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A multidisciplinary journal for publication of medical and biomedical research findings on issues pertinent to improving family health and related issues of public health

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A multidisciplinary journal for publication of medical and biomedical research findings on issues pertinent to improving family health and related issues of public health

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# SECTION 1: INVITED PAPERS

## STEM CELLS AND REGENERATIVE MEDICINE

**Clement E. ANYIWO**
(MD, M. Sc. Zeugnis immun, FMCPath, FWACP, FICS)

School of Health Sciences, North America Higher Education Centre for postgraduate studies, San Antonio, Texas, USA
(Email: clemany@gmail.com)

**ABSTRACT:**

Stem cells derived from the embryo (embryonic stem cells), bone marrow or umbilical cord blood (adult stem cells) has the potential to differentiate into many cell types in the human body. The embryonic stem cell are called "pluripotent" because they can become any of the body's 220 cell types including bone, muscle, skin, nervous tissue as well as immune cells. Those derived from bone marrow and cord blood are considered "multipotent" because they can differentiate only to specific families of cell types such as those of muscle and bone and also replenish mature cells within tissue. Despite the potential that they have in repairing or regenerating human tissue damaged by injury or disease, embryonic stem cells have been under a lot of criticisms due to ethical, religious as well as political considerations. The development of induced pluripotent stem cells (iPSCs) from adult stem cells, derived from mouse and man by cellular reprogramming seems to have produced embryonic-like stem cells without destroying the embryo-which critics say it is a deliberate attempt to take away human life because it contains human DNA. As promising as they seem the iPSCs would still have to pass the test of skepticism by certain stem cell scientists and screening tests of functionality when subjected to the usual development and applications cues. These prerequisites are needed before they can be certified as truly pluripotential and therefore could be applied in the treatment of human diseases particularly those that have defied cure such as Parkinson's disease, multiple sclerosis and insulin-dependent diabetes mellitus. Applications of stem cells in rejuvenating diseased or destroyed human parts are largely experimental because of various constraints. However, neurons have been generated from iPSCs made from the skin cells of patients with Parkinson's disease. There are also other success stories in the treatment of degenerative diseases particularly in Germany where stem cells have been employed in treating many patients suffering from diverse diseases.

Even if development of iPSCs has not offered much at this time towards repairing of tissues damaged by disease, it has at least circumvented the technical complexities of cloning thus avoiding most of the ethical and legal constraints associated with human embryo cell research. It has also refuted the old dogma that cells once differentiated cannot be dedifferentiated into primordial stage of development. Embryonic stem cells remain the gold standard in stem cell research while more studies are needed to gain better insight into the pluripotency of iPSCs. Whether derived from the embryo or adult cell, stem cells hold a huge promise in regenerative medicine in the near future.

Key words: Stem cells, research, regenerative medicine

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**INTRODUCTION:**

There is a long list of controversial topics in medical science today and this includes human cloning-an attempt to reproduce man(thus "imitating" God), cryonics-preserving a dead body waiting for an advance in science to bring it back to life (similar to cell preservation in liquid nitrogen) and euthanasia-mercy killing of terminally ill and helpless patient. Then stem cell research. All these are still under the scourge of political, as well as ethical criticism and bedeviled by superstition, religious and mystical beliefs. As useful as autopsy is today we learn that the first surgeon that performed a post mortem in Italy was publicly executed for interfering with a departed soul! [1] Resistance to blood transfusion by some religious persuasions is too well known to be mentioned and this has been underscored by the Grail message which states inter alia that : "if a different blood group, was used in the case of blood transfusion, then the soul living in such a body would find itself prevented from fully developing its volition, would perhaps be entirely cut off from it because with the blood of different composition the radiation also changes and is then no longer adapted to the soul." Be that as it may blood transfusion has continued to save lives [2]

The term "stem cell" was first proposed for scientific use by the Russian histologist, Alexander Maksimov in 1908. He postulated the existence of haemopoetic stem cells. Research in stem cells was born out of the observations of Ernest McCulloch and James Till of the University of Toronto In the 1960s. Thereafter many other scientists including Joseph Altman and Gopel Das started research on stem cells [3,4] Stem

cells consist of two broad types of mammalian cells: Embryonic stem cells, isolated from the inner mass of blastocyts and adult stem cells found in the adult tissue. The precursor of the embryonic stem cells is the totipotent ( omnipotent) morula which can differentiate into many different cell types down to the pluripotent and the unipotent which can produce only one cell type (see figure)We also have oligopotent stem cells having ability to differentiate into only a few cell types such as lymphoid or myeloid cells.

Stem cells research is based mainly on embryonic cells which have the potential to differentiate into any of the 220 cell types in the human body including bone, muscle, skin and nervous tissue as well as blood cells. Because of this they are called pluripotent [5] Man is therefore a progeny (offspring) of stem cells and this is the central logic behind reparative or regenerative medicine using stem cells. One of Buddha's favorite quotations that say it all states "Believe nothing, no matter where you read it or who said it, even if I told you unless it agrees with your reason and common sense". It is therefore sensible to suggest that one day embryonic stem cells will be the answer for the repair of human tissue damaged either by injury or disease. There is of course adult stem cells which are considered "multipotent" because they can give rise only to specific families of cell types such as those in muscle and bone and replenish mature cells within a tissue [5]

**RESEARCH:**

Work in embryonic stem cells started over 30 years ago in mice particularly the phenomenon of "pluripotency" but it was not until 1998 with the first isolation of human embryonic stem cells that studies commenced in humans [5] These cells are extracted from the embryo, in the early stage of its development, and cultured in petri dishes in the laboratory thus giving rise to embryonic stem cells. Their pluripotency has to be confirmed when they glow on addition of a fluorescent dye. An alternative source of stem cells has been identified as umbilical cord blood which is capable of differentiating into all blood cell types. Among the advantages is the ease of collection [6].

Embryonic stem cell research continues to be regarded by critics as a deliberate taking away of human life by destroying its embryo. Which they say contains human DNA. With the recent breakthrough in stem cell research this criticism will be a thing of the past soon. In 2006 Yamanaka and his colleagues of the University of Kyoto published a groundbreaking study in which they created what they called induced pluripotent stem cells ( iPSCs) from the skin

cells of mice [5]. As should be expected this has to pass the test of skepticism by other stem cell scientists. The good news is that today thousands of them, using the formula developed by the Japanese researchers as a paradigm, are working to understand how they can translate the study and apply it in the treatment of human diseases that have defied treatment.

What did the Japanese scientists do? They reversed the development of mouse cells by returning them to the embryonic state! This is called cellular reprogramming. It is analogous to reversing the biological clock or what chemists call back titration. Mammalian cells under normal circumstances can only differentiate progressively with time but they never dedifferentiate or revert into a more primitive type. This was what the Japanese did by using a few select genes to change adult cells' identity and making them embryonic and therefore truly pluripotent! Before the ingenious approach of the Japanese scientists cellular reprogramming has been on but with a different technology.

This was done by injecting the genetic material from an adult cell into an egg devoid of its DNA. The resultant DNA-egg hybrid develops into an early- stage embryo from which pluripotent stem cell can be extracted. Dolly the sheep was created in 1997 by this nuclear transfer method otherwise known as cloning. Yamanaka and his colleagues circumvented this approach by directly changing adult cells into pluripotent cells without the use of eggs or embryos. Rather "they reasoned that introducing the genes normally active only in embryos into the adult cell might be sufficient to reprogram that cell into an embryo-like state"[5] They screened a total of twenty- four genes that are turned on in pluripotent cells but silent in adult cells and came up with four*: Oct 4,Sox2,Klf4, and c-Myc* that are actually necessary to produce iPSCs.They then used a modified retrovirus as a delivery vehicle to inject the four genes into the DNA of mouse skin cells.

Other researchers were able to replicate this study in mouse and human cells and even with the proteins encoded by the four reprogramming genes directly into the cells. One of such studies was conducted by Sommer and Mostaslavsky [7] in which they produced transgene-free induced pluripotent stem cells. Another approach that was contemplated was to reprogram body cells into iPSCs without using viruses just as the Japanese group circumvented the nuclear DNA-egg hybrid technique. Pioneers in this alternative approach used a cocktail of four drugs that can mimic the effect of the reprogramming genes but they were unable to generate pluripotent cells.

So far about a dozen adult cell types have been reprogrammed into iPSCs from both man and animals.

Next to the promulgation of a new truth the second best thing I think scientists should do is to appraise their work in light of foreseeable problems. Konrad Hochedlinger of the Department of Stem Cell and Regenerative Biology at Harvard University has done that quite eloquently.

Some of the new pluripotent stem cells though look like embryonic stem cells microscopically, do not pass screening tests of functionality to which the embryonic stem cells have also been subjected. These tests are "the ability to produce a wide variety of body cells types in petri dish when exposed to the appropriate developmental cues; the ability of stem cells to produce a teratoma (a type of tumour containing cells from all embryonic tissue lineages) when injected under the skin of mouse; and the capacity, when injected into an early-stage mouse embryo, to contribute to the development of all tissue lineages, including germ cells, in the resulting new born mouse"[5] Another problem observed was that the retroviruses used to deliver the four reprogramming genes are not completely "silenced" or properly shut off, and the important genes in the cells' original DNA are not properly turned on.

This results in cells that have lost both sides: losing their identity without gaining their pluripotency. All viruses are intracellular pathogens and utilize the host genome for their replication. It is not therefore surprising that the Japanese group was able to discover that some of the mice that they injected with iPSCs in their developing embryos formed cancers as a consequence of DNA damage caused by a residual effect of retroviral activity. Even if all these problems are overcome another particular one that is glaring in the face is the issue of rejection of transplanted stem cells because of histo-incompatibility [8].

**Therapy with stem cells**

It was Paracelsus, renowned as father of pharmacology that aptly said that "all that man needs for health and healing has been provided by God and nature, the challenge of science is to find it."Applications of stem cells to treat or cure diseases are progressing in two areas: diseases modeling and cell therapy. The latter in mouse and the former in man [5].

What is being manipulated here is the potential ability of embryonic stem cells (ESC) and iPSCs to produce "spare parts" for organs or systems damaged by injury or disease as can happen in myocardial infarction, neurological disorder such as multiple sclerosis [9] or spinal cord injury,

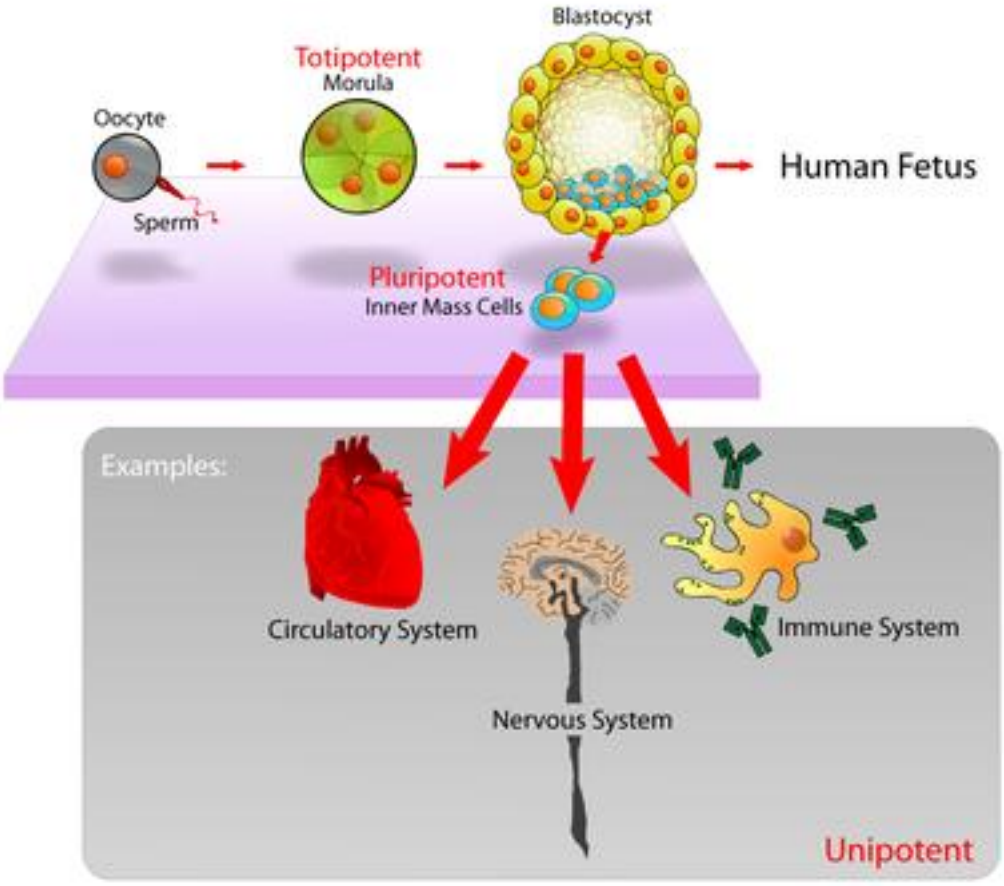
cancer, sickle cell anaemia or insulin-dependent diabetes mellitus. Recent studies have shown that delivering bone-marrow-derived stem cells to infarcted patients could reduce mortality rates and clinical symptom, but improvement s in the myocardial delivery and stem cell optimization are required to realize this potential [10] Also by use of reprogrammed skin cells from mice into iPSCs it was possible to cure sickle cell anaemia in mice with iPSC –derived blood progenitor cells with corrected sickle cell anaemia genes [5]. The healthy precursors now produced normal red blood cells. A version of Parkinson's disease in rats has also been treated by transplanting iPSC-derived neurons, but not without complications for as much as 20% of them died from brain tumours (David Prentice, personal communication). In a span of ten years when Noseworthy and colleagues speculated that it may be possible to enhance remyelination by transplanting oligodendroglial precursor cells into discrete, clinically important lesions in multiple sclerosis [9]. Sundberg and coworkers have shown that both oligodendrocyte precursor cells and oligodendrocytes can be used in repairing myelin in multiple sclerosis and spinal cord injury [11].

Applications of stem cell in regenerating diseased or destroyed human parts are largely experimental because of various constraints already mentioned. Skin cells as well as blood cells are easily accessible and could be transformed into iPSCs and later into any cell type to be used as "repair kits" to cure disease. If that happens adult cells from patient can be turned into pluripotent cells and then tailored into desired cells into the ailing tissue of that patient. This is analogous to auto transfusion and therefore there will be a perfect match both genetically and immunologically. As good and promising as this technique sounds it is a daunting task because the precursor cells are difficult to grow or manipulate outside the body. However, neurons have been generated from iPSCs made from the skin cells of patients with Parkinson's disease. Cancers can be caused by stem cells gone berserk because they have lost contact inhibition. If that proves to be correct it should revolutionize cancer therapy by using replacement cell transplant. So far the best known stem cell therapy to date is bone marrow transplant used to treat leukaemia which is a cancer of white blood cells.

Doctors in the X-Cell Centre at the Institute of Regenerative Medicine in Germany have employed stem cells in treating more than 3500 patients suffering from diverse

diseases. They treated patients with their own (autologous) adult stem cells. These included Alzheimer disease (a brain disorder causing intellectual impairment and memory loss), Liver disease, stroke, multiple sclerosis, muscular dystrophy and cerebral palsy [12]. Also more than 1500 patients received either intravenous or subcutaneous transplantation of fetal stem cells for the treatment of stroke, epilepsy as well as multiple sclerosis [13].

**Figure: Diagram downloaded from Wikipaedia showing embryonic stem cells isolated from inner mass of blastocyts**




{www.Wikipaedia.com}

## DISCUSSION AND CONCLUSION:

Embryonic stem cells (ESC) as well as iPSC research have a lot to offer in rejuvenating the body which hitherto has been done by people through cosmetic surgery, balanced nutritious diet and physical exercise. Unfortunately resentment towards ESC research is still on. Recently in the United States, following a federal judge's decision to temporarily block federal funding for embryonic stem cell research, the Obama administration appealed the judge's decision, and got the injunction lifted, because it issued guidelines in 2009 permitting the National Institutes of Health to conduct embryonic stem cell research and allowed federal funding for it. If this injunction is not lifted very promising research studies that are potentially life-saving for millions of people around the world will be halted. The law suit was against National Institutes of Health and filed by researchers opposed to the use of ESC, a group that seeks adoptive parents for human embryos created by in-vitro fertilization and a non -profit Christian Medical Association. The ruling said that all ESC research involves destroying embryos, which violates the Dickey-Wicker Amendment which unambiguously prohibits the use of federal funds for all research in which human embryo is destroyed [14].

The good news is the discovery of iPSCs which circumvents the technical complexities of cloning and avoids most of the ethical and legal constraints associated with human embryo stem cell research. However the problems with reprogramming genes using retroviruses still remain. Because these viruses integrate themselves permanently and remain active in the host genome they can cause DNA damage which may trigger cancerous changes in the cells as mentioned earlier. Many scientists including researchers in Harvard University have developed methods that avoid permanent genetic manipulation of cells including the use of a modified type of adenovirus, which resides inside the cells for a short time enough to convert them into iPSCs, to deliver the four reprogramming genes mentioned earlier. It is believed that this discovery will be a stepping stone towards the application of virus-free and tumour-free iPSCs for human therapy- thus passing the test of quality control. Another source of encouragement is the umbilical cord blood which is an alternative source of haemoepoetic stem cells discovered in 1978. They have the advantage of higher chances of finding donors and lower risk to recipients since cord blood does not have to match recipient's tissue being immature. But its limitation is that it is only capable of

differentiating into blood cell types including those of the immune system [6]. Also stem cells have been found in amniotic fluid. They are multipotent and can differentiate into cells of adipogenic (fat cells), osteogenic (bone cells), myogenic ( muscle cells),endothelial, hepatic and neuronal cells (nerve cells) [15]. A Vatican paper " Osservatore Romano" called stem cells from the amniotic fluid the "Future of Medicine" because they can be harvested without destroying the embryo [16].

Two more hurdles facing ESCs or iPSCs are whether the conversion of body cells into iPSCs and the subsequent conversion of iPSCs into therapeutically relevant cell types can be made efficient enough for widespread use. The other hurdle is whether iPSCs retain any memory of the body cell type from which they are derived. This is reminiscent of anamnestic response exhibited by T and B lymphocytes. The question here is, for example, will it be possible to produce a custom-made cardiac muscle tissue to replace the myocardium in case of myocardial infarct? The risk here is that a iatrogenic disease may ensue due to graft-versus-host reaction because of an immunological attack mounted by donor cells on the host lymphoid tissue [17]. The observation of Cowan [18] that ESC stored for a long time have been shown to create the type of chromosome anomalies that lead to cancer is yet another rider to worry about. I am aware that there is now a commercially marketed product made from human iPSCs-a heart cell line called iCell Cardiomyocytes intended for use by pharmaceutical companies to test the effect of heart drugs. But not to replace the damaged heart muscle yet.

Because of all the problems with iPSCs mentioned above, embryonic stem cells remain the reference point in stem cell research while more research is needed to gain more insight into the pluripotency of iPSCs and answer questions surrounding their applications in treating many human diseases. Having said that, the discovery of iPSCs by cellular reprogramming is a great breakthrough that has refuted the accepted dogma that cells once differentiated cannot be dedifferentiated into primordial stage. Critics of embryonic stem cell research-the bone of contention- have to rethink. Blastocyte-the stage before embryo's cells begin to differentiate and from which the so-called embryonic cells are derived has no human features and so this procedure cannot be tantamount to taking away human life. Also new stem cell lines already exist due to the common practice of in-vitro fertilization. Addressing the other unexpressed concern of the critics, advocates of embryonic stem cell research guarantee that new human life will not be

created even for the purposes of experimentation- the type that created Dolly the sheep in 1997.The concern is genuine because embryonic stem cells, theoretically speaking, can be conditioned to generate sperm and ovum to produce human embryo through the traditional in-vitro fertilization. Stem cells, whether from the adult or embryo, umbilicus or amnion, hold a huge promise in regenerative medicine in years to come.

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# HISTORY AND MEDICINE IN HISTORY, 450BC – 1450 AD

**Peter J. YEARWOOD,** PhD.

History Strand, School of Humanities and Social Sciences,
University of Papua New Guinea
cliopjy@yahoo.co.uk, yearwopj@upng.ac.pg.

**ABSTRACT:**

This article examines the parallel histories of medicine and history to about 1450. They emerged together as part of the shift from poetry to prose in Greek culture in the fifth century BC. They each pursued similar strategies of observation, compilation, and analysis. Hippocratic medicine provided a paradigm for Thucydides' development of analytic history. Medicine was further systematised by Galen in the second century AD.

After the collapse and division of the Roman Empire, the *Dar al-Islam* became the main area of intellectual advance. Its scholars had little interest in Graeco-Roman historians, but they translated and used the scientific and medical writers. In both history and medicine they tried to create sciences based on Aristotelian philosophy. The article looks in particular at Avicenna's attempt to reconcile Aristotle and Galen, and compares this with the eighteenth century debate between preformationists and epigeneticists. It emphasises the need to look at such arguments in the context of their times, and notes the continuing tension between the simplicity of theory and the messiness of data.

The transfer of learning from the *Dar al-Islam* into Western Europe paralleled that from the Graeco-Roman world into the *Dar al-Islam*. Again, historical writing was overlooked, but philosophical, scientific, and medical writers were translated. They would be the basis for the development of modern science.

Key words: History of Medicine, Hippocrates, Herodotus, Thucydides, Aristotle, Galen, Avicenna.
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**History and Medicine in History, 450BC – 1450 AD**

Philosopher/historian of science Thomas S. Kuhn has noted a striking difference between the training and therefore the outlooks of those in the natural sciences and those in the humanities. For natural scientists "textbooks are systematically substituted for the creative scientific literature that made them possible" [1]. The great figures of the past are by no means ignored, indeed they are venerated, but for their input into the existing paradigms of what Kuhn calls "normal science". The student of natural science is seldom required to read the actual works of, say, Newton, Faraday, or Einstein, and certainly not those of the scientists whose ideas they pushed aside and replaced. By contrast the student of the humanities rapidly moves beyond the textbook to the works which the textbook summarises and discusses. Whereas natural scientists read about Galileo and Galen, humanists read Shakespeare and Sophocles. Historians are quickly taught to move away from derivative "secondary" sources to "primary" ones, the documents and artefacts of the time we are researching. On that basis each historian reconstructs the past and tries to convince others of the plausibility of that reconstruction.

This difference in training means that natural scientists are seldom conscious of the intellectual world in which the great figures of the past operated and of the inwardness of the controversies in which they were involved. An historian interested in the mechanisms of intellectual development, and particularly in how one set of ideas is displaced by another may therefore be able to look at early medicine in a different way from a current practitioner, but, I would hope in a way which he or she may still find interesting. What we may broadly call the western world derives intellectually, and has a consciousness of deriving, from the classical civilisations of the Mediterranean basin. History and medicine emerged together and interacted fruitfully. They are the earliest products of Greek prose. Before their emergence the Greek world had been mythological and had expressed itself in poetry of great beauty and power. Prose marked a new approach to the world: questioning, investigative, and sceptical. In the second half of the fifth century BC Hippocrates emerged as the "Father of Medicine" promoting an approach which tried to explain health and disease as natural phenomena. At about the same time Herodotus began his "investigations" – the Greek word is *histories* – which won him the title "Father of History". No other disciplines know their own fathers so well.

Hippocrates was the "Father of Medicine" largely because he established a profession of healers bound together by the famous Hippocratic Oath. The works ascribed to him were in fact written by several authors over a long period of time. His school worked within a tradition which saw health and illness in terms of balances or imbalances between, especially, the four bodily fluids (*khymoi* in Greek, translated into Latin as *humores*): blood, phlegm, bile (*khole*), and black bile (*melankhole*). Hippocrates himself seems to have been little interested in such theories. He and his followers recognised that diseases might have many causes. They emphasised instead careful observation, compiling case histories, and developing effective practices.

While doctors continued to revere Hippocrates, historians, by contrast, largely disavowed Herodotus. His prose style was admired for its clarity, its simplicity, and its effectiveness. When his *Histories* came to be divided into nine books, each was given the name of one of the Muses. Very unusually for a Greek historian, all nine books survive complete. Nevertheless, his ready acceptance of what were often tall tales from distant lands was dismissed as credulity; his willingness to consider non-Greek viewpoints was scorned as softness to barbarians by an increasingly xenophobic culture, while his lack of intellectual rigour was apparent to all. A generation later Thucydides announced a new approach: "The absence of romance [*mythodes*] in my history will, I fear, detract somewhat from its interest; but if it be judged useful by those inquirers who desire an exact knowledge of the past as an aid to the interpretation of the future, which in the course of human things must resemble it if it does not reflect it, I shall be happy" [2]. That medical science was his paradigm for this new history appears from the introduction to his account of the plague of Athens of 430 BC: "All speculation as to its origins and causes, if causes can be found adequate to produce so great a disturbance, I leave to other writers, whether lay or professional; for myself I shall simply set down its nature, and explain the symptoms by which it may be recognized by the student if it should ever break out again" [2]. Both in history and in medicine, accurate accounts of relevant phenomena are seen as essential for taking right decisions in the future. Thucydides' account of the collapse of trust and of social relations in the plague is closely paralleled by his description of a similar breakdown during the violent revolution in Korkyra (Corfu) in 427 BC. War and disease are presented as similar phenomena to be analysed in a similar manner. History is the product of human nature, which can best be understood by its pathology.

Thucydides is generally recognised as having been the greatest analytic historian of classical antiquity. His work was unfinished, but, as with Herodotus, all of it survives. He continues to inspire the "realist" school in the modern discipline of International Relations. While Thucydides overshadowed his successors, medicine developed beyond Hippocrates. Aristotle in the fourth century BC systematised many of what we would now call the scientific disciplines. Whereas his philosophical predecessor Plato had looked to mathematics, especially geometry, for paradigms, Aristotle looked to biology, especially embryology. He was particularly interested in questions of growth and development, building up a model of a purposeful, teleological universe.

The Greek world would eventually be incorporated into the political structure of the Roman Empire. Nevertheless, Greek, rather than Latin, remained the language of intellectual endeavour. The medical tradition culminated in the work of Galen in the second century AD. He saw himself as the systematiser of medicine, laying out the subject like the roads of the Empire, and connecting it to logic, physics, and ethics. In an age where Neo-Platonism dominated philosophy, he readily linked the characteristics of the body (microcosm) with those of the universe (macrocosm), establishing paradigms which would last for more than a thousand years. He developed the system of the four humours, paying particular attention to blood, which in its venous form came from the liver and in its arterial form from the heart. The balance of health might most often be restored by blood-letting, which for almost two thousand years would be seen as the equivalent of taking a couple of aspirins. Improved knowledge of anatomy led Galen to reject the Aristotelian view of the primacy of the heart, and to ascribe comparable roles to the liver and to the brain.

After Galen the Roman world divided. Always intellectually lagging, the Latin west underwent barbarian invasion and almost complete cultural collapse, as knowledge of Greek was lost. Graeco-Roman civilisation survived in the east, where the Roman Empire, now based on Constantinople, continued, but where the southern portion was incorporated into the Arab Caliphate. For almost a millennium this would be the most dynamic area of intellectual advance. Historians, especially historians of science, and even more historians of medicine, know this well; but it is only dimly, if at all, perceived by the wider public. There is also a problem with terms. Writers refer either to the "Arab" or to the "Islamic" world, but the *Dar al-Islam*, like the Roman Empire, but

unlike Western Europe, was a multicultural world. Very few of its leading thinkers were in fact Arabs; many were Persian. Islam was indeed the dominant religion, but by no means the only one. Christians and Jews remained, and contributed to the development of the civilisation. Thinkers in the *Dar al-Islam*, like those in Christendom, had to reconcile the claims of a revealed monotheism with a heritage of secular learning which had emerged in a polytheistic culture. There was a tension, often creative, between the demands of theology and the conclusions of *falsafa* (philosophy). To speak of "Islamic science", or "Islamic medicine", is therefore misleading. The historian Marshall Hodgson has proposed the term "Islamicate" [3]. Though this has so far failed to catch on, I shall make use of it.

Whatever terms are used, the scale of cultural transfer was astounding. To a large degree it was promoted by the Caliphs of the ninth century, especially al-Mamun with his House of Translation, *Bayt al-Hikma*. The process was selective. The translators were not interested in Greek literature or Greek historiography. Homer and Herodotus did not pass into Arabic. History writing in the *Dar al-Islam* was essentially a new departure. Instead it was the works of philosophy, science, and medicine which were translated, assimilated, and made the bases of fresh intellectual advance.

Islamicate thinkers sought to develop new sciences, providing philosophical backing for studies which previously had been, or were seen as having been, excessively empirical. Writing in the late fourteenth, early fifteenth centuries ibn Khaldun contrasted a surface history which elegantly presented information about events in the remote past with a different kind of history which "involves speculation and an attempt to get at the truth, subtle explanation of the causes and origins of existing things and deep knowledge of the how and why of events" This would be rooted in philosophy and be a branch of it, dealing with big themes such as "the origin of races and dynasties, the synchronism of the earliest nations, the reasons for change and variation in past periods and within religious groups; also concerning dynasties and religious groups, towns and hamlets, strength and humiliation, large numbers and small numbers, sciences and crafts, gains and losses, changing general conditions, nomadic and sedentary life, actual events and future events – all things expected to occur in civilization" [4].

Ibn Khaldun's history was grounded in Aristotelian philosophy. So also was the medicine of the *Dar al-Islam*. The eleventh

century thinker ibn Sina, who is better known in the west as Avicenna, tried to reconcile what he saw as the essentially empirical work of Galen with the natural philosophy of Aristotle, of which he was an authoritative exponent. His task was complicated by the fact that Galen's knowledge of anatomy was clearly far superior to that of Aristotle. Both for Aristotle and for Avicenna, questions of reproduction were central, as it was through reproduction that animals came to be organised. Embryonic development was therefore the key to a deep understanding of biology.

Reproduction had long been controversial. As offspring obviously had resemblances to each of their parents, Hippocrates had argued that seed was generated both by males and by females. Aristotle rejected this. Heat was central to his analysis. He believed that women were cool and wet. They could provide passive material, which he identified with menstrual blood. The active, organising principle came entirely from the man through the semen, a blood residue which could be created by the male's superior heat. The ovaries had been discovered by the time of Galen, who saw them as female testicles. Therefore, he argued, both men and women could produce semen as a blood residue, and each contributed to the foetus. Avicenna had to accept Galen's anatomy, and with it the idea of female semen, but insisted that this had only a passive role. For medicine to be properly scientific, natural philosophy had to trump observation. With ibn Rushd (known in the west as Averroes) in the thirteenth century, Aristotelian orthodoxy on reproduction was taken further: ovaries were as functional as male nipples. There could be no active female principle in reproduction.

It is all too easy to jeer at the parties to debates based on premises and paradigms which we now know to be false as the result of greater empirical knowledge. Arabic litterateurs had great fun with Aristotle's belief that testicles functioned as pulleys. Even more dangerous is the distortion that results from using hindsight to pick winners and losers based on superficial similarities to our own understandings. This is why we need historians of science. Embryology involved deep questions about phenomena which were difficult to observe even with instruments such as microscopes, which were developed from the seventeenth century. European scientists in the eighteenth century divided themselves between preformationists, who believed that all structures were present in the embryo from the beginning, and epigeneticists, who believed that structures differentiated themselves out of an initial simplicity. Preformationists found themselves committed to the conclusion that all

subsequent embryos had to have been contained inside the first human egg or spermatozoon. Several claimed to have seen such homunculi nestled within spermatozoa or ova. As the ovum, was not discovered until1826, the "egg" they were examining would have been the Graafian follicle. Epigeneticists, on the other hand, could explain the process of differentiation of structures only by invoking a rather mysterious "life force" As Stephen Jay Gould has pointed out, without a conception of coded instructions, eighteenth century biologists could not see that it was programmes rather than parts or forces which were transferred from one generation to the next [5]. Scientists of the *Dar al-Islam* did not have microscopes. They did have different paradigms from those of eighteenth century Europe. But two aspects of the problem were the same. One was the technical question of the role of the sexes in reproduction. Did males provide the crucial organising principle, as argued by Aristotle, his Islamicate followers, and preformationists seeing homunculi in spermatozoa? or did something equally important come from the female, as argued by Hippocrates and Galen? or was it all female, according to preformationists who looked at what they thought was the egg? If active principles came from each sex, how could they be so successfully combined in the embryo? The second question involved the balance between theory, which seeks simplicity, and the messiness of empirical data. We may now have the concepts to deal with the first question, but the second will remain as long as men continue to look at the world and try to make sense of it. In the *Dar al-Islam, falsafa* had often been regarded with suspicion as a threat to religious orthodoxy. After, roughly, the time of ibn Khaldun it lost its vigour. By then its creative impulse had stimulated western Christendom.

The incorporation of Graeco-Roman science into the *Dar al-Islam* was paralleled centuries later by the transfer of Islamicate learning into Western Europe. Unlike the earlier process, this owed little to the initiative of rulers. From the eleventh to the thirteenth centuries individual western European scholars journeyed to southern Italy and to Spain to acquire and translate Arabic manuscripts. They started the process of intellectual revival and development which has continued to the present day. As with the earlier process, the transfer of culture was selective. Western European had no interest in Arabic literature or historiography. Ibn Khaldun would not be known in the West until the nineteenth century. Admittedly, he wrote almost two hundred years after Western scholars had stopped reading contemporary Arabic writers, but they might have translated some

of the great historians of the eighth to tenth centuries whom ibn Khaldun would acknowledge as masters and whose tradition he sought to revive: ibn Ishaq, at Tabari, and al Masudi. They did not do so. The development of historiography in the west would owe nothing to the historians of the *Dar al-Islam*. In fact ibn Khaldun remains the only one of those historians whose work is easily available in English. Compared with the ready accessibility of classical Greek and Latin writers this constitutes a serious cultural weakness and an impediment to popular understanding of the development of our intellectual tradition.

What did pass from the *Dar al-Islam* was philosophy, natural philosophy (what we would now call science), and, outstandingly, medicine. Islamicate Aristotelianism posed a major challenge to Western Christendom, which was intellectually Platonic insofar as it was anything. Christianity emerged in a Neo-Platonic world and is most readily compatible with it. The attempts of St Thomas Aquinas to reconcile revealed monotheism with Aristotle's philosophy followed in the tradition of the *faylasufs* (philosophers) of the *Dar al-Islam*: al Kindi, al Farabi, ibn Sina, ibn Rushd, and the Jewish ibn Maimun (Maimonides). The impact of Islamicate medical thinking on the West was, if anything, even greater. It would remain authoritative until well into the eighteenth century. Arabic, along with Latin, and later on Greek, was recognised as one of the major languages of scholarship. Like Plato (Platon), Aristotle (Aristotles), and Ptolemy (Ptolemaios), the names of the major Arabic writers were bent into forms convenient for western Europeans: Rhazes (al Razi), Avicenna, Averroes, Avenzoar (ibn Zuhr). The acclimatisation of these names shows how central the thinkers of the *Dar al-Islam* were to us.

To carry this discussion forward into the European Renaissance and beyond would produce either a sweeping, superficial account, or a dull plod through facts and dates. In general we may note that medicine has subdivided itself into many disciplines, and in so doing acquired greater rigour. At first this had little effect on its efficacy. In the mid nineteenth century the English novelist Trollope gave his characters a choice of Doctor Philgrave and Doctor Killchild [6]. A stroll through any Victorian cemetery shows how right he was. Therefore this would be a golden age of quackery and snake-oil salesmanship, which indeed pioneered modern advertising techniques. A century later scientific medicine could at last cure diseases, but many in the wider society would worry that it ignored the patient as an integrated human being. Such worries have partly fuelled the remarkable growth of "alternative medicine".

The expansion and subdivision of medicine has contrasted with shrinkage of history. New disciplines emerged – economics, sociology, political sciences, international relations – each asserting an intellectual rigour which, it was claimed, history lacked. Partly in response, history tried to be "scientific". There was even cliometry, which appeared to show, through the use of sophisticated statistical techniques, that American slavery was not oppressive – on average slaves were likely to be whipped only once in their lifetime –, and railways contributed little to nineteenth century economic development. Despite this, history still appeared somewhat as an elderly poor relation among the social sciences. As science lost its prestige by the 1980s, many historians took a "linguistic turn", looking towards the humanities and especially to literary analysis in which examination of texts trumped whatever problematic reality old-fashioned historians might want to see behind them. Despite the obvious excesses and absurdities, which are so easy to caricature, "scientific" and "literary" approaches have each enriched and continue to enrich our understanding of history. Nevertheless, the core of the discipline remains the process of investigation and analysis which emerged with Hippocratic medicine in the fifth century BC.

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# SOCIETY – THE FOUNDRY OF HUMAN MINDS

**Olga TEMPLE,** MA

Linguistics & Modern Languages, School of Humanities and Social Sciences,

University of Papua New Guinea

(Email: olga.temple77@gmail.com)

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John Stuart Mill (1806-1873) [1], widely regarded as the 'most influential English-speaking philosopher of the nineteenth century' [1], examined the relationship between the society and the individual, focusing on the "nature and limits of the power which can be legitimately exercised by society over the individual" in his famous essay On Liberty [2]. Though the issue of Civil Liberty had "divided mankind, almost from the remotest ages," he felt that, in his time, it required a "different and more fundamental treatment," because it had begun to "profoundly influence the practical controversies of the age" [2]. He also predicted that the controversy over 'social liberty' was "likely soon to make itself recognized as the vital question of the future" [2].

We live in that future now, and the perceived contradiction between social and individual interests has become the biggest bone of contention in current ideological debates. Mill's argument for individual liberty, insofar as it does not encroach on the liberty of others, still holds sway:

"The sole end for which mankind is warranted in interfering with the liberty of action of any of their number is to prevent harm to others. His own good, either physical or moral, is not sufficient warrant" [2]

However, individual actions are so interwoven into the fabric of social life that it is difficult to define or quantify their effects on 'others.' In the twenty-first century, we are torn between arguments for individual freedom, epitomized in the idea of 'free market,' and calls for government regulation to assure social cohesion and social justice. This issue has become further complicated by a dangerous paradox

of our time: on the one hand, individuals have acquired unprecedented power to impact the society through the use of modern science and technology (tools for doing both 'good' and 'bad,' depending on one's perspective).

On the other hand, the rise of corporations in our profit-driven economy has marginalized individuals in society – the "Invisible Hand" of the 'free market' controls the ebb and flow of our lives. Capitalism, built on the promise of individual freedom, has subjugated individuals to the economic interests of organizations. Profits rule, while individuals chase after the mirage of personal freedom.

Individuals are becoming more aware of their technologically enhanced power to impact the society; however, there is a general lack of understanding of individuals' visceral interconnectedness / interdependence with the society. Egocentric thinking conflicts with our technological maturity – that is why the relationship between the society and individuals requires now even more fundamental treatment. A deeper understanding of how the foundry of society casts all individuals (including individualists) may help us discover our common purpose and the objective laws of social harmony. I hope to contribute to this ongoing enquiry by highlighting the fundamental role of society in 'humanizing' our individual minds. But first, what is being 'human'?

**The '*Human*' Difference**

We have been asking this question since the dawn of human consciousness. In Ancient Greece, Aristotle (384-323 BC) [3] pointed out that it was 'connected experience' that enabled man to abstract similarity between things:

"By nature, animals are born with the faculty of sensation, and from sensation memory is produced in some of them, though not in others. And therefore the former are more intelligent and apt at learning than those which cannot remember. … The animals other than man live by appearances and memories, and have but little of connected experience; …from memory, experience is produced in men; for several memories of the same thing produce finally the capacity for a single experience" [3].

This human ability to abstract similarities from experiences,

connected in our memory, is the root of all abstract ideas and knowledge – generalization. Through our senses, we experience the physical world; our concrete experiences, connected in memory, are the soil from which meaning grows in our minds. Human understanding, to Aristotle, was 'knowledge about the principles and causes':

"We do not regard any of the senses as Wisdom; yet surely these give the most authoritative knowledge of particulars. But they do not tell us the **'why'** of anything – e.g., why fire is hot; they only say that it is hot. … Wisdom is knowledge about certain principles and causes" [3]

Because thoughts have no physical substance and so cannot be perceived directly, their nature (despite Aristotle's incisive analysis) remained wrapped in mystery until quite recently, when David Hume (1711-1776) [4] became interested in the *mechanism* of human thinking. In his *Enquiry Concerning Human Understanding* (1748), he claimed that humans 'in all times and places' make sense of things by connecting ideas. These ***connections*** we make between ideas reflect our perception of how things *relate* to each other in terms of their similarity or difference, time and space, and cause and/or effect:

"It is evident that there is a principle of connexion between the different thoughts or ideas of the mind, and that in their appearance to the memory or imagination, they introduce each other with a certain degree of method and regularity. In our more serious thinking or discourse, this is so observable that any particular thought, which breaks in upon the regular tract or chain of ideas, is immediately remarked and rejected. And even in our …dreams, we shall find … a connexion upheld among the different ideas, which succeeded each other. Were the loosest and freest conversation to be transcribed, there would immediately be observed something which connected it in all its transitions. … Among different languages, even where we cannot suspect the least connexion or communication, it is found, that the words, expressive of ideas, the most compounded, do yet nearly correspond to each other: a certain proof that the simple ideas, comprehended in the compound ones, were bound together by some universal principle, which had an equal influence on all mankind.

Though it be too obvious to escape observation, that different ideas are connected together; I do not find that any philosopher has attempted to enumerate or class all the principles of association; a subject, however, that seems worthy of curiosity. To me, there appear to be only three principles of connexion among ideas, namely, Resemblance, Contiguity in time or place, and Cause or Effect.

That these principles serve to connect ideas will not, I believe, be much doubted. A picture naturally leads our thoughts to the original: **[a]** the mention of one apartment in a building naturally introduces an enquiry or discourse concerning the others: **[b]** and if we think of a wound, we can scarcely forbear reflecting on the pain which follows it. **[c]** … The more instances we examine, and the more care we employ, the more assurance shall we acquire, that the enumeration, which we form from the whole, is complete and entire: [a] Resemblance; [b] Contiguity; [c] Cause and effect

… Contrast or Contrariety is also a connexion among Ideas: but it may perhaps, be considered as a mixture of Causation and Resemblance. Where two objects are contrary, the one destroys the other; that is the cause of its annihilation, and the idea of the annihilation of an object, implies the idea of its former existence" [4]

David Hume thus, for the first time in recorded history, described the universal principles of human thought. His claim that human minds connect ideas by resemblance, contiguity and cause/ effect gave us an insight into the inner workings of our minds. All three types of association are necessary for generalization – they form the matrix of our understanding.

This ability to abstract meaning from concrete experiences is the distinguishing feature of the human race. According to T.W. Deacon, Professor of Biological Anthropology and Neuroscience at the University of California, 'Biologically, we are just another ape. Mentally, we are a new phylum of organisms' [5].

Karl Marx also commented on the 'human difference' in *Das Kapital* [6]: "The spider makes operations resembling the operations of the weaver, and the bee creating its waxen cells disgraces some architects. But from the very beginning, the worst architect differs from the best bee in

that before building the cell of wax, he already has built it in his head. The result, which is received at the end of the process of work, already exists in the beginning of this process in an ideal form in a representation of a person. The person does not only change the form given by nature, but in what is given by nature he … realises his conscious purpose, which as a law determines the way and character of his actions and to which he must subordinate his will" [6].

Lev Vygotsky (1896-1934) [7], the brilliant Russian psychologist, defined the 'human difference' in even broader terms: 'Man cannot be distinguished by a single feature (intellect, will), but in principle by his relation to reality' [7]. We share physical sensation and most of our feelings with other intelligent animals. But, unlike animals who passively adapt to their environment, we are conscious of our separateness from the world and act deliberately to transform it to our liking.

Despite certain similarities, there is a fundamental difference between human and animal behavior: human actions are voluntary / intentional, whereas animals act by instinct. "Will implies freedom from the situation," argued Vygotsky. "***Man wants the stick, the ape wants the fruit***" [7]. Animals, even when they seem to use a tool intelligently, do not want the tool for its own sake; for them, it is just a means to satisfy an instinctive wish. The concept of ***tool*** requires abstraction from the situation; 'tool' is the ***meaning*** of an object. The use of tools requires the ability to 'make sense' of things, to give them ***meaning***.

The 'human difference' is, first and foremost, in the way the human mind ***thinks*** – through abstracting 'principles and causes' from our connected experience. But why did we, biological apes, leap into consciousness? How did our distant ancestors start to think 'Human'? And what makes consciousness sprout in each of our individual minds, forming a relationship between us, and the world? In essence,

**Where do human minds come from?**

The short answer to this question is 'from the society.' The collective mind of the society has crafted Language, the tool all individuals use to think 'Human' and to communicate their thoughts. We are relatively small and fragile creatures, living in a world full of

dangers – our individual survival depends on the cooperation of others in the group (the society). Cooperation is impossible without communication; no wonder, then, that the process of natural selection favored groups who could communicate (and, therefore, cooperate amongst themselves) more effectively. Thus, in the course of human biological evolution, our brains developed the high-speed networking capability required for connecting our experiences in memory and abstracting from them their common features – to produce, finally, what Aristotle called a 'single experience' – the ***idea*** of similar experiences [3].

Thus, human adaptation in response to our existential need for cooperation resulted in the emergence of the most ingenious tool of all time – Language, the tool for constructing meaning. Basically, Language is a system of signs (those 'single experiences' abstracted in the collective mind of the society). What we call words are ***signs*** of conventional meanings. Like all signs, they have a double function: intellectual (to carry meaning) and social (to communicate).

Language, thus, is a social *means* of thought – a TOOL societies use for generating and communicating an infinite variety of complex meanings. How does it work?

**The Mechanism of Language**

The Language tool consists of a set of conventional word-meanings and rules for putting them together into sentences to create complex meanings (thoughts). Artists can create any kind of mosaic images by arranging colored tiles in a particular way (See Figures 1 and 2).

Speakers are all like artists, in that sense – they create all kinds of complex images (meanings) by arranging words into sentences. Words are like tiles of different colors – the same brown tile may be part of a flower (Fig. 1), a sucker on a frog's toe (Fig. 2), or anything else, depending on how we ***use*** it in our mosaic.

Each tile in any mosaic acquires its 'meaning' only in the context of the other tiles that make up the whole image. In the same way, each word acquires its true meaning only in the nexus of the proposition whose meaning, in turn, is more than the sum of its words – it also depends on how they have been put together (*The Earth*

*orbits the Sun* ≠ *The Sun orbits the Earth*).

Word-meanings are fluid in use – so fluid, indeed, that words and their meanings are relatively independent of each other in the thought mosaics we make. It is practically impossible to 'fix' meanings in use, because ideas exist only in our minds. We perceive the world's mosaics with our own eyes and ears, and we make sense of them only in our own heads. Each mind's eye views the world from its own perspective; its clarity of vision depends on many factors, such as our experience, disposition, context of communication, etc. The 'image' (sentence meaning) different people see with their mind's eye, therefore, may not be the same; it depends as much on the 'color' and patterns of the word-meanings making up the 'image' (proposition), as on their subjective perception – this is why ambiguity is so inherent in all human languages. We 'think' by connecting ideas into complex mosaics of meaning. Just as the process of breathing involves both inhalation and exhalation, so also the process of thinking involves both synthesis and analysis of ideas. We use language (our 'thinking tool') to spin our 'webs of significance' by synthesizing ideas into conventional sentence structure (the nexus of the Subject, Verb and Object) and analyzing or specifying any of the major sentence constituents.

**The Rational Mechanism of Language**

The mechanism of human understanding – generalization – is thus embodied in the syntax of all human languages. In order to understand something /form a concept, we must be able not only to connect, but also to *abstract*, to *single out* characteristic elements, and to view them separately from the "totality of the concrete experience in which they are embedded" [8]. Every word of language is already a generalization; every statement in every human language is a structural embodiment of its

- ***Synthesis***, connecting word-meanings into the ***nexus*** of the proposition (mosaic), and
- ***Analysis***, focusing on ***parts*** of the sentence (recursion) – i.e., describing them by associating parts of the whole with other ideas, based on some Resemblance, Contiguity, or Cause/ Effect relationship.

Figure 1. http://www.firelily.com/samples/images/mosaic.lily.html

Figure 2.
http://www.lineartgallery.com/web/Artist/Strachan/art_mosaics/art_mosaic_photos/art_mosaic_frog.jpg

Generalization, thus, is the universal matrix of human thought which finds expression in the diverse grammars of the world's languages, all shaped by it. This idea is not new; the epistemology of the great Islamic philosopher of the tenth century, al Farabi, postulated 'universal reason' (i.e., universal principles of human understanding):

[Logic] "shares something with grammar in that it provides rules for expressions, yet it differs in that grammar only provides rules specific to the expressions of a given community, whereas the science of logic provides common rules that are general for the expressions of every community" [9].

Logic is universal – two plus two is four, no matter where you come from. It constitutes the Rational Language Mechanism (generalization, which has created (and finds expression in) all human languages. However, logic is not inborn – how do we, individuals, learn to think?

**Stages of Our Cognitive Development**

**Jean Piaget** (1896-1980) [10], the outstanding Swiss psychologist, showed that consciousness germinates and develops in our minds in the process of cognitive development during our so-called 'formative' years. He argued that the difference between child and adult thinking is ***qualitative***, not just quantitative; that a child is not a miniature adult, and that his mind is not the mind of an adult on a small scale. Through observation and experiments, he proved that the human brain continues to develop after birth, and matures only by adolescence. Piaget contended that we generally go through four stages of cognitive development, driven by biological changes in our brains [10]:

- **The Sensorimotor Stage** occurs between birth and age two. Babies are born with no thinking structures and develop them through 'soaking up' language and exploring their environment through their senses. At his stage, humans are incapable of coherent logical thought, even though most babies begin to speak their first words long before they are two. 'The child's babbling, crying, even his first words, are quite clearly stages of speech development that have nothing to do with the development of thinking' (Vygotsky) [7]. The child's first

words are not really words, but rather expressions of feelings that are communicated not by the words, but by the child's whole behaviour at the time (like pointing, reaching out to something or pushing it away, etc.). The word *mama*, for example, could mean anything from *Mama, give me* or *Mama, come here*, or *Mama, hold me*, etc.

- **The Pre-Operational Stage** (approximately two to seven years of age). At a point in this stage, children make the greatest discovery of their lives – that each thing has its name! They begin to ask, '*What is this?*' about every new thing they come across. They rapidly develop language skills and, concurrently, the underlying thinking structures. Children also develop personal traits and character, but are yet incapable of mature reasoning. For example, the concept of conservation is above the average toddler (conservation implies the understanding that actual amounts of any substance may remain constant, even if their shapes may change). Preoperational kids are egocentric – they cannot see things from another person's perspective. Both conservation and de-centering are basic requirements for logical thinking.

- **The Concrete Operational Stage**: ages approximately seven to adolescence. Children begin to grasp conservation and de-centering, although word-meanings, in their understanding, still remain the names of concrete things. That is why children cannot really understand some abstract thoughts, even if they know the necessary words – the adequately generalized concept may still be lacking.

Word-meanings develop in the child's mind together with his thinking ability – by the end of the pre-operational stage, they have fully learnt the structures of language, and their thinking becomes more abstract. They

begin to reason logically, but only on a concrete level, not hypothetically or abstractly. They solve problems logically, but not systematically or consistently. As opposed to preoperational children, kids in the concrete operations stage are able to take another's point of view and take into account more than one perspective simultaneously. Although they can understand concrete problems, they cannot yet consider all of the logically possible outcomes.

- **The Formal Operations Stage**: adolescence or above. The concrete operational and the formal operational stages differ mostly by the degree of abstraction in the way we think and the amount of experience on which we draw. The mind is now capable of sophisticated logical thought. It can think abstractly, hypothetically and can solve problems using the logic of combinations. Piaget considered this to be the ultimate stage of cognitive development. Not all people reach the formal operation stage; research shows that only about 25% of all adults use formal operations on a regular basis; these require significant training and cognitive discipline.

Piaget's research showed that, at birth, there is no significant difference between us and other intelligent animals [10]. Babies have no self-awareness and are governed entirely by instincts and feelings; self-awareness and logical thinking are contingent on the child's biological development and social interaction.

**Lev Vygotsky** [7] went even further to claim that there is no genetic link between speech (vocalization) and abstract thought. He graphically represented speech and thought as two separate circles in both animals and human babies:

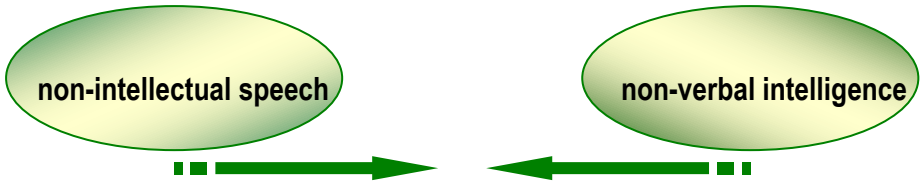


Fig. 3: Speech and Thought spring from different roots – they do not overlap at birth

During the first stage of our cognitive development, we 'soak up' the sounds and words of language used by the people around us through our physical senses of hearing and sight. Gradually, we begin to 'connect' the sounds of certain words with concrete objects around us; when that connection 'clicks,' we begin to *use* those sounds to refer to things around us. Babies' first words are not yet abstract thought, claimed Vygotsky; rather, they are expressions of wishes and feelings [8]. It is not so much the words as the whole behavior of the child that communicates meaning – much like a dog's barking may signal anger, fear, pain or joy. Gradually, thought germinates from feelings: from the people around us, we learn to connect single words together, creating more complex meanings (phrases and sentences). Vygotsky described the process of language acquisition as the prerequisite for abstract thought [8]:

In mastering external speech, the child starts from one word, then connects two or three words; a little later, he advances from simple sentences to more complicated ones, and finally to coherent speech made up of series of such sentences; in other words, he proceeds from a part to the whole. In regard to meaning, on the other hand, the first word of the child is a whole sentence. Semantically, the child starts from the whole, from a meaningful complex, and only later begins to master the separate semantic units, the meanings of words, and to divide his formerly undifferentiated thought into those units. The external and the semantic aspects of speech develop in opposite directions – one from the particular to the whole, from word to sentence, and the other from the whole

to the particular, from sentence to word. A child's thought, precisely because it is born as a dim, amorphous whole, must find expression in a single word. As his thought becomes more differentiated, the child is less apt to express it in single words but constructs a composite whole. Conversely, progress in speech to the differentiated whole of a sentence helps the child's thoughts to progress from a homogeneous whole to well-defined parts [8].

For the child, words are the *names* of concrete objects: "signification independent of naming, and meaning independent of reference, appear later … Only when this development is completed does the child become fully able to formulate his own thought and to understand the speech of others. Until then, his usage of words coincides with that of adults in its objective reference but not in its meaning" [8]

Thought and speech in children begin to intersect in the course of language acquisition. At a certain point, children discover that everything around them has a name; this revelation triggers a qualitative change in their behavior:

Before this turning point, the child does (like some animals) recognize a small number of words which substitute … for objects, persons, actions, states, or desires. At that age, the child knows only the words supplied to him by other people. Now the situation changes: The child feels the need for words and, through his questions, actively tries to learn the signs attached to objects. He seems to have discovered the symbolic function of words. Speech … enters the intellectual phase. The lines of speech and thought development have met [8].

Language, according to both Vygotsky and Piaget, precedes logic [8, 10]. The vocabulary of the child grows with the grasping of new concepts; if a concept is too abstract for the child's mind to grasp, that word will not 'enter' the child's consciousness. The same holds true for us at any age: if the concept is not formed in the brain, the word will not 'make sense' to us. The dynamics of thought development follows the dialectics of *synthesis* and *analysis*, for abstract thought (generalization) is *both*:

… the advanced concept presupposes more than unification. To form such a concept, it is also necessary to abstract, to single out elements, and to

view the abstracted elements apart from the totality of the concrete experience in which they are embedded. In genuine concept formation, it is equally important to unite and to separate: synthesis and analysis presuppose each other, as inhalation presupposes exhalation [8]. In adults, the overlap where thought and speech coincide represents ***verbal thought*** (Figure 4):

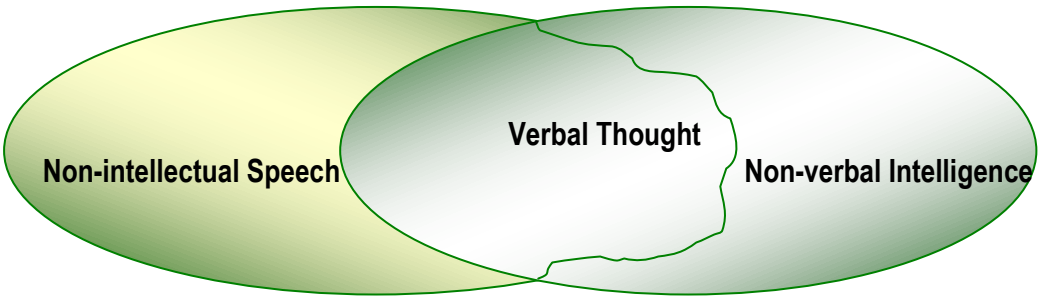


Fig. 4: The relationship between speech and intelligence in mature humans

This diagram shows that a lot of our intelligence is non-verbal, just as much of our speech (vocalization) is non-intellectual:

"Schematically, we may imagine thought and speech as two intersecting circles. In their overlapping parts, thought and speech coincide to produce what is called verbal thought. Verbal thought, however, does not by any means include all forms of thought or all forms of speech. There is a vast area of thought that has no direct relation to speech. The thinking manifested in the use of tools belongs in this area, as does practical intellect in general" [8].

We can express our physical feelings and emotions, most of which we share with animals, non-verbally – we all laugh and cry in the same way. Symbolic representation – abstract meaning – is uniquely human, and it is the society, the people around us, who make us human by giving us language, the social means of thought. The words of language are units of 'both generalising thought and social interchange' [3]. A clear correlation exists between our social and cognitive

development, between our *social interaction* and our *thinking ability* [8, 10, 11]. By installing the software of language in our brains through social interaction, the society creates human individuals. The more social stimulation we receive, the better we become at using the language tool to think.

**CONCLUSION**:

Research indicates that individual human minds are inconceivable outside of the society – not only because individuals cannot survive physically in total isolation, but because human minds are only forged in the foundry of society. We acquire our 'human difference' – abstract thought, embodied in language – only in the course of socialization.

Collective human welfare must, therefore, define the natural limits of individual freedom in society, because the natural interests of humankind (physical survival being the most basic one amongst them) are, in fact, the natural interests of every individual. We can draw a valid analogy between cancer cells, which greedily deprive other body cells of nutrients, and individuals/ organizations, who pursue their egocentric interests outside of the context of the objective public good; the outcome of both scenarios is grim.

In what is given to us by nature, we must realize our conscious purpose – our common survival. Collectively, we must de-center from egocentric nationalist concerns and begin to think in global terms; we must recognize our common purpose as a law which must determine the way and character of our actions – if we are to survive.

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## BENEFITS OF BIOLOGICAL ANTHROPOLOGY FOR PUBLIC HEALTH

**Matthew LEAVESLEY,** PhD.

Anthropology & Sociology Strand, School of Humanities and Social Sciences,
University of Papua New Guinea
(Email: matthew.leavesley@gmail.com)
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In recent years, Papua New Guinea (PNG) has seen a resurgence of interest from biological anthropologists wishing to carry out research projects. Generally speaking, biological anthropology (bio anth) draws on medical datasets in order to consider notions of human evolution. However, occasionally, in developing economies, questions are raised about the morality of collecting medical data to investigate evolution, when commonly national governments struggle to fund what might be considered routine public health programmes. Here we emphasise the point that bio anth, can produce interesting and productive synergies, including mutually beneficial datasets.

Broadly speaking, biological anthropology seeks to understand humans the world over, past and present, from an evolutionary perspective. This encompasses an understanding of humans in the context of other animals, in particular primates, the behaviour and biology of humans

throughout their evolutionary history and the study of human populations today in terms of their growth, development and health [1]. On the basis of this definition, there is clearly scope for overlapping research interests with medical sciences. In spite of this, bio anth is occasionally challenged to justify itself in the light of legitimate concerns; particularly in developing economies like PNG, questions such as "why are we allocating funds to biological anthropology when deaths during child-birth are at an unacceptably high level?" come to be asked. While this kind of emotive narrative is, of course, justified in discussions surrounding the national health policy, it can sometimes be a distraction.

There is, from time to time, a common misconception that bio anth projects draw funds away from pure medical research projects. However, in reality, grants for bio anth generally come from different funding agencies to those funding pure medical research. Or, if they do, as in the case of the National Science Foundation (US), the bio anth funds come from a different pool. One such organisation that funds bio anth and not medical science is The Wenner Gren Foundation. Therefore, bio anth can, in fact, expand the pool of money from which medical science and public health programmes can potentially benefit. What follows is a brief outline of some current and potential bio anth projects that could produce data sets of possible interest to medical science and public health.

**Studies into lifestyle diseases:**

McDade is a biological anthropologist with an interest in human biology in relation to the surrounding cultural ecological contexts, particularly in relation to human life course, especially amongst youths. His research has developed a range of biomarker methods amenable to the constraints imposed by rural field research conditions [2]. In particular, McDade has investigated the relationship between stress and cultural change amongst Samoan youths. In an extremely successful study, he brought together methodological tools from psychoneuralimmunology with anthropological models of cultural change to investigate stress and its impact on society [3, 4].

In another recent study, this time situated in New Ireland (PNG), sputum samples were collected from which levels of cortisol were measured as an indicator of relative stress. This allowed the researchers to conceive of notions of the role of stress as an indicator of human adaptability within an evolutionary framework. Suffice it to say, mental health is commonly neglected and could consequently benefit from more attention.

From a public health perspective, studies like these might allow medical scientists to gain a snapshot into the mental health baseline for the study area that might, given stringent assessment, be usefully applied or used as a guide for other parts of the country.

**Deoxyribonucleic Acid (DNA):**

In recent years, interest from both medical science and anthropology in DNA has dramatically expanded. From the perspective of anthropology, DNA is an extremely useful tool in confirming and creating genealogies. It allows biological anthropologists to interrogate notions of human evolution through charting human ancestry at a variety of scales, including the village (differentiate between families), the region, nations, as well as global population dispersals. For instance, DNA analyses have played a significant role in differentiating between the 'regional continuity' and 'out of Africa' models of human evolution and dispersals. While these studies clearly focus on anthropological questions, the data can equally be applied to issues in contemporary public health. In particular, much medical research has been undertaken into identifying specific genes that give individuals greater or lesser capacity/resistance to specific diseases. Some knowledge of the genetic character of the PNG citizenry is essential, if we are to take full advantage of any future advances in this research. Curiously enough, a significant proportion of DNA research in PNG has been undertaken by researchers such as Kayser [5], Friedlander [6] and Matisoo-Smith [7], all of whom have a keen interest in biological anthropology. Notably, the latter is a collaborating Principal Investigator on the Genographic Project designed to gain a holistic view of the human genome. Once again, this project was funded by the National Geographic, an organisation which does not commonly fund medical research. Therefore, this project, and others like it, is generating valuable data supported by non-traditional medical science funders.

Additionally, an anecdotal experience reported from a community in the Bismarck Archipelago adds an unexpected twist in support of DNA research. Recently, a Professor of biological anthropology, on the occasion of returning the results of a study to the respective community, remarked that, upon receiving the results (accompanied by extensive explanation), a community leader remarked that it seemed strange that two neighbouring communities were in the middle of a dispute, given that they were so closely genetically related.

Effectively, the results of the study where interpreted as a political avenue to unite two factions and end hostilities. In this context, the results of a bio anth driven DNA study were co-otped into a peace process that might in turn be seen as a preventative health mechanism.

**Studies into the evolution of body size (stature):**

Recent studies into human physiological stature based on data from a number of communities across the world have provided some interesting interpretative avenues in terms of human evolution. The interpretations were based on morphometric (and DNA) data reflecting human growth rates. Previously, short stature has been interpreted as a result of thermoregulatory, nutritional and/or locomotory adaptations to life in tropical forests. More recently, it has been suggested that short stature is the outcome of a life history trade-off in high mortality environments, where high mortality rates select for early reproduction - early sexual maturation and, consequently, early growth cessation [8]. This conclusion can immediately be considered in relation to our national health programme. This is because it readily identifies communities with a history of high mortality. Secondly, and by extension, the data acquired in support of this study can also be extremely useful in planning for health service delivery. Given the genetic and environmental diversity of PNG, it is particularly important to ensure that public health is tailored to the specific needs of each community. Childhood growth is of particular importance to public health. In order to prescribe the best treatment to a particular patient, it is important to have some notion of the general health characteristics of the community from which they originate. In this context, the population profile provided by bio anth could be extremely helpful.

Elsewhere, biological anthropologists are forging ahead with studies into biomechanics. In particular, Shaw and Stock [9] and Shaw [10] sought to determine the relationship between bone morphology and muscle properties in order to gain insights into muscular strength, metabolic rate and other energetic attributes that might in turn be used as an analogue to model the physiology of extinct *hominins*. Shaw used peripheral quantitative computed tomography (pQCT) to measure long-bone cross-sections of European athletes as an analogue for highly active populations [10].

The pQCT technique of data collection has great potential to contribute useful data within bio anth. Conceivably, it could be applied to consideration of issues such as the impact of rural urban drift on skeletal robusticity within an evolutionary framework.

Given the propensity for bio anth to collect statistically relevant datasets, we would indirectly gain datasets that might give us some baseline data on the proportion of diseases such as osteoporosis in the sample population. This, in turn, might be useful in planning for, and directing future medical science research into aged care.

There are also other useful spin-off benefits. On some occasions, bio anth researchers have put together teams of researchers that included a Medical Doctor. In one notable case, the project proposal included expenses for a practicing MD to visit a remote community on a regular basis (albeit for the 3 year life of the project). The MD was assigned two roles in the project. First was to assist in the bio anth data collection and, ultimately, in its interpretation; and, secondly, it allowed the community in question to have access to an MD - for the first time ever.

Lastly, and possibly most significant, is that bio anth considers medical data from both a biological and anthropological perspective. The ultimate output is, therefore, conceived from both medical and anthropological perspectives.

This cultural framework is important because, in some significant ways, culture plays an important role in public health through life-style choices. Culture also plays an important role in considering how a given community is going to respond to medical services in terms of its receptiveness to treatment. Possibly one of the few areas where this is beginning to become well understood is in relation to HIV/AIDS in PNG.

**Future directions in biological anthropology:**

A recent conference at the McDonald Institute for Archaeological Research at the University of Cambridge brought together archaeologists, evolutionary theorists and neuroscientists with the notion of forging a road towards investigating what archaeologists call the sapient paradox [11].

In a nutshell, while biological anthropologists have determined the approximate timing of the speciation event that gave rise to *Homo sapiens sapiens*, the archaeological evidence for actual modern human behaviour does not begin until significantly later. As these collaborations are in their infancy, only time will tell what spin-off benefits will arise.

**CONCLUSION:**

When properly co-ordinated, biological anthropology projects can be mutually beneficial to medical science in a variety of ways. The examples described here

illustrate how biological anthropology research can have a net benefit for anthropological studies into human evolution, medical science and Public Health Programmes by producing datasets of relevance in all these fields. The crucial step then is to carefully co-ordinate biological anthropology results so as to maximise these benefits.

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## SECTION II: ORIGINAL RESEARCH

# DIFFERENCES IN SUBMERGENCE TIME AND ALAM REACTION RATE IN *Anopheles stephensi*, *Aedes egypti* and *Culex pipiens* FOURTH INSTAR LARVAE IN RESPONSE TO VIBRATION STIMULUS

**Rodney L ITAKI**[*1] MBBS, B. Med Sci. and **Setsuo SUGURI**[2] PhD

[1]Immanuel Lutheran Rural Hospital, P.O Box 420, Wabag, Enga Province;
[2]International Medical Zoology, Faculty of Medicine, Kagawa University, Miki, Kita, Kagawa, 761-0793 Japan
*Corresponding author: email. itaki7@gmail.com

**ABSTRACT:**

Studying mosquito larvae behavior can provide insights into developing new ways in controlling vector borne diseases. Feeding is an aspect of larval behavior that has been studied extensively. Surface and underwater feeding behaviors are linked by diving. The larvae use dive behavior to feed underwater or to escape predators. We elicited dive responses in fourth instar larvae of three different mosquito species using vibration stimulus and compared their response rate and submergence time. Marked differences in the response rate and submergence time were recorded for the three species of mosquitoes. These differences can be exploited to develop new larvae control methods to reduce mosquito borne diseases.

Key words: dive behavior, *Anopheles stephensi, Culex pipiens, Aedes aegypti*, vibration stimulus, alarm reaction
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## INTRODUCTION:

In an effort to develop new ways to control mosquito borne diseases, larval behavior has been studied extensively [1-5]. Walker and Merritt [2] made an exhaustive list of larval behaviors. Generally, larval behaviors consist of a group of surface behaviors and a group of under water behaviors [2, 6]. These two groups of behaviors are linked by dive and rise behaviors. A mosquito larva after diving may remain submerged for some time. This period is known as the submergence time [2, 6]. Some factors that may determine the submergence time of a

mosquito larva include, gas exchange requirements, presence of a predator on the surface of the water and the need to feed while underwater; also included are the difference in anatomical structures and the physiological needs of different mosquito species [6].

A number of studies have examined the diving behavior or alarm reaction [1, 3, 7, 9]. To elicit a dive behavior various stimulus have been used to provoke a mosquito larva to dive [6]. It has also been found that if a stimulus is given continuously, the mosquito larva becomes desensitized to that stimulus [6]. After diving, a mosquito larva tends to stay submerged for some time before re-surfacing (personal observation). Studies examining dive behavior have established that different mosquito species may have different submergence time [1, 3, 6, 7, 9], but published data comparing the submergence time of different mosquito species under the same experimental conditions is scanty.

In the present study vibration stimulus was used to elicit dive response (alarm reaction) in fourth instar larvae of three different mosquito species (*Anopheles stephensi*, *Aedes albopictus* and *Culex pipiens*). The submergence time under controlled environment and the response rates for each of the three species of mosquitoes were calculated.

**MATERIALS and METHODS:**

Larvae and adults were maintained using standard protocols in an insectary with temperature maintained at 26$^{o}$C and relative humidity 65 % with a 15 hours 8 hours day night cycle [2, 6]. Light was provided by four 40-watt fluorescent light bulbs. Eggs were hatched in 250ml of de-chlorinated tap water in plastic cups (surface area = 95 cm$^2$). At the late second to early third instar stage, larvae were transferred to 33- x 24- x 7-cm pans. Larvae were fed on ®Tetra Min baby fish food. Water was changed every other day. Only fourth instar larvae were used in this study.

All observations were done in a cylindrical tube, length = 200cm and internal diameter = 14.9cm, water surface area = 174.28cm$^2$ and containing 374.28cm$^3$ of water. The tube was filled with de-chlorinated tap water and incubated for 1 week to permit growth of microorganisms on the walls including the floor, in the water column and at the air-water interface [2]. Larvae at the fourth instar stage were individually pipetted into the glass tube and allowed to acclimatize for one hour. A total of 65 larvae (*Anopheles stephensi* = 23, *Aedes egypti* = 21, *Culex pipiens* = 21) were used.

The vibration stimulus used was a single tap made on the side of the tube. When the

larvae did not dive, the taping was done in quick successions. A non response was recorded if the larva did not dive after two minutes of tapping.

Preliminary observations showed that *Anopheles* larvae required longer period for the stimulus to be applied before eliciting a dive response. Thus, unlike the other five minutes was allowed before recording non response for the *Anopheles* larvae. Hand held stop watch was used to record the submergence time.

**RESULTS and DISCUSSION:**

Table 1 shows fourth instar *Aedes aegypti* (100%) larvae were more sensitive to the vibration stimulus compared to *Anopheles stephensi* (0.17%) and *Culex pipiens* (4.91%) larvae.

Preliminary experiments showed that *Aedes aegypti* larvae used the entire microcosm to feed while *Culex pipiens* larvae fed by filtering while attached to the water's surface with occasional dives to feed on the walls of the cylinder. Diving in response to a vibration stimulus was a rare behavior in fourth instar *Anopheles stephensi* larvae. This species spent most of its time at the water's surface.

Although *Anopheles* larvae had the lowest response rate (0.17%), they typically remained submerged for almost five minutes compared to *Culex* and *Aedes* larvae which remained for 1.75 and 2.38 minutes respectively.

*Anopheles* larvae remained under water twice as long as *Aedes* and nearly three times as long *Culex* larvae. It was also noted that giving the stimulus intermittently did not evoke further diving response in larvae that responded to the initial stimulus. This is in line with previous studies showing mosquito larvae becoming accustomed to a stimulus if given repeatedly [6].

Larva of *Anopheles* species have a higher density compared to *Culex* and *Aedes* species [6]. The higher density could be one reason for their longer submerged time. *Culex* and *Aedes* species are less dense thus they tend to float up to the water's surface.

The *Anopheles* larvae sank to the bottom of the observation cylinder when the larva was not actively swim to the surface. *Aedes* and *Culex* larvae floated back to the water's surface even when not actively swimming. The larvae of these two species swam actively to stay submerged.

Table 1: Alarm reaction response rate and mean submergence time in fourth instar *Anopheles stephensi, Culex pipiens* and *Aedes aegypti* larvae

| Variables | *Anopheles stephensi* (n = 21) | *Culex pipiens* (n = 21) | *Aedes aeygypti* (n = 21) |
|---|---|---|---|
| Mean submergence time (minutes) | 4.25 | 1.75 | 2.38 |
| Response rate (%) | 0.17 | 40.91 | 100.0 |
| Non-response rate (%) | 83.0 | 50.09 | 0.00 |

The difference in the alignment of the larvae to the water surface may also be a reason why there was a marked difference in the response rate. *Aedes* and *Culex* larvae hang obliquely to the water surface compared to *Anopheles* species which lie parallel to the surface. This difference in orientation may result in differences in the surface tension force acting on the siphon of the larvae. As a result, *Anopheles* larvae may require a slightly greater force to break free from the water surface compared to *Aedes* and *Culex* larvae which may need less strength. A simple wriggle of their body may be sufficient to break free from the water's surface. Differences in feeding behavior between the three species are well documented [1, 2]. The difference noted in the response rate and submergence time may be a reflection of the difference in feeding behavior. Differences in gas exchange requirements influence the duration that the larvae stay submerged. Gray and Bradly [10] have shown that adult mosquitoes breathe discontinuously. Hetz and Bradly [11] further hypothesized that the discontinuous breathing may be a mechanism to prevent injury from oxygen radicals. It is not known if mosquito larvae also breathe discontinuously. However, it is possible that a similar mechanism observed in insects that live underground [12] may exist in mosquito larvae and this mechanism may be different in the larvae of different mosquito species. Thus the differences observed in the larvae of the three mosquito species in the present study may reflect differences in gas exchange requirements.

The differences in surface and submerged time could be determined by frequency in diving episodes (preliminary observations). This study did not examine the time larvae spent at the water surface. However, we can speculate that larval species that tend to spend most of their time feeding at the water surface may be affected by larvicides that float while larval species that dive frequently and or spend most of their time submerged may be affected by larvicides that are dissolved in water. Thus according to some researchers [13-15], this explains some of the differences observed in natural oil toxicity against mosquito larvae.

One can suggest that laboratory experiments studying larvicides need to consider differences in larval behavior in different mosquito species and incorporate these differences in their experiment designs. In addition, users and manufacturers of commercially available larvicides need to consider differences in larval behavior, then reformulate the constituents and vary the application protocols to suit targeted mosquito species.

The changes in the use of commercially available larvicides may allow larvae of specific mosquito species to be targeted.

**CONCLUSION:**

Our results showed difference in the alarm reaction rate of *Anopheles*, *Culex* and *Aedes* fourth instar larvae in response to vibration stimulus. The three species also showed differences in their mean submergence time. These findings confirmed pervious studies on mosquito larva behavior [1-9].

The difference in submergence time and alarm response rate may be due to differences in the anatomy and physiological requirements in the species studied. The differences observed can be exploited to develop new larval control methods or to improve existing ones. More detailed studies should be done to re-examine mosquito larvae behavior.

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# ASSESSMENT OF SERUM CHOLINESTERASE (PSEUDOCHOLINESTERASE) AND DIBUCAINE NUMBER AMONG STUDENTS IN THE UNIVERSITY OF PAPUA NEW GUINEA

**Rachael ROWE, Wesley PURI, Victor J. TEMPLE*, Harry AIGEELENG ** and Samson GRANT***

Division of Health Sciences, *Division of Basic Medical Sciences and **Division of Clinical Sciences, School of Medicine and Health Sciences, University of Papua New Guinea
{Funded by research grant from: Office of Higher Education, Research, Science and Technology Papua New Guinea}

(Corresponding Author: VJ. Temple: templevictor@gmail.com)

**ABSTRACT**:

Serum Cholinesterase (SChE) or Pseudocholinesterase can be used for assessing the sensitivity to Succinylcholine (Scoline) or as indicator for insecticide and pesticide poisoning.
This cross-sectional study assessed the SChE and Dibucaine number (DN) among students in the University of Papua New Guinea.

The single proportion formula was used to estimate the sample size. A total of 276 apparently healthy students were selected by simple random sampling. The "Linear Chemicals" reagent kit for assay of SChE was used to determine the SChE activity. The DN was estimated by assay of the SChE with and without the inhibitor Dibucaine. Signed informed consent was obtained from 217 students, which consisted of 99 (45.6%) males and 118 (54.4%) females. The mean ages for the male and female students were 26.0 ± 6.5years and 23.8 ± 5.3 years respectively.

The median SChE activity was 3.74KU/L for the males and 3.51KU/L for the females. The Interquartile Ranges (IQR) of the SChE activities for the male and female students was 2.80 – 4.48KU/L and 3.02 – 4.13KU/L respectively. Although there was no statistically significant difference between the SChE activity of the male and female students, considerable variation was observed in the SChE activity in both the male and female students.

The median DN for the male students was 85.43 % and the IQR was 81.6% – 88.2%. For the female students the median was 86.1% and the IQR was 83.5% – 88.0%. No statistically significant difference was obtained between the DN for the male and female students.

The data strongly supports the need for screening of students and other patients before administering Scoline which is the muscle relaxant commonly used in the Port Moresby General Hospital and other hospitals in National Capital District in Papua New Guinea.

Key words: Serum, Cholinesterase, Pseudocholinesterase, Dibucaine, Scoline, Students,
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**INTRODUCTION:**

The most physiologically important Cholinesterase (ChE) is Acetylcholinesterase (AChE) located in the presynaptic knobs of cholinergic neurones, red blood cells, lungs and spleen [1, 2]. The functions of AChE are well defined [1, 2, 3]. The other ChE is Serum Cholinesterase (SChE), which is mainly located in the plasma and serum [1, 2, 3]. The alternative names for SChE are Pseudocholinesterase, Acetylcholine Acylhydrolase, Benzoyl Cholinesterase and Cholinesterase II [1, 2]. Although the major biological function of SChE is not well defined, several researchers have suggested that it catalyzes the hydrolysis of acetylcholine and other esters of Choline in the blood, such as, Succinylcholine (Suxamethonium) and Mivacurium used as muscle relaxants during surgical procedures [2 – 5]. Some ester type compounds like Procaine, Chloroprocaine, Tetracaine, Cocaine and Heroin are hydrolysed by SChE [6, 7].

Both AChE and SChE activities are inhibited by organophosphorus compounds in pesticides and insecticides [1, 2]. During exposure to these toxicants the SChE activity usually falls more rapidly compared to AChE, thus clinical signs or symptoms are not detected under limited exposures [1, 2]. The change in SChE activity is used as a sensitive biochemical parameter for assessing the exposure of an individual to organophosphorus compounds and sensitivity to Succinylcholine [2 – 5].

Two major genetic variants (“atypical” and “normal”) of SChE are known [2,3,6]. This gave rise to three forms of SChE. Individuals with the “atypical variant” form of SChE are homozygous for the “atypical” gene; those with the “normal variant” form are homozygous for the normal gene [2, 3, 6]. The third variant are individuals that are heterozygous for SChE [2,3,6].

The SChE activity is usually low in an individual with the atypical variant compared to the high SChE activity in an individual

with the normal variant [2,3,6]. For the heterozygous individual the SChE activity is between these two extremes [2,3,6]. These three variant forms of SChE can be identified by reaction with Dibucaine, which is an inhibitor of SChE [3, 7 – 9]. Dibucaine (Nupercaine) is a local anaesthetic [7]. Irrespective of the level of SChE in blood the activity of the normal variant is greatly inhibited by Dibucaine compared to the activity of the atypical variant [7, 8, 9]. Dibucaine inhibits the activity of the normal variant form of SChE by 80%, compared to a 20% inhibition of the atypical variant form of SChE [7, 8, 10]. Dibucaine inhibits the heterozygous variant form of SChE by between 40 to 60% [7, 8, 10].

The Dibucaine number (DN) is the percentage inhibition of SChE by Dibucaine under specified conditions [7 – 10]. The DN is an important diagnostic characteristic of an individual because it is independent of the amount of SChE in the blood [7]. Thus the adequacy of SChE in an individual can be assessed both quantitatively and qualitatively. The SChE activity is used as the parameter for the quantitative assessment and the DN is used as the parameter for the qualitative assessment [7].

The low SChE activity in some individuals may be an inherited enzyme abnormality that results in abnormally slow degradation of the acetylcholine like esters. These individuals may be Suxamethonium-sensitive and may have qualitatively low SChE level, which is a genetically determined trait. The clinical consequence of this may include prolonged paralysis of the respiratory and other skeletal muscles after administration of a standard dose of Succinylcholine [4 – 7, 10]. It is therefore important to assess the SChE activity and DN prior to the administration of Suxamethonium, to minimise the risk of prolonged muscular relaxation and apnoea in susceptible individuals [4 – 7, 9].

In most developed countries the assay of SChE is part of the routine laboratory tests carried out either for assessing the sensitivity to Succinylcholine or for assessing liver function or as an indicator for insecticide and pesticide poisoning [5]. The lack of such routine laboratory tests in most developing countries with high incidence of malaria is worrisome. This is because the predominant types of insecticides and pesticides used to reduce the spread of the malaria parasites are organophosphates and carbamates that are highly toxic to both humans and other organisms [9].

A review of the literature indicates that published data on the prevailing SChE and

the SChE variants (DN) in the Papua New Guinea (PNG) population is scanty [11]. The use of a variety of insecticides in the form of sprays and coils for protection against mosquito bites is a common practice among students in the University of PNG (UPNG). Chronic exposure to these insecticides can results in depression of SChE activity without affecting the DN. This study was prompted by the apparent lack of data to characterize SChE among students in the UPNG.

The aim of this study was to assess the SChE activity and DN among students in the University of Papua New Guinea. Knowledge of the SChE activity and variants that are prevalent among the students can be used by appropriate authorities to make informed recommendations for the screening of students and other patients before using Scoline as muscle relaxant in the Port Moresby General Hospital (PMGH), which serve as the major general, specialist and reference hospital in NCD and PNG.

## SUBJECTS AND METHODS

This was a prospective cross-sectional study carried out in the NCD, which is the incorporated area around Port Moresby the capital city of PNG. The sampling sites included both campuses (Waigani and Taurama) of the University of Papua New Guinea (UPNG). This study was conducted among students because of their easy accessibility and willingness to donate blood samples for research purpose.

The single proportion formula was used to estimate the sample size, which was calculated using a design effect of one, a relative precision of 7.0% and a confidence level of 95%. An assumed prevalence rate of 25% was used because of the non availability of data on the characteristics of SChE among student in the UPNG. The sample size of about 270 apparently healthy students was considered adequate for a study, with a predicted non-response rate of 20%.

A total of 276 apparently healthy students (subjects) were selected by simple random sampling. The purpose of the study was appropriately explained to each of them before requesting their signed informed consent. All students had been living in the UPNG for at least twelve weeks before sample collection, which is less than the fifteen weeks academic session in the UPNG. Blood sample was collected only once from a consented student. About 0.5ml of blood was collected into a plain sterile microtainer after finger-stick using contacted activated single-use lancet. Each clotted blood was centrifuged to obtain the serum

which was then decanted and stored frozen at – 70°C, until required for analysis.

The "Linear Chemicals" reagent kit for assay of SChE (total and inhibited) was used [10]. All additional reagents used were of analytical grade. The assay of SChE and determination of the DN were carried out as indicated in the manufacturer's protocol [10]. The SChE catalyzes hydrolysis of the substrate Butyryl-Thio-Choline, forming Butyrate and Thiocholine. The Thiocholine interacts with 5,5`-Mercaptobis-2-Nitrobenzoic acid (DMNB) to form a yellow coloured compound {5-Mercapto-2-Nitrobenzoate (5-MNBA)}. The progress of the reaction (at 30$^{o}$ Celsius) was monitored at 405nm and the change in Absorbance ($\Delta$A) recorded ever 30 seconds [10]. The SChE activity was calculated thus: SChE Activity (KU/L) = ($\Delta$A/30sec) x (23111/1000) [10].

The DN was estimated by assay of the SChE with and without the inhibitor Dibucaine. The percent inhibition of the SChE was calculated as follows: One minus the ratio of inhibited to un-inhibited SChE expressed as percent. The percent inhibition is the DN of SChE in the sample assayed [10]. Internal bench quality control (QC) was carried out using the normal and abnormal QC samples supplied by the manufacturer [10].

Data analysis was by the Microsoft Excel Data Pack and Statistical Package for Social Sciences (SPSS) softwares. Kolmogorov-Smirnov test for normality was used to assess the distribution of the data. Chi-square test, Mann-Whitney test and Wilcoxon U tests were used as appropriate.

Ethical clearance and approval for this project were obtained from the Research and Ethics Committee in the School of Medicine and Health Sciences UPNG and the Chief Executive Officer PMGH.

**RESULTS:**

Blood samples were collected from 217 of the 276 apparently health students randomly selected for participation in this study. This gave a consent rate of 78.6%. The 217 students consisted of 99 (45.6%) males and 118 (54.4%) females. Table 1 shows the summary statistics of the age for the male and female students. The mean age for the male students was 26.0 ± 6.5 years (Mean ± STD), the median age was 23.0 years and the Interquartile Range (IQR) was 22.0 – 30.0 years. For the female students the mean age was 23.8 ± 5.3 years, the median was 22.0 years and the IQR was 21.0 – 24.0 years.

The Kruskal-Wallis and Chi-square tests indicated that the mean age of the male

students was significantly (p = 0.001) higher than that of the female students. The Mann-Whitney and Wilcoxon U tests also indicated statistically significant (p = 0.001) differences between the mean ages of the male and female students.

The Kolmogorov-Smirnov test for normality of distribution indicates that the SChE activities (KU/L) obtained for the male and female students were not normally distributed. This was confirmed by the outliers shown in the Box-plots of the SChE activities (KU/L) for the males and females presented in Fig. 1. Thus, non-parametric statistics were used for analyzing the data.

Table 2 shows the summary statistics of SChE activities (KU/L) for the male and female students. The median SChE activity was 3.74KU/L for the males and 3.51KU/L for the females. The Interquartile Ranges (IQR) of the SChE activities for the male and female students was 2.80 – 4.48KU/L and 3.02 – 4.13KU/L respectively.

Mann-Whitney and Wilcoxon U tests (p = 0.432), Kruskal-Wallis test (p = 0.432) and Chi-Square test (p = 0.379) indicated that the SChE activity (KU/L) of the male students was not statistically different from that of the female students. Analysis of Variance (ANOVA) after Post-Hoc analysis (Scheffe) also indicated that there was no statistically significant (p > 0.05) difference between the SChE activity of the male and female students.

Table 1: Summary statistics of the age (years) for the male and female students

| Parameters | Male students (n = 99) | Females students (n = 118) |
|---|---|---|
| Mean (years) | 26.0 | 23.8 |
| Median (years) | 23.0 | 22.0 |
| 95% Confidence Interval (95% CI) | 24.6 – 27.3 | 22.8 – 24.8 |
| Standard Deviation (STD) | 6.5 | 5.3 |
| Range (years) | 15.0 – 56.0 | 14.0 – 51.0 |
| Interquartile Range (IQR) (years) | 22.0 – 30.0 | 21.0 – 24.0 |

Fig. 1: Box-plots of Serum Cholinesterase activity (KU.L)

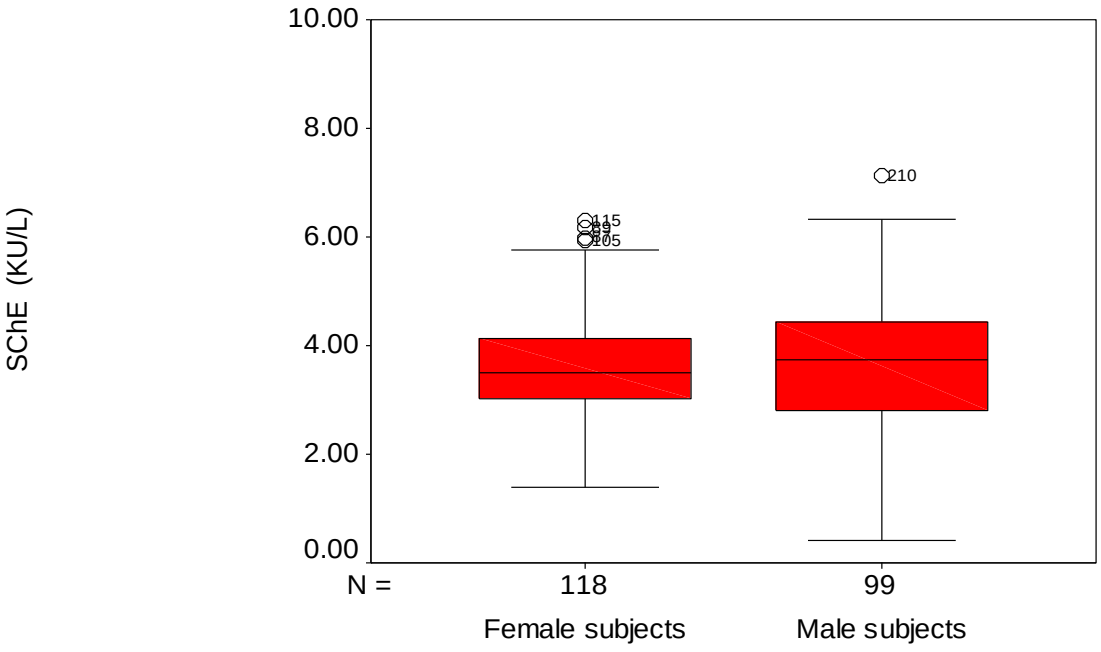


Table 2:
Summary statistics of the Serum Cholinesterase activity (KU/L) for male and female students

| SChE activity (KU/L) | Male students (n = 99) | Female students (n = 118) |
|---|---|---|
| Median | 3.74 | 3.51 |
| IQR | 2.80 – 4.48 | 3.02 – 4.13 |
| Mean | 3.64 | 3.36 |
| 95% CI | 3.37 – 3.90 | 3.44 – 3.81 |
| STD | 1.32 | 1.02 |
| Range | 0.51 – 7.13 | 1.40 – 8.76 |
| Calculated Reference Ranges | 0.67 – 6.43 | 0.85 – 6.13 |

Table 3:
Percentile cut-offs for Serum Cholinesterase activity (KU/L) for male and female students

| Percentiles | Male students (n = 99) | Female students (n = 118) |
|---|---|---|
| 5th | 1.16 | 2.11 |
| 10th | 1.91 | 2.62 |
| 25th | 2.8 | 3.02 |
| 50th | 3.74 | 3.51 |
| 75th | 4.48 | 4.13 |
| 90th | 5.17 | 4.49 |
| 95th | 6.02 | 5.76 |

Table 4:
Distribution of male and female students according to their Serum Cholinesterase Activity (KU/L)

| SChE activity (KU/L) [10] | Male students (n = 99) | Female students (n = 118) |
|---|---|---|
| < 2.4 | 14 (14.1%) | 7 (5.9%) |
| 2.4 – 8.5 | 85 (85.9%) | 110 (93.2%) |
| > 8.5 | 0 | 1 (0.9%) |

*Reference 10

The percentile cut-offs for the SChE activities for the male and female students are presented in Table 3. The 5th to 25th Percentile cut-offs for the males were lower than that of the females. This trend changed for the 50th to 95th Percentile cut-offs were the values for the males were higher than the values for the females.

The distribution of the male and female subjects according to their SChE activity is presented in Table 4. Normal SChE activity was prevalent in 85.9% of males and 93.2%

of females. The SChE activity was below the lower limit of normal in 14.1% of males and 5.9% of females. When asked about the use of mosquito coils and sprays positive responses were obtained from 4.0% of males and 7.6% of females. Most of the male and female students had no knowledge about the content of the mosquito coils and sprays that they are using against malaria.

The Spearman's rho (r = - 0.03, p = 0.76) indicated weak non-statistically significant inverse relationship between the SChE activity and age among the male students. A similar relationship was indicated by the Pearson coefficient of correlation (r = - 0.085, p = 0.4).

For the female students, Spearman's rho (r = 0.06, p = 0.516) showed weak linear non-statistically significant relationship between the SChE activity and age. The Pearson coefficient of correlation also indicated weak (r = 0.06) linear non-significant (p = 5.1) relationship between the SChE activity and age.

The reference range (Mean ± 2 STD) of the SChE activity (KU/L) was calculated after log-transforming the SChE data. The calculated Reference Range of the SChE activity for the males was 0.67 – 6.43KU/L and for the females was 0.85 – 6.13KU/L.

Test for normality of distribution of the Dibucaine Numbers (DN) for the male and female students indicates that the DN was not normally distributed. The Box-plots of the DN data for the male and female students are shown in Fig 2. Outliers are indicated in the Box-plot for only the female students.

The summary statistics of the DN for both male and female students are presented in Table 5. The median DN for the males was 85.43 % and the IQR was 81.6% – 88.2%. For the females the median was 86.1% and the IQR was 83.5% – 88.0%. No significant difference was obtained between the DN for the male and female students, when tested statistically using the Mann-Whitney and Wilcoxon U tests (p = 0.225), the Kruskal-Wallis test (p = 0.225) and Chi-Square test (p = 0.304). Similar trend was observed (p = 0.34) using the analysis of variance (ANOVA) after Post-Hoc analysis (Scheffe).

The percentile cut-offs obtained for the DN for the male and female students are presented in Table 6. The 5$^{th}$ to 50$^{th}$ Percentile cut-offs for the males were lower than that of the females.

Fig. 2: Dibucaine Numbers (%) for male and female subjects

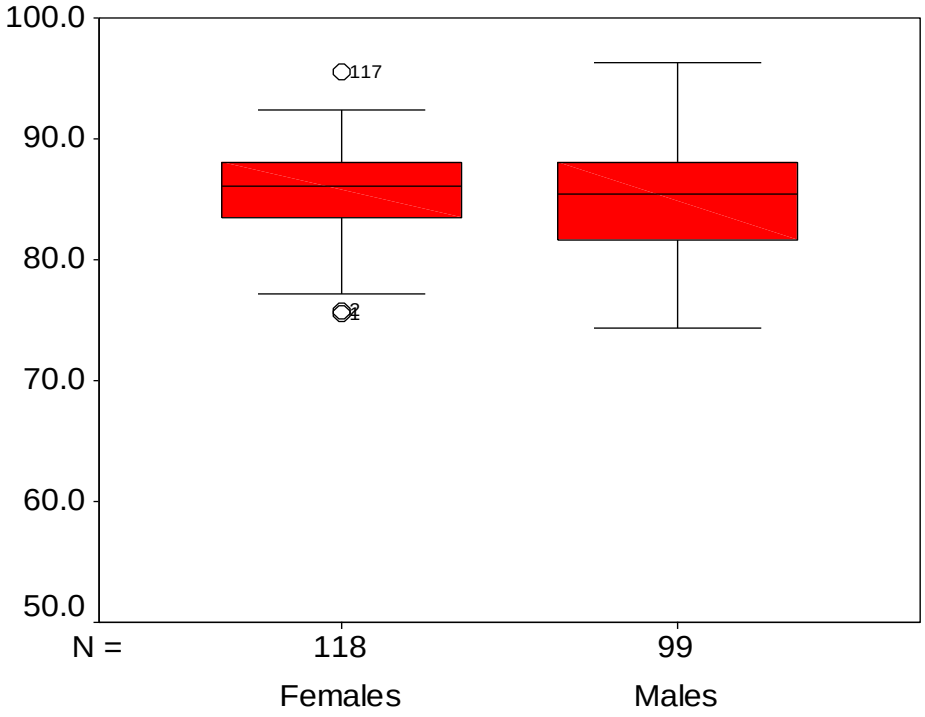


Table 5: Summary statistics of the Dibucaine Number (%) for male and female students

| Dibucaine Number (%) | Male students (n = 99) | Female students (n = 118) |
|---|---|---|
| Median | 85.4 | 86.1 |
| IQR | 81.6 – 88.2 | 83.5 – 88.0 |
| Mean | 84.4 | 85.6 |
| 95% CI | 83.7 – 85.8 | 85.0 – 86.3 |
| Std Dev | 5.1 | 3.6 |
| Range | 61.9 – 96.3 | 75.5 – 95.5 |

Table 6: Percentile cut-offs for Dibucaine Numbers (DN) for the male and female students

| Percentiles | Male students (n = 99) | Female students (n = 118) |
|---|---|---|
| 5th | 77.0 | 77.6 |
| 10th | 77.8 | 80.8 |
| 25th | 81.6 | 83.5 |
| 50th | 85.4 | 86.1 |
| 75th | 88.2 | 88.0 |
| 90th | 90.4 | 89.6 |
| 95th | 92.5 | 90.8 |

Table 7: Distribution of the male and female students according to their Dibucaine Number (variant form) of Serum Cholinesterase

| *Dibucaine Number (DN %) [10] | Male students (%) | Female students (%) |
|---|---|---|
| 0 – 20 (Atypical variant) | 0 | 0 |
| 20 – 69 (Heterozygous) | 1.0 (1.0%) | 0 |
| 70 – 90 (Normal variant) | 86.0 (86.9%) | 109.0 (92.4%) |
| > 90 | 12.0 (12.1%) | 9.0 (7.6%) |

*Reference 10

The distribution of the male and female students according to their DN is presented in Table 7. The atypical variant SChE (DN between 0 – 20%) was not detected in both the male and female students. Normal variant SChE (DN between 70 – 90%) was prevalent in 86 (86.9%) of males and 109 (92.4%) of females. The DN was greater than 90% in 12 (12.1%) males and 9 (7.6%) females.

The Bivariate (2-tailed) correlation tests between the SChE activities and DN indicated strong linear statistically significant

relationship for the males students (Spearman's rho = 0.453, p = 0.01; and Pearson coefficient of correlation r = 0.454, p = 0.01). Similar trends were obtained for the female students, Spearman's rho = 0.359, p = 0.01; and Pearson coefficient of correlation r = 0.382, p = 0.01.

The Spearman's coefficient of correlation (rho = 0.083) indicated weak linear non-statistically significant (p = 0.414) relationship between the DN and age of the males students. A weak (rho = - 0.081) inverse non-statistically significant (p = 0.38) relationship between the DN and age of the female subjects was indicated by the Spearman's coefficient of correlation.

**DISCUSSION:**

The unit for expressing SChE activity depends on the procedure and the method used to assay the enzyme [7, 8, 10 – 13]. Thus, there is no universally acceptable normal reference range that can be used for the comparison of the SChE activity obtained from laboratories using different assay techniques [7, 8, 10 – 13]. However, most commercial kits for assay of SChE activity tend to use the International Unit (U) under specified conditions [8, 10, 13].

In the present study the results were interpreted using the reference range for SChE activity and the cut-off points for the DN recommended by the manufacturer ("Linear Chemicals") of the analytical kits [10].

The total non-response rate of 21.4% (59 students) was slightly higher that the predicted non-response rate of 20.0% used in the calculation of the sample size for this study.

The mean SChE activity for the male (3.64KU/L) and female (3.63KU/L) students were within the normal range (2.4 – 8.5KU/L) recommended by the manufacturer of the SChE analytic kits [10] used in the present study. Both values were also within the normal range (2.2 – 5.2KU/L) reported by other authors that have used similar analytical procedure [14]. Although there was no statistically significant difference between the SChE activity of the male and female students in the present study, the SChE activity of 14.1% of the males was below the lower limit of the normal range compared to 5.9% of the females. Our data supports the findings reported by Curtain et al [11] that no individuals were found with extremely low or zero SChE activity and that considerable variation was observed in the SChE activity between the male and female students in Papua New Guinea [11]. The 10$^{th}$ percentile SChE activity (1.91KU/L) for the male

students was significantly lower (p = 0.01) than the corresponding value (2.61KU/L) for the female students and was also below the lower limit of the normal range (2.4KU/L).

The 5th and 95th Percentile SChE activities for the male and female students (Table 3) were within the calculated reference ranges obtained for these groups in the present study (Table2). Several researchers have suggested that when reference ranges are established locally, SChE activity becomes a sensitive indicator and of pesticide poisoning, loss of hepatic biosynthetic capacity and unusual sensitivity to Succinylcholine [12 – 14]. The calculated reference ranges obtained for the male and female students in the present study can server as guidelines for the quantitative assessment of SChE activity among the population in NCD. The lower SChE reference range can serve as the exposure index for assessing low-level and chronic residual exposures among students that regularly use mosquito coils and sprays. There is the need to advocate for improve knowledge and awareness among students about the negative impact on health of the long term use of insecticides that contain organophosphates and carbamates.

The DN below 20% which is characteristic of the atypical variant of SChE was not present in the male and female students. This result supports the earlier report by Curtain et al [11] that homozygote for the atypical variant of SChE was not found in peoples in Papua New Guinea. The non-detection (zero percent) of individuals with the atypical variant of SChE in our present study and that of Curtain et al may be due to the low number of subjects (217 students in our study and 2144 individuals in that of Curtain et al) studied. Pestel et al [15] in a study of 24,830 Europeans reported a prevalence of 0.07% atypical homozygous variant which gave an incidence rate of 1:1400. Pestel et al also reported 1.23% prevalence of the heterozygous variant amount Europeans. This is higher than the 0.46% prevalence of the heterozygous variant among the 217 subjects in our present study. The 12.1% and 7.6% of male and female students respectively with DN greater than 90% which is above the upper limit of the cut-off points for the normal variant (DN 70 – 90%) requires further investigation. Several authors [7, 16] have indicated that appearance of additional variant forms of SChE may be caused by a variety of mutation on the gene locus encoding for the enzyme.

**CONCLUSION**:

Although there was no statistically significant difference between the SChE activity of the male and female students, considerable variation was observed in the

SChE activity in both the male and female students. However, none of these students were found with extremely low or zero SChE activity. No statistically significant difference was obtained between the DN of the male and female students.

Our data strongly supports the need for screening of students and other patients before administering Scoline which is the muscle relaxant commonly used in the PMGH and other hospitals in NCD. The SChE activity is the parameter for the quantitative assessment and the DN is the parameter for the qualitative assessment.

There is the need to advocate for improve knowledge and awareness among students about the negative impact on health of the long term use of insecticides that contain organophosphates and carbamates.

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## CIGARETTE SMOKING AMONG NIGERIAN ADOLESCENT PUBLIC SENIOR SECONDARY SCHOOL BOYS: PREVALENCE, CHARACTERISTICS AND ATTITUDES

**Alphonsus N. Onyiriuka *[1] and Rita C. Onyiriuka[2]**

[1] Department of Child Health, University of Benin Teaching Hospital, PMB 1111, Benin City, Nigeria.
[2] Department of Economics and Statistics, Faculty of Social Sciences, University of Benin, Benin City, Nigeria.
(*Correspondence Author: Dr. A. N. Onyiriuka; E-mail: alpndiony@yahoo.com; didiruka@gmail.com)

**ABSTRACT:**

Although recent trends indicate a rising cigarette-smoking prevalence among adolescents in African countries, data on characteristics and attitudes of these African adolescent smokers are scarce despite the peculiar socio-cultural and economic patterns of African countries compared to Western and Asian countries. This study sought to determine the prevalence of cigarette smoking among public senior secondary (SS) school boys in Oredo Local Government Area (LGA) of Edo State and identify the associated risk factors and behaviors.

In this school-based cross-sectional study, anonymous self-administered questionnaire was used in obtaining data on cigarette smoking from 1,060 students in the two males-only secondary schools located in the LGA. The prevalence of cigarette smoking was 12.0%, 22.4% and 17.1% among first-year (SS1), second-year (SS2) and third-year (SS3) students respectively, with an overall prevalence of 16.5%. The mean age at which smoking began was 16.9 $\pm$ 1.2 years (95% confidence interval, CI = 16.7 – 17.1). A significantly higher risk of smoking was associated with having parents, elder siblings or best friends who smoke. Tolerant attitude of parents and best friends towards smoking by their sons/friends encouraged

smoking among students. The two major reasons for taking up smoking were peer pressure effect (36.5%) and the need to mix freely with peers in social situations (22.9%). All the smokers were "light smokers" (smoking less than 10 sticks of cigarette per day). 3.4% of the smokers smoked their first cigarette within 30 minutes after waking up in the morning. Among the smokers, 80.6% admitted smoking was harmful and 60% said they would like to quit smoking but lacked the will power and were uncertain how to quit. Considering that the prevalence of smoking among adolescents tend to increase with age, school-based smoking control intervention programs should start in the primary/junior secondary schools and they must also address family influences on adolescent smoking. Empowering adolescents with skills to resist peer pressure is advocated.

Keywords: Cigarette, smoking, adolescents, boys, public schools.
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## INTRODUCTION

Cigarette-smoking which often begins during adolescence is on the rise and constitutes a public health problem, not only because of the preventable morbidity and mortality it causes, but also, because of its socioeconomic implications [1, 2]. The prevalence rate of cigarette smoking is declining in developed countries but rising in developing countries [3, 4]. Despite this trend, there is paucity of information on cigarette smoking among adolescents in most developing countries [5].

Cigarette smoking commonly begins during adolescence when several factors such as peer pressure, family influences, social class, curiosity, defiance of social norms and the desire to imitate others influence a youth to start smoking and maintain the habit [6, 7].

Adverse effects of cigarette smoking may occur during adolescence. For instance, increased prevalence of chronic cough, sputum production, wheezing, bad breath and yellowish discoloration of the teeth have been documented [8]. Some components in cigarette smoke are known to induce xenobiotic enzymes in the smooth endoplasmic reticulum in hepatocytes [8]. This process can influence metabolism of some drugs such as phenacetin, theophylline and imipramine, including affecting the rate in some endogenously produced

hormones [8]. Studies have shown that adolescent cigarette smokers are at increased risk of other forms of drug misuse [9, 11]. Inhalation of cigarette smoke (passive smoking) may predispose adolescents to early pharmacological dependence on cigarette [10].

Adolescent smokers may become nicotine dependent by smoking fewer cigarette sticks per day compared to adult smokers [8]. More than 90% of adolescent smokers may become adult smokers [8].

One of the signs of nicotine dependence is smoking more than 10 sticks of cigarette per day or feeling a craving to smoke their first cigarette within 30 minutes after waking up in the morning [12, 13].

Prevalence of cigarette smoking among male secondary school students varies from country to country; Republic of Yemen 21.9%, Saudi Arabia 20.0%, Syria 15.9%, Portugal 21.5% , and Bahrain 21.0% [9, 14 – 17]. In Nigeria, there is paucity of information on the prevalence of cigarette smoking, smoking behaviors and attitudes toward smoking among adolescent secondary school students [18]. Ibe and Ele [19] recently studied only female secondary school students in Nigeria despite the reported higher prevalence among male students by other authors [1, 5, 9, 20].

The present study, therefore, sought to determine the prevalence of cigarette smoking among adolescent senior secondary school male students in Oredo LGA and to assess the characteristics and attitudes of the smokers.

## PARTICIPANTS AND METHODS

This cross-sectional study was conducted in two public senior secondary schools for male students in Oredo LGA, Edo State, Nigeria. According to Edo State Ministry of Education Statistics, there are nine public senior secondary (SS) schools in the LGA comprising 4 females-only, 3 co-educational and 2 Males-only [21]. Students in all the classes in the two males-only public SS schools were recruited for this study. Consent for the study was obtained from the school authorities.

Data was collected between January and February 2009, using a modified anonymous self-administered

questionnaire based on WHO guidelines for conducting surveys on smoking prevalence and behavior [22]. In the survey design, we emphasized to the students that the questionnaire was anonymous and their participation was entirely voluntary in order to protect the students' privacy and minimize underreporting.

In this survey, information was collected on socio-demographic characteristics, smoking behavior which included questions on: smoking status, age at which smoking started, number of cigarettes smoked per day, reasons for smoking, place of smoking, place of procurement of cigarette, whether or not younger siblings go to purchase cigarette for them and the time they smoke their first cigarette on waking up in the morning and whether or not they smoke in the presence of their parents. The attitude of smokers towards the harmful nature of smoking and quitting of smoking was also assessed. The disapproving attitude or otherwise of the smokers' close contact toward smoking was documented. The socio-economic classification of each student was determined using a scoring system suggested by Ogunlesi et al, [23], which combined the highest educational attainment, occupation and income of the parents.

The scores for both parents were used to assign their children to socio-economic classes (I to V). Social classes I and II represent the high social class, social class III represents the middle social class, and social classes IV and V represent the low social class [23].

The individual academic performance of each student was assessed by classifying them into three levels, "never failed", "failed once" and "failed more than once". In this study, each failure refers to having to repeat one year at school. The smokers were also assessed for signs of nicotine dependence by identifying those who smoked 10 or more sticks of cigarette per day or feel a craving to smoke their first cigarette within 30 minutes after waking up in the morning; these signs were used to indicate potential nicotine dependence requiring initiation of treatment [12, 13].

Data management and analysis were performed using the SPSS statistical program. Chi-square test was used for assessing the significance of differences between smoking status

and study variables among respondents. A p-value <0.05 was considered significant. Odd ratio (OR) and 95% confidence intervals (CI) were calculated.

{Smokers were those who smoked at the time of the study, whether daily, weekly or less than weekly. Ex-smokers were those who have smoked before but were not smoking at the time of the study. "Non smokers" were those who have never smoked and ex-smokers combined [22]. Those who smoked less than 10 sticks of cigarette per day were classified as "light smokers" while those who smoked 10 or more sticks of cigarette per day were classified as "heavy smokers"}.

**RESULTS:**

At the time of this survey, a total of 1073 male students (974 in school A and 99 in school B) were attending the two males-only public SS schools in the LGA. The response rates were 99.4% in school A and 96.0% in school B. Overall response rate was 99.1%.

The questionnaires of 3 students were excluded from the analysis because they were incompletely filled, thereby leaving a total of 1060 questionnaires for data analysis. Students in both schools had similar socio-demographic characteristics, thus further analysis of data was carried out for the combined group of students.

Of the 1060 respondents, 175 (16.5%) were smokers and 885 (83.5%) were non-smokers. Among the non-smokers 79 (8.9%) were ex-smokers and 806 (91.1%) were never-smokers.

Table 1 shows the socio-demographic variables and prevalence of cigarette smoking for the respondents. Prevalence was higher among students in their third year (SS3) and older (aged between 17 and 21 years) and from families in the middle social class. The mean age at which smoking commenced was 16.9 ± 1.2 years (95% confidence interval, CI = 16.7 – 17.1).

Both smokers and non-smokers had similar economic and demographic profiles. Results obtained for the characteristics and attitudes towards smoking by the 175 smokers are presented in Table 2. Peer group pressure and the need to mix freely with peers in social situations together accounted for 59.4% of the reasons for smoking by respondents.

All the smokers were "light smokers" (smoked less than 10 sticks of cigarette per day) but 3.4% smoked their first cigarette within 30 minutes after waking up in the morning.

The majority of smokers (84.5%) and non-smokers (87.2%) lived with both parents. The remainder lived with either one parent or other relatives.

**Table 1:** Socio-demographic variables and prevalence of cigarette-smoking for the respondents

| Socio demographic variables | No of respondents (%) | No of smokers | Prevalence in percent |
|---|---|---|---|
| **School year** | | | |
| First year (SS1) | 366 (34.5) | 37 | 10.1 |
| Second year (SS2) | 359 (33.9) | 53 | 14.8 |
| Third year (SS3) | 335 (31.6) | 85 | 25.4 |
| **Total** | **1060 (100)** | **175** | **16.5** |
| **Age group (years)** | | | |
| 13 – 15 | 170 (16.0) | 17 | 10.0 |
| 16 – 17 | 715 (67.5) | 111 | 15.5 |
| 18 –21 | 175 (16.5) | 117 | 66.9 |
| **Total** | **1060 (100)** | **175** | **16.5** |
| **Social class of family*** | | | |
| High social class | 335 (61.6) | 33 | 9.9 |
| Middle social class | 412 (38.9) | 74 | 18.0 |
| Low social class | 313 (29.5) | 47 | 15.0 |
| **Total** | **1060 (100)** | **164** | **15.5** |

*Eleven (6.3%) of the smokers have lost one of their parents, as a result were excluded from the social class determination.

One hundred and forty one (80.6%) smokers were aware that cigarette smoking is harmful to the body through the warning during advertisements (radio/bill boards). The smokers enjoyed smoking most in the following circumstances: Social gathering with peers/friends (60.0%); During manual work e.g., farming (15.0%); At home behind the house (11.0%); In the bedroom (10.0%) and Anywhere (8.0%). A higher percentage of non-smokers (58.6%) than smokers (41.3%) expressed the desire to continue their education after secondary school. Out of the 175 smokers, 154 (88.0%) purchased their cigarette from either a nearby shop or hawker. Thirty percent of smokers regularly sent their younger siblings to

purchase cigarette for them. Fifty (28.6%) of the smokers stated that they would like to stop smoking but their main obstacles were lack of will power, uncertainty about how to quit and fear of being regarded as timid or weakling by friends who continued to smoke.

**Table 2:** Characteristics and attitudes towards smoking by 175 smokers

| Characteristics and attitudes | No (%) |
|---|---|
| **Reasons given by respondents for smoking** | |
| Peer group pressure | 64 (36.5) |
| Mix freely with peers in social situations | 40 (22.9) |
| Imitate others | 23 (13.1) |
| Boost self-confidence | 21 (12.0) |
| Curiosity | 15 (8.6) |
| Relax/Relieve anger and frustration | 12 (6.9) |
| **Opinion about smoking** | |
| Harmful | 141 (80.6) |
| Harmless | 20 (11.4) |
| Uncertain | 14 (8.0) |
| **Do you smoke in the presence of your parents/guardian?** | |
| Yes | 38 (21.7) |
| No | 137 (78.3) |
| **Do you want to quit smoking?** | |
| Yes | 105 (60.0) |
| No | 51 (29.1) |
| Uncertain | 19 (10.9) |
| **Regular source of cigarette** | |
| Nearby shop | 120 (68.6) |
| Hawkers | 34 (19.4) |
| Friends | 21 (12.0) |
| **Do you send your younger siblings to purchase cigarette for you?** | |
| Yes | 53 (30.3) |
| No | 122 (69.7) |
| **Number of cigarette sticks smoked per day** | |
| 1 – 4 | 124 (70.9) |
| 5 – 9 | 51 (29.1) |
| $\geq$ 10 | 0 (0) |

| | |
|---|---|
| **Do you smoke your first cigarette within 30 minutes of waking up in the morning?** | |
| Yes | 6 (3.4) |
| No | 169 (96.6) |
| **Academic performance of individual smoker** | |
| Never failed | 29 (16.6) |
| Failed once | 96 (54.8) |
| Failed more than once | 50 (28.6) |

**Table 3:** Prevalence of smoking among students in close contact with other smokers

| *Contacts | Smokers (%) n=175 | Non smokers (%) n=885 | Odds ratio | 95% confidence interval |
|---|---|---|---|---|
| Father | 40.1 | 26.5 | 1.84 | 1.23 – 2.57 |
| Mother | 0.3 | 0.1 | 0.83 | 0.40 – 0.90 |
| Brother/Sister | 36.5 | 15.3 | 1.76 | 1.11 – 2.68 |
| Best friend | 47.6 | 11.9 | 4.34 | 2.88 – 6.48 |

*In some cases, more than one students' close contact is a smoker

**Table 4:** Disapproving attitude of respondent's close contacts toward respondent smoking

| *Contact | Disapproving (%) | | Odds ratio | 95% confidence interval |
|---|---|---|---|---|
| | Smokers n=175 | Non-smokers n=885 | | |
| Parents | 84.6 | 93.5 | 1.90 | 1.08 – 3.01 |
| Elder siblings | 68.9 | 84.8 | 1.98 | 1.36 – 2.89 |
| Best friend | 33.5 | 70.2 | 3.54 | 2.63 – 5.48 |

*In some cases, more than one students' close contact disapproved of smoking

The prevalence of smoking among students in close contact with other smokers is shown in Table 3. Smokers were four times more likely to have smokers among their best friends compared to non-smokers. The smoking status of fathers and elder siblings were significantly different

between smokers and non-smokers. As shown in Table 4, the highest odd ratio was found in the disapproving attitude of best friends. Higher percentage of parents and elder siblings of non-smokers disapproved of smoking by student.

**DISCUSSION:**

The overall prevalence rate of cigarette smoking (16.5%) among adolescent boys in public senior secondary school reported in this study was lower than the corresponding figures reported for Saudi Arabia (20.0%), Bahrain (21.0%), Portugal (21.5%), and Yemen (21.9%) but comparable to 15.9% reported for Syria [9, 14, 15, 17]. In the United Kingdom, overall smoking rates have not changed appreciably over the last 20 years with 21 to 26% of adolescents admitting to smoking regularly [11]. A much higher smoking rate was reported among French adolescent students [24]. Differences in socioeconomic and cultural factors may account for relatively lower smoking rate in our study. The prevalence rate of smoking obtained in this study was two times higher than the figure (7.7%) reported among secondary school girls in South East Nigeria, indicating that cigarette smoking was more prevalent among boys than girls in Nigeria [19]. The reported prevalence of cigarette smoking in the general adult population in Nigeria vary from 17.6% to 24.4%, which suggests that about 90% of adolescent smokers may become adult smokers [1, 8, 18].

Prevalence of smoking was higher among third-year (SS3) than first-year (SS1) students. This finding agrees with reports by other researchers that the rate of cigarette smoking increases with age [9, 17, 25]. The opposite was found in a study in Bahrain [17]. The lower prevalence among first-year students in this study may be due to their younger age and that the intensity of peer pressure has not reached its peak.

In the present study smoking commenced at a mean age of 16.9 $\pm$ 1.2 years, which is similar to the mean age (16.8 $\pm$ 1.1 years) reported for students in Bahrain [17], but different for the mean age (13.4 $\pm$ 2.1 years)

reported for high school boys in Portugal [9]. This suggests that cigarette smoking by students in high schools starts at an earlier age in Portugal compared to Nigeria [9]. Among adult rural dwellers in South West Nigeria, the reported mean age of commencement of smoking was 18.9 ± 5.6 years, which is higher than the 16.9 ± 1.2 years obtained for public SS male students in the present study [18]. This difference in the results may be due to the type of study population used in both studies; adolescents versus adults, rural dwellers versus urban dwellers. The implication of the age of commencement of smoking reported in this study is that preventive strategies should, at least, start from the junior secondary schools, or better still, from the primary schools.

Most (59.1%) of the smokers in the present study took up smoking either because they were urged on by peers and friends or simply to mix freely with peers in social situations. Available studies among adolescent secondary school boys did not report the reason given by their respondents [9, 17]. In contrast, the leading reasons for smoking among Kuwaiti adults were to relieve boredom and to feel relaxed [26]; these are two of the uncommon reasons among Nigerian adolescents. In the Nigerian context, therefore, cigarette smoking control programs should include equipping adolescents with skills to resist peer pressure.

In consonance with other studies [16,17, 26] having parents, elder sibling(s) or best friends who smoke encouraged adolescents to take up smoking, suggesting that parents and elder siblings of smokers provided early negative role models for these adolescents. The likelihood of smoking was higher among students whose parents and elder siblings have tolerant attitude toward smoking by their sons or younger brothers. Similar findings have been reported by other investigators [17, 27]. In this regard, for school cigarette smoking control programs to succeed, it must target not only the student, but also, their families.

Majority of the smokers in this study have repeated one or more school year due to failures, which supports the findings reported by others that poor academic performance promote up take of smoking [9, 17]. Our results indicate that majority (68.6%) of the smokers purchased their cigarette from nearby shops and that 30.3% of the

smokers sent their young siblings to purchase cigarette for them. Other researchers in Nigeria reported that nearby shops were the commonest regular sources of cigarette for smokers [18]. This situation is worrisome, because cigarette seems to enjoy an excellent distribution system making procurement very easy, thereby escalating rate of consumption. The practice of sending younger siblings to purchase cigarette may expose them and make them potential smokers in the near future, further fueling the cigarette smoking epidemic in developing countries.

All the smokers in our study were "light smokers", which support the findings reported by Soresi et al that "light" smoking is common among high school students [28]. It is possible that financial constraint limited the number of cigarette sticks consumed daily by these students, because in the Oredo LGA, each a stick of cigarette cost between 10 and 15 Naira (between 0.07 – 0.1 USA Dollars). Despite the "light" smoking habits observed among the smokers in the present study, the negative impact on their health cannot be overemphasized. According to Soresi et al ,[18] occurrence of respiratory symptoms such as cough with phlegm and breathlessness were similar when "light" and "heavy" smokers were compared [28]. They also showed that nicotine dependence can occur even in adolescent high school students who are "light" smokers [28]. Only 3.4% of the smokers in our study smoked their first cigarette within 30 minutes of waking up in the morning, suggesting a low risk of nicotine dependence. Further studies are needed to fully assess the pattern of smoking behavior among other students in the various categories of schools in Nigeria. The data obtained in the present study can be used to assess the magnitude of the problem of smoking among male adolescent secondary school students in Nigeria.

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**SECTION III**

## SHORT COMMUNICATION

# PHARMACOLOGICAL EVALUATION OF *Alstonia scholaris* Linn R. Br. FROM PAPUA NEW GUINEA

**Gelenta SALOPUKA Prem P. RAI, Paul CONNELLAN*, Dion THOMPSON*, Teatulohi MATAINAHO**

Discipline of Pharmacy, School of Medicine and Health Sciences, University of Papua New Guinea,
*Centre for Phytochemistry & Pharmacology, Southern Cross University, Lismore, NSW, Australia

(Corresponding Author: Prem P. Rai: Email: raipp@yahoo.com)
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*Alstonia scholaris* Linn. R. Br. (Apocynaceae) is a large evergreen tree commonly distributed throughout the lowlands of PNG and is an inhabitant of primary and secondary forests, lower montane rainforests, monsoon forests and savannah woodlands [1]. It is used as a traditional medicine in many parts of PNG for the treatment of severe fevers including malarial fever, and other conditions such as diarrhoea, dysentery, headaches, stomach ache, cough, shortness of breath (SOB), TB, pain and as an oral contraceptive [2]. In other parts of the world, it is used as febrifuge and tonic to aid digestion and stimulate appetite, as remedy for liver and intestinal problems, for treatment of chronic malaria with enlarged spleen, against diarrhoea and as wash for haemorrhoids; as anti-diabetic, anti-helminthic and anti-epileptic agents [2, 3]. *A scholaris* has been reported to contain numerous Indole Alkaloids [4-6], some major ones being echitamine, akuammigine and akuammicine [4], Manilamine, $N^4$-methyl angustilobine B, Angustilobine B $N^4$-oxide, 19, 20-(*E*)-vallesamine, 20S-Tubotaiwine and 6,7-seco angustilobine B [7]. Triterpenoids [8,9,10], Flavonoids and Glycosides [10,11] and beta-sitosterol (a phytosterol) [10]. Some of its reported

pharmacological activities include Anti-anxiety [12], Anti-plasmodial [13], Immuno-stimulating effect [14], Anti-cancer [15], Hepato-protective [16], Anti-nociceptive [17], Anti-inflammatory [17] and Anti-ulcerogenic [18]. Toxicity studies on the acute and sub-acute effects in mice and rats have revealed dose dependant effects [19].

The pharmacological evaluation of *A. scholaris* comprises one aspect of a project on standardization of herbal medicine derived from *A. scholaris.* Standardization ensures that the preparation is stable, has correct potency and is reproducible when administered by the herbal practitioner within the settings of the traditional use of the plant. Hence, it is essential in verifying that the plant elicits the activity for which it is used traditionally by subjecting extracts of the leaves and bark of the plant from various locations in PNG to the following bioassays: (a) Cytotoxicity, (b) Nitric oxide inhibition, (c) PGE2 inhibition, and (d) Phagocytosis. Sites of sample collection included Brown River in Central, Losuia in Kiriwina, Taurama campus of University of PNG in NCD and Lorengau in Manus.

Cytotoxicity effect of *A. scholaris* extracts was carried out on Mouse lymphoblast (P388), Mouse Swiss albino embryonic fibroblast (3T3) and Mouse leukemic monocytes macrophage (RAW) cell lines. P388 (mouse lymphoblast) was used mainly as an initial screen for general toxicity from which a base-line concentration was determined for carrying out the other assays. In general, results from this assay indicated relatively high percentage (%) inhibition of P388 cells by leaf water extracts at 200ug/mL in comparison to the bark extracts in water. The un-dried leaf extract from Taurama, displayed the highest activity at the same concentration. 200ug/mL of the methanol extract of un-dried leaves from the same location also showed the highest inhibition against P388 cell line relative to other locations in the study.

Additionally, values for the concentration at which 50% of the activity is inhibited (the $IC_{50}$) were determined using the methanol extracts with a concentration of 200ug/mL, against the mouse lymphoblast cells. IC50s ranged from 120.25 to 408.19ug/mL, indicating that all dried leaf and bark extracts had relatively high $IC_{50}$ and hence low toxicity as compared to one of the positive controls, Curcumin, having an IC50 of 15.55ug/mL. Moreover, percentage inhibition of 3T3 and RAW

cells revealed the dried leaf extract in methanol from Lorengau (Manus Province) as having the highest inhibitory effect against both cell lines. Leaf extracts displayed relatively higher inhibition against 3T3 cells than the bark extracts. RAW cells seemed to show relatively greater sensitivity to both leaf and bark extracts at the concentration assayed.

Results from the $PGE_2$ inhibition assay showed that all aqueous leaf and bark extracts of *A. scholaris* from all the study sites displayed no inhibitory effect on $PGE_2$ production as compared to the positive inhibitor control. With respect to phagocytosis activity, water and methanol extracts of the un-dried and dried leaves of the tree from the Taurama campus revealed that the aqueous extract of un-dried leaves had the highest positive phagocytic activity in human blood. Methanol extracts had the lowest phagocytic effect.

The water extracts of un-dried leaves and bark of *A. scholaris* from Losuia and Brown River exhibited phagocytic activity however, the bark had relatively higher activity for both locations. The water extracts of un-dried leaves and bark from Lorengau displayed positive phagocytosis with the latter displaying relatively higher activity.

In conclusion, pharmacological evaluation of *A. scholaris* extracts has to some extent provided scientific rationale for its traditional use in the treatment of fever. Water extracts displayed less inhibitory effect on cell growth at 200ug/mL relative to methanol extracts at the same concentration however, $IC_{50}$ values of the latter revealed relatively high values as compared to one of the controls (curcumin). Methanolic and aqueous extracts prepared through boiling, did not possess any inhibitory effect on $PGE_2$ production in the initial experiments. Un-dried leaf and bark extracts in hot water demonstrated positive phagocytosis activity wherein the bark extracts from all locations exhibited relatively high activity (approx. 30% increase). Hence results of the bioassays indicate that the fever-reducing property of *A. scholaris* is most probably propagated through phagocytosis, the innate component of the body's defence system. It is recommended that the un-dried bark extract from Taurama campus be subjected to phagocytosis assay since it was not included in the initial assay. Establishment of a thin layer chromatographic profile for the extract

exhibiting the highest relative phagocytosis activity can be utilized as a reference against which extracts of *A. scholaris* from the other locations can be compared.

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