fruit/vegetable consumption and missing/delaying meals which increased the risk of peptic ulcer disease, other food habits examined in this study showed no association.

Studies have shown that aspirin has a significant impact on both duodenal ulcer and gastric ulcer (38). Even though smoking has not being a significant risk factor among the group of patients used in this study, it is a well established risk factor for peptic ulcer disease among both men and women (39, 40). In this analysis, those who belonged to the middle social class had a less chance of having a peptic ulcer disease. One reason for this could be due to the less stress they face by being in the middle social class. Räihä *et al*(40) suggested that stress is a significant predictor of peptic ulcer disease. They reported an association between the self – reported stress involved with daily activities and peptic ulcer disease. So the less mental stress generated by being in the middle social class may have been a protective factor.

Adaptation of healthy practices had no significant protective effect against peptic ulcer disease among our patients. In this study, healthy practices such as personal hygiene, proper disposal of faeces and hygienic garbage disposal were considered. Deficiency of most of these practices has an effect on transmission of *H.pylori* infection. There are many studies that highlighted the importance of sanitary practices on transmission of *H.pylori* infection (41, 42).

Gastro-oesophageal reflux disease

Gastro-oesophageal reflux disease was diagnosed according to the criteria described by Devault and Castell (43).There were 142 patients with gastro-oesophageal reflux (Male/Female: 75/67) and they were compared with 269 controls (Male/female: 147/122).

When compared with the higher socioeconomic class, subjects in the middle class had a lesser risk in developing the reflux disease. Compared with regular consumers of boiled cooled water, people who never consumed boiled cooled water had a lesser risk of getting the disease. While smoking had no effect, alcohol consumption increased the risk of reflux. Consumption of tea more than twice a day reduced the risk of reflux while intake of anti-rheumatics, analgesics or antibiotics and missing/delaying meals predisposed the reflux disease. While frequent consumption of fruit/vegetables had no effect, frequent intake of spicy or starchy food increased the risk of the disease.

Obesity has long been considered to cause gastro-oesophageal reflux. The mechanism by which obesity causes reflux is not clear, although there is some limited data suggesting that hiatus hernia may be the causal link between obesity and reflux (44). However in this study there is no significant difference between the mean body weight of the cases and controls.

Consistent with the studies reported, alcohol consumption has been a significant risk factor in our group of patients (45).

Among the bad food practices, missing or delaying meals frequently was a significant risk factor identified in this study. Many studies have shown an association between food habits and reflux disease. In some studies, the prevalence of reflux disease was significantly lower in subjects taking fruit and vegetables frequently (45, 46). But this association was not seen among our subjects.

Sedentary life style was identified as a significant risk factor in this study. Compared to very active category, subjects in less active groups had more chance of developing reflux disease. Studies carried out elsewhere support this protective role of physical activity on reflux disease (44, 45). Some suggest that physical activity at work appears to be a risk factor for frequent reflux symptoms, whereas recreational physical activity appears to be beneficial against the development of reflux disease (47).

Being in the middle social class was a protective factor for gastro-oesophageal reflux disease. Less stress associated with middle class life style would be the plausible explanation. In previous studies, psychological, physical and social stresses were known to increase reflux symptoms (46, 48).

Gastritis

Apart from the etiological agent *H. pylori*, other risk factors of gastritis are not well known. Factors that promote non *H.pylori* gastritis except alcohol have not been studied extensively. There are different beliefs among people that some life style practices induce gastritis.

There were 217 patients with histologically confirmed gastritis (Male/Female: 117/100).They were compared with 286 controls (Male/Female: 160/126).

In our sample of patients, being in the middle social class, frequent consumption of tea and healthy practices during day to day activities were significant protective factors against the development of gastritis. Bad food practices such as frequent consumption of acidic/spicy food, frequent missing or delaying meals and frequent consumption of starchy foods were seen as significant risk factors for the development of gastritis. Sedentary life style and consumption of alcohol too were significant risk factors for gastritis. Drinking of ordinary water instead of boiled cooled water had a significant protective effect against the development of gastritis. Use of drugs in the recent past such as anti-rheumatic drugs, analgesics and antibiotics appeared to be significant risk factors for the development of gastritis. Alcohol induced gastritis is a well known phenomenon in etiology of gastritis as shown in many studies (49, 50). This study also showed that alcohol is a significant risk factor.

There are some studies that favour the increased association between acidic and spicy foods with gastritis. Atisook *et al* (51) showed that dietary habits of Thai had a greater influence on gastritis. Intake of hot and spicy food and capsicum was related to gastritis in the Thai population. They also suggested that seafood and fruit may be protective against mucosal injury induced by hot and spicy food.

The use of drugs such as NSAIDs and analgesics has been shown to increase the risk of gastritis. Atisook *et al* (51) has identified NSAIDs as another risk factor inducing gastritis among Thais. Their study suggested that the nationwide incidence of pangastritis correlated well with common use of aspirin and non prescribed medications.

There are no studies to support that physical activity has a direct relationship with gastritis. Yet there are evidence to suggest that excessive physical exertion result in upper gastrointestinal disturbances that has been observed in long distance runners (52). Since recreational runners were not affected by such disturbances, regular physical activity may not generate gastrointestinal disturbances (53). The psychological stress associated with sedentary life style may have contributory effects on gastritis. In the group of gastritis patients used for the study, those who belonged to the middle social class produced less stress and therefore more protective against gastritis.

The association of drug usage, social habits and food habits with the three upper gastrointestinal diseases is summarized in tables 3, 4 and 5 respectively.

Measurement of quality of life in patients with upper gastrointestinal symptoms; a comparative study

Upper gastrointestinal symptoms are very common in clinical practice. However the effects of these long term symptoms on the physical, psychological, social and environmental well being are not well known. There are sufficient studies to indicate that upper gastrointestinal symptoms are associated with poor QOL in affected individuals. However head to head comparisons of these studies are not possible as they have different entry criteria and have used different scales. In this study, the validated Sinhala translation of World Health Organization Quality of Life BREF (WHO QOL BREF) questionnaire was used on 126 patients of the same patient group and 200 controls to study the impact of these symptoms (54). As it contains only 26 questions representing the four domains, this scale is convenient to be used in busy endoscopy setups. Further, it takes less time to fill the information and the patient acceptance was also good.

Both groups completed the questionnaire that asses QOL in four domains; physical, psychological, environmental and social relationship.

The overall QOL for patient group was 59.9 whereas the overall QOL for control group was 66.6 (p <0.001). Results showed that in all four domains, patients scored less than controls indicating that patients had poor QOL when compared to controls. Differences in physical (p <0.001) and psychological (p <0.001) domains were wider and were statistically significant. Although the differences in social relationship and environment domains showed similar trends, they were not statistically significant (Table 6). Most of the available scales were confined to measure the quality of life in specific disease categories such as the gastro-oesophageal reflux disease, but not in a broader spectrum of people with common symptoms. Madisch *et al* (55) described the impact of heartburn on patients' Health-Related Quality of Life (HRQOL) using the validated Quality of Life in Reflux and Dyspepsia questionnaire (QOLRAD). The questionnaire had domains for daily functioning, impaired vitality, emotional distress and sleep disturbance. They found that overall HRQOL was impaired across all domains and stated that there was consistent evidence to believe that heartburn substantially impairs all aspects of health-related quality of life. Kinoshita *et al* (56) also reported that as in Western countries, QOL of the Japanese patients with gastro-oesophageal reflux disease was significantly decreased in comparison to those of healthy individuals.

The effect of density of *H.pylori* colonization on quality of life.

The association between *H.pylori* density and quality of life of the patients within the group was also studied. The density of *H.pylori* colonization within the patient group was graded as none, mild and moderate.

There was no significant difference in the mean scores of physical (P=0.31) domain. However there was a significant difference in the mean scores of social (P<0.001), environmental (P=0.01) and psychological (P=0.044) domains depending on the degree of *H.pylori* colonization (Table7). This might be due to the known association of *H.pylori* infection with poor socioeconomic status and unhealthy environment (57, 58). People who belonged to less privileged groups probably had higher tendency to carry *H.pylori* and therefore when their QOL was analyzed the particular association of poor socioeconomic status and unhealthy environment with the disease might have been revealed.

The significant effect of *H.pylori* density on the psychological domain could be related to the stress. We can hypothesize that when the *H.pylori* density is more, the severity of dyspeptic symptoms would be more resulting in psychological stress.

Moayyedi *et al* (59) found that *H. pylori* is significantly associated with dyspepsia and suggested that the organism may be responsible for 5% of upper gastrointestinal symptoms in the community. Animal studies have demonstrated that *H. pylori* infection influences the development of gastric mucosal injury in the early phase of stress exposure (60). Therefore stress and *H.pylori* seem to exert their effects in a vicious cycle. Yet a controversial theory put forward by Stone et al (61) indicates that *H. pylori* infection does not play an important role in overall symptoms of dyspepsia in a community.

Conclusions and recommendations

This study reveals that more than 80 % of the patients referred for upper gastrointestinal endoscopy had an abnormality. Although national guidelines for endoscopy were not developed, our findings show that current referral system is appropriate and yields more positive than negative results. This reflects that clinicians use the endoscopy service judiciously to avoid unnecessary referrals. Furthermore, endoscopy provided information which could influence the short term and long term management of many patients. Therefore it is a very beneficial investigation for our clinical settings.

Among all the abnormalities detected, gastric abnormalities were commoner. Most common abnormalities found were gastritis and gastric ulcers. The third most common abnormality was gastro-oesophageal reflux disease. Duodenal abnormalities were rare among our patients.

In the sample population, 86% of patients had histological evidence of gastritis but endoscopy missed the diagnosis in about 60% of the patients with histological gastritis. Hence endoscopically normal mucosa does not exclude gastritis. If gastritis is suspected on clinical grounds or is considered in the differential diagnosis, a biopsy should be obtained for histological examination even if gastric mucosa appears to be normal on endoscopy.

The overall prevalence of *H.pylori* infection among the patients was 49.4%. This is very much less than expected. Most of the developing countries in Asia especially our neighbouring countries such as India, Pakistan and Bangladesh record high prevalence rates. Even more affluent countries in the region such as China and Japan also have reported relatively high prevalence rates. Our prevalence figures are closer to those of Western countries.

The prevalence of infection among different disease categories was found to be low in the sample studied. Reports from other countries, both developed and less developed, indicate that the infection is highly prevalent among the patients with gastritis and gastric ulcers. In the sample population, the prevalence of infection was around 50% for both disease categories. The low prevalence of infection, question the role of *H.pylori* as an etiological agent in upper gastrointestinal diseases in our country. Factors that prevent *H.pylori* colonization of the gastric mucosa should be explored further. If food or hygiene related factors are found to be responsible for the low prevalence of infection among Sri Lankans, they can be used to control the infection in other populations.

Generally the disease is known to be related to low socio-economic status and poor sanitation. Improvement of socio-economic status and sanitary practices in the recent past may have limited the transmission of infection. Most Sri Lankans have easy access to healthy drinking water. In all three case control studies, people who drank water without boiling had a low prevalence of major three upper gastrointestinal diseases. Whether unboiled water has a factor which prevent *H.pylori* colonization and whether it gets destroyed during the process of boiling should be considered.

There may be certain genetic factors that prevent the infection. Since the majority of the study sample constituted of Sinhalese, a genetic factor protecting them from infection is a possibility. However there is no such evidence to prove this theory. Certain culinary and medicinal herbs used in Sri Lanka might play a role against *H.pylori*. As demonstrated by studies their bactericidal and anti- adhesive properties against *H.pylori* could be protecting Sri Lankan people from colonization by this bacterium. We recommend further studies in this area.

Since *H.pylori* is associated with a little more than 50% of people with gastritis and gastric ulcers, blind anti *H.pylori* therapy in these cases is not justifiable. Therapy should be given only to those with demonstrable *H.pylori* preferably in the histology specimen stained by the modified Gimsa staining. Histology appears to be the most reliable way of detecting the organism in our settings. Endoscopist should make an attempt to obtain multiple biopsies representing different areas of the stomach to increase the detection of *H.pylori* and to get a better idea about the musocal changes. If endoscopist has a practical limitation in obtaining multiple biopsies, single biopsy obtained from antrum and stained with modified Gimasa can be recommended. The appropriateness of the urease test which is the second most common method of detecting *H.pylori* in our hospitals needs to be reconsidered. Although histology takes more time and relatively expensive, it appears to be the best method for the detection of the organism. The yield of the histology can be augmented by having the services of a histopathologist who has a special interest in this area due to the difficulty in recognizing the slender, thin organism unless special attention is given to locate them.

Many risk factors for gastritis other than *H.pylori* were identified in this study. There are commonly used drug groups such as analgesics, anti-rheumatics and antibiotics that were identified as significant risk factors for gastritis in the sample population. Many of these drugs can be obtained in Sri Lanka without a doctor's prescription. Therefore inappropriate use of these drugs is very common among Sri Lankan people. Alcohol was identified as a significant risk factor for gastritis. Many bad food habits practiced by Sri Lankans also act as significant risk factors. Skipping and delaying meals, frequent use of spicy and acidic foods, and consumption of starchy foods contributed significantly. This study shows that there are many factors that can be used to prevent people from getting gastritis other than eradicating *H.pylori*. Discouraging the indiscriminate use of drugs, modification of lifestyle practices and proper eating habits are important components in the management of gastritis. Skipping and delaying meals are common practices among school children and working class. Reflux symptoms are common among these groups and they should be advised to take meals at regular intervals.

According to the study presented here, upper gastrointestinal symptoms cause significant morbidity. The symptoms have a significant impact on the physical and psychological aspects of quality of life. This aspect of life is often forgotten when dealing with these patients. They should be treated to relieve their symptoms promptly and should be followed up to make sure that symptoms are kept under control. This would minimize their symptoms and improve the quality of life. Further these patients should be motivated during consultations to improve their morale.

The findings of this study would be helpful in planning the management of individual patient, improving the yield of endoscopic examination and advising the community on measures to be taken to prevent or minimize upper gastrointestinal diseases. The information could be useful to clinicians and health policy makers. This information can be passed on to the teachers and parents who can educate and reinforce proper eating habits among children. Furthermore this data can be used in planning future studies in this area.

Future studies

In Sri Lankan setup the most widely used investigation to diagnose *H.pylroi* infection is histology using routine H & E staining. The test is invasive, requires endoscopy and therefore an additional burden to the hospital setup where there is usually long queues at endoscopy units. Furthermore, histology is time consuming and costly even though it is being done at almost all tertiary care units free of charge. Introduction of a non invasive test is beneficial to diagnose patients without endoscopy. Using such a test asymptomatic people can be screened, the response to treatment can be monitored and subsequently patient compliance will be better. Value of serology in this context is poor since it has yielded low sensitivity and specificity values. *H.pylori* stool antigen test is such a non invasive test that is widely used in other countries where prevalence of infection is low. The validity of this test is currently being studied.

We also intend to study the prevalence of *H.pylori* infection in different geographic locations i.e Eastern Province of Sri Lanka and among a different ethnic group; Muslims. In multi-ethnic countries *H.pylori* has been studied extensively. There has been a variation in prevalence figures, strains responsible, virulence markers, antimicrobial susceptibility etc. Therefore, it will be an important aspect of *H.pylori* infection to be studied in Sri Lanka.

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Review

Bone turnover markers and prediction of bone loss in elderly women

Lenora J
Department of Physiology, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.

Abstract

Around 70,000 osteoporosis-related fractures occur in Sweden annually and approximately half of the women in western world will sustain a fragility fracture after the age of 50 years. Fracture preventive efforts require the identification of individuals who are at high risk. Biochemical markers of bone turnover (BTMs) have shown some degree of fracture predictability. There is also a correlation between rate of decrease of areal bone mineral density (aBMD) and incident fractures.

In this study, the correlation between BTMs and rate of bone loss (change of aBMD and ultrasound variables) over 5 years was investigated in the Malmö OPRA cohort of 75-year old women (n = 506 to 601). In addition, correlation of BTMs and bone metabolism, as assessed by scintigraphy, was tested in postmenopausal women (n = 22). Finally, the effect of precision error on the longitudinal monitoring of change in aBMD was assessed in elderly women (n = 690) and in elderly men (n = 211).

There was a strong correlation between all bone turnover markers and the results of scintigraphy (total skeletal uptake of ^{99m}Tc-labelled methylene diphosphonate), with no significant difference between the markers of bone formation bone resorption. BTMs were correlated to the 5-year rate of change of aBMD, especially in the legs and the total body, and 5-year change in speed of ultrasound. When serial measurements of BTMs were analysed, the mean value of measurements were correlated more strongly to aBMD change than single measurements, and women with constantly high levels of BTMs had higher rates of bone loss. Precision error of aBMD measurement by dual-energy X-ray absorptiometry has an influence on the detection of individuals with aBMD change exceeding the least significant level. The calculated follow-up interval for detection of a change in aBMD beyond the least significant level in more than half of the elderly individuals ranged from 3–32 years, and was dependent on the equipment used and the skeletal site tested.

The results of this study indicate that currently available BTMs are associated with future bone loss. However, these correlations may not be strong enough to be predictive of bone loss at the individual level. DXA also has some limitations when used in the longitudinal setting in elderly individuals. DXA is therefore of limited use in the longitudinal monitoring of bone loss. Further studies with novel bone turnover markers may improve the ability of BTMs to predict bone loss.

Corresponding author: Lenora J

<rsjlenora@yahoo.co.uk>

This study was performed in Malmö University Hospital, Lund University, Sweden and the results were included in a thesis with five published papers for a PhD degree with the Lund University, Sweden and defended the thesis on 23rd of October 2009.

Introduction

Bone is a dynamically and metabolically active organ that is continuously subjected to resorption and formation by coordinated action of osteoclasts and osteoblasts on the surface of trabecular bone and in the Haversian canals (1). These two processes are collectively called bone turnover or bone remodeling and they are coupled in time and space. About 10 % of the skeleton is remodeled each year (2), allowing the skeleton to adjust its strength to mechanical stress and to repair any microdamage (3, 4). Bone remodeling is also necessary for maintaining the metabolic function of the skeleton and calcium homeostasis (5).

During the growth period in childhood and in adolescence bone formation predominates; increasing the bone size and strength until the maximum bone mass (peak bone mass) is reached in the 2nd or the 3rd decade of life (6, 7). After reaching the peak bone mass, there is a state of equilibrium, where the rate of bone formation equals the rate of bone resorption. After the age of 40 years the bone resorption starts to predominate over formation. The aging process

includes endosteal resorption, periosteal apposition, trabecularization of cortical bone and increase in cortical porosity. In women this process is accelerated in the first few years after the menopause due to estrogen deficiency (8). Postmenopausal women decrease the aBMD or lose bone at a rate of 2-5% per year (8). Individuals who lose bone at a fast rate can develop osteoporosis and get fragility fractures at early ages.

Osteoporosis is a systemic skeletal disease characterized by low bone mass, micro architectural deterioration of bone tissue leading to increased risk of fragility fracture, most commonly affecting postmenopausal women and elderly men. After 50 years of age more than 40% of women and 13% of men in western countries are at a risk of developing a fragility fractures at any site during the rest of their life time (9). Osteoporosis is diagnosed by measuring bone mineral density (aBMD) using dual energy X-ray absorptiometry (DXA) and defined as aBMD value 2.5 standard deviations or more below the mean of young female adult population. It is important to identify individuals with osteoporosis and individuals with fast bone loss to take preventive measures to avoid fractures. Fast bone losers are detected using DXA, measuring aBMD at least one to two years apart. It is expensive and consumes time during which the woman loses bone further.

Bone turnover markers, (BTMs) or biochemical markers of bone turnover, are bone tissue proteins or their fragments, or enzymes released from bone cells during bone turnover. Proteins can be by-products of collagen formation or products of collagen degradation, or non-collagenous proteins such as osteocalcin or bone sialoprotein. Enzymes, such as bone-specific alkaline phosphatase and tartrate-resistant acid phosphatase 5b, can also be used as bone turnover markers. Bone turnover markers can be detected in serum or urine. Ideally, they should reflect only the activity of osteoblasts or osteoclasts. Bone turnover markers that are released predominantly during bone formation or resorption are known as bone formation or resorption markers, respectively (10). Bone formation and bone resorption are usually tightly coupled in time and space and therefore, any marker reflects the overall rate of bone turnover (11). Certain bone turnover markers may reflect different stages of formation and resorption but they cannot reflect disease-specific processes or for instance distinguish between the activities at cortical or trabecular bone (11).

The main objective of this study was to investigate the possibility of predication of bone loss over five years using baseline levels of BTMs as well as a serial measurement of BTMs. The effect of precision error of DXA measurements on the assessment of repeated bone densitometry in elderly women and men was also studied. In the first study it was aimed to study whether bone turnover, as assessed by total skeletal uptake of Technetium 99-labelled methylene diphosphonate, correlate more to bone formation markers or to resorption markers.

Materials and methods

These studies were conducted in Malmö University Hospital, Lund University, Malmö in Southern Sweden.

Participants in Study I

For this study (12) we recruited 22 post menopausal women (aged 52–80 years) out of the women who sought medical advice or treatment for minor orthopaedic problems, and who were free from the condition that had originally brought them to the clinic, from the registers of the orthopaedic clinic at Malmö University Hospital, Sweden. By the time the study was started, the women who had ever been treated with bisphosphanates or women who had been treated with oestrogens or corticosteroids within the previous year and women with bone active disease were excluded.

Participants from the Malmö OPRA cohort, Study II–V

The Malmö Osteoporosis Prospective Risk Assessment (OPRA) cohort consisted of elderly women who were randomly recruited from the population registry of Malmö city (13-16). For the baseline investigation, 1604 women were invited by mail one week after their seventy-fifth birthday. Baseline recruitments took place between November 1995 and May 1999. Of the 1,604 women invited, 1,044 (65%) participated at baseline. Of the 560 women who did not participate, 13 had died shortly after the invitation, 139 could not come because of illness, 376 were not interested or could not attend for reasons other than illness, and 32 women could not be reached despite repeated letters and telephone calls. Baseline DXA was performed on 995 individuals. The women were invited for prospective follow-up visits after 1, 3 and 5 years. At the 5-year follow-up, 691 had second aBMD measurements performed at least one site, and 551 women had completed both baseline and 5-year quantitative ultrasound scan (QUS) measurements.

In **Study II,** 601 women who had attended both the baseline and the 5-year DXA measurements were included (13). These women had baseline serum and/or urine samples available and had not taken hormone replacement therapy or bisphosphonates during the study period.

In **Study III,** 506 women who had attended both the baseline and the 5-year Quantitative Ultrasound Scan of calcaneus (QUS) measurements were included (14). These women had baseline serum and/or urine samples available and had not taken hormone replacement therapy or bisphosphonates during the study period.

In **Study IV,** 573 women were included (16). They attended both the baseline and the 5-year DXA measurements, and had given serum and/or urine samples at baseline and at the 1, 3 and 5-year follow up measurements. The women included had not taken hormone replacement therapy or bisphosphonates during the study period.

In Study V, 691 women were included (15). These women had a baseline and 5-year follow-up DXA measurements available. In addition, 211 men from the Malmö part of the MrOs study (the Osteoporotic Fractures in Men Study) who attended DXA measurements at baseline and at the 5-year follow-up were included. The MrOs study is an international multi-centre study on risk factors for osteoporosis and fracture in elderly men. The men in the Malmö cohort of the MrOs study were recruited from the population registers of Malmö city.

Bone density measurements

Dual-energy X-ray absorptiometry

The total body, the total hip, the femoral neck and the lumbar spine aBMD and bone mineral content (BMC) measurements in the women were performed by using a Lunar DPX-L scanner (Lunar DPX-L; Lunar Corporation, Madison, USA) at baseline (**Study I–V**) and after 5 years **(Studies II, III, IV and V)**. Men were measured at the same regions of interest using a Lunar Prodigy scanner (Lunar Prodigy, Madison, USA), which uses the fan beam technique.

Quantitative ultrasound of the calcaneus

Ultrasound measurements were performed in elderly women at baseline and after five years with a Lunar Achilles® scanner (Lunar Corporation, Madison, USA) for the right calcaneus. The results were obtained as speed of sound (SoS), broadband ultrasound attenuation (BUA) and the stiffness index **(Study III)**.

Serum and urine samples

Serum and urine samples were collected for the analysis of markers of bone turnover at baseline (age 75 years, **Studies II, III and IV**), and follow-ups after 1, 3, and 5 years (**Study IV**). Non-fasting blood samples were collected between 0800 and 1300 hour, and serum was separated and stored within two hours. First morning void urine samples were also collected. Serum and urine samples were stored at -80°C. For **Study I**, non-fasting serum and urine samples were collected at 0900 hour. The analyses for each bone metabolic marker were done at the same time in order to minimise inter-assay variability.

Measurement of bone turnover markers

Markers of bone formation

Bone-specific alkaline phosphatase (S-Bone ALP) was determined by using Metra BAP immunoassay (Quidel Corporation), with an intra- and inter-assay coefficient of variation (CV) of 3.6% and 4.4%, respectively. Serum intact and N-mid osteocalcin (S-Total OC(N-Mid®)) were determined by using the Elecsys N-MID Osteocalcin Immunoassay (S-Total OC; N-MID®; Roche Diagnostics), with intra- and inter-assay CV of 2.3% and 2.4%, respectively. Serum intact osteocalcin (S-OC[1-49]), serum total osteocalcin (S-Total OC) and serum total carboxylated osteocalcin (S-cOC) were determined by previously described, in-house protocols with intra- and inter-assay CV of less than 5% and 8% respectively, for all the assays (17). Briefly, protocols are two-site assays based on two monoclonal antibodies (Mabs) in the combinations 3G8/2H9 (for S-OC[1-49]), 2H9/6F9 (for S-TotalOC) and 6F9/3H8 (for S-cOC). Mab 3G8 is specific for intact OC, Mab 6H9 binds to fragment Gly^{7}-Arg^{19}, Mab 2H9 recognizes fragment Arg^{20}-Arg^{43} and Mab 3H8 binds to the same fragment (Arg^{20}-Arg^{43}) but prefers OC-containing gamma-carboxyglutamic acid (Gla), with only 9% cross-reactivity with non-Gla-containing OC (18).

Markers of bone resorption

Serum C-terminal cross-linking telopeptides of type I collagen (S-CTX-I) was determined by Elecsys β-Cross Laps® immunoassay (Roche Diagnostics) with intra- and inter-assay CV of 5.9% and 5.8%, respectively. Serum tartrate-resistant acid phosphatase 5b (S-TRACP5b) was assessed by a solid phase, immunofixed enzyme activity assay as described earlier (19) with an intra- and inter-assay CV of 1.8% and 2.2%, respectively.

Urinary deoxypyridinoline (U-DPD) was measured by the Metra DPD Immunoassay (Quidel Corporation, San Diego, CA, USA) with an intra- and inter-assay CV of less than 12% and 10%, respectively.

Urinary osteocalcin

Urinary osteocalcin (U-OC) consists of fragments less than thirty residues in length from the middle region of

the molecule (20). Three assays were used to analyse various molecular forms of U-OC as described previously (21). Assays were based on the same Mabs as the assays for serum OC (18). Briefly, the two-site assay U-MidOC consisted of Mabs 6F9 and 3H8 and recognized the most abundant mid-molecule fragments of U-OC (spanning residues 7–31, 7–29, 6–29, 9–31, 7–32 and 7–33). Two-site assay U-LongOC (2H9/6F9) detects only the longest U-OC fragments (7–32, 7–33) with low affinity. Competitive assay U-TotalOC (3H8) also measures (in addition to the same mid-molecule fragments) more truncated U-OC fragments, starting from residue Asp^{14}. The intra- and inter-assay CVs were 1.7% and < 12% (for U-MidOC), 4.3% and < 14% (for U-LongOC), and 14% and < 27% (for U-TotalOC), respectively (21).

Urinary creatinine

Urinary creatinine was measured by the kinetic Jaffe reaction with a Beckman synchron LX20-4, with CVs of 3% or less. All the measurements of urinary bone markers were corrected for urinary creatinine and expressed as ratios (**Studies I, II, III, and IV**).

Bone Scintigraphy

Bone scintigraphy procedure was performed within 28 days after the DXA scanning (**Study I**) according to a method described by Brenner *et al.* (22). An intravenous injection of 520 (517 ± 15) MBq of ^{99m}Tc-MDP (Medronate®, Amersham International) was given at 0900 hour. Whole body imaging was performed directly (3 minutes) after injection and 5 hours after injection (1400 hour). A double-headed gamma camera system (Siemens Multispect 2) equipped with low-energy high-resolution collimators was used for the scan.

Total skeletal uptake (TSU) of ^{99m}Tc-MDP was calculated using three minute images and five hour images, excluding the urinary bladder and the soft tissue uptake as described by Brenner *et al.* (22).

Results

Results- Study I

Bone turnover markers are correlated with total skeletal uptake of ^{99m}Tc-methylene diphosphonate (^{99m}Tc-MDP)

The median TSU of ^{99m}Tc-MDP was 23% (range 5–48%). There was a significant correlation between all bone turnover markers, with r-values from 0.52 ($p = 0.013$) to 0.90 ($p < 0.001$). The two bone resorption markers had numerically higher correlations (S-TRACP5b: $r = 0.90$; and S-CTX-I: $r = 0.80$) than the bone formation markers (S-Total OC: $r = 0.72$; and S-Bone ALP: $r = 0.66$), but the differences were not statistically significant. There was no correlation between the TSU of ^{99m}Tc-MDP and age, weight, body mass index or total body aBMD.

Results- Study II.

Prediction of bone loss using biochemical markers of bone turnover

Annual change in aBMD varied between +0.4% (spine) and -2.0% (femoral neck). Significant associations ($p < 0.01$) in the aBMD change of the leg region (derived from the total body measurement) were found for four different S-OCs (standardized regression coefficient -0.20 to -0.22), U-DPD (-0.19), S-TRACP5b (-0.19), S-CTX-I (-0.21), two of the three U-OC/crea (-0.16).

After adjustment for baseline total body BMC (bone mineral content), associations were found for all S-OC:s (-0.11 to -0.15), two of the three U-OC:s (-0.14 to -0.16) and aBMD change at the total hip, and for three of the four S-OC:s (-0.14 to -0.15), S-TRACP5b (-0.11), two of the three U-OC:s (-0.14 to -0.15) and aBMD rate of change at the femoral neck. There were no significant associations concerning change in aBMD at the lumbar spine.

Results- Study III.

Bone turnover markers are correlated with quantitative ultrasound of the calcaneus: 5-year longitudinal data

There was a correlation between all markers and baseline QUS measurements ($Beta_{std}$ values from -0.07 [$p < 0.05$] to -0.23 [$p < 0.001$]). When we evaluated the correlations between the baseline bone markers and 5-year prospective changes in QUS, all three molecular forms of serum osteocalcins showed correlations with changes of SoS and stiffness index (unadjusted and adjusted for baseline body weight) ($Beta_{std} = –0.10$ [$p < 0.05$] to -0.17 [$p < 0.001$]). S-CTX-I showed a correlation with changes in SoS (unadjusted and adjusted for weight) and unadjusted stiffness index ($Beta_{std} = –0.09$ to $–0.10$ [$p < 0.05$]). S-TRACP 5b and U-MidOC/crea showed correlations with unadjusted changes in SoS ($Beta_{std} = -0.10$ [$p < 0.05$]). S-Bone ALP did not show any correlation with any of the prospective changes in QUS, and none of the bone turnover markers correlated with prospective changes in BUA before or after adjustment of baseline body weight.

Results- Study IV

Serial assessment of serum bone turnover markers identifies women with the highest rate of bone loss and osteoporosis risk

Baseline BTMs showed a weak correlation with change in total body aBMD, but the association was more pronounced when we used the average of two measurements of each marker (standardised regression coefficient from –0.12 to –0.23, $p < 0.01$). Adding a third and a fourth measurement further strengthened the correlation (with coefficients of up to -0.30, $p < 0.001$). Changes in BTMs did not correlate to bone loss as strongly as the average values. Women with constantly high turnover lost significantly more bone at total body (-2.6%) than women with intermediate (-1.6%) or low turnover (-0.2%, p for trend < 0.001). They also had greater bone loss at the hip (-8.3%, -6.0% and -5.1%, respectively; $p = 0.01$). Results were similar in the subgroup of women with osteopenia.

Results - Study V.

Effect of precision on longitudinal follow-up of bone mineral density measurements in elderly women and men

At baseline, aBMD (SD) in g/cm^2 for women was: total body (TB) 1.008 (0.093), total hip (TH) 0.857 (0.147) and lumbar spine (LS) 0.987 (0.190); in men, TB 1.187 (0.097), TH 0.982 (0.138) and LS 1.240 (0.190). Precision error (in g/cm^2) for Lunar DPX-L in women was 0.010 (TB), 0.028 (TH) and 0.016 (LS). Precision error using Lunar Prodigy for women was 0.009 (TB), 0.009 (TH) and 0.039 (LS). Precision error using Lunar Prodigy for men was 0.007 (TB), 0.014 (TH), and 0.031 (LS).

Mean change in aBMD (in g/cm^2) per year in women was, for TB -0.003 (0.007), for TH -0.011 (0.016) and for LS 0.004 (0.015). Corresponding results in men were -0.003 (0.006), -0.006 (0.009) and 0.005 (0.016) at TB, TH and LS respectively.

The number of individuals with 5-year aBMD change at TB that exceeded the LSC was 244 women (38.6%) and 73 men (35.6%). The corresponding results at TH were 265 women (41.4%) and 78 men (38.6%); at LS the numbers were 303 women (45.0%) and 51 men (24.6%).

Monitoring time interval (i.e. LSC/median rate of change in aBMD) for both populations was 8 years (for TH aBMD) and 13 years (for LS aBMD). Based on Prodigy precision data, the monitoring time intervals for women were 3 and 32 years for TH and LS, respectively.

Discussion

To the best of my knowledge, this study as part of the Malmö OPRA study has been the largest study in elderly women to assess the ability to predict bone loss over several years. The design of the OPRA study has several advantages: it has (i) a well-defined population, (ii) a high attendance rate, (iii) a thorough ascertainment of fracture, (iv) a long follow-up, and (v) the use of novel and established bone turnover markers. The overall aim of the work described in this study was to improve the prevention of fragility fractures in the future. There are numerous risk factors for fragility fracture. Bone mineral density is one of the most important risk factors that is potentially modifiable. For diagnostic purposes, a diagnostic threshold is used for bone density test results, below which the term osteoporosis is used. However, a large proportion of individuals who sustain a fragility fracture are not osteoporotic (4, 23, 24). Apart from the fact that they do not take other risk factors into account, bone density test results only reveal the current situation. They do not show the ongoing bone turnover; thus, they do not provide information on future changes in bone density.

There are several reasons for the development and use of bone turnover markers. The work in these studies illustrates efforts to find ways of assessing future bone loss by the measurement of bone turnover markers (**Study II and III**), of how to improve this assessment (**Study IV**), and to investigate whether some markers are more specific than others (**Study I–IV**). Since the time required to assess bone density changes with bone density equipment is very long (**Study V**), it seems unreasonable to follow up compliance and effect of anti-osteoporotic medication by repeated bone density measurements.

Currently, bone turnover markers are being used extensively in research applications and also being tested as tools for the management of metabolic bone diseases such as osteoporosis and Paget's disease in clinical practice, because these markers are non-invasive and relatively inexpensive. Monitoring of the efficacy of bone-active drugs is currently the most promising clinical application of bone turnover markers, because of the possibility of detecting a change in the levels of bone turnover markers within a few weeks of treatment (25-28). Some markers, particularly resorption markers such as S-TRACP5b, S-CTX-I, U-CTX-I, U-NTX-I and U-DPD, and some bone formation markers such as S-bone ALP and S-OC, have shown some degree of fracture predictability in different populations (10), but the prediction is not strong enough to use in individual patients. The fracture predictability afforded by bone turnover markers is weaker than the predictability afforded by DXA (29), but it is somewhat inconsistent between studies (30-34).

A high rate of bone turnover is associated with a high rate of bone loss and osteoporosis (35, 36). Early

detection of individuals who are at high risk of developing osteoporosis could be important for clinical decision-making. In particular, individuals with osteopenia and individuals with a high rate of bone loss may need more careful follow-up.

In **Study II** and **III**, baseline bone turnover markers, in particular S-OCs, U-DPD/crea, S-TRACP5b, S-CTX-I, U-LongOC/crea and U-MidOC/crea could be correlated to the rate of change of aBMD in the legs. To some degree, there were correlations with rate of change of aBMD in the arms, in the total body, in part of the body, in the total hip and in the femoral neck. None of the markers were found to be correlated to the rate of change of aBMD at the lumbar spine; nor did S-Bone ALP and U-TotalOC/crea show any correlation with rate of change of aBMD. When the correlation between bone turnover markers and 5-year change of QUS variables was examined, all markers except S-Bone ALP showed correlations with changes in SoS, while none of the markers showed any correlation with changes in BUA **(Study III)**. When the mean of serial measurement of bone turnover markers was used instead of baseline measurement, the correlations became stronger as the number of samples used increased, and the women with constantly elevated levels of bone turnover markers had a significantly higher rate of bone loss **(Study IV)**.

In general, the correlation between bone turnover markers and the change in aBMD was not strong. The strongest correlation coefficients were 0.22 when the baseline levels were used and they were 0.32 when the mean of four serial measurements was used. None of the markers proved to be superior to the others. Bone formation and resorption markers had almost similar magnitudes of correlations. This could be due to the tight coupling of bone formation and resorption. This idea is supported by the results of **Study I**, in which no difference between bone formation markers and resorption markers in TSU of ^{99m}Tc-MDP was found. Bone turnover markers are released from the whole skeleton. This may be the reason for higher correlations with bone turnover markers at large skeletal sites including the total body, the partial body and the legs, than smaller sites such as the femoral neck and the lumbar spine (**Study II and IV**).

Many other factors also affect the clinical usefulness of bone turnover markers. Pre-analytical conditions affecting bone turnover markers such as age, gender, menopausal state, ethnicity and recent fracture are not controllable, whereas other factors such as the effect of food intake, physical activity and circadian rhythm can be controlled (37). The OPRA study was designed to control factors such as age, gender, ethnicity and menstrual status. Samples were taken in the morning in the non-fasting state, which could have affected the results, mainly the S-CTX-I levels (38). Many other factors such as time of the day, recent fracture and level of physical activity may have an effect on bone turnover markers. The study design was deliberately not changed during the study period, and all samples were collected in the same manner to make comparisons possible within the cohort.

Bone density has a smaller annual change or response to anti-resorptive and anabolic treatment compared to the response of bone turnover markers. Precision has an effect on the shortest follow-up interval between repeated scans. In the population-based cohorts in **Study V**, several years were needed to detect a significant change between measurements. The estimated monitoring time intervals (i.e. least significant change / median rate of change in aBMD) were between 3 and 32 years, depending on the site of measurement and the equipment used. Only when a high degree of bone loss is expected may a shorter follow-up time be useful. Thus, DXA has shortcomings in detecting rapid losers and individuals with a high risk of developing osteoporosis.

Single measurements of bone turnover markers and follow-up measurements of DXA both have limitations in their ability to detect individuals with rapid bone loss. Serial assessments of bone turnover markers can substantially improve the ability to find individuals with increased loss of bone density. Whether or not intervals shorter than one year could be used to improve the predictive ability of bone turnover markers remains to be evaluated.

Conclusions

There is a correlation between levels of bone turnover markers and the rate of bone loss in elderly women, with varying degrees of correlation coefficients at different skeletal regions. In general, bone turnover markers correlate better with change in aBMD at large skeletal sites, such as the total body, and weight-bearing sites such as the legs, than with aBMD change at specific clinically important regions such as the femoral neck and the total hip. Correlations between bone turnover markers and rate of bone loss become stronger when serial measurements of bone turnover markers are used. The individuals with constantly high levels of bone turnover markers have higher change in aBMD. However, these correlations may not be strong enough to be predictive of bone loss at the level of the individual patient. DXA is used to monitor change in aBMD to aid in treatment decisions. However, long durations of follow-up are needed to detect aBMD changes in elderly women and men that exceed the least significant change. DXA is therefore of limited use in the longitudinal monitoring of bone loss.

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Review

Improving micronutrient status of the Sri Lankan population: effect of iron and zinc fortified rice flour

Hettiarachchi M
Nuclear Medicine Unit, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.

Abstract

In Asia, rice provides more than 70% of the energy in the diet. Rice is the single most important crop occupying 34.0% of the total cultivated area in Sri Lanka. About 1.8 million farm families are engaged in paddy cultivation island-wide, produce 2.7 million tonnes of paddy annually and satisfy around 95.0% of the domestic requirement. As such, rice can be a good candidate for fortification with micronutrients because it is the staple diet of all sectors of the population, consumed almost daily in large amounts in Sri Lanka. This intervention programme describes the feasibility of rice flour fortification to increase the micronutrient intake and thereby to improve growth and status of iron, zinc and folate of Sri Lankan population.

Corresponding author: Hettiarachchi M

<manjula.hettiarachchi@gmail.com>

This study was performed in University of Ruhuna, Sri Lanka and the results were included in a thesis with eight published papers. Further, two orations were done at the University Academic Sessions & Sri Lanka Medical Association respectively and recognized as best post graduate research by the Hiran Thilakerathne fund of the University Grants Commission, Sri Lanka Association for Advancement of Science (SLAAS) and Thrid World Academy of Sciences (TWAS) & National Science Foundation of Sri Lanka during respective years and defended the thesis on 01 of February 2007

Introduction

Food fortification is an important aspect of a nutritional intervention programme. The fortified food products are expected to become the main source of the specific added nutrient in the targeted population, and help prevent inadequacies of those nutrients in groups at risk. The effectiveness of such a programme depends on whether or not the fortified food is accepted, purchased, and consumed by them. Further, factors such as the quality, taste, and price of the fortified products also will play important roles. Fortification programmes make a range of effective investments that improve intake and status of micronutrients, contribute to size and stature of the population (direct effects), and improve cognitive ability as well as scholastic achievements (indirect effects). Further, economic growth and poverty reduction can also be achieved through iron and zinc deficiency reduction (1).

As in the other countries in Asia, Sri Lanka faces widespread health and nutritional problems. Micronutrient deficiencies strike both children and women and surveys showed that deficiencies in iron, iodine, and vitamin A are the most damaging forms (2). Anaemia is a major public health problem in Sri Lanka, affecting all segments of the population and contributing to increased morbidity and mortality rates. In 2001 the prevalence was estimated as 32% among non-pregnant women, 30% among pregnant women, 22% among adolescents, 21% among primary schoolchildren, and 30% among preschool children (3). There have been no reports on zinc status in the Sri Lankan school children until we undertook the present study (4). However, folic acid status among adolescent girls and non-pregnant, non-lactating young women in Colombo has been studied and published recently (5) which showed that 43% had low serum folic acid concentrations and 47% had low iron stores with an anaemia prevalence of 12.9 %. Therefore, to combat these deficiencies a proper long term nutritional intervention is a necessity.

In addition to the National salt fortification programme, a fortification of wheat flour with iron was tested out in 1998-1999. Wheat flour fortified with 66mg/Kg of electrolytic or reduced iron was tested in a double blind, controlled trial in the estate sector of Sri Lanka with an aim to reduce the prevalence of anaemia (6). After two years of intervention neither electrolytic nor reduced iron had an effect on haemoglobin concentration among study subjects. The findings from this study suggested that fortification of wheat flour with iron was not beneficial

in reducing anaemia in the population studied. Wheat, however is an imported food item and as such, not feasible for a long term national food fortification. We therefore, selected a locally produced cereal, the rice as a suitable vehicle for fortification.

In most parts of the world, the average diet is predominantly cereal, with wheat, rice and maize being the staples. In Asia, rice provides more than 70% of the energy in the diet (7). Rice is the single most important crop occupying 34 percent (0.77 million hectares) of the total cultivated area in Sri Lanka. About 1.8 million farm families are engaged in paddy cultivation island-wide. Sri Lanka currently produces 2.7 million tonnes of paddy annually and satisfies around 95 percent of the domestic requirement (8). Rice provides 45% of total energy and 40% of total protein requirement of an average Sri Lankan. As such, rice can be a good candidate for fortification with micronutrients because it is the staple diet of all sectors of the population, consumed almost daily in large amounts in Sri Lanka. The intervention programme was concerned with a rice flour fortification to increase the micronutrient intake and thereby to improve growth and status of iron, zinc and folate of Sri Lankan population was conducted with the approval from the Ethical Review Committee of the Faculty of Medicine.

Materials and Methods

Investigation I Survey on rice flour consumption pattern The rice, as flour is being used by Sri Lankans in the preparation of their meals. But no data are available on the level of consumption of rice flour *per se*. Therefore, a survey by using a pre-tested questionnaire to study the patterns of purchasing, consumption and acceptability of meals made out of rice flour in a representative sample from Galle district was conducted. The aim of this cross sectional survey was to determine the actual intake of rice and rice flour based meals, as well as study factors that influence the dietary intake of rice flour.

A two-stage stratified random sampling design was used in the survey. Urban, semi-urban and rural households of the Galle district were the domains for stratification. Randomly selected Public Health Midwifery (PHM) areas (n=29) were the primary census blocks. The income and expenditure survey in 2002 carried out by Department of Census and Statistics, Sri Lanka (9), has calculated that a sample size of 1050 housing units would represent the Galle District. We randomly selected streets in each PHM areas and then all housing units on that street were included in the study. On average there were 35-38 households per street in the selected areas, so that a total of 1096 households were randomly selected for the survey.

The survey was implemented in March and April 2004 and the interviews were undertaken after obtaining written informed consent. A trained interviewer visited each house and either the head of the household or the spouse or both were interviewed. The questionnaire was used to obtain information on rice consumption pattern -especially the quantity of rice and rice flour purchased and utilized by the household during the preceding month. The ownership of the rice growing fields as well as meal preparation practices with rice flour in the household was also documented. The social class of each family was defined by a scoring system after considering the occupation, aggregate family income, educational status of chief house holder and the housing conditions (3). Social classes were classified as class 1 (cumulative score of 19-20); class 2 (cumulative score of 17-18); class 3 (cumulative score of 13-16); class 4 (cumulative score of 9-12); and class 5 (cumulative score of 4-8). Intakes of rice and rice flour were calculated using following formula; '*amount consumed x frequency per day x (number of days per week/month)*'

Investigation II Fortification of rice flour and consumer acceptance of fortified rice flour based meals Rice flour was prepared at the Agro Food Technology (AFT) division of the Industrial Technology Institute (ITI), Colombo by grinding brown country rice (well polished 6-8 % weight reduction on polishing -Grade 2) to 300- 500 mesh size using an electric grinder. Because of established high bioavailability and proven efficacy (10), the ferrous sulfate and zinc oxide were selected as fortificants to be used in this study. Fortificant levels used were 60 mg of elemental mineral (i.e., iron, zinc) to 1 kilo gram of rice flour, based on guidelines adapted in other countries (11, 12). To overcome the inhibitory effects of phytates in rice, disodiumEDTA was included at a molar ratio of 1:1 of iron:EDTA, a ratio that has been shown to be both efficacious and safe in previous studies (13). Folic acid was added to achieve the concentration of 2 mg per kilo of rice flour and 162.85mg of dried ferrous sulfate ($FeSO_4$) and 74.12mg of zinc oxide (ZnO) powder were added to each kg of rice flour to obtain the desired level of fortification. In addition, 385.08mg of disodiumEDTA in dry powder form was added to each kg of rice flour in appropriate groups in order to achieve a molar ratio of 1:1 elemental Fe:disodiumEDTA and 0.7:1 of elemental zinc:disodiumEDTA. The fortificants, $FeSO_4$ and ZnO, were supplied by Dr. Paul Lohmann, Germany; disodiumEDTA by AKZO NOBEL, Netherlands and folic acid by Glaxo Welcome through

their local agent Greenfields International, Sri Lanka. Blending the fortificants with rice flour was accomplished at the ITI using a ribbon blender. Iron and zinc levels in the fortified rice flour were measured by flame atomic absorption spectrometry (f-AAS) in random samples of rice flour to confirm proper mixing.

Experimental meals were prepared at the Nutrition Research Laboratory, Department of Community Medicine, Faculty of Medicine, Galle where the fortified flour was stored at ambient condition. Temperature ranged from 30°-40° C and relative humidity on or above 80%. The meals made out of the fortified flour at baseline, and then at monthly intervals up to three months of storage, were subjected to sensory evaluation for taste, odour, acceptance and texture. Duplicate fortified and control flour (non-fortified) samples were tested by panelists (n=13), in the laboratory. The evaluation panel consisted of the volunteers from the staff (academic (n=3), non-academic (n=5) and students (n=5)) of Faculty of Medicine. They were convened to test one type of food per day on three days. On each day dishes made out of fortified flour of different storage levels and the control flour were tested. Three commonly consumed rice flour dishes that were identified at the consumption survey i.e. *pittu*, string hoppers and *aluwa* were used.

Pittu is a flat small 'droplet' prepared by mixing rice flour with water and coconut and steamed on a pre-heated water pan. String hoppers are thin noodles made out of preheated rice flour and mixed with water and then steamed. *Aluwa* is sweet cake made from rice flour mixed with tricle/ sugar and water, cooked and spreaded on a board. The formulae of the meals used in this study are the ones commonly practiced by Sri Lankan people.

The sensory evaluation was based on testing for multiple sample difference in quality attributes following the procedures used in India (14). The panelists evaluated the coded meals based on taste, colour, odour and acceptability, using a hedonic scale of -5 to +5, with zero being the value assigned to the control food made with unfortified flour. The panelists were also invited to make comments on the evaluation forms. In addition, the technicians who were responsible for preparing the meals kept records of their observations on the characteristics of the uncooked flour during their preparations.

Investigation III: Determination of the bioavailability of iron and zinc from folate-fortified rice flour and study of the enhancing effect of disodium ethylene- diamine- tetraacetic-acid (disodiumEDTA) on bioavailability Children between the ages of 7-10 years were recruited for this study through a meeting of potential participants and their families from University field training area. Informed written consent was obtained from the parents after explaining in detail the purpose, risks and benefits of the study. Fifty seven (57) children came and 7 were excluded (3 parents refused phlebotomy; 2 children were taking supplements; venous access was not possible in one child; and another child had a history of seizures). Based on a study for iron absorption from fortified wheat flour seen in a similar population in Indonesia (13) a sample of 18 was predicted to give greater than 90% power in detecting the assumed difference of about 8% between the $FeSO_4$ group and the $FeSO_4$+ disodiumEDTA group. Adding 6 more subjects to each group to accommodate drop-outs, the minimum requirement was 24 subjects in an arm. To assess the interaction of zinc with iron, each arm was divided into two resulting in four groups of investigation.

Arm 1 – Without EDTA
Group 1- $FeSO_4$ and folate (n=13);
Group 2- $FeSO_4$, ZnO and folate (n=12);

Arm 2 – With EDTA
Group 3 - $FeSO_4$, disodiumEDTA and folate (n=13);
Group 4 - $FeSO_4$, disodiumEDTA, ZnO and folate (n=12).

Stable isotopes that were used in this study (^{57}Fe, ^{58}Fe, ^{67}Zn and ^{70}Zn) were purchased from Trace Sciences International, Toronto, Canada. The ^{67}Zn and ^{70}Zn (90 % enrichment by mass) were obtained as zinc oxide dry powder, and prepared each in an aqueous solution of 0.085 mg per ml and then tested for sterility and pyrogenicity using the quantitative chromogenic limulus amebocyte lystate test (QCL-1000 kit from BioWhittaker, BioWhittaker Molecular Applications) at the Investigational Pharmacy of Texas Children's Hospital, Houston, Texas. ^{57}Fe (95% enrichment by mass) and ^{58}Fe (96% enrichment by mass) were provided in elemental metal form. Iron isotope solution for administration was prepared, as the sulfate, in our laboratory, by dissolving metals in 0.03 ml of 7M nitric acid and 0.125 ml of 0.5M sulfuric acid for every mg of elemental iron. The solutions were dried at 120°C, at 230°C, and finally at 500°C for 30 minutes each in a muffle furnace. After cooling, the final products were re-suspended in 0.2M sulfuric acid at 0.24 ml for every mg of iron. Deionized water was added to produce a solution yielding a unit dose of 1.5 mg elemental iron for each 2.5 ml solution.

Each test meal, '*halapa*', was individually prepared. Rice flour (fortified with folic acid only) was weighed on a calibrated scale within ±0.1g of the desired weight (25g). Each portion of rice flour was mixed with 15mL of doubly distilled water and kneaded for 2-3

minutes until a smooth dough was produced. The dough was flattened on a leaf (*Macaranga peltata*) that is commonly used for flavoring in Sri Lanka. Next, 1.5mg of elemental iron in the form of $^{58}FeSO_4$ (all 4 groups), 9.627mg of disodiumEDTA (group 3 and 4) and 1.5mg of elemental zinc in the form of ^{67}ZnO (group 2 and 4) were spreaded over the dough. The exact dose was carefully measured and recorded with the subject's identification number. The amount of isotope added was in the same proportion of the elements present in the fortified flour. A mixture of grated coconut (12g) cooked in sugar syrup (10g) was spreaded on each of the dough, and then the *halapa* was folded and steamed for 10 minutes. The steamed product was stored in a refrigerator and heated in the microwave oven just before the administration of the meal. The levels of the iron and zinc in the preparations were confirmed by f-AAS, and the phytate level was measured by Association of Official Analytical Chemists (AOAC) method and found to be 22.5mg per *halapa*.

On day one, all children were asked to arrive at the testing site (Faculty of Medicine) at 0630 hours after fasting overnight except for water. A reference iron isotope dose that consisted of 5 mg ^{57}Fe as iron sulfate dissolved in orange juice with 50mg of ascorbic acid added was administered to them. Orange juice and ascorbic acid were used as enhancers of iron absorption. The children were asked to avoid any meal for two more hours to prevent any interaction with the contents of such a meal. On the following morning, again after an overnight fast, each subject was given a test meal according to one's group allocation. They fasted for an additional 2 hours and then resumed their usual diet. Subsequently, a reference dose of ^{70}Zn was given intravenously to subjects of arms 2 and 4 who had been receiving a meal made out of zinc-fortified rice flour.

Approximately 72 hours after the test meal, 25mL of urine was collected in to urine collection bags from subjects in arms 2 and 4 to measure fractional excretion of zinc. Two children dropped out at this stage and as such, 48 children completed the trial. Fourteen days after the isotope administration a venous blood sample (5mL) was obtained from all and red cells were separated to measure red blood cell (RBC) iron isotope enrichment. Of the blood samples taken for measurement of ^{57}Fe and ^{58}Fe, three were contaminated during transportation. In addition, ^{58}Fe was not available to provide an adequate dose to two subjects. Therefore, 45 samples for ^{57}Fe, and 43 samples for ^{58}Fe were analyzed. Twenty four samples were evaluated for zinc absorption from zinc groups.

Iron isotope ratio was measured using a thermal ionization magnetic sector mass spectrometer (MAT 261; Finnigan ThermoQuest, Bremen, Germany). The results were expressed as the ratio of ^{58}Fe to non administered iron isotope i.e. ^{56}Fe. The ratio of two non-administered isotopes (^{56}Fe and ^{54}Fe) was used to correct for temperature-specific differences in fractionation. Iron absorption was calculated from iron incorporation, based on the assumption that 90% of the absorbed iron was incorporated into red blood cells. Urinary zinc isotope enrichments were measured similarly to iron isotopes. Isotope ratios were expressed with respect to a non-administered isotope (^{66}Zn) and corrected for differences in fractionation with the use of ^{64}Zn to ^{66}Zn ratio (two non administered zinc isotopes). Zinc absorption was calculated from the relative fractional excretion of the oral and intravenous isotope doses in 72-hour urine samples. All the isotope measurements were done at the Children's Nutrition Research center, Baylor College of Medicine, Houston, Texas, USA.

Investigation IV: Efficacy of fortified rice flour fortification in improving iron, zinc and folate status and anthropometry of children As the final step of this research programme, a pilot efficacy study over a period of one month was carried out as a prelude to a future placebo controlled study on efficacy of fortified rice flour in a Sri Lankan population sample. The subjects used for the absorption study were recruited for this study as well after obtaining informed written consent from their parents. The subjects were randomized into four groups based on type of fortification as mentioned in the previous investigation. The weight and the height were recorded and a medical history and a physical examination were performed on each subject before the fortified rice flour was given. All subjects were given 75g per day of fortified rice flour prepared according to their group allocation, to be consumed daily for a period of 4 weeks. Parents were instructed to prepare this as a common food item for the whole family (families were supplemented with fortified rice flour packets containing 75g/ per person/ day). A venous blood sample (5mL) was obtained from each subject for the determination of Hb and serum levels of ferritin, zinc and folate at the beginning and at the end of the trial and their weight and height of each was also recorded. Baseline and final biochemical and anthropometry parameters of the subjects were compared.

Results

Investigation I Of the total sample of 1096 families 314 were from urban communities, 485 represented semi-urban and 297 were from rural settings of the

Galle District (Table 1). The mean monthly consumption of rice per family was 36.5Kg. The mean rice consumption per person per meal observed in the different sectors in this study population was not significantly different from each other (137.71g in urban; 138.95g in semi-urban; 134.44g in rural; p=0.40). Subjects in the urban sector had significantly lower per capita rice flour consumption (130.3 g/meal; p=0.05) when compared with semi-urban (142.57 g/meal) and rural (148.67 g/meal) sectors. Rice flour consumption patterns by the social classes are illustrated in Table 2. Families of social class 2 had higher rice consumption than in the other social classes. But no such difference was observed in the intake of rice flour. 48.8% of families in class 1 and 67.8% of families in class 2 had purchased rice flour (Table 2) whereas only 18% of the lowest income group (class 5) purchased rice flour in the preceding month.

Table 1 Rice flour consumption patterns according to sector[1]

		Average consumption in grams (mean, 95% CI)	
Sector	n	rice flour (person/day)	rice flour (family/day)
Urban	314	130.34 (121.8; 138.9) [a]	836.52 (782.3; 890.7)
Semi-urban	485	142.57 (134.1; 157.0) [b]	836.47 (797.0; 876.0)
Rural	297	148.67 (136.2; 161.1) [b]	897.52 (837.7; 957.3)
All	1096	140.72 (135.1; 146.3)	853.03 (824.6; 881.4)

[1] Results tabulated as mean and 95% confidence Interval
[a,b] values with same superscripts in a column are not significantly different (p<0.05)

Table 2 Rice and rice flour consumption patterns according to social class[1]

		Average consumption (grams)% of families purchasing rice flour		
	n	rice (person/meal) mean (95% CI)	rice flour (person/day) mean (95% CI)	
Class 1	41	131.45 (118.1-144.8)[a]	132.56 (117.7; 153.4)	48.8
Class 2	90	150.44 (139.4-161.5)[b]	134.91 (118.5; 151.3)	67.8
Class 3	567	136.95 (133.5-140.4)[a]	142.82 (135.0; 150.6)	39
Class 4	371	117.83 (131.9-142.0)[a]	141.99 (131.6; 152.4)	33.4
Class 5	27	137.37 (105.6-130.1)[a]	110.78 (92.2; 129.4)	18.5

[1] Groups with different superscript letters (a, b) different significantly (p<0.05)

Average family size of the study sample was 4.1; majority of the families were of social classes 3 and 4. Overall, 86% of households consumed rice flour and two out of five households purchased rice flour from the market (Table 3). Urban (44.5%) and semi-urban (43.1%) households purchased rice flour, at a 15% higher rate than the rural (27.2%) sector (Table 3). Nearly half of the households (49.8%) bought readymade food items made out of rice flour. Common rice-based meals eaten were string hoppers, pittu, hoppers and sweet cakes. 12.8% of the study population had their own paddy fields; the majority belonging to rural communities.

Investigation II The external characteristics of cooked food items showed that all types of flour had similar properties to that of the control after one month of storage. Since the flour was made up of brown country rice there was no obvious darkness or discoloration in the fortified flour due to oxidation of ferrous sulfate. However, when the flour was stored for more than two months, black spots were observed

Table 3 Popularity of rice flour based food consumption according to area of living[1]

		Urban sector	Semi-urban sector	Rural sector	All
Families (n)		314	485	297	1096
Rice flour	-home made	174 (55.4%)	276 (56.9%)	216 (72.7%)	666 (60.8%)
	-buy	140 (44.6%)	209 (43.1%)	81 (27.3%)	430 (39.3%)
Rice flour consumption	Frequently	276 (87.9%)	416 (85.8%6)	251 (84.5%)	943 (86.0%)
Reasons for infrequent consumption	-price	10 (3.2%)	17 (3.5%)	16 (5.4%)	43 (3.9%)
	-taste	14 (4.5%)	33 (6.8%)	9 (3.0%)	56 (5.1%)
	-prep:	4 (1.3%)	6 (1.2%)	13 (4.4%)	23 (2.1%)
	-other	10 (3.2%)	13 (2.7%)	8 (2.7%)	31 (2.8%)
Purchase of ready-made meals		173 (55.4%)	248 (51.1%)	125 (42.1%)	546 (49.8%)

[1] Results were expressed as number of families (percentage)

Table 4 Sensory attributes of meal [1]

	Product	Mean sensory value			
Sensory attribute		0 month	1 month	2 month	3 month
Acceptability[1]	S. Hoppers	1.41	1.06	0.53	0.31
	Pittu	2	1.44	1	0.92
	Aluwa	1.18	0.77	0.44	0.31
	P-value	*0.23*	*0.61*	*0.49*	
Texture	S. Hoppers	2.06	1.06	0.84	0.69
	Pittu	1.39	0.94	0.68	0.62
	Aluwa	1.53	1.12	1	0.82
	P-value	*0.23*	*0.6*	*0.08*	
Odour[2]	S. Hoppers	1.77	1.12	1.05	0.85
	Pittu	2.17	1.56	1.05	1.08
	Aluwa	0.94	0.53	0.63	0.69
	P-value	*0.14*	*0.11*	*0.21*	
Taste	S. Hoppers	1.41	1	0.74	0.46
	Pittu	2.17	1.44	0.95	0.92
	Aluwa	1.47	0.94	0.58	0.39
	P-value	*0.11*	*0.52*	*0.33*	

[1] There is a significant difference in acceptability of Pittu over Aluwa (p=0.028)
[2] There is a significant difference in odour of Pittu over Aluwa (p=0.009); and String hoppers (p=0.013); Meals of second month were significant from meals at the end of first month (p=0.004)

Table 5 Percentage iron absorption from the reference dose and the test dose

	Ref. dose	Test dose	P-value[1]			
	(^{57}Fe) Absorption	(^{58}Fe) Absorption	Grp 1	Grp 2	Grp 3	Grp 4
Group 1	31.4 % ± 11.7	2.5% ± 1.5	---	0.23	0.32	0.03
Group 2	30.3 % ± 12.8	3.5% ± 2.0	0.23	---	0.03	0.1
Group 3	31.9 % ± 08.0	1.9% ± 1.1	0.32	0.03	---	0.005
Group 4	30.4 % ± 12.6	6.1% ± 4.4	0.03	0.09	0.005	---
Mean	31.0 % ± 10.9	3.4 % ± 2.9				
P-value	0.98	0.003				

[1] Derived from the Analysis of variance (ANOVA)

Table 6 The baseline anthropometry and serum biochemistry of the study subjects[1]

Parameter	Group 1	Group 2	Group 3	Group 4	Overall
Sex	M 6; F 8	M 7; F 6	M 6; F 7	M 6; F 7	M 25; F 28
Age (months)	88.0 ± 18.7	94.9 ± 19.5	94.8 ± 19.2	100.9 ± 15.9	94.1 ± 18.4
Weight (Kg)	19.9 ± 3.8	20.5 ± 3.6	20.1 ± 3.9	21.7 ± 4.5	20.5 ± 3.9
Height (cm)	119.2 ± 7.3	122.3 ± 9.2	121.0 ± 8.2	124.5 ± 9.3	121.8 ± 8.5
BMI	13.9 ± 1.7	13.6 ± 0.9	13.6 ± 1.3	13.8 ± 1.5	13.8 ± 1.4
Haemoglobin (g/dL)	11.9 ± 1.1	12.2 ± 0.9	12.3 ± 1.2	12.2 ± 1.1	12.2 ±1.1
Serum Ferritin (µg/L)	47.3 ± 30.4	52.9 ± 36.8	51.9 ± 27.1	42.0 ± 23.9	48.5 ± 29.4
Serum Folate (nmol/L)	12.8 ± 4.9	12.4 ± 4.1	12.1 ± 5.6	13.0 ± 8.1	12.6 ± 5.7
Serum Zinc (µmol/L)	12.1 ± 2.7	11.8 ± 2.4	11.8 ± 2.3	12.1 ± 1.7	11.9 ± 2.2

[1] Group 1-(Fe+folate); group 2- (Fe+EDTA+folate); group 3- (Fe+Zn+folate); group 4- (Fe+EDTA+Zn+folate); results presented in mean ± SD

Table 7 The anthropometry of the children following intervention[1]

Parameter		Group 1	Group 2	Group 3	Group 4	Overall
Weight (Kg)	Pre	19.9 ± 3.8	20.5 ± 3.6	20.1 ± 3.9	21.7 ± 4.5	20.5 ± 3.9
	Post	19.9 ± 2.7	20.7 ± 3.0	20.9 ± 4.3	22.0 ± 4.7	20.9 ± 3.7
	P-value	0.01	0.03	<0.001	<0.001	<0.001
Height (cms)	Pre	119.2 ± 7.3	122.0 ± 9.2	121.0 ± 8.2	124.5 ± 9.3	121.8 ± 8.5
	Post	120.7 ± 7.0	122.4 ± 8.2	122.2 ± 8.0	125.1 ± 9.8	122.6 ± 8.2
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001
BMI	Pre	13.9 ± 1.7	13.6 ± 0.9	13.6 ± 1.3	13.8 ± 1.5	13.8 ± 1.4
	Post	13.6 ± 0.9	13.7 ± 0.9	13.7 ± 1.4	13.9 ± 1.5	13.8 ± 1.2
	P-value	0.2	0.26	0.04	0.003	<0.001

[1]Group1-(Fe+folate); group2- (Fe+EDTA+folate); group3- (Fe+Zn+folate); group4- (Fe+EDTA+Zn+folate); results presented in mean ± SD; p-value from the paired t-test comparing pre and post intervention

Table 8 The effect on haematology following intervention[1]

Parameter		Group 1	Group 2	Group 3	Group 4	Overall
Hb (g/L)	Pre	119.9 ± 11.3	121.9 ± 9.8	123.1 ± 12.2	121.9 ± 11.1	121.7 ± 10.9
	Post	125.0 ± 07.8	127.0 ± 10.3	129.8 ± 06.3	128.6 ± 08.3	127.6 ± 8.2
	P-value	0.64	0.11	0.02	0.008	<0.001
Ferritin (µg/L)	Pre	47.3 ± 30.4	52.9 ± 36.8	51.9 ± 27.1	42.0 ± 23.9	48.5 ± 29.4
	Post	48.5 ± 25.1	55.4 ± 32.1	49.5 ± 21.2	46.8 ± 35.4	50.0 ± 28.1
	P-value	0.93	0.55	0.76	0.59	0.66
Folate (nmol/L)	Pre	12.8 ± 4.9	12.4 ± 4.1	12.1 ± 5.6	13.0 ± 8.1	12.6 ± 5.7
	Post	17.5 ± 3.6	19.4 ± 9.6	17.4 ± 6.3	21.3 ± 9.9	18.8 ± 7.7
	P-value	0.03	0.02	0.02	<0.001	<0.001
Zinc (µmol/L)	Pre	12.1 ± 2.7	11.8 ± 2.4	11.8 ± 2.3	12.1 ± 1.7	11.9 ± 2.2
	Post	12.3 ± 2.8	12.1 ± 2.2	13.0 ± 2.5	12.7 ± 2.6	12.5 ± 2.5
	P-value	0.61	0.56	0.15	0.27	0.06

[1]Group 1-(Fe+folate); group2- (Fe+EDTA+folate); group3- (Fe+Zn+folate); group4- (Fe+EDTA+Zn+folate); results presented in mean ± SD; p-value from the paired t-test comparing pre and post intervention

in string hoppers and *aluwa*. There were no obvious signs of surface dehydration (stiff-textured or sticky in consistency) that could result in the products.
All sensory characteristics of foods remained positive during the whole period of flour storage (Table 4). Nevertheless, compared with the baseline the acceptability declined over storage time although there was no consistency in the direction of change. A significant difference in acceptability of *pittu* over *aluwa* (p=0.03) was observed during the course of the trial whereas there was no difference between acceptance of *aluwa* over string hoppers (p= 0.79) and *pittu* over string hoppers (p= 0.05). There was no difference in acceptability with the duration of flour storage (p= 0.434). Texture of the meals did not show any significant differences over the type of food (p=0.43), or the duration of storage (p=0.31). Compared with the control meals there was a significant difference in the odour of *pittu* over *aluwa* (p=0.009) and string hoppers (p=0.01). A significant difference in odour between the first month and the second month was seen in all 3 meals (p=0.004). There was no significant difference in taste between the type of meals (p=0.99) and duration of storage (p=0.74).

Investigation III Iron absorption from the reference dose of ^{57}Fe was similar among the 4 groups, with a mean of 31.0% (Table 5). The overall absorption of ^{58}Fe from the meal in all groups was 3.4 ±2.5% (geometric mean of 2.8%). Significant intra-group differences were seen for absorption of ^{58}Fe. The highest fractional absorption of Fe from the meal was in group 4 (6.1±4.4%) and lowest in group 3 (1.9±1.1%; Table 5).

The presence of zinc did not adversely affect absorption of ^{58}Fe from the meal. In fact, the absorption was greater in subjects groups 3 and 4 who received zinc (3.8 ±3.7%) than in groups 1 and 2 who did not receive zinc (3.0 ±1.8%) although this difference was not statistically significant (p>0.10). Absorption of ^{58}Fe from a meal was significantly greater (p<0.05) in groups 2 and 4 (4.7 ±3.6%) than those in groups 1 and 3 (2.2 ±1.3%). However, when group data were combined, those who consumed disodiumEDTA (groups 2 and 4) and those who did not (groups 1 and 3), there was a significant negative interaction between zinc and disodiumEDTA. In the groups not consuming zinc (groups 1 and 2), there was no significant difference in iron absorption (p<0.10) with or without disodiumEDTA. Among the groups that consumed meals containing zinc (groups 3 and 4), a highly significant increase (p=0.005) was seen in group 4 that was given disodiumEDTA.

In a multivariate model of fractional absorption of ^{58}Fe included the group, initial ferritin and haemoglobin levels, age, sex, and weight, only the group (p<0.01) and sex (p=0.02) were significant. Males (4.4 ±3.7%) had a higher fractional absorption than females (2.5 ±1.5%). Based on the iron content in the fortified rice flour (60mg/kg), the absorption of added iron was highest in group 4 (6.1 ±4.4%). Children in this group had average absorption of 92µg (±66.3 µg) of iron from the 25g of rice flour in the test meal. This represents about 13% (range, 4-22%) of the estimated absorbed requirement (0.7 to 0.8mg) of iron in this age group (15).

Fractional zinc absorption averaged 10.9 ±5.1% overall in those groups who received a meal made with zinc-fortified rice flour. Fractional absorption of zinc was 13.5 ±6.0% in group 4 and 8.8 ±2.0% in group 3. The difference between the two groups was statistically significant (p= 0.04). In a multivariate model to predict fractional zinc absorption including the group, initial zinc level, sex, age, and weight; only the group was statistically significant (p= 0.04). Average absorption of added zinc in the meal was 202µg (±89.8 µg) in group 4 and 132µg (±30µg) in group 3 respectively. This constitutes approximately 17% (±8.0%) and 11% (±2.5%) of the 1200 µg of recommended absorbed zinc needs of an 8 year old child (16).

Investigation IV The sample consisted of 25 male and 28 female subjects with an age range of 7 to 10 years. There were no significant differences between subgroups in terms of age, anthropometrics and biochemical data (Hb, ferritin, zinc, and folate) at the beginning of the study (Table 6). At baseline 21% were underweight and 17.0 % were stunted. Further, baseline data from this study showed that 38.0% of the subjects were anaemic, 8.0% had low SF, 36.0% were deficient in folate and 15.0% had zinc deficiency. statistically significant improvement in anthropometry was observed with the intervention from their respective baseline value (Table 7, p<0.001). However, BMI was not significantly improved in groups 1 and 2. The zinc supplemented groups (groups 3 & 4) had a mean weight gain of 780g whereas non-zinc treatment groups (groups 1 & 2) had only 570g. These values were not significant (p= 0.205).

Haemoglobin and serum folate levels significantly improved (p<0.001) during the intervention (Table 8). When the sub-group analysis was made, all four groups had significant improvement in serum folate from their respective baseline value (overall

6.28nmol/L, $p<0.001$). Groups 3 and 4 had shown a significant improvement in Hb concentration ($p=0.01$). Overall, levels of SF and serum zinc improved (by 1.51µg/L and 0.59µmol/L respectively) during this short period of time, but these changes were not significant ($p=0.10$). In the groups that received disodiumEDTA when compared with those that did not receive a greater increase in SF and Hb in the EDTA groups was evident. However, the increased was statistically significant in Hb only ($p=0.03$). The groups that received zinc-fortified meals showed a gain in serum zinc, but did not reach a statistically significant level.

Discussion

Food fortification provides maximum benefit for minimum investment (17) and is generally recognized as being the most efficient as well as the most cost-effective means of eliminating micronutrient deficiencies when compared with supplementation. The results of fortification are broad and sustainable when compared with supplementation. A staple food that is consumed regularly by the majority of the population is generally used as a vehicle for fortification and as such, high population coverage can be easily achieved.

The observed monthly per family consumption of rice in our study is close to the finding (35.3Kg) of the national survey (9). According to the national survey 70% of households spent more than half of their total expenditure for food and drink in Sri Lanka and *per capita* rice flour consumption of 70.75g with 296.45g per household. However, our findings are of double this amount (140.7g). This could be due to the fact that our results are based not only on rice flour purchased but also on home made as well. The social class difference in rice flour purchasing patterns explains, in part, that there is an avenue to popularize fortified rice flour and food products of fortified flour among poorer segments of the population. We did not quantify the common rice flour based food products that the national survey had identified. However, in our study we found that string hoppers, hoppers and *piitu* were the most frequently consumed rice flour based food items in Sri Lanka. Urban households appear to have better access to prepared rice-based foods; hence a lesser quantity of rice flour based foods are prepared at home. Rural households (72%) prefer home-made rice flour. It may be due to poor access for ready-made flour and prepared food outlets and also due to cultural reasons. Small proportions of households claimed that rice flour based meals are not tasty (5.0%), or high in cost (3.9%) and need longer preparation time (2.1%).

There are some theoretical as well as practical advantages on using rice flour to deliver micronutrients to children through complementary feeding. It is unlikely that other kinds of flour especially wheat would be readily available to children of low-income families at a low cost in our country if the government discontinues the subsidiary programme on wheat. On the other hand it is the rice that is being used as the first cereal in the preparation of complementary foods for babies by the Sri Lankan mothers. The findings of this survey show that rice flour appears to be an appropriate vehicle for fortification with micronutrients. Since most of home-made complementary foods in Sri Lanka are also based on either rice or rice flour, our findings suggest that toddlers too can benefit from such fortification.

It was evident during this consumer acceptance trial that the quality of flour did not get deteriorated with time although rancidity of ferrous sulfate was reported in a previous study on wheat flour fortification in Sri Lanka (18), where wheat flour fortified with sodiumironEDTA and ferrous fumarate became less acceptable sooner than the flour fortified with reduced iron and electrolytic iron. But in our trial as we did a multiple micronutrient fortification i.e. ferrous sulfate with disodiumEDTA, zinc oxide and folic acid, its' sensory characteristics could have been different from the previous wheat flour trial. The effect of rancidity/ discoloration of ferrous sulfate may have been masked by the presence of disodiumEDTA (19). The quality of flour although supposed to be deteriorated with time, the taste, texture and acceptability remained within the desired limits at the third month of storage as well. The ambient flour storage conditions in Sri Lanka are harsh, with high temperatures and humidity. Flour under such conditions requires relatively rapid consumption. The results obtained by this investigation confirm that fortified rice flour stored upto a period of 3 months was accepted by the subjects for preparation of their meals. It can be concluded that the odour, acceptance, taste and texture of meals made out of rice flour with 60 mg/kg fortification level of iron and zinc had satisfactory sensory attributes and therefore, enrichment of rice flour could be considered as a feasible strategy in Sri Lanka.

This is the first-ever trial in Sri Lanka examining the absorption of micronutrients from the fortified rice flour. The fractional absorption of both iron and zinc from fortified rice flour was lower than predicted.

However, it was evident that disodiumEDTA enhanced the absorption of both micronutrients. The rice flour used in this study had relatively low phytate concentrations (i.e. 110±10 mg/100g). The phytic acid content in the local rice is variety-dependent (250 to 530 mg/100g with an average of 320 mg/100g), and polishing (6-8% in our rice) will further reduce the phytate to a range of 80 to 330 mg/100g (20). The phytic acid:zinc molar ratio of this study was 15:1. The low fractional absorption in this study can be attributed solely to high phytate levels. However, the zinc status of the individuals also needs to be considered. Zinc absorption increases with increasing zinc deficiency (16). The relative adequacy of the zinc status of the population studied may have contributed to the low bioavailability of zinc observed.

Analysis of the results demonstrated that regular consumption of micronutrient-fortified rice flour for four weeks led to improvements in weight, height and several measures of micronutrient status in semi urban school children in Galle. The improved growth (weight and height) in the study groups cannot be assumed to be due to a higher energy intake from the rice flour itself, as a control group was not included in the study. But there are other potential explanations. One of the cardinal features of zinc deficiency is growth failure; thus, it is possible that the increase in zinc intake augmented lean tissue accretion or increased utilization of the energy provided. Such a possibility is described in the response of BMI in this study as it significantly improved only in zinc supplemented groups. Secondly, it is possible that it stimulated an increase in appetite of the children and thereby increasing their energy intake. This could have resulted in the improvement in growth and general status of health of these children. Thirdly, improvement in iron stores, and enhanced serum folate and zinc status *per se* may have had an impact in limiting morbidity, which in turn can lead to improvement in growth and development of children.

The mean improvement in haemoglobin was higher in zinc supplemented groups. Thus, it was demonstrated that the added zinc had no deleterious effect on iron stores. The increase in serum zinc and folate concentrations observed at the end of the study strongly suggests a beneficial effect on zinc and folate status of primary school children as a result of consumption of the fortified rice flour. This is of great importance as improvement in iron, zinc and folate status have been shown to enhance growth and decrease morbidity and mortality from infectious diseases in school children in developing countries (21).

The present pilot efficacy study is limited by the small size of the study population. Further, the duration of supplementation may not be sufficient to draw definite conclusions on the effectiveness of the fortification formula used. It is not possible to definitively evaluate the outcomes without a control group, although our results are likely to reflect the true benefit of the fortified rice flour. Therefore, a definitive randomized, placebo-controlled, double blind study covering multiple age groups over an extended period should be conducted in order to evaluate the effectiveness of the proposed rice flour fortification before implementing at national level.

Conclusions

In this research programme, rice flour was evaluated as a potential vehicle for fortification with micronutrients such as iron, zinc and folate. One of the limiting factors in fortifying a dietary staple is the lack of simple and affordable technology to fortify with stable and bioavailable nutrients without compromising commonly accepted taste and appearance. A meal made out of 50g of fortified rice flour, one fourth of the daily recommended absorbed iron and zinc content of a primary school child can be met. In order to evaluate efficacy and effectiveness of rice flour fortification, a definitive randomized, placebo-controlled, double blind studies in multiple age groups over an extended period is necessary. Therefore multi-sector approach to be adopted in the establishment of any food-fortification programme, encompassing the participation of relevant governmental organizations, food industry, trade organizations, consumers, academic and research facilities, marketing specialists and any involved international organizations and agencies is recommended.

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Review

Physical Activity, Bone Mass and Bone Structure in Pre-pubertal Children

Alwis G

Department of Physiology, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.

Abstract

Physical activity (PA) has been described as one of the best strategies to optimize skeletal development during growth. In this study, at baseline 81 boys and 53 girls aged 7–9 years were included in a curriculum-based exercise intervention program comprising 40 minutes of PA per school day. Age and gender-matched 57 boys and 50 girls, assigned to the general Swedish school curriculum of 60 minutes PA per week, served as controls. Both boys and girls in the intervention group had significantly higher accrual of bone mineral content and larger gain in bone size in the lumbar vertebrae. No exercise-induced bone mineral accrual or structural changes were observed at the femoral neck. The PA measured by accelerometers was high so that all children reached the international recommended level of 60 minutes of moderate to vigorous PA per day. Children who participated in the exercise intervention groups were reported to experience more of the highest intensities of physical activities. This study has identified that a school-based exercise intervention program in pre-pubertal children enhances the skeletal benefits at the lumbar spine but not bone mineral accrual or structural changes at the femoral neck.

Corresponding author: Alwis G

<**alwisgayani@yahoo.com**>

This study was performed in University of Lund, Sweden and the results were included in a thesis with five published papers for a PhD degree with the University of Lund, Sweden and defended the thesis on 7th of May 2009

Introduction

Low bone mineral density (BMD) is a risk factor for fragility fractures and much of the fracture preventive effort today is devoted to preventing low BMD. The peak bone mass (PBM) is also to be considered as an important determinant of bone strength and fracture prevention in old age because around 50% of bone mass at the age of 70 years is estimated to be predicted by PBM (1, 2). Theoretical analyses estimate that a 10% increase in PBM could delay the development of osteoporosis by 13 years (3). Physical activity (PA) has been described as one of the lifestyle factors that could optimize gain in bone mass and bone strength during growth. During childhood and adolescence, both high-intensity and moderate-intensity PA increases PBM and bone structure (4, 5). The pre- and early peri-pubertal period, a period with fast skeletal growth, is usually considered as the best opportunity to enhance skeletal strength by exercise (6).

The specific effects of PA on bone health have been investigated in numerous cross-sectional studies, prospective observational studies, prospective controlled intervention and prospective randomized controlled trials (RCT). Reports suggest that resistance and high-impact exercise is the type of training that confers the most obvious benefits during these ages (7). However, most of the studies have included volunteers and used specifically designed high-impact PA programs, such as jumping down from a height, and organized sports activities (8-11). One problem with such monotonic programs is the high dropout frequency (12). Exercise interventions have also evaluated whether school-based physical education (PE) classes that use a variety of physical activities, jumping activities or circuit training, influence bone mass and the femoral neck (FN) structure using hip structural analysis (HSA) (13-15).

The current knowledge in this thesis is whether moderately intense exercise intervention programs could improve bone mass and bone structure (16). PA could possibly be used as a prevention strategy against low bone mass and low bone structure because studies have shown that exercise-induced skeletal benefits during growth and young adulthood persist into adulthood, assisting in fracture reduction (17).

Based on the consistency of literature, PA during growth has been described as one of the best strategies to optimize skeletal development during growth. However, most exercise intervention studies in children have involved the use of volunteers and specifically designed high impact exercise programs. Therefore, this study aimed to evaluate whether a general school-curriculum-based, moderately intense exercise intervention program and the mode of transportation to school could influence bone mass and bone structure in a group of pre pubertal children.

Study design and Methodology

The study subjects were recruited from first two years' evaluations of the Paediatric Osteoporosis Prevention (POP) cohort. The POP Study, in Sweden, is a prospective controlled exercise intervention study in school children that annually assesses musculoskeletal development in school children. Four neighboring schools that were government-funded and located in the same socioeconomic background were invited to the study. One school was invited to participate as the intervention school and the other three schools served as control schools. In the intervention school, the normal mean Swedish curriculum of 60 minutes per week was increased to 40 minutes per day (200 minutes per week). Children from the control schools were also assigned to similar activities as performed in the intervention school, but limited to the mean duration of PE classes in Swedish school, one or two sessions per week or mean 60 minutes per week. The physical educational curriculum in Swedish schools includes PE classes with a variety of activities, such as ball games, running, jumping and climbing. The intervention was deliberately designed not to be a specific osteogenic exercise program. The physical educational classes were conducted under supervision by the ordinary class teacher, typical of PE classes in Swedish schools. The studies were approved by the Ethics Committee of Lund University and the Radiographic Committee at Malmo University Hospital, Malmo, Sweden. Also, the studies were conducted according to the Helsinki Declaration and informed written consent was obtained from parents or guardians of participants prior to the commencement of the study.

Anthropometric measurements

Body weight was measured with an electric scale to the nearest 0.1 kg and body height by a wall-tapered height meter to the nearest 0.5 cm. The children were measured in light clothes in sock soles. BMI; kg/m^2 was calculated as weight in kilograms divided by height in meters squared. The TB fat mass and TB lean mass was measured by a DXA TB scan (DXA, DPX-L version 1.3z, Lunar®, Madison, WI).

Dual energy X-ray absorptiometry (DXA)

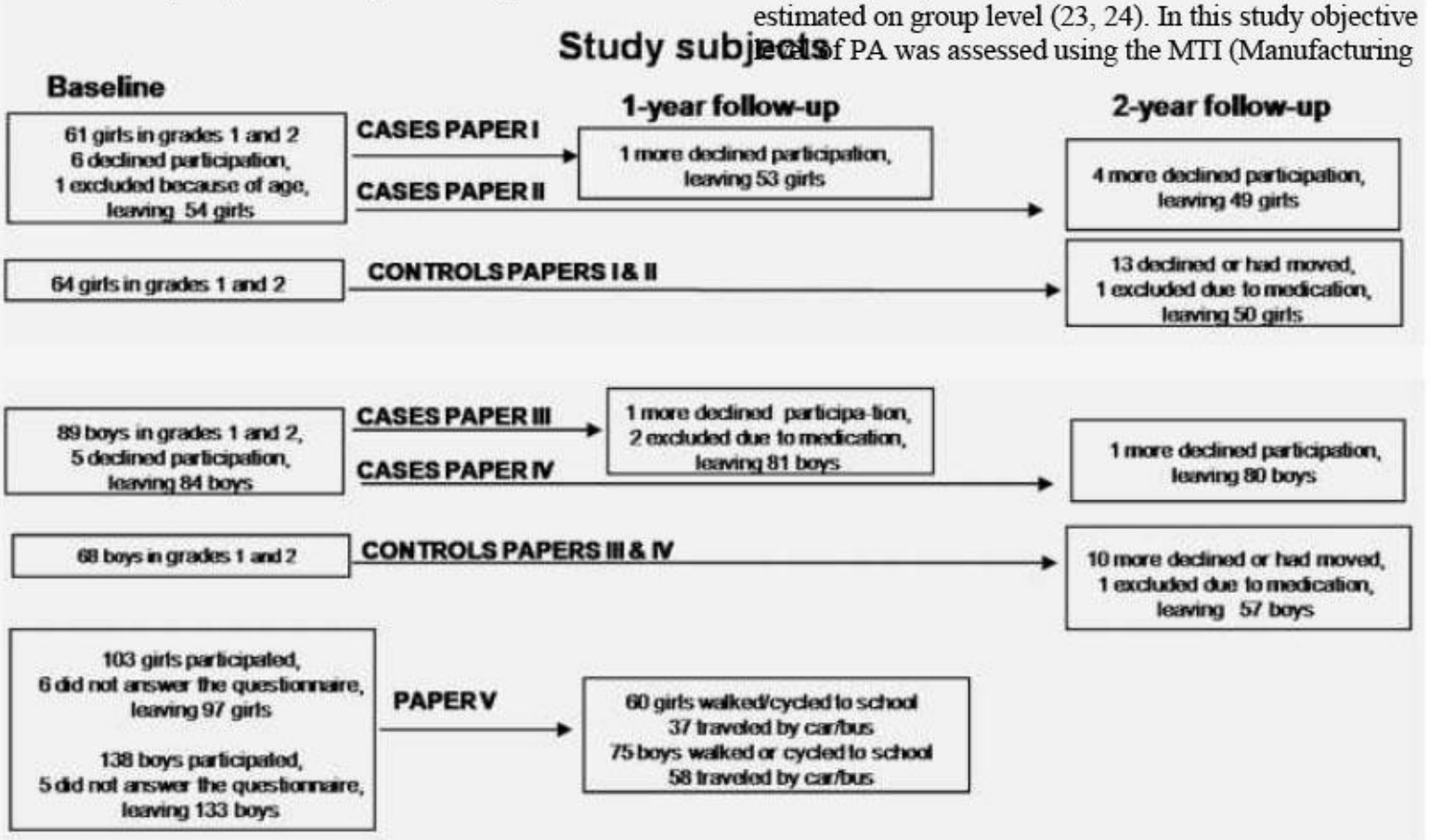
DXA (DXA, DPX-L version 1.3z, Lunar®, Madison, WI) was used to assess development in the children. During the measurements the children were dressed in light clothes and no shoes. Paediatric software was used in children below 35 kg in weight. Bone mineral content (BMC) and areal bone mineral density (aBMD) were evaluated for the total body (TB), the LS (L2–L4 vertebrae), third lumbar vertebra (L3) and FN. The width of the L3 was estimated from the LS scans and the width of the FN was estimated from the hip scans. DXA is the most commonly available bone densitometry technique and considered the gold standard in the measurement of BMC and BMD. BMC is expressed in grams. BMD (g/cm^2) is calculated by dividing the total amount of bone mineral by the projected bone area. The high precision, the non invasive short scan time and the low radiation dose which is less than the daily dose of radiation from the natural background radiation are the advantages of the DXA technique (18). However, DXA measurements do not estimate the material composition or the structure of the bone, which is a disadvantage because it is well known that bone material composition, bone geometry and bone micro-architecture are also important for bone strength. One field that has gained interest during the last few years is the DXA based hip geometry, specific software-- the hip structural analysis (HSA), where the three-dimensional structure of the hip is estimated from the two-dimensional hip DXA scan (19). Cross-sectional moment of inertia (CSMI, cm^4) is derived from the integral of the bone mass profile across the minimum bone cross-section together with its center of mass. CSMI estimates the ability of FN to withstand bending forces and is calculated using the mass of the absorptive curve (20).

Hip structural analysis (HSA)

Hip structure has been evaluated by the DXA-derived HSA software provided by Lunar Instruments Corporation (Madison, WI). All standard image files of the proximal femur were analyzed by one technician, using the HSA software. Using this software the X-ray absorption data of the proximal femur is extracted from the output image data file and the BMC, aBMD and its distribution within the FN calculated. First the operator has to manually define the center of the femoral head and place the FN axis as accurately as possible along the FN. Thereafter, the region of interest in the FN is placed in the proximal part of the FN, and finally the femoral shaft axis is defined centrally along the shaft. The

software will then iteratively assess all cross-sections in the femoral neck region of interest (FN ROI) and identify the plane with the least CSMI. The CSMI estimated by DXA has been found to be highly correlated with the CSMI measured directly on adult cadaver specimens (r^2 = 0.96) (20). Automatic identification of the weakest cross-section of the FN is a fundamental feature of the HSA software, and this cross-sectional level is then used for subsequent calculation of Z and CSA. The Z is computed as CSMI divided by half the width of the FN. The endosteal diameter was estimated using the algorithm described by Thomas J. Beck (21). Mean cortical thickness was calculated as the difference between the periosteal and the endosteal diameter divided by two.

Flow of study subjects through the study



Objective assessment of physical activity

Subjective level of PA and lifestyle factors were assessed using an interviewer administered questionnaire. Because of the difficulties in subjectively assessing the level of PA in children, accelerometers have been increasingly used for objective measurements of PA in children (22). Objective assessment of PA by accelerometers provide valid and reliable measures of intensity, frequency, duration and total amount of PA especially in epidemiological studies when PA is to be estimated on group level (23, 24). In this study objective level of PA was assessed using the MTI (Manufacturing Technology Incorporated, Fort Walton Beach, FL, USA) accelerometer, model 7164. The accelerometer measurements were performed for four consecutive days at the two-year follow-up evaluation. Accelerometer data are averaged over a period called an epoch. A recording epoch of ten seconds was selected for this study. SAS-based software (SAS Institute Inc, Cary, NC, USA) was used to analyze all accelerometer data. This software automatically deletes missing data, defined as continuous sequences of 60 consecutive epochs (i.e. 10 minutes) or more with zero counts. In order to minimize inter-instrumental variation, all accelerometers were calibrated against a standardized vertical movement. Mean activity was considered to be the total accelerometer counts per valid minute of monitoring (mean counts/min; cpm). Age- and body-mass-specific cut-off points exist for accelerometer counts representing activity of varying intensities, and these cut-off points made it possible to roughly estimate

the number of minutes the child was engaged in activity above a specific intensity threshold. Time spent performing above three Metabolic Equivalents (METs) was considered to reflect moderate-to-vigorous physical activity (MVPA), and time spent above six METs was considered to reflect vigorous physical activity (VPA). Cut-off points used for all children were >167 counts/epoch for MVPA and >583 counts/epoch for VPA (25). As both animal and human studies suggest that a mechanical load inserted with a high load, a fast load and for the skeletal an unusual load confers the highest anabolic skeletal response (7, 26). we also wanted to compare the duration of the most intense activities between the groups. For this reason we also report the duration of activities >1000 counts/10-second epoch or >6000 counts per minutes per day and > 1667 counts/10-second epoch or >10,000 counts per minute per day (27, 28).

Results

At baseline, the two groups did not differ with regard to lifestyle factors, age, anthropometrics or bone parameters. When compared the annual changes, both boys and girls in the intervention group had significantly higher accrual of BMC and larger gain in bone size in the lumbar vertebrae. No between-group differences were observed for annual changes in the FN bone mineral accrual or hip structural changes measured. The positive effects in the lumbar spine were less in absolute values in the boys than in the girls (Table1-3).

Total duration of PA estimated by questionnaire was higher in the intervention group than in the control group. In contrast, at follow-up, there was no difference in the total amount of daily activity measured by accelerometers, while the intervention group was presented with a higher proportion of the most intense activities, above 10,000 cpm (counts per minute). No differences were reported in activity level when comparing children who walked or cycled to school with those who went by car or bus. The PA measured by accelerometers was high such that all children reached the international recommended level of 60 minutes of moderate to vigorous PA per day (Table 4-5).

Discussion

This study evaluates a prospective controlled school-based PE intervention program in pre-pubertal children aged 7 to 9 years at baseline. The study reports that the intervention was not associated with improved accrual of bone mineral or beneficial structural changes at FN during the first two years when the inferences were drawn based on measurements with the DXA-derived HSA analyses (27-29). In contrast, the exercise intervention was associated with a significant positive influence in the accrual of bone mineral in L2-L4 and gain in bone size in the L3 in both girls and boys during the one- and two-year period. (27, 30-32). The positive effects in LS in the boys were less in both absolute and relative values when compared with the changes in the girls. Why there was an exercise-induced effect in LS but not in FN and why the benefits were more obvious in girls is not clear. The proportion of trabecular bone varies from being about 25% in the distal radius and femoral neck to 66–75% in the vertebral body (33).The larger proportion of trabecular bone in a lumbar vertebral body than in FN could partly explain the discrepancy, as skeletal response to mechanical load is more often seen in trabecular than cortical regions due to the larger surface-to-volume ratio in trabecular bone.

The children were on a high level of habitual PA as observed by the accelerometer data, that all children, both girls and boys, reached the international recommended level of PA per day (34). Thus, if the children were already physically active, the amount of additional school-based training contributed proportionately less to the total amount of PA than in cohorts with sedentary children (35). The extra amount of exercise gained by the intervention could be enough to lead to benefits in a predominantly trabecular region, such as LS, but not in FN.

Differences between boys and girls in the level of PA could also explain the more obvious effects in the girls. As the boys generally had a higher level of activity than the girls, the intervention in the girls contributed proportionally more to the total amount of PA than in the boys (27, 31). Girls with the same chronological age as boys are generally closer to puberty than boys and the changes in bone mineral accrual and bone size in the lumbar spine is more obvious in girls than the boys (36). Because of the earlier onset of puberty, girls reach peak BMC velocity roughly 1.5 years earlier than boys (6). Thus, the observation that LS bone mass increases initially more in girls than in boys of the same chronological age is consistent with the literature (36, 37).

The HSA is another technique trying to assess hip geometry, but exercise intervention studies have so far reported conflicting results. Some studies have reported that everyday PA during growth predicts hip structure (38, 39), while other trials have opposed this view (40).

The discrepancies in the conclusions when comparing the trials could be based on differences in the study designs. The maturational level and gender of the children was different in the cited trials, and it is known that exercise-induced skeletal benefits are easiest to reach in the early pubertal period. Because of the study

Table 1 Annual changes boys 1 year follow-up

	Cases (N=81)	Controls (N=57)	P-value
Height (cm)	5.6 ± 1.1	5.7 ± 0.6	0.45
Weight (kg)	3.2 ± 1.8	3.3 ± 1.2	0.75
Lean mass (kg)	2.2 ± 0.6	2.1 ± 0.4	0.38
Fat mass (kg)	1.3 ± 1.5	1.0 ± 0.9	0.14
Lumbar spine	**Cases (N=76)**	**Controls (N=51)**	
L3 BMC (g)	0.87 ± 0.55	0.58 ± 0.26	**0.0007**
L3 BMD (g/cm^2)	0.041 ± 0.036	0.027 ± 0.015	**0.01**
L3 vBMD (g/cm^3)	0.003 ± 0.017	0.002 ± 0.007	0.82
L3 width (cm)	0.14 ± 0.14	0.07 ± 0.07	**0.0010**
L2L4 BMC (g)	2.46 ± 1.20	1.94 ± 0.51	**0.004**
L2L4 BMD (g/cm^2)	0.041 ± 0.024	0.028 ± 0.011	**0.0005**
Femoral neck	**Cases (N=74)**	**Controls (N=48)**	
width (cm)	0.10 ± 0.16	0.09 ± 0.08	0.80
CSMI (cm^4)	0.081 ± 0.119	0.082 ± 0.046	0.95
BMD (g/cm^2)	0.036 ± 0.07	0.037 ± 0.03	0.92
BMC (g)	0.211 ± 0.42	0.292 ± 0.19	0.21
CSA (cm^2)	0.098 ± 0.13	0.093 ± 0.06	0.81
Section modulus (cm^3)	0.051 ± 0.07	0.051 ± 0.03	0.99
Endosteal diameter (cm)	0.09 ± 0.16	0.08 ± 0.08	0.83

Values are mean ± SD

Table 2 Annual changes boys at 2 year follow-up

	Cases (N=80)	Controls (N=57)	P-value
Height (cm)	5.6 ± 0.7	5.7 ± 0.6	0.19
Weight (kg)	3.4 ± 1.6	3.3 ± 1.2	0.57
Lean mass (kg)	2.2 ± 0.5	2.1 ± 0.4	0.92
Fat mass (kg)	1.3 ± 1.4	1.0 ± 0.9	0.10
Lumbar spine	**Cases (N=76)**	**Controls (N=51)**	
L3 BMC (g)	0.72 ± 0.30	0.58 ± 0.26	**0.009**
L3 BMD (g/cm^2)	0.031 ± 0.02	0.027 ± 0.02	0.30
L3 vBMD (g/cm^3)	0.001 ± 0.010	0.002 ± 0.007	0.46
L3 width (cm)	0.11 ± 0.06	0.07 ± 0.07	**0.0015**
L2L4 BMC (g)	2.06 ± 0.76	1.94 ± 0.51	0.30
L2L4 BMD (g/cm^2)	0.030 ± 0.015	0.028 ± 0.011	0.37
Femoral neck	**Cases (N=73)**	**Controls (N=48)**	
Neck width (cm)	0.108± 0.084	0.098± 0.060	0.49
CSMI (cm^4)	0.078 ± 0.067	0.082 ± 0.046	0.70
BMD (g/cm^2)	0.025 ± 0.038	0.037 ± 0.032	0.06
BMC (g)	0.231 ± 0.213	0.281 ± 0.205	0.20
CSA (cm^2)	0.080 ± 0.079	0.093 ± 0.060	0.31

Section modulus (cm^3)	0.044 ± 0.040	0.050 ± 0.027	0.39
Endosteal diameter (cm)	0.103 ± 0.081	0.090 ± 0.057	0.35

Values are mean ± SD

Table 3 Annual changes girls at 1 year follow-up

	Cases (N=53)	**Controls (N=50)**	**P-value**
Weight (kg)	3.5 ± 2.2	3.2 ± 1.3	0.42
Height (cm)	6 ± 1.2	5.7 ± 0.96	0.19
Lean mass (kg)	2.2 ± 0.75	1.9 ± 0.56	**0.01**
Fat mass (kg)	1.9 ± 1.5	1 ± 0.86	**<0.001**
Lumbar spine	**Cases (N=50)**	**Controls (N=48)**	
L3 BMC (g)	0.91 ± 0.61	0.54 ± 0.24	**<0.001**
L3 BMD (g/cm^2)	0.047 ± 0.035	0.024 ± 0.014	**<0.001**
L3 vBMD (g/cm^3)	0.004 ± 0.015	0.001 ± 0.007	0.22
L3 width (cm)	0.16 ± 0.10	0.09 ± 0.05	**<0.001**
L2L4 BMC (g)	2.35 ± 1.13	1.77 ± 0.55	**0.0019**
L2L4 BMD (g/cm^2)	0.042 ± 0.026	0.026 ± 0.01	**<0.001**
Femoral neck	**Cases (N=42)**	**Controls (N=43)**	
width (cm)	0.086 ± 0.247	0.067 ± 0.113	0.64
CSMI cm^4)	0.069 ± 0.167	0.074 ± 0.067	0.86
CSA (cm^2)	0.084 ± 0.180	0.091 ± 0.078	0.82
Section modulus (cm^3)	0.044 ± 0.088	0.049 ± 0.042	0.73
Endostealdiameter	0.078 ± 0.240	0.057 ± 0.114	0.59
Mean Cortical thickness	0.004 ± 0.008	0.005 ± 0.005	0.44

Values are mean ± SD

Table 4 Physical activity in boys evaluated by accelerometers

At 2 year follow-up in boys	**Intervention (N=72)**	**Controls (N=55)**
Recording time (hrs/day)	12 (1)	13(1)
Mean activity (counts/min)	770 (267)	728 (211)
>3METs (min/day)	211(55)	209 (45)
>6 METS (min/day)	44 (21)	48 (19)
>6000 counts/min (min/day)	16 (10)	15 (9)
>10000counts/min (min/day)	4 (3), p=0.01	2 (3)

Values are mean ± SD

Table 5 Physical activity in girls evaluated by accelerometers

At 2-year follow-up	**Intervention (N=41)**	**Controls (N=40)**
Recording time (hrs/day)	12 (1)	12 (1)
Mean activity (counts/min)	644 (184)	590 (115)
>3 METS (min/day)	194 (45)	185 (35)
>6 METS (min/day)	34 (15)	35 (12)

>6000 counts/min (min/day)	13 (8)	11(6)
>10000 counts/min (min/day)	3(3), p<0.001	1(1)

Values are mean ± SD

design, this study cannot rule out that a more intense training program or a program spanning a longer period could lead to structural and bone mineral benefits also in FN. For example, soccer training for three years in pre-pubertal boys has been associated with obvious bone mass benefits also in FN (35). To report that high-intensity osteogenic programs in volunteers are associated with skeletal benefits does not increase our knowledge. Instead this study was designed to evaluate whether a general school-based PE intervention program on a population-based level, also including children with little or no interest in sports, could be used as a preventive strategy against low bone strength. Furthermore, we wanted to evaluate an intervention at a level so that all children could participate.

One cross-sectional publication has reported that a higher level of daily PA is associated with beneficial structural changes in the hip evaluated by the HSA software in pre-pubertal children at a mean age of 5.2 years (38). Two observational studies with 6 and 7 years of follow-up in children and adolescents support the view that higher level of PA is associated with the higher CSA and Z of the FN compared to less active peers (39, 41). One randomized school-based jumping intervention program, including three sessions of ten minutes per week over seven months, reported that there were no beneficial effects found in the gain in hip structure in 43 pre-pubertal girls in the training group in comparison with 25 matched girls in the control group. In contrast, in 43 early pubertal girls in the same study, the gain in FN BMD was 2.6% and gain in Z 4.0% larger, with a p value < 0.05, in intervention girls in comparison to the gain in 63 early pubertal girls without this intervention program (13). Another randomized prospective controlled trial evaluating a school-based exercise intervention program followed during 20 months, with the intervention consisting of circuit training given for 12 minutes, three times a week in pre-pubertal boys, reported that the 31 boys in the intervention group had increased FN BMC 4.3% more ($p <0.01$) and Z 7.5% more ($p <0.05$), than the 33 boys in the control group (42). The differences in gender, follow-up period and maturational status may influence the conclusions in these two papers.

DXA-derived BMD is a measure that estimates the amount of bone mineral in a three-dimensional bone structure but projected on a two-dimensional area of the bone. Because the third dimension of the bone, the depth, cannot be estimated by DXA, BMD is also often called areal BMD (aBMD; g/cm^2), which includes the total amount of bone mineral within the scanned area (43). This is one problem when trying to estimate the accrual of bone mineral in a skeleton that changes in size, as in growing children (43).

The large individual variation in changes in bone size and body composition (fat mass and lean body mass) that occur during growth can also obscure results when prospectively following growing children (44). The total amount of soft tissue, the distribution of fat mass and the ratio between the fat and the lean mass are also important when estimating the actual BMD level. It is known that differences in fat in the bone marrow or the soft tissue above, below or around the bones may affect the DXA bone variables (45, 46). Thus, the changes in lean tissue and fat content during the study period could also influence the estimated level of BMC. The finding of higher fat mass in control group in the present study contradicts other studies in pre-pubertal boys that report participation in sport to be associated with reduced fat mass and improved gain in lean mass (46).

DXA scanners were designed to measure bone density, not the structure of a bone, so poor spatial resolution complicates the detection of bone dimensions (19). Inconsistent positioning of the limb, especially in anteversion of the hip or inaccurate placement of the ROI, may also result in a measurement error (47). This especially accounts for prospective studies when individual changes are followed by repeated scans in the same individual. The DXA-derived HSA is a two-dimensional technique that is transferred to a three-dimensional assessment of the bone. For example, CSMI measured in a single plane of a DXA-projected area does not represent the bending strength of whole bone because it measures CSMI in one direction only (19). Even a smaller error in this estimate will lead to a greater error as CSMI is proportional to the fourth power of the radius of the femoral neck (16). The two-dimensional estimation of the periosteal dimension also gives rise to problems, as the skeleton can respond to mechanical load by expansion in different directions (9) and may be not captured by the HSA analyses. The calculated CSMI value, derived from a single slice in one plane, may then not reflect the true CSMI. The bending strength of FN would therefore be inaccurately estimated. The cortical thickness and the endosteal diameter are estimated after making assumptions of a homogenous porosity in the cortical shell, a homogenous cross-sectional shape that assumes FN to be cylindrical, not elliptical (21).

In spite of these limitations, HSA should be regarded as one method to focus interest not only on the amount of bone mineral but also FN structure when trying to better understand the skeletal response during mechanical load and PA. The discrepancy when comparing the literature highlights the requirement of further prospective investigations in children and adolescences, with the use of objectively measured intervention programs and

three-dimensional imaging techniques such as CT and MRI when evaluating the hip structural changes.

Walking and cycling to school could be an important regular source of PA in growing children. Cross-sectional studies support this view when reporting that walking and cycling to school are associated with a higher level of PA compared to traveling by vehicle (48, 49). However, this prospective study (50) reported that a physically active mode of transport to school was not, as evaluated by accelerometers, associated with higher overall levels of PA than transportation by car or bus. Also, there were no differences in the accrual of bone mineral or gain in bone size when the two transportation groups were compared. The discrepancy could be due to fact that the children in the POP cohort were on a general high level of PA so that the additional PA provided by active school transportation was of no biological significance. Another possible explanation is that the children lowered their leisure time PA if they practiced active school transportation supporting a previous report which infers the total amount of PA in children to be constant (51). That is, if children increase one activity they decrease another. Accelerometer data supported that there actually was no different level of PA between the two transportation groups (50). Furthermore, the distance from home to school was in general short in the POP cohort, 0.5 to 1.7 kilometers. In other words, active school transportation could be of importance in cohorts with a low habitual level of PA or a longer distance to school. Whether or not active school transportation provides skeletal benefits, as advocated over the years, as well as other health-related benefits must also be evaluated in further follow-up studies within this cohort.

The intervention program in the POP cohort, as estimated by the questionnaire, was associated with a higher duration of exercise per week. In contrast, the accelerometer data could not verify this (27, 28). As previously shown, the children in the POP study were all on a relatively high level of PA, irrespective of being in the intervention group or not and irrespective of having active or passive school transportation (27, 28, 50). In fact, all children reached the international recommended level of 60 minutes of MVPA per day set by the United Kingdom Expert Consensus Group (34). The present study supports that the accelerometer-measured total level of PA was no different between the intervention and control group, while the most intense activity, above 10,000 counts per minute, was higher in the intervention group could possibly be influenced by an intervention program (27, 28). The finding is of considerable interest as high-intensity activities with few daily repetitions are more important than a long duration of exercise if the purpose is to reach skeletal benefits (7, 26, 52).

Conclusions

A prospective, controlled, moderate exercise intervention program in the general school curriculum for pre-pubertal children aged 7 to 9 years at baseline suggests that the increaseed duration of PE classes has a positive influence on BMC accrual and bone size in the lumbar vertebrae. However, the program does not influence the bone mineral accrual or structural changes at the FN during the first two years. Also, the positive effects in the LS are less in absolute values in the boys than in the girls during the first two years of follow-up. The actual self-reported significant differences in the total duration of PA between the intervention and control group were not found when the two groups were compared with the accelerometers for total level of PA. Children in the exercise intervention groups, however, both girls and boys, were reported with more of the highest intensities (> 10,000 counts per minute) of PA as detected by accelerometers. Regardless of the mode of school transportation, all girls and boys reached above the international recommended level of PA per day. The relatively low amount of additional PA contributed by active school transportation seems not to influence bone health.

Perspectives

Further studies are required to determine whether an exercise program exceeding two years and in children closer to puberty could be beneficial also for the FN structure. In addition, trials with a longer follow-up period should be performed so as to evaluate whether the benefits measured at LS are transitory or additive with each year over the entire growth, thus rendering benefits in PBM and bone size of biological significance. More advanced techniques such as pQCT and MRI should be applied for the assessment of bone structure in order to add more knowledge within this field.

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Review

Peak bone mass as measured by phalangeal bone mineral density and its association with nutritional status, socioeconomic status and physical activity: A community based-cross-sectional study in Galle district

Rodrigo PM

Department of Anatomy, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.

Abstract

Current knowledge on bone mineral density (BMD) changes during adolescence is based on the studies done in Western populations. Obvious differences in determinants of bone health between Asians and Europeans would not allow such a comparison. Knowledge of the age in which BMD attains its peak is important in planning health promotional activities in a country. Current recommendations on bone health in Sri Lanka are based on studies conducted in Western populations but geographical variations in BMD accrual would limit such an application. This project examines phalangeal BMD (pBMD) in subjects selected from the Galle district to ascertain the timing of BMD peak and its associations with nutritional status, socioeconomic status and physical activity, in a cross-sectional manner. The age at which the peak phalangeal BMD is achieved was determined in 657 healthy men and women, aged between 20-49 years, selected by stratified randomization from the Galle District. The peak phalangeal BMD was seen in men and women between 30-39 years. Females of this age group (i.e. 30-39 years, n=582) were further studied to examine the associations of their bone mineral density and bone mineral content (BMC)with anthropometry, physical activity (current and past), socioeconomic status, dietary intake and biochemical markers of bone health.

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Corresponding author: Rodrigo PM

< **mahindarodrigo@yahoo.co.uk**>

Introduction

Peak bone mass (PBM) can be defined as the amount of bony tissue present at the end of skeletal maturation. The bone mass of a given part of the skeleton is directly dependent upon both its volume (size) and the density of the mineralized tissue contained within the periosteal envelope (1). It has been generally accepted that peak bone mass at any skeletal site is attained in both sexes during mid-thirties (1). It is considered that bone mass increases rapidly during childhood and adolescence to 90% of adult levels and reaches the peak in the third decade (1). Thereafter, bone mass starts to decline where minerals and collagen matrix are removed from bone more rapidly than new bone tissue is added. The rate of bone loss, however, varies between two genders and in different age groups (1). Peak bone mass and the subsequent rate of loss would determine the amount of bone mass left in old age. From youth to old age, women typically lose half of trabecular and one-third of cortical bone, whereas men lose only one-third of trabecular bone and one-fifth of cortical bone (2). Therefore, Frankie, in 2004 (2) suggested that optimizing PBM at the point of skeletal maturity is one method of ensuring higher bone mass in later life as it means that loss begins at a higher point and it would take a longer time before bone mass becomes critical.

All the studies on PBM have used either BMD or BMC as the in-vivo methods of quantifying bone mass and despite subtle differences they have used these terms interchangeably in studies on PBM (3,4). The importance of achieving an ideal peak bone mass for bone strength in later life has been suggested by previous observational and longitudinal studies (2,4). They have suggested that individuals with a high peak bone mass are likely to have a high bone mass in old age, so that, osteoporosis-prone individuals could be identified much earlier in life if bone density was low for age. Osteoporosis is a systemic skeletal disease

that is mainly characterized and quantified by low BMD. Although a great emphasis has been placed on understanding the mechanisms of bone loss in postmenopausal women and older men, it is now known that early life events are equally important in the pathogenesis of osteoporosis (4).

Several variables, more or less independent, are supposed to influence bone mass accumulation during growth; heredity, sex, dietary components, endocrine factors, mechanical forces, and exposure to risk factors (5). Quantitatively, the most prominent factor appears to be the genetic determinant, as estimated by studies comparing monozygotic and dizygotic twins (7). With respect to nutrition, the quantitative importance of calcium intake in bone mass accumulation during growth, particularly at sites prone to osteoporotic fractures, remains to be clearly determined; the same can be said for the impact of physical activity. Finally, the crucial years when these external factors will be particularly effective on bone mass accumulation remain to be determined by longitudinal prospective studies in order to produce credible and well targeted recommendations for the setting up of osteoporosis prevention programs aimed at maximizing peak bone mass (6).

The mass of bone tissue present at any time during adult life is the difference between the amount achieved at maturity and the loss due to aging. Hence there is a growing interest among researchers to investigate how bone mass evolves during development and to identify the important factors that influence its rate of accumulation (8). Experimentally, bone mass is assessed by burning a piece of bone and estimating the weight of ash. Using pork carcasses, Michell *et al*., in 1998 (9) found a strong correlation between the ash content and both BMD and BMC measured by Dual Energy Xray Absorptiometry (DXA). Nagy *et al*., in 2002 (10), found 100% accuracy of DXA in estimating bone mass when compared with weight of ash. Hence, both BMD and BMC are used as surrogate measurements of bone mass in clinical applications and in-vivo studies.

There are many methods available for bone mass measurement. They are not much used in clinical practice. Most of them are used only for research purposes. For example, single-proton absorptiometry (SPA), dual proton absorptiometry (DPA), single energy X-ray absorptiometry (SXA) and radiographic photodensitometry (11,12) The most widely used technique to measure bone density is DXA.

Measurement of bone mineral content and bone mineral density

BMC is measured in grams of bone size where as BMD is expressed as the BMC over the antero-posterior projected bone area (BA) measured in square centimetres. $\mathbf{BMD} = \frac{\mathbf{BMC}}{\mathbf{BA}}$ $\mathbf{g/cm^2}$

There are several different machines that measure bone density. Central machines measure density in the hip, spine and total body. Peripheral machines measure density in the finger, wrist, kneecap and heel.

Dual energy X-ray absorptiometry

Radioigraphic absorptiometry is the modern-day descendent of radiographic photodensitometry (11). The underlying principle of Dual – energy X-ray absorpotiometry (DXA) is to determine the bone density in *vivo* by passing a monochromatic or dual energy photon through bone and soft tissue (11). The amount of mineral encountered by the beam could be quantified by subtracting the beam intensity after passage through the region of interest from the initial beam intensity. In radiology, attenuation refers to a reduction in an X-ray beam's intensity. The differences in tissue densities are responsible for creating the images seen on an X-ray. When the beam was passed through a region of the body containing both bone and soft tissue, attenuation of the photon beam occur at both energy peaks (12).

The regions most often measured with DXA are the hip (proximal femur) and lumbar spine (second lumbar vertebra to fourth lumbar vertebra), but it can also be used for measurement of other peripheral sites such as radius, calcaneous or even the total body. From a total body scan, lean mass, fat mass and even regional bone masses like arms, legs, head, and trunk can be obtained. Hip measurement is the best site for predicting hip fracture where as the spine measurement is the best site for the prediction of vertebral fracture. Central DXA (lumbar spine and proximal femur) is used for research and making therapeutic decisions, whereas peripheral DXA (finger and or heel), is used for research and screening purposes (12). Dual energy X-ray technology is also being employed in portable devices dedicated to the measurement of one or two appendicular sites. As such, these devices are characterized as peripheral DXA devices (12).

Method

Investigation 1

The eligible men and women were subjected to the measurement of BMD and BMC of the middle phalanx of the middle finger of the non-dominant hand (14) using the accuDXA (Shick technology, New York, USA), portable densitometer with DXA. Its scan time

is less than one minute and gives BMD as g/cm^2 and BMC in grams. Machine has a precision of less than 1.0% with an inbuilt quality control procedure, hence no user intervention is required (12). It initially obtains a digital picture of the middle phalanx to make sure the proper positioning of the region of interest in the scanning area and then measures both bone mineral content and bone mineral density. All scans were done with the help of one technician who had training on accuDXA and the accuracy and precision of the machine was tested on each scanning day using the in built software in the machine. Machine was kept in a cool environment during scanning to ensure the accuracy of estimations.

Seven hundred and forty five (745) individuals of 20 to 49 years of age were invited and 702 subjects (response rate 94.2%) attended the interview (352 males and 350 females). Forty five subjects (6%) were excluded from the sample due to the presence of diseases or having received medications, which could affect bone metabolism.

Investigation 2

After analyzing the results of the investigation 1, (table 1.1 & 1.2 above) it was found that both men and women reached peak bone mass between 30-39 years. BMD among women in this age group was further studied to examine the association of BMD with physical activity, social status, dietary intake, nutritional status assessed by anthropometry and biochemical markers of bone health.

Table 1.1 Mean BMD (SD) of 324 males and 333 females according to the age categories[1]

	20-29 yrs	30-39 yrs	40-49 yrs	*P-value
Male	0.595 (0.057)	0.603 (0.061)	0.591 (0.066)	0.32
Female	0.495 (0.057)	0.506 (0.062)	0.502 (0.064)	0.42

[1]There were 108 males and 108 females in 20-29 yrs category, 105 males and 110 females in 30-39 yrs category and 111 males and 115 females in 40-49 yrs category

*p compares the mean BMDs in three age groups and calculated using ANOVA

Measurements of the BMD and BMC

Measurements of the BMD and BMC were done by using AccuDXA (Shick technology, New York, USA). Measurements were taken by the same technician who participated in the investigation 1, and precautions described earlier were taken to ensure the accuracy of the measurements.

Table 1.2 Mean BMC (SD) of 324 males and 333 females according to the age categories[1]

	20-29 yrs	30-39 yrs	40-49 yrs	*P-value
Male	2.15 (0.34)	2.21 (0.36)	2.17 (0.39)	0.48
Female	1.48 (0.28)	1.55 (0.31)	1.55 (0.31)	0.15

[1]There were 108 males and 108 females in 20-29 yrs category, 105 males and 110 females in 30-39 yrs category and 111 males and 115 females in 40-49 yrs category

*p compares the mean BMCs in three age groups and calculated using ANOVA

Biochemical analysis

The subjects were instructed to be present at designated places at a given time (i.e. 0800hours in the morning) in batches with overnight fasting to obtain a sample of venous blood (5ml) for the biochemical assessment of serum vitamin D, parathyroid hormone and alkaline phosphatase. Blood was drawn from the median cubital vein under sterile conditions using disposable syringes. Specimens were brought immediately to the radioimmuneassay (RIA) laboratory of the Nuclear Medicine Unit, Faculty of Medicine in a box containing ice packs and serum separation was done without a delay and the separated sera were stored in a freezer at -80°C.

Results

Bone mineral density (BMD) and bone mineral content (BMC)

Mean (SD) BMD of the study sample was 0.493 (0.06) g/cm^2 and mean BMC was 1.49 (SD 0.28) g. BMD in the sample ranged from 0.230 to 0. 660 g/cm^2 while BMC ranged from 0.85 to 2.36 g. characteristics of study subjects were shown in Table 2.1

Effect of anthropometry on BMD/ BMC

Simple linear regression was performed with BMD as the dependent variable and all anthropometric measurements separately as independent variables. When all measures were included in the model and

Table 2.1 Characteristics of the subjects under study (n=582)

Measurement	Unit	Mean	SD
Weight	kg	51.06	8.9
Height	cm	154	5.6
BMI[1]	kg/m^2	21.61	3.6
Abd girth[2]	cm	74.4	8.9
Chest circum[3]	cm	86	8.3
Hip circum[4]	cm	89.97	8.3
SFT[5]	mm	15.72	6.4
Foot length	cm	23.48	7.9

[1]BMI – body mass index (weight / height^2)
[2]Abd girth - Abdominal girth
[3]Chest circum -Chest circumference
[4]Hip circum -Hip circumference
[5]SFT- Skin fold thickness

Table 2.2 The association between BMD and anthropometric measurements[1]

Measurement	r	r^2	Regression Co-efficient	p-value
Weight (kg)	0.29	0.08	0.002	<0.001
Height (cm)	0.34	0.12	0.004	<0.001
BMI (kg/m^2)	0.15	0.02	0.003	<0.001
Abd girth (cm)	0.2	0.04	0.001	<0.001
Chest circum (cm)	0.2	0.04	0.001	<0.001
Hip circum (cm)	0.18	0.03	0.001	<0.001
SFT (cm)	0.01	0.0001	0.0001	p=0.80
Foot length (cm)	0.03	0.001	0.0002	p=0.49

[1]n= 582

weak associations were excluded in step-wise fashion, height remained the strongest predictor of BMD (regression coefficient 0.004; SE 0.0004; p<0.001). In this model, height alone explained 12% of variation in BMD (Table 2.2).

Other measurements such as weight; abdominal circumference, chest circumference, and hip circumference, when taken individually were able to explain only 3-8% of variation in BMD. A one centimeter difference in height was associated with 0.004 g/cm^2 difference in BMD. Similarly, a change of one kilogram was associated with 0.002 g/cm^2 change in BMD. A unit increase in BMI was associated with an increase in BMD of 0.003 g/cm^2 (Table 2.2).

Socioeconomic status on BMD/ BMC

Classification of subjects according to the current socio-economic status is shown in Table 3. None were qualified to be included in Class 1 while 242 women were in social Class 2. There was no. difference in BMC/ BMD according to their social class indicating that socioeconomic status of these women had no significant influence on the peak bone mass observed

Physical activity on BMD/ BMC

They were categorized according to their physical activities as very active, moderately active, and less active depending on the type and duration of their physical activities. None of the participants were qualified to be named very active based on their current physical activity. BMD and BMC results according to their physical activity are presented in Table 4.

Women who were very active in their school days had the highest BMD irrespective of their current activities (mean BMD of 0.533 in very active women in the past). Women who were less active both in the past and at present had the lowest BMD (mean level of 0.448). In general, there was a positive association between BMD and the intensity of physical activity, in which BMDs of the moderately active women in the past were in between those of less and very active women irrespective of their current activities (p<0.001).

Dietary intake on BMD/ BMC

The intake of macro and some micro nutrients were assessed by a 24-hour dietary recall on three non consecutive days. The dietary survey data sheets that lacked information on any of the three days were excluded (n=5). Furthermore, incomplete recalls (n=3) or unrealistic data (n=53) that could not be corrected reliably were also rejected. Exclusion of unrealistic data was done with the agreement of the specialist on nutrition who oversaw the data collection. 524 study subjects or 90.0% of the total sample were able to provide complete dietary records. After recording and summarizing the recalls, the food intake data were converted into nutritional values with the help of Food Composition Tables (15). Daily energy and nutrient intakes were calculated in the three-day intake and presented as the average intake (mean, SD, median and inter-quartile range) in Table 5.

Table 3 BMC and BMD according to the socioeconomic status[1]

Category		n	BMC mean	BMC SD	BMD mean	BMD SD
Social Class[2]	Class- 2	242	1.485	0.269	0.49	0.057
	Class -3	136	1.483	0.285	0.489	0.059
	Class -4	155	1.523	0.298	0.497	0.063
	Class- 5	50	1.512	0.298	0.501	0.062
			(f=0.87; p=0.46)		(f=0.74; p=0.53)	
Marital status	Unmarried	202	1.507	0.231	0.496	0.052
	Married	380	1.492	0.308	0.491	0.064
			(f=0.90; p=0.34)		(f=0.38; p=0.54)	
Education[3]	Upto O/L	198	1.55	0.368	0.504	0.074
	A/L	324	1.45	0.221	0.484	0.051
	Above A/L	60	1.572	0.208	0.509	0.045
			(f=10.35; p<0.001)		(f=9.85; p<0.001)	
Occupation[4]	Professional	254	1.444	0.227	0.482	0.052
	Clerical	133	1.541	0.225	0.502	0.048
	Skilled	132	1.333	0.24	0.463	0.053
	Unemployed	63	1.961	0.142	0.58	0.032
			(f=121.55; p<0.001)		(f=85.86; p<0.001)	
Income[5]	< Rs.5,000.00	20	1.784	0.369	0.548	0.078
	Upto Rs.10,000.00	162	1.601	0.278	0.515	0.058
	> Rs. 10,000.00	400	1.44	0.26	0.481	0.056
			(f=32.25; p<0.001)		(f=28.98; p<0.001)	

[1]n= 582, p and f -values were calculated from analysis of variance (ANOVA)
[2]Classification of social classes were given in Chapter 2, section 2.2.2.4, page 24
[3]Educational level was grouped as those who did not complete Advanced Level (A/L) examination, an upto Ordinary level (O/L), those who had at least one attempt in A/L to A/L group and those who had either a diploma or University degree in above A/L group 4 Income was calculated for the gross earnings of the family

Table 4 Mean BMD (SD) by past and present physical activities

		Past activities		
		Very active	Moderately active	Less active
Present activities	Moderately active	0.533 (0.050)[a] (n=127)	0.491 (0.06)[b] (n=134)	0.471 (0.05)[c] (n=97)
	Less active	0.533 (0.04)[a] (n=40)	0.477 (0.05)[b] (n=142)	0.448 (0.07)[c] (n=42)

[a b c] values with different superscript in a row are significantly different (ANOVA, p<0.001)

Table 5 The average daily energy and nutrient intakes[1]

Parameter	Mean intake	SD	Median	IQR
Energy (Kcals/day)	2268.6	515.8	2285.57	1910 -2600
Protein (g/day)	27.72	14.5	24.39	20.60 -28.40
Fat (g/day)	43	26.8	38.03	30.10 - 50.40
Calcium (mg/day)	783.49	461.6	659.33	450.00 - 1028.00
Phosphate (mg/day)	1042.39	289.3	1009.42	841.00 -1216.00
Iron (mg/day)	20.04	12.3	18.38	14.30 -23.40
[2]Vitamin D (µg/day)	4.98	5.53	2.5	1.5 - 25.96
[3]Vitamin K (µg/day)	0.88	1.43	4.03	0.21 - 2.42
[4] Vitamin A (µg/day)	266.9	1.72	275.62	192.10 - 387.60

[1] calculated from the 3-day intake and presented as mean intake, standard deviation (SD), median and inter quartile range (IQR); n=524
[2] data on vitamin D intake obtained from 363 subjects
[3] data on vitamin K intake obtained from 78 subjects only
[4] data on vitamin A intake obtained from 413 subjects

Table 6 Daily intake of nutrients (according to % Recommended Daily Energy Intake)[1]

	Below 75% (n=68)	75-100% (n=190)	above 100% (n=324)	F-test[2]	P-value[2]
Energy intake (Kcals)	1406.55 (165.8)	1949.02(150.6)	2636.90(327.2)	809.91	<0.001
Protein intake (gm)	23.63(12.6)	25.47(13.6)	29.89(14.9)	8.89	<0.001
Fat intake (gm)	32.64(20.4)	37.76(25.1)	48.17(27.7)	15.52	<0.001
Calcium intake (mg)	468.72(251.5)	718.86(410.3)	856.30(487.2)	27.84	<0.001
Phosphate intake (mg)	722.97(170.6)	911.37(196.0)	1186.27(264.1)	154.27	<0.001
Iron Intake (mg)	13.94(8.2)	17.03(6.0)	23.08(14.5)	26.25	<0.001
Vitamin D(µg)	6.55(36.16)	24.68(43.5)	20.82(41.6)	0.021	0.487
Vitamin K(µg)	2.63(1.4)	2.04(3.1)	1.48(1.7)	1.104	0.337
Vitamin A (µg)	318.64(165.0)	291.49(137.6)	306.03(143.4)	0.794	0.453

[1]The recommended daily intake (RDI) of energy was taken as 2200 Kcals/day based on recommendations of WHO/FAO expert panels and published in Nutrition Guide by Department of Health Services in 2000 (16)
[2] The f-test and p-value from analysis of variance (ANOVA)

Table 7 BMC/BMD in different categories of energy intake (n=524)

	Subjects who met daily energy requirement				
	< 75% (n=68)	75-100% (n=190)	>100% (n=324)	F-test	P-value
BMD	0.484 (0.05)	0.488 (0.06)	0.497 (0.06)	2.22	0.11
BMC	1.433 (0.27)[a]	1.468 (0.28)[a]	1.527 (0.28)[b]	4.62	0.01

F-test and p-value from analysis of variance
[a b] values with different superscript in a row are significantly different (p<0.01)

Table 8 Mean BMC/ BMD of different categories determined by consumption of calcium from dairy products [1]

	Daily (n=343)	2-5 times per week (n=206)	Less than 2 times per week (n=30)	F-test	P-value
BMC	1.498 (0.015)[a]	1.512 (0.020)	1.373 (0.053)[b]	3.114	0.045
BMD	0.492 (0.003)	0.497 (0.004)	0.472 (0.011)	2.317	0.099

[1] corrected for calcium intake from non-dairy products; results expressed as mean (SE)
[a b] values with different superscript in a row are significantly different (ANOVA; $p<0.01$)

Table 9 Mean BMC/ BMD of different categories determined by consumption of calcium from non-dairy products[1]

	Daily (n=172)	2-5 times per week (n=304)	Less than 2 times per week (n=102)	F-test	P-value
BMC	1.508 (0.022)	1.507 (0.016)	1.446 (0.029)	1.881	0.164
BMD	0.495 (0.005)	0.495 (0.003)	0.482 (0.006)	1.87	0.155

[1] corrected for calcium intake from dairy products; results expressed as mean (SE); f-test and p-value from analysis of variance (ANOVA)

Table 10 Mean BMD (SD) according to the frequency of dairy and non-dairy calcium consumption[1]

		Dairy calcium		
		Daily	2-5 times wk	< 2 times wk
Non dairy calcium	Daily	0.493 (0.07 n=171	0.497 (0.07) n=169	0.508 (0.07) n=101
	2-5 times wk	0.495 (0.06) n=323	0.496 (0.06) n=250	0.484 (0.06) n=125
	<2 times wk	0.476 (0.05) n=222	0.495 (0.06) n=154	0.452 (0.05) n=66

[1]BMD of women who consumed less calcium (either in the form of dairy or non dairy) was significantly lower ($p<0.05$ by ANOVA)

Table 11 Mean values (SD) of serum 25(OH) D, i-PTH and ALP in 434 subjects

Parameter	unit	mean	SD
Serum parathyroid hormone	pg/ml	49.97	24.64
Serum 25(OH) D	nmol/L	35.32	24.71
Serum Alkaline Phosphatase	IU/L	64.18	27.51

Table 12 Pearson correlation coefficient (r) between BMD/BMC and serum measurements[1]

	BMD		BMC	
Measurement	**r**	**p-value**	**r**	**P-value**
25(OH) D	0.127	0.008	0.124	0.01
i-PTH	-0.164	0.001	-0.152	0.002
ALP	-0.016	0.74	-0.03	0.52

[1] n=434; serum 25(OH) D has shown a significant positive correlation with BMD/ BMC whereas intact parathyroid hormone has shown a significant negative correlations ($p<0.05$)

Table 13 Anthropometry, BMD and other serum measurements (PTH, ALP) in the thirds of 25(OH) D concentrations[1]

Measurement	Lower third of 25(OH)D	Middle third of 25(OH)D	Upper third of 25(OH)D	f-test	P-value
BMC	1.475 (0.29)	1.489 (0.28)	1.542 (0.27)	2.147	0.12
BMD	0.485 (0.06)[a]	0.493 (0.05)[a]	0.503 (0.06)[b]	3.449	0.03
Weight	50.57 (9.2)	51.17 (9.2)	51.02 (8.1)	0.111	0.9
Height	155.28 (5.0)[a]	153.50 (6.0)[b]	152.59 (5.2)[b]	8.047	0.03
BMI	20.98 (3.7)	21.75 (3.8)	21.93 (3.3)	2.262	0.11
Foot length	23.28 (1.3)	24.46 (1.7)	23.13 (1.0)	0.725	0.49
Abd girth	74.22 (8.8)	74.90 (9.2)	74.43 (9.2)	0.186	0.83
Chest circum	85.78 (7.9)	86.29 (8.5)	86.30 (8.1)	0.162	0.85
Hip circum	90.36 (8.0)	90.75 (8.3)	88.84 (8.8)	1.93	0.15
SFT	15.21 (5.9)	16.01 (6.4)	15.86 (6.9)	0.533	0.59
Serum i-PTH	69.61 (16.7)[a]	53.10 (21.6)[b]	30.11 (17.7)[c]	141.91	<0.001
Serum ALP	61.72 (24.6)	66.52 (25.7)	64.50 (31.2)	1.119	0.328

[1] there were 145 subjects in each thirds of serum vitamin D

[a, b,c] values with different superscript in a row are significantly different (ANOVA; $p<0.05$)

The intake of different nutrients and the recommended daily intake (RDI) for Sri Lankan (16) in this age group were examined. It was evident that only 324 women (55.7%) meeting the recommended daily energy intake of 2200 Kcals/ day. 514 women (88.3%) met up to 75% of daily energy requirement and in 11.7% of women's diet contained below 75% (Table 6) of the requirement. It was also shown that the intakes of other dietary components (protein, fat, calcium, iron and phosphate) were significantly improved with the energy intake except in the case of dietary vitamin A, D and K (Table 6). In the categorical analysis (Table 7), mean (SD) BMD and BMC values in the three categories of energy intake, defined as a percentage of total energy intakes, showed a positive trend where women in the highest energy category had the highest BMD/BMC while women in the lowest energy intake category had the lowest BMD and BMC values. Difference of BMD (2.6%) between the lowest and the highest was not statistically significant while difference of BMC (6.6%) was significant.

Effect of calcium on BMD/BMC

In addition to the survey by 24-hour recall method, the calcium intake among women was assessed by using food frequency questionnaire. Among the good sources of calcium, dairy products (milk, yoghurt, curd and cheese) as well as non dairy calcium sources such as green leaves, legumes, and small fish were considered and the frequency of their intake was documented. For the analysis, subjects were categorized as those who consumed these foods daily, 2-5 times per week and less than 2 times per week.

Effect of the consumption of dairy products on BMD/ BMC was examined after adjusting for non dairy calcium intake. Consumption of dairy calcium daily or more than 2-5 times per week was associated with significantly higher BMC (p=0.05) when compared with those who consumed dairy calcium less than 2 times per week (Table 8). A Similar relationship was found with BMD but the difference did not reach statistical significance. Further, effects of the consumption of non dairy calcium on BMD/ BMC were examined after adjusting for dairy calcium. Even though it was evident that BMC/ BMD levels were low in those who consumed non dairy calcium less than 2 times per week, the difference was not statistically significant (Table 9).

The effect of both dairy and non-dairy calcium on BMD was examined in 3x3 table using ANOVA with categories of dairy and non-diary calcium as fixed factors (Table 10). There was no significant difference in BMD in women who consumed any source of calcium more than 2 times a week. However, women who consumed both dairy and non-diary calcium less than 2 times per week had the lowest BMD.

Biochemical measurements

Serum samples obtained from study subjects were analyzed for 25-hydroxy vitamin D {25(OH) D}and intact-parathyroid hormone (i-PTH) using radioimmunoassay technology. Serum total alkaline phosphatase (ALP) levels were also estimated using kinetic method. Blood was not able to collect from 62 subjects as they did not present for blood drawing sessions. Furthermore, 86 serum samples that were spoilt during storage had to be discarded. As a result, 434 serum samples were finally analyzed for 25(OH) D, i-PTH, ALP and the results are illustrated in Table 11. Mean (SD) level of 25(OH) D was 35.32 (24.7) nmol/L while median and IQR were 30.84 and 15.75-52.36 respectively. Severe vitamin D deficiency below 12.5nmol/L defined according to the Lips (2001) classification (127) (below 12.5nmol/L) was seen in 21.4% of subjects. 19.1% subjects had moderate (12.5- 25.0nmol/L) and 15.7% had mild (25.0-35.0nmol/L) vitamin D deficiency.

Mean (SD) i-PTH concentration of the study sample was 49.97 (24.64) while median and IQR were 52.00 and 30.87 -70.00 pg/mL respectively. Elevated serum i-PTH concentrations (defined as a serum i-PTH concentration >65.0pgm/L) was observed in 142 (33.5%) subjects. Mean (SD) serum ALP concentration was 64.18 (27.51) while median and IQR were 61.75 and 44.99-78.00 IU/L respectively. Elevated serum ALP (defined as serum ALP >95.00 IU/L) was observed in 51 (12.1%) subjects.

Effect of serum 25(OH) D and i-PTH on BMC/BMD

Serum 25(OH) D showed a significant positive correlation with BMD (r=0.13, p=0.008) and BMC (r=0.124, p=0.010) (Figure 3.1) and a significant negative correlation with i-PTH (r= -0.624, p=0.000). Serum i-PTH showed a significant negative correlation with BMD(r= -0.164, p=0.001 and BMC(r= - 0.152, p=0.002) (Figure 3.2). Although ALP had negative correlations with BMD and BMC, they were not statistically significant (Table 12).

Correlation of serum 25(OH) D and i-PTH

Serum 25(OH) D had a negative correlation with i-PTH. Regression model was fitted with i-PTH as the dependent variable and 25(OH) D as the independent variable to asses the relationship of these two variables and the following formula was developed. .

$Y = a + bc$ (a = intercept = 73.08; b = slope = -0.62)

When i-PTH value of 65.0pg/ml was considered as the cut-off value which demarcates the elevated serum parathyroid response, above formula was used to calculate the serum 25 (OH) D values that would initiate the rise of PTH level: Where

Y = cut-off for elevated i-PTH (65pg/ml); c = 25(OH) D levels i-PTH rise

When the results were applied to the above formula:

$Y=a + bc$ $(65 = 73.08 + (-0.62 \times c)$ $c = 13.02)$

The initiation of rise in i-PTH in the sample was seen at 25(OH) D level of 13.02nmol/l

Hence this level would demarcate 25 (OH) D insufficiencies among the subjects in the sample. The present study revealed that only 22.1% (n=96) of subjects had 25(OH) D levels below 13.02nmol/l. In this subgroup, 76% of subjects (n=73) had elevated i-PTH (above 65pg/ml). Other 24 % (n=23) had mean i-PTH concentration of 51.64pg/ml (median of 58.00pgm/l).

Further, BMC/BMD, anthropometric indices and other serum measurements were analyzed in the tertiles of 25 (OH) D concentrations (Table 13). In contrast to women in the lower tertile of 25(OH) D, women in the upper tertile were shorter, had higher BMD and lower i-PTH level. BMC, weight and BMI showed no difference in the tertiles of 25(OH) D. Women with lower 25(OH) D had higher i-PTH level and women with higher 25(OH) D had lower i-PTH level. Furthermore, i-PTH showed a trend across the tertiles of 25(OH) D. ALP which was tested as a surrogate of hypovitaminoses D did not show a difference in the tertiles of 25(OH) D (Table 13).

Discussion

This study provides information on BMD of appendicular skeleton in a group of healthy Sri Lankan men and women. Results of the present study indicate that phalangeal peak bone mass is achieved between 30 and 39 years in both sexes. Compared to women of the same age category, men had a higher (19%) bone mineral density (17).These findings are comparable with the Third National Health and Nutrition Examination Survey (NHANES-III) conducted in the USA, which showed higher BMD in men than in women in all age groups (17). Puberty is the period during which the gender differences in BMD both in the axial and appendicular skeleton begin to appear (19). It is also well known that men have bigger bones than women (24). Gender difference in BMD partly depends on the bone size as the differences in BMD were minimized when they were adjusted for bone volume (19). Furthermore, the volumetric bone mineral density appears to be similar in the female and male newborns (19),

According to Bonjour *et al*, in 1994 (1) the age at which peak bone mass is achieved has been shown to vary in different study populations and in different skeletal sites. In the present study, men and women reached the peak bone mass between 30 and 39 years. In the USA, all three major ethnic groups (non-Hispanic White, non-Hispanic Black and, Mexican American) reached the peak hip BMD between 20 and 30 years (17). Delays in reaching the peak bone mass in populations outside the USA have been reported. Saudi women reached peak spine BMD around 35 years (18). Similarly in Greek women, while spinal BMD reached its peak between 30 and 35 years, femoral neck peak BMD was seen between 25 and 30 years (20). In Chinese women, although peak BMD in proximal femur was seen between 20-24 years, the peak BMD in the forearm bones was not seen until 40-44 years (21). The exact reasons for the delay in reaching the peak bone mass in certain populations are not known. As suggested by Bonjour *et al*., 1994 (1) variations in genetic, social, and nutritional states in different populations may have played a role. It is also possible that BMD trends in different skeletal sites are under different genetic control mechanisms. A recent study conducted in Southern Sri Lanka (22) among a group of community dwelling healthy women, showed an age discrepancy in achieving PBM in two central skeletal sites. Although this study was not reassigned to study the time of PBM, the maximum spine BMD was observed between 30-39 years while hip BMD reached the maximum between 40-49 years. This delay in PBM in Sri Lankan subjects may be due to several reasons. Genetic factors and nutrition appear to be the most plausible explanation. The average daily income in Sri Lanka is below SLR 500.00 (USD 3.88) and it is well below the figures of other countries in the region and outside. According to the population census in 2004, nearly 20% of households were below the National Poverty Line (23). Nutrition and poverty are linked in many ways and poor nutrition may play a role in timing of PBM in Sri Lankan population. The results of this study could not be compared quantity wise with the PBM observed elsewhere as there are no data available on pBMD from other countries. However when calculations were done based on BMD and T scores of two individuals, the mean and SD used as the reference values in the machine were estimated to be 0.512 and 0.06 g/cm2 respectively. Our results were highly comparable with these figures. While SD's were the same, mean BMD of the reference population was only 2.0% higher.

Conclusions

This project examines the peak bone mass in a peripheral skeletal site which is rich in cortical bone, with regards to its timing and association with historical determinants of bone health in a group of subjects from the Galle district selected by stratified randomization method. The main aim of the present study is to determine the most appropriate interventions which can be used in the community level to improve peak bone mass among Sri Lankans. The study was conducted in the Galle district which has most of the characteristics that describe the cross-section of normal Sri Lankan population. The rural-urban mix, the full spectrum of socio-economic strata, and agricultural base with supplementary industries are some of these key characteristics.

It is hoped that findings of this project would help health policy makers when formulating guidelines and recommending interventions in promoting bone health especially among adolescents and preadolescents in the country. Findings of this study can be used as a baseline for future studies in this area.

Based on the findings of this project, the following conclusions can be made

Phalangeal BMD/BMC reaches its peak between 30-39 years in both males and females.

Males have a higher BMD/BMC when compared with females in the same age group.

In women, height, weight, body mass index, hip circumference, chest circumference and abdominal girth showed significant associations with BMD and BMC

Of the anthropometric indices examined, height was the best predictor of the phalangeal BMD in women participated in this study.

In women, physical activity during adolescence has a positive and long lasting effect on bone mineral density.

Dietary calcium has no linear relationship with peak BMD/ BMC in women. However, phalangeal BMD was lower among women who consumed both dairy and non-dairy calcium rich food infrequently.

The peak BMD/ BMC showed a variation in different socio-economic classes partly due to inequalities of income and degree of physical activity

In contrast to the widely held belief, hypovitaminosis D is prevalent among these community living healthy women. Hypovitaminosis D was a significant determinant of BMD/ BMC in this group of women

i-PTH among women in this study showed an inverse correlation with vitamin D levels. The rise of i-PTH occurs when 25 (OH) D levels is reduced below 13.02 nmol/L.

Serum alkaline phosphatase, the widely used surrogate marker of vitamin D deficiency in clinical evaluation of patients, showed poor correlation with vitamin D and PTH levels in women in this study indicating that it is not reflective of serum vitamin D level.

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Review

The assessment of accuracy of cytobrush technique and spatula technique when compared to the histopathology in the diagnosis of oral premalignant and malignant lesions

Nanayakkara PGCL
Department of Anatomy, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.

Abstract
This comparative study was conducted to analyze the cytological smears prepared from oral premalignant and malignant lesions by the use of two cytological techniques.
The smears taken with spatula from oral premalignant lesions showed epithelial cells predominantly from superficial and intermediate cell layers. A few parabasal cells were also seen in smears taken from ulcerative leukoplakia lesions. But smears taken with the cytobrush showed cells from all three layers of the epithelium with a good cell harvest.
In analysis, we found a statistically significant difference between the two cytological techniques used for the diagnosis of squamous cell carcinoma and dysplasia. The the cytobrush technique appeared to be as accurate as the histopathological technique.

Corresponding author: Nanayakkara PGCL

<pgclnana@med.ruh.ac.lk>

Introduction

Oral cancer is a serious disease in which many cases have a disastrous outcome. Annually 20,000 new cancer patients are detected in Sri Lanka, from which 1500-2000 cases are oral cancers. It is the highest-ranking malignant tumor in Sri Lanka. Deaths form oral cancer in Sri Lanka is 1095 per year. Therefore, it is a major health problem in Sri Lanka (1).

Population screening is an accepted method of reducing the morbidity & mortality. But clinically differentiating the premalignant and malignant lesions from similar looking benign lesions at an early stage is difficult (2). As scalpel biopsy is invasive & associated with potential morbidity, many oral lesions are subjected to biopsy only when they display the features of malignancy, while innocuous looking early stage cancerous lesions are merely observed clinically. This may be the reason, in part, for more than 50% of oral cancers to be diagnosed in the advanced stage. Therefore, early detection of apparently innocuous oral cancers is mandatory. The above situation leads to the necessity of a simple technique to diagnose oral cancer in its early stages and to predict the behavior of epithelial dysplasia.

Since 1992, a number of cytological studies has been conducted using the cytobrush technique for the above purpose, but all these studies concentrated on the test diagnostic characteristics like sensitivity and specificity. Even the largest study conducted in 1999 (3) is incomplete as the cytological diagnosis of 618 smears were not compared with their histopathological diagnoses. Therefore, it is necessary to do further studies before using the cytobrush as a diagnostic instrument (screening technique) by comparing the diagnosis made by the cytobrush technique with the histopathological technique in the diagnosis of malignant and premalignant lesions.

Almost all the above studies using cytobrush technique were conducted in western countries where the majority of leukoplakia lesions are due to smoking (4) and not due to chewing betel as in India and Sri Lanka (5). Normally, the keratotic layer of leukoplakia lesions due to chewing betel is thicker than that due to smoking tobacco. Therefore, it was decided to find out whether the same cytobrush technique will be equally effective in diagnosing the leukoplakia lesions in the Sri Lankan population.

Methodology

Subjects were selected according to their clinical presentation as oral premalignant lesions and oral carcinoma from Oral and Maxillo-facial units of Teaching Hospitals, Galle, Kandy, Peradeniya and

General Hospital, Matara. Prior to the study informed written consent was taken from the subjects.

Two cytological smears were taken from the same lesion by using a cytobrush plus cell collector and a metal spatula by complete randomization procedure. Subsequently a surgical biopsy was taken from the same lesion for the tissue diagnosis.

Cytological smears were stained with modified papanicolaou staining technique and histological sections with H & E. The cytological smears were assessed using a light microscope. In assessment of a cytological smear, the whole cytological smear was examined and the gradings were performed under high power (x40). According to the findings diagnosis was made as Normal, Inflammation, Keratosis / Hyperkeratosis, Dyskaryosis (mild, moderate, severe), Carcinoma and Inadequate smear.

Methods of statistical analysis

We evaluated these different techniques by calculating sensitivity, specificity, accuracy, positive and negative predictive values and also used Chi-squared goodness of fit test to test whether an observed frequency distribution of diagnoses by two cytological techniques deferred significantly from the histopathological diagnosis.

Results

Cytological diagnosis of clinically malignant lesions

Final results of the histological and cytological analysis are show an in the Table 1. In statistical analysis, 4 non-squamous cell carcinoma lesions and 3 inadequate cytological smears were removed. Therefore the number of clinically malignant lesions studied was 69.

1. **Comparison of spatula and cytobrush techniques with the biopsy technique in the diagnosis of clinically malignant lesions** (Diagnosed as Severe, Moderate, Mild epithelial dysplasia, Squamous cell carcinoma and others) A statistically significant difference was observed in between the spatula technique ($X^2 = 23.685$; **d.f. = 4**; **p = 0.000**) and the biopsy technique in the diagnosis of oral carcinoma lesions. There was no statistically significant difference between the cytobrush technique ($X^2 = 2.307$; **d.f. = 4**; **p = 0.680**) and the biopsy technique.

2. **Comparison of spatula and cytobrush techniques with the biopsy technique in the diagnosis of clinically malignant lesions** (Diagnosed as precancer, cancer and others) A statistically significant difference was observed between the spatula technique ($X^2 = 22.306$; **d.f. = 2**; **p = 0.000**) and the biopsy technique in the diagnosis of clinically malignant lesions. There was no statistically significant difference between the cytobrush technique ($X^2 = 1.910$; **d.f. = 2**; **p = 0.385**) and the biopsy technique.

3. **Comparison of spatula technique with cytobrush technique in the diagnosis of clinically malignant lesions** (Diagnosed as Severe, Moderate, Mild epithelial dysplasia, Squamous cell carcinoma and others) A statistically significant difference was observed between the use of spatula technique ($X^2 = 11.397$; **d.f. = 4**; **p = 0.022**) and the cytobrush techniques in the diagnosis of clinically malignant lesions.

4. **Comparison of spatula technique with the cytobrush technique in the diagnosis of clinically malignant lesions** (Diagnosed as pecancer, cancer and others) A statistically significant difference was observed between the use of spatula ($X^2 = 10.524$; **d.f. = 2**; **p = 0.005**) and the cytobrush techniques in the diagnosis of clinically malignant lesions.

Therefore, in the diagnosis of oral cancer and precancerous lesions, the cytobrush technique is as effective as the biopsy technique. The cytobrush technique is also more effective than the spatula technique in the diagnosis oral malignant and premalignant lesions.

Evaluation of diagnosis of clinically malignant lesions

To evaluate the cytological diagnosis of oral squamous cell carcinoma by the spatula and the cytobrush techniques, the sensitivity, specificity and the accuracy rates were calculated. Thereafter the chi-squared test was applied to find out whether the observed differences of these figures were due to chance alone or not.

Spatula technique

(a) **Evaluation of diagnosis of precancers in clinically malignant lesions**

Sensitivity = 88.89%, Specificity = 64.71%, Accuracy = 71.01%, PPV = 47.06%, NPV = 94.29%

(b) **Evaluation of diagnosis of cancers in clinically malignant lesions**

Sensitivity = 60.42%, Specificity = 95.24%, Accuracy = 71.01%, PPV =96.67% NPV = 51.28%

Table 1 Histopathological and cytological diagnosis of clinically malignant lesions

Histopathological Diagnosis	No. of cases	Cytological Diagnosis	Spatula	Cytobrush
Chronic Abscess	01	Inflammation	01	01
Mucus Cyst	01	Normal	01	01
Keratosis	01	Keratosis	01	01
Non-Squamous cell carcinoma	04	Dyskaryosis	04	04
Severe Epithelial Dysplasia	07	Severe Dyskaryosis	03	07
		Moderate Dyskaryosis	02	00
		Mild Dyskaryosis	01	00
		Squamous cell carcinoma	01	00
Moderate Epithelial Dysplasia	09	Moderate Dyskaryosis	07	08
		Mild Dyskaryosis	02	00
		Inadequate Smear	00	01
Mild Epithelial Dysplasia	03	Mild Dyskaryosis	02	03
		Normal	01	00
Squamous cell carcinoma	50	Severe Dyskaryosis	10	03
		Moderate Dyskaryosis	08	02
		Squamous cell carcinoma	29	44
		Keratosis	01	00
		Inadequate Smear	02	01
Total	76		76	76

Table 2 Histopathological and cytological diagnosis of leukoplakia lesions

Histopathological Diagnosis	No. of cases	Cytological Diagnosis Diagnosis	Spatula	Cytobrush
Hyperkeratosis	08	Keratosis	07	07
		Inflammation	01	01
Hyperplasia & Acanthosis	03	Keratosis	03	03
Severe Epithelial Dysplasia	19	Normal	01	00
		Keratosis	01	00
		Severe Dyskaryosis	10	14
		Moderate Dyskaryosis	04	02
		Mild Dyskaryosis	03	02
		Squamous cell carcinoma	00	01
Moderate Epithelial Dysplasia	30	Keratosis	02	01
		Inflammation	01	00
		Moderate Dyskaryosis	15	26
		Mild Dyskaryosis	11	03
		Inadequate smear	01	00
Mild Epithelial Dysplasia	28	Normal	03	00
		Keratosis	01	00
		Inflammation	00	01
		Moderate Dyskaryosis	01	03
		Mild Dyskaryosis	21	24
		Squamous cell carcinoma	01	00
		Inadequate smear	01	00
Squamous cell carcinoma	28	Normal	01	00
		Keratosis	01	00
		Severe Dyskaryosis	01	05
		Moderate Dyskaryosis	05	01
		Mild Dyskaryosis	04	01
		Squamous cell carcinoma	14	21
		Inadequate smear	02	00
Total	116		116	116

Cytobrush technique

(a) Evaluation of diagnosis of precancers in clinically malignant lesions

Sensitivity = 100%, Specificity = 90.20%, Accuracy = 92.75%, PPV = 78.26%, NPV = 100%

(b) Evaluation of diagnosis of cancers in clinically malignant lesions

Sensitivity = 89.58%, Specificity = 100%, Accuracy = 92.75%, PPV = 100%, NPV = 80.77%

The above sensitivity, specificity and accuracy figures were compared with the 'gold standard' to find out whether there is any statistically significant difference between them.

1. **Lesions diagnosed as dysplasia by the biopsy technique**
   - **(A) Spatula technique** – Sensitivity was not significant, Specificity was highly significant and accuracy was significant.
   - **(B) Cytobrush technique** – Sensitivity, Specificity and accuracy were not significant.
2. **Lesions diagnosed as carcinoma by the biopsy technique**
   - **(A) Spatula technique** - Sensitivity was highly significant, Specificity was not significant and accuracy was significant.
   - **(B) Cytobrush technique** – Sensitivity, Specificity and accuracy were not significant.

Evaluation of diagnosis of oral leukoplakia

Final results of the histological and cytological analysis are shown in the Table 2.

In statistical analysis, 4 inadequate cytological smears were removed. Therefore, number of leukoplakia lesions studied was 112.

Spatula Technique

(a) Evaluation of diagnosis of precancers (dyskaryosis) in leukoplakia.

Sensitivity = 86.67%, Specificity = 72.97%, Accuracy = 82.14%, PPV = 86.67% , NPV = 72.97%

(b) Evaluation of diagnosis of cancers in leukoplakia.

Sensitivity = 53.85%, Specificity = 98.84%, Accuracy = 88.39%, PPV = 93.33%, NPV = 87.63%

Cytobrush Technique

(a) Evaluation of diagnosis of precancers (dyskaryosis) in leukoplakia.

Sensitivity = 96%, Specificity = 83.78%, Accuracy = 91.96%, PPV = 92.31%, NPV = 91.18%

(b) **Evaluation of diagnosis of cancerous lesions in leukoplakia.**

Sensitivity = 76.92%, Specificity = 98.84%, Accuracy = 93.75%, PPV = 95.24%, NPV = 93.41%

The above sensitivity, specificity and accuracy figures were compared with the "gold standard" to find out whether there was any statistically significant difference between them.

1. **Lesions diagnosed as dysplasia by the surgical biopsy technique**
   - **(A) Spatula technique** - Sensitivity was not significant, Specificity was significant and accuracy was not significant.
   - **(B) Cytobrush technique** – Sensitivity, Specificity and accuracy were not significant.
2. **Lesions diagnosed as carcinoma by the surgical biopsy technique.**
   - **(A) Spatula technique** - Sensitivity was highly significant, Specificity was not significant and accuracy was not significant.
   - **(B) Cytobrush technique** - Sensitivity, Specificity and accuracy were not significant.

Inter-examiner variability

In order to ascertain the diagnostic consistency in the interpretation of cytological smears, forty randomly selected cytological smears were evaluated by the two consultant oral pathologists and by the author himself and they were assessed by the use of chi-squared test. The difference seen in reporting of cytological smears by the three observers were not statistically significant.

DISCUSSION

Even though the oral exfoliative cytology is a simple, painless, economical, rapid and non-invasive procedure, its main disadvantage is the low sensitivity due to inadequate sampling and technical errors (6). The main reason for the inadequate sampling is the inability to get an adequate number of cells from all the cell layers of the epithelium (7). Even though the smears obtained from ulcerated or reddish areas of oral

lesions give an adequate number of cells, in keratotic lesions, the number of cells coming from parabasal and basal layers are few as they are buried beneath the thick mantles of keratinaceous debris.
As this is a comparative study we did not match or controlled the following factors that may have had an effect on the nuclear and cell diameters, i.e. the site of lesion, sex of the patient, presence or absence of anemia or tobacco habits.

Exfoliative cytology of clinically malignant lesions

Use of spatula technique
In the smears prepared by using the spatula technique, the total number of cells present in each smear were less than that of smears prepared by the use of a cytobrush. This finding is similar to the one observed by Ogden et al. in 1992 (8). The majority of cells in those smears were intermediate cells, but parabasal cells were also seen in some smears (9).
Two smears prepared from histopathologically confirmed squamous cell carcinoma were inadequate. One of them was from the inferior surface of the tongue where the lesion was very fragile and less pressure was applied in taking the smear and the other from the right buccal mucosa close to the retromolar area, a difficult area to reach with the spatula.
There were 02 false negative smears and one of them was from the vermilion border of the lip and the other from the hard palate. The reasons for this may be presence of a thick keratin layer in the lip and the hard palate which is lined with masticatory mucosa to form a protective barrier and less shedding of cells from it as stated by Ogden et al. (8).
One false positive case was reported as squamous cell carcinoma, but histopathologically it was lesion with severe epithelial dysplasia. This finding may be due to misinterpretation by the author because the relevant smear taken by the cytobrush had been diagnosed as severe dyskaryosis.
The results obtained with the uses of spatula technique showed a poor degree of compatibility in the diagnosis of dysplasia and dyskaryosis, similar to the findings reported by Ramesh et al.(9). This poor degree of compatibility may be due to:
Poor cell harvest gained by the use of cytology technique. Only the superficial cell layers are removed with few or no deeply placed epithelial cells. Overlapping and clumping of the epithelial cells on a smear causes difficulty in interpretation of a smear and as the spatula is non-flexible, smears taken from most of the inaccessible sites of the mouth had only a few epithelial cells for a diagnosis. Use of Cytobrush technique In smears prepared by the use of cytobrush, the total number of cells present in a smear were very much higher than that of a smear prepared by the spatula technique. In addition to that, in more than 80% of the smears, parabasal cells could be identified.
Out of the two inadequate smears, one smear was taken from the inferior surface of the tongue and the other was from the left lip and commissure. As the lesion in the inferior surface of the tongue was very fragile, less pressure was applied over the lesion in taking smears and the thick keratin layer forming a crust over the lesion on the lip may be the cause for inadequacy as there is less epithelial cell exfoliation of the lip and the commissure as reported by Folsom et al. (10).
No false negative cases were reported when using the cytobrush technique in our study. But Scuibba (3) reported two inadequate smears in his study of 298 patients.
The compatibility of dysplasia and dyskaryosis was high in our study with the use of the cytobrush technique. This high degree of compatibility of dysplasia and dyskaryosis and the zero false negatives obtained may be due to the good cell harvest gained by the presence of large number of bristles in the brush, touching a larger surface area of the lesion. The number of cells coming from the deeper cell layers was more and less clumping and less overlapping resulted in more accurate microscopical interpretation. As the cytobrush was somewhat flexible, smears taken from most of the inaccessible sites of the mouth had an adequate number of cells for the diagnosis.

Exfoliative cytology of oral leukoplakia

Spatula technique
Four smears were inadequate and according to their site of lesion, one lesion was from the inferior surface of the tongue close its base, one from the alveolar ridge and two were from the left buccal mucosa. The reason for this may be the lesions found on the inferior surface of the tongue and the alveolar ridge was both inaccessible to the spatula and the thick keratin layer over the lesions. A similar type of poor cell yield was also reported by Ogden et al. (8) from smears taken from ventral surface of the tongue.
The inadequacy of the two smears taken from the right and left buccal mucosa may be due to the presence of thick keratin layer over the lesion preventing exfoliation of cells.
Five smears were diagnosed falsely as keratotic; three of them came from the buccal cheek and one each from the right and left commissure. Again the presence of thick keratin layer may have interfered with the exfoliation of deeply placed epithelial cells or the dysplastic change confined to the deeper layers may be the cause for incorrect diagnosis.

Five smears were diagnosed falsely as normal smears. According to their location, 02 smears were from right commissure and one each from the lip, hard palate and dorsum of the tongue. In the commissure, the lip and the dorsum of the tongue, the thick keratin layer may have prevented the exfoliation and the thick masticatory mucosa over the hard palate might be the reason for false negative smear from the hard palate. In this instance atypical cells may be lying in the lower strata or the dysplastic change may be confined to the deeper layers.

One smear was diagnosed falsely as an inflammatory lesion that was taken from the bucco-alveolar sulcus. Therefore, 11 lesions gave false negative results in our study. These false negatives may be due to the presence of thick keratinized layer impeding the emergence of deeper keratotic cells as stated by Mehta et al. (11). Removal of the superficial keratotic layer as much as possible before taking a smear from a lesion and proper selection of the area to harvest may be the reasons for the low false negative rate in our study as compared to the previous studies.

The degree of dyskaryosis of the smears and the degree of dysplasia of the histology were not closely compatible, a finding similar to this was also stated by Ramesh et al. (09). The reasons for this disparity may be the reasons described previously under the use of spatula technique in the diagnosis of oral squamous cell carcinoma. One mild epithelial dysplasia lesion was diagnosed as squamous cell carcinoma by the use of spatula technique. This may be due to the following; the biopsy tissue might have not been taken from the representative area of the lesion leading to a negative result, only a small segment of the lesion has undergone malignant change, and unless multiple biopsies are taken, the malignant component could be missed. On the other hand, the smears permit sampling of the entire lesions enabling even a small focus of malignancy to be picked up. Thirdly, a technical error occurring in sectioning of the biopsy tissue.

In interpreting cytological smears, subjectivity is a common problem (12). To overcome this problem, inter-observer variation study was done and we found a good agreement of the results among three observers similar to the agreement of grading of dysplasia reported by Lippman et al. (1993).

In our study, the overall diagnostic accuracy of spatula technique in the diagnosis of oral leukoplakia was over 80%. However, in most of studies conducted by various authors, the sensitivity of cytological diagnosis of atypia in leukoplakia were poor. Nevertheless, the studies conducted by Ramesh et al. (09) (92%) and Banoczy (13) (90%) showed a high degree of diagnostic accuracy than the previously mentioned studies.

In our study, taking smears from ulcerated or fissured areas and scraping out the superficial surface before taking a cytological smear may be the reason for the high accuracy of this technique in the diagnosis of leukoplakia lesions. The other reason may be that in 50% of the samples, cytobrush was used to take the first smear and then the second smear was taken by the spatula technique. Therefore, when the smear was attempted for the second time from the same site, the second smear would have harvested more cells from deeper cell layers.

Cytobrush technique

None of the smears was reported as inadequate, but two smears gave false negative results. One cytological smear taken from the right commissure contained only the superficial anucleated squames cells and the other taken from the alveolar ridge showed chronic inflammatory cells. In the former, the thick keratin layer may be the reason for the false negative result and in the latter inability to reach the lesion using the cytobrush may be the reason.

Comparative study of cytobrush and spatula in the diagnosis of precancerous and cancerous lesions

Evaluation of cytological diagnosis of oral squamous cell carcinoma

(a) Spatula technique

Spatula technique is a highly sensitive in the diagnosis of dyskaryosis in clinically malignant lesions but its accuracy and specificity is poor.

Spatula technique is not a sensitive and accurate method in the diagnosis of oral squamous cell carcinoma in clinically malignant lesions. However it can be used to differentiate non-cancerous lesions from cancerous lesions in clinically malignant lesions.

There are several reports attesting to the sensitivity of cytological diagnosis of clinically malignant lesions of the mouth and oropharynx (Montgomerry and Von Haam 1951, Pomeranz and Stahl 1953, Peters 1958, Sandler and Stahl 1958 and Sliverman et al. 1958 with sensitivity ranging from 86.7 per cent to 94.5 per cent. But in all of these studies cytologically diagnosed dyskaryotic and cancer smears were grouped as true positives. Therefore, it is difficult to compare these findings with the results of our study.

(b) Cytobrush technique

In the diagnosis of dyskaryosis and squamous cell carcinoma in clinically malignant lesions, cytobrush technique is a sensitive and accurate method. These findings are similar to those of Remmerbach et al. (14).

Evaluation of cytological diagnosis of leukoplakia

(a) Spatula technique

Spatula technique is not a highly sensitive technique to diagnose carcinomatous lesions in leukoplakia, but it is good to separate out non-cancerous lesions in leukoplakia.

However, spatula technique is a sensitive and accurate method to differentiate dyskaryotic lesions from the cancerous lesions.

The accuracy of exfoliative cytology in the diagnosis of oral leukoplakia was different from 36.1% stated by Mehta et al. (11) to 90% stated by Banoczy (13). But very recently Mishra et al. (5) reported a good agreement between cytology and histopathological diagnosis in 86.2% of dysplastic lesions and 95.2% in malignant lesions. However, in most of the previous studies done by various authors, dysplastic and cancerous lesions were grouped together as true positives. Therefore, it is difficult to compare their finding with the results of our study.

(b) Cytobrush technique

Cytobrush technique is a sensitive, specific and accurate method in the diagnosis of squamous cell carcinoma and dyskaryosis in leukoplakia. It is difficult to compare the results of this study with results of the previous studies done by various authors as they have grouped both dysplastic and cancerous lesions together as true positives.

Evaluation of the diagnostic ability of spatula and cytobrush techniques in differentiating non-cancerous and non-dysplastic lesions from cancerous and dysplastic lesions

Leukoplakia

(a) Spatula technique

When the spatula technique was used to differentiate cancerous and dysplastic lesions from non-cancerous and non-dysplastic lesions, no statistically significant difference was reported. Therefore, the spatula can be used to differentiate cancerous and dysplastic lesions from non-cancerous and non-dysplastic lesions in leukoplakia. If the test is positive, the final diagnosis will be very accurate. However, it is not the same when the results are negative.

(b) Cytobrush technique

When the cytobrush technique was used to differentiate cancerous and dysplastic lesions from non-cancerous and non-dysplastic lesions, there was no statistically significantly difference with those of histopathological diagnosis. This suggests that the cytobrush can be used to differentiate cancerous and dysplastic lesions from non-cancerous and non-dysplastic lesions in leukoplakia. Therefore, unlike in the case of spatula both positive and negative test results lead to a much reliable diagnosis with the use of cytobrush.

But in a previous study done by Poate et al. (2004) to investigate the uses of cytobrush technique in the diagnosis of oral epithelial dysplasia in potentially malignant lesions a sensitivity of 71.4%, specificity of 32%, PPV of 44.1% and NPV of 60% was reported.

Scheifele et al. (2004) using oral CDx technique reported a high sensitivity (92.3%) and specificity (94.3%) figures in their study on detecting dysplasia and oral squamous cell carcinoma in premalignant lesions were similar to our results.

Squamous cell carcinoma

(a) Spatula technique

When the spatula technique was used to differentiate cancerous and dysplastic lesions from non-cancerous and non-dysplastic lesions, only the specificity was significantly different. Therefore, the spatula technique does not appear to differentiate cancerous and dysplastic lesions from non-cancerous and non-dysplastic lesions in clinically malignant lesions. Therefore, if the test is positive, 98.46% positive test results are either dysplastic or cancerous. However, if the test is negative, only a 50% of the test results are true negatives.

According to the previous comparative studies done by Peters (15) to Ramesh et al. (9), the calculated values of sensitivity, specificity, accuracy, positive predictive value vary and negative predictive value are similar to our study except the finding for specificity which is only 50%. That may be due to 11 false negatives reported in our study.

(b) Cytobrush technique

When the cytobrush technique was used to differentiate cancerous and dysplastic lesions from non-cancerous and non-dysplastic lesions, no statistically significant difference was reported. Therefore, the cytobrush can be used to differentiate cancerous and dysplastic lesions from non-cancerous and non-dysplastic lesions in oral carcinoma. Therefore if the test is positive, all the positive test results are either dysplastic or cancerous and if the test is negative all the negative results are non-cancerous or non-

dysplastic. According to the study done by Scuibba (3), cytological diagnosis of clinically malignant lesions by the use of cytobrush technique, sensitivity, specificity, accuracy, positive predictive value and negative predictive value were 100%, 92.86%, 95.3%, 87.93% and 100% respectively.

According to the above findings of our study, cytobrush is a good cytological instrument for the diagnosis of both oral premalignant and malignant lesions as it is a very simple, user friendly and less time consuming procedure compared to more time consuming quantitative exfoliative cytology techniques or highly expensive molecular biology techniques. As stated by Henry et al. (1960), the greatest value of cytological examination lies in its ability to disclose the presence of intra-epithelial or non-invasive carcinoma when the clinical appearance is relatively innocent and cancer is not suspected. Its utilization prior to biopsy provides greater assurance of definitive diagnosis, as cancer cells can be detected in the cytological smear even though the biopsy specimen may be inadequate for a diagnosis. Moreover, the diagnosis of oral cancer at an earlier stage can improve the prognosis as stated by Epstein in 1992. It is also beneficial in certain clinical situations where the other diagnostic techniques are impractical to use such as when the patient is medically compromised or has received radiotherapy or may be refusing to under go a biopsy (16) or in an anxious patient who insists on, having a treatment for an apparently innocuous lesion, to use as a screening method in patients who have been treated for oral cancer. In a case of multiple mucosal lesions, exfoliative cytology can be used to locate the most appropriate area for the biopsy. If the lesion is located in a region that presents a surgical risk, then the exfoliative cytology is beneficial to come to a diagnosis.

But some disadvantages are also there like only the individual cells could be studied (Therefore, the pathognomonic features of the disease must be present in or on the cells themselves), furthermore only surface epithelial cells can be obtained (Hence, characteristic pathological changes must extend to the surface for accurate diagnosis), cells cannot be studied in their proper tissue relationship to one another as in surgical biopsy specimens. If the surface of the lesion is heavily keratinized, a typical character of the lesion will not be demonstrated. Treatment cannot be predicted on a positive smear. White lesions can be produced by many diseases other than the cancer (Those white lesions are not precancerous. They cannot be diagnosed from exfoliated cells and even though the technique is simple, if not accomplished adequately then the material is not representative, giving an inadequate sample.

Conclusions

According to our findings, the cytological diagnosis of clinically malignant lesions and premalignant lesions by the cytobrush technique is accurate. But, as it gives no information about the presence or extent of invasion, it should not replace the routine histopathological examination. As stated by Umiker et al. (7), the major role of exfoliative cytology is a supportive one for the histopathological examination.

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ABS.No. 01

Abstracts of Oral Presentation

A descriptive study on homicides in Galle, Sri Lanka

Rathnaweera RHAI

Department of Forensic Medicine, Faculty of Medicine, University of Ruhuna.

Introduction: The intentional killing of a human being by another is the ultimate crime. Its indisputable physical consequences manifested in the form of a dead body also make it the most categorical and calculable. **Objective:** To find out epidemiological, socio-economic and postmortem data on homicides and compare them with the findings of previous studies.
Materials and Methods: A retrospective study was carried out at the Teaching hospital, Karapitiya during the year 2011. All the potential homicide cases referred during the study period were analyzed.
Results: Forty (34 males) homicides were studied. The majority (62.5%) was in the 21-40 age group and 85% of them were married. Most of the homicides were reported from Meetiyagoda (20%), Elpitiya(15%) and Karandeniya(15%). A sharp force was the commonest method (n = 18; 45%). The number of homicides by fire arm and blunt force were 13 (30%) and 9 (25%) respectively. In studies done in the year 2006 and 2008, firearm was the commonest method used. All assailant unknown (20%) cases were firearm deaths. Knife was the commonest weapon used (50%) in sharp force trauma followed by swords (44%). Chest remains the commonest site of injury in both sharp force (16) and firearm (entry wound 14, exit wound 09) deaths. In blunt force trauma, head and face remains the targeted sites (100%).
Conclusions: Majority were young married males. Sharp cutting weapons have become more popular. Swords are being used as often as the knives. There is a definite change in the method used for homicides in the war era and post-war era. Firearms are being used in cases where the assailant is unidentified.

ABS.No. 02

Abstracts of Oral Presentation

An assessment of the pre mass treatment prevalence of Soil Transmitted Helminths (STHs) in Bope-Poddala Medical Officer of Health (MOH) area

Harsha MPDH[1], Malini D[2], Weerasekera S[3], Galappaththi RU[3], Vithanage IC[3], and YahathugodaTC[3]

[1] *Allied Health Science Degree Programme, Faculty of Medicine, University of Ruhuna.*
[2] *MOH office, Bope-Poddala division, Galle, Sri Lanka.*
[3] *Department of Parasitology, Faculty of Medicine, University of Ruhuna.*

Background: The Ministry of Health has issued a new circular to control STH infections (*General Circular Letter-02-172/2012*). Bope-Poddala MOH area is considered as moderate risk areas (prevalence 10% - 20%) and was requested to conduct annual de-worming programmes including children and pregnant women. There was no indication to do pre helminth surveys prior to mass treatment in the issued circular.
Objective: To emphasize the fact that pre-STH surveys are necessary before implementing new de-worming guidelines.
Methods: The highest risk group—pre-school children—was considered as the study population in this survey. All 38 registered pre-schools were visited. All the parents of students presented on the day of survey were asked to collect a sample of feaces. Personnel and STH related data were collected by direct administration of a questionnaire. Each sample was examined twice by the first and third author using direct normal saline and iodine smears. All positive cases were confirmed by the last author.
Results: Sample recovery rate was 74.8% (314/420). Mean age was 4.4(SD=0.6) years. Overall STH prevalence was 1.6% (CI=1.39; n=5). All five cases were due to *Trichuris trichiura* (whipworm). Four cases of *Enterobius vermicularis* (pinworm-not a STH) were accidently detected. More than 60% of children have consumed at least one dose of anthelmintics within past six months. Almost all (97.5%) had disposed their children's feaces properly.
Conclusions: In 2000, Bope-Poddala had more than 8% of STH prevalence. Recent low prevalence may be due to (i) anti-filariasis mass treatment which included diethylcarbamazine and albendazole conducted in the entire area annually between 2002 and 2006; (ii) often unprescribed anthelmintic treatment to pre-schoolers and the improvement of socio-economic background. Accumulation of trichuriasis is possible due to drug resistance.
Recommendations: We strongly believe that each MOH office should conduct pre-surveys before starting de-worming guidelines to prevent unnecessary expenses and emergence of new drug resistant strains.

ABS.No. 03

Abstracts of Oral Presentation

Sexual violence towards females at Matara

Rathnaweera RHAI

Department of Forensic Medicine, Faculty of Medicine, University of Ruhuna.

Introduction: Statistics on sexual violence towards females are limited and underestimated as many cases go unrecorded.
Objective: The study was designated to find out epidemiological and socio-economic statistics of the victims of sexual violence and the medico-legal aspects of injuries due to sexual violence.
Materials and Methods: All the sexual violence cases referred to the Judicial Medical officer's office, General hospital, Matara form 1st of January 2012 to 1st of December 2012 were retrospectively analyzed.
Results: There were 260 victims referred for medico-legal examination. The age of the victims ranged from 4 years to 62 years. From the total 202 (77.5%) victims were below 16 years. Among them 177 (68%) belong to the age group of 11-16 yars. 234 (90%) of the victims were from low socio-economic class and 210 (80%) of them had studied below grade 10. The alleged incident had taken place at the victims' house in 110 (42%) cases. In 148 (57%) cases, the victim had given the "consent" for the alleged act. The pregnancy was confirmed in 22 (8%) at the time of examination. Hymenal tears were observed in 100(38%) cases and 11 had fresh tears. In 149 (57%) cases the assailant was the boy friend of the victim. Extragenital injuries were present only in 4 (1.5%) cases.
Conclusions: Majority of the victims were below 16 years of age belonging to low socio-economic class. Majority had given "consent" for the alleged act and this could be a reason for lack of extragenital injuries. This data highlights the magnitude of juvenile sexual abuse especially by the boy friends of the victims.

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