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TABLE OF CONTENTS

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Towards Knowledge Management for Healthcare: Effects of Latest Medical Information for the Quality of Healthcare in the Developing Countries: A Case Study

Amaraarachchi J.L[1], Perera H.S.C[2], Pulasinghe K[3]
[1]*Faculty of Computing, Sri Lanka Institute of Information Technology, Sri Lanka;*
[2]*Faculty of Engineering, University of Moratuwa, Sri Lanka;*
[3]*Faculty of Graduate Studies, Sri Lanka Institute of Information Technology, Sri Lanka*
jayantha@sliit.lk, hscp@mot.mrt.ac.lk, Koliya@sliit.lk

ABSTRACT

In the last 2 decades, the Information and Communication Technologies (ICTs) revolution has redefined the structure of the 21st century healthcare organizations. It is clear that the 21st century healthcare organizations in developing countries will bring new healthcare services and the traditional management & technological concepts would not be the appropriate conduit for disseminating these new healthcare services.

The fundamental challenge faced by the 21st century clinical practitioner in a developing country is to acquire proficiency in understanding and interpreting clinical information so as to update knowledge that leverages the quality of decisions made at the clinics. An additional challenge must be considered by the clinical practitioners to make potentially life-saving decisions whilst attempting to deal with large amounts of clinical data & Information. Since the Clinical Knowledge Management Systems (CKMS) consist of most related Data, Information and Knowledge, it could be utilized to achieve the above challenges.

According to the current economies, developing countries cannot afford to buy CKMS which needs a proper IT backbone and knowledge culture to run it. Medical practitioners (MPs) currently have no proper facilities to access the latest medical Information resources to make effective clinical diagnosis. Shortage of medical experts and MPs in Healthcare Institutions located in rural and remote areas in developing countries are also a massive problem which affects the quality of healthcare badly. By implementing and providing proper facilities for MPs to access KMS, this problem can be alleviated substantially.

The objective of this paper is to investigate the importance of the latest medical Information for making quality clinical decisions which improves the quality of healthcare. Findings of the research have shown that there is a strong linkage between accessing and using latest

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Information/knowledge in clinical activities and the quality of healthcare. This research used a case study methodology for achieving the research objectives. Rural and remote areas in Sri Lanka were used for the case study, since Sri Lanka is one of the developing countries situated in the Asian region. As the first step of a solution to the Information problem, a KM framework for Healthcare Institutions to create Knowledge Management Systems was introduced.

Keywords: KM-Knowledge Management, HI-Healthcare Institute, MP-Medical Practitioner, KMS-Knowledge Management System, HC-Health Care, KW-Knowledge

1. INTRODUCTION

Healthcare professionals face information overload and they come across paradoxical information. They are overwhelmed by information but cannot find a particular piece of information when and where they need it [1]. Technologies have increased the dissemination of information, but worsened the problem of unwanted information.

On average, a physician today spends about 25 percent of their time managing medical Information and has to learn 2 million clinical specifics [2]. This is further compounded by the fact that biomedical literature is doubling every 19 years. In UK each physician receives about 15 kg of clinical guidance per annum [3]. These indicators illustrate how difficult it is for Healthcare Institutions (HIs) and Healthcare Stakeholders (HSs) to successfully meet the healthcare Information needs that are growing at an exponential rate.

As stated earlier, Healthcare professionals in the developed countries meet their medical information requirements with the support of ICT. But Healthcare professionals currently working in the rural and remote areas of healthcare Institutions hardly meet their medical Information requirement or do not meet at all. Clinical decisions made on inaccurate or incomplete information, inevitably mean either loss of lives or effect patients badly.

Majority of the population in Sri Lanka still live in rural and remote areas while the majority of medical experts are stationed in the urban areas. But, low level medical facilities have been given to rural and remote medical centers to serve the majority of the population. Sri Lanka has 80% of its main healthcare centers located in cities and host only 30% of the total population [4]. A 70% of the Sri Lankan population is rural and remote which is served by only 20% of medical practitioners [3]. Therefore rural communities are at a far greater disadvantage such as late discovery of ailment, transport time to reach urban healthcare facilities and inexperienced primary healthcare providers in rural areas. In some cases, rural patients are sent or they willingly visit hospitals in developed (urban) areas at considerable expense.

The first half of figure 1 depicts the number of physicians per 100,000 people in the period of 2008-2012, in six developed countries and the other half depicts the same information for five developing countries including Sri Lanka [5]. Sri Lanka has 55 physicians per 100,000 people while, a developed country like Italy has 350 physicians per 100,000 people. According to the annual health bulletin published by the Ministry of Health, Sri Lanka had only 55 physicians

/100,000 populations in 2006, which is well below that of many countries with equivalent levels of income [6]. According to the survey conducted in Kandy district, over 60% practitioners have to treat more than 75 patients per day. This indicates the physicians in developing countries have to serve a larger number of patients per day, thereby the quality of Healthcare may not be preserved.

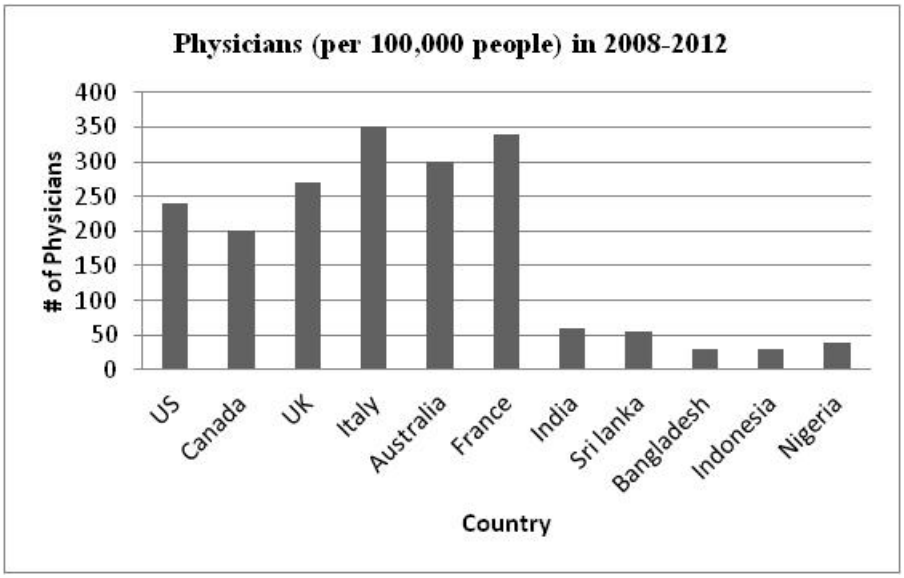

Figure 1: Physicians per 100,000 people in 2008-2012 (Source: The World Bank-World Development Indicators-2013)

The lack of medical experts in rural health Institutions has a direct impact to the quality of healthcare. According to the health statistics, 72.7% of medical specialists serve in urban centers and 25.1% in semi urban areas and only 2.2% serve in rural areas. But comparatively majority of the patients come from rural areas [4]. This implies there is a huge shortage of medical specialists in rural and remote areas. Therefore the specialist health coverage to majority of the population who live in rural and remote areas is still a distant dream [6].

The advancement in Information Technology (IT) and in particular Telecommunication Technology has brought about fundamental changes throughout the healthcare process [7]. Further interaction between the twin revolutions of Information and Communication Technologies (ICTs) and Telecommunications have enabled healthcare technologies based on concepts such as Electronic Patient Records (EPR) and Electronic Health Records (EHR)[8-16]. By incorporating modern technologies to healthcare management, it helps to reduce the workload of physicians which leads to increase the quality of healthcare. A study was done by the authors to investigate, the Information and knowledge needed by medical practitioners.

The study has revealed the knowledge needs of medical practitioners have not been fulfilled specially those who are located in rural and remote areas in Sri Lanka. If the knowledge

requirement is readily accessible, then the knowledge gap between medical practitioners and the medical experts could be reduced.

2. ROLE OF ICT IN HEALTHCARE

2.1 Healthcare Information

Delivering healthcare services to the patients is a complex process that highly depends on healthcare related Information and Healthcare professionals' experience. Health resources, Healthcare provision, Healthcare utilization, Healthcare coverage etc, are the types of healthcare information required by the healthcare professionals, managers and policy makers at each level of the healthcare system. To identify the utilization of healthcare services provided at primary, secondary and tertiary level, healthcare managers require the information in relation to healthcare utilization, healthcare coverage etc. One of the major impediments in hospital progress toward, efficiency and cost-effectiveness is the difficulty in sharing Information among healthcare organizations [13].

A key element to implement Health Information Technology (HIT) is to understand what to collect, where to collect, whom to report to and how this information will be used and by whom because these are used to provide curative, preventive, rehabilitative and palliative care to the population. Considering this, it is required to determine the information needs, tools for data collection and levels of data generation. Once these are defined and determined, it is easy for the managers and providers to proceed with the implementation process in a better way.

Over the past decade, the healthcare Industry has increasingly tried to embrace new IT, such as telephony, computer and Internet associated technologies, as it searches for opportunities for higher quality care [14]. E-health is currently evolving, which refers to the delivery of healthcare services involving the electronic transfer of health-related Information using electronic-based technologies [15]. The Healthcare Information and Management System Society (HIMSS) broadly defined e-health as an IT-enabled healthcare system that improves the access, efficiency, effectiveness and equality of clinical and business processes utilized by healthcare organizations, practitioners, and patients.

Growing use of Electronic Medical Record (EMR) systems in Europe and the United States (US) has been driven by the belief that these systems can help to improve the quality of healthcare. Decision support systems, particularly for drug order entry are becoming important tools in reducing medical errors.

2.2 Knowledge Management in Healthcare

In a healthcare context, it can be argued that Knowledge Management (KM) is the formal management of knowledge for facilitating the creation, identification, acquisition, development, dissemination, utilization, and preservation of a healthcare enterprise's knowledge using advanced technology [16-17]. More so, KM also involves:

converting knowledge from the healthcare enterprise's sources (individuals, groups, data and text), and connecting healthcare participants—healthcare professionals, management and patients—with that knowledge [18].

A typical KM structure can be split in to two arms *Knowledge Management Process* and *Knowledge Management Enablers*. K*nowledge Management enablers* are considered to be the factors that influence the development of the *knowledge management process*.

2.3 Knowledge Management Processes

KM processes consist of a number of events that form into a cyclic arrangement, i.e. the preceding process providing input to or influencing the subsequent KM process. For our discussion, *creation is regarded as* the initial process of the KM framework.

Create: Responsible for the creation of healthcare knowledge, possibly through trial-and-error or blind variation and selective retention methods.

Identify: Determines the existence of useful healthcare knowledge from the knowledge created in the earlier process. This can be achieved through mining efforts similar to that of data mining and knowledge discovery.

Collect/Acquire: Once useful knowledge has been identified, next follows the process of acquiring the knowledge.

Organize/Develop/Preserve: This can be viewed as a form of 'knowledge processing' whereby the knowledge is transformed, represented, and organized in a defined format. This process also concentrates on the explication of tacit knowledge which is supported by expert systems, issue-based information systems, best-practice databases, and lessons learnt archives. Similarly, knowledge capitalization aims to allow the reuse of knowledge of a given domain previously stored and modeled in order to perform new tasks [19].

Share/Disseminate: Provides the mechanisms to disseminate the stored knowledge to all participants of the healthcare enterprise and possibly to other healthcare enterprises.

Adapt: This process is typically the responsibility of healthcare professionals in their practice. Upon introspection of the 'created' knowledge healthcare professionals may then need to tailor it to ensure appropriateness, currency and accuracy.

Apply/Utilize: Knowledge when not used is equally, if not more, useless and again, this process is typically the responsibility of healthcare professionals. The success of a healthcare KM framework depends on its success in providing knowledge that is being used effectively to meet the demands of the healthcare enterprise

2.4 KMS for Clinical Activities

Clinical responsibilities of a Medical Practitioner have been defined as: "The diagnosis and treatment of human responses to actual or potential health problems". The practices (steps) in the clinical process are identified as an assessment, diagnosis, treatment and evaluation. They are cyclic, overlapping and interrelated.

- Step 1, **Assessment**, is the most critical step. Try to identify the actual problem or the potential problem with the patient. This step involves collecting, organizing, and analyzing Information/data about the patient. The methods of data collection can be observation, interview, and examination.
- Step 2, **Diagnosis**, is a statement that describes a specific human response to an actual or potential health problem.
- Step 3, **Treatment**, is prescribing medications, therapies, undertaking surgeries and other treatment.
- Step 4, **Evaluation**, compare the patient with the stated patient goals and has three different operations or purposes. Evaluation of the quality of the written care. Plan and evaluation of the client's progress.

Medical professionals especially use the healthcare knowledge combined with their know-how and experience to deliver healthcare services. Today, this work can be enhanced by enabling technologies such as a KMS that provides up to date tacit and explicit knowledge of medical experts.

3. METHODOLOGY

The research design, process, and methods used for data collection of the research are discussed in this section. The methodology used to develop the conceptual clinical KM framework is described in section 5. The basis of selection of the case study is also discussed. The research design was guided by a qualitative philosophy and used the case study methodology to achieve the study objective, "To Investigate the Effects of Latest Medical Information for the Quality of Healthcare in the Developing Countries."

The survey has been conducted by covering the Health Institutions located in Rural and Remote areas in Kandy district in Sri Lanka. A medical practitioner from a Health Institution was considered for the survey. Practitioners in these areas play a significant role for the patients in the healthcare.

The conceptual research model was tested using the data collected by the questionnaire. The survey helped to capture beliefs, experiences and perceptions of medical professionals on Information and knowledge management which they currently use for their clinical activities. A total of 136 questionnaires were given to medical practitioners in 105 health Institutions in Rural and Remote areas in Kandy district. The effective response rate was 55.2%. The questionnaire was organized in a manner that has covered four main sections: (1) to investigate

the needs of the latest medical Information for clinical activities, (2) to study how medical practitioners in rural and remote areas currently meet their Information and knowledge requirements for clinical activities, (3) to examine how medical practitioners in rural and remote areas can be motivated to use KMSs for clinical activities and (4) to make an assessment on existing infrastructure facilities in healthcare centers located in rural and remote areas.

Survey items been identified in the questionnaire have been used in the data analysis to identify their relationships. In addition to the survey, several meetings and interviews were conducted with some selected medical professionals to get more understanding and clarify certain issues about the healthcare in rural and remote areas.

4. DATA ANALYSIS

Data collected from the main case-survey was analyzed in two phases. First analyzed the data obtained from descriptive statistics to identify the factors effect on accessing knowledge/Information in the clinical activities. Secondly the effect of latest medical Information/Knowledge with the quality of healthcare was tested [20]. The table 1 is a representation of how medical practitioners' access the information. Among the mode of access it seems that more than 21% of people use e–Learning and Training Courses. More than 57% of medical practitioners' say the resources are not available. It can also be seen that more than 65% of practitioners do not use any mode of access to information. This shows that there is an insufficiency in information to them.

Table 1: Mode of Access*Preference of Information Retrieval cross tabulation

Type of Information Possible to Access * Mode of preference Crosstabulation								
			Mode of preference					
			Strongly-agree	Agree	Disagree	Strongly-disagree	Dn/Cs	Total
Type of Information Possible to Access	Patient records, test results, prescriptions	% within Type of Information Possible to Access	37.3%	34.7%	4.0%	1.3%	#REF!	100.0%
		% within Mode of preference	30.8%	21.0%	25.0%	100.0%	#REF!	25.0%
		% of Total	9.3%	8.7%	1.0%	.3%	5.7%	25.0%
	Electronic medical research information	% within Type of Information Possible to Access	28.0%	44.0%	2.7%		25.3%	100.0%
		% within Mode of preference	23.1%	26.6%	16.7%		21.4%	25.0%
		% of Total	7.0%	11.0%	.7%		6.3%	25.0%
	Electronic drug information	% within Type of Information Possible to Access	30.7%	48.0%	2.7%		18.7%	100.0%
		% within Mode of preference	25.3%	29.0%	16.7%		35.0%	25.0%
		% of Total	7.7%	12.0%	.7%		4.7%	25.0%
	Disease information electronically	% within Type of Information Possible to Access	25.3%	38.7%	6.7%		29.3%	100.0%
		% within Mode of preference	20.9%	23.4%	41.7%		54.2%	25.0%
		% of Total	6.3%	9.7%	1.7%		7.3%	25.0%
Total		% within Type of Information Possible to Access	30.3%	41.3%	4.0%	.3%	24.0%	100.0%
		% within Mode of preference	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
		% of Total	30.3%	41.3%	4.0%	.3%	24.0%	100.0%

Table 2: Type of Information Possible to Access*Mode of Preference Crosstabulation

Mode Of Access * Preference of Information Retrieval Crosstabulation								
			Preference of Information Retrieval					
			Strongly-agree	Agree	Disagree	Strongly-disagree	Dn/Cs	Total
Mode Of Access	Text Books	% within Mode Of Access	36.0%	60.0%	4.0%			100.0%
		% of Total	7.2%	12.0%	.8%			20.0%
	Internet	% within Mode Of Access	49.3%	37.3%	8.0%	4.0%	1.3%	100.0%
		% of Total	9.9%	7.5%	1.6%	.8%	.3%	20.0%
	E - Learning	% within Mode Of Access	57.3%	10.7%	21.3%	8.0%	2.7%	100.0%
		% of Total	11.5%	2.1%	4.3%	1.6%	.5%	20.0%
	Training Courses	% within Mode Of Access	33.3%	52.0%	12.0%	2.7%		100.0%
		% of Total	6.7%	10.4%	2.4%	.5%		20.0%
	No resources available	% within Mode Of Access	57.3%	6.7%	20.0%	12.0%	4.0%	100.0%
		% of Total	11.5%	1.3%	4.0%	2.4%	.8%	20.0%
Total		% within Mode Of Access	46.7%	33.3%	13.1%	5.3%	1.6%	100.0%
		% of Total	46.7%	33.3%	13.1%	5.3%	1.6%	100.0%

The table 2 represents the Type of Information Possible to Access*Mode of Preference crosstabulation. It can be seen that there are more than 71% of practitioners who hope that there is a possibility that they can access new medical information by having information through an electronic information system.

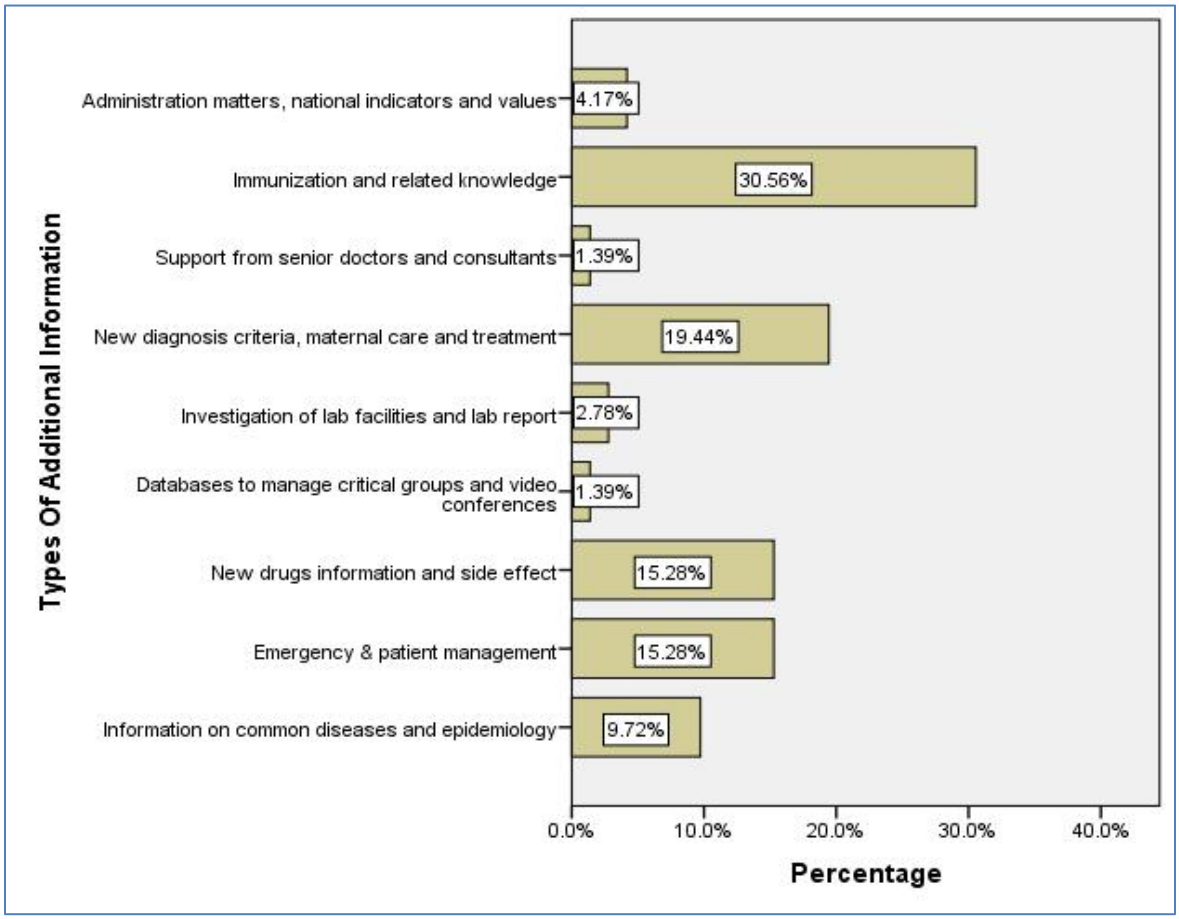

Figure 2: Graph 1-Types of Additional Information

The bar graph 1 is a representation of what types of additional information is referred by medical practitioners. It can be seen that more than 30% of MPs' need to refer information on an immunization and related knowledge while 19% of MP's refer information on new diagnosis criteria, maternal care and treatment. Also bar chart shows that more than 15% MPs refer to information on an emergency & patient management and/or new drugs information & side effects.

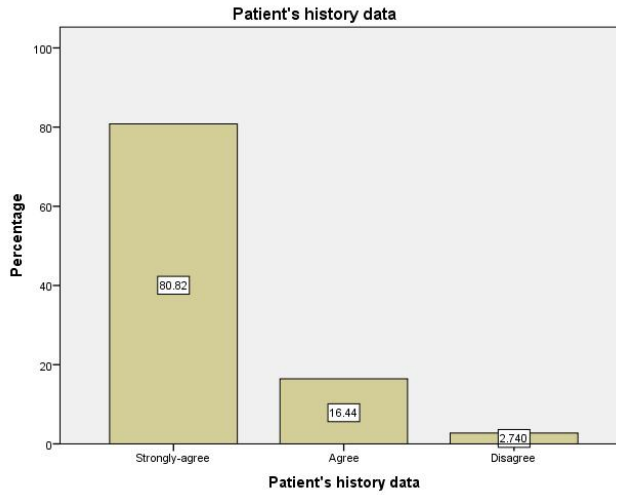

Figure 3: Graph 2-Patient's History data

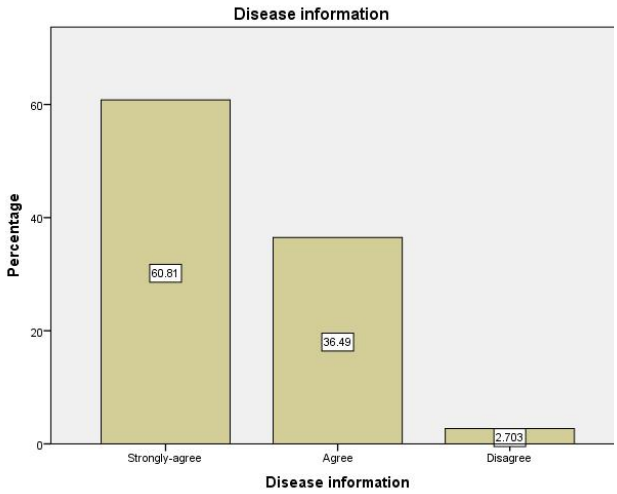

Figure 4: Graph 3-Disease Information

Graph 2 represents patient's history data. It shows more than 97% of MPs like to refer this information electronically for their clinical activities. Graph 3 represents disease information. It can be seen that more than 97% of MPs need to refer disease information system for their clinical activities.

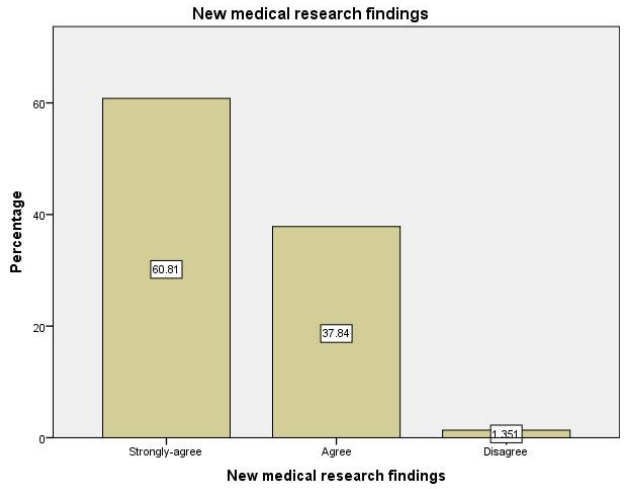

Figure 5: Graph 4-New Medical Research Finding

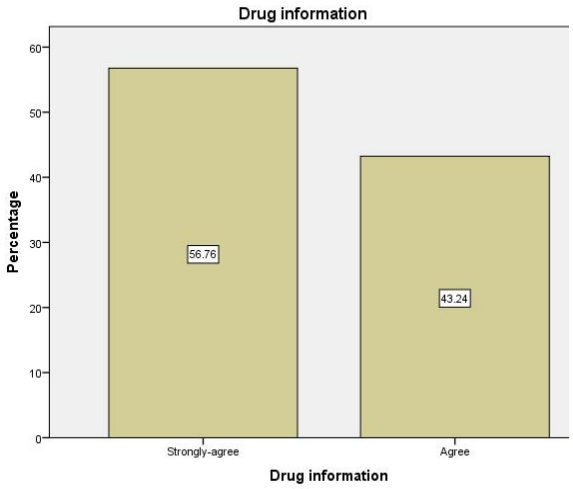

Figure 6: Graph 5-Drugs Information

Graph 4 represents the information on new medical research findings. According to the graph, more than 98% of MPs like to refer this information electronically for clinical activities. Graph 5 represents drugs information. It shows all MPs like to refer drugs information system electronically for clinical activities.

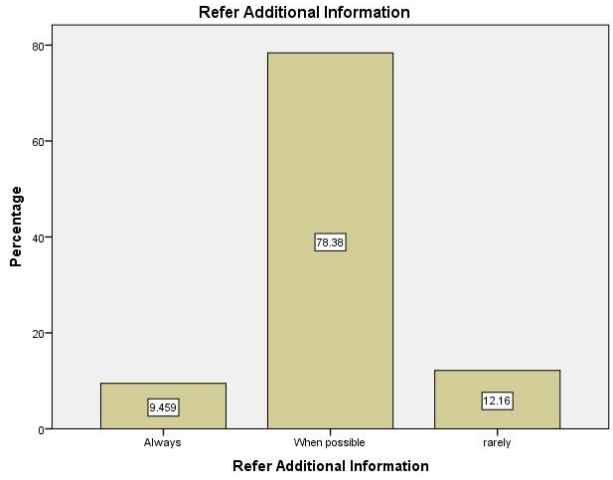

Figure 7: Graph 6-Refer Additional Information

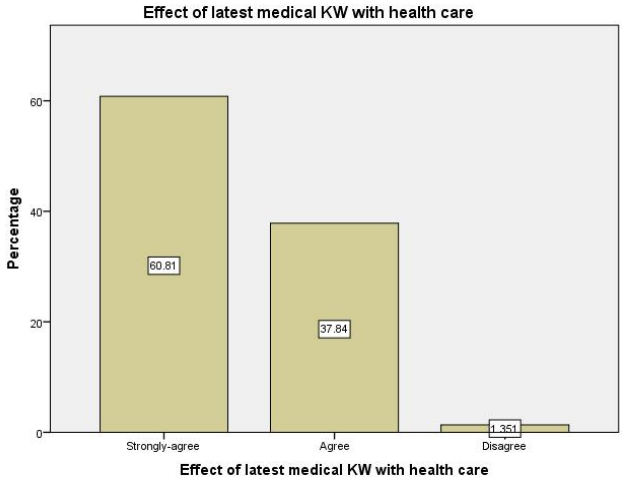

Figure 8: Graph 7-Effect of Latest Medical KW with HC

Graph 6 represents the statistics about 'Referring additional Information' by MPs. It shows more than 9% of MPs like to refer additional information always. But the majorities (78%) of MPs are referring additional information 'when possible'. Reasons for this would be that MPs are fully overloaded with patients or they have not given proper facilities to access necessary information.

Graph 7 represents the statistics about the 'effect of latest medical information/knowledge with the quality of healthcare'. According to the graph, more than 98% MPs have agreed that there is a big impact on the quality of healthcare, if they can access the latest medical information and/or knowledge systems electronically.

Table 3: Factors Effect on Latest Medical KW with Healthcare

		Effect Of Latest Medical KW with health care
New Medical Research Findings	Contingency Coefficient	0.816
	Significance	0.000
Disease Information	Contingency Coefficient	0.759
	Significance	0.000
Drug Information	Contingency Coefficient	0.665
	Significance	0.020
Decision Support Facility	Contingency Coefficient	0.616
	Significance	0.038

The table 3 is a representation of how the above analyzed data would be related to the latest medical KW with healthcare

It seems that new medical research findings are one of the important factors that should be included in the knowledge as it has the highest contingency coefficient among the other factors. From the table 3 of contingency, it can be concluded with 95% level of confidence that new medical research findings, disease information, drugs information and decision support

facility are highly related with the effect of latest medical Information/Knowledge with the quality of healthcare.

5. CONCEPTUAL CLINICAL KM FRAMEWORK

The methodology used to develop the conceptual clinical KM framework for the healthcare Institutions is described here. The graph 7 has shown that the 'latest medical information' shows a relationship with the quality of healthcare. Thus Medical Practitioners must provide access to medical information systems which cover the latest medical information and knowledge. A conceptual clinical KM framework has been proposed. The framework transforms the conceptual ideas of KM into a customizable working program with defined objectives, using existing industry techniques. The framework also supports the designing, building, and maintenance of a knowledge - sharing platform, both from an IT and organizational point.

This study contributes to the existing body of knowledge on the linkage between three multidisciplinary research themes;

- Healthcare Management Concepts (HMC)
- Information and Communication Technology (ICT)
- Knowledge Management (KM)

The final outcome would be a conceptual KM framework for clinical activities in the healthcare Institutions. This is the basis of designing a clinical KMS for medical practitioners to access latest medical Information to make better clinical decisions that leads to raise the quality of healthcare.

The development of conceptual clinical KM framework comprised with two steps to follow.

- Develop the first level conceptual clinical KM framework with core features
- Develop the second level detailed conceptual clinical KM framework, on the basis of the revision of first level conceptual clinical KM framework

5.1 The First Level Conceptual Clinical KM Framework

A KMS design framework should integrate business processes and IT with associated functions to facilitate the KMS design [21]. It emphasizes the needs of understanding the organizational KM activities being supported and signals a shift from technology-led to knowledge–led systems. To develop a successful clinical KMS, a conceptual framework is required. A clinical KMS will be an IT based system developed to enhance the knowledge creation, codification, transfer, and application to support the clinical activities such as Assessment, Diagnosis, Treatment and Evaluation. .

Empirical data was gathered via the questionnaire which is the main case. The findings of this case, in conjunction with inputs from the literature review have formed in to an iterative

process. The analysis of the collated data resulted in a first level conceptual clinical KM framework for Healthcare organizations in the developing countries. It can therefore be argued that the dimensions of the clinical KMS design include both clinical processes and KM technologies which are driven by the e-health environment.

5.2 The Second Level Conceptual Clinical KM Framework

The data gathered from ten National Health Institutions (sub cases) has supported the main case study. The analysis of the additional data has been used to validate the findings obtained. The data obtained from the sub cases has been used to revise the first conceptual clinical KM framework, leading to the second conceptual clinical KM framework. The ultimate result was a revised and final conceptual clinical KM framework as depicted in Figure 9.

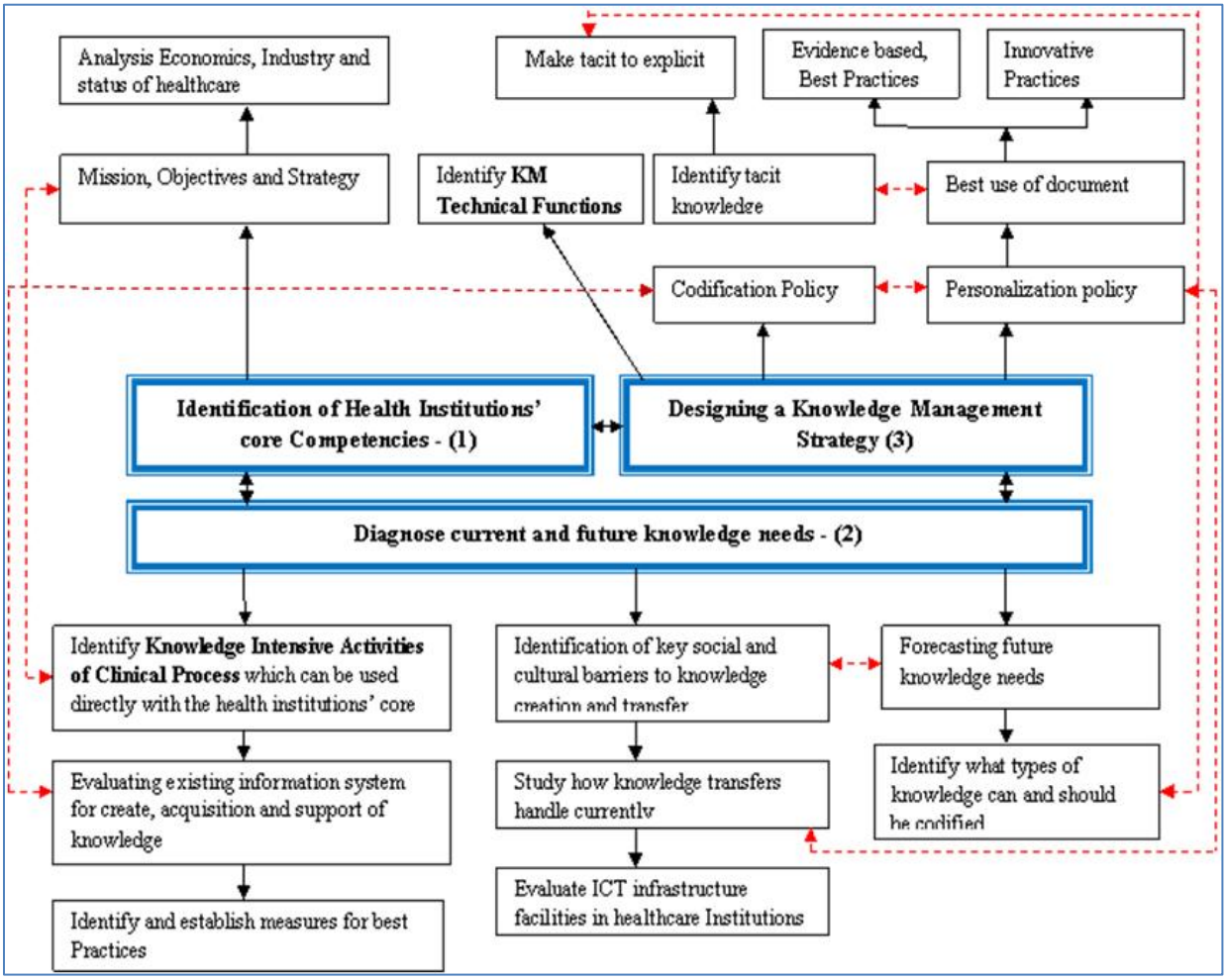

Figure 9: Final Conceptual KM Framework for Clinical Activities.

The final version of clinical KM framework is a three layered system. It is based on empirical data serving as a basic utensil for healthcare institutions for understanding how to develop KMSs.

5.2.1 Identification of Health Institutions' Core Competencies

The first step (cage (1) in Fig. 9) in formulating KM strategy involves the identification of the core competencies in healthcare Institutions. This process will enable healthcare Institutions to be clear about its mission, objectives, and strategy. It will create awareness of the mission, objectives and strategy in the context to its economic, industry and status of healthcare outlook.

5.2.2 Diagnose Current and Future Knowledge Needs

The second step is the diagnosis in Healthcare Institution's current and future knowledge needs (cage (2) in Fig. 9). This section of the framework analyzed the current technological infrastructure (ICT related facilities and e-health applications) in place for supporting knowledge transfer. After completion of this process, HIs need to identify knowledge intensive activities and assess what knowledge is to be codified (cage (2) in Fig. 9). It results in the decision to adopt a KM strategy with emphasis on either personalization or codification. Irrespective of the strategy adopted, HIs must identify what represents in the best clinical practices. This will help in capturing tacit knowledge of clinical specialists up to some extent.

5.2.3 Designing a Knowledge Management Strategy

In the last section (cage (3) in Fig.9), a KM strategy would be identified for HIs. Each HI can adopt a KM strategy which emphasizes on tacit knowledge. This resides in its resources or on a KM strategy that emphasizes the organizational processes. If decided to adopt a codification-led KM strategy, then the classification have to be taken on Artificial Intelligent components. This is leading to identification of relationships that exists between different types of knowledge (tacit or explicit) being transferred and dissemination practices. Then the result in a spiral transfer between the processes marked with the broken arrow signs in Fig. 9. Finally the knowledge identified, created and generated is then modeled in to knowledge repositories.

This was aimed to develop a conceptual clinical KM framework to identify the key factors involved in the development of Knowledge Management Systems (KMSs) for clinical activities. 'Access latest medical Information for clinical activities' is one of the key factors that led for the development of conceptual clinical KM framework. This framework will provide a systematic guideline for KMS designers to adopt IT and the needed KM technical functions to support the activities in clinical processes when designing a KMS for clinical activities. By developing KMSs for clinical activities, medical practitioners will get opportunities to access latest medical information which leads to improve the quality of healthcare in the developing countries.

6. DISCUSSION AND CONCLUSION

The research outcome has shown that there are no specialist doctors or very few medical specialist who serve in the HIs located in rural and remote areas in developing countries. Further the majority of practitioners who serve in HIs in rural and remote areas are overloaded

with patients and over 60% practitioners have to handle more than 75 patients per day. These factors have a negative impact on the quality of healthcare. According to data analysis, it can be seen that more than 97% of medical practitioners have agreed that latest medical information would affect the quality of healthcare. But due to many constraints they cannot access the latest medical information. It also revealed by the study that some medical practitioners serve in the healthcare Institutions located in rural and remote areas, gather medical information/knowledge from pharmaceutical representatives. Therefore the medical practitioners should have access to the latest medical Information & Knowledge system for clinical activities that help to update their medical knowledge.

Findings of the study also revealed the importance of medical information & knowledge for quality healthcare. This emphasized the needs of complete & reliable clinical KMS to use for clinical activities that improve the quality of healthcare. Further it has discovered some facts that will affect Information and knowledge sharing in the clinical activities. By providing IT Infrastructure, reliable communication network, information network and an e-Health technology can initiate this process in HIs. These factors may facilitate utilizing Information and knowledge in a much effective way which helps to break barriers in the knowledge culture in HIs. Knowledge Management training and education can fill any gaps and convert the Health Organization to a learning organization.

This paper reports statistically that there is a strong association between accessing latest medical Information for clinical activities and quality of Healthcare. Attitude of medical practitioners, Infrastructure facilities, Information systems software, patient association and diagnosis, patient treatment and staff benefits play a significant role in enabling Knowledge Management in Healthcare Institutions in the developing countries.

The authors conclude with the statement that the clinical KM framework will provide a benchmark for all future KM implementations in healthcare which provides access to the Latest Medical Information systems. Clinical KMSs orient all future efforts on sharing tacit-to-tacit knowledge in order to avoid knowledge gaps and leverage all available resources.

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Temporal decoupling of oxy- and deoxy-hemoglobin hemodynamic responses detected by functional near-infrared spectroscopy (fNIRS)

Nicoladie D. Tam[a], and George Zouridakis[b]

[a] *Department of Biological Sciences, University of North Texas, Denton, TX 76023, USA*

[b] *Departments of Engineering Technology, Computer Science, and Electrical & Computer Engineering, University of Houston, Houston, TX 77204, USA;*

nicoladie.tam@unt.edu, zouridakis@uh.edu

ABSTRACT

This study provides experimental evidence that there is temporal decoupling between the hemodynamic responses of oxy- and deoxy-hemoglobin (Hb) as detected by functional near-infrared spectroscopy (fNIRS). Using 64 spatially distributed optrodes to record motor cortical activities during a free arm movement task (right-left and front-back movements), we detected that the temporal profile of oxy- and deoxy-Hb responses are desynchronized and decoupled (i.e., oxy- and deoxy-Hb levels do not rise and fall at the same time). We correlated four different measures of hemodynamic profiles with the arm movements, namely, oxy- (HbO_2) and deoxy-hemoglobin (Hb) and their summation ($HbO_2 + Hb$) and difference ($HbO_2 - Hb$) signals. These measures correspond to the changes in oxygen delivery, oxygen extraction, total blood volume delivered, and total oxygenation with specific movement directions, respectively. They revealed different components of the hemodynamic response in a localized neuronal population in the motor cortex. The results suggested that, by using these four measures, oxygen delivery and oxygen extraction can be coupled in one movement direction, but decoupled in another movement direction for the same human subject executing the same movement task. Oxygen delivery and oxygen extraction do not always co-vary together temporally. Thus, using a single measure of oxygen delivery or extraction alone may not be sufficient to determine whether the cortical area is activated or deactivated. Rather, a combination of all four measures of hemodynamic signals that represent temporal coupling and decoupling of oxygen delivery and extraction is needed to differentiate the temporal profiles of neural activation and deactivation. It demonstrated that different hemodynamic measures can reveal temporally decoupled activation/deactivation patterns differentially during the right-left and front-back motor task. Therefore, relying on a single measure of deoxy-Hb may be insufficient to characterize the neural responses without the oxy-Hb measure. Orthogonal arm

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movement (right-left vs. front-back) directions can be differentiated based on the differential temporally coupled and decoupled hemodynamics.

Keywords: Functional near-infrared spectroscopy, fNIRS, hemodynamics, motor control, neural activation, temporal coupling.

1. INTRODUCTION

This study describes how functional near-infrared spectroscopy (fNIRS) can be used to reveal oxy- and deoxy-hemoglobin (Hb) hemodynamic responses can be decoupled temporally during a cognitive motor task. fNIRS has been used as a noninvasive optical imaging technique to detect neural activation according to the hemodynamic response of the imaged neural tissues [1-4]. Hemoglobin molecules is known to absorb light in the near-infrared (NIR) spectral region, with a characteristic different absorption spectra for oxy-Hb (HbO_2) and deoxy-Hb (Hb) based on the molecular chemical bonds [4]. Using fNIRS, real-time detection of brain activation can be inferred from the metabolic activities of the underlying neural tissues. The hemodynamic response is deduced from the temporal profile of oxygen delivery and extraction in the neural tissues, which is dependent on the metabolic activity and oxygen consumption in real time. It is ideal to detect cortical activity since NIR light can penetrate tissues up a depth of 2 cm without significant loss of the optical signals. Using finite element simulation methods, the depth of recorded neural activation can be computed from the absorption variations [5]. Thus, localized cortical activity can be deduced from the hemodynamic profile in real time [1-3], but it is often assumed that oxygen delivery and oxygen extraction are coupled and co-varied together. This study will verify whether such oxy- and deoxy-Hb hemodynamic profiles are coupled or decoupled temporally during a cognitive motor task.

As a brief review, HbO_2 and Hb levels are detected optically, and computed from the modified Beer-Lambert law [4] based on refracted light scattered by the tissue. fNIRS can detect not just HbO_2 level but also Hb level, since the difference in spectral absorption between oxy- and deoxy-Hb molecules provides direct estimation of oxy- and deoxy-Hb levels. In contrast, functional magnetic resonance imaging (fMRI) detects primarily deoxy-Hb level, but not oxy-Hb level, because it is based on the blood oxygen level dependent (BOLD) activity. This is because deoxy-Hb molecules are more paramagnetic than oxy-Hb molecules, and the magnetic susceptibility of blood increases linearly with increasing oxygenation. BOLD signal is more sensitive to venous changes than arterial changes because arterial blood is usually fully oxygenated whereas venous blood is more deoxygenated. Functional NIRS not only detects both oxy- and deoxy-Hb levels, it also detects both arterial and venous blood oxy- and deoxygenation. In contrast, fMRI detects only deoxygenation in the venous return. Functional NIRS can detect high-resolution temporal signals in msec [1-3] providing real-time monitoring of changes in hemodynamic responses, whereas fMRI usually acquires low-resolution temporal signals when it takes 1 sec to scan the whole brain.

It is often assumed that relative oxygenation changes with the amount of deoxygenation, but this assumption has not been validated experimentally using fNIRS measurements. There is evidence that BOLD response is shown to reflect pooled local field potential activity [6]. Although neuronal activation and vascular responses are correlated by the "neurovascular coupling" [7], the coupling between oxy- and deoxy-Hb hemodynamic responses have yet to be determined experimentally. It has been shown that transient BOLD signals in fMRI may not necessarily reflect cerebral blood flow (CBF), cerebral metabolic rate of oxygen (CMRO2), or cerebral blood volume (CBV) in simulation model and experimental recording from primary motor area (M1) and supplementary motor area (SMA) [8]. Although there is a high correlation between ASL (arterial spin labeling)-based fMRI, BOLD, and NIRS during an event-related motor activity in human subjects [9], we will show in this study that complex dynamics of temporal coupling and decoupling can occur differentially in the NIRS hemodynamic response. We will use various differential measures of fNIRS hemodynamic signals to quantify the temporal profiles of neurovascular responses (oxygen delivery vs. oxygen extraction, and total blood volume vs. total oxygenation) in real time with respect to the demand of a cognitive task.

A brief review of the computation of the hemodynamic response is provided, applying the modified Beer-Lambert Law [4] to extract the HbO_2 and Hb signals:

$$I = I_0 10^{-(\alpha_{\mathrm{Hb}}\Delta c_{\mathrm{Hb}} + \alpha_{\mathrm{HbO}}\Delta c_{\mathrm{HbO}})L} G \tag{1}$$

and

$$OD = -\log\frac{I}{I_0} = \mu_a L D_{PF} + G = \left(\alpha_{\mathrm{Hb}}\Delta c_{\mathrm{Hb}} + \alpha_{\mathrm{HbO}}\Delta c_{\mathrm{HbO}}\right)L \tag{2}$$

where *OD* is the optical density of the sample (as determined by the negative log ratio of the detected intensity of light *I* with respect to the incident intensity of light I_o), μ_a is the absorption coefficient of the tissue, *L* is the net distance traveled by the light from the light emitter to the NIR sensor, D_{PF} is the differential path-length factor, and *G* is the geometry factor. D_{PF} accounts for the extra total distance that light travels through the tissue due to scattering. *G* accounts for light attenuation because of the geometry of the sample. By inducing a small change in μ_a between time t_1 and t_2, the change in the optical density will be:

$$\Delta OD = -\log(\mathrm{t}_1/\mathrm{t}_2) = \Delta\mu_a L D_{PF} \tag{3}$$

The total blood volume delivered to the tissue is given by the sum of changes in optical density:

$$\text{Total Blood Volume} = \Delta c_{\mathrm{HbO}} + \Delta c_{\mathrm{Hb}} \tag{4}$$

and the amount of oxygenation is given by the difference between changes in optical density:

$$\text{Total Oxygenation} = \Delta c_{\text{HbO}} - \Delta c_{\text{Hb}} \quad (5)$$

We will denote HbO_2 level to represent Δc_{HbO}, and Hb level to represent Δc_{Hb}, since the Δc_{HbO} and Δc_{Hb} values are computed as the output optical signals in the optical recording instruments. The total blood volume delivered to the tissue is the sum of HbO_2 and Hb (HbO_2 + Hb), and the total oxygenation extraction is the difference between HbO_2 and Hb (HbO_2 – Hb). We will use these two derived measures to characterize the hemodynamic responses, in addition to the convention oxygen delivery (HbO_2) and oxygen extraction (Hb) measures to quantify the neural activation/deactivation temporal profiles.

We will employ a motor execution task to detect whether there is a temporal decoupling between oxy- and deoxy-Hb temporal hemodynamic responses. It is well known that movement directions can be decoded from the tuning curve representing their preferred movement direction [10-14] using microelectrode recordings of identified neurons in the motor cortex. We will extend this correlation of neural activation with motor execution commands to confirm the hemodynamic response associated with arm movements optically. Since the resultant movement direction can be decoded computationally by the population vector sum of the movement related neurons in a localized region of the cortex electrically [10-14], we will provide the empirical evidence to show that the hemodynamic changes can also be used to decode movement directions optically. We will demonstrate that decoding movement directions requires all four hemodynamic measures of oxygen delivery, extraction, total blood volume and total oxygenation (based on HbO_2, Hb, (HbO_2 + Hb), and (HbO_2 – Hb)), while relying on a single measure of deoxy-Hb is insufficient to differentiate the movement direction adequately [15-17].

Functional NIRS recordings have the advantage of sampling at a higher temporal resolution (in KHz) than fMRI recordings (in Hz), even though most hemodynamic responses occur at a much slower rate than the fNIRS sampling frequency [18]. Nonetheless, the higher temporal resolution of hemodynamic signals is essential for detecting dynamic movement activity, such as the high frequency movements that require maximal effort (ME) [19]. Although previous fNIRS studies had revealed movement related hemodynamic response to motor tasks, most of these studies focused on repetitive motor activation tasks rather than directional arm movements. These studies included finger tapping or oscillator movements [7, 20-24], walking movements [25], movement imagery [26] and activity while solving an anagram task [27]. We will examine how hemodynamic responses in the motor cortex can be used to differentiate the difference between orthogonal arm movement directions by the temporal coupling/decoupling of oxy- and deoxy-Hb signals.

2. MATERIALS AND METHODS

We recorded the optical signals of hemodynamic response in fourteen human subjects while executing a directional arm movement task. Human subjects were asked to execute volitional freehand arm movements in the horizontal *xy*-plane parallel to a desk surface. We included fourteen healthy subjects in this study. All subjects were instructed to initiate right-left (*x*-direction) or front-back (*y*-direction) hand movements between 30-cm targets in a horizontal plane by a sound tone. A 64-channel ISS Imagent™ optical system is used to record the hemodynamic signals (HbO_2 and Hb) from both motor cortices bilaterally during the above motor task. We recorded multiple front-back (or right-left) movements (repeating the movement sequence for a period of 5 min) so that signal-averaging analysis is used to improve the signal-to-noise ratio. The experimental protocol was approved by the Institutional Review Board.

3. RESULTS

Figs. 1 and 2 shows the hemodynamic signals during a backward (–*y* direction) arm movement (in a front-to-back direction) 30 cm in front of the human subject. The graph shows changes in oxy-hemoglobin (HbO_2) profile (in red) and deoxy-hemoglobin (Hb) profile (in blue), which is correlated temporally with this backward (front-to-back) movement direction. It revealed a continual *decrease* of oxygen delivery (HbO_2), but a slight *increase* in oxygen extraction (Hb) for this arm movement. That is, oxygen delivery and extraction are decoupled temporally with a decrease (deactivation) for oxy-Hb and a slight increase (activation) for deoxy-Hb. This shows that oxygen delivery decreases while oxygen extraction increases simultaneously.

This result shows that, in contrary to the usual assumption, oxy-Hb (HbO_2) and deoxy-Hb (Hb) are always changing in the same direction temporally. That is, oxygen delivery and oxygen extraction do not increase (or decrease) together. Instead, one hemodynamic measure (oxy-Hb) activates while the other measure (deoxy-Hb) deactivates simultaneously during the same arm movement. This temporal decoupling is revealed by the fNIRS measures (unlike fMRI BOLD signals, which only measures deoxy-Hb level but not oxy-Hb level).

Fig. 2 shows the sum of oxy-Hb and deoxy-Hb (HbO_2 + Hb) (in red) (which represents the total blood volume delivery to the brain tissues in motor cortex), and the difference of oxy-Hb and deoxy-Hb (HbO_2 – Hb) (in blue) (which represents total oxygenation in the tissue). These two derived hemodynamics measures reveal that both blood volume and oxygenation decreased continually during the backward arm movement. This suggests a deactivation (decrease) in the total blood delivery and total oxygenation, which are coupled temporally. In contrast, the oxygen delivery (oxy-Hb) decreased, while oxygen extraction (deoxy-Hb) increased (see Fig. 1), suggesting that oxygen demand was so high that oxygen delivery could not keep up with oxygen extraction.

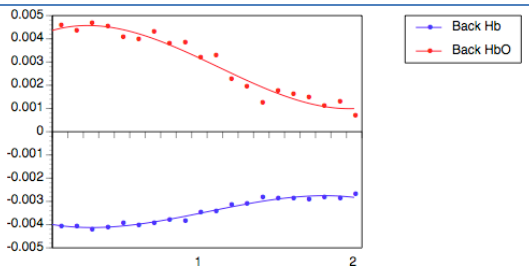

Figure 1: Oxy- and deoxy-Hb hemodynamic responses of arm movement toward backward (front-to-back) in –*y* direction. Sound tone is used to instruct movement onset is at time 0. [HbO_2 signal is shown in red, and Hb in blue. *X*-axis is in sec, *y*-axis is in decoded optical intensity units.]

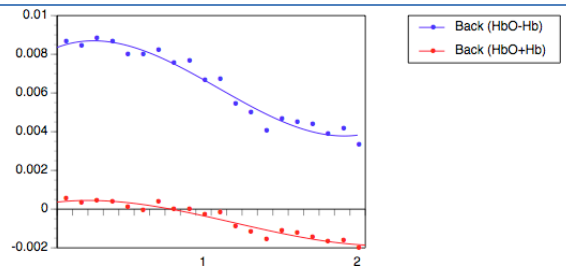

Figure 2: Hemodynamic responses as measured by the sum of HbO_2 and Hb (HbO_2 + Hb), and difference of HbO_2 and Hb (HbO_2 – Hb) for the same backward arm movement shown in Fig. 1. [(HbO_2 + Hb) signal is shown in red, (HbO_2 – Hb) signal is shown in blue.]

This analysis shows that three of the hemodynamic variables – oxygen delivery (HbO_2), total blood volume (HbO_2 + Hb), and total oxygenation (HbO_2 – Hb) – all decreased in the backward movement direction (front-to-back direction), while oxygen extraction (Hb) level increased slightly. This shows a temporally decoupled response of oxygen extraction from the above three hemodynamic variables. Thus, the optical recordings revealed that not all four hemodynamic measures (HbO_2, Hb, (HbO_2 + Hb), and (HbO_2 – Hb)) are coupled temporally (or activated simultaneously) in a task related response.

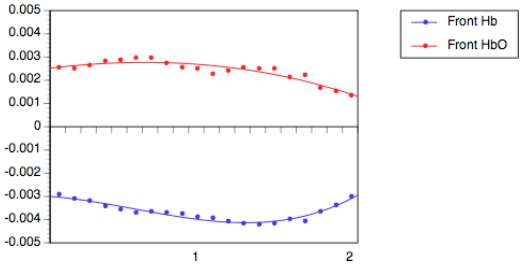

Figure 3: Oxy- and deoxy-Hb hemodynamic responses of arm movement toward frontward direction (back-to-front) in +*y* direction.

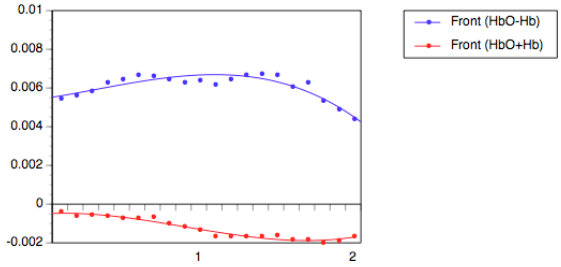

Figure 4: Hemodynamic responses as measured by the sum of HbO_2 and Hb (HbO_2 + Hb), and difference of HbO_2 and Hb (HbO_2 – Hb) for the same frontward arm movement shown in Fig. 3.

In contrast, frontward (back-to-front) arm movement in +*y* direction (Fig. 3) shows an opposite change in the hemodynamic response. It shows a slight increase (then a decrease) in the oxy-Hb level, but an opposite temporal profile (a slight decrease and then an increase) in deoxy-Hb level. That is, a temporal decoupling between oxygen delivery and oxygen extraction occurred for the frontward movement. But for the derived measures of total blood volume and oxygenation (Fig. 4), i.e., (HbO_2 + Hb) and (HbO_2 – Hb), they showed opposite changes temporally, unlike the simultaneous deactivation for backward arm movements. This shows that the same motor cortical area was activated/deactivated differently for both frontward and backward arm movements as revealed temporally by the opposite changes

(increasing/decreasing) in oxygen delivery, extraction, total oxygenation and total blood volume (by comparing Figs. 1-4).

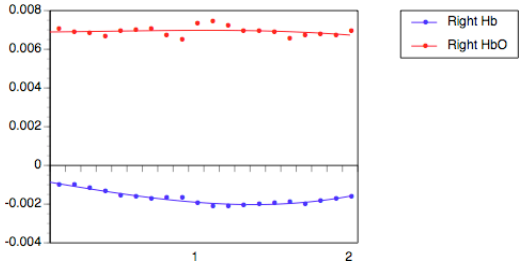

Figure 5: Oxy- and deoxy-Hb hemodynamic responses of arm movement toward rightward direction (right-to-left) in +*x* direction.

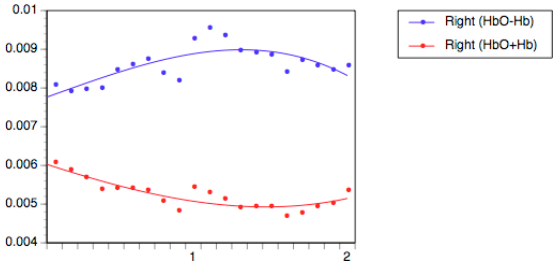

Figure 6: Hemodynamic responses as measured by the sum of HbO_2 and Hb (HbO_2 + Hb), and difference of HbO_2 and Hb (HbO_2 – Hb) for the same rightward arm movement shown in Fig. 5.

In order to illustrate the differences in temporal hemodynamic profiles between front-back and right-left movements, Figs. 5-8 show the hemodynamic responses for the right and left movements (compared to Figs. 1-4 for the front and back movements recorded from the same optrode in the same human subject). Fig. 5 shows the temporal decoupling response with no change in oxy-Hb, but a slight decrease in deoxy-Hb level for the rightward (+*x* direction) arm movement. But for the derived measures of total blood volume and oxygenation (Fig. 6), i.e., (HbO_2 + Hb) and (HbO_2 – Hb), they showed opposite changes temporally. They are decoupled temporally.

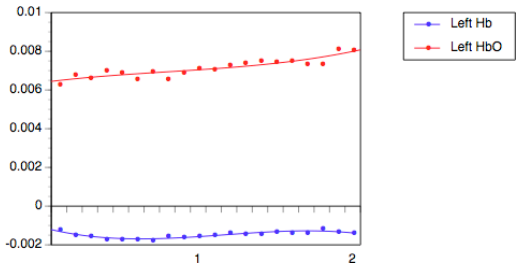

Figure 7: Oxy- and deoxy-Hb hemodynamic responses of arm movement toward leftward direction (left-to-right) in –*x* direction.

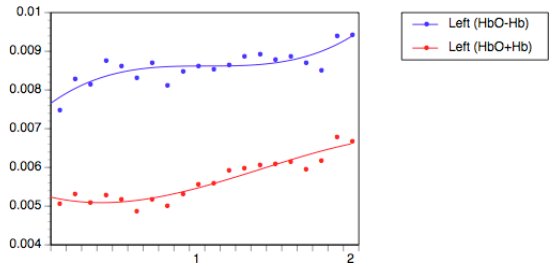

Figure 8: Hemodynamic responses as measured by the sum of HbO_2 and Hb (HbO_2 + Hb), and difference of HbO_2 and Hb (HbO_2 – Hb) for the same leftward arm movement shown in Fig. 7.

For leftward (–*x* direction) arm movements, Fig. 7 shows there is a slight increase in oxy-Hb with no change in deoxy-Hb level. If oxy-Hb and deoxy-Hb levels were to use as an indicator to differentiate between right and left arm movements by comparing Fig. 5 and Fig. 7, then activation of oxy-Hb suggests leftward movement while deactivation of deoxy-Hb suggests rightward movement. But if the derived measures of total blood volume and oxygenation were used to compare the difference, i.e., (HbO_2 + Hb) and (HbO_2 – Hb), then the temporal profile for the above two measures are decoupled for rightward movement (see Fig. 6) but coupled for leftward movement (see Fig. 8).

Thus, the activation/deactivation patterns vary depending on which temporal hemodynamic profile is used as the criterion to characterize the neural response. The temporal changes in hemodynamic response for the neural population under the same optrode vary depending on whether the arm movement is right, left, front or back (i.e., *+x*, *–x*, *+y* or *–y* directions). They can be temporally coupled (or temporally decoupled) in any combination among the four hemodynamic measures [HbO_2, Hb, (HbO_2 + Hb) and (HbO_2 – Hb)] we examined in this study.

4. DISCUSSION

This study demonstrated empirically that oxygen delivery and extraction can be coupled (and decoupled) temporally by the same neural tissue on demands, depending on the specific movement execution directions. This dynamical change in temporal profiles shows the complexity (as well as the unique differential changes) in oxy-Hb and deoxy-Hb signals associated with a specific movement direction. Analysis of the fNIRS signals representing the hemodynamic responses demonstrated that different measures of hemodynamic responses showed differential changes temporally to the same motor task. Specifically, the differential activation and deactivation of hemodynamic responses cannot be assumed to occur simultaneously without actual measurements of the oxy-Hb and deoxy-Hb signals.

The analysis revealed that various measures of hemodynamic vascular responses exhibit decoupled responses temporally during the same movement direction, while the hemodynamic responses are not always opposite for the opposite movement direction. Oxygen delivery, extraction, total blood volume, and total oxygenation all can be coupled/decoupled temporally in the same movement direction, but partially decoupled/coupled in opposite movement direction. The temporal decoupling may not occur in opposite manner (i.e., activation vs. deactivation) for the opposite movement direction. Similar temporal decouplings were also reported [16, 17].

The decoupling is likely due to a transient supply of oxygen not keeping up with the oxygen demand in the neural tissue. Although oxygen consumption increases with metabolic activity, it can be due to increased metabolism in supportive cells, such as glial cells that regulate ionic concentration and neurotransmitter metabolism. Hemodynamic response is known to be associated with oxygen demand and metabolic activity, but may not necessarily coupled with neuronal activity directly [28]. Since we did not record both electrical and optical signals simultaneously, it is unknown in this study whether hemodynamic vascular response is related temporally to the electrical firing activities or synaptic events.

Most importantly, the differential changes in the temporal hemodynamic responses revealed that oxygen demands can be accomplished by two independent processes: oxygen extraction and oxygen delivery. Total oxygen delivery can be maximized by an increase in total blood volume delivered (HbO_2 + Hb), and by an increase in partial pressure of oxygen (P_{O2})

saturation in the hemoglobin molecules. Oxygen extraction is accomplished by unloading oxygen molecules from hemoglobin molecules, which is detected by a decrease in P_{O2} in the hemodynamic signal.

The decoupled temporal dynamics is a phenomenon often observed during physical exercise in the peripheral system (during high muscle oxygen demand), when oxygen consumption is so high that oxygen extraction exceeds the ability for oxygen to be delivered to the target tissue by blood. It is only when oxygen demand is not intense that oxygen delivery is sufficient to meet the demand by the total blood volume delivered under normal non-hypoxic circumstances. This phenomenon is observed during non-demanding physical exercise with P_{O2} maintaining at 96–99% saturation normally. Hypoxic condition can occur during strenuous exercise when metabolic demand is so high that P_{O2} saturation in hemoglobin molecules will drop below 96%, creating the temporally decoupled hemodynamic phenomenon.

It can be deduced that, when neural metabolic demand is so high, micro-hypoxic condition can occur locally at the activation site. It is only during normal (non-hypoxic) condition that, without exceeding this limit, the oxygen delivery can keep up with extraction. This could account for the above differential temporal decoupled phenomenon in the hemodynamic responses illustrated earlier.

This interpretation is consistent with the complex transient biphasic temporal cerebral oxygenation responses in response to intense oscillatory motor stimulation [21]. It is also consistent with similar findings of regional cerebral blood oxygenation (rCBO) in the motor cortex and blood flow velocity changes (CBFV) in the middle cerebral artery in a combined NIRS and transcranial Doppler Sonography (TCD) study for sequential finger opposition task [23]. The size of hemodynamic response also reported [22] to be dependent on the resting period between sequential motor executions, which peaked maximally with a 30-sec resting period in finger tapping task.

These findings are consistent with our findings that the hemodynamic responses are complex dynamical interactions among many various vascular components to produce the temporal changes [16, 17]. This shows that oxygen delivery and extraction can change independently and may decouple temporally. So if the neuronal activation/deactivation response is characterized by only one of these hemodynamic measures (either oxy-Hb or deoxy-Hb) without the other, it will miss the complete description of the temporal response that represents both oxygen delivery and extraction dynamics.

Most importantly, if only deoxy-Hb level were used to infer the hemodynamic response as neural activation (as in fMRI BOLD signal analysis), it could mischaracterize the neural response since fNIRS signals can reveal a simultaneous increase in oxy-Hb level but a decrease in deoxy-Hb level (or vice versa) during the same motor task. Thus, no single measure of the hemodynamic response is sufficient to characterize the neural activation/deactivation pattern temporally. Since fNIRS recordings can detect a much higher temporal resolution (in msec)

than fMRI (in sec), the hemodynamic response can change so rapidly that it can increase and then decrease within the time frame of less than 1 sec (which is less than the typical scanning time required for fMRI recordings). It remains to be confirmed with simultaneous recordings of both fNIRS and fMRI in subsequent studies to determine whether any direct evidence will support the above findings experimentally.

5. CONCLUSION

This study demonstrated the experimental evidence that differential temporal changes in hemodynamic measures of HbO_2, Hb, (HbO_2 + Hb) and (HbO_2 – Hb) can occur in NIRS signals during the same motor task. The increase/decrease in the above hemodynamic measures can be decoupled from each other temporally. The temporal changes in hemodynamic response are not necessarily opposite for arm movements that were made in the opposite direction. If neural activation were deduced based on monitoring the deoxy-Hb level in the BOLD signals alone without monitoring the oxy-Hb level, it may represent a partial record of the hemodynamic activities compared to the more complete record of both oxy- and deoxy-Hb signals recorded by the fNIRS signals.

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Role of Neural Analysis in Epileptic Attacks with Special Reference to Trace Elemental and Immunological Findings

[1]Sanjeev Kumar, [1]Vinod Kumar, [2]Reena

[1]Department of Physics, Medical Physics Research Laboratory, D.A.V. (P.G.) College, Muzaffar Nagar, U.P India

[2]Department of Mathematics, Shri. K. K. Jain College, Khatauli, Muzaffarnagar, U.P, India

[1]sanjeev1962kumar@yahoo.co.in; [2]reena_math@rediffmail.com

ABSTRACT

Neural circuits that are involved in feeding behavior show precise coordination with brain centre that modulate energy homeostasis and cognitive function. The effects of food on cognition and emotions can start before act of feeding itself. The recollection of foods through olfactory and visual sensory inputs alerts the emotional status of the brain. The ingestion of foods triggers the release of hormones or peptides. These hormones or peptides can reach centre such as the hypothalamus and the hippocampus and activate signal – transduction pathways. Epileptic attacks or seizures may be regarded as an emergent property of a network where the underlying physiology oscillatory coordination has given way to excessive coordination. It may be significant that the medial temporal cortex, an area where high amplitude oscillation appears to play a role in episodic memory. It is susceptible to seizure. The trace elements such as Cu, Zn, Fe, Ca, Mg, Na and K are found in the traces in our blood .The excess and deficiency cause different body disorders and affect the immunity of human beings. If the immunity is disturbed in our system many diseases affect our CNS. In the present study we have tried to show how the trace elements and other abnormalities are related to neural system.

Keywords: Neuron , Brain ,Excitable cell, Epilepsy and Nervous system.

1. INTRODUCTION

We may giving an idea of a branch of science which is called Neurophysics comes under Biophysics stream. It can be easily understand Biophysics. Biophysics is today the youngest daughter of general Physiology, a sister to Biochemistry and Pharmacology. Subject matter is not very well defined in this stream. Although the basic skeleton of the approach of this stream

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is very clear. It is a good thought to the engineers and physicist to give a suitable concept of a system, which is twisted and molded to describe the living things. Biochemistry and Biophysics may have an attempt to describe and interpret the chemical as well as physical processes of biological materials in terms of the principles and theories of organic chemistry, physical chemistry and physics. Biophysics is concerned with the unexplained questions about the physics of biological systems. Biophysics has advantages of low complexity and more certainty than the biological subjects. It has main disadvantage of being limited to only specific aspects of the whole living system.

Biophysics for human being can be thought of as providing a description of the whole physical system from the particular point of view of physics.

The scope of biophysics now a day is rather very broad. Hill [1] a Noble prize winner has published a research article in 1910. The use of physical techniques or ideas alone for investigation of biological problems does not make Biophysics. The study of biological function, organization and structure by physical and physiochemical ideas and methods may be put as a subject and then hastens to emphasize.

It has been established that the natural systems are of enormous complexity. It is clearly advisable to subdivide the problem in two phases:

- The first part of the problem is the structure and functioning of such elementary units individually.
- The second part of the problem consists of understanding regarding the orientations of these elements into whole system and how the functioning of the whole system is expressed in terms of these elements.

We would like to introduce a new term 'the cell' here and it has been found that the number of cells in the human body is in general of the order of 10^{15} or 10^{16}. These cells are called neurons and situated in human brain. The number of neurons in the Central Nervous System (CNS) is somewhere in the order of 10^{10}. All of artificial automata made by man have number of parts of the order of 10^{3} to 10^{6}.

Davied [2] has described in detail for the introductory part of nervous system in terms of nerve cells. Organisms, which are large divided into a number of units called cells. Every cell is progeny of another cell. This statement holds good and constitutes the cell theory. Every cell is bounded by a cell membrane and contains a nucleus in which the genetic material is found. The main part of the living matter of the cell is a highly organized system called cytoplasm, which is concerned with activity of the cell. The cell membrane separates this highly organized system inside from the relative chaos that exists outside the cell.

Every cell requires a continual supply of energy in order to increase and maintain its high degree of organization and in order to respond to and alter its environment. This energy must

be derived ultimately from the environment. This energy is found in the form of chemical energy. It can be extracted by the cell from glucose molecules.

We can describe here the mechanism of the cell in thermo dynamical terms, which is an open system and maintained in a rather improbable steady state by the continual expenditure of energy. It's life is a continual battle against the second law of thermodynamics. The cells of nervous system are called neurons. Their primary function is the handling of information. Within the cells this mainly takes the form of changes in the electric potential across the cell membrane, whereas information is passed between cells largely in the form of chemical messages.

The idea and mechanism tell us about the nervous system is composed of discrete cells. It is known as the theory of neuron development. This is a particular application of the cell theory and it was developed in the nineteenth century.

Neurons have functional regions specialized for different purposes. The places or sites where one neuron contacts another cell called another neuron and transmits or receives some useful or un-useful information are called as synapses. Synaptic transmission is called one way scheme and it works from the pre -synaptic cell to the postsynaptic cell.

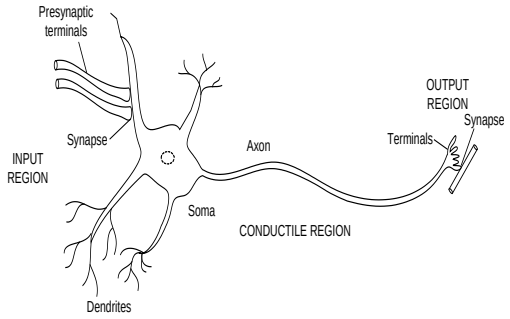

Figure 1: The main region of neuron.

The input region commonly consists of branched processes and it is called dendrites, it may include the surface of the cell body. This cell body is called soma and contains the cell nucleus of the neuron. The postsynaptic responses in the input region may be sufficient to produce excitation in the conductile region of the neuron, whose activity consists of unitary events called nerve impulses or action potentials. The conductile region is a long process and called the axon. The axon terminates in fine branches that make synaptic contact with other cells, such as other neurons or muscle cells. These terminals are pre synaptic and form the output region of the neuron. They secrete a chemical substance, which is called neurotransmitter, where an action potential arrives along the axon. This carries all informations across the synapses to the post-synaptic cell.

If we would like to define a living material, which is a function of the proteins, it must be taken into account and considered. Proteins are composed of chain made up from different combinations of twenty different amino acids, and their properties depend upon the sequence in which these amino acids are arranged. Immunoglobulins are also proteins.

The protein's amino sequences is specified by the nucleotide base sequence in the DNA molecules, which form the genetic material of the cell. Proteins may be considered as the product of evolution.

The shape of many protein molecules changes when they react with smaller molecules or other proteins. Changes of this type underline much protein activity, such as enzymatic hydrolysis, opening of membrane channels, and muscular contraction. The day to day activity of the cell can be described largely in terms of the actions of proteins.

2. CONDUCTION OF IMPULSES BY NERVES AND ROLE OF NERVOUS SYSTEM

Glasser [3] has described the fundamental concepts of Biophysics and given a great knowledge regarding the explanation of neurons to nervous system with all possible difficulties. The rapid co ordinations and response in human brain shows that the CNS must transmit information in an electrical or magnetic forms. The problems of the nervous system are similar to those of transmitting telephone messages over long distances. Either there must be many parallel low frequency channels, or fewer high frequency channels, each modulated by many separate signals. The number of channels has a tendency to increase with the complexity of living organism. The in formations may be transformed by electrical pulses.

Biophysicists have studied both the nervous and endocrine systems. Both the system lend themselves to the application of complex physical technique, and these can be analysed by the type of reasoning common to Physics and Electronics. This is true for the interaction between group of neurons, endocrine glands and also of the neuron-endocrine interactions. Feedback loops exist in which the effect produced alters the behavior of the neurons or endocrine glands producing these effects. Physicists and electrical engineers refer to these types of control mechanisms as negative feedback. Physiologists have called many of them **homeostatic** mechanisms because they tend to keep the state of organism constant.

Human body is a complex suspension of proteins in fluid. Actually the body, being a very special kind of electrolyte with many discontinuities in the form of membranes of different types, generates potentials. This potential difference between points of the body works as a normal function of the living organism.

Milton et al. [4] have studied epilepsy and suggested this disease is a dynamic from the clinical computation- list point of view. A neurologist can approach to a patient with epilepsy is

to first classify the epilepsy and then try to formulate a treatment plan. Epilepsy may be treated fundamentally as a dynamical disease. Time-dependent phenomena may occur on time scales, which have a range from milliseconds to hours to days and years may too have important consequences both for the occurrence and recurrence of seizures. Treatment strategies must take into account the evolving dynamics of this disease. The fundamental computational challenge in epilepsy is to understand the relationship between the structure of CNS and its dynamics. A cost effective and more effective treatment strategies may be developed by interdisciplinary work.

All the dynamical systems can be easily described by an appropriate mathematical model. Clinicians and Neuro-physiological and Neuro-psychological members are the legally investigators entitled to directly record from the human brain of a patient with epilepsy and measure the responses of the brain to various treatment strategies including electrical stimulation and surgical removal of the epileptic focus.

Scientists who study dynamical systems may think only about this challengeable disease. The discussion begins with the success of computational neuroscience. The description of the generation of the action potential by a single, isolated neuron must be introduced in the process of evolution.

Hodkin-Huxley has developed an equation for the excitable cell. These equations can be easily derived using the basic principles of electricity. Persinger et al. [5] have suggested that all brain functions and their associated experience may be easily studied and determined by physical principles. John [6] suggested that the complexity of brain function can be derived from a small number of basic algorithms.

Graben [7] has introduced Neurophysics and studied the different aspects of brain functions in terms of some specific challenges related to digital computer electronics purposes.

Metaphorically, the brain is compared with a digital computer that runs software algorithms in order to perform cognitive computations. Digital computers consist of circuit boards with chips, transistors, resistors, capacitances, power supplies, and other electronic components wired together. Digital computer mechanisms are non-linear physical systems in nature.

Brain consists of 80% of water contained in cells and also surroundings cells. Physical wet-ware substrate supports computational dynamics. We can start our work from the physiological facts about neurons, their cell membranes, electrolytes and ions. Biophysical principles of neural computation is parallel to those of computation in electronic circuits may be taken into consideration. Physiological properties may be described by electric equivalent circuits.

Equivalent circuit of the membrane allows for derivation of the action potentials, which are the basic building blocks of neural conductance models. Travelling along the axon, pre synaptic terminals reached at this stage. Hodgkin-Huxley equations have to be supplemented by

additional terms describing the dynamics of voltage gated calcium channels. Calcium flowing into the terminal causes the release of transmitter vesicles that pour their content of neurotransmitter into the synaptic cleft of a chemical synapse. Then, at the post synapse, transmitter molecules dock onto receptor molecules, which indirectly open other ion channels. The kinetics of these reactions give rise to the impulse response functions of the postsynaptic membranes. These membranes behave almost passively , a linear differential equation describes the emergence of post-synaptic potentials by the convulsion product of the postsynaptic pulse response with the spike train, i.e., the sequence of action potentials. Post-synaptic potentials propagate along the dendrites and the soma of the neuron and superimpose likely to a resulting signal that eventually arrives at the axon hillock.

Scott [8] has written in his monograph regarding the sodium and potassium ion currents. These currents respond non-linearly to changes of voltage across the membrane. The behavior of these non-linear currents has been described in a simple way. Lytton [9] studied computer modeling in epilepsy in detail with the detail of single neuron. This study has a considerable progress in developing realistic models within anatomically detailed morphology. Neural networks are also moderately realistic simulations. These networks are responsible for the oscillations of brain mechanism. They may be helpful in providing clues to pathophysiology of the disease. All the neurological disorders such as epilepsy, paraplegia, migraine, Alzheimer's disease, muscular dystrophy and other diseases can be better understood with the progress of these networks in neurological science.

The theory of immune network provides the network view that lymphocytes are mutually and dynamically connected by antigen antibody interaction .Not only antigen but also antibody generated by lymphocytes will act as an antigen against the other lymphocytes , thus persuading internal image of antigen .Both antigen and its internal image active the same specific type of lymphocytes .This network view may help in the direction of the immune memory in the network .If it is disturbed by an antigen , the network in an equilibrium will move to the other equilibrium point , We are giving some of the main features of the immune network theory as well as process is controlled by the interaction among antigens . The neural network use electric signals for communication ,while the immune system uses chemical ingredients the neural networks are used for the general pattern recognition ,the immune system is for special pattern recognition .The neural networks form a hierarchical systems , while the immune systems form a network without a center . The neural networks assume homogenous units, while immune systems assume heterogeneous agents. The immune system uses the diversity and self identification .

The mathematical modeling can reduce the gaps between the objects of neuroscience study from the level of chemical concentrations to the neurons and networks.

It is established that the brain's business is information only. Information content may be predicted or inferred at different levels of investigation, from ion concentration up to differential cortical perfusion patterns. We would like to add here that a variety of information theoretical tools for assessing the information content of signals.

The human brain is a complex nervous system with many individual components, neurons, synapses, acting in concert. The brain is said to show emergent properties. These properties can not be explained by considering the properties of the underlying units. We may use a property of such as thermodynamics. Large scale properties emerge from Newtonian approximations of the behavior of individual particles.

In the neural system, the emergent properties are seen in distributed representation. Information is parceled out among many units. The individual unit does not carry individually interpretable information or an individually interpretable processing task.

Neurons have great anatomical and physiological complexity. It remains an open question as to how much of this complexity is used for information processing by the cell and how much is simply a consequence of having to support its life process. Living cells are very complicated. The complexity of the individual neuron makes it an information processing device made up of multiple units. Dendritic sub regions encompassing groups of synapses would serve as processing units. The processing units at this stage are heavy interdependent and dynamically linked. We are unable to separate the units conceptually or informatically. Single neuron is simply computationally a single processing unit. Single neuron may be regarded as a point neuron with only a single and particular state. Neurons differ vastly across different brain areas and across types within an area.

Human body is a complex suspension of proteins in fluid .We can learn by observing their behavior in the presence of an electric field . It is natural to make such kind of experiment on the human body . The body is substantially different from many technological systems. If we have small current in our body then it behaves more or less like the inert circuit elements , which are used by electrical engineers as resistance , captaincies and electrolytes . Our body is made up of special kind of electrolyte with discontinuities in the form of membranes of various types , generates potentials and potential differences between different points of the body as a normal function of the living organism. If we apply some current on the body , tissues are caused to respond actively in such a fashion that is not very simple .On the other hand if current of very large magnitude passed through the body ,shock or death may result . Physiological systems are assumed to be representable as combinations of resistances and capacitors .tissue does not have the property representable as an inductance . This has been established that the appearance of tissue under the microscopic examination often suggests an appropriate circuit diagram .We can identify the tissue electrical properties with peculiarities of cell structure in plausible fashion . When a steady direct current is passed through tissue , the

tissue behaves like a resistance . A proper model for material is an electrolyte , or a suspension of one type of electrolyte in another .

We are using the theory of Hodgkin-Huxley models[10] directly in the present proposed work , which is applied to neurons and a brief idea is given here. If we see an increase of sodium conductance, which leads to a more positive membrane potential, or, to a depolarization, while an increasing conductance either of potassium or of chloride entails a further negativity, or hyper polarization of the membrane potential in case of epileptic attacks . We may achieve these effects with the help of voltage-gated sodium and potassium channels. They can be taken as AN and AK. We can presume that these are into the cell membrane and given by the equivalent circuit , which is given below.

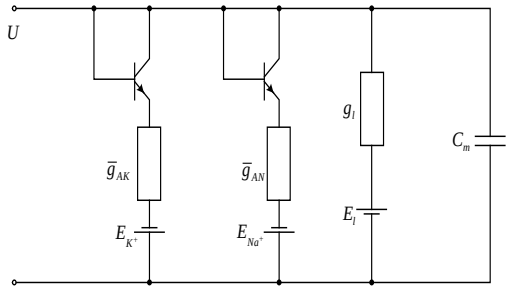

Figure 2: Equivalent circuit for the Hodgkin-Huxley equations.

The first and second mesh represents the voltage-gated potassium and sodium channels. Third mesh is taken from stationary descriptions of leakage potential. The capacitance is now needed for the dynamics of the membrane potential.

Apply Kirchoff's law. Total current through the circuit added upto an injected current I_m

$$I_{AK} + I_{AN} + I_e + I_c = I_m \tag{1}$$

We have some other types of currents called partial given below

$$I_{AK} = P_{AK}\ \bar{g}AK\ (U - E_{K^+}) \tag{2}$$

$$I_{AN} = P_{AN}\ \bar{g}AN\ (U - E_{Na^+}) \tag{3}$$

$$I_l = g_l\ (U - E_l) \tag{4}$$

$$I_c = C_m \frac{dU}{dt} \tag{5}$$

Now membrane potential $U(t)$ will obey the differential equation as

$$C_m \frac{dU}{dt} + P_{AK}\ \bar{g}_{AK}(U - E_{K^+}) + P_{AN}\ \bar{g}_{AN}(U - E_{Na^+}) + g_l\ (U - E_l) = I_m \tag{6}$$

Now equation (6) has to be supplemented by the following equations for open probabilities P_{AK} and P_{AN}

$$\frac{dp_K}{dt} = a_K\,(1-P_K(t)) - \beta_K P_K(t) \tag{7}$$

and the rate equation

$$\alpha_K(U) = e^{\frac{W_0(C\to 0)+QU}{K_B T}} \tag{8}$$

For α_{AK}, α_{AN}

Hodgkin and Huxley reported two other relations

$$P_{AK} = n^4,\ P_{AN} = m^3 h \tag{9}$$

Now, n, m and h obey three master equations

$$\frac{dn}{dt} = \alpha_n(1-n) - \beta_n n \tag{10}$$

$$\frac{dm}{dt} = \alpha_m(1-n) - \beta_m m \tag{11}$$

$$\frac{dh}{dt} = \alpha_h(1-n) - \beta_h h \tag{12}$$

Now, equation (6) and (10), (11), (12) are called required Hodgkin-Huxley equations. They constitute a four-dimensional non-linear dynamical system controlled by the parameter I_m.

The emergence of an action potential results from different kinetics of the ion channels. If the cell membrane is slightly depolarized by the current I_m, the opening rate α_n for the sodium channels increases. Further depolarization of the membrane is found.

A spike train travels along the axon and after the several branches reaches to presynaptic terminals, the composition of membrane is totally changed. Voltage-gated calcium channels are seen in addition to the voltage gated potassium and sodium channels.

Now we have

$$I_{AC} = l^5\,\bar{g}_{AC}\,(U - E_{Ca^{2+}}) \tag{13}$$

where l obeys another master equation

$$\frac{dl}{dt} = \alpha_l\,(1-l) - B_l\,l \tag{14}$$

In the absence of an injected current (I_m = 0), the presynaptic potential $U(t)$ is introduced in the equation given below

$$C_m \frac{dU}{dt} + I_{AK} + I_{AN} + I_{AC} + I_l = 0 \tag{15}$$

If the calcium leakage is very low can be neglected. We have an enhancement of the intracellular concentration

$$\frac{d\,|\,Ca^{2+}\,|_{\text{int}}}{dt} = \frac{-I_{AC}}{q\,N_A V} \tag{16}$$

$q = 2e$ is the charge of calcium ion; N_A = Avogadro number

The accumulation of calcium in the cell plasma gives rise to a cascade of metabolic reactions. Calcium does not act as an electric signal. It acts as an important messenger and chemical reagent enabling or disenabling the functions of enzymes.

3. MECHANISM OF EXCITATION

There are a number of factors involved in active response, which remain obscure because they occur at atomic dimensions.

Tissues will respond to stimuli which are electrical, mechanical, thermal and chemical. The electrical impulses are easy to grade, time, shape and record. We can describe the mechanism of excitation phenomena in terms of characteristics of electrical impulse.

A cell is excited if the current is caused to flow from the inside of the cell outward (i.e., electrons flow from outside inward), and if the current flow is from outside inward, the threshold for a second stimulus is increased. If a pair of electrodes is placed on the surface of a cell (e.g. nerve axon), excitation will appear at the cathode. A tissue may also originate a response at the anode.

4. THEORY OF EXCITATION OF NEURONS

The existing theory of excitation is based on the empirical observations. If we assume that there is a quantity, which is called excitation and increases at a rate which is proportional to the applied voltage to a cell. At the same time, there is also a decrement in excitation, which occurs at a rate which is proportional to the amount of excitation. We can say that a rise in excitation is exponential in nature.

Hill[1] has proposed a theory of excitation. He pointed out one every significant factor in excitation. Threshold may rise at the same time the local excitatory state is building up as a result of passage of an electric current. The threshold for a second stimulus is the difference between the excitatory state and the existing absolute threshold.

Now, if we assume that the absolute threshold begins to increase upon the application of the current and that it does so exponentially, with a time constant λ.

This increase may be associated with the accumulation of K^+ ions in the medium immediately surrounding the cell. A decay of the threshold occurs when the exciting current is

removed. If the local excitatory state before stimulation is V_0 and the excitatory state at time θ is V, the build up is given by the relation

$$V - V_0 = b\, e^{-t/K} \int_{\theta=0}^{\theta=t} I_\theta\, e^{\theta/K}\, d\theta \tag{17}$$

Where b is a constant; K is the decay constant for the excitatory state; I_θ is the current, which is a function of θ.

Similarly, the buildup of threshold which occurs may be expressed by the following relation

$$U - U_0 = \frac{e^{-t/\lambda}}{\lambda} \int_{\theta=0}^{\theta=t} (V - V_0)\, e^{\theta/\lambda}\, d\theta \tag{18}$$

Here, U is the threshold at time θ; U_0 is the resting threshold; λ is the time constant of threshold decay.

When U and V are equal in nature, the excitation occurs. It has been experimentally verified that a frog's modulated nerve K is about 0.25 m sec and λ is of the order of magnitude of 50 m sec.

The impedance of cells and tissues may be assumed to be a largely function of the capacitive reactance of the cell membranes. It has been already established that the membranes change their characteristics as physiological conditions fluctuate .The measurement of whole body impedance may be considerable interest on clinical point of view. Cole and Curtis [11] have summarized analyses of current distributions in nerve based on the cable theory. According to this theory the nerve consists of a large number of electrical elements connected by a conducting medium inside the cell and outside the cell. Equivalent circuit is given below

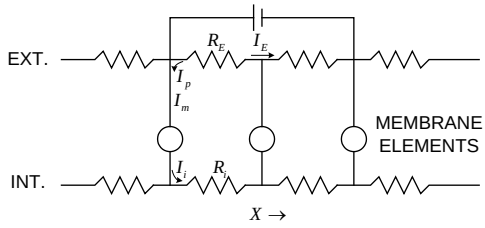

Figure 3: Systematic diagram of the theoretical system involved in cable theory.

If r_1 is the resistance per unit length of the medium bathing the cell, r_2 is the resistance per unit length of the material inside the cell, z_m is the membrane impedance per unit length, i_1 is the current through r_1, i_2 is the current through r_2, i_m is the current through z_m, v_1 and v_2 are the potentials of the outside of the resting portion and the inside of the resting portion.

Apply Ohm's law

$$\frac{\partial V_1}{\partial x} = -r_1\, i_1 \tag{19}$$

also $$\frac{\partial V_2}{\partial x} = -r_2\, i_2 \tag{20}$$

and $$V_1 - V_2 = -z_m\, i_m \tag{21}$$

If there is no current flow in this system

$$i_1 + i_2 = 0 \quad \text{and} \quad \frac{\partial i_1}{\partial x} = -\frac{\partial i_2}{\partial x} = i_m \tag{22}$$

$$\frac{\partial^2 V}{\partial x^2} = -(r_1 + r_2)\, i_n \tag{23}$$

$$V = V_1 - V_2$$

Membrane current is proportional to the second derivative of the voltage. Now the equivalent circuit of the individual membrane element contains a capacitance C_m in parallel with a resistance r_4 and a battery of potential E in series.

If i_1 is the current in the C_m, i_4 is the current through r_4 and t is the time.

$$\left.\begin{aligned} i_3 &= -C_m \frac{\partial V}{\partial t} \\ i_4 &= \frac{E - V}{r_4} \end{aligned}\right\} \tag{24}$$

The total current through this element is

$$i_n = i_3 + i_4 = -C_m \frac{\partial U}{\partial t} + \frac{E - V}{r_4} \tag{25}$$

Now, use equation (23) and substitute the value of i_m from (25) in (23), we get

$$\frac{\partial^2 V}{\partial x^2} = -\frac{r_1 + r_2}{r_4}\left[E - V - r_4 C_m \frac{\partial V}{\partial t}\right] \tag{26}$$

For convenience, we use two terms such as characteristic length of the cell (λ) and time constant of the membrane (τ_m)

$$X = \sqrt{r_4 / (r_1 + r_2)} \tag{27}$$

$$\text{and} \quad \tau_m = r_4\, C_m \tag{28}$$

Now substitute these values in (26), we get

$$\lambda^2 \frac{\partial^2 V}{\partial x^2} - \tau_m \frac{\partial V}{\partial t} - V = -E \tag{29}$$

If the cell responds actively, the impulse is propagated at a velocity the cell.

We wish to examine the distribution of currents about the active area, we may use a coordinate system and an equation is given below

$$y = x - Vt \tag{30}$$

$$\frac{\partial V}{\partial x} = \frac{\partial V}{\partial y} \tag{31}$$

$$\frac{\partial^2 V}{\partial x^2} = \frac{\partial^2 V}{\partial y^2} \tag{32}$$

$$\frac{\partial V}{\partial t} = -V \frac{\partial V}{\partial y} \tag{33}$$

Substitute these values in (29), we get

$$\lambda^2 \frac{\partial^2 U}{\partial y^2} + V \tau_m \frac{\partial V}{\partial y} - V = -E \tag{34}$$

$$E = V - \beta \lambda^2 \left[\frac{\partial^2 V}{\partial y^2} + \frac{1}{\lambda_0} \frac{dV}{dy} \right] \tag{35}$$

where β is defined as the ratio of membrane resistances in activity to that at rest, λ_c is the characteristic length of a non-conducting membrane and is equal to

$$\lambda_0 = \frac{1}{V} (r_1 + r_2) C_m \tag{36}$$

$\bar{\lambda}$ is the characteristic length due to membrane conductances, λ_0 is the property of the membrane capacitance.

The propagation amounts to the spread of a localized short circuit, so that E and r_4 are zero at $y = 0$, and we obtain their resting values at all other points.

Now if e is the voltage spread from the active area, V is equal to $E - e$ and we get

$$\bar{\lambda}^2 \frac{\partial^2 e}{\partial y^2} + U \tau_m \frac{de}{dy} - e = 0 \tag{37}$$

Except at $y = 0$, $\bar{\lambda}$ and λ_e are constants so that

$$e = e_0 e^{-(y/\lambda_a)} \tag{38}$$

where λ_a is the space constant, a characteristic length of the membrane outside the active area.

It can be expected that the current flow through the membrane in the inactive region adjacent to an active area would fall off exponentially with distance from the edge of the active

areas. The excitatory effect of an active area on adjacent areas would reach to a point at which the membrane current had fallen off threshold value.

It has been experimentally verified that the cable equation hold good at the weak sub threshold stimulus level. If the stronger shock of short duration exist, an excitation will appear with the condition that the cathodic response try to reach a certain level, rather than when a pulse of constant energy flow through the cell.

5. MATERIALS AND METHODS

Blood samples of epileptic patients along with normal healthy control were collected from the Department of Neurology, Safdarjang Hospital, and New Delhi 110016 after the approval of ethical committee of the hospital. 10 ml freshly drawn blood from each patient was collected in clean and dry test tube without any anti-coagulant. The test tube was kept for 45 minutes at room temperature ($22 \pm 2°C$) for the formation of clot. Sera of different patients were separated by centrifugation at 1500 r.p.m. upto 15 minutes and were collected in screw capped test tubes.

The immunological parameters (IgA, IgG, IgM, C_3 & C_4) were quantitated by using singles radial immunodiffusion method of Mancini et al. [12] using commercially available antibody-agar plates. The plates were standardized with purified immunoglobulins.

The atomic absorption spectral estimation of the serum samples from normal persons and epileptic patients were carried out on atomic absorption spectrophotometer Model No. AA-6300 of Shimadzu Japan, at Deptt. Of Environmental study University of Delhi 110007.

6. ROLE OF FOOD AND EFFECTS OF NEUTRITION

Gómez [13] has supplied some information related to brain foods. Effects of nutrients on brain function have also been studied. He has reported in the study with a suspicion that the relative abundances of specific nutrients can affect cognitive processes and emotions. It has been studied the influences of dietary factors on neural function and synaptic plasticity may reveal some of the vital mechanisms. These mechanisms are responsible for the action of diet on brain health and mental function. There are so many gut hormones may enter the brain. These hormones may be produced in the brain itself. These can influence cognitive ability of the brain. Regulators of synaptic plasticity such as brain derived neurotrophic factor can function a metabolic modulators. These are responsible to peripheral signals such as food intake. If we are at the stage of understanding the molecular basis of the effects of food on cognition, we can help the community of research to determine the possibility of increase the resistance of neurons through the adjusting the dietary food habits. These thoughts can promote mental fitness in the human body. Energy homeostasis and cognition is shown in the Figure. 4.

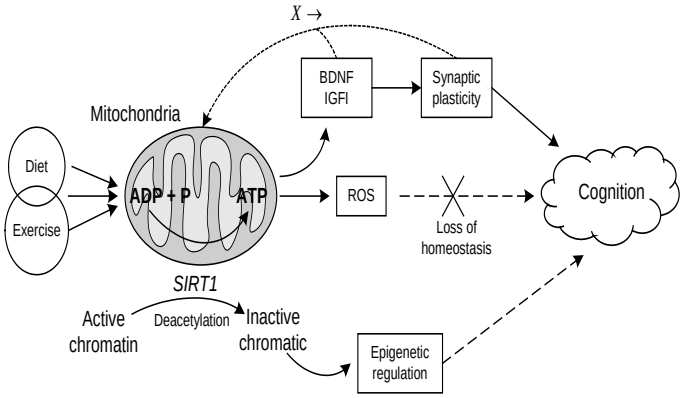

Figure 4: Energy homeostasis and cognition.

It has been established that the diet and exercise can help in affecting the mitochondrial energy production. This production is very important to maintain neuronal excitability and function of synaptic. The arrangement of some definite diets and exercise can have additional effect on synaptic plasticity and cognitive function. Adenosine triphosphate (ATP) is produced by mitochondria. ATP is responsible for the activation of brain-derived neurotrophic factor and insulin like growth factor. This ATP supports synaptic plasticity and cognitive function.

Excess energy production may be caused by very high calorific intake or strenuous-exercise results in the production of reactive oxygen species. If this level goes up the buffering capacity of the cell, synaptic plasticity and cognitive function are compromised. A reduction in the actions of signal transduction may be found.

7. EFFECTS OF FEEDING ON COGNITION

Neural circuits that are involved in feeding behavior show precise coordination with brain centre that modulate energy homeostasis and cognitive function. The effects of food on cognition and emotions can start before act of feeding itself. The recollection of foods through olfactory and visual sensory inputs alerts the emotional status of the brain. The ingestion of foods triggers the release of hormones or peptides. These hormones or peptides can reach centre such as the hypothalamus and the hippocampus and activate signal – transduction pathways. These two can promote synaptic activity and contribute to learning and memory. A schematic diagram of feeding on cognition is shown in Figure. 5.

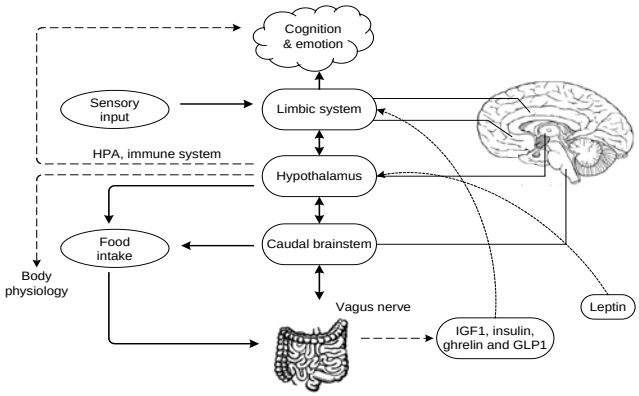

Figure 5: Effects of feeding on cognition.

8. EXPLANATION OF EPILEPTIC JERKS ON NEURAL NETWORKS

It is debatable that the oscillations in the brain are evidence of noise or reflect a vital component of signaling or information processing. The firing of neurons at a particular phase of a cycle would serve to associate the activity of that neuron with others on the same phase. It is easy to define a neural ensemble through which a set of cells could form a distributed representation or perform distributed processing.

The seizure in epilepsy is a specific symptom related to neural activity of abnormal processing in the human brain. Such nerve cells (neurons) are strengthened with the element which we take in the form of food. The trace elements such as Cu, Zn, Fe, Ca, Mg, Na and K are found in the traces in our blood given in Table 1 and regression equations with correlation coefficients of normal and epileptic persons are given in Table 2and Table 3. The excess and deficiency may lead to different body disorders and affect the immunity of human beings. We have measured immunological parameters and given in Table 4. and regression equations with correlation coefficients of normal and epileptic persons are given in Table 5and Table 6. If the immunity is disturbed in our system many diseases affect our CNS. The abnormal behavior of nerve cells comes into play which is responsible for the epileptic seizures. Computational neuroscience is a helpful tool to present broad picture of the neural activity. A simulation on the basis of elemental supplementation may be proposed and can be demonstrated in future.

Table 1 Mean ± Standard deviation of Cu, Fe, Zn, Na, K, Ca and Mg in epileptic patients and normal healthy controls.

S. No.	Element	Type of sample	Mean + S.D.	Disease
1	Copper	Serum	(0.1767 ± 0.1087) mg/l	Epilepsy
2	Copper	Serum	(0.1329 ± 0.0380) mg/l	Control
3	Iron	Serum	(1.8483 ± 1.8079) mg/l	Epilepsy
4	Iron	Serum	(1.1826 ± 1.7671) mg/l	Control
5	Zinc	Serum	(1.6875 ± 1.8156) mg/l	Epilepsy
6	Zinc	Serum	(1.0267 ± 0.6347) mg/l	Control
7	Sodium	Serum	(6.2271 ± 0.0956) mg/l	Epilepsy
8	Sodium	Serum	(6.1203 ± 0.0708) mg/l	Control
9	Potassium	Serum	(0.1572 ± 0.0098) mg/l	Epilepsy
10	Potassium	Serum	(0.1443 ± 0.0043) mg/l	Control
11	Calcium	Serum	(4.0285 ± 1.0521) ml/l	Epilepsy
12	Calcium	Serum	(0.0483 ± 0.0284) ml/l	Controls
13	Magnesium	Serum	(4.7017 ± 0.9548) mg/l	Epilepsy
14	Magnesium	Serum	(0.1098 ± 0.0310) mg/l	Control

Table .2: Regression and correlation coefficient studies on Na, K, Ca, Mg, Zn, Cu and Fe in normal samples

Diagnosis	Regression coefficients	Regression equations	Coefficient of correlation	Coefficient of partial correlation	Multiple correlation coefficient
Normal	$b_{Na.K}$ = 4.1282 $b_{K.Na}$ = 0.0156	Na = 4.1282K + 2.524 K = 0.0156Na + 0.0955	r_{NaK} = 0.2539	$r_{CaNa.K}$ = -0.5293 $r_{CaK.Na}$ = 0.6316	$R_{Na.KCa}$ = 0.6016 $R_{K.NaCa}$ = 0.6616
	$b_{Ca.Mg}$ = -0.0090 $b_{Mg.Ca}$ = 0.0107	Ca = -0.0090Mg+0.04923 Mg = -0.0107Ca + 0.1103	r_{CaMg} =- 0.0098	$r_{NaK.Ca}$ = 0.5187 $r_{MgNa.K}$ = -0.0663	$R_{Ca.KNa}$ = 0.6894 $R_{Mg.NaK}$ = 0.1196
	$b_{Cu.Fe}$ = 0.0048 $b_{Fe.Cu}$ = 10.3828	Cu = 0.0048Fe + 0.1272 Fe = 10.3828Cu – 0.1974	r_{CuFe} = 0.2236	$r_{MgK.Na}$ =-0.0801 $r_{NaK.Mg}$ =0.2472	$R_{Na.MgK}$ = 0.2618 $R_{K.MgNa}$ = 0.2654
	$b_{Cu.Zn}$ = 0.0109 $b_{Zn.Cu}$ = 3.0297	Cu = 0.0109Zn + 0.1217 Zn = 3.0297Cu + 0.6239	r_{ZnFe} = -0.4814	$r_{ZnCu.Fe}$ = 0.3388 $r_{ZnFe.Cu}$ = -0.5447	$R_{Cu.FeZn}$ = 0.3988 $R_{Fe.CuZn}$ = 0.5761
	$b_{Fe.Zn}$ =-1.3404 $b_{Zn.Fe}$ =-0.1729	Fe = -1.3404Zn + 2.5589 Zn = -0.1729Fe + 1.2320	r_{CuZn} = 0.1817	$r_{CuFe.Zn}$ = 0.3610	$R_{Zn.FeCu}$ = 0.5656

Table .3: Regression and correlation coefficient studies on Na, K, Ca, Mg, Zn, Cu and Fe in epilepsy

Diagnosis	Regression coefficients	Regression equations	Coefficient of correlation	Coefficient of partial correlation	Multiple correlation coefficient
Epilepsy	$b_{Na.K}$ = 0.0888 $b_{K.Na}$ = 0.0009	Na = 0.0888 K + 3.213 K = 0.0009Na + 0.1541	r_{NaK} = 0.0091	$r_{CaNa.K}$ = -0.4992 $r_{CaK.Na}$ = -0.7019	$R_{Na.KCa}$ = 0.4993 $R_{K.NaCa}$ = 0.7019
	$b_{Ca.Mg}$ = -0.1603 $b_{Mg.Ca}$ = -0.1320	Ca = -0.1603Mg + 4.7821 Mg = -0.1320Ca + 5.2338	r_{CaMg} = - 0.14	$r_{NaK.Ca}$ = 0.3561 $r_{MgNa.K}$ = -0.5366	$R_{Ca.KNa}$ = 0.7509 $R_{Mg.NaK}$ = 0.6206
	$b_{Cu.Fe}$ = 0.0254 $b_{Fe.Cu}$ = 7.0330	Cu = 0.0254Fe + 0.1296 Fe = 7.0330Cu + 0.6055	r_{CuFe} = 0.42	$r_{MgK.Na}$ =-0.4218 $r_{NaK.Mg}$ =-0.2194	$R_{Na.MgK}$ = 0.5367 $R_{K.MgNa}$ = 0.4219
	$b_{Cu.Zn}$ = 0.0357 $b_{Zn.Cu}$ = 9.9642	Cu = 0.0357Zn + 0.1163 Zn = 9.9642Cu – 0.0731	r_{ZnFe} = 0.33	$r_{ZnCu.Fe}$ = 0.5318 $r_{ZnFe.Cu}$ = 0.1192	$R_{Cu.FeZn}$ = 0.6412 $R_{Fe.CuZn}$ = 0.4366
	$b_{Fe.Zn}$ = 0.2404 $b_{Zn.Fe}$ = 0.4785	Fe = 0.2404Zn + 1.4426 Zn = 0.4785Fe + 0.8030	r_{CuZn} = 0.59	$r_{CuFe.Zn}$ = 0.2922	$R_{Zn.FeCu}$ = 0.6044

Table 4: Mean levels and standard deviation of C_3, C_4, IgG, IgM, IgA in epileptic patient and normal healthy control.

S.No.	Immunological Parameter	Types of Samples	Mean ±S.D Unit	Disease/ Control
1	IgG	Serum	(18.18 ± 4.87) gm/l	E
2	IgG	Serum	(17.24 ±3.07) gm/l	C
3	IgM	Serum	(1.38 ± 0.32) gm/l	E
4	IgM	Serum	(7.42 ± 1.64) gm/l	C
5	IgA	Serum	(7.05 ± 1.17) gm/l	E
6	IgA	Serum	(7.83 ± 0.68) gm/l	C
7	C_3	Serum	(1.53 ± 0.26) gm/l	E
8	C_3	Serum	(1.58 ± 0.15) gm/l	C
9	C_4	Serum	(0.26 ± 0.13) gm/l	E
10	C_4	Serum	(1.28 ± 0.10) gm/l	C

Table 5: Regression and correlation coefficient studies on C_3, C_4, IgG, IgM and IgA in normal blood samples.

S.No	Correlation Coefficients	Regression Coefficients	Regression Equations	Coefficients of Partial Correlation	Multiple Correlation Coefficients
C_3	C_3C_4 = – 0.0256	b_{C3C4}= – 0.0394 $b_{C4\ C3}$= – 0.0167	C_3 = – 0.0394 C4 + 1.5966	--	
C_4	C_4C_3 = – 0.0256		C_4 = – 0.0167 C3 + 0.3087	--	
IgG	IgG IgM = 0.4814	b_{GM}= 0.9006 b_{MG}= 0.2574	IgG = 0.9006 IgM + 15.0577 IgM = 0.2574 IgG – 2.0109	$r_{GM.A}$ = 0.4201	$R_{G.MA}$ = 0.2386
IgM	IgM IgA = 0.4140	b_{MA}= 1.0214 b_{AM}= 0.1678	IgA = 0.1678 IgM + 2.4291 IgM = 1.0214 IgA – 0.4698	$r_{AG.M}$ = 0.0943	$R_{A.GM}$ = 0.1788
IgA	IgG IgA = 0.2746	b_{GA}= 1.2673 b_{AG}= 0.0595	IgG = 1.2673 IgA + 13.6493 IgA = 0.0595 IgG + 1.8102	$r_{MA.G}$ = 0.3344	$R_{M.AG}$ = 0.3177

Table .6: Regression and correlation coefficient studies on C_3, C_4, IgG, IgM and IgA in epileptic blood samples.

S.No	Correlation Coefficients .	Regression Coefficients	*tion*	Coefficient of Partial correlation	Multiple Correlation Coefficients
C3	C_3C_4= 0.5566	$b_{C3.C4}$ = 1.0887	C_3 = 1.0887 C_4 + 1.2436	--	
C4	C_4C_3= 0.5566	$b_{C4.C3}$ = 0.2845	C_4 = 0.2845 C_3 - 0.1684	--	
IgG	IgG IgM= 0.1404	$b_{G.M.}$ = 2.0907 $b_{M.G.}$ = 0.0094	IgG = 2.0907 IgM + 5.2891 IgM = 0.0094 IgG + 1.2146	$r_{GM.A}$ = 0.0609	$R_{G\cdot MA}$ = 0.0778
IgM	IgM IgA= 0.3103	$b_{M.A.}$ = 0.0867 $b_{A.M.}$ = 1. 1104	IgM = 0.0867 IgA + 1.2077 IgA = 1.1104 IgM + 0.5193	$r_{AG.M}$ = 0.2436	$R_{A.GM}$ = 0.1499
IgA	IgG IgA= 0.2728	$b_{G.A}$ = 1.1351 $b_{A.G.}$ = 0.0655	IgG = 1.1351 IgA + 15.8504 IgA = 0.0655 IgG + 0.8658	$r_{MA.G}$ = 0.2855	$R_{M.AG}$ = 0.0996

Epileptic attacks or seizures may be regarded as an emergent property of a network where the underlying physiology oscillatory coordination has given way to excessive coordination. It may be significant that the medial temporal cortex, an area where high amplitude oscillation appears to play a role in episodic memory. It is susceptible to seizure. Abnormalities in cognitive coordination would in turn be manifestations of abnormalities in neural coordination associated with either excessive or inadequate involvement of neural subsets in oscillation defined ensembles.

The trace elements such as Cu, Zn, Fe, Ca, Mg, Na and K are found in the traces in our blood .The excess and deficiency cause different body disorders and affect the immunity of human beings . If the immunity is disturbed in our system many diseases affect our CNS. The abnormal behavior of nerve cells comes into play which is responsible for the epileptic seizures. Computational neuroscience is a helpful tool to present broad picture of the neural activity. Chandra[14] has written somewhere else in literature that the interaction between nutrition and immunity focused on protein –energy malnutrition . The absorption , transfer , and distribution of many trace elements are not independent on specific binding and transport of proteins .Thus it is not surprising that changes in the concentration of trace elements exert large impact on immune responses . The process of inflammations increases vascular permeability and allows anti body, complement and other proteins to pass out the circulation and enter the extravaseular space. It may also induce inflammatory cells including lymphocytes to cross the vascular endothelium and accumulate in the tissues. Total net effect is to deploy all the resources of the immune system at the site of injury. Cells antibody and complement leave the blood and go into action where the demand is high. It may be in the affected tissue outside the vessel wall .The effect is to abrogate in CNS, if only temporarily, its isolation from the immune processes of the body. The barrier, which excludes plasma proteins from the brain breaks down, allowing antibody to enter the extra vascular space. The amount of proteins in CSF increases and with it the level of immunoglobulin. Immunocompetent cells enter the CNS. The

CNS now becomes capable to generating an immune response [15]. We have proposed the current study in the light of some findings on immunological and trace elemental estimations . We can say that the approach of this study may be use full in the direction of neural network problems of epileptic attacks. Neurons are definitely attached with the brain area and everything is related to our food , which we eat . Suitable diet and other types of food may protect our body from diseases.

9. CONCLUSION

It is very important to understand that seizure can result from many different pathologic mechanisms. It may upset (disturb) the balance between inhibition and excitation. Epilepsy is outcome of the processes, which disturb extracellular ion homeostasis, change energy metabolism, alter receptor function, or alter transmitter uptake. The outcome of synchronous bursting of cortical neurons may superficially appear to have a similar phenotype. Seizure phenotype can be altered by the location and function of neuronal network, recruited into the synchronous bursting than by underlying patho physiologies. Computer scientists have used complex neural network system to model the brain activity. Some of the simulations may use many separate data structures to represent individual brain cells, or neurons .Each neuron can receive information in the form of an electrical pulse from the neurons (of the order 10^3 to 10^4).Scientist are required powerful computer to handling all of the interconnections of neurons.

Neural network models may provide a way to piece things together to understand how epileptic behavior translates from the action of just a few neurons to a behavior affecting the human brain? Human brain contains about 10^{10} neurons .Neural network which made earlier treated each neuron as a fixed entity. A neuron can exist only in one of two state such as firing or inactive. Some researchers treated each neuron as a pathway into itself. The route of electrical signal from fibrous dendrites into the cell body and out through the axon to the other neurons. Due to this fact a data chain of the neurons can be build up.

Neural network provide a glimpse into epilepsy that completes information obtainable through clinical or laboratory studies.

A successful seizure prediction requires a combination of further modeling and experimental work in term of measuring the immunological and trace elemental parameters . Multifactorial causes of epilepsy can be understand with the programming of computer models. These models can encapsulate so many conspiring and counteracting causes. We can also extend these complexities to the therapeutic domain. We know that many drugs have multiple binding sites and multiple effects. A new anticonvulsant therapy can be suggested with the help of computer simulation. A further detailed study in the computer modeling related to epileptic attacks can persuade. We have to develop a device that simply alert patients with a

period of high seizure probability. Due to advancement of computer, it is now possible to use super computers to run massive simulations, again with the hope of providing greater verisimilitude by more closely approximating the large numbers of cells in brain areas.

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Study of immunoglobulin 'G' with ultra violet spectroscopy in Duchene muscular dystrophy and Alzheimer's disease

Sanjeev Kumar* , Sweety and Shweta Chaudhary
Department of Physics, Medical Physics Research Laboratory, D.A.V. (P.G.) College
Muzaffar Nagar – 251 001 U.P.(India.
*sanjeev1962kumar@yahoo.co.in; *sanjeev1962kumar@rediffmail.com

ABSTRACT

In the present paper, we have studied human IgG in Duchene Muscular Dystrophy (DMD) using ultra violet spectroscopy. A comparison with normal controls is also made. We have found three types of bands in the spectra of IgG of DMD patients and normal healthy controls. First and second band regions cover the ultraviolet behavior of proteins but third band does not show absorbance of proteins because the absorbance intensity greater than 310 nm. Third band does not contain protein without tryptophan. We have to concentrate on the region of two bands below 300nm. We have first region in the range 200 nm to 227.38 nm in DMD patients and 200nm to 229.60 nm in normal controls. Second region starts from 244.40nm to 297.70nm in patients and 244.40 nm to 297.70 nm in controls only. Third band did not show any protein absorbance in all the cases of study. It has been seen that the variation in AD samples is on the decreasing pattern in comparison with healthy controls.

Keywords : Immunoglobulin G (IgG), Duchene muscular dystrophy (DMD), Epilepsy (E), Normal (N) , Ultra violet (UV) and Alzheimer's disease (AD).

1. INTRODUCTION

One of the most important and exciting advances in modern biochemistry has been the application of spectroscopic method, which measure the emission and absorption of energy of different frequencies by molecules and atoms. Spectroscopic studies of proteins, nucleic acids and other biomolecules are providing many new insights into the structure and dynamic process in these molecules.

Ultra violet visible (UV-Vis) spectroscopy is a different tool of spectroscopy. It is used in the solid state to prove the formation of the complexes [1]. If there is any shifting of the bands can indicate the hydrogen bonding with in the complexes. This technique is used to identify the

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charge transfer reaction. It is very important to note that the results of ultra violet spectroscopy are of relatively little important if not studied infrared spectroscopy along with Nuclear magnetic resonance techniques. On the combined factual view of all the three branches of spectroscopy a valid structural proposal can be made. This is the main reason why the other sections of the work focus on both IR and NMR along with other useful structural determination techniques.

Molecules have the ability to absorb ultraviolet or visible light. Absorption corresponds to excitation of outer electrons in the molecules. These transitions of electrons are important to understand. If a molecule absorbs energy an electron may get promotion from the highest occupied molecular orbital to the lowest unoccupied molecular orbital. The energy differences between electronic levels in most of the molecules vary from 125 to 650 KJ/mole [2].

It has been established that occupied molecular orbitals with the lowest energy are the σ orbitals. If the energy is slightly higher than π orbitals come into play a role. Non-bonding orbitals are at still higher energy levels. The highest energy orbitals belong to π* and σ* . These are called unoccupied or ant bonding orbitals. The schematic diagram to represent the electronic transition of the energy levels is given in the Fig. 1.

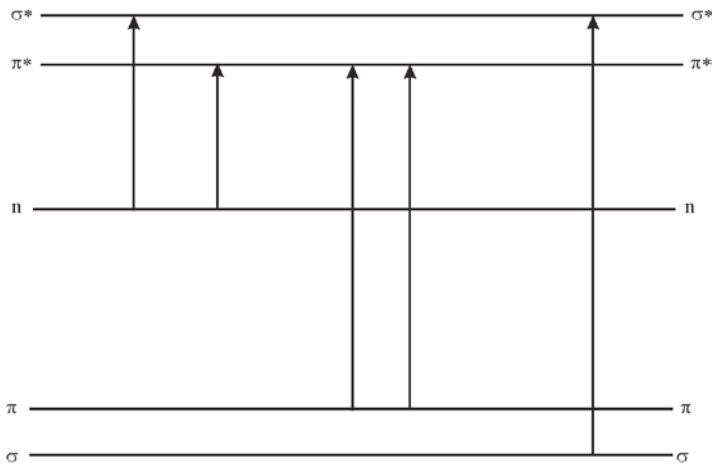

Figure 1: Electronic energy levels and transition

Molecular absorption in the ultraviolet and visible region of the spectrum is dependent on the electronic structure of the molecule. Absorption of energy is quantized. Due to this fact the elevation of electrons from orbitals in the ground state to higher energy orbitals is an excited state. Ultraviolet spectroscopy is limited to conjugated systems for the most part of the electromagnetic spectrum. Ultraviolet spectroscopy provides the facility of recognition of very complexities in molecules. A large portion of a complex molecule may be transparent in the ultraviolet light. A spectrum obtained is similar to that of a much simpler molecule.

An ultraviolet visible spectrum is a graph of light absorbance verses wavelength in a range of ultraviolet or visible regions. Such type of a spectrum can be produced by a more sophisticated spectrophotometer. Data can be collected one wavelength at a time by simple instruments.

The wavelengths of absorption peaks can be correlated with the types of bands in a given molecule. The wavelength is valuable in determining the functional groups within a molecule. Ultraviolet and visible absorption is not a specific test for any given compound. The nature of the solvent, the pH of solution, temperature, high electrolyte concentrations, and the presence of interfering substance can influences the absorption spectra of compounds. The variations can be made in width of the slit for band within the spectrophotometer.

Ultraviolet visible spectroscopy is routinely used in the quantitative determination of solutions of transition metal ions and highly conjugated organic compounds.

Solutions of transition metal ions can be coloured. These can absorb visible light. The 'd' electrons within the metal atoms can be excited from one electronic state to another state. The colour of metal ion solutions is strongly affected by the presence of other species. These may be certain anions or ligands. Organic compounds with a high degree of conjugation also absorb light in the ultraviolet or visible regions of electromagnetic spectrum. Solvents for these determinations are generally water; it can be used for water soluble compounds. Ethanol can be used for organic soluble compounds. The radiations which move with the speed of light are called "electromagnetic radiations". Radiation vibrates perpendicular to the direction of propagation with wave motion.

It is broken completely into the several portions called as electromagnetic (EM) spectrum. Different regions of this spectrum provide different kinds of information as a result of interactions. Light may be considered to have both waved and particle character shown in Fig. 2.

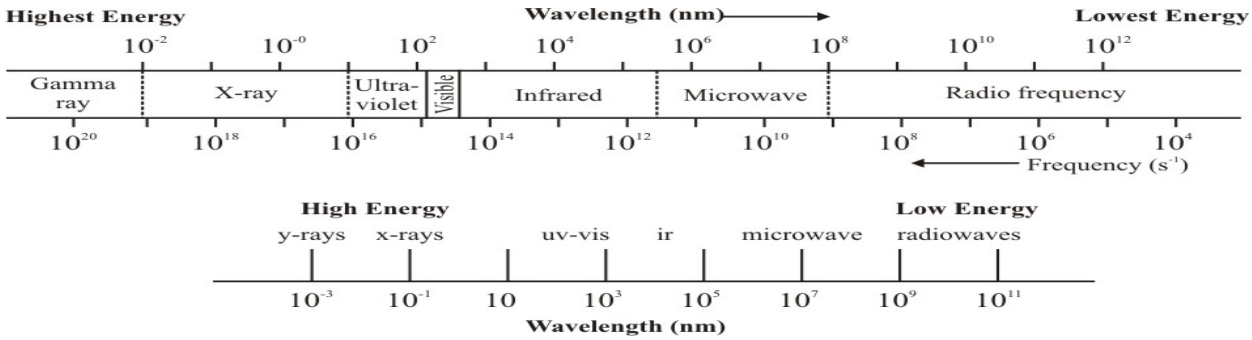

Figure 2: Energy spectrum of different wavelengths to show the different regions.

The part of the e.m. radiation spectrum with the visible light is most familiar to us. This is the small portion of all the possible types. Molecular absorption spectroscopy in the ultraviolet and visible is concerned with the measured absorption of radiation in its passage through a solid

liquid or a gas. The wave length region is used for this type of spectroscopy is from 190 nm to 1000 nm. The absorbing medium is kept at room temperature. Ultraviolet and visible spectroscopy is used to measure the multiple bonds or aromatic conjugation within the molecule. UV-Vis region is subdivided further as vacuum UV (100 nm to 200 nm), near UV (200 nm to 400 nm) and visible region (400 nm to 750 nm). Vacuum UV is called because molecules of air absorb radiations in this region. Molecule absorption spectra are more meaningfully considered as a function of wave number (Cm^{-1}). We use wave length (nm) to measure the quantity of absorption.

UV-Vis spectroscopy involves the absorption of UV and visible light by the molecule causing the promotion of an electron from ground electronic state to an excited electronic state of the transition. Absorption of this high energy light causes electronic excitation. The easily accessible part of the region is from 200 nm to 800 nm. This shows absorption only if conjugated π-electron systems are present. Ultra violet radiation having wavelength less than 200 nm is more difficult to handle. The energies are sufficient to promote or excite a molecular electron to a higher energy orbital. Ultra-violet region is only a small part of the electromagnetic spectrum and it extends from 190 nm to 300 nm. We are interested in the spectrum, which obtained when ultra-violet light passes through medium and not in the source spectrum. The interaction of ultraviolet radiations with matter may result either in the emission or in the absorption spectrum. UV radiations excite the transparent substance from the lower to higher energy state via electronic transitions. The transitions induced by ultra violet are not common to all electronic structures; in fact these take place in conjugated systems. We are in a position to recognize the characteristic groups because the UV spectra are highly sensitive to environmental factors in biological macromolecules. As the energies involved in electronic spectra are large, these are associated with changes in rotational and vibrational states, which blur the observed spectrum, rendering it characterless in liquids. However, even this highly characteristic of a particular molecular group lies both in its frequency and intensity.

The spectra have the absorption peak position and the intensity of the peak. We can express the peak position in wave length (nm), frequency (Hz) or wave number (Cm^{-1}).

The intensity is dependent on the interaction probability and hence the dipole moment of transition. This parameter depends on the electronic charge distribution in transition. Low probability transitions are forbidden.

The polarity of the exited state also affects the intensity. Light absorption laws in transparent medium were formulated by Lambert [3] and the intensity of transmitted light was given by

$$I = I_0 e^{-\alpha \ell} \quad (1)$$

where I_o is the intensity of the incident light, the characteristic coefficient of absorption for the medium and ℓ is thickness of the absorbing medium. We can calculate the Bunson-Rosioe extinction coefficient (K) by the formula

$$K = \left[\log_{10}\left(\frac{I_0}{I}\right)\right]\frac{1}{\ell}\,, \tag{2}$$

$$\mathrm{K}\ell = \log_{10}\left(\frac{\mathrm{I}_0}{\mathrm{I}}\right),$$

$$(\log_e 10)(\mathrm{K}\ell) = \left[\log_{10}\left(\frac{\mathrm{I}_0}{\mathrm{I}}\right)\right](\log_e 10),$$

$$2.3026\ \mathrm{K}\ell = \log_e\left(\frac{\mathrm{I}_0}{\mathrm{I}}\right),$$

or $$e^{2.3026}\mathrm{K}\ell = \frac{\mathrm{I}_0}{\mathrm{I}},$$

$$\mathrm{I} = \mathrm{I}_0 e^{-2.3026\mathrm{KI}} \tag{3}$$

Comparing (1) and (3), we get

$$\alpha = 2.3026\ \mathrm{K}$$

The intensity of transmitted light is given by Lambert – Beer Law [3],

$$\frac{I}{I_0} = e^{-KC\ell} \tag{4}$$

or $$\mathrm{KC}\ell = \log_e\left(\frac{\mathrm{I}_0}{\mathrm{I}}\right) = \mathrm{A}, \tag{5}$$

The absorbing molecules having concentration C. K is constant characteristic of the solute and *l* is the solution path length. A is known as absorbance or absorptivity.

When the constituents of the absorbing molecule are unknown, intensity is expressed as the absorption by the solution of 1 gm/100 ml concentration and 1 centimeter path length of the sample $\left(\mathrm{E}_{1\mathrm{cm}}^{1\%}\mathrm{or}\mathrm{A}_{1\mathrm{cm}}^{1\%}\right)$

Thus $$\left(E_{1cm}^{1\%} = \frac{A}{C\ell}\right) \tag{6}$$

The corresponding energy is given below

$$E\left(\frac{kcal}{mol}\right) = Nh\upsilon = \frac{28.587}{\lambda(nm)\times 10^3} \quad (7)$$

The total energy of molecule is given by

$$E = E_e + E_v + E_r, \quad (8)$$

where E_e is the electronic energy, E_v the vibrational energy and E_r the rotational energy.

Transitions of electrons take place from filled molecular orbits in ground state involving bonding (σ and π) or non bonding (n) orbits (electrons), to higher energy involving the anti-bonding σ^* or π^* orbitals. These remain empty in an unexcited state. It is customary to discuss and to describe the intensities of absorption bands in terms of molar absorptivities $\in_{max}$ (molar extinction coefficients). The coefficients can be obtained from the determination of the wavelength of maximum absorption. Accurate measurement of the absorbance at this one wavelength can provide these coefficients. $\in_{max}$ is not directly related to any quantity.

Changes in the energies accompany the changes in electronic energy and give rise to the familiar band spectra. A single electronic transition comprises many individual lines (bands), and the quantity, i.e., total energy transferred should be sum of the contributions from all these lines (bands).

The quantity of interest in ultraviolet spectroscopy is not $\in_{max}$ but it is the integrated (absolute) intensity (I). This is equal to the area under the absorption curve. Ultraviolet spectra rarely contain isolated bands, and we must extrapolate the band to the abscissa in arbitrary manner.

Now, experimental integrated intensity is mathematically defined by the integral

$$I = \int \in d\nu \quad (9)$$

In UV region, strongly overlapping bands are frequently encountered. Evaluation of the integrated intensity of such bands requires analysis into separate bands.

Calculation of Absorption Intensity

Calculation of absolute intensity of an absorption (or emission) bands is not difficult. Mulliken [4] has shown probabilities of emission (A) and absorption (B) between two electronic states from initial (i) to final (f) by the relation

$$A_{if} = (64\,\pi^4\,\nu^3\,e^2/3h)\,G_f\,D_{if} \quad (10)$$

$$B_{if} = (8\,\pi^3\,e^2/3h^2\,c)\,G_f\,D_{if} \quad (11)$$

where, e is the electronic charge of electron, h is Planck's constant, c is the velocity of light, ν is the frequency of emission, G_f is the statistical weight of the final state, D_{if} is the dipole strength.

B_{if} can be transformed into a measure of intensity, the oscillator strength f, which is given by

$$f = \left(\frac{8\pi^2 mc}{3h}\right) G_f \nu D_{if} = 1.096 \times 10^{11} G_f \nu D_{if} \quad (12)$$

and is related to the absolute intensity

$$f = 0.102(mc^2/N\pi e^2) \int \in d\nu = 4.315 \times 10^{-9} \int \in d\nu \quad (13)$$

where m is the mass of the electron, N is Avogadro's number, $\in$ is the molar absorption coefficient, G_f is considered as unity.

The dipole strength (D) is proportional to the intensity and is given by the relation

$$I^2 \ \alpha \ D^2 = \int \psi_i \ M \ \psi_f \ d\tau \quad (14)$$

ψ_i and ψ_f are the total wave functions of the initial and final states of the molecule respectively, $d\tau$ are represents the product of the volume elements in the coordinates of all the nuclei and electrons, $\overline{M}$ is called dipole moment vector.

According to quantum mechanics, desired average can be obtained as

$$\int \psi_i \sum e \, r \psi_i \, dT = \left(\psi_i \, M \psi_f \, dT\right) \quad (15)$$

where, $\overline{M} = \Sigma e \, \bar{r}$ is called the dipole moment vector.

Now, $\overline{M}$ in 3-Cartesian coordinates axes is given by the relation as described below

$$M^2 = M_x^2 + M_y^2 + M_z^2 \quad (16)$$

D^2 can be given by the following relation

$$D^2 = \int \psi_i \, M \, \psi_f \, dT \quad (17)$$

where ψ_i and ψ_f are the total electronic wave functions of the initial and final states, $d\tau$ is the product of the volume elements in terms of the coordinates of all the electrons.

If the spectral shape of a ligand spectrum does not change but the magnitude changes in such a way that it is proportional to the concentration of bound ligand, then the spectral data can be utilized to estimate the equilibrium binding constant, K. The ligands are binding in one binding mode or in constant proportions in more than one mode.

If we consider the equilibrium

$$L_b \overset{k}{\underset{\leftarrow}{\rightarrow}} L_f + S_f \tag{18}$$

where L_b is a bound ligand and L_f is a free ligand, S_f is a free site.

If we treat a macromolecule, which is a series of binding sites of n residues in size, then the total site concentration is given by the following relation

$$\frac{[M]}{n} = S_{tot} \tag{19}$$

Here, [M] is the concentration of the macromolecule residue.

It has been reported that for proteins and sometimes preferable to take as concentration of molecules rather than residues.

$$S_{tot} = n'[M] \tag{20}$$

Here, n' is the number of binding sites per protein.

We have the calculation for k which is given below

$$k = \frac{nC_b}{C_f[M]} \tag{21}$$

Ultraviolet spectroscopy can be used for multi component analysis of mixture consisting of n absorbing analytes is possible provided the Beer-Lambert Law is valid and holds, by measuring the absorbance at k suitable wavelengths with k > n, For each wavelength λ_a. This 'a' can have the values 1, 2, ...k – 1, k.

Total absorbance is given by the relation

$$A(\lambda_a) = b\sum_{i=1}^{n} \varepsilon_i(\lambda_a) C_i \tag{22}$$

where $\varepsilon_i(\lambda_a)$ is the molar (decadic) absorption coefficient of analyte *i* at the wavelength λ.

C_i is its molar concentration.

The ratio $A(\lambda_a)/b$ is called linear decadic absorption coefficient. This quantity is also called linear absorbance.

The concentrations of the n analytes may then be calculated from k simultaneously equations.

The use of derivative spectroscopy decreases the signal to noise ratio. It can improve the detection of a sharp band in a broad back ground, or a narrow shoulder on a broad band. Gradual changes in spectral back ground or source flux will be less pronounced in derivative spectroscopy.

Derivative spectra are generally obtained by digital differentiation or by wavelength modulation of the radiation entering the sample cell. The wavelength modulation interval has to be much less than the band width of any absorption band in the spectrum.

The derivative spectrum in terms of wave number can be calculated from the spectrum in terms of wavelength by

$$\frac{dA(\bar{\nu})}{d\bar{\nu}} = \left(-\lambda^2\right) \frac{dA(\lambda)}{d_\lambda} \tag{23}$$

$$\frac{d^2A(\bar{\nu})}{d\bar{\nu}^2} = \left(\lambda^4\right) \frac{d^2A(\lambda)}{d\lambda^2} + 2\lambda^3 \frac{dA(\lambda)}{d\lambda} \tag{24}$$

The first and second derivatives of the Beer' - Lambert Law are. If $\frac{d\phi_0}{d\bar{\nu}}$ =0 then we can write these derivatives as

$$\frac{\frac{d\phi_t}{d\bar{\nu}}}{\phi_t} = -2.303\,c\,b\frac{d\varepsilon}{d\bar{\nu}} \tag{25}$$

$$\frac{\frac{d^2\phi_t}{d\bar{\nu}^2}}{\phi_t} = \left[2.303\,c\,b\frac{d\varepsilon}{d\bar{\nu}}\right]^2 - 2.303\,c\,b\frac{d^2\varepsilon}{d\bar{\nu}^2} \tag{26}$$

Second derivative spectra are predominantly used in quantitative analysis.

Molecular absorption spectroscopy in the ultra violet and visible is concerned with the measured absorption of radiation in its passage through a gas, liquid or a solid. Ultra violet spectroscopy allows for the determination of the concentration of protein in a sample as the absorbance at 280 nm and it is directly proportional to the concentration of protein. The protein sample has tryptophan or tyrosine content, which absorb at 280 nm. Determination of protein concentration can be done by the absorbance at 205 nm. Peptide bonds are analyzed directly by the using a method of Scoopes [5].

Jagger [6] has reported that the important molecular components of proteins that are expected to absorb ultra violet are the aromatic amino acids and peptide bond. Tryptophan and tyrosine are the major components, which absorb ultra violet above 250 nm. Phenylalanine, cystine are also important components because most of the other amino acids do not absorb in this region. These two components may be major absorbers in a protein with low tryptophan – tyrosine content. Disulfide bonds are very crucial structures. Their presence increases the UV liability of proteins. Peptide bond (– CONH –) has some double bond character. It is a relatively weak absorber and is only important below 240 nm. A peptide bond for every amino acid residue in a protein makes its contribution to protein absorption below 240 nm.

There is an interaction in a protein among amino acids. The total absorbancy of a protein solution is quite similar to the sum of the absorbencies of solutions of the component amino acids. Proteins usually exhibit an absorption peak around 280 nm. Scopes, R. K. [7] has measured proteins at 205 nm and given a formula regarding the average absorbance due to tryptophan and tyrosine at 205 nm is given here. as

$$\left[3.6+\left(\frac{4.6-3.6}{4}\right)\right]\times A_{280}=3.85\times A_{280} \tag{27}$$

We can write an expression

$$\in_{205}^{1mg/ml} = C + 3.85 \times \in_{280}^{1mg/ml} \tag{28}$$

C is the extinction coefficient due to the polypeptide chain in its native configuration, plus contributions from phenylalanine, histidine and other residue side chains but excluding tyrosine and tryptophan.

For a protein solution of unknown concentrations, values of the absorbance. A_{280} and A_{205} are noted. The expression $\in_{205}^{1mg/ml}$ can be replaced by $\in_{205}^{1mg/ml} \times A_{280} / A_{250}$.

We can write two possibilities of approxmamation as

$$\in_{205}^{1mg/ml} = 31 \tag{29}$$

$$\in_{205}^{1mg/ml} = C + 120 \times (A_{280} / A_{250}) \tag{30}$$

and if we rearrange eq^{n} (28) as

$$\in_{205}^{1mg/ml} = \frac{C}{1-3.85\times\left(A_{280}/A_{205}\right)} \tag{31}$$

If we substitute C = 27.0

We can have the best values as

$$\in_{205}^{1mg/ml} = 27.0+120\times\left(A_{280}/A_{205}\right) \tag{32}$$

$$\in_{205}^{1mg/ml} = \frac{27.0}{1-3.85\times\left(A_{280}/A_{205}\right)} \tag{33}$$

Perkampus [8] has provided some of the features of bond analysis with the Gaussian and Lorentzian functions. The bands to be separated in most of the cases can be approximated by Gaussian curves and the extinction coefficient or absorbance can be written here as.

$$A = A_{max}.\ \overline{e}^{\left\{B\left(\bar{\nu}_0\ -\ \bar{\nu}\right)^2\right\}} \tag{34}$$

$\bar{\nu}_0$ is the wave number at the band maximum.

The parameter B is connected to the full width at half the maximum intensity of the band by the following eq^n.

$$B = \frac{4\log_2}{\Delta\bar{\nu}_{1/2}^2} \tag{35}$$

Eq^{n}s (34) and (35) can be written as

$$\left[\log\left(\frac{A_{max}}{A}\right)\right]^{1/2} = \left(B\bar{\nu}\right)^{1/2} - \left(B\bar{\nu}_0\right)^{1/2} \tag{36}$$

Eq^n (36) is the basis for a linear regression for fitting the theoretical to experimental curve.

2. REVIEW OF THE LITERATURE

The application of UV absorption spectroscopic technique for the examination of the concentration of protein molecules has undergone significant change during the past couple of years. Changes in conformation with altered temperature or with the addition of organic molecules to an aqueous solvent medium, are usually associated with positional shifts and

intensity changes in ultra-violet absorption bands. These variations can be explained on the basis of the energy level transitions and empirical correlations with information obtained by other methods.

Proteins interact with ultra violet light with a consequent absorption of energy giving rise to absorption spectrum [8]. Not all molecules are found to exhibit this absorption. This phenomenon is confined to the molecules with double bonds and longer wavelength to conjugated bonds. The energy absorbed by the molecule causes electronic transition from ground state to one of the molecular excited states involving an electron a π orbital or a non bonding p-type orbital into a higher anti-bonding π^*-orbital. Thus the transition between the ground state of entire system and the excited state of the chromosphere proves quite helpful in understanding the structure of proteins. The absorption spectrum in ultra violet region is influenced by a number of factors including the state of bonding and nature of the bonds involved. The shift in the position of absorption maxima and change in the area under the absorption curve help in the recognition of a characteristic group or its environments. The excitation of electrons in the frequency above 50,000 cm^{-1} has been used to determine the conformation of peptide chains which has characteristic absorption maxima at 185 nm.

Many properties of proteins including their primary structure, modification of sequence of amino acids in polypeptide chains and covalently attached prosthetic groups influencing the nature and function of proteins have been studied in the past by using this technique.

This study also took into consideration the question of inter atomic distances and the bond angle from the shift in absorption peaks on their intensities. The plasma proteins in general give absorption bands between 200 nm to 400 nm. Aromatic proteins absorb in the range 260 nm to 280 nm. The 280 nm absorption is due to tryptophan and tyrosine residues in the aromatic proteins. Phenylalanine absorbs at slightly lower wave length ~255 nm. The free amino acid contents of plasma show concentration of phenylalanine as 0.95 mg/100ml, tyrosine as 0.91 mg/100 ml and tryptophan as 0.98 mg/ 100 ml of plasma.

Most of the previous investigations of ultra-violet spectra of protein measured in the region ranges from 250 nm to 300 nm. In this region absorption is due almost entirely to the aromatic side chains of tryptophan, tyrosine and phenylalanine. The stronger absorption bands of these side chains between 190 nm to 240 nm, not studied rigorously till now. One can attribute this to technical problems and still more because of the overlapping absorption in this region from histidine , methionine, cystine and ionized cystine and the long wave length end of the absorption due to the peptide bond itself.

Absorption bands located below 200 nm have been of great interest. Absorption band of the peptide linkage is centered near 190 nm. The transition from a helix to a random coil is associated with significant increase of ultra violet absorption in this region. The work of

Rosenheck and Doty [9] has given importance to the absorption studies in the ultra violet region. The main factor for this lies in the strong connection between the ultra violet absorption bands and the optical rotatory dispersion (ORD) changes associated with the helix coil transition. The intermediate region of 200 nm to 250 nm has been studied in some of earlier publications [10, 11]. Difference spectra in neutral and acid solutions of several proteins exhibit a significant line near 235 nm. This peak is higher compared to the signals due to tyrosine residues near 278 nm to 287 nm or those due to tryptophan at slightly larger wave lengths.

So far as the structure of native globular proteins is concerned, the amino acids side chains may emerge on the outer side towards the solvent from the main peptide chain or may be folded into the interior of the molecule and enclosed by surrounding groups of the protein itself. Native globular proteins are immunoglobulins such as IgG, IgA, IgM, IgD and IgE. Intermediate situations may also present. An amino acid side chain is likely to be partly covered near the surface of the molecule but flexible enough to appear out occasionally and have interactions with the surrounding solvent. Some proteins are also likely to have water molecules trapped in the interior, so that internal groups may still have association with water.

There is a possibility of some sort of fissures or crevices at the surface of the protein molecule. Small solvent molecules can go through these fissures. The amino acids composition of globulin in the decreasing order or proportion is given below:

(1)	Leucine	(2)	Valine	(3)	Aspartic acid	(4)	Alanine
(5)	Lysine	(6)	Histidine	(7)	Phenylalanine	(8)	Glutamic acid
(9)	Theonine	(10)	Proline	(11)	Glycine	(12)	Serine
(13)	Tyrosine	(14)	Arginine	(15)	Tryptophan	(16)	Methionine
(17)	Cysteine						

Jiang *et. al.* [12] studied this spectroscopy technique for the protein back bone transitions in aqueous solution. They have made some calculations to simulate and backbone transitions of protein. One can find such type of transitions in the region 180nm to 220 nm. These findings are very sensitive and provide a probe for secondary structure of proteins. They have characterized fine structure of UV spectra accurately for the identification of secondary structure of proteins.

Amoldus *et. al.* [13] have studied the thermal stability of immunoglobulin and measured the protein concentration at 280 nm. The study of the structure of immunoglobulins in detail reveals that these proteins are composed of four polypeptide chains which are connected by disulphide bonds and non-covalent forces. The four polypeptide chains are grouped together in different fragments, two identical F_{ab} segments and one F_c segment forming a Y shaped conformation. The antigen binding sites are located on the far ends of the F_{ab} segments. The F_{ab}

segments are linked to the F_c by the hinge region, which varies in length and flexibility in the different antibody classes and isotypes.

Motrescu *et. al.* [14] have studied spectrophotometric analysis of the blood plasma for different mammals to study and establish a correlation between protein concentration and their absorbance in the ultraviolet region. Protein absorbance band at 280 nm determines the characteristic spectrum of the blood plasma and the absorption maximum strongly depends on the blood plasma protein concentration.

Kanagathara, N. *et. al.* [15] have applied UV-visible spectral study on normal blood samples and found that there is a linear relationship between the protein content and the maximum absorption in the ultraviolet region. A band at 280 nm helps in determining the characteristic spectrum of the blood plasma. They have also reported that the absorption spectrum of the diseased samples of blood show same changes from normal blood sample. It may help us and treated as an evidence for the manifestation of the disease.

Gunasekaran *et. al.* [16] have also studied the ultraviolet spectroscopy on normal and jaundice blood samples and reported their findings as proteins in the sera absorb strongly at 280 nm. It is due to amino acids like tyrosine and tryptophan. The amide backbone of the proteins in the blood shows a band at 210 nm [14].

Yuette *et. al.* [17] have used this technique to characterize and differentiate the types of blood. Yuette *et. al.* [18] have also used the spectroscopy for the quantitative investigation of platelet quality. The optical properties for isolated platelets, platelet rich plasma and leukopleted platelet rich plasma have also been determined.

Akihisa [19] used this technique for the characterization of red blood cells. A Successful simulation of experimental red cell spectra, which contain various amounts of hemoglobin, were also carried out. A quantitative interpretation of the red blood cell spectra was also achieved in context of corpuscular hemoglobin concentration, corpuscular volume, and cell count.

A very important class of proteins that conform to a common submit structures are immunoglobulins. These molecules have domains which are structurally independent, compact globular regions consisting of continuous stretches of the polypeptide chain of hundred amino acids long, with a characteristic fold. This fold contains two β-sheets and essentially no α-helices. These globular protein structures have a unique feature regarding non-polar residues which are sequestered into a core.They avoid contact with water at this stage. Immunoglobulins or antibodies show a very strong structure function relation in the different domains. Due to this property these molecules have an excellent system for various diagnostic tests. The domains of the antibodies with a high specificity to bind the analyte assure that these immunoglobulins can be used for a reliable and fast determination of low concentrations of

analyte. Other domains of immunoglobulin promote protein binding to a surface in the proper orientation. Its binding sites are easily accessible to the antigen.

Welhelm *et. al.* [20] have studied ultraviolet absorption spectra of blood serum and certain amino acids and reported that the absorption band around 280 nm of blood serum was due to the presence of proteins. They were mainly the tyrosine and tryptophan constituents of the proteins are responsible for a nice and clear band. A mixture of tyrosine, tryptophan, phenylalanine, cystine, glycine, leucine and glutamic acid in proportion indicated by analysis of blood serum (albumin and globulin) gives the ultraviolet visible spectra.

Malvin [21] has studied ultraviolet absorption spectra of certain physiological fluids, such as human bile, saliva, pericardial fluid, uric acid, urine, albumin, pseudo globulin euglobulin and O-O blood serum and plotted some graphs, which are valuable to the clinician and researchers. Edward *et. al.* [22] have studied the middle ultraviolet spectra of proteins and found that major absorption band of the aromatic compound was due to a $\pi \rightarrow \pi^*$ transition. Minor transition was involved in tyrosine spectrum.

Alastair *et. al.* [23] have determined protein by UV absorption and found that absorption of radiation in the near UV by proteins depends on the tyrosine and tryptophan content and to a very small extent on the amount of phenylaline and disulfide bands. They have also reported that the extinction of nucleic acid in the 280 nm region may be as much as ten times that of protein at their same wavelength. Nucleic acid in small percentage can influence the absorption.

Yong *et. al.* [24] have studied the effect of ultraviolet irradiation on molecular properties and immunoglobulin production regular activity of β-lactoglobin and found that ultraviolet irradiation is effective for altercation of molecular properties and anti-genicities of β-lactoglobulin. Low allergenic foods may be prepared with the help of such types of treatment.

Henry *et. al.* [26] have studied application of ultraviolet absorption spectroscopy to the analysis of biopharmaceuticals. They have suggested that the non-destructive technique depends on contributions from ultraviolet absorption of aromatic amino acids, cystine and light scattering. This technique can be used for determination of concentration and detection of impurities as well as assessment of aggregation state.

Mc Crathy *et. al.* [27] have studied this spectroscopy on IgG aggregate formation and found that few changes were observed in treated IgG. These changes remained un-aggregated but so many significant size dependent changes were also seen in aggregated IgG.

Edelhoch [28] has studied spectroscopic determination of tryptophan and tyrosine in proteins and presented a rapid method for the determination of tryptophan in proteins and it was based on absorbance measurements at 288 nm and 280 nm. of the protein.

Mansor *et. al.* [29] have studied FTIR and UV-Vis study of chemically engineered biomaterial surfaces for protein immobilization. They suggested that proteins have a very particular chain configurations and conformations that promote high levels of specificity during chemical interactions. They have also studied the phonomenous of protein immobilization onto biomaterial with chemically engineered surface. They have succeeded to put forward the views such that all the surface modeled material has shown acute hypoglycemic peak response associated with the insulin bioactivity.

Mirian *et. al.* [30] have studied ultraviolet spectroscopy of hemes in electron carrier C-type cytrochromes and its model compounds and found that some of the compounds have shown their spectra at different wavelength such as cytrochrome C at 406 and 530 nm. Cytrochromes C-557 at 409, 525 nm, Cytochrane C has shown two peaks at 408 and 530 nm. MP-9 at 395, 507 and 622 nm. Hemin has shown two peaks at 383 and 613 nm. These compounds were tested at 7.2 pH.

Sanii *et. al.* [31] have studied UV-Visible spectroscopy of the native membrane and the structure of retinal and found those two bands for protein bacterierhodospin at 280 nm and at 570 nm. The 280 nm absorption band is due to the $\pi \rightarrow \pi^*$ transition of the tyrosine and tryptophan amino acid.

3. MATERIAL AND METHODS

The blood samples of Alzheimer's disease patients were collected from the Department of Neurology, Safdarjang Hospital, New Delhi. Twenty milliliters freshly drawn blood from each patient was collected in clean and dry test tube without any anti-coagulant. The test tube was kept for 45 minutes at room temperature (22 ± 2°C) for the formation of clot. Sera of different patients were separated by centrifugation at 1500 r.p.m. upto 15 minutes and were collected in screw capped test tubes. IgG sample were prepared on protein A-Sepharose method [32].

The IgG binding properties of protein A, make affinity chromatography with protein A-sepharose CL-4B a very simple method for preparing IgG. 1.5 g protein-A sepharose CL-4B was swollen in 10 ml phosphate buffered saline (PBS) for 1 hour at room temperature and then packed into a small chromatography column. 10 ml human serum was diluted with an equal volume of PBS. The serum was filtered through the column at a flow rate of 30 ml/h. Washing was done through unbound protein with PBS. Until no more protein left the column (the protein was monitored with a UV flow cell).

The bound IgG was eluted with glycine-HCL buffer having a pH value of 2.8. The pH of purified IgG solution was titrated to near neutrality with NaOH and dialyzed against PBS. The column was regenerated by washing with 2 column bed volume of PBS. The column was stored at 4°C.

The protein A content of the swollen gel is 2 mg/ml and the binding capacity for human IgG is approximately 25 mg/ml of packed gel. As binding of protein A to IgG involves tyrosine residues on the protein A glycosyl tyrosine (0.1 M in 2 NaCl) can be used to elude the IgG rather than the glycine – HCl buffer.

The ultra-violet spectra of the IgG extracted from normal person and Alzheimer's disease patients were recorded on Shimadzu UV visible recording Spectrophotometer UV-260. Normal saline was used as a reference. Block diagram of ultraviolet spectrophotometer is shown in Fig. 3

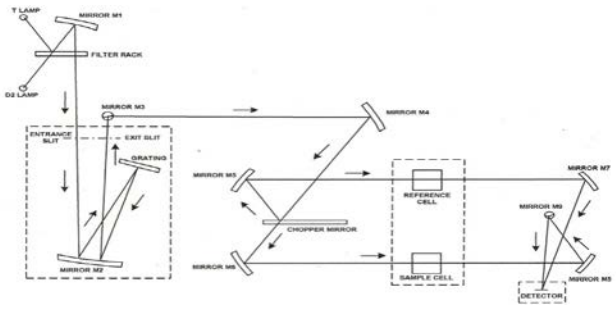

Figure 3: Block diagram of ultraviolet spectrophotometer

4. RESULTS

Ultraviolet absorption spectra of Alzheimer's disease patients were recorded and compared with normal healthy controls. We observe peaks at different wavelength in all samples. A comparative detail of the work is given in Table-1. Typical ultra violet spectra of DMD , AD and control person are given in Fig.4 to Fig.6.

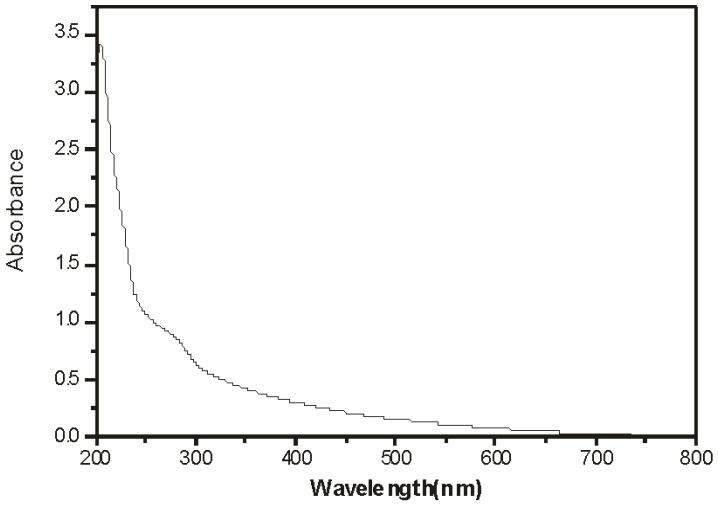

Figure 4 : Ultra Violet spectrum of Normal child

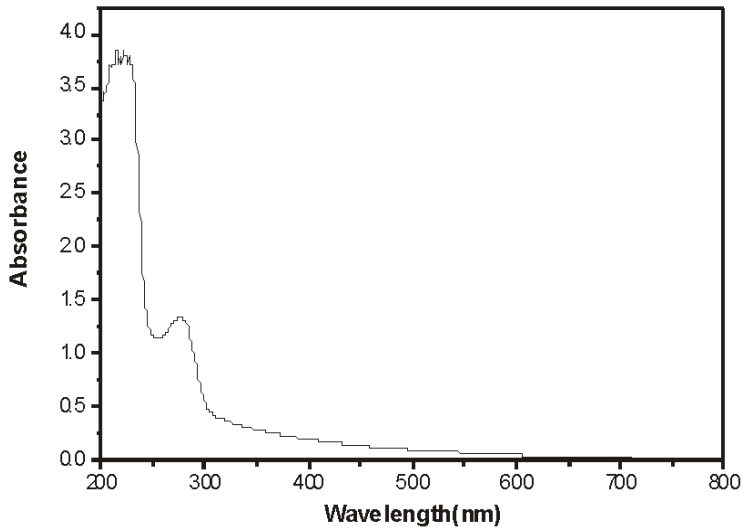

Figure 5 : Ultra Violet spectrum of DMD Child

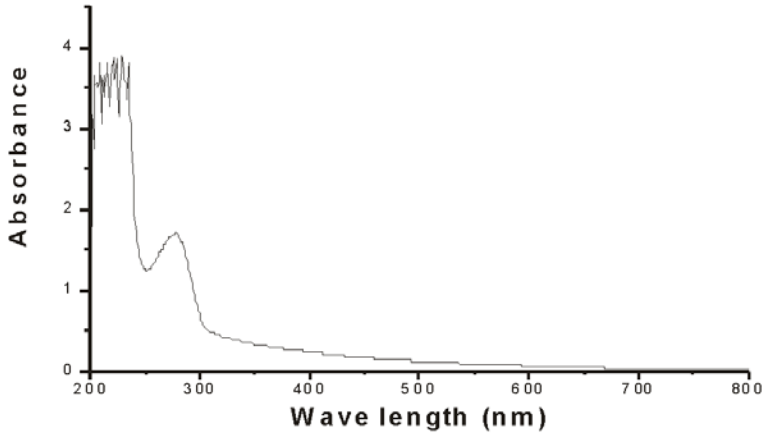

Figure 6 : Ultra Violet spectrum of Alzheimer's diseased person

Table. 1: Experimental findings on the immunoglobulin G molecule of Alzheimer's disease patients and healthy controls and compared with epileptic samples.

E-Epilepsy N-Normals and GME-Grand Mal Epilepsy, AD- Alzheimer's disease, NAD Controls for Alzheimer's. DMD- Duchenne Muscular Dystrophy , NDMD -Controls for DMD

S.No.	Type of sample	Peak 1 nm.	Peak 2 nm.	Peak 3 nm.	References
1	N	205.80	243.08	299.96	Kumar [33]
2	N	208.32	232.97	275.95	
3	N	205.6	260.6	278.6	
4	N	206.6	260.4	277.8	
5	N	207.2	243.2	279.2	
6	N	208.1	244.1	280.1	
7	N	206.0	244.1	281.0	

8	N	207.8	246.8	280.8	
9	N	203.6	243.2	276.6	
10	N	207.2	241.4	284.6	
11	N	205.4	244.1	282.8	
12	N	207.4	230.44	278.48	
13	GME	205.8	260.8	278.8	
14	GME	205.8	268.4	283.4	
15	GME	205.2	259.8	278.6	
16	GME	205.2	260.8	278.4	
17	GME	206.6	268.6	278.6	
18	GME	205.8	260.4	278.6	
19	GME	205.0	268.8	277.4	
20	GME	205.8	260.8	278.8	
21	GME	206.0	268.8	278.4	
22	GME	205.4	261.0	278.6	
23	GME	206.2	261.2	278.6	
24	GME	205.6	261.0	278.4	
25	GME	205.8	259.9	278.6	
26	E	207.71	237.41	279.13	
27	E	206.45	231.73	280.39	
28	E	202.45	231.73	280.03	
29	E	202.65	232.99	280.03	
30	E	202.65	234.25	281.02	
31	E	203.20	231.71	280.37	
32	E	200.01	231.61	278.38	Kumar [33]
33	E	202.20	234.24	279.11	
34	E	202.40	235.50	279.74	
35	E	203.17	235.40	279.01	
36	E	201.27	235.40	279.01	
37	E	202.65	238.68	279.01	
38	E	202.65	238.05	279.01	
39	AD	232.92	276.65	335.05	Present work
40	AD	229.20	269.35	305.58	
41	AD	229.20	270.00	298.55	
42	AD	225.55	267.16	305.85	
43	AD	228.47	269.35	313.15	
44	AD	229.20	267.16	313.15	
45	AD	225.55	273.00	308.77	
46	AD	235.77	269.35	298.55	
47	AD	222.63	244.53	274.46	
48	AD	225.55	268.62	301.47	
49	AD	221.90	278.89	296.36	

50	AD	224.82	262.05	300.01	Present work
51	NAD	230.66	270.08	306.58	
52	NAD	228.47	267.89	303.66	
53	NAD	235.77	268.62	318.99	
54	NAD	230.66	267.16	311.69	
55	NAD	228.47	270.00	297.82	
56	NAD	221.17	260.59	301.47	
57	NAD	224.82	271.54	297.82	
58	NAD	228.47	272.27	305.12	
59	NAD	235.77	267.16	301.47	
60	NAD	228.47	272.27	303.66	
61	DMD	222.22	275.85	311.92	
62	DMD	222.20	277.70	303.60	
63	DMD	220.35	273.63	311.00	
64	DMD	204.14	255.50	296.20	
65	DMD	211.10	259.20	307.30	
66	DMD	212.58	264.38	308.78	
67	DMD	227.38	281.40	325.80	
68	DMD	200.00	244.40	286.95	
69	N DMD	225.90	285.10	314.70	
70	N DMD	225.90	297.70	307.30	
71	N DMD	229.60	287.69	329.50	
72	N DMD	200.00	245.51	302.86	
73	N DMD	200.00	245.51	303.60	
74	N DMD	200.00	249.58	296.20	
75	N DMD	200.00	244.40	299.90	
76	N DMD	221.83	281.40	318.40	
77	N DMD	200.00	246.25	351.70	
78	N DMD	220.35	279.55	309.15	

We have found three types of bands in the spectra of IgG of DMD patients and normal healthy controls. First and second band regions cover the ultraviolet behavior of proteins but third band does not show absorbance of proteins because the absorbance intensity greater than 310 nm. Third band does not contain protein without tryptophan. We have to concentrate on the region of two bands below 300nm. We have first region in the range 200 nm. to 227.38 nm. in DMD patients and 200nm. to 229.60 nm. in normal controls .Second region starts from 244.40nm. to 297.70nm.in patients and 244.40 nm. to 297.70 nm . in controls only. Third band did not show any protein absorbance in all the cases of study. We have found Ist band ranges from (221.17 nm^{-1} to 235.77 nm^{-1}) in all the cases of AD and healthy subjects.

The second band ranges from (244.53 nm^{-1} to 278.89 nm^{-1}) in AD and normal and available at (260.59 nm^{-1} to 272.27 nm^{-1}). There is a variation between these two categories for this band region. There is a shift in the lower side of the band in AD cases. On the other hand a shift in the right side of the band is also observed.

The third band was found in the range from (274.46 nm^{-1} to 313.55 nm^{-1}) in AD samples while in NAD it starts from 297.52 nm^{-1} to 318.99 nm^{-1}. It has been seen that the variation in AD samples is on the decreasing pattern in comparison with healthy controls.

The band near at 275 nm is due to tyrosine amino acid residues of protein. It has been established that this band corresponds to a $\pi \rightarrow \pi^*$ transition. The increase in absorptivity and the long wave shift of the spectrum of tyrosine with ionization of the phenolic hydrogen provides the basis for studying the hydrogen ion equilibria of tyrosyl groups in proteins.

A band at 258 nm is associated with phenylalanine amino acid residues of protein. The low intensity absorption peak centered slightly below 260 nm corresponds to a forbidden $\pi \rightarrow \pi^*$ transition. Vibrational fine structure is found in the region. Aside from increased blurring of the fine structure on passing into successively more polar media, change of solvent effects little change in the general size, shape and location of the absorption envelope. Forbidden transition occurs due to coupling of the electronic transition with different molecular vibrations in a way which removes the symmetry of the barrier.

The band of phenylalanine is weak and it is obscured in proteins by much stronger tyrosine and tryptophan absorptions. Phenylanaline is occasionally visualized in protein spectra as ripples or we may say fine structure in the region 250 nm – 270 nm. These ripples can be amplified by the difference spectral method.

It is very important to point out that the absorptivity of phenylanaline is very small around 260 nm. It is the strongest absorbers in the region of the peptide absorption.

The spectrum of tryptophan appears in the range 270 nm to 290 nm. The absorptivity of tryptophan is greater than unionized tyrosine and about twice as great as ionized tyrosine.

5. DISCUSSION

The ultraviolet spectra of proteins have been made the subject of study. It has been well established that the spectra of amino acids show proteins have a high intensity absorption band in the neighborhood area of 190 nm. A similar band is found in simple peptides, with absorptivity increasing with increasing chain length in oligopeptides. The band at 190 nm is not available with aliphatic amino acids.

It has been seen in several proteins the molar absorptivity per peptide bond at 205 nm falls in the range 260 nm to 310 nm. This shows that a contribution of seventy percent of total

absorptivity, the peptide absorptivity is conformation dependent. The absorption spectrum of proteins is of great interest and made easy to study.

A detailed study for absorbance on proteins was carried out by some scientist and is given in Table-2 (Rosenheck, K. *et. al.* [9]).

Table. 2: Comparison of different proteins.

S.No.	Type of sample	Peak 1 nm.	Peak 2 nm.	Peak 3 nm.	References
1	Carbonic anhydrase	280.0	-	-	
2	Carboxy peptidase A	278.0	-	-	Neurath [34]
3	Chymotrypsinogen	282.0	-	-	Chervenka [35]
4	α-Lactalbumin	280.0	-	-	
5	β-Lactoglobin	280.0	-	-	Gorden *et. al.* [36], Piez*t* [37]
6	Lysozyme	281.0	-	-	Formageot *et. al.* [38], Wetlaufer*et. al.* [39]
7	Papain	278.0	-	-	Glazer *et. al.* [40]
8	Ribonuclease	277.5	-	-	Hermans *et. al.* [41]
9	Bovine serum mercaptalbumin	280.0	-	-	
10	Human serum mercaptalbumin	279.0	-	-	
11	Δ-3-Ketosteroid isomerase	277.0	-	-	Kawahara *et.al.* [42]
12	Tryptophan	267.2	274.7	281.6	Coulter *et. al.* [43], William *et. al.* [44]
			275.0		
13	Tryptophan	269.4	279.4	288.8	
			278.9	287.5	
14	Phenylalanine	271.4	-	-	
		263.3	257.6	251.6	
15	Tryptophan	280.0	-	-	Franz [45]
16	Tyrosine	275.0	-	-	
17	Phenylalanine	258.0	-	-	

There is a broad band with a peak found in the spectrum of protein at 280 nm and a minimum at about 250 nm on the shorter wave length side. This band may be correlated with the presence of tyrosine tryptophan and phenylalanine [46]. The absorption band which is found at wave length above 280 nm is due to non protein occur chromophore. Nucleo proteins

shows maximum in the region of 260 nm. This may be associated with the presence of purine and pyrimidine nuclei. Bovine serum albumin (BSA) shows two characteristics UV bands around 280 nm and 215 nm respectively.

Some of spectra reported for proteins end at 235 nm on the shorter wavelength side of the main 280 nm peak. The absorption curves rise rapidly in this region. Steep rise may provide some information regarding the region of continuous absorption. Specific structure of a protein distinguish it from other compounds in the presence of a large number of peptide bands. UV spectra of anhydrides, esters and fatty acids and acid chloride show a broad band in the region of 200 nm. Ultra violet spectroscopic studies below 240 nm are of interest. Ultra violet spectroscopic studies of amino acids were also carried out by Coulter*et. al.* [43] and they have reported that aliphatic amino acids do not show absorption above 250 nm. Tyrosine tryptophan and phenylalanine show spectrum of absorption in the same spectral region as the proteins. The ultra violet absorption band of proteins has in consequence been attributed to the content of amino acids.

It has been seen that the peptide group of the protein main chain absorb the light energy in the range from 180 nm to 230 nm. Aromatic side chains of tryptophan tyrosine and phenylalanine also absorb light in this region. These residues of proteins may absorb light in the region from 240 nm to 300 nm. Disulfide bond also shows a absorption character near to 260 nm. The aromatic amino acids do not absorb any light above 310 nm. The protein absorbance above 310 nm is zero. The proteins without tryptophan residues do not show any absorption spectrum above 300 nm.

There is a region which starts from 285 nm to 295 nm shows several bands which originate from the nine tyrosine and the single tryptophan residue.

We would like to make a point of inference that at 205 nm the side chains of amino acids make relatively small contribution to the total absorption of proteins and it is absent in all AD and normal subjects.

Absorption coefficient for the peptide bond is of an order of magnitude many times higher than that of amide or carboxylic group. It can be said that the side chain of amino acids.

Our data did not support the work of Scopes [5]. Tyrosine absorbance at 205 nm is a fundamental property of the proteins but we could not get any absorbance at this level. It might be due to the aging factor of the human subjects in our study. The diet has also play an important key role in the metabolism of the body. If we take diet in rich proteins and other compounds then there is a possibility to have such types of absorbance due to tyrosine. It might be the change due to some other factors of the body. Our main aim was to throw light on the molecular level of immunoglobulin G molecule of proteins. We have also succeeded in giving our views related to ultraviolet spectroscopy. Now days ultraviolet spectroscopic studies are not

in bulk but few studies are also available at the present period of research. One research group of this ultraviolet activity is engaged in determining all the biological fluids such as plasma, hemoglobin, CSF, urine etc. to give a definite contribution to the total absorption of proteins. It can be taken into consideration for the study of conformation of structure of proteins [47].

Franz [45] studied the ultra violet spectroscopic behavior of biological molecules and supplied suitable information for the general character of proteins such as detection of conformational changes and ligand binding etc.

On the basis of presence finding we can safely said that the tryptophan content is a good parameter for the conformation of structure of IgG.

6. CONCLUSION

Proteins and nucleic acids are supposed to be linear polymers [48]. It has been seen that in the polymers a limited set of residues are bound together with the help of amide or phosphodiester bonds. We have a situation like carbohydrates bound through the linking options.

The UV absorbance spectrum for a biomolecule is sum of the spectra of component parts. The UV absorbance for nucleic acids is found from 200 nm to 300 nm. This spectrum is due to transitions of the purine and pyrimidine bases.

The backbone starts to contribute at about 190 nm. The region, which is accessible and required below 200 nm. It is found to be dominated by

$A \rightarrow A^*$ transition of the bases. Oxygen absorption interferes with the spectrum. The UV spectra of the bases look like simple bands. Each band is a composite of more than one transition.

The base transitions are disturbed significantly by $\pi \rightarrow \pi$ stacking interactions. The wavelength maxima and transition intensities vary depending on the base sequence and structure.

In the case of peptides and proteins the spectroscopy of amide bands, the side chains and any prosthetic group determines the observed UV-visible absorption spectrum. The intensities and wavelengths for nucleic acids can be disturbed by the local environment of the groups. UV spectra for proteins can be divided into two regions like near and far UV region. The near UV zone is started from 250 nm to 300 nm and is also described as the aromatic region. Transition of disulphide bonds also contribute to the total absorption intensity in the said region. The far UV above 250 nm is directly dominated by the transition of the peptide backbone of the protein, but transitions from some side chains may also contribute to the spectrum below 250 nm.

The aromatic side chain, phenylanaline, tyrosine and tryptophan all have transition in the near UV region.

Tryptophans have most intense transitions. Many proteins have few tryptophans compared with the other aromatic groups. These transitions are not dominated in the near ultraviolet regions.

The peptide chromophore gives transitions in the region from 180 nm to 240 nm. It has non bonding electrons on the oxygen and also on the nitrogen atoms. This chromophore has π-electrons which are delocalized to some extent over the carbon, oxygen and nitrogen atoms. This peptide chromophore has the transitions of σ bonding electrons. The lowest energy transition of the peptide chromophore is a $\pi \rightarrow \pi^*$ transition. This transition is analogous to ketones.

The $\pi \rightarrow \pi^*$ transition is dominated by the carbonyl π-bond. This bond is also affected by the involvement of the nitrogen in the π-orbitals. The electric dipole transition moment is also polarized some where near the line between the oxygen and nitrogen atoms. This transition has a centre at 190 nm. It has been seen that in an α-helix, the coupling of the $\pi \rightarrow \pi^*$ transition moments in each amide chromophore results a component at about 208 nm, which contribute to the characteristic α-helix.

Protein absorbances can be seen as dominated by tryptophan residues and a clear out absorbance found at 280 nm. The other aromatic residues also absorb at 280 nm. This absorbance may be used to give an estimate of protein concentrations. It has been experimentally verified and checked by the researchers that 1 mg cm^{-3} protein solution has an absorbance of 1 absorbance unit in a 1 cm path length cell. This is due to the fact that many proteins have a similar percentage of aromatic amino acid residues.

The Bear-Lambert law for the determination of concentration of nucleic acids and proteins is based on the fact that the samples are hundred percent pure. If nucleic acids are found, the interference of these with the protein concentration determination may appear. They also absorb at 280 nm.

Phenylalanine residues contribute fine structure such as wiggles to the spectrum from 250 nm to 260 nm. The aromatic amino acids do not absorb above 310 nm. Neither proteins nor nucleic acids ultraviolet absorbance at 320 nm. It has been well documented that protein absorbance should be zero at wavelengths greater than 310 nm. Proteins without tryptophan residues do not absorb above 300 nm.

The studies of peptides are suggestive consideration of protein structure. Electrostatic effects are very small. The peptide absorption coefficients for a protein should depend on the amino acid composition. It should be greater than triglycine.

We conclude this method is reliable and efficient to detect the changes at the molecular level. Specific changes could be seen in the structure of protein molecule with the help of detailed theory of ultraviolet spectroscopy. This spectroscopy confirms that the absorbance by proteins above 300 nm is not possible and no protein absorb at this wavelength. We have found the absorbance peaks above this wavelength. Other spectroscopic techniques may help in the study of this absorbance. The ultraviolet spectroscopy is reliable and usable technique and more predictive related to protein determination.

Our study shows a clear cut indication regarding protein absorbance that the proteins are damaged in the region of third band completely. We have to study more to get the cause of the non-availability of absorbance of proteins by other suitable method of spectroscopy.

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