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JOURNAL OF BIOMEDICAL INVESTIGATION, DECEMBER, 2008, Vol. 6, No. 2

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Journal of Biomedical Investigation, 2008; 6(2): 1-4.

Review Article

Evolution of the Prototype of the Concept of Teaching Clinical Occupational Medicine at Undergraduate Level in South-Eastern Nigeria.

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SUMMARY

This concept paper narrates the activities that started in 1995, in the course of developing the concept of teaching clinical occupational medicine in a Nigerian Medical School. The setting was a medical school in South East, Nigeria. The activities included, conception of the idea, development of the concept, the practical application of the concept in teaching medical students both in the University and the clinical teaching in the teaching hospital; development of the concept to the level of prototype; testing the concept over a period of time to see that it really works; scientific research to validate the concept. This was done in 2006 for current practice in medical education stipulate not less than ten years, from the inception of the concept. In this presentation, the introduction discussed the issues in current medical practice in Nigeria, in particular and other parts of the world in general. The discussion explained the issues raised in the development and implementation of the concept. The conclusion included recommendations on how best to apply the prototype of the concept in other medical schools where clinical occupational medicine is not yet being taught at undergraduate level. In this respect, the forming of linkages with universities in developed countries, where clinical occupational medicine is taught at undergraduate level, is emphasized. One thousand and fifty medical graduates of the aforementioned medical school have benefited from this concept. This means that they can diagnose and manage common occupational diseases.

Key Words: Prototype; Concept; Occupational; Medicine.

INTRODUCTION

The setting is in the Department of Community Medicine, of a Medical School and her sister institution, the Teaching Hospital in South Eastern, Nigeria. The clinics run by the department in the teaching hospital came under focus in 1995 and the absence of an Occupational Medicine clinic was glaring.[1]

The curriculum of the medical students for Occupational Health was also, reviewed critically. It was obvious that clinical occupational medicine was included in the curriculum. In this era of globalization it is clear that medical graduates in Nigeria should be well versed in clinical occupational medicine, since these medical graduates could work in any part of the world when they graduate. It is therefore necessary to include the clinical component in the teaching of occupational health, in our medical school.,[2] A request was made to the management of the aforementioned Teaching Hospital to establish an occupational medicine clinic. This was promptly granted in 1996.

It now became possible to augment teaching of clinical occupational medicine to medical students with clinical exposure in the occupational medicine clinic. Eventually the idea/concept was developed to a prototype which can be duplicated in any medical school if the

concept is not already in place. In public interest, the prototype of the concept of teaching clinical occupational medicine at undergraduate level is hereby stated.[3]

OBJECTIVE OF THE CONCEPT

The overall objective of this concept is to produce medical graduates who have clinical occupational medicine skills that will enable them to recognize and manage common occupational diseases seen in our environment.[4]

INSTITUTIONAL ARRANGEMENT

Teaching of clinical occupational medicine is done by Faculty members of Department of Community Medicine of the medical school, who also hold the complimentary position of Honorary consultant in community medicine, (which includes occupational medicine) in aforementioned sister institution, the Teaching Hospital.[5] 4, 5th and 6th (final) year Medical students are involved. About one thousand have graduated since the concept started in 1996.

TRAINING PROGRAMME

400 Level: Taking of detailed occupational history of patients in the Occupational Medicine clinic, and the wards is emphasized. Also recognition of simple signs and symptoms of occupational diseases.

500 Level: Students with Faculty members manage patients (in the casualty, wards and clinic) who have common occupational diseases (in the out patient department and the outreaches. Viz: Occupational skin diseases, noise induced hearing loss, accident and trauma in the work place, HIV/AIDS in the workplace, occupational lung diseases and/health problems of agricultural workers and small scale industrialist.

600 Level- the Final Year: Students are taught management of occupational diseases at the primary healthcare level and also providing Health services for health care professionals.[7]

EVOLUTION OF THE CONCEPT

Development of the concept of teaching clinical occupational medicine at undergraduate level in the said Medical School started in 1996 after the establishment of the occupational medicine clinic. The constraint in starting the clinic was the fact that only consultants in community medicine interested in Occupational Medicine as a sub-specialty, participate in running of the clinic. In Nigeria and the whole of the West African sub-region, specialization in occupational medicine is within community medicine. Infact in the specialty of community medicine, occupational medicine is only one of the about eleven sub-specialties.[8,9] The author who has occupational medicine as his sub-specialty actually sought and obtained management approval for establishment of the occupational medicine clinic in the said Teaching Hospital in 1996. The concept was religiously implemented as stated in the prototype (or model). The first set of medical graduates of the medical school who benefited from the programme according to the final assessment before graduation were found to be competent in the skill of clinical occupational medicine.

From the inception in 1996 till date, 2008, about one thousand and fifty medical graduates of the university have benefited from the concept.[10]

CONSTRAINTS IN IMPLEMENTATION OF THE CONCEPT MANPOWER

As stated earlier, since occupational medicine in Nigeria and the West African sub-region is only one of the sub-specialties in community medicine, not all the community medicine specialists are interested in the practice of clinical occupational medicine. The same goes for resident doctors, who also assist in guiding the medical students in their clinical postings.

RESOURCES

There is no financial allocation to the occupational medicine unit. All requests pass through the head of community medicine department. Consequently, if the head of community medicine department is not interested, the occupational medicine unit gets next to nothing in terms of equipment and other facilities.

TIME ALLOCATION

Allocation of teaching time for occupational medicine is at the discretion of the head of community medicine department and has been observed; reduction in time allocation affected the implementation of this concept. These changes worry medical students.

ATTITUDE TOWARDS OCCUPATIONAL MEDICINE

In Nigeria and the rest of the West African sub-region, some medical experts think that developing countries do not need clinical occupational medicine and that the emphasis should be on infectious diseases like malaria etc. however research carried out by the occupation medicine unit, have shown tremendous exposure of our people especially the small scale industrialists to numerous occupational hazards; and also that indeed many of them come down with occupational diseases and occupationally related diseases.[12]

EFFORTS MADE TO OVERCOME THE CONSTRAINS.

Medical Equipment: Although presently the occupational medicine clinic is not equipped, for reasons already stated above, the author harnesses equipment in use in other units in the hospital. For example, request for x-rays are made from the x-ray department; request for lung function tests are made from respiratory internal medicine clinic. Although occasionally such patients are lost to follow-up, the occupational medicine clinic would have recorded the patients work history and made a provisional diagnosis, and of course would have the records of the patient. The same is the case for other investigations done in the teaching hospital. Occasionally it had been necessary for the clinic to refer patients for investigations in the occupation medicine clinics of some oil companies in Nigeria, which are well equipped for clinical occupational medicine practice.[13,14]

OTHER VITAL COMPONENTS OF THE CONCEPT

MATURITY: After the concept was developed to a prototype, the preliminary report was presented in conferences of learned societies at local and thereafter regional and international levels. Comments by peers in such conferences helped in improving the concept. However this write-up is being presented for publication in a journal ten years, after the concept was developed to a prototype. The reason for the delay is to allow for maturity of the idea and to see that it really works. This is in line with requirements of concepts in medical education being developed to level of prototype.[15]

RESEARCH: Many research projects were carried out at various stages in the evolution of the concept. There was preliminary research that validated the need to develop the concept in the first place. Later there were research projects to validate the concept, and others to evaluate the development of the concept to a prototype. All these are for publication in appropriate scientific journals.

The research projects are also requirements, in line with such medical education ideas.[1]

DISCUSSION

The write up so far has been a sort of narrative of events in the course of development and evolution of the concept of teaching clinical occupational medicine at under graduate level in a medical school. Relevant literature as they apply to various aspects of the concept are now reviewed.

Over the years, there have been calls for improvement of the teaching and practice of clinical medicine. In Nigeria, experts in community medicine have repeatedly made such calls. They have even specifically called for according more attention to the practice of occupational health and for more emphasis on the teaching and practice of clinical community medicine generally and clinical occupational medicine in particular.[1] Political leaders and medical experts in political positions have also observed that for the much desired improvement in medicare to be achieved, intellectuals, must be allowed to exhibit their flair for innovation.[16] It is worthy of note that teaching of clinical occupational medicine at undergraduate level is emphasized in the medical curriculum of medical schools, as stipulated by the medical and dental council of Nigeria. The fact that it is not taught is actually as a result of, Nigerian medical schools applying the so called "hidden curriculum." Medical education experts recognize the existence of the "hidden curriculum", which actually influences the implementation of the real curriculum.[17]

CONCLUSION

Incidence of occupational diseases and occupationally related diseases are on the increase in developing countries including Nigeria. Unfortunately, the diagnosis is left for only the specialists in community medicine and occupational medicine, but these experts are very few in Nigeria and other developing countries.

There is therefore the need to inculcate in all fresh medical graduates, the skill to recognize and commence management of common occupational diseases and occupationally related diseases. This can only be possible if students are taught clinical occupational medicine at the undergraduate level, with emphasis on obtaining a detailed work history.[4,7] Some medical schools, in developing countries have linkages with medical schools and teaching hospitals in developed countries, where clinical occupational medicine is taught at undergraduate level. Medical students going for such postings in developed countries should all do a clinical rotation in clinical occupational medicine. Opportunities for doing such positing in other institutions abroad should be explored especially by medical schools in developing countries who wish to adopt this prototype.[18]

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Review Article

Review of diseases in which occupational cause is likely in medical practice.

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ABSTRACT

Whatever a medical doctor's specialty, he or she may come across diseases related to occupation. The commonest of such diseases seen by the general practitioner are stress reactions. The orthopaedic surgeon is seen by patients with complaint of symptoms of over use syndromes, in the limbs and also back and waist problems. People who work with vibrating tools may come down with Raynaud's syndrome and need to consult general surgeons. Workers in noisy work places may suffer noise induced hearing loss, or deafness or other symptoms like tinitus. All these people need the expert care of E.N.T (Ear, Nose and throat) surgeons. Females whose work exposes them to chemicals may suffer gynarcologic and endocrine reproductive problems many of such women suffer from infertility and need treatment by gynaecologists before they can have children. Dusty work places make workers come down with occupational lung diseases, and need treatment in respiratory medicine clinics. Some chemicals, encountered in some work environment cause renal and bladder problems. These conditions may need to be treated by internal physicians or even surgeons with bias for urology. There are also many non-specific symptoms that maybe related to the work place. The diagnosis of the above conditions depends on the medical doctor being alert to the possibility of an occupational cause and being able to take an appropriate occupational history.

Key words: Review; Occupational; Diseases; Medical Practice

INTRODUCTION

Occupational medicine is a specialty that crosses traditional medical boundaries into other scientific fields and the world of industry. New medical graduates are often completely ignorant of occupational medicine, yet most soon realize that some knowledge of the subject is desirable if the patient's needs are to be adequately served.

Diseases in which occupational cause is likely in medical practice:

Skin Diseases: Main symptoms of skin diseases are itch, appearance of a rash or lump and occasionally pigmentary changes. The main symptoms of occupational skin diseases are the same as for other skin diseases. However, all cases of occupational skin diseases occur initially at the site of contact, with the offending agent. In order to diagnose an occupational disease, a careful history of the evolution of the lesion is necessary. The chemicals and other materials a patient came in contact with need to be reviewed. The doctor should find out if others in the patients work place have similar problems. This is because occupational skin disease often occurs in clusters.

Contact Dermatitis: Most common occupational skin disease seen in clinical practice is contact dermatitis. It can be irritant in nature or allergic irritant dermatitis can be caused by cement as seen in construction workers etc. Other causes include oils as seen in operators of machinery; solvents, used in painting and cleaning,

detergents, used in house work, hair dressing, cleaning; and fibre glass used in insulation and in building. Allergic dermatitis can be caused by the following:-. rubber gloves used by surgeons, technicians, nurses etc; epoxy resin hardners used in electrical manufacture, joinery, and repair work. cobalt used in making glass and pottery, dyes and perms used in hairdressing; woods especially hardwoods used by joiners and carpenters, also cause contact derinatitis. Other occupational skin diseases include skin infections[2].

Lung Diseases: Presentation of both occupational and non-occupational lung disease are the same. The patient presents with the following symptoms: breathlessness persistent cough, chest pain. There is a report of an abnormal chest radiograph. Occasionally there is anxiety of being harmed by something at work. Most of the well-known syndromes of lung disease have one or more occupational causes. The key to their detection is occupational history.

Occupational Asthma: Diagnosis is easily missed unless the following key questions are asked. Does the wheeze change at weekends or on holiday? Does anything at work affect your chest?[3]

Occupational causes of lung disease: Occupational causes of lung diseases are numerous and include the following: Asthma, which can be caused by contact with animals, hardwoods and grains [3,4,7] cigarette smoke and coal dust cause allergic alveolitis[8]. , while radiation, and asbestos cause bronchial carcinoma [8,16] Beryllium causes sarcoidosis [8,22] whereas silica, coal and asbestos cause diffuse or nochular fibrosis of the lungs. Healthcare workers who come in contact with Mycobacterium tuberculosis in the course of their duty could develop tuberculosis [3,7].

Toxic fumes like those of cadmium cause pneumonitis [8,16,18,20,] Pneumonia caused by legionella species is seen in workers working on faulty air – conditioning systems[4]. Asbestos causes many occupational lung diseases as mentioned above and also pleural fibrosis and mesothelioma; same goes for silicia which in addition also causes pleural fibrosis[4].

Bronchial Carcinoma: If a patient presents with a rare cancer or a common cancer in unusual circumstances (e.g oat cell carcinoma in a young non-smoker) always suspect an environmental or occupational cause. Also make inquires if someone with cancer has worked in the chemical industry. Patients with silicosis also have an elevated risk. No features distinguish occupational lung cancer from the disease associated with cigarette smoking. Asbestos, radiation and other causes add to the risk from smoking. Smokers develop cancer relatively late in life, but those exposed to chemical carcinogens often develop the disease rather early—Just about ten years after the start of exposure in case of chloromethylethers.[5]

Acute Attack of Breathlessness: An acute attack of breathlessness is most commonly due to asthma or left ventricular failure. In occupational diseases, acute allergic alveolitis and inhalation of toxic gas, cause acute attack of breathlessness.

Chronic Breathlessness: Chronic, steady worsening breathlessness is usually caused by irreversible or partly reversible airflow obstruction, cigarette smoking being the main cause. Less commonly, restrictive lung disease usually due to pulmonary fibrosis is responsible. Occupational factors may be involved in either of these syndromes. Non-asthmatic chronic airflow obstruction is usually caused pathologically by emphysema. High level of dust in work places especially coal mines may contribute to the development of emphysema and airflow obstruction. Asbestosis is a progressive disease

with lower zone fibrosis spreading to the lungs silicosis is also progressive.[5]

No Symptoms, But Abnormal Chest Film: Some occupational lung diseases cause abnormalities of the chest radiograph but no associated symptoms or functional abnormality. Pleural plaques, which often calcify may be quite extensive as a result of asbestos exposure. These are harmless. Coal workers pneumoconiosis also may cause a heavy profusion of spots on the film without any functional, impairment. Other pneumoconiosis due to inhalation of tin refining fumes, iron oxide in welding and metal polishing and barium sulphate in its production cause dramatic X-ray abnormalities and no harm.[6]

Chest Pain: The asbestos related diseases may present with chest pain, usually associated with breathlessness.

Mesothelioma is a malignant disease of the pleura (rarely of the peritoneum) caused mainly by exposure to crocodolite asbestos in ship wards and insulation work, decades previously. [7,8] Pneumonia can be acquired from the work environment. A doctor seing a patient with pneumonia should inquire about the work place and especially about the possibility of aerosols of recirculated or contaminated water. The best known cause is legionella disease spread by circulation of droplets from contaminated air conditioners and cooling systems. [9]

The hand, arm and back disorders: Vibrating white finger is due primarily to vascular spasm induced by using tools vibrating mainly in the range 20-400Hz. It may become a permanent disability even after use of the tools. Thus any manual worker presenting with features of Raynauld's disease should be questioned about the use of tools. [10]

Upper Limb Strain Disorders: On movement of the upper arm, pain in the shoulder may be due to frozen shoulder or, rotator cuff syndrome. Pain in the elbow tennis elbow, may be due to or golder's elbow. Forearm pain may be due to (tenosynovitis) or if in the hand due to trigger finger, de Quervain's syndrome in all these cases patient's occupation maybe an aetiological factor, so details of the work performed must be sought[11]. Often repetitive movements, in awkward positions of the joints are the usual cause. People most at risk are keyboard and visual display unit operators, hairdressers, cleaners, musicians and music teachers. [11]

The Painful Back: Most problematic from occupational point of view is low back pain. There are two syndromes—acute disc prolapses provoked by sudden strain on the lumbar spine and which is associated with sciatic pain, limitation of spinal flexion, and straight leg raising and often neurological signs. The condition occurs frequently as a result of awkward lifting or sudden back strains in the work place. The incidence of the condition may be reduced by proper design of the workplace and of the tasks. Provision of appropriate aids and training in lifting techniques. [10]

Chronically Painful Back: The second syndrome is the chronically painful back with limitation of movement, occurring in older male workers, and associated with degenerative changes in the spinal joints. This condition is due to repetitive trauma of the back. These two syndromes can be alleviated by surgery. [10]

Disorders of The Hip: Osteoarthritis of the hip is a very common condition of the elderly and may be contributed to by occupational factors. Farmers are at increased risk. [12]

EAR, EYE and NOSE disorders

Hearing loss and tinnitus. The damage is measured by audiometry. Workers at special risk of hearing damage are usually those in heavy productive industry, such as metal work, drilling and quarrying, stone cutting or use of noisy machinery, as in textiles, printing, wood cutting, transportation and agriculture. Noise above 90 decibels (as measured with special instruments that are electronically weighted to mimic loudness functions of the human ear) cause hearing loss and tinnitus. The harmful effect of noise are cumulative. Frequenting discos has resulted in young people having some degree of hearing loss before even they start work. In addition to protecting workers from noise, many companies now carry out pre-employment audiometry. [13, 14]

Disorders of The Eye (Sore Eyes): Complaints about sore or aching eyes are among the most common symptoms encountered in occupational medicine in organization, which use visual display units. These are rarely serious. If there is also evidence of a defect in refraction; then it indicates poor design of the work place, in terms of lighting, screen reflectance or even posture at the desk. On the other hand eye injury due to foreign body or burns is potentially very serious and common. [15] Though easily preventable. Eye injury may also occur as a result of exposure to light, Kerotitis is conjunctivitis caused by inadequate eye protection in arc welding, while accidental exposure to laser beams (some times reflected inadvertently off a polished surface) may cause serious corneal or retinal burns. Protection from lasers depends critically on the wavelength of the light which the laser beams, and goggles must be specific to the laser being used. Itchy eyes are frequent complaint in workers exposed to organic antigen, such as grain dusts. The symptom is often a herald of occupational asthma. Chlorine or formaldehyde on the other hand cause non-allergic eye irritation. [3]

Rhinitis—Allergic rhinitis is a common herald of occupational asthma, usually in association with exposure to organic antigen from vegetable matter or from animals. Sometimes rhinitis is the sole manifestation of such allergy. In severe cases nasal polyposis occur.

Septal Ulceration—Uncontrolled exposure to metal fume or dust, chromates (sometimes in cement) cause ulceration of the nasal septum an often painless condition.

Carcinoma—Exposure to dust in furniture and leather goods manufacture cause carcinoma (adenocarcinoma being the usual histological type).

The nervous system: Solvents, are lipid soluble so can be retained in nervous tissue which contain a lot of lipids. Painting, cleaning, carpet-tile laying, laboratory and chemical work, degrading operations and those whose jobs expose them to petroleum products stand the risk of toxic chemical entering their brain tissue. Only substance abuse, alcoholism, glue-sniffing and other solvent exposure can contribute to or cause neuropsychiatric disease. This ranges from a syndrome headache, loss of concentration and short term memory and depression, full-blown dementia or neurological disease. Whenever a doctor sees patients with diseases such as motor neuron disease, the possibility of occupational causation should be borne in mind.[17, 18] Peripheral neuropathy is a motor neuropathy, characterized with wrist drop and is a classical feature of lead poisoning. Parkinsonism is caused by chronic manganese poisoning, prolonged exposure to carbon disulphide and acute carbon monoxide, poisoning. Poisoning may lead to the development of a parkinsonian syndrome, as well as other central nervous system damage. [5]

Stress Reactions: Majority of patients seen in occupational medical practice with neuro-psychiatric symptoms are suffering from psychological rather then chemical stress. Such patient, present with anxiety and depression, and an array of minor physical symptoms, a history of adverse reaction to stress at previous times is often present and causes are usually multiple involving home, and family as well as work. Poorly paid job with little prospect of satisfaction or promotion. [19]

Psychological Breakdown: Psychological breakdown can occur in a worker with recent promotion beyond capacity, conflicts due to multiple responsibilities, too many demands on time, [17, 19] A tiring shift pattern, excess overtime, too little or new technology, can also cause psychological breakdown[19]. If a new or unreasonable boss increases productivity targets, or there is threat of redundancy, sexual harassment or bullying, high sickness absence in colleagues. The workers in such work places can also suffer psychological breakdown.

Disease of the liver and gastro intestinal tract

A wide range of liver diseases have occupational causes but are rare. The gastrointestinal tract is almost untouched by occupational disease although oesophageal and stomach cancers are seen more in rubber vulcanizers and coal miners respectively.The liver transforms lipid-soluble chemical into water soluble ones, with the usual result of a less toxic metabolite. Occasionally the reverse occurs e.g acute hepatic necrosis caused by carbon tetrachloride as occurs in dry-cleaning workers.

However, it is seen that even in workers in a chemical industry, abnormalities in liver function are more likely to be related to alcohol or other causes than to occupational factors[20]

Urinary tract diseases: Occupational causes of acute renal failure, are high level exposure to cadmium dust or fumes released by cutting metal alloys, making pigments and battery manufacture Chronic renal failure following damage to nephrons is caused by carbon disulphide and a wide range of solvents. Bladder cancer is seen in those working in rubber tyre industry and in the manufacture of organic dyes.[21]

Disorders of the reproductive system: Factors at work can cause infertility, miscarriage, and foetal abnormalities. Heavy physical work during pregnancy may have a harmful effect on the outcome. Toxic substances affect the process of gametogenesis, fertilization and pregnancy. Exposure to ionizing radiation and handling of cytotoxic drugs adversely affect the reproductive organs of males and females. In females, organic mercury is potent reproductive poison that causes infertility, miscarriage and foetal abnormalities, [19,23].

Occupational blood disorders: Benzene causes marrow aplasia, with normocytic anaemia as clinical feature. Gamma radiation also causes marrow aplasia but with neutropaemia as clinical feature. Lead causes anaemia with impaired haemosynthesis and stripped red cells as clinical features. Aniline and some of its analogue nitrites cause mthemoglobinaemia, with cynosis as clinical feature, cyahosis. This is reversed by ethylene blue. Arsine causes haemolysis whose clinical feature is intravascular haemoglobin. Gamma radiation and benzene cause leukaemia usually the chronic myeloid type. Toluene causes thrombocytopenia, with bleeding disorders as clinical features. [5,22]

Cardiac Disease: There is strong evidence of association between risk of heart disease and specific occupations. In these circumstances workers exposed to carbon disulphide in manufacture of viscous rayon have an increased

likelihood of death from coronary artery disease. People exposed to nitrates such as glyceryl trinitrate and ethylene glycol dinitrate in manufacture of explosives and of pharmaceuticals have an increased risk of angina and infarction. Those exposed to high levels of halogenated organic solvents such as tricholroethelene may suffer sudden death, probably related to ventricular fibrillation.[18, 1, 2, 16]

General ill-health and infection: The most difficult patients to investigate and manage in medical practice are often those with non-specific symptoms of malaise and general ill-health. As with all other symptom complexes, the occupational history may lead to diagnosis and appropriate management.

The most important occupational factors are psychological problems and physical problems associated with the building in which the person works, the two not infrequently interacting. Less commonly chronic poisoning or occupational infection may sometimes be severe as when legionella species contaminate an air conditioner and cause out breaks of pneumonia or when a farmer is infected with leptospira species and dies of hepatorenal failure. Recurrent over dosage with pesticides and solvents may be seen often leading to nonspecific symptoms. Farmers and fruit growers may easily spray selves with carbamate or organophosphorus insecticides and manifest symptoms of anticholinesterase poisoning, headache, blurred vision, weakness sweating and tremor. Recurrent exposure to solvents is particularly liable to occur in the self employed or in people employed in small companies involving painting and floor covering with flexible vinyl materials. Headaches and a feeling of drunkenness are the usual features with the threat of long-term neurological damage. [7, 22]

CONCLUSION

It is obvious from the text of the paper that occupational diseases are the same disease that affect various parts of the body and the systems. The only difference is that they have occupational or environmental causes. Their management is the same but removal of the patient from the occupational environment that caused the disease, in most cases leads to dramatic recovery of the patient. It is therefore recommended that medical doctors in their practice, no matter the specialty or area of interest should always consider possible occupational causes while managing any disease/disorder in any patient. In this respect the taking of a detailed work (occupational) history will be most appropriate for an accurate diagnosis. If this is done the otherwise missed cases of occupationally related diseases will be recognized and appropriately managed. [9,23,24,]

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Original Article

Prevalence and Intensity of Malaria among HIV Seropositive Patients in Nnewi, Anambra state.

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ABSTRACT

The prevalence and intensity of malaria parasite in HIV patients were determined. A total of 300 subjects comprising of 150 HIV seropositive subjects and 150 HIV seronegative control subjects were recruited for the study. The prevalence and intensity of malaria were determined from the blood samples collected from the subjects. The total prevalence and mean intensity of malaria in HIV seropositive subjects were 64.7% and $2.1x10^3$ parasites/μl and in control HIV seronegative subjects were 58.7% and $1.6x10^3$ parasites/μl. The age group of 18-30years recorded the highest prevalence and intensity of malaria parasites in both the HIV-seropositive and HIV seronegative subjects. The infection status of the male and female subjects showed no significant difference ($p>0.05$). Consideration of the socio-economic status of the dually infected patients in terms of parasitic prevalence showed significant result ($p<0.05$), where those with primary education had the highest prevalence. Prevalence and intensity by occupation yielded no significant result ($p>0.05$). There is high prevalence and intensity of malaria infection in HIV seropositive population. On a population basis, an increased prevalence of malaria and increased parasite intensity in HIV infected individuals could lead to increased malaria transmission affecting both HIV seropositive and seronegative individuals.

Key words: Malaria, Human Immunodeficiency Virus.

INTRODUCTION

Malaria and Human Immunodeficiency Virus (HIV) are among the two most important global health problems of our time. They are the two of the most important infectious diseases in the tropics and any interaction between the two diseases is expected to have far-reaching public health implications, in particular, in countries with constrained resources, co-infecting large numbers of people. Together, HIV/AIDS and malaria kill four million people each year, with additional four million new HIV infection and three hundred to five hundred million infections annually as well[1]. Transmission of both malaria and HIV can result from improper blood transfusion practices and unsafe injections.[2,3] In some settings, policies to minimize HIV transmission through transfusion exist but are not implemented. In area with improper blood transfusion practices, malaria may indirectly increase the risk of HIV transmission, as severe malaria-associated anaemia often leads to blood transfusion particularly in children. For example, in the Democratic Republic of Congo, malaria accounts for almost 90% of blood transfusions administered to children[4]. It is estimated that each year between 5,300 and 8,500 children in areas of stable malaria in Africa acquire HIV infection from blood transfusions given for severe malaria[5].

Co-infection of malaria and HIV is common where the two diseases co-exist in general population or in specific high groups. Given this extensive overlap with resulting high levels of co-infection, interactions between the two diseases have major implications for the treatment, care and prevention of both. For

pregnant women in areas of unstable malaria, the risk of developing severe malaria is 2-3 times higher than that of non-pregnant women living in the same area. In these areas, malaria may result in low birth weight infants, spontaneous abortion or neonatal death. In areas of stable malaria, most adult women have developed some immunity and the principle impact of malaria infection in these pregnant women is malaria-related anaemia which increases the risk of low birth weight infants[1]. Since HIV infection is associated with malaria, it is expected that prevalence of malaria will rise too with its attendant deleterious consequences[6]. In African, only between 8 and 25% of malaria infected patients visit hospital to seek medical treatment[7]. They are mainly those patients that experience febrile malaria attacks and severe malaria anaemia[8]. The remainder embark on self medication; eventually culminating to anti-malaria drug resistance due to inadequate dosage[9]. Some patients infected with HIV also, do not know their retroviral status, but apparently refuse to go for medical check-up. However, the delay leads to a steady immune depletion indicated by a steady decline in $CD4^+$ T-cells[10]. By the time the infection is detected, the immune system may have been compromised. Hence, the present study was designed to evaluate the prevalence and intensity of malaria in HIV seropositive subjects.

MATERIALS AND METHOD

Study Area: This study was conducted at the HIV Special Treatment Centre (STC) in Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State, Nigeria. The Centre serves almost the South-eastern part of the Country.

Selection of Patients: The patients recruited into the study include HIV seropositive individuals attending the RVD clinic (Special Treatment Centre-STC) at the Nnamdi Azikiwe University Teaching Hospital, Nnewi (n=150) and HIV seronegative subjects (n=150) were also recruited for the study. Blood samples were collected from the 300 participants for HIV screening and confirmation and for detection and malaria parasite count. Informed consent was obtained from the patients studied before blood collection.

METHODS: HIV screening was by immunochromatography kit method (ACON Laboratories Inc. USA). The procedure was as described by the manufacturer. In brief, 25ul of serum samples was dispensed into the "specimen pad" of the test strip. 80ul of buffer was added. The reaction was allowed for 5 minutes, the appearance of distinct red lines at test region and control region of the kit suggest positive HIV test while one distinct red line in the region of the control suggest HIV sero negative test. The appearance of the distinct red line of the control region validates the result without which the kit is assumed to be non functional. Malaria screening as well as parasite count was done using the World Health Organization method - Parasite per microlitre of blood[11]. Other information such as age, sex, occupation and socioeconomic status were also collected from each subject; as well as the symptoms the HIV patients. Those who complained of symptoms like intense fever, severe headache, cough, internal body heat, night sweating, body rash, pains in the joints and other parts of the body, fatigue, loss of appetite, diarrhea and so on were termed symptomatic where as those who had no symptom though they are HIV positive patients were termed asymptomatic.

RESULT

A total of 300 patients made up of 150 HIV positive and 150 HIV negative (Control) were examined. The HIV positive patients recorded a malaria prevalence of 64.7% and a mean parasite load of $2.1x10^3$ parasites per microlitre of blood against the control group who recorded 58.7% and a mean parasite load of $1.6x10^3$ per microlitre respectively. The prevalence and parasite intensity according to sex showed that the HIV positive female subjects have higher prevalence (65.8%) and mean parasite load ($2.2x10^3/\mu l$) respectively than their HIV positive male counterpart. However, the prevalence and parasite intensity were similar between the control female group

and their control male counterpart, (p>0.05). (See Table 1). Considering the socio-economic status of the subjects and with respect to their literacy level, those who attained primary school education have the highest malaria prevalence but not intensity for both HIV positive and control subjects. (See Table 2). Meanwhile, prevalence and intensity by occupation gave no significant result (p>0.05) (See Table 3).

DISCUSSION

The present results revealed interactions between HIV and malaria in dually infected patients. Despite recent initiative by the Roll Back Malaria (RBM) to halve malaria burden by the year 2010, the malaria burden still persists at an alarming level [12]. In sub-Saharan Africa, the state of malaria is increasing due to additional burden as a result of HIV pandemic, which has assumed unimaginable proportion in the region. Elsewhere in Africa, studies have established association between HIV infection and increased susceptibility to malaria infection [13-15].

In this present study, a total malaria prevalence of 64.7% and a mean parasite intensity of $2.1x10^3/\mu l$ of blood recorded in HIV infected subjects are comparably higher than those of Control HIV negative subjects who recorded 58.7% and $1.6x10^3/\mu l$ respectively. This can be attributed to adverse impact that HIV has on malaria or vice versa. This is one angle through which association reported between HIV and malaria drives the prevalence of the latter forward. This agrees with the works of Amuta and Ikpa, in Markudi, [16] Uneke et al in Jos. [17]

The humoral and cell-mediated immunity to malaria parasite are reported to be developed in areas of stable malaria transmission [18]. This immunity can be altered in HIV infected persons and could influence the frequency and course of malaria infection. [19] [20] This may explain the high prevalence and intensity of malaria infection in HIV positive population. However, there may be need to further investigate other risk factors that may be associated with high prevalence of HIV and malaria interactions such as presence of opportunistic infection, nutritional status and viral load. On a population basis, an increased prevalence of malaria and increased parasite density in HIV infected individuals could lead to increased malaria transmission affecting both HIV positive and negative individuals [21].

The age group of 18-30 years has the highest prevalence and intensity of malaria in both HIV positive and control groups. This is in contrast to Uneke *et al,* [17] who recorded higher prevalence among the older age groups (51-60) of HIV sero-positive population. This high prevalence of malaria of the old age may be attributed the deteriorations of the immune cells at old age. Malaria is an important cause of adolescent hospital admissions in many sub-Saharan African Countries with stable malaria transmission. HIV infection increases malaria prevalence especially in the young age groups [22]. This may partly relate to reduce acquisition of acquired malaria immunity in young individual [23].

Table 1: Prevalence and intensity of malaria according to sex

	HIV POSITIVE			HIV NEGATIVE (CONTROL)		
Sex	number examined	% positive	mean malaria load ($x10^3/\mu l$)	Number Examined	% positive	mean malaria load ($x10^3/\mu l$)
Male	45	28(62.2)	1.9	77	44(57.1)	1.4
Female	105	69(65.7)	2.2	73	44(60.3)	1.2
Total	150	97(64.7)		150	88(58.7)	

Table 2: Prevalence and intensity of malaria with respect to the socio-economic status (Quantified by literacy level).

	HIV POSITIVE			HIV NEGATIVE (CONTROL)		
Status	number Examined	% positive	mean malaria load ($x10^3/\mu l$)	Number Examined	% positive	mean malaria load ($x10^3/\mu l$)
Primary	41	28(68.3)	1.9	44	32(72.2)	2.4
Secondary	82	53(64.6)	2.0	77	48(62.3)	1.2
Tertiary	27	16(59.3)	2.8	29	8(27.6)	1.9
Total	150	97(64.7)		150	88(58.7)	

Prevalence: significant ($p<0.05$), Intensity: not significant ($p>0.05$)

Table 3: Prevalence and intensity by occupation.

	HIV POSITIVE			HIV NEGATIVE (CONTROL)		
Occupation	number Examined	% positive	mean malaria load ($x10^3/\mu l$)	Number Examined	% positive	mean malaria load ($x10^3/\mu l$)
Student	15	9(64.3)	2.7	25	18(72.0)	1.2
Traders	85	56(65.9)	1.7	64	41(64.0)	1.4
Civil Servants	20	12(60.0)	2.3	29	10(34.0)	1.5
Others	30	20(66.7)	2.9	32	19(59.4)	1.2
Total	150	97(64.7)		150	88(58.7)	

Prevalence and Intensity: not significant ($p>0.05$)
Note:Others include: housewives, labourers, cyclists, applicants, hairdressers, conductors, masons and drivers.

This may explain why there is higher prevalence and intensity of malaria in the younger age groups. However, malaria tends to affect mainly children and pregnant women, especially in rural areas, whereas HIV is more common among sexually active adults in urban centers [24]. According to the Centre for Disease Control and Prevention (CDC), HIV infection is the leading cause of death in Africa, especially in women [10]. HIV infected women remain susceptible to the effects of malaria whether or not they are pregnant. In sub-Saharan Africa, the highest overlap between malaria and HIV infections occur in females [22]. HIV and malaria are responsible for much of the disease burden affecting females who suffer disproportionately from these combined infections relative to others [22].This may attribute to higher prevalence and intensity recorded in HIV sero-positive women, although, there was no significant difference between the infection status of male and female patients studied.

Further stratification of the patients according to their socio-economic status gave a significant result ($p<0.05$), which means that, there may be a relationship between HIV/malaria co-infection and socio-economic status of the patients. The highest prevalence of malaria infection was found in those who had primary education. This group of people may have been placed in the least salary scale in their occupational setting, having low educational background; thus belonging to lower socio-economic class. According to WHO [1], malaria and HIV are both diseases of poverty as well as cause of poverty and they share determinants of vulnerability to

infection. Many of the circumstances that give rise to such vulnerability are present in sub-Saharan Africa. Given the wide geographical overlap in occurrence and the resulting co-infection, the interaction between the two diseases clearly has major public health implications. These diseases are part of the poverty trap in Africa. People get sick because they are poor and they get poorer because they are sick. The people in lower socio-economic class spend greater part of their income in healthcare services, transportation as well as feeding (which may not be adequate). The cost may be too much to bear due to lack of fund. The health condition may get worse until they can no longer work. Soon the entire family is malnourished as a result of illness. Hence, there is serious loss to the economy. World Health Organization reported that the overall consequences of malaria on the lives of Africans translate into an annual loss of as much as 1.3% points of growth as compared with countries without malaria. Malaria and HIV contributed immensely to Nigerians impoverished state and will no doubt continue to keep Nigerians poor.[25] Prevalence and intensity by occupation had no significant result ($p>0.05$).

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Original Article

Sibling species distribution of the vectors of human Onchocerciasis in South East Nigeria, epidemiological implications and the control of Onchocerciasis in Nigeria.

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ABSTRACT

Cytogenetic studies were carried out on the members of the *Simulium damnosum* complex in South East Nigeria. The studies were undertaken for a period of 12 months. Sample collections were carried out in the different bioecological zones (typical rainforest, mosaic forest, mosaic/guinea savanna and forest interphase/transitional zone) to ascertain the geographical distribution and variation in the species composition of the different vectors of Onchocerciasis in the area. *Simulium* larvae were fixed in Carnoy's solution and cytogenetic analysis were carried out. The geographical distribution of the different cytospecies of *Simulium damnosum* complex was mapped. A total of 334 cytological identifications were made. Three out of the 9 West African sibling species, belonging to two of the *Simulium damnosum* complex: the *S. squamosum* sub-complex (228) and the *S. damnosum* sub-complex (56) were observed. The ICb variant of *Simulium squamosum, which* had been described from the River Sanaga (Cameroon), was observed for the first time in Nigeria. This suggests a probable change, in the nearest future, of the epidemiological pattern of Onchocerciasis in the study area.

Key words: Simulium damnosum, cytogenetic, polytene chromosomes, epidemiology.

INTRODUCTION

Human Onchocerciasis, more commonly known as river blindness, is a chronic parasitic blinding disease of man [1]. It is endemic in 38 countries worldwide, including areas of Africa and the Middle East [2, 3]. About 123 million people live in endemic areas and an estimated 18 million people are currently infected, out of which about 270,000 are blind and an additional 500,000 have visual impairment [4]. Nigeria is an African country with the largest people blinded by Onchocerciasis, accounting for over 40% of the world's cases of the disease [5, 4, 6, 7].

The sibling species composition and distribution of the vectors of human Onchocerciasis and also the clinical features of the disease have been known to vary considerably from one geographical region to another and even between different bioclimatic zones within a single region [8].

This in turn affects patterns of epidemiology which are associated with different strains of the parasite (forest v. savanna) [9, 10] and different vectors (cytospecies or species)[11], the abundance of the vectors and degree of variation in composition, their vectorial and migratory capacity. [12] It is therefore imperative that the taxonomy, ecology and behaviour of the *Simulium* vector species and their relationship to the parasite they transmit and the environment be properly understood. The bioecological structure, the South eastern Nigeria is quite varied [13, 14] and not much is known about the role of the different sibling species of the *S. damnosum* complex in Onchocerciasis transmission in this region in particular and in

Nigeria in general. Although the Rapid Epidemiological Mapping of Onchocerciasis (REMO)/Atlas geographical information system (GIS), developed by WHO in collaboration with the Nigerian National Onchocerciasis Programme (NOCP) is good at assessing the current status of the disease in the human population through the analysis of easily detected symptoms, [15, 16] they are less efficient at rapid detection of changes in the epidemiology of the disease. Early detection of such changes requires information on the vector cytospecies composition, seasonal abundance and geographical distribution, which will be provided by cytotaxonomic identifications. The accurate and reliable identification of *Simulium* species transmitting the different species and strains of *O.volvulus* and their distribution in studies of epidemiology of Onchocerciasis vectors and in the planning and execution of control measures has therefore been repeatedly emphasized. [17, 18] The aim of the present study is to use cytotaxonomy to identify and determine the identities of the different strains of vectors of human Onchocerciasis in the study area. The variation in their seasonal and geographical distribution will be determined and such will be related to the disease epidemiological pattern and potential for consequent control by vector eradication. It is hoped that this will also allow the filling in of the obvious gap in the understanding of the Onchocerciasis vector ecology and dynamics in Nigeria, and also contribute to the rapid accumulation of baseline data on which a nationwide Onchocerciasis control can be based eventually.

MATERIALS AND METHODS

For the purpose of clarity in this research, the study area is divided into three natural zones. Zone A consists of the coastal areas i.e. Cross River and Akwa Ibom States (mainly forest), zone B consists of Imo and Abia States (a mixture of mosaic and degraded forest) while zone C comprises of Enugu, Ebonyi (forest-savanna, mosaic/guinea savanna) and Benue (forest interphase and a transitional zone between forest and savanna bioclimes) States. Vector breeding sites were selected from the different bioclimatic zones based on available prevalence data [16, 18] and preliminary sampling for *Simulium* in the area.

The collection of *S.damnosum* complex larvae for cytogenetic determination of sibling species were carried out, between December - March for the dry season and May - October for the wet season, from ten sites in eight endemic communities in the study area. Final and penultimate stage larvae were collected from their riverine breeding sites and fixed in Carnoy's solution (3:1 absolute ethanol: glacial acetic acid) and polytene chromosome preparations were made by dissection and staining in FLP-Orcein according to standard procedures [19]. Chromosomes were mapped and cytospecies identifications were made according to previously described criteria [19]. Autoshapes in Microsoft Word were employed to indicate the geographical distribution of the different cytospecies of *Simulium damnosum* complex in the study area and this was related to the known epidemiological pattern of Onchocerciasis in the South east of Nigeria.

RESULTS

A total of 334 cytological identifications were made. These new identifications, together with details of the geographical location of sampling sites, their bioclimatic zones and season are presented in Table 1. The new cytological identifications consisted of *S.squamosum* and *S.yahense* belonging to the *S.squamosum* sub-complex and *S.damnosum* belonging to the *S.damnosum* sub-complex. No new fixed inversion differences were observed. *S.squamosum* was recorded from all the bioclimatic zones, breeding in alongside *S.yahense* or *S.damnosum* in most areas. Two variants of *S.squamosum*, ICs and ICb were observed and the latter is being observed in Nigeria for the first time. [20] *S.yahense* was found breeding in the mosaic forest and forest areas, alongside *S.squamosum* in all cases. An allopatric breeding situation was observed to be exhibited by the *S.squamosum* species at only one sample site, the Atan River.

DISCUSSION

The correct identification of individual member species of the *S.damnosum* complex in any locality is needed for a better understanding of epidemiology and also in the practice of control operations. In the latter case, precise identification can be important for inferring the possible provenance of flies and for delimiting control zones.

The map that emerged from the nationwide Rapid Epidemiological Mapping of Onchocerciasis (REMO) in Nigeria [16] was reasonably detailed and was consistent with the known disease pattern particularly across the international border with Cameroon in South eastern Nigeria.[21] The result showed that the South eastern part of Nigeria is hyperendemic [18] and most of the area is included among those for which *mectizan* treatment is definitely required. However, the level of endemicity has not been precisely ascertained in most communities in the area as the above result is based on REMO. An exception is the communities around Enugu, Udi and Oji River where the disease is known to be mesoendemic.[13]

Again, the result of the strain distribution of *O.volvulus* in Nigeria had suggested that the non-blinding strain is found mainly in southern Nigeria while in central and northern Nigeria, the blinding strain predominates.[22] This pattern of distribution follows the local ecology of the area. An attempt is being made from the findings of this study to correlate the known epidemiological pattern of Onchocerciasis in the study area with the presence of different species of the *S.damnosum* complex at the different locations.

The South east of Nigeria is a rainforest bioclime, which begins to change to a forest savanna mosaic just north of Enugu. In the typical forest areas of zone A, where the epidemiology of Onchocerciasis is known to be forest type,[23] *S.squamosum* and *S.yahense* appear to be the dominant vectors although it is not certain which of them is the more efficient and important vector.

The situation is similar in the degraded mosaic forest area in zone B which has also been known to show a predominantly forest-type epidemiology.[18] It seems however that there is a gradual invasion of these areas by the savanna species, *S.damnosum*, probably as a result of the ongoing forest degradation in these areas. If continued, this will pose a great risk because it is possible that a savanna blinding Onchocerciasis could get down into this bioclimatic zone. The obvious mixture of savanna and forest vectors, *S.squamosum* and *S.damnosum* in zone C correlates well with the known disease pattern in this transitional zone.[24]

The allopatric breeding situation observed in Atan River could be significant in the provision of sources of "pure" larvae with which larvicidal and transmission experiments could be conceived, tested and successfully applied in the campaign against Onchocerciasis as was the case in the OCP area.[2] The movement of the ICb variant of *S.squamosum* across the Cameroonian border as far as Idodo river (Emene) in zone C, a distance of about 750km should also be of a great concern not only because *S.squamosum* had been known to be seldom migratory and the great possibility of re-invasion from Cameroon in the event of a nationwide vector control, but because the northern part of Cameroon is infested with a serious blinding form of Onchocerciasis.[25] There is a great probability that this form of Onchocerciasis could sooner or later gradually invade the South eastern part of Nigeria through the Cameroonian border.

The evidence that migratory movement of adult females of the *S.damnosum s.l.* occurs over hundreds of kilometers, at least under some meteorological conditions, makes it essential to consider the situation of any country where control is planned in relation to its neighbouring areas. When considering a national vector control programme, Nigeria has the advantage that on the whole, she is free from the possibility of re-infestation of controlled areas from either the south, i.e. the Bight of Bonny or the north.

This is because there is no known breeding site of *S.damnosum s.l.* in the semi-desert areas of the Republic of Niger lying to the north of Nigeria. Again, the Benin Republic on the west of Nigeria and also the Republic of Chad has already been covered by the OCP.[3, 2] The nation is however vulnerable to re-infestation at least on the long run, from the East because of the existence of breeding foci in the Republic of Cameroon. In view of all these, the South - east of Nigeria becomes a very important zone to watch in the event of a nationwide Onchocerciasis control, especially with regards to the break in transmission through vector control.

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Table 1: Cytospecies identification and geographical distribution of *S.damnosum complex in the study area.*

LOCALITY					CYTOSPECIES				Number
Site No.	Name of River	Geographical coordinates (Lat./Long.)	Bioclimatic Zone	Season	*squa*		*yah*	*dam*	(n)
					ICs	ICb			
1	ATAN	7°50',5°08'	Forest	D	27	-	-	-	27
2	AKPA-IKPONG	8°05',5°04'	Forest	D	19	-	-	4	23
3	IMO	7°23',5°42'	Mosaic Forest	D	39	-	-	3	42
4	IBII	7°22',5°55'	Mosaic Forest	D	5	1	18	-	24
				W	9	2	14	-	25
5	IBII	7°23',5°57'	Forest	D	9	17	-	-	26
				W	14	4	-	-	18
6	IBU	7°22',5°57'	Forest	D	7	-	13	-	20
7	UGBI	7°18',5°56'	Forest	D	14	4	2	-	20
				W	4	-	6	2	12
8	IYI-AKA	7°20',6°00'	Forest	D	10	-	14	-	24
9	IDODO	7°42',6°35'	Mosaic/Guinea Savanna	D	10	5	-	17	32
10	OKPOKWU	7°52',6°50'	Forest Interphase/Transitional Zone	D	5	-	-	7	12
				W	6	-	-	23	29

(D) = dry season, (W)= wet season, (squa) = squamosum, (yah) = yahense, (dam)=damnosum, (ICb) = short asynaptic centromeric region and (ICs) = synaptic centromeric region.

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Original Article.

Isolation, Characterization and Propagation of Bakers Yeast from Local Palmwine *(Elaeis Guinensis).*

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ABSTRACT

Bakers yeast was isolated, characterized, and propagated from local palmwine *Elaeis guinensis,* collected from two different stations, namely: Mgbakwu, in Awka North Local Government Area and Nchagbo, in Ihiala Local Government Area, both in Anambra State. This was carried out with the aid of Saubraud Dextrose Agar (SDA), using a Pour Plate Technique (PPT). The inoculation was carried out and incubated for three days at room temperature. The mixed yeast colonies obtained at the end of the incubation period were purified into pure cultures by sub-culturing into a fresh SDA, using Streaking Plate Technology (SPT). The characterization of yeast was carried out on the basis of the cultural, microscopic and biochemical examination of the isolates of yeast. The yeast finally isolated, characterized and identified include: *Saccharomyces cerevisiae, Schizosaccharomyce pombe* and *Saccharomyces exigus.* Pure cultures of *S. cerevisiae* were propagated and multiplied using cane molasses to obtain a good deal of biomass.

Keywords: Organisms, Isolation, Analysis.

INTRODUCTION

Yeast, a form of fungi, like other micro-organisms, exist in or on most organic living matter. A common example of yeast is the bloom observed on grapes. Yeasts have simple nutritional needs and are unable to carry out photosynthesis. Yeast are used in the fermentation industry especially in the anaerobic conversion of simple sugar to ethyl alcohol and carbon dioxide, otherwise called alcohol fermentation. Yeast belongs to the protascomycetes family characterized by building as a means of reproduction. The yeast family has two groups: Endomycatacaea and Saccharomycetacea; called the true yeasts. A typical example is *Saccharomyces,*[1] Bakers yeast is commonly used for baking flour and powder and *S cerevisiae* is the yeast of choice[2].

Bakers yeast is of three types: compressed yeast, active dry yeast and instant active yeast. Media for growth of yeast contains molasses and various salts including ammonium and phosphate salts. The medium is adjusted to P^H 4.5-5.0, sugar level between 0.5 to 1.5%, while temperature is 30^0C or less. Yeast reproduces by budding or sporulation.[3]

Yeasts are found in all habitats that contain fermentable sugar materials, liquid extracts and secretions. They have been isolated from places like human body, water, air, fruits, vegetable materials and the like[4] but for the purpose of this work, palmwine is the source of interest. Palmwine is a sweet milky white effervescence liquid with a sweet taste that was obtained from oil palm tree *Elaeis guinensis* and the raphia palm tree *Raphia hookeri*__or *Raphia verifera* respectively. Yeast and yeast like fungi are widely distributed in nature. True inhabitants are those that may reproduce in the habitat.[5] Fate of yeast is affected by factors like P^H, water content (Aw), oxygen, carbon, nitrogen and vitamin sources available as well as other living organisms like the plant and animal host, filamentous fungi; bacteria, algae and insects.[6] Nutritional needs of yeast range from simple sugar (carbon sources) to ammonia (Nitrogen source). Other nutritional needs of yeast include: growth factors, hormones (biotin) and trace elements.[7] Some of their uses include: bread making and other bakery product,[8] while derived food yeast is used as supplements for humans, production of B – vitamin, for

production of single cell protein (SCP). Some species are used to remove petroleum as a pollutant from environment; in fermentation, for research and studying eg. NAD, RNA, ATP. They are also a rich source of protein amino acid.[9] Some are infectious eg. *Candida albicans, Cryptococcus neoformans*. Bakers yeast is used mainly for baked foods,[2] and the yeast of choice is *S cerevisae* used as a leaving agent. It exhibits active growth in the dough medium; utilizing fermentable sugar in the medium to yield other compounds like CO_2 and alcohol. Thus the functions of yeast in the dough are leavening, maturation and development in dough. *S cerevisae* has been noted for their ability to ferment and tolerate high ethanol concentration. Thus, high tolerance, flavouring, aroma flocculation, food sugar utilization and acid tolerance are characteristic nature of bakers yeast.[10]

Palmwine analysis shows that palmwine extract contains about 57% of yeast, mostly *S cerevesiae.* The mass product of bakers yeast made locally from palmwine will reduce the amount spent on importation of this organism from other countries because it affects our foreign reserves. The production of this bakers yeast in large quantity will reduce drastically the use of chemical leavening agents like bromides and bicarbonates used as substrate bakers yeast; for these cause diseases like cancer, kidney failure among others.[11] Also, production of bakers yeast will reduce cost of raw materials for the bakery industry. The objective of this study is therefore to identify, isolate, concentrate and harvest the yeasts *S cerevesiae* which is the major yeast of the bakery industry. Comparative study was also carried out between the produced and the commercial yeast.

MATERIALS & METHODS

Media used include: Saubraud Dextrose Aga (SDA), Corn meal Agar, Presporulation medium, post ssporulation medium, peptone water, starch medium, non sugar dough and sugar dough.

Each media was prepared according to the directions given for preparation; they were autoclaved at 121^{0}C for 15 minutes and allowed to cool to 45^{0}C.

SAMPLE COLLECTION

Palmwine samples were collected from Mgbakwu in Awka North and Nchagbo in Ihiala Local Government Areas. Samples were collected in sterile containers from local palmwine tappers in the study area.

ISOLATION PROCEDURE

1ml of each wine sample was added to 9ml of sterile distilled water using a sterile pipette to make a 1 in 10 dilution,[8] Fowel (1969). Subsequent dilutions were made to obtain 10^{-5} and 10^{-6} dilutions of each sample. 1ml of 10^{-5} and 10^{-6} dilutions of each sample were inoculated into SDA, that was cooled to 45^{0}C in McCarthy bottles and poured aseptically into sterile petri dishes. Plates were incubated at room temperature for 3 days, after which the plates were observed for microbial growth. Growth colonies were picked at random and sub-cultured to obtain a pure culture. Colonial morphology, cellular characteristics were studied. Isolates were identified according to criteria described by[11] Lodder (1970).

IDENTIFICATION TESTS

Wet mount using lactophenol cotton blue test, sugar fermentation tests for the production of acid and gas, starch hydrolysis, ascospore production ie the sporulation test. Spore staining test using malachite green was done according to[8].

PREPARATION OF MEDIA FOR PROPAGATION

The molasses, containing about 50% sugar was heated and filtered, decanted and diluted with sterile water to provide a sugar concentration of 1.5%. Sugar level was deliberately low to favour cell multiplication. Hydrometer bulb was used to ascertain the sugar concentration. After dilution, P^H of medium was adjusted to 4.5 using dilution. Medium was finally sterilized at 121^{0}C for 10 minutes before it was allowed to cool.

CULTIVATION OF YEAST CELLS

After sterilization, the media was inoculated with 2 x 10^5 cells of *S. cerevisieae* in 250ml of molasses at 30^0C for 3 days. Fermented medium was centrifuged at 10,000rpm under aseptic conditions.

FERMENTATION ABILITY IN DOUGH

The fermentation ability of the bakers yeast isolated was investigated in various dough compositions on both sugar and non-sugar dough. This was compared with commercial bakers yeast.

STATISTICAL ANALYSIS

Statistical data was done using the students t – test, to know the relationship that exist between the palmwine bakers yeast and the commercial yeast.

RESULTS

Three yeast isolates were obtained from the palm wine samples. These are: *Saccharomyces cerevisiae, .S pombe* and *.S exigus.* These are coded PW_1, PW_2, and PW_3, respectively. They were isolated accordingly.[11] The result shows that the fermentative ability on sugar dough is also statistically not significant. Comparisms were also made in the CO_2 level produced by the commercial yeast and palm wine yeast on both sugar and non sugar dough. The result also showed no significant difference.

DISCUSSION

Palmwine in bakers yeast exhibited an impressive fermentative ability on both sugar and non sugar doughs as illustrated in table IV & V respectively.

Table I – Test for non sugar dough

Time	CBY	CBY^2	PWY	PWY^2
20	38	1444	30	900
30	50	2500	35	1225
40	55	3025	36	1290
50	62	3844	40	1600
60	71	5041	45	2025
70	75	5625	51	2601
80	78	6084	56	3136
90	79	6241	61	3721
100	82	6724	64	4096
110	84	7056	68	4624
120	87	7569	70	4900
	761	55153	556	30120

t = 2.913 Critical t value = 0.181

Table II – Test for sugar dough

Time	CBY	CBY^2	PWY	PWY^2
20	48	2304	40	1600
30	58	3364	42	1764
40	68	4624	48	2304
50	72	5184	50	2500
60	78	6084	56	3136
70	82	6724	58	3364
80	84	7056	62	3844
90	85	7225	68	4624
100	86	7396	70	4900
110	86	7396	74	5476
120	88	7744	78	6084
	853	65101	648	39596

t value = 3.1 Critical t value = 1.181

From table V, palmwine yeast and commercial bakers yeast performed well on dough containing mild quantity of sugar depending on weight of flour. Also, it was observed that there is drop in the rate of CO_2 produced after about 1 hr. 20 mins for commercial yeast and $1^1/_2$hrs for palmwine yeast. This happens when the free sugar in the dough is depreciated.[2] Looking at tables IV & V again, the rate of gas production by palmwine yeast and commercial yeast is different for both yeast types. This is due to the fact that the commercial bakers yeast has undergone some changes, purification and concentration, hence it cannot be said to be ordinary. Palmwine yeast may contain some impurities; hence higher technology is needed for the purification and concentration.

From the t-test analysis, it was observed that the t-value calculated is higher than the critical t-value. This is indicative that the commercial yeast is better than the palm wine yeast product

at (P>0.05). The same is true in non sugar and sugar dough's. This may be because the commercial bakers yeast must have undergone some modifications, concentrations and improvement, but the palm wine in bakers yeast produced is good enough for bakers and if commercialized, will reduce the bulk of money spent on importation of yeast. Also with the addition of little technology, the palm wine yeast produced will be concentrated and purified in which case it can compete favorably with the commercial yeast. Bakers yeast from the palm wine has excellent aroma which it produces when mixed with dough; which is lacking in commercial bakers yeast.

Table III – Shows the results and characteristics of the isolates.

Isolate Code	**PW1**	**PW2**	**PW3**
Colour of Colony	Creamy White	Creamy	Creamy
Cellular morphology	Oval or round	Cylindrical	Oval
Cultural morphology	Smooth, round smooth, large, raised entire, soft.	Smooth surface, convex	Rough surface, raised, circular surface.
Motility test	+VE	+VE	+VE
Ascopore formation	+VE	+VE	+VE
Nitrate reduction	+VE	+VE	+VE
Starch hydrolysis	-VE	-VE	-VE
Urease test	-VE	-VE	-VE
Glucose	+VE	-VE	-VE
Maltose	+VE	+VE	+VE
Sucrose	+VE	+VE	+VE
Arabinose	+VE	-VE	+VE
Melebiose	-VE	-VE	-VE
Galactose	+VE	+VE	+VE
Lactose	-VE	-VE	-VE
Culture on Corn meal Agar	Pseudomycelium not present	Pseudomyclium present	Pseudomycelum present
Probable identities	*Saccharomyces cerevisae*	*Saccharomyces pombe*	*Saccharomyces exgus*

Key: +ve = Positive result that is presence of acid and gas.

-ve = negative result that is no presence of acid and gas.

Table IV – Shows the fermentative ability of commercial and palm wine yeast on non sugar dough.

	Time	20	30	40	50	60	70	80	90	100	110	120	$\bar{X}$
CBY	Level	38	50	55	62	71	75	78	79	82	84	87	69.20
PWBY	Level	30	35	36	40	45	51	56	61	64	68	70	50.50

The result shows that the fermentative ability of both yeast in non sugar dough are not statistically significant.

Table V – Shows the fermentative ability of both types of yeast on sugar dough.

	Time	20	30	40	50	60	70	80	90	100	110	120	$\bar{X}$
CBY	Level	48	58	68	72	78	82	84	85	86	86	88	75.91
PWBY	Level	40	42	48	50	56	58	62	68	70	74	78	58.72

CONCLUSION

Due to the increase in the price and cost of importation of the commercial yeast, bakers now divert to the use of chemicals as alternatives. These chemicals have been proven to be disadvantageous to man. With the production of the bakers yeast from palmwine, and molasses, the problem seems to have a solution underway. The use of palmwine and molasses are economical, for both are cheap and readily available.

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Short Communication.

Relationship between Chlamydia Seropositivity and Presence of Symptoms of Sexually Transmitted Infections among Students of Nnamdi Azikiwe University, Awka.

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ABSTRACT

In an attempt to determine the prevalence of Chlamydia antibodies among students of higher institution, 84 students of a University in South-Eastern Nigeria were screened for Chlamydia seropositivity. Volunteers were selected and questionnaires distributed. The questionnaires determined demographic data, level of sexuality, marital status, health care patronage and symptomatic presentation among the students selected. Of 84 students recruited, 26(31%) were males while the rest, 58(69%) were females. The antibody test was carried out using world wide diagnostics Chlamydia IgG quantitative test. The overall prevalence of Chlamydia seropositivity among the population studied was 6.0%,females being more affected (6.9%) than males (3.8%). Frequency of symptom was higher among females 41(89.1%) than was the case among males 5(10.9%) in all the 46 symptomatic cases investigated. Lower abdominal pain and discharge were more frequently associated with Seropositivity. The highest incidence of C. trachomatis Seropositivity was among students who had begun sexual activity under 25 years of age, had multiple sexual partners and with little or no access to health care.

Key words: Chlamydia, Population, Frequency.

INTRODUCTION

Chlamydia infection is caused by the bacterium Chlamydia trachomatis. The symptoms of the disease resemble that of other sexually transmitted diseases Chlamydia infection was not recognized as a sexually transmitted disease until recently[1]. The organism is an obligate intracellular parasite that exclusively infects humans (it cannot synthesize its own ATP or grow on artificial medium) ,it was once thought to be a virus Chlamydia infection is the most prevalent sexually transmitted disease in the United States[2]. There are roughly four million cases annually, most occurring in men and women under the age of 25years. Direct and indirect costs of Chlamydia (mainly costs for complications) total 24 billion US dollars a year. This is most likely an under estimate, since many people with Chlamydia infection likely have gonorrhea as well. Hence, costs to diagnose and treat the later sexually transmitted disease must be included.

MATERIALS AND METHODS

The study population consists of student volunteers in a University in South-Eastern Nigeria. The study was carried out between October and December 2003 and the Volunteers were asked to submit their blood samples. A total of 84 students both male and female were sampled within this period. Using a standardized questionnaire the subjects were interviewed either in their hostels or in classrooms. The following basic demographic data and information were collected: access to health care, Gynaecologic and obstetric history, history of STDs, sexual behavior, condom use

and history of sexual coercion. Blood samples were collected from volunteers who complained of symptoms of sexual diseases and from females, mostly those with a history of sexually transmitted infections. The samples were screened for presence of antibodies to chlamydia using IgG (Worldwide Diagnostics) enzyme linked immunosorbent assay (ELISA).

RESULTS

Incidence of Chlamydia seropositive students according to age and sex: Five (6%) of the study population were seropositive. Seropositivity was more frequently encountered among females 4 (4.8%) than males 1 (1.2%). Seropositivity was not recorded among individuals of 25 - 28years and 37 - 40years.

Prevalence and nature of sexually transmitted infection manifested by students: A wide range of symptomatic display was noted among seropositive cases. This ranged from dysuria, discharge, lower abdominal pain with discharge. Frequency of symptom was higher among females 41(89.1%) than was the case among males 5(10.9%) in all the 46 symptomatic cases investigated. The least displayed symptom was lower abdominal pain associated with dysuria which had only 2 cases found in women. Dysuria was more common in men, while women displayed all the observed symptoms in various degrees, men only exhibited two i.e dysuria and discharge respectively. With respect to seropositivity, discharge and lower abdominal pain were the associated symptoms. See table 1.

DISCUSSION

The study has shown that the overall incidence of the disease was 6.0% among the population studied. This is a bit higher than the 4.0% prevalence obtained by Ngandijio et al.[3] among Camerounian students. More women 58(69.0%) were selected than men 26(31.0%) since women are usually more predisposed to infection and therefore are at higher risk group than men. The result indicates that there is a slight prevalence of Chlamydia seropositivity among students of Nnamdi Azikiwe University. Symptoms associated with sexually transmitted infections (STI) were higher among females 41(49%) than was the case among their male counterparts 5(6%).

TABLE I: Prevalence and nature of STI symptom manifested by students of NAU

	SEROPOSITIVE		SERONEGATIVE		
SYMPTOMS	MALE N(%)	FEMALE N(%)	MALE N(%)	FEMALE N(%)	TOTAL N(%)
Dysuria	0(O%)	0(0%)	4(8.7%)	6(13.1%)	10(21.8%)
Discharge	0(O%)	1(2.2%)	1(2.2%)	8(17.3%)	10(21.8%)
Dispaerunia	0(O(%)	0(0%)	0(0%)	3(6.5%)	3(6.5%)
Lower abdominal pain	0(O%)	2(4.3%)	0(0%)	12(26.1%)	14(30.4%)
Lower abdominal pain with dysuria	0(O%)	0(0%)	0(0%)	2(4.3%)	2(4.3%)
Lower abdominal pain with discharge	0(0%)	0(0%)	0(0%)	7(15.2%)	7(15.2%)
TOTAL	0(0%)	3(6.5%)	5(10.9%)	38(82.6%)	46(100%)

This is not surprising since women are more prone to infection than men. The result also shows that 2(40%) of 5 seropositive cases never displayed any symptom. It is not clear how infectious asymptomatic Chlamydia seropositive individuals are, and thus how relevant they are compared with symptomatic cases. Three factors may contribute to the transmission dynamics: first, case management is poor. Those given inadequate drug treatment probably remain

seropositive and may spread the infection. Biodata analysis showed that 2 of 5 seropostive students were asymptomatic; this is likely to perpetuate transmission.

Asymptomatic persons may not realize that they are infected and hence they do not seek care. If symptoms presented are common and non specific the patient is not likely to suspect an infection (mostly in women).This is so in pregnancy when urogenital symptoms not due to infection are common[2]. Conversely, some people may suspect an infection, but decide not to seek care because of perceived shame, cost, or unpleasant services.

Improving people recognition of abnormal urogenital symptoms and encouraging presentation for syndrome management are potentially important for reducing the level of Chlamydia Seropositivity.

It is also observed that a wide range of symptomatic display was noted among seropositive cases. This ranged from dysuria, discharge, lower abdominal pain, dyspaerunia to combined symptoms such as lower abdominal pain with discharge. It is important to note that these symptoms are not directly associated with Chlamydia Seropositivity but may be evidence of infection by other organisms. It is worthy to note that frequency of symptom was more among females 41(89.1%) than was the case among males 5(10.9%). However, only discharge and lower abdominal pain was present in the symptomatic and seropositive cases. It was also observed that the possibility of displaying a symptom increased with number of sex partners. This appears to be in harmony with the view[2] that most people who suffer from STI have more than one sex partner. In this study, unmarried women displayed more symptom than any other group.

Chlamydia infection can be regarded as a socially transmitted disease. Thus medical intervention cannot be the only solution to control infection rates. Social factors, including behavioral changes and consistent access to quality healthcare, need to be included to eradicate this preventable disease.

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Original Article

Pattern of Biochemical Tests in A New Suburban Nigerian Teaching Hospital

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ABSTRACT

The utilization of biochemical tests is well established and justified in modern medicine. This study highlights findings in the Chemical Pathology Department of a suburban teaching hospital in eastern Nigeria. Our study highlighted annual variation of patients visit, with more visit between the months of March and August. This implies more visits during the rainy season. In this area, the most frequently requested biochemical tests includes; creatine (18.3%), urea (18.2%), glucose (15.2%), and electrolytes (14.8%). Others include urinalysis (7.9%), total protein (3.4%), albumin (3.1%), aspartate amino transferase-AST (2.6%), alkaline amino transferase-ALT (2.5%), lipids (2.5%), alkaline phosphatase (1.8%), calcium (1.6%), and prostate specific antigen-PSA (1.2%). The hormone tests such as tri-iodothyronine(T^3), thyroxine(T^4), and thyroid stimulating hormone(TSH), luetinizing hormone(LH), follicle stimulating hormone(FSH), prolactin, progesterone, testosterone, and estradiol are not commonly requested (0.4% each).The tumor markers alpha-feto protein(AFP) and beta- human chorionic gonadotrophin (bHCG) are rarely requested. This study reflects the overall activity of Chemical Pathology laboratory, revealing the tests more frequently requested; and also showing the period of the year when patients visit is more. Considering the high cluster of patients visit, between March and August, it appears people in this area presents with health problems needing medical attention more in the rainy season.

Keywords: Biochemical tests; chemical pathology; teaching hospital laboratory.

INTRODUCTION.

The clinician's main task is to make reasoned decision about patient care, despite imperfect clinical information and uncertainties about clinical outcomes. While date elicited from the history and physical examination are often sufficient for making a diagnosis or as a guide to therapy, more information may be required. In these situations, clinicians often turn to diagnostic tests for help. The importance of biochemical tests in the management of diseases cannot be overemphasized. Clinical biochemical studies elucidate changes that occur in diseases in the chemical constituents of the body and biochemical mechanism of the body[1]. These changes may either be a cause[2-4] or effect[5-7] of a disorder. Biochemical tests may also be utilized in the monitoring of disease patterns and prevalence in any given community. The pattern of diseases in this community is far from being known. This study shows in a way the prevalent diseases and a pattern of their occurrences in this community.

METHOD AND SCOPE OF THE STUDY.

The study was restricted to the chemical pathology laboratory of the Imo State University Teaching Hospital, Orlu-IMSUTH. The annual returns of biochemical investigations from the chemical pathology laboratory were obtained covering a

period of three years-2005-2007. The data was evaluated manually to derive; number of patients visit per month, work output per month, annual workload, and the percentage distribution of tests done in the department. The Imo State University Teaching Hospital transformed from a state general hospital into a teaching hospital in April, 2004. It was officially commissioned on Tuesday November 30, 2004; with total bed occupancy of three hundred and sixty beds. At that time, it was constrained by poor and inadequate equipment, facilities and staffing. As such, the chemical pathology laboratory could not do much investigation. By 2005, following the acquisition of basic laboratory equipment, the department could do basically all routine tests such as, blood glucose, urea, creatine, lipid profile, liver function test, uric acid, and calcium etc. Following subsequent acquisition of equipment, the scope of services in the department was further broadened to include such tests as tumor markers (prostate specific antigen-PSA, beta human chorionic gonadothophin-bHCG, and alfa-feto protein-AFP), thyroid function test (T3,T4,TSH), fertility hormones LH, FSH, progesterone, estradiol, testosterone, prolactin. As at the time of this study, the chemical pathology laboratory of the hospital also have facilities for creatine kinase (CK-MB), lactate dehydrogenase (LDH), cortisol, and dehydroepiandesterone DHEA.

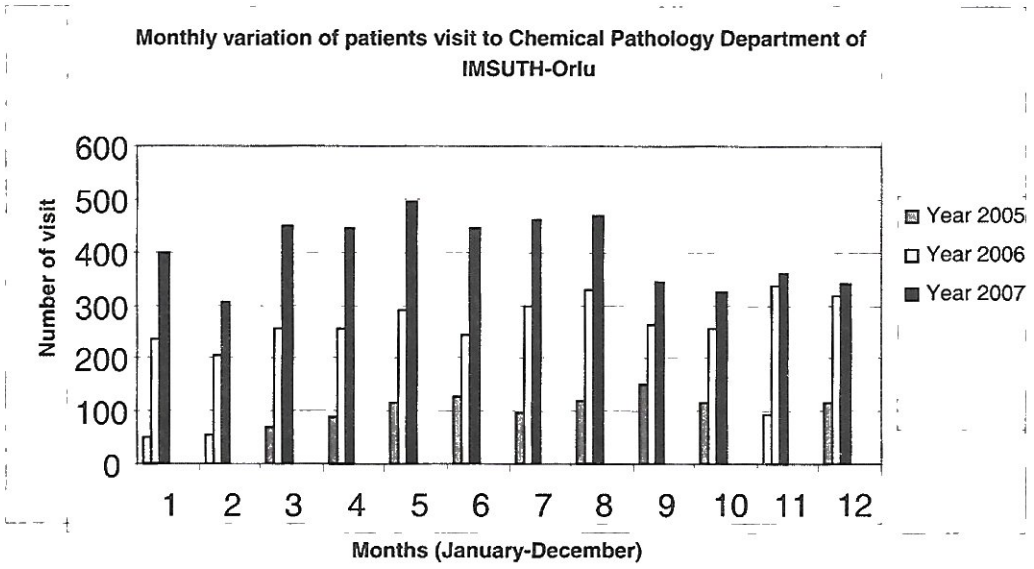
RESULT

Figure 1 shows the monthly variation of patients visit to Chemical Pathology Department of Imo State University Teaching hospital, Orlu. This showed a steadily increasing patients visit between the year 2005 and 2007. The total patients visit in 2005 was 1,194 with average monthly and daily visits of 100 and 3 respectively.

In 2006, the total patients visit was 3,289, with average monthly and daily visits of 274 and 9 respectively. The total visit increased to 4,842 in 2007 with subsequent increases in the average monthly and daily visits of 404 and 14 respectively. The pattern of visits shows peak patients visit between the months of March and August of each year. Figure 2 shows the monthly variation of work output (work load) in the Department. The work output varied proportionally with patients visit. Figure 3 shows the annual work output of the Chemical Pathology Department. Work output increased steadily from 4,869 per annum in 2005, to 8,617 per annum in 2006, and to 11,288 per annum in 2007. There was peak work output between March and August of each year studied. The results of this study indicate more patients visit during the rainy season. Also, there was increasing relevance of the hospital Chemical Pathology laboratory over the years studied, as evidenced by the increasing work output. Figure 4 shows the percentage distribution (frequency) of biochemical tests performed in Chemical Pathology laboratory of IMSUTH-Orlu.

The most frequently requested biochemical tests included; creatine (18.3%), urea (18.2%), glucose (15.2%), and electrolytes (14.8%). Others include urinalysis (7.9%), total protein (3.4%), albumin (3.1%), AST (2.6%), ALT (2.5%), lipids (2.5%), alkaline phosphatase (1.8%), calcium (1.6%), and PSA (1.2%). The hormone tests such as T^3, T^4, and TSH, luetinizing hormone, follicle stimulating hormone, prolactin, progesterone, testosterone, and estradiol were not commonly requested (0.4% each).The tumor markers alfa-feto protein and beta HCG were rarely requested (<0.1% each). The hormonal assays T^3, T^4, and TSH, luetinizing hormone, follicle stimulating hormone, prolactin, progesterone, testosterone, and estradiol, together with the tumor markers alfa-feto protein and beta HCG cumulatively make constituted about 3.2% of total tests done in the department. While as creatine, urea, glucose, and electrolyte were the most frequently requested tests. Electrolyte was requested as a profile comprising sodium, potassium, chloride, and bicarbonate. Similarly, lipid profile was requested as a profile comprising triglyceride, total cholesterol, high density cholesterol, low density cholesterol, and very low density cholesterol.

Figure 1



DISCUSSION

The result of this study shows more patients visit during the rainy season. The cause of this trend is not clearly identified in this study. However, it may be easily adduced that, rainy season poses a lot of health challenge to the people. This period encourages the breeding of many vectors of disease transmission such as mosquito, housefly, sun fly, sand fly[8-11] etc.

Figure 2

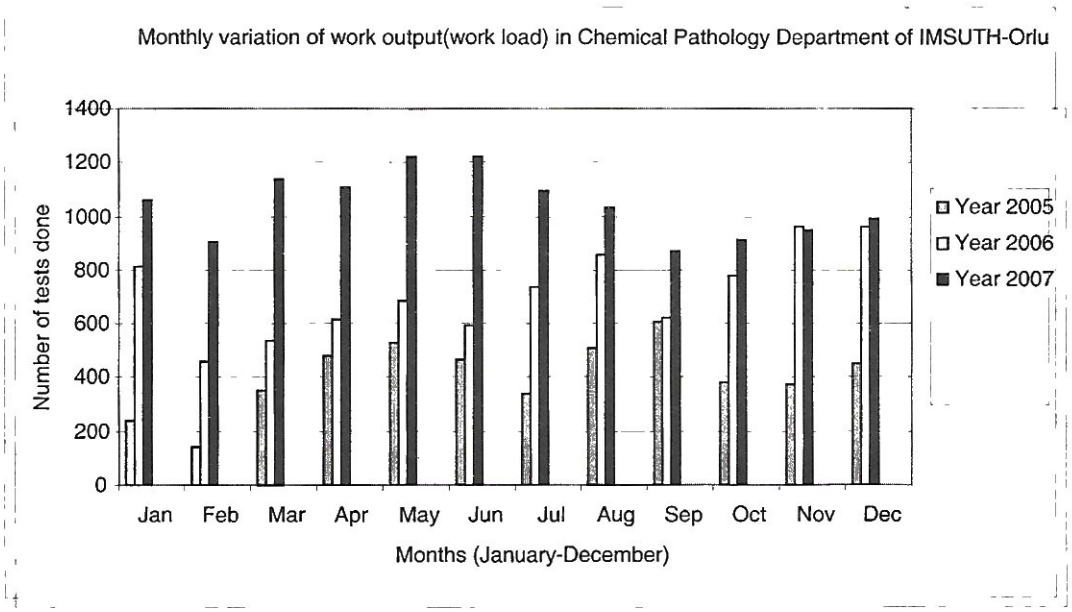


Figure 3

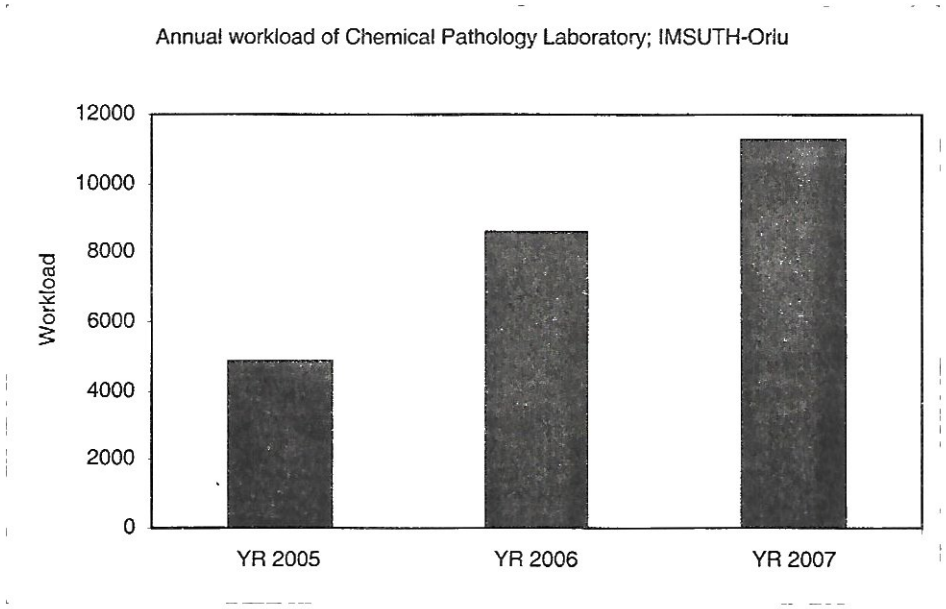
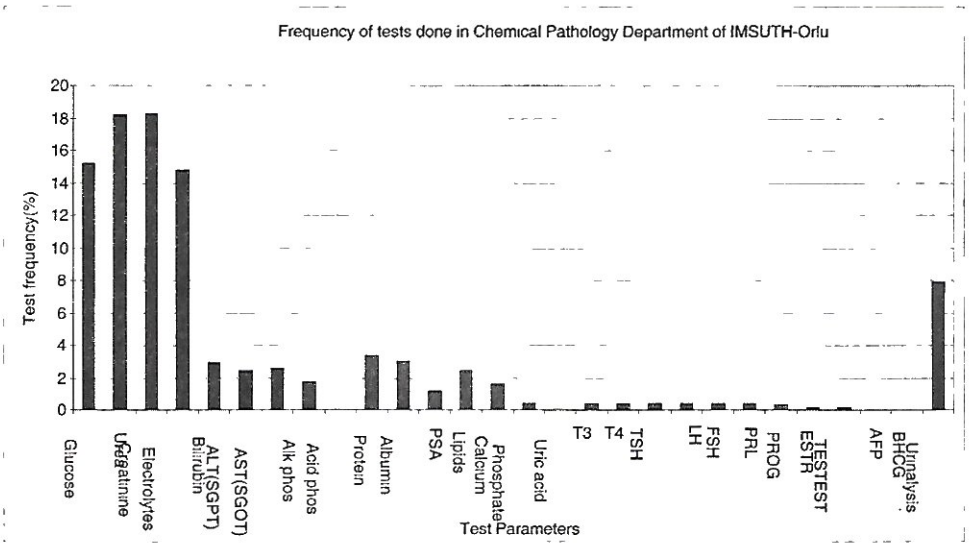


Figure 4



Also, the people may be easily overwhelmed by their high indulgence in farming activities which is at its peak during this period with most our patients drawn from this suburban community. This habit of overindulgence in farming activity with out commensurate attention to personal health needs predisposes the individual to infections, stress and stress related complications.

The work output of the laboratory observed a steady increase all through the period of this study. The hospital embarked on a number of awareness exercises that enhanced its services. Particularly, the free surgery exercise during which some medical experts are invited from United States of America to assist in various kinds of surgical procedures. This is usually done in April and December of every year since inception of this initiative. It attracts a lot of people from within and outside the state, who on their own are unable to bear the cost of their surgical needs. The outcome of the surgeries is marked with high success rate, thus building-up confidence among the people, and there by increasing the hospital's acceptability and patronage. Often times too, the hospital permitted

free medical services to the people-especially children and antenatal women. These efforts highlight the importance of awareness campaign, and elaborate information strategy in the enhancement of services of a growing hospital[12]. The hospital also became more attractive among the general practitioners and primary health care physicians around its vicinity with patronage from outreach referrals also increasing.

Within this period of growth, the hospital acquired chemistry auto-analyzers and employed more laboratory personnel. This approach enhanced service delivery. This observation supports the simultaneous expansion of facility and manpower with increasing work load.

This prevents putting excess pressure on existing facility; to forestall breakdown. Services were also enhanced. In recent years, some fundamental changes in clinical medicine, including new trends in laboratory investigation have become evident. There is increased dependence on laboratory services. Consequently, the physician is faced with array of laboratory tests, some of which are newly developed and may entail substantial expenditure. Where as a rising trend in the dependence on biochemical tests is the feature of most industrialized societies,[13-16, 17-19] the reverse is the case in Nigeria.[20] Poverty is a feature of tests underutilization. The hospital is located in a suburban area with majority of patients belonging to medium or low social economic class. It is observed that when the cost of laboratory test is high, the patient tend to decline from doing the test. The high profile tests such as the hormonal assays and the tumor markers are relatively rarely done in this center. They are the most expensive tests, and consequently the least conducted. Undoubtedly, tests that incur substantial expenses on patients discourage elaborate biochemical testing essential for proper diagnosis. On the other hand, incentives from government and non-governmental agencies by way of discounted charges, and outright provision of free facilities encourage easy assess to health facilities, with a resultant enhancement of health status of the people.

Blood urea and creatine determination are clinically very useful in the assessment of renal function.[21] Urea and creatine top the list of frequently requested tests. This may not be unconnected with high prevalence of renal complications in this environment. Further studies however will be needed to show the extent of this discovery. Glucose determination is often performed in the evaluation of disorder of carbohydrate metabolism. High prevalence of diabetes mellitus accounts for high demand for glucose determination[22-24]. The relative high demand for electrolytes determination underscores the manifold role of electrolytes in human physiology. Thus, an abnormal electrolyte level is a frequent consequence of a variety of disorders. [25-41]

Considering the high cluster of patients visit between March and August, it appears people in this area get sick more in the rainy season. There should be more studies to uncover the underlying factors. This study also suggests more emphasis to be given to the facility for urea, creatine, glucose, and electrolytes tests in the planning of a Chemical Pathology laboratory. Also provision should be made for expansion of workload in terms of commensurate enhancement of manpower and facilities for effective sustenance of medical services. The finding from this study may be of value in the establishment of referral hospital with chemical pathology facility in locations similar to the one reported here.

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Original Article

Iron Indices in School children in Ceres District of the Western Cape South Africa.

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ABSTRACT

Iron status of children from communities in South Africa has been a subject of debate and the lack of single biochemical indicator makes it more challenging, hence this study was designed to assess the iron status of children in a Ceres district of the Western Cape in South Africa. For this study, 265 children were randomly recruited and blood sample collected from the participating children was used for the analysis of the following parameters; soluble transferrin receptors (sTfR), haemoglobin (Hb), mass cell volume (MCV) and ferritin (Fert).

The result showed that the mean serum iron level of 13.45 umol/l, mean serum ferritin level of 1.85mg/l, mean serum transferring receptor of 30.11 ug/l were recorded in >75% of the participating children. The Haemoglobin level of less than 12.5 g/l and mass cell volume of 83.10 ft were observed in >50%, of participating children. There were no gender differences for any of the iron measure. The haemoglobin level showed marginal iron deficiency while only serum iron was significantly correlated with the heamoglobin level. The serum transferrin receptors, ferritin and MCV did not indicate possible iron deficiency. The results of other factors such as socioeconomic status and demographic data did not show any significant interference either. The implication of the finding is discussed.

Key Words: Blood, iron, children, Hb, STR, (MCV), (Fert).

Introduction

Iron deficiency is the most common nutritional deficiency in Africa, Asia and most developing world; affecting as much as 66–80% of the world's population (1, 2) and is the leading nutritional cause of anemia (3). It is easily noticed in anemia and those particularly at risk include: children, pregnant women, women with heavy menstruation and people with mal-absorption problems.

Although over the years, iron status of children has improved according to data published by (National Health and Nutrition Examination Survey 111 or NHANES 111) and (NHANES 11) (4), despite these improvement, iron deficiency is still common in most countries.

Infants and young children are especially vulnerable to iron deficiency because of their rapid growth and increased physiologic demands for iron (3) in other to fulfill major body functions. Also, diets high in cereals and low in meat and fish products may cause iron deficiency due to poor dietary iron bioavailability. Studies also show that ascorbic acid increases iron absorption (5). Of particular significance is the fact that iron deficiency in children can adversely affect cognitive and psychomotor development during vulnerable periods such as the toddler years (6). Iron deficiency during childhood has multiple consequences like neurochemistry disorder; alteration of dopamine receptors (7) and decreased monoamine oxidize activities (8).

Dietary intake requirement, inflammation and most biochemical indicators are age-related and constitute a major factor influencing iron concentration in children (9). Iron deficiencies in children are generally estimated from studies

using healthy adults, but they differ in many ways from that of adults. In children, the most likely cause is an inadequate amount of iron in the diet, coupled with the extra requirement for iron because of growth, also children present particular problem as they are highly susceptible to diseases and require more nutrients than adults (9). Other features are non-hematological but are clinically important and are clearly defined in children than in adults. Presently, there is no single biochemical indicator available to reliably access iron inadequacy in children (10).

This study focused on determination of such parameters as serum ferritin, serum transferrin receptor, MCV, and Haemoglobin values as possible indices for assessment of iron status in the Western Cape.

Subjects

This paper presents the biochemical indicators of iron in school children in Ceres district. A total number of 265 grade one learners aged between 7-9 years in six primary schools in Ceres district of the Western Cape, South Africa, were involved in this survey. These schools serve communities that duly represent the diverse ethnic groupings as well as socio-economic groupings within the bigger South African community. Informed consent was received.

Exclusion and inclusive criteria

Children with CRP concentration greater than 10mg/l were excluded while those with CRP concentration less than 10mg/l were included. The CRP concentration was used to include participants as a sign of apparent healthy state and to exclude participants as a sign of inflammation and ill health. The 265 pupils met the criteria of ≥ abnormal iron measure.

Data collection

Sample and data collection took place during school hours at each school over a period of one week in 2003 and 2004 respectively. Samples were prepared for analyses within one week of sample collections according to the following methodology:

Blood analysis: 5mL of whole venous blood was collected in a zinc free heparinised tube[12]. This process was carried out by a trained community staff nurse. Through the process care was taken to avoid any health risk situation that will endanger the subject or the nurse through blood contamination, infections or psychological situation. All blood samples collected were adequately marked and labelled. The blood samples were then stored in coolers under ice bags at 0 to - 4 °C, for usually < 24hrs[13].

In the laboratory the whole sample was then centrifuged to separate the serum. The serum was then stored at –15°C for all analyses. Prior to each analysis the serum was then thawed once only.[14]. Iron reagent was used to measure the iron concentration by a timed-endpoint method. In the reaction, iron is released from transferrin by acetic acid and is reduced to the ferrous state by hydroxylamine and thioglycolate. The ferrous ion is immediately complexed with the FerroZine Iron Reagent. The SYNCHRON LX ^{R} i725 (15) system automatically proportions the appropriate sample and reagent volumes into a cuvette. The ratio used is one part sample to 8 parts reagent. The system monitors the change in absorbance at 560 nm. The change in absorbance is directly proportional to the concentration of iron in the sample and is used by the SYNCHRON LX (15, 16) system to calculate and express the iron concentration.

Serum ferritin measurement was based on radio-imunometric assay (Pathcare laboratory Ltd. Cape Town). Combined measurement of ferritin, soluble transferrin receptor, and C-reactive protein was performed by a sandwich enzyme-linked immunosorbent assay technique. The external standard for ferritin consisted of the IBS standard diluted to 19.2 µg/l ferritin. The coefficient of variation for within assay-variation was < 4.5% for all ELISA assays, with a between-batch coefficient of variation of 6.2–7.4%.

All infants had data for Hb (hemoglobin), MCV (mean cell volume). Values for transferrin

saturation, ferritin, TfR, and body iron were available for all infants. A total of 236 infants had complete data for the measures used to determine iron status in NHANES II or III (i.e., Hb, MCV, transferrin saturation, and ferritin) (13, 16). Missing data were due to technical problems, such as trouble obtaining sufficient blood, samples with CRPC conc. >10mg/L and ethical exceptions.
For CRP, within-batch variation was <3% and between batches, it was <5%.

Precautions: Blood specimen storage and stability
Tubes of blood were kept closed at all times in a vertical, stopper-up position. Serum was physically separated from contact with cells as soon as possible. A maximum limit of two hours from the time of collection is needed for the assay to expire if not used (17). Separated serum should not remain at 15-30°C longer than 8hrs. Serum assays should be completed within 8hrs and the separated samples should be stored at 2-8°C. If the assays are not completed within 24hrs, serum samples should be re-centrifuged and separated from precipitates before testing. Frozen samples should be thawed only once. Analysis deterioration may occur in samples that are repeatedly refrozen and thawed (17).

Ethical considerations
The Senate Research committee of the University of the Western Cape provided ethical approval for this study (SHD of 2004/6). The participation of learners was voluntary following informed consent by parents or guardians. The participants were free to terminate participation at their convenience. Confidentiality of the data collected and subsequent findings were assured by using only code numbers for each participant.

Statistical analysis
Statistical analyses were performed using SAS Version 8.12 (18). The theory of Oliver Jean Dun (1971) was used in comparing of test of quality of dependent correlation coefficient (Pearson Correlation Coefficients) between blood indexes, food, vegetables and water samples. The health of learners plays a major role in determining the response of learners to elements utilization, therefore, paired student t-tests were used to relate variability to physical factors as well as objective observational conditions.

Results:
The male: female ratio in this study is 1:1. The mean age was 7.73 ±0.60yrs. The average weight and height of the children are 21.93 ±4.8kg and 118.69 ±7.2cm, respectively. The median household income contributors were 2 persons and that of income was R250–R999 per month (Table 1).

The mean value of the serum iron (umol/l) of the participating children was within the range of the standard reference but 20%, 75% and 5% of the children presented with serum iron value below, within and above the standard reference range. This showed no significant drop in serum iron P>0.05. See table 2.

Similarly, the mean soluble transferrin receptor (mg/l) and mean serum ferritin of the participating children were within the range of the standard reference. However, 24%, 75% and 1% of the children presented with values below, within and above the standard reference range in each case. This value did not show any significant drop in soluble transferrin value P>0.05). See table 2.

The mean haemoglobin (g/dl) was within the range of the standard reference but 5%, 95%, and none of the children presented with values below, within and above the standard reference range. There was no significant drop in haemoglobin P>0.05. See table 2.

The mean mass cell volume (ft) of the participating children was also within the range of the standard reference, however, 15% and 85% of the children presented with values below and within the standard reference range. There was no significant drop in MCV P>0.05. See table 2.

In comparing each biochemical indicator against serum iron using SAS (Pearson correlation

coefficient), there was a significant (P<0.01) correlation between serum iron and hemoglobin, as shown in Graph 1 with few out-layers. Removing all out-layers (Graph 2) a strong correlation could be seen, indicating marginal serum iron decrease in children investigated and the lack of consistence of serum iron decrease was also observed.

The ferritin model utilized in NHANES II (transferrin saturation, and ferritin) resulted in a considerably lower estimate of 25% iron deficiency for children aged 7 to 9yrs (11). Corresponding estimates of iron deficiency with anemia were high, respectively, using Hb < 13 g/dl, the cutoff in NHANES II and 111 (4). The approach recommended by a world health Organization (WHO) Expert Committee at ≤14yrs of age (NHANES 11) and the Sweden/Honduras study (NHANES 111), with more stringent cutoffs given for five iron status measures and Hb, yielded prevalence estimates of 5% for iron deficiency and >50% for iron deficiency anemia (graph 2). There are yet no established norms for TfR in infants, but the Sweden/Honduras study (NHANES 111) considered "abnormal" TfR > 11 mg/l. Only one infant in our study had a value above this cutoff. MCV showed a corresponding level of low iron deficiency. Graph 3 shows a detail description of iron source and potential iron deficiency and anemia complications.

Discussion:

The results have clearly showed a non prevalent of iron deficiency situation, with two or more biochemical indicators (indices) not showing abnormal levels of iron in serum as noticed in whole serum iron. This finding is in agreement with the theory that at least two biochemical indicators must be abnormal to ascertain iron deficiency[10]. Also that iron deficiency is the most common nutritional deficiency in the world, affecting as much as 66–80% of the world's population[19] and is the leading nutritional cause of anemia in the developing world[3]. Anemia been the third stage of iron deficiency may occur only when the total iron hemoglobin level is reduced below normal which is ≤13 for infants [10, 20].

It is contrary to most findings that showed high level of iron deficiency among disadvantage

Table 1: Socio-economic characteristics of participating children and family

		Age of participants (years)	Weight (kg)	Height (cm)	Family members contributing to household income	Family average wage
Phase One		Average in yrs	(Kg)	(Cm)	Income *	
1	November 2003	7.60	20.46	118.71	4.82	2.19
2	September 2004	7.84	22.48	118.62	4.12	1.84
1 & 2	2003/04	7.73 **SD** 0.60	21.93 **SD** 4.82	118.69 **SD** 7.23	4.73 **SD** 1.28	2.73 **SD** 1.24

Table 2: Blood and serum biochemical makers for iron

Parameters	Mean ± Sd	**Standard reference range for category of participants	Number of participants with values within, above and below the standard reference range			SEM
			Below (%)	Within (%)	Above (%)	
Serum iron (umol/l) (n=236)	13.45 ± 5.19	9.5 – 21.3	20	75	5	0.34
sTfR (mg/l) (n=235)	1.83 ± 0.67	0.8 – 2.3	24	75	1	0.04
Ferritin (ug/l) (n=235)	30.11 ± 15.52	20 - 100	24	75	1	1.01
HB (g/dl) (n=232)	12.53 ± 1.10	11.5 – 15 5	5	95	0	0.07
MCV (ft) (n=232)	83.10 ± 4.42	77 - 95	15	85	0	0.29

sTfR = Soluble transferrin receptor, Hb = Hemoglobin, MCV = Mass cell volume, **Ref Std (12)

children, although other factors might have been responsible but the appropriate cutoffs for iron deficiency in infant and children will remain controversial. Individual iron index levels showed a non-perfect distribution in comparison with the laboratory references, except in MCV and Hb where a strong relationship can be observed and statistical significance showed a strong correlation between samples mean and standard means, as observed in serum iron and hemoglobin, sTfR, and MCV.

One of the factors that is regularly overlooked when assessing iron status of children is the concentration of several nutrients such as zinc, vitamin A and iodine. Dietary intake requirement, inflammation and most biochemical indicators are age-related[6]. Although there were higher levels of sTfR and very low Ferritin and MCV observed in one of the samples, the results did not show exact relationship between both, but need further investigation especially when there are very high levels of sTfR which might result in lowering of both Ferritin and MCV. However, the proportion meeting criteria for iron deficiency was considered low (≤5%) and regular iron absorption and storage is not fully developed in infants therefore making it difficult to interpret measures connected to iron storage such as ferritin and sTfR and standards used for children results will tend to lack consistency.

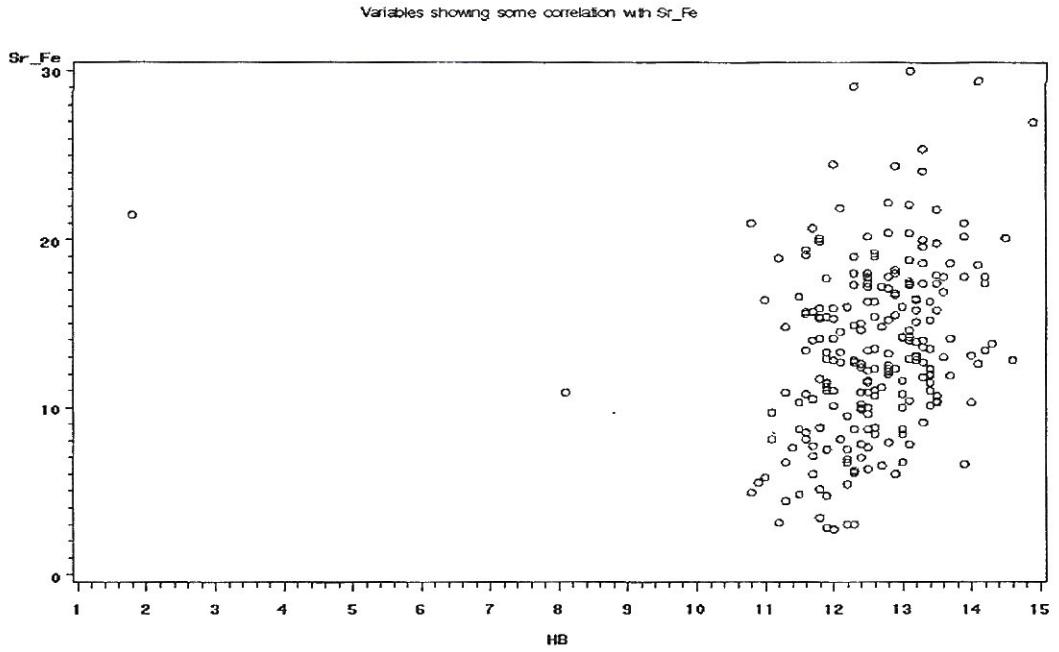
Other results showed no differences between male and female children on any individual iron measure or the proportion meeting criteria for iron deficiency, this result is a contrast to gender difference observed in some other studies [21,22,23]. Although the explanation for the differing finding is not readily available, iron food fortification might have played an important part.

Conclusions

Caution should be taken in drawing conclusion on the nutritional status (iron) of children, as

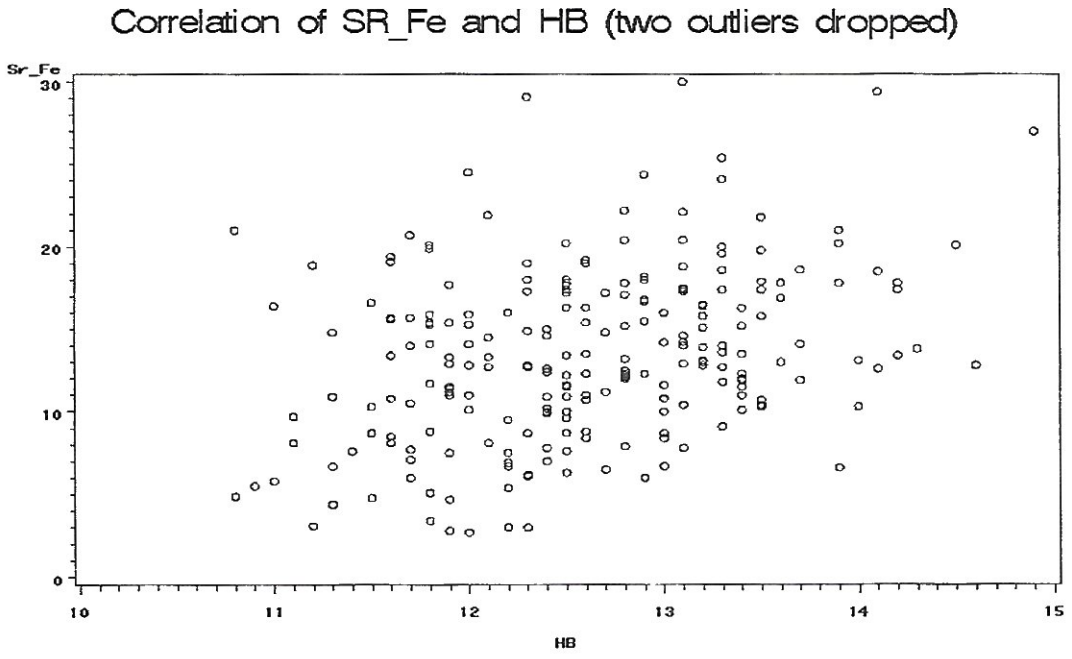
many factors can easily influence the low biochemical iron indicators. Children's immune systems are still developing and there are higher frequencies of sickness than in adults. Sub-clinical inflammation in apparently-healthy children, ethnic life style, and state of other nutrients can easily lead to misinterpretation of iron status and overestimation of those with deficiency.

Graph: 1



(11.5 ----------15.5g/dl)

Graph: 2



(11.5 ----------15.5g/dl)

These results had shown marginal iron deficiency in the children population and might have been influenced by non active infection (inflammation and worms). Hence, the need for more investigation on their involvements prior to treatment is very important as most direct intervention has not been effective. Iron deficiency can easily be corrected with supplementation and food rich in iron before it gets to a clinical deficiency state.

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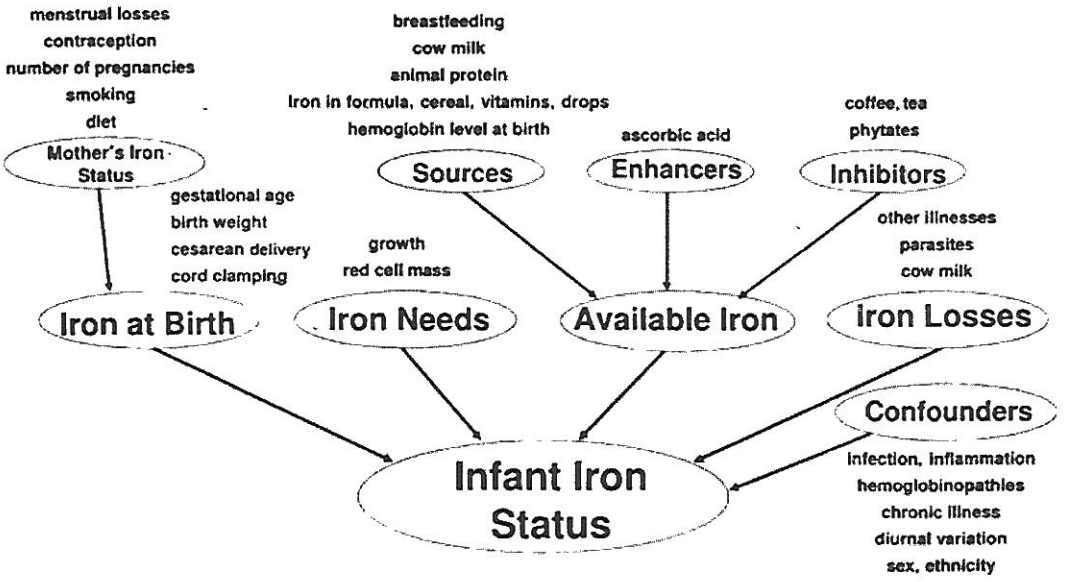
Graph 3.

PREDICTING IRON DEFICIENCY IN INFANTS



Estimated iron sources and deficiencies in infants (4)

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Original Article

Trace Element Indices in Hair and Saliva of School Children

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ABSTRACT

Evaluation of essential trace elements in hair and saliva is gradually being accepted as a useful tool in both scientific research and the diagnoses of disease. The normal concentration of these trace elements in hair and saliva; iron, zinc, copper, manganese were determined in 265 healthy children (7-9y) and their relationship to the health and the learning ability of the children were also investigation. The concentration of these trace elements in hair and saliva were in (Hair: Fe 28.47±0.70mg/kg; Zn 172.08±4.49mg/kg; Cu 21.03±0.79kg; Mn 1.30±0.05kg and Saliva: Fe 1.06±0.03mg; Zn 0.64±0.39mg; Cu 0.19±0.09mg; Mn 0.11±0.00 nmol/L). There was a significant high levels of trace element in hair but reduced concentration in saliva samples when compared with the reference means ($P<0.05$ in each case). There is a strong correlation between Zn concentration in saliva and Zn concentration in hair ($P<0.05$). Also there is a significant relatively low saliva Mn levels compared with the proportion that meets the thresholds (0.11±0.003nmol/l). In comparing samples with same parameters there was no significant correlation in most of the parameters. Anthropometric data and socioeconomic status had no effect on the levels of these elements in hair and saliva. The results indicate the possibility of assessing the presence of these elements using non-invasive methods in the absence of contamination and substantiate the potential of hair and saliva as a biomarker but could not ascertain the exact tolerable levels of the elements.

Key Words: Trace Elements, Children, Hair, Saliva and Ceres.

INTRODUCTION

Hair and saliva has become one of the most valuable and effective tools in analyzing trace elements status in human[1]. It can help to facilitate the identification of any abnormities be it toxic or deficiency, thereby helping in identifying the possible immune status of children. Information on the levels of trace elements in biological specimens in children is rear, especially in sub Sahara Africa[2]. At present, the use of trace elements concentrations in plasma or serum is widely accepted as norm but same can not be said of other tissue indices; hair, saliva, urine and nails.[3,4,5] The use of multi-element analysis to assess an individual's nutritional health or predisposition to disease has been controversial, but more studies which correlate concentrations of essential elements in organs like hair to deficiencies in tissue and body levels, has made it more acceptable due to more evidence is now available which document the presence of toxic elements such as Arsenic, Cadmium, Lead and Mercury in hair to excess exposure, and serves as a useful diagnostic index of toxic elements.[6,7,8] Experts now agree that diagnosis and prevention of disease using saliva assay is possible due to steady progress made over years. Prompting more institutions, laboratories and medical practitioners to be involved in various researches using saliva, thereby making it more acceptable and due to the convenience and easy of collection, no pains involve during collection like the use of blood[9]. This technique (noninvasive) stands out as an important tool in assisting the determination of trace elements status, especially in rural communities that might not afford the invasive method or as a prerequisite for the use invasive method. However, research has shown that the human body cannot synthesize trace elements as

in case of copper, where the human diet must supply regular amounts for absorption[10]. Excess copper lowers zinc levels and produces hair loss, insomnia, depression and schizophrenia, while low copper levels are associated with Menke's and Wilson's disease[11]. Copper deficiency, in turn, is known to provoke iron deficiency and anemia. Zinc, a nutritionally essential element, may influence both the absorption and the toxicity of lead[11]. The interactions between environmental lead and environmental zinc levels and blood lead concentrations suggest that zinc may influence the association between soil and dust lead and corresponding blood lead levels.[12]

Hair copper usually reflects tissue levels except in copper transport diseases such as Menke's and Wilson's disease, which may present with low copper concentrations and is mostly associated with water leached from copper pipes, and from certain dyes and hair bleaching agents[11]. Zinc is an enzyme and is essential for most metabolic pathways, and should be available in reasonable amount likewise iron and manganese as they are essential for metabolic activities and physiological functioning of the human body[13]. Hence, the knowledge of trace element levels is very essential in the growth and development of children. It has been scientifically proven that hair trace mineral analysis is useful for the evaluation of person's general state of nutrient and health, and has been confirmed in most studies, including these study also it is valuable in detecting predisposition to disease and will assist the Doctor in determining if a patient is suffering from mineral deficiency or mineral in-balance or heavy metal pollutants in the body which may be responsible for a particular illness[14] and to establish an understanding between essential trace elements in hair and the same elements in organs[15]. Trace elements deficiency may not affect learning ability directly but may affect children through its impact on resistance to infection and in turn affect their school attendance and subsequent poor learning ability[16], as indicated in this study, it will best address the course and discrepancies associated learning difficulties.

METHODOLOGY

Study design

This pilot study aimed at assessing the levels of iron, zinc, copper and manganese in hair and saliva (noninvasive). This technique is widely used for the determination of trace elements in matrices, especially biological materials, and making them useful for analyzing iron, zinc, copper and manganese in grade one learners in primary schools in Ceres, Western Cape, South Africa. Prior to the data collection parents of the children under investigation were asked through questioners on whether special shampoos, relaxers or cream were used on their hairs, and also if they had any direct contact with objects that might increase their venerability to these elements contamination. Where the answer is yes, the samples collected were discarded and not included in the analysis.

Study population and study sample

The study population consisted of all grade one learners attending the six primary schools in Ceres in 2003 and 2004. The study sample was selected randomly from each school, using random number tables, with proportionate representation of each school. A total number of 265 learners were included in this study (Table 1) following informed consent, 62% of those asked to participate, signed consent forms.

Data collection

Sample and data collection took place during school hours over a period of one week towards the end of each year. Samples were prepared for analyses within one week of sample collection.

Determination of iron, zinc, copper and manganese in hair and saliva samples

The determination of trace elements (iron, zinc, copper and manganese) in hair and saliva, using the conventional *aqua regia* digestion procedure, consist of dissolving of samples in a 3:1 mixture of HCl and HNO_3 and digested in a hotplate for about 3hrs[17]. A photometric method was used in analyzing the digested samples using atomic absorption spectrophotometer (AAS) (Unicam AAS Type solar)[18,19].

Spectrophotometry: About 5mL of digested and made up samples was analyzed using the AAS machine at a wavelength most suitable to the particular element been analyzed with minimum or no interference. Precaution was taken throughout the experiments to avoid contamination of the samples, reagents and chemicals used. The samples were weighed accurately. Extra care was taken to avoid errors in reading coursed by acid interference, common with *aqua regia* method. In order to obtain reproductive results, it was important that we maintain constantly optimal aspiration and furnace condition. All machine readings were repeated twice.

HAIR: Approximately 0.5g of hair, obtained mostly from the back of the head close to the neck, was obtained from each learner using a sterilized stainless steel scissors. The scissors were cleaned with surgical spirits after each hair collection.

Hair samples were then washed with non-ionic detergent and rinsed with distilled water, oven dried for four (4) days at 60-70°C and stored in an airtight plastic bag. A well-mixed 0.25g of dried hair was weighed into a beaker and digested in 12mL of *aqua regia* (1/3 HNO_3 and 3/4HCl) acid, heated in a Gerhardt (Trace metal digestion units, DIN 38414) heated digestion block. The maximum digestion temperature was 120°C and to avoid loss of materials each beaker had a glass lid. Digestion continued until a clear and almost colorless solution was obtained. Each sample took 2-3 hrs to digest. The clear solution obtained was allowed to cool, filtered with Whatman no. 42 paper and diluted to a final volume of 100mL with di-ionized distilled (DDW) water[20]. This solution was stored in a plastic container until analysis using AAS was performed.

SALIVA: At least 5.0mL of mixed saliva was collected from the learners into a detergent washed polypropylene vial by direct spouting collection. The samples were checked for food and blood or nasal discharge contamination and contaminated samples were discarded. Four samples that did not have matching hair samples were discarded. The mixed saliva was then frozen and stored in a freezer at 0°C to 4°C.

Prior to the sample preparation, the saliva samples were defrosted and allowed to equilibrate to room temperature before being rechecked for any trace of contaminants. Five mL of saliva was then measured into a beaker and 20mL of 2% nitric acid (HNO_3). This solution was then filtered with Whatman no. 42 filter paper into a measuring cylinder, diluted to a final volume of 100mL with DDW[20]. The 100mL solution was then stored in a plastic container until analysis with AAS for zinc, iron, copper and manganese was done.

Ethical considerations

The Senate Research committee of the University of the Western Cape provided ethical approval for this study (SHD of 2004/6). The participation of learners was voluntarily following informed consent by parents or guardians. The participants were free to terminate participation at their convenience. Confidentiality of the data collected and subsequent findings were assured by using only code numbers for each participant.

Statistical analysis:

The data were analyzed using SAS version 8.12[21]. The results are presented as mean, standard deviation, in all samples, correlation coefficient (Pearson Correlation Coefficients) between zinc, copper in hair and saliva, also manganese in hair and saliva. The P-values <0.01 was considered statistically significant.

Results:

Table 1 shows the selection process, sixty-two percents of study samples consented to participate in the experiment. Representing 265 of the total 426 grade one learner over the study period (Table 1) with a male: female ratio 1:1, at an average age of learner was 7.73±0.60yrs. The average weight and height of the learners were 21.93 ±4.8kg and 118.69 ±7.2cm, respectively. The median household income contributor were 2 persons and that of income was R250-R99 per month (Table 2).

Table 3 shows the total number of samples collected from each participant, the average levels of zinc, iron, copper and manganese the hair and saliva samples and their reference values. The average concentration of all elements in hair include; Hr_Zn 172.08mg/kg with a standard reference of 150-250mg/kg and 95% of samples below the reference, Hr_Fe is 8.47mg/kg with the reference 6-15mg/kg and 86% of samples are within the reference, Hr_Cu 21.03mg/kg the reference is 15 – 35mk/kg and 60% within the reference and Hr_Mn 1.30mg/kg is high when compared with reference (0.2 - 0.8mg/kg) and 95% above the reference values. The saliva showed a contrary results to that of hair with Sl_Fe 1.06mg/l and the reference 1.52 - 5.72mg/l, 94% of values are below the reference, similar results can be found with Sl_Cu 0.20mg/l and the reference 1.26 – 2.99mg/l likewise Sl_Zn 0.64mg/l and reference 0.5 – 1.20mg/l with 52% of samples below the reference.

When compared with the standard value of ≤1mg/kg and contrary result was observed in saliva with most of the samples concentration are within reference value in both iron and copper (Sl_Fe 1.06±0.03mg/l and Sl_Cu 0.19±0.09mg/l) with the standard been Fe 1.52 – 5.72mg/l and

Table 1: Participants Data One: Number of participant and their percentages over the two Phases.

S/N	School Code	No. Of pupils in the school	Number of participants	Number of participants	Percentage of participant	Ration of Girls/Boys
Phase one		Learners	Selected	Consent	Response rate%	(M/f)
1	November. 2003	544	200	120	60.0	43/57
2	September 2004	688	226	150	64.5	54/46

Table 2: Demographic and Socio-economic data of learners

		Age of participants	Weight	Height	Family members contributing to household income	Family average wage
Phase One		in yrs	(Kg)	(Cm)	Ave numbers of persons *	Income per month **
1	November 2003	7.60	20.46	118.71	(2 - 3)	(R250 – R3000) Ave income (R800)
2	September 2004	7.84	22.48	118.62	(2 -3)	(R250 – R3000) Ave income (R800)
	Total	7.73 +/- 0.60	21.93 +/- 4.82	118.69 +/- 7.23	(2 – 3)	(R250 – R999) Ave income (R800)

No. of people contributing to household income, **Average family wages per month in Rands is (R 250 – R3000) per month

Cu 1.26 – 2.99mg/l but the concentration in Sl_Zn 0.64±0.39mg/l was low when compared with standard at 0.5 -1.20mg/l. Sl_Mn was likewise low 0.11nmol/l when compared with the reference <40 nmol/L and showing a P value of 1 in both 95% upper and 75% lower confidence levels when compared with proportion meeting thresholds.

Comparing means against standards, there is a significant difference (P<0.01) between hair manganese and saliva manganese, also hair copper and saliva copper. Pearson correlation Coefficient showed a strong correlation between Sl_Mn and Hr_Mn (P<0.01), as shown in fig (1).

In other results levels of zinc, iron, copper and manganese were found in samples; water, cultivated vegetable and most commonly eaten foods, although these levels were high but within the range of reference values. Other factors like weight and height showed no significant difference within anthropometric data, although there was a significant difference in the socio-economic status of children but these did not influence the levels of these elements; zinc, iron, copper and manganese in both hair and saliva of learners investigated.

Discussion

This study has highlighted the possibility of using noninvasive method in investigating the presence of different elements in humans and can be compared with other researchers[1,22]. Hair provides more accurate and relatively permanent information on levels of exposure and the implication of the lack or excess of one trace element being able to interfere with the metabolic utilization of another element present as observed with excess copper lowering zinc levels and produces hair loss, insomnia, depression and schizophrenia and this can be found in different correlation factors found with and between parameters[11]. Same can be observed in saliva where more laboratories and clinic are relying on saliva for diagnosing and treatment of different diseases and due to the relative easy with which samples can be collected and analyzed, and confirm the relationship between salivary trace element concentrations and dietary intake as reported in literatures[9,23].

The cost-effectiveness of this method when compared to blood analysis in disadvantage communities has sparked our interest in the potential valuation of zinc, iron, copper and manganese in hair and saliva as an assessment index for trace elements[24].

Table 3. Iron, zinc, copper, manganese in hair and saliva samples.

		Levels of trace elements hair and saliva samples and references					
Trace element	**No. of Children**	**Trace Element index Conc.**	**Ref. standard Scale**	**% within standard range, below and above**			**Standard Deviation**
*	**N**		**	**Within (%)**	**Below (%)**	**Above (%)**	
Hr_Fe in mg/kg	193	8.47	6 - 15	86	10	4	0.99
Hr_Zn in mg/kg	193	172.08	150 - 250	3	95	2	0.44
Hr_Cu in mg/kg	193	12.7	15 - 35	60	20	20	1.31
Hr_Mn in mg/kg	193	0.67	0.2 - 0.8	3	2	95	0.44

Sl_Fe in mg/L	247	1.06	1.52 - 5.72	5	94	1	0.49
Sl_Zn in mg/L	249	0.64	0.5 – 1.20	45	52	3	0.39
Sl_Cu in mg/L	249	0.20	1.26 – 2.99	10	85	5	0.14
Sl_Mn in nmol/L	249	0.11	<40	100	0	0	0.05

*Hr_ Pb = Hair lead, Hr_Cd = Hair cadmium, Sl_Pb = Saliva lead, Sl_Cd = Saliva cadmium, (**)Ref range. Assaf and chung 1984, N = number of children involved in each analysis.

The result has show a strong correlation with manganese in both hair and saliva, with high percentages within reference which can indicates a disease free condition (manganism) resulting from lack or chronic manganese exposure, as characterized by different symptoms,[25] possibility of adverse effects of Mn accumulated *in utero* on 3-year-old children's psychomotor performances[26] which might be observe in later years.

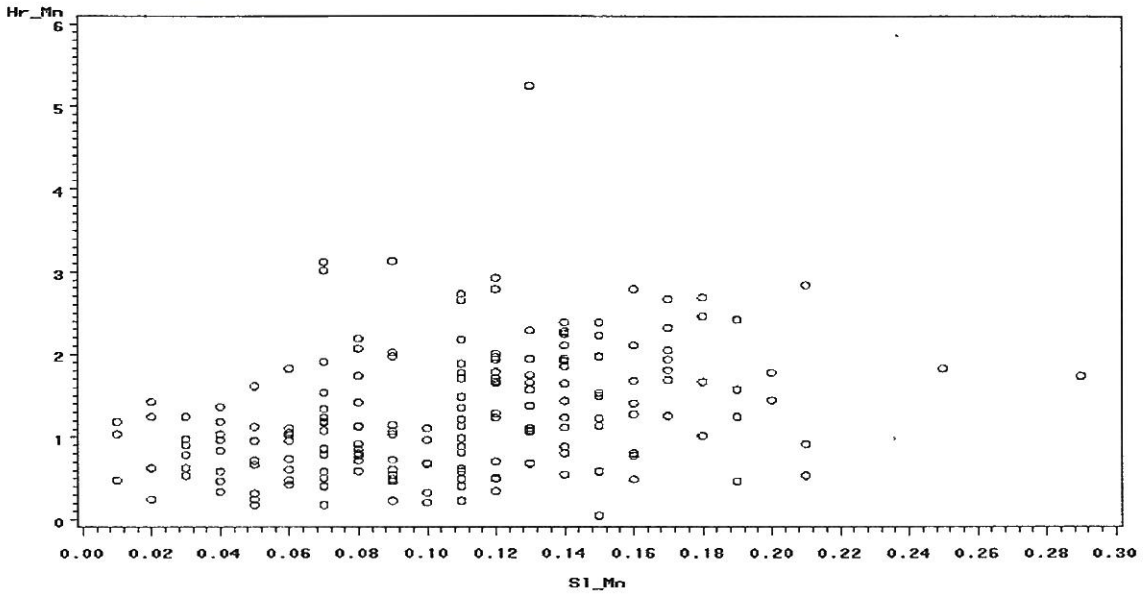

Figure 1: Graph of dispersion for hair Mn and saliva Mn showing strong correlation.

The addition of large amounts of zinc to a diet interferes with intestinal copper absorption that results in copper deficiency in spite of adequate copper intake. Hence, zinc is said to antagonize copper absorption. This is in compliance with the levels of these elements found in both food and cultivated vegetables which might have balanced the utilization in children samples. The potential Iron deficiency during childhood observed in developing countries although not observed in this survey has multiple consequences like neurochemistry disorder; alteration of dopamine receptors[27] and decreased monoamine oxidize activities[28]. Studies have linked iron deficiency with lead toxicity, and have provided evidence that these two conditions are related. [29]

This result provides a very significant study as the both indicators showed high level of iron and lead, complementing the growing evidence that high iron intake and iron-replete status may reduce lead absorption in children,[30] thus, the prevention of iron deficiency may represent a potential public health intervention for reducing lead exposure in humans,[31] and this can be

observed in levels of lead found in hair samples of children investigated.[32]
Studies like these will enable researchers pre-empt the possible outcome of subsequent findings but caution most be taken as concentration of an element in parameter might not indicate the same with others (saliva, blood and urine) and they might exhibit different concentrations even though the samples are collected at the same time.[32] The lack of standard procedure for sample collection, washing, treatment and analytical quality control protocol arouses considerable resistance towards the reliability of noninvasive techniques.[33]

The role of external factors responsible for varied levels of these elements found in children cannot be underestimated. Hence, the influence of the dietary intake is very significant (e.g. commonly eaten food, locally cultivated vegetables). The influence of other factors like weight, height and age showed no significant effect likewise the socioeconomic status did not play any significant role and can be attributed to the lifestyle of the people.

CONCLUSION

All over the world, trace element deficient has been a major issue of problem and critical evaluation of these elements will assist in diagnosing most food related illness, but not finding easier and less expensive technique will hinders this process, especially in rural communities were poverty is high. The use of noninvasive parameters as indices of trace element evaluation as shown in this study will ameliorates this problem. Although, this methodology needs further investigation and conclusions drawn on best approach, precautions, standards and references will enable it becomes workable process.

The reliability of trace elements in hair and saliva as indices of trace element status could be markedly enhanced when combined with other related indices measured simultaneously. Hence, the use of blood and other parameters should be encouraged. The interaction of trace elements in the body may have a dramatic impact on the utilization of other nutrients. Be it essential/non essential or micro/macro nutrients and help define the outcome of dietary improvement mechanism now adopted by various governments (dietary supplementation and food fortification programmes).

However, careful observation and diagnostic examination of cases with abnormal results will establish the presence or evidence of some form of diseases such as inflammation, infection and malignancy undermining the health status of the children, and this can be triggered by low nutrient intake resulting from poverty or poor nutrient intake. Trace element can be best evaluated through a simultaneous comparison of a variety of biochemical and physiological parameters of trace elements, Anorexia, short stature, and low level of hair nutrient.

Limitations

Despite the limitations of this study, such as self-reported subjective estimations of exposure, our observations suggest that environmental anthropogenic sources, especially environmental contamination, eating habits, acid interference and human error may contribute to increase in levels of these trace elements, it remains potentially sensitive fraction of the general population. More research is needed to objectively identify these sources and their associations with increased in some elements found in some samples and to assess the effect of their intake and subsequent child development taking into account the role of trace elements in metabolic activities in human body.

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