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Effect of Oral Iron Chelator Deferiprone on Skeletal Radiography of Thalassemia Major Patients

By Dr. Anita Saxena & Dr. Archana Gupta
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Abstract - Regular blood transfusion along with iron chelation therapy is a supportive treatment for thalassemia major. Chelation therapy too has its side effects. The most common adverse effects associated with administration of deferiprone are agranulocytosis, neutropenia and arthralgia, primarily, of the large joints.
Objective : The study was undertaken to examine the effect of deferiprone on the large bone joints of thalassemia major patients. Material and Methods: Thalassemia major patients (62) on hyper-transfusion treatment regime aged between 4 19 years were assigned to three groups. Group I included 42 patient taking deferiprone, Group II included 10 patients on deferoxamine, and Group III included 10 patients who were not taking chelation therapy.

Keywords : *Thalassemia, iron chelation, deferoxamine, bdeferiprone, musculo-skeleton.*

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Effect of Oral Iron Chelator Deferiprone on Skeletal Radiography of Thalassemia Major Patients

Dr. Anita Saxena [α] & Dr. Archana Gupta [σ]

Abstract - Regular blood transfusion along with iron chelation therapy is a supportive treatment for thalassemia major. Chelation therapy too has its side effects. The most common adverse effects associated with administration of deferiprone are agranulocytosis, neutropenia and arthralgia, primarily, of the large joints.

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Results : Radiographs of 19 (43%) patient from group I showed peri-articular changes in the knee joint which were clinically correlated with complaints of joint pain, stiffness and consequently limping, swelling inability to squat and/or climb stairs. Peri-articular changes were also present in the wrist (9/42patients), elbow (9/42) and ankle (4/42), reduced joint space in elbow (1/42) and soft tissue swelling in another.

Conclusion : The findings of the study are suggestive of the fact that arthritis changes observed in the patients were related to deferiprone therapy.

Keywords : Thalassemia, iron chelation, deferoxamine, bdeferiprone, musculo-skeleton.

I. Introduction

β-thalassaemia major is the commonest lethal single gene disorder in India with a prevalence of 1-17% in different population groups (mean prevalence is 3.3%). This disease has a spectrum of clinical severity which is associated with ineffective erythropoeisis, bone marrow expansion and repaid destruction of erythrocytes. Anemia demands frequent blood transfusion to maintain life while hemosiderosis and other complications of the disease require a continuous and distressing treatment regime that includes iron chelation treatment regular medical supervision, request admissions to the hospital and on many occasions surgery. This autosomal recessive haematological anemia, caused by abnormality of beta globin synthesis, is fatal in infancy without transfusions but is fatal in adolescence even with them. The only curative treatment for this disease is bone marrow transplantation (BMT) which is expensive, not easily affordable by a common Indian family and with variable success rate of BMT 60-70%.

Regular blood transfusion followed by iron chelation therapy is just a supportive treatment for this disease which is associated with serious complications. The cost of supporting a thalasemic child varies from few thousand rupees to Rs.1,00,000 a years depending upon the kind of treatment opted by the family. The excess iron causes diffuse organ damage, usually resulting in fatal cardiac toxicity. In supportive treatment, because the magnitude of the body iron burden seems to be the principal determinant of clinical outcome[2-4] the prime goal of iron-chelating therapy in patents with thalassemia major is to control iron overload. The optimal body iron should minimize both the risk of adverse effects from the iron-chelating agent and the risk of complications from iron overload. With stable transfusion requirements and in the absence of other confounding factors, the lower the level of body iron is desired, the higher the dose of iron chelator is required., The advent of treatment with subcutaneous deferoxamine has, however, changed the gloomy prognosis of the disease. Studies have demonstrated that over 90% of patients who comply with the difficult and expensive regimen of deferoxamine treatment survive without heart disease [4.5] and with minimal toxic effects (deferoxamine induced bony changes are well documented [6-10]) if dose is tailored to the iron burden [11].

The successes achieved with deferoxamine, as well as the limitations of this treatment, have stimulated the design of alternative strategies of iron chelating therapy, including orally active iron chelators. Only a few of the many hundreds of potentially useful oral chelators have been found suitable for clinical studies. The development of the most promising of these deferiprone (1, 2 dimethy 1-3-hydroxypryridin-4-one or LI) has progressed rapidly and data from several trails have provided direct and supportive evidence for its short-term efficacy[12]. Deferiprone is able to promote iron excretion, although its effect on serum ferritin level is variable [13-15]. However, at the same time the toxicity of

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this agent mandates a careful evaluation of the balance between risk and benefit of deferiprone in patients with thalassemia in most of whom long-term deferoxamine is safe and efficacious therapy. The most common adverse effect associated with administration of deferiprone has been arthralgias, primarily, of the large joints [413,15-18] the etiology of which remains elusive bringing into question its long term use in humans[19], neutropenia or agranulocytosis first reported in 1989[20]. This study was undertaken to examine the effect of deferiprone on the large bone joints of thalassemia major patients.

II. Material and Methods

The study was conducted at Sanjay Gandhi Post Graduate Institute of Medical Sciences, Luckknow between year 2001 and year 2002 on 62 thalassemia major patients on hyper-transfusion treatment regime aged between 4 and 19 years registered with department of Medical Genetics. The patients visited hospital every 3 to 4 weeks for blood transfusion. Patients were divided into three groups based on which chelation therapy they were taking. Group I included 42 patients taking deferiprone (duration 6 months to 6 years), Patients in Group II were on deferoxamine infusion and belonged to rich Indian class who could afford good and expensive treatment. Group III included 10 patients who were not taking chelation therapy because of financial constraints. Patients from Group I and III belonged to low and middle socio-economic groups who could not afford expensive treatment. Clinical history of the patient is given in Table 1. Prior to starting deferiprone or deferoxamine complete blood count (CBC), serum ferritin, ,liver function test (serum bilirubin, AST, ALT, serum alkaline phosphate), HIV I & II antibodies, and blood sugar fasting and post meal were tested. All the patient were immunized for hepatitis B. Growth assessment of the patients was done once in three months.

Routine Tests : Pre-transfusion hemoglobin was tested on every visit and serum ferritin levels every six months. Yearly evaluation of endocrine glands included TSH, T4, serum Cortisol, GTT, Calcium and Phosphorus.

Deferiprone : Patients were given deferiprone after written consent had been obtained from their parents. The parents were given detailed information on efficacy, safety and potential side effects of deferiprone (marketed in India as Kelfer, Cipla Ltd.). Dose was prescribed as follows: starting dose 50 mg/kg body weight, which was gradually increased ton 60 mg, 75mg and finally to 1000 mg/kg body weight. CBC was checked every month. Deferiprone was discontinued if patient developed high fever, prolonged or abnormal bleeding, or if total leucocyte count was <4000/l or platelets <1000,0001/1. Deferiprone was stopped in case patient developed bone joint related problems. The patient was then managed on non-steroidal anti-inflammatory drugs. After the symptoms resolved, the drug was restarted.

Deferoxamine : Starting dose of deferoxamine was 25 mg/kg body weight which was gradually increased up to 45 mg/kg body weight. Patients on this drugs were advised to take Vitamin C on the day of infusion.

Radiograph : To examine bone age, bone density, bone expansion, peri-articular changes (arthritic changes, soft tissue swelling, loose bodies and reduction in joint space) antero-posterior and lateral views of wrist, knee and ankle joints were taken and analyzed and clinically correlated. Bone age more than 2 years below the chronological age was taken as delayed age.

Deaths : After the completion of the study 5 patients died: 3 from Group II and I each from Groups I and III.

Statistical analysis was done using SPSS 10.0 for Windows. Frequencies for hemoglobin and serum ferritin were calculated. Paired t test and one-way analysis of variance were used for further analysis of data.

III. Results

Hemoglobin, Serum Ferritin and Other Conditions: Patients in all the three groups had low hemoglobin and high serum ferritin level. Only 6 patients had hemoglobin more than 9 but less than 10.0 mg/dL and another 6 had serum ferritin ≤ 2500 ng/L. Analysis of variance showed that there were significant differences (P>.001) between the hemoglobin and serum ferritin levels of the three groups. There was significant difference in these two parameters when group I was compared with group III (paired t test P>0.001) and when group II was compared with group III (paired t test P>0.001). There was no significant difference between the hemoglobin and serum ferritin levels of group I and II. Decline in serum ferritin was observed within first ;year of starting chelation therapy. Compliance to chelation therapy was generally good. Only two girls attained sexual maturity (one each from group II and III). During study 2 patients were diagnosed to be suffering from hypothyroidism, 4 from cortisol deficiency, 2 from epilepsy and another 2 from congestive heart failure. None of the patients were HIV positive. Three patients had undergone splenectomy and 11 were sufferening from hypersplenism.

IV. Radiological Findings

Bone age, bone density and bone expansion : Radiographs showed that the boned age of 4 patients (1 patient from Group II and 3 patients from group III) was delayed. Bone age of rest of the patients was either the same as the chronological age or 1 or 2 years

less than it. X-rays of 3 patients (4.9%) from Group I (n=1) and II (n=2) were normal. Rest (96%) of the patients had mild to moderate expansion and reduction in bone density. Three patients (4.9%) 2 from Group I and 1 from group II had severe expansion and reduction in bone density. Erylmeyer flasking was observed in 5 (8%) patients: in the knee joints of 4 patients and in all the three joints elbow, knee, and ankle of 1 patient.

V. Peri-Articular Changes

Group I (Deferiprone) : Radiographs of 19 (43%) patients showed peri-articular changes in the knee joint which were clinically correlated with complaints of joint pain, stiffness, (consequently of limping), swelling, inability to squat and climb stirs (Table 2). Out of these 19 patients, 7 patients had involvement of both the knees. Due to severe arthritis in the knee joints 2 patients were unable to walk and hence confined to bed. Peri-articular changes were present in the wrist joint of 9 (21%) patients, elbow joint of another 9 (21%) and in the ankle of 4 patients (10%). X-ray of 1 patient showed reduced space in elbow joint which clinically correlated with the patient's inability to flex arms. Soft tissue swelling was observed in one radiograph of the knee.

In 6 patients (14%), more than one join was affected. Arthritic changes were present in all the four joints of 2 patients, three joints (wrist, elbow and knee) of 1 patient, two joints (wrist and elbow) of 3 patients and wrist and knee of another 1 patient. Deferiprone of one patient was stopped due to severe arthralgia and swelling in knee joint (no chelation presently) and of another due to thrombocytopenia.

Knee joint was the most commonly affected joint and the most frequent symptom was pain and swelling in the joint. These symptoms appeared within first two years of starting deferiprone therapy.

Group II (Deferoxamine) and Group IIII (No Chelation): Radiographs of patients in this group did not show any periarticular changes. None of the patients complained of joint pains or showed changes in total blood counts.

VI. Discussion

One of the major concerns with clinical use of L1 is the risk of associated toxicity[19]. Issues regarding safety of deferiprone have been discussed by the International Study Group for Oral Iron Chelators (ISGOIC), a group of about 40 scientists and clinical investigators with extensive experience in the management of thalassemia patients in 1993 in Nicosia[21]. Consensus was that there is an urgent need for further well controlled clinical studies of deferiprone in sufficient number of patients in order to enable proper judgment of its suitability for general long – term clinical use. The relative effectiveness and safety of and compliance with deferiprone and deferoxamine were compared in a prospective randomized trial begun in Canada in 1993.

The most common adverse effect associated with administration of deferiprone has been arthralgias, primarily, of the large joints the etiology of which remains elusive. The most serious adverse effect associated with the administration of deferiprone was severe neutropenia or agranulocytosis, first reported in 1980[20]. Till 1997 this complication had been reported in 13 patients, of whom 10 were thalassemia major patients[20, 22, 23], as early as 6 weeks and up to 21 months after initiation of deferiprone. In five patients in whom rechallenge with deferiprone was attempted after white blood cell counts returned to normal, a second decrease in neutrophil count was observed[23]. The mechanism of deferiprone-induced neutropenia is unknown. Although studies in animals and early reports in humans suggested that this effect might be related to administration of high doses of deferiprone, at least 7 patients have developed agranulocytosis during administration of the standard daily dose of 75 mg/kg body weight; this adverse effect appears not to be dose-dependent, but idiosyncratic and unpredictable.

Results of a long term-term deferiprone therapy show that 1 per 100 patients developed agranulocytosis, which was reversible. Other significant complications in decreasing order of incidence were: transient liver enzyme abnormalities (44%), arthropathy (21%) , zinc deficiency (14%) and nausea 80%)24. Joint symptoms in association with deferiprone therapy are known25. Joint symptoms occurred in up to 33% of patient in Indian trial15. In another study26, arthropathy caused discontinuation of deferiprone during second or third years of therapy. Joint symptoms were present for several weeks and did not improve with lowering the dose of deferiprone (to 50 mg/kg/d). The knee joints were mainly affected and the clinical symptoms were stiffness, crepitus, and effusion. However, despite conducting several tests pathophysiology of arthropathy could not be known[26]. Some degree of joint or muscle stiffness and pain affecting shoulder, back ankle, knee joint and osteoarthritis of the knees was reported in patients on deferiprone[25]. The explanation for joint symptoms was unclear.

Osteoarthropathy is well recognized in thalassemia major, usually in the second or third decade and is attributed to underlying bone changes[27] and lower limb pains are particularly frequent in thalassemia[28]. Other adverse effects reported with deferiprone administration include dermatologic changes associated with decreases in serum zinc concentration which resolve with oral zinc supplementation[29],[30], nausea, and transient or sustained liver enzyme abnormalities[24]. Author has also conducted a study on serum zinc levels in thalassemia major pathients.

In the present study, although chelation therapy had brought down serum ferritin levels but they were still very high[31]. Only 6 patients were well chelated as they had serum ferritin ≤ 2500ng/dL. Results of this study show expected bone changes (reduced bone density and bone expansion) in almost all the patients which can be attributed to persistently low hemoglobin levels. Involvement of knee joint (peri-articular) which supports the findings of previous studies[25, 26] was the main finding of the study although these changes were observed in patients from group I only, that is, those patients who were on deferiprone and they were be clinically correlated (complains of pain, stiffness, swelling etc.). Knee joint involvement in this study is the highest reported so far[15,24]. Three patients suffered from join problems the most. In the first two cases, arthritic changes in the knee joints of two patients were so severe that they were unable to walk and were disabled. One of these patients died due to multiple organ involvement. In the third case, due to reduced joint space in the elbows the patients was unable to flex the arms. In a lesser degree of disablement, three more patients who had changes suggestive of arthritis in the knees, were unable to squat due to pain in the knees. Other serious adverse effect of deferiprone were repeated thrombocytopenia in 2 patients, leucopenia in 2 patients and bone marrow suppression in one. The patient developed hypersplenism, followed by thrombocytopenia (while still on deferiprone therapy) and finally bone marrow suppression. This patient switched over to deferoxamine therapy approximate 11 months prior to his death. However, after the study was completed, 7 patients switched over from deferiprone to deferoxamine therapy firstly due to join pains (3 males) and secondly in order to avoid any other associated complications (3 males and 1 female).

Deferiprone has a much lower therapeutic ratio than deferoxamine, for two reasons. First, deferiprone is considerably more toxic and regularly depresses the granulocyte count in both normal and iron-overloaded animals[32], deferoxamine in contrast does not depress the marrow. In clinical studies deferiprone has caused both agranulocytosis and arthralgia or arthritis[33]. Second, Oliveri and her collegues clearly demonstrated that deferiprone can reduce iron stores to lower, if still elevated, levels in patients with severe iron overload, the drug has a concentration-dependent affinity for iron[34]. Three molecules of deferiprone are required to bind one molecule of iron, whereas deferoxamine binds iron tightly in a 1:1 ratio. For this reason, deferprone must be present at very high concentrations (close to toxic levels) to be effective. It dissociates from iron when the concentration of iron in body fluids falls to the level achieved just few hours after oral adminstration[34]. Hence as demonstrated by Olivieri and her colleagues, deferiprone does not readily reduce excessive body iron stores below a certain level. It is, therefore, not clear if the drug will provide long term protection from disease. Deferiprone is now well known to cause adverse effects on musculo-skeleton[4]., though in some studies the symptoms have resolved on discontinuation of the drug.

Our study emphasizes the fact that patients who were on deferoxamine and those who were not on iron chelation did not suffer from arthritic problems. Since a lost of studies have reported similar musculo-skeletal pains and osteoarthritis in patients on deferiprone therapy, it suggests that these symptoms are related to deferiprone therapy. An immunological mechanism could be responsible for these symptoms[25]. It is also possible that soluble LI-iron complexes of metabolites formed in the joints or transported there from plasma or LI itself may be implicated[25]. It is important that for future clinical studies, patients with preexisting clinical complications are included so that possible adverse effects of the drug can be easily distinguished from the progression of the underlying disease[25].

The results of our study show that long-term iron chelation therapy is feasible using deferiprone but it is associated with serious side effects. Our study confirms the findings of previous studies in which different side effects of deferiprone have been reported [13,15,29].

Keeping in mind financial constraints of low and middle socio-economic Indian families deferoxamine therapy has two main limitations: firstly, it is an expensive drug (both oral as well as infusion) not easily affordable by Indian families and secondly it calls for 10-12 hours of continuous subcutaneous infusion causing discomfort to the patient [35,36] and hence poor compliance. On an average the annual expenditure of a patient on deferoxamine is Rs.1,00,000/- and that of a patient on deferiprone is Rs.12,000/-. From this is evident that deferiprone is relatively inexpensive compared to deferoxamine and hence, deferiprone is the only option for thalassemia major patient from low and middle socio-economic strata (since there is no national health policy supported by Indian Government) since this drug decreases the iron overload to a measurable extent. However, toxicity of deferiprone mandates a careful evaluation of the balance between risk and benefit to the patients with thalassemia who require life long iron chelation bringing into question its long term use in humans.

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Table 1 : Clinical Details of Thalassemia Major Pateints.

Clinical Details	Group I (42)		Gourp II (N=10)		Group III (N=10)	
	Boys	Girls	Boys	Girls	Boys	Girls
Sample	29	13	6	4	5	5
Mean Age (years)	9.57	8.69	15.5	13.2	8.25	11.0
Delayed bone age	-	-	1	-	-	3
Hemoglobin X ±SD Minimum Maximum	7.6 ±.88 5.4 9.0	8.1 ±.95 6.3 9.7	8.3 ±.59 7.3 9.1	8.5 ±.88 7.3 9.3	6.2 ±1.0 5.0 7.8	6.9 ±.97 6.0 8.4
Serum Ferritin X ±SD Minimum Maximum	5322.5 ±2657.7 2,000 10,073	3744.5 ±1838.2 1326. 6803.00	5145.1 ±2533.3 2859 8,730	3893.7 ±2866.5 1398 8,000	8800 ±2683.2 4,000 10,000	7552.6 ±2121.8 4210.00 9,625
HIV	-	-	-	-	-	-
HCV Positive	5	1	1	1	-	1
Hypersplenism	11		1	1	1	3
Splenectomy	-	2	1	-	-	-
Thrombocytopenia	2	-	-	-	-	-

Leucopenia	2	-	-	-	-	-
Bone Marrow Suppression	1	-	-	-	-	-
Hypothroidism	1	-	1	-	-	-
Cortisol Deficiency	1	-	3	-	-	-
Diabetes Mellitus	-	-	1	-	-	-
CHF*	1	-	1	-	1	-
Epilepsy	1	-	-	1	-	-
Sexual Maturation	-	-	-	1	-	1

- *CHF Congestive Heat Failure.*

Table 2 : Changes In Bone Joiints As Depicted By Radiographs.

								PARTICULR CHANGES					
	Bone Density				Bone Expansion				Arthritic Changes		Reduced Joint Space		STS
	Norm	-1	-2	-3	Norm	+1	+2	+3	Norm	Present	Norm	Present	
Hand with Writs	3	36	21	2	3	38	18	3	33	9 Carpometacarpal 3 7.1 Radial epiphysis 4 7.3 Ulnar Epiphysis 1 1.0	ALL		Nill
%	4.9	61.0	58.0	3.2	4.9	61.0	29.0	4.9	78.0				
Elbow	3	46	9	4	4	44	12 (Flasking in 1)	2	33	9 Resorption of olecranon 2 4.8 Radial+Ulnar epiphysis 2 2.4 Himerus 2 4.8 Loose bodies 1 2.4		1	1
%	4.9	74.0	14.5	6.4	6.5	70.0	19.3	3.2	78.0			2.4	
Knee	6	43	9	6	5	39	13 (Flasking in 7)	3	24	Medical condyles 19	ALL		Nill
%	9.6	78.1	14.5	9. 6	3.1	62.0	20.9	3.1	57	43.0			
Ankle	6	44	8	4	6	49	4 (Flasking in 2)	3	26	4	ALL		Nill
%	9.6	70	12.9	6.4	9.6%	79	6.4	9.3	81.2	9			

Reduced Bone Density: Normal, -1 mild, -2 moderate, -3 severe.
Bone Expansion: normal, +1 mild, +2 Moderate, +3 Severe.
STS : Soft Tissue Swelling

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Effect of Daily Treatment with Tadalafil on Serum Prorenin, Nephrin, Plasminogen Activator Inhibitor-1 and Interleukin-6 Levels in Type 2 Diabetics in Relation to Microalbuminuria

By Ziena Salah Najeb, Shatha H. Ali & Kalid Ibrahem Al-Lheby
Baghdad University, Iraq

Abstract - Tadalafil is a phosphodiesterase-5(PDE-5) inhibitor, currently marketed for treating erectile dysfunction, as well as, for the treatment of pulmonary arterial hypertension.These are orally available drugs acting via intracellular signaling pathways, specifically the cyclic nucleotide monophosphate cyclic GMP, represents an important mediator in controlling of the outflow in region (kidney). The use of phosphodiesterase (PDE) inhibitor-5, tadalafil, is known to restrain the degradation of cyclic GMP, could offers a great opportunities in the treatment of kidney dysfunction associated with diabetes .Which may be assessed by testing for microalbuminuria that mostly precedes the development of overt diabetic nephropathy.

Keywords : Tadalafil, diabetic nephropathy, prorenin, nephrine, interleukin-6, plasminogen activator inhibitor-1, microalbuminuria.

GJMR-B Classification : NLMC Code: WK 825, WK 840, WK 870

Strictly as per the compliance and regulations of :

Effect of Daily Treatment with Tadalafil on Serum Prorenin, Nephrin, Plasminogen Activator Inhibitor-1 and Interleukin-6 Levels in Type 2 Diabetics in Relation to Microalbuminuria

Ziena Salah Najeb [α], Shatha H. Ali [σ] & Kalid Ibrahem Al-Lheby [ρ]

Abstract - Tadalafil is a phosphodiesterase-5(PDE-5) inhibitor, currently marketed for treating erectile dysfunction, as well as, for the treatment of pulmonary arterial hypertension. These are orally available drugs acting via intracellular signaling pathways, specifically the cyclic nucleotide monophosphate cyclic GMP, represents an important mediator in controlling of the outflow in region (kidney). The use of phosphodiesterase (PDE) inhibitor-5, tadalafil, is known to restrain the degradation of cyclic GMP, could offers a great opportunities in the treatment of kidney dysfunction associated with diabetes. Which may be assessed by testing for microalbuminuria that mostly precedes the development of overt diabetic nephropathy. The study was designed to evaluate the effect of *daily treatment* with 2.5mg *tadalafilfor 21 days* on some inflammatory markers [interleukin -6 (IL -6), plasminogen activator inhibitor-1 (PAI-1)]inrelation to diabetic nephropathy (nephrin levels –a newly introduced biomarker of nephropathy) in relation to serum prorenin levels in type 2 diabetics, both with & without microalbuminuria.

Twenty-three type2 DM patients (16 male, 7 female) were selected from patients attended the Specialized Center of Diabetes and Endocrinology in AL-Kindy Teaching Hospital/Baghdad, in addition to sixteen non diabetic participants (11 male, 5 female). The ages of selected subjects were within 30-60 years old. Assessment of the serum levels of fasting glucose, prorenin, nephrin, interleukin-6, plasminogen activator inhibitor-1. In addition to detecting the presence of microalbuminuria. These investigations had been performed at basal time and after treatment with 2.5mg tadalafil daily for 21 days.

The association of microalbuminuria with each of the studied parameters (FSG, HbA1c, IL-6, PAI-1, prorenin, nephrin) at basal time for both groups were non-significant ($p>0.05$). After treatment with tadalafil for 21 days, the same association remains non-significant ($p>0.05$) for (IL-6, PAI-1, prorenin, nephrin), except for FSG ($p=0.014$) in diabetics. Comparing results obtained from diabetics to those of controls before starting treatment with tadalafil exertnon significant variation from those of controls (nephrin, IL-6,PAI-1), except for those related to glycemia : fasting serum glucose & Hb A1C, as well as for microalbuminuria, in addition to serum prorenin elevation. Whereas, values obtained after treatment with Tadalafil in both groups (controls & diabetics) indicate that daily treatment with Tadalafil, could produce a preferable modifications in serum nephrinprorenin, PAI-1 and IL-6, as well as in microalbuminuria.

In conclusion because of the complex pathogenicity of diabetic nephropathy, new therapeutic interventions targeting primary mechanisms contributing to renal damage are critical for the future treatment of diabetic nephropathy. The use of phosphodiesterase (PDE) inhibitors, tadalafil, could offers great opportunities in the treatment of kidney dysfunction, as presented this study by improving serum levels of IL-6,and PAI-1, indicating a possible anti-inflammatory effect by tadalafil therapy, mediated through its vascular effects. Furthermore, it improves serum levels of prorenin & nephrin - the new early indicator of nephropathy. Generally, PDE inhibitors are regarded as efficacious, have a rapid onset of action and favorable effect-to-side effect ratio.

Keywords : Tadalafil, diabetic nephropathy, prorenin, nephrine, interleukin-6, plasminogen activator inhibitor-1, microalbuminuria.

I. Introduction

The prevalence of diabetes for all age-groups worldwide was estimated to increase from 2.8% in 2000 to about 4.4% in 2030, with a total number of about 366 million in 2030.[1] Diabetes mellitus (type 2) encompasses individuals who have insulin resistance and usually have a relative (rather than absolute) insulin deficiency.[2] Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance.[3]

Several mechanisms were proposed to be involved in pathogenesis of diabetes as well as it' complications, among these oxidative stress which plays a central role in the onset ofdiabetes mellitus as well as in the development of vascular and neurologic complications.[4] The source of oxidative stress is a cascade of reactive oxygen species (ROS) leaking from the mitochondria[5], such process could be associated with the onset of type 2 diabetes via insulin resistance.[6] Meanwhile, the advanced glycation end products (AGEs), which is believed to play a causative role in vascular complications of diabetes mellitus[7], through

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increasing vascular permeability, inhibition of vascular dilation by interfering with nitric oxide, oxidizing LDL, (8)binding cells including : macrophage, endothelial, and mesangial cells to induce the secretion of a variety of cytokines and enhancing oxidative stress.[8,9] However,the underlying mechanisms behind onset of diabetes are complex because hyperglycemia may be both the cause and effect of increased oxidative stress[10], since chronic hyperglycemia in diabetes result in specific long-term damage, dysfunction, and failure of different organs can affect, especially the eyes, kidneys, nerves, heart and blood vessels.[11]

Diabetic nephropathy is the main cause of end-stage renal disease.[12,13] Although poor glycemic control for long periods is amajor factor in the development of diabetic nephropathy,hypertension, hyperlipidemia, in addition to predisposing genetic factors.[14] The molecular mechanisms that underlie the pathogenesis of diabetic nephropathy remain unclear. Recent studies have highlighted the hyperglycemia- induced production of reactive oxygen species (ROS) to be involved in diabetic microangiopathy,[15] via activation of protein kinase C (PKC) pathway,[16] formation of advanced glycation end products (AGEs)[17,18] , and activation of transcription factors such as nuclear factor-B,[19] genes associated with oxidative stress.[20,21] Diabetic nephropathy is characterized by thickening of the glomerular basement membrane,[22] mesangial expansion, and glomerular sclerosis. These changes cause glomerular hypertension and progressive decline in glomerular filtration rate (GFR). First sign of development of diabetic nephropathy is microalbuminuria (urinary albumin < 200 μ/min). The disease is usually asymptomatic until nephrotic syndrome or renal failure develops.[23]

Phosphodiesterase type-5 Inhibitor (PDE5-i) was first marketed in 1998 (sildenafil) as a demanded treatment of male erectile dysfunction (ED).[24] Since 2003 tadalafil had been introduced as a selective phosphodiesterase enzyme (PDE5) inhibitor,[25] (this enzyme is responsible for the degradation of cyclic guanosine monophosphate cGMP) which in turn increases intracellularguanosine monophosphate levels.[26] Cyclic GMP is primarily responsiblefor controlling the size of blood vessels carrying blood. PDE5 is an enzyme found in the corpus cavernosum smooth muscle, vascular and visceral smooth muscle, skeletal muscle, platelets, kidneys, lungs, and cerebellum.[27] Endothelial dysfunction is a major defect that could contribute to vascular disease in diabetes and is mostly associated with insulin resistant.[28,29] PDE5-I shows an improvement in endothelial dysfunction after acute and chronic treatment.[30]

Type1- plasminogen activator inhibitor (PAI-1) is a 43 KDaserpin family member that inhibits tissue- and urokinase-type plasminogen activators (t-PA, u-PA). This protein appears to be an important regulator of plasminogen activator by t-PA and extracellular proteolysis by u-PA.PAI-1 is upregulated in human diabetic nephropathy.[31] Whereas, Prorenin, the precursor of renin, exists in circulating blood at concentrations that are≈5 to 10× higher than those of renin. For many years, prorenin was considered to be an inactive form of renin with no physiological role.[32] Later, in the mid-80s of the last century, the levels of circulating prorenin (but not renin) were found to be increase in diabetic subjects.[33] Subsequent studies revealed that such high levels correlated with prediction of microalbuminuria.[34] (Pro)renin receptors were recently detected in human kidneys, and their distribution included the mesangium and podocytes. Prorenin and the (pro)renin receptor could play a pivotal role in the pathophysiology of diabetic nephropathy.[35] Furthermore human nephrin is a protein necessary for the proper function of renal filtration barrier. Which is consist of fenestrated endothelial cells , the glomerular basement membrane, and the podocytes of epithelial cells. Because nephrin is a transmembrane protein involved with the slit diaphragm, in the Podocyte, is found to be dysregulated in diabetic nephropathy, though the extent of their expression loss that may be subjected to different regulatory factors. Quantifying the degree of loss may help identify the most useful protein to use as an early biomarker of diabetic nephropathy.[36]

Human interleukin-6 (IL-6) acts as a pleiotropic polypeptides regulating inflammatory and immune responses through actions on cells. It can provide important signals in the pathophysiology of a range of diseases, including diabetes mellitus. Achronic low-grade inflammation and activation of the innate immune system are closely involved in the pathogenesis of diabetes[37],[38] and its microvascular complications.[39] However inflammatory cytokine, IL-6,can be involved in the development and progression of diabetic nephropathy.[40] Additionally, IL-6 is also produced by adipocyte which might induce hepatic CRP synthesis ,and is thought to be a reason why obese individuals have higher endogenous level of CRP.[41] *This study was designed to evaluate the effect of daily treatment with 2.5mg tadalafil for 21 days on some inflammatory markers (interleukin - 6, plasminogen activator inhibitor-1) on nephrin levels, a newly introduced biomarker of diabetic nephropathy, in relation to serum prorenin levels in type 2 diabetic patient with and without microalbumiuria.*

II. Material and Methods

Twenty-three type 2 diabetic patients (16 male, 7 female) were selected under the supervision of a specialized physician from diabetics whom diagnosed to have diabetes according to American Diabetes Association Criteria[42], attending the Specialized Center of Diabetes and Endocrinology in AL-kindy Teaching Hospital, during the period from *January* till *June*/ 2012

with ages (mean+SEM) of 47.61±7.94 years. We excluded those patients whom suffering from cardiac problems such as : angina, heart failure, those with severe renal and hepatic disease. Patients were maintained on their current oral hypoglycemic drugs (Daonil indoses range of 5-15mg and Glucophage in dosesrange of 850-1500mg daily) during the study period. And sixteen ageand sex-matched participants to be consider as controls, were selected from our family, friends and staff member of the center (age 44.00±5.93years). The study was approved by *The Local Research Ethics Committee* and all subjects weregiven awritten informed consent to participate in this study. All of the participants in this study (diabetics & controls) received 2.5 mg daily dose of Tadalafil (Cialis®) continuously for 21 days. Subjects descriptive characteristics are illustrated in table-1.

Venous blood samples were collectedafter an overnight fasting at basal time, before starting tadalafil therapy, and after 3 weeks as post period time from both groups (diabetics & control), to estimate the effect of daily oral dose of tadalafil (2.5mg) on some biochemical markers (FSG : for assessment of glycemic control, serum prorenin[44] & nephrin[43] : for nephrotic assessment, and serum interleukin-6 (IL-6)[46] & plasminogen activator inhibitor-1 (PAI-1)[45] : for assessment of inflammatory state, in relation to diabetic nephropathy. HbA1c level was estimated to determine glycemic state, utilizing whole blood. Microalbuminuria was performed on fresh urine first morning specimens by a rapid Accu-check strips, purchased from MICRAL-TEST/Roche-Franceutilysing immunoassay of the gold-labeled antibodies to detect albumin in urine samples[47]. Statistics for data was obtained by applying SPSS 17.

III. Results

Data of diabetic patients with positive microalbuminuria, and those with negative microalbuminuria were compared to those subjects of the control group, at basal timeas summarized in table-2. Additionally, after 3 weeks of treatment with tadalafil results were analyzed, We found that thirteenof the patients (out of 23) remain with positive microalbuminuria compared to the pretreatment number of diabetics with positive microalbuminuria (16 out of 23), while from the sixteen controls; twelve were with negative microalbuminuria after tadalafil treatment compared to pretreatment number of subjects (11) out of the total controls number(16), indicating an increase in the percentage of negative micrroalbuminuria from 30.43% to 43.47% after treatment of diabetics with tadalafil, and from 68.75% to 75.0% for controls, as shown in *table- 2 & table-3,* respectively.

The association of microalbuminuria with each of the studied parameters (FSG, HbA1c, IL-6, PAI1, prorenin, nephrin) at basal time for both groups were non-significant ($p>0.05$). After treatment with tadalafil for 21 days. The same association remains non-significant ($p>0.05$) for (IL-6, PAI-1, prorenin, nephrin), except for FSG ($p=0.014$) in diabetics, as shown in table-2 and table-3, respectively. Comparing results obtained from diabetics to those of controls before starting treatment with tadalafil, exert non-significant variation from those of controls (nephrin, IL-6, PAI-1), except for those related to glycemia : fasting serum glucose & Hb A1C, as well as for microalbuminuria, in addition to serum prorenin elevation (figure-1). Whereas, values obtained after treatment with Tadalafil in both groups (controls & diabetics) indicate that daily treatment with Tadalafil for 21 days, could produce a preferable modifications in serum nephrinprorenin, PAI-1 and IL-6, as well as in microalbuminuria, as illustrated in *Figure -2.*

Table 1 : Statistical Correlations Between The Two Dichotomous Responding (Neg. & Pos.) Microalbuminuria In The (Diabetic & Control) Groups At Pre- And Post- Treatment With Tadalafil Periods At Some Descriptive Parameters.

Period	Some Related Parameters	Diabetic		C.S.	Control		C.S.
		C.C.	P-value		C.C.	P-value	
Pre-treatment	Age (Years)	0.332	0.240	NS	0.363	0.296	NS
	Gender (male/female)	0.226	0.266	NS	0.161	0.513	NS
	BMI(kg/m^2)	0.236	0.507	NS	0.382	0.434	NS
	Duration (Years)	0.411	0.197	NS	-	-	NS
Post-treatment	Age(Years)	0.181	0.676	NS	0.373	0.274	NS
	Gender(male/female)	0.195	0.340	NS	0.078	0.755	NS
	BMI(kg/m^2)	0.333	0.237	NS	0.422	0.325	NS
	Duration(Years)	0.392	0.244	NS	-	-	NS

NS:Non-significant ($p \geq 0.05$) ; C.C : Contingency Coefficient (Correlation ship), BMI =Body Mass Index.

Table 2 : Results Of Studied Parameters According To Response (-Ve&+Ve.) To Microalbuminuria Test In (Diabetic And Control) Groups Before Treatment With Tadalafil.

Parameters	Diabetic					Control			
	Microalbuminuria mg/L	No.	Mean	Std.Error Mean	P-Value)	No.	Mean	Std. Error Mean	P-Value
HbA1c %	Neg.	7	8.05	0.96	0.573	11	4.75	0.13	0.397
	Pos.	16	8.52	0.35		5	5.37	0.65	
FSG mmol/L	Neg.	7	11.08	3.30	0.992	11	4.61	0.22	0.236
	Pos.	16	11.11	0.76		5	5.91	0.93	
FSInsulin ng/ml	Neg.	7	16.73	4.34	0.135	11	7.77	1.16	0.272
	Pos.	16	9.17	0.90		5	11.50	4.25	
IL-6 pg/ml	Neg.	7	7.15	0.47	0.454	11	6.50	0.43	0.587
	Pos.	16	6.73	0.30		5	6.05	0.76	
PAI-1 ng/ml	Neg.	7	1.51	0.36	0.752	11	1.48	0.36	0.270
	Pos.	16	1.36	0.27		5	0.83	0.27	
Nephrinng/ml	Neg.	7	9.17	1.24	0.638	11	9.00	1.09	0.447
	Pos.	16	10.12	1.19		5	10.49	1.48	
Proreninng/ml	Neg.	7	167.5	32.9	0.125	11	91.74	18.12	0.704
	Pos.	16	115.14	16.34		5	78.97	28.45	

HbA1c=GlycatedHemoglobin ,FSG=Fasting Serum Glucose ,FSInsulin=Fasting Serum Insulin,IL-6 = Interleukin-6, PAI-1=Plasminogen Activator Inhihitor-1.

Table 3 : Results Of Studied Parameters According To Response (-ve&+ve.) to Microalbuminuria Test (Diabetic And Control) Groups After Treatment with Tadalafil.

Parameters	Diabetic					Control			
	Micro albumin-uria mg/L	No.	Mean	Std.Error Mean	P-Value)	No.	Mean	Std. Error Mean	P-Value)
FSG mmol/l	Neg.	10	8.39	0.75	0.014*	12	4.48	0.13	0.210
	Pos.	13	11.61	0.89		4	7.20	1.71	
FSInsulinng/ml	Neg.	10	17.05	3.51	0.600	12	12.25	1.71	0.761
	Pos.	13	14.76	2.62		4	13.73	6.88	
IL-6 pg/ml	Neg.	10	5.80	0.34	0.337	12	7.86	0.52	0.393
	Pos.	13	5.43	0.21		4	8.94	1.47	
PAI-1 ng/ml	Neg.	10	3.20	0.64	0.764	12	3.73	0.65	0.435
	Pos.	13	2.95	0.54		4	2.72	0.95	
Nephrinng/ml	Neg.	10	13.15	1.29	0.154	12	13.96	1.15	0.154
	Pos.	13	15.75	1.17		4	13.78	2.67	
Proreninng/ml	Neg.	10	83.50	15.92	0.745	12	46.66	10.51	0.745
	Pos.	13	75.87	16.19		4	48.39	24.19	

**: significant difference between +ve&-vemicroalbuminuria within each group (p<0.05),FSG=Fasting Serum Glucose ,FSInsulin=Fasting Serum Insulin,IL-6 = Interleukin-6, PAI-1=Plasminogen Activator Inhihitor-1.*

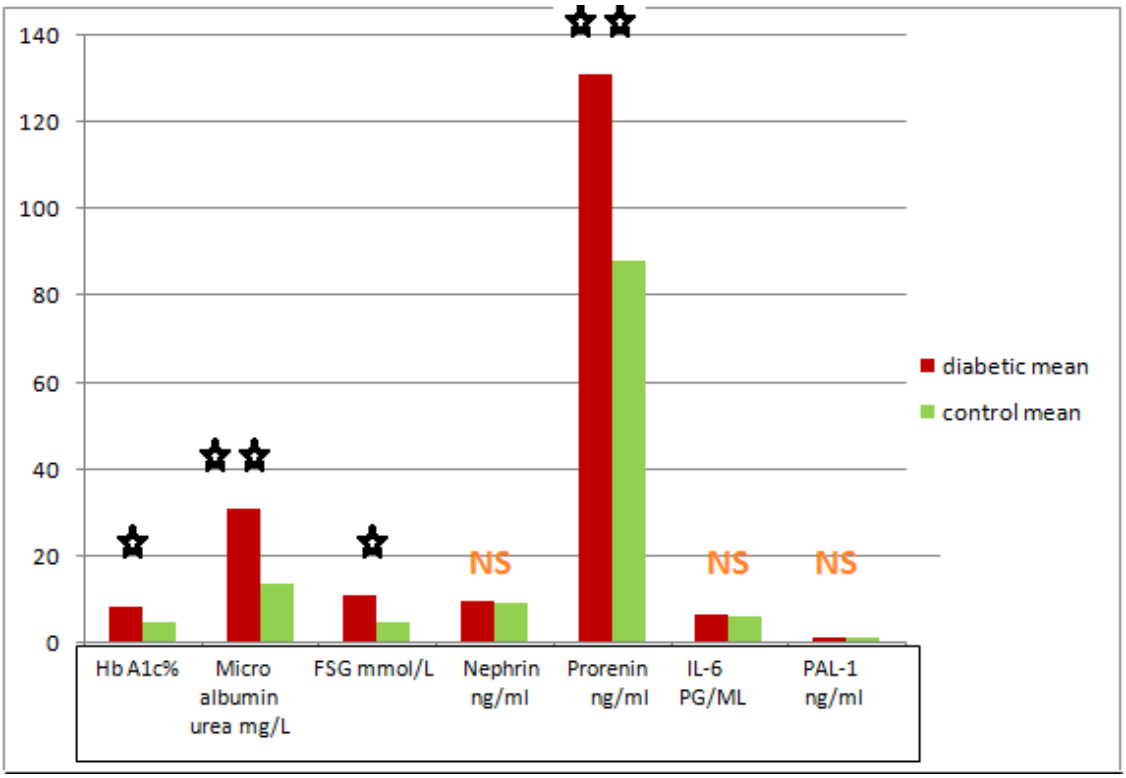

Figure 1 : Descriptive Statistics Of Studied Parameters For The Two Independent Groups (Diabetic And Control) Before Treatment With Tadalafil .

NS=Non significant difference from control , ★ = significantly different from control (p<0.05).

★★ significantly different from control (p<0.001),

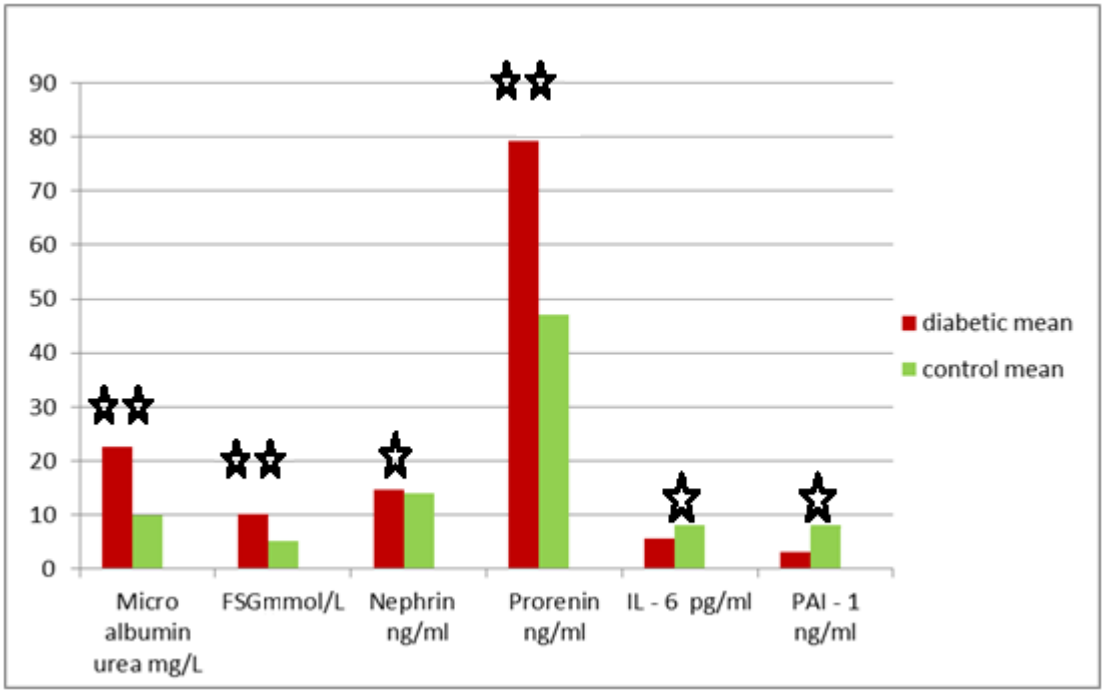

Figure 2 : Descriptive Statistics Of Studied Parameters For The Two Independent Groups (Diabetic And Control) After Treatment with Tadalafil

☆ = significantly different from control (P<0.05), ☆☆ = significantly different from control (p<0.001).

IV. Discussion

Data analysis by considering testing for microalbuminuria (negativeor positive) within each of diabeticsand control, in relation to age, gender, body mass index and duration of diabetes showed statistically non-significant differences among the studied groups at both the basal and post treatment period (p>0.05) as shown in *table-1.* The presence of microalbuminuria may be due to the fact that those patient were on irregularanti diabetic treatment leading to poor glycemic control.[48] Whereas, in non-diabetic subjects the % of microalbuminuria increases with increased age or due to exercise.[49]

There ishighly significant difference at FSG (p=0.05) and A1C (p=0.05) levels in diabetic group (shown in figure-1) at basal time. Since glucose combines with many proteins in circulation and in tissues via a nonenzymatic, irreversible process to form advanced glycation end products (AGEs). The best known of these is glycated hemoglobin, A1c (HbA1c) is a specific member of this group and is useful as an indicator of average glycemia during the 2-3 months before its measurement[50]. Other AGEs are presumed to contribute to the complications of diabetes, such as glycosylated proteins of the basement membrane of the renal glomerulus[51], which could be attributed for developing nephropathies of diabetes. Furthermore, after treatment with Tadalafil, significant differences for FSG (p=0.001) and microalbuminuria (p=0.001), remain to be detected between the two groups as seen in Figure-2, indicating no detectable effects on glycemic indices by tadalafil treatment for 21 days.

Whereas, a non- significant relation seen in the outcomes of coincidence testing between the two groups considering PAI-1 levels at the basal values (figure-1), although the normal human kidneys do not express PAI-1 receptors but PAI-1 receptors were over expressed in pathologic condition as that associated with diabetes nephropathy. Meanwhile, reactive oxygen species mediated PAI-1 up-regulation in renal cells cultured under high glucose, hypoxia, and TGF-beta 1.[52] However, a significantly lowered values of serum PAI-1 (p=0.05) after 21 days treatment with, asseen in Figure-2.

Although, IL-6 is one of the main cytokines involved in pathogenesis of diabetes was IL-6.[53] anon significant differences of IL-6 levels at the pre-treatment period as compared to controls (figure-1) may due to small size of tested group in this study. Cytokines can stimulate the cells that produce them, or adjacent cells, or even can intervene through direct cell–cell interaction; and, finally, cytokines may induce the expression of other cytokines and cytokine receptors.[54] IL-6 was significantly decreased in diabetic group after tadalafil treatment, which may be due to the effect of tadalafil and/or better commitment of patients with their treatment during period of study, or due to higher

urinary excretion as shown in Figure-2. While, no significancy that detected in control group values, after treatment with tadalafil, may be due to the absence of IL-6 expression which was already elevated in diabetic patients.[55,56] The involvement of PDE isozymes in regulating inflammatory cytokines has been reported, as reported by a previous study, tadalafil was the only PDE5-I to show a potentially anti-inflammatory effect. Chronic dosing of PDE-5 inhibitor (daily, low dose) safely used with maximum efficacy in improving endothelial dysfunction. PDE-5 inhibitor decrease expression of endothelial nitrous oxide synthase (NOS) impairing (NO) release or NO destruction precludes sufficient cyclic guanosine monophosphate (cGMP) formation and eliminate PDE-5inhibitor efficacy.[57]

As presented in figure-1, serum prorenin levels were significantly elevated in diabetics as compared to controls ($p<0.001$), whereas, serum nephrin levels didn't express such differences. But after treatment values expressed significantalterations by tadalafil treatment as compared to controls, (at $p<0.05$) fornephrinand, prorenin ($p=0.001$) as seen in Figure-2. Because, the tested treatment of diabetic nephropathy focus on orally available drugs acting via intracellular signaling pathways. Specifically, the cyclic nucleotide monophosphate cyclic GMP, represents an important mediator in the control of the outflow region (kidney).[58] Where, the expression of nephrin shows characteristic changes in diabetes, which inconsistent with this study (fiure-1). In vitro studies on human cultured podocytes demonstrated that glycated albumin and angiotensin- II reduced nephrin expression. Glycated albumin inhibited nephrin synthesis through the engagement of receptor for advanced glycation end products.[59] Whilst, proreninlevels revealed that these high levels correlated with the presence of microvascular complications, and it was proposed that prorenin might be used to predict the occurrence of microalbuminuria in diabetics.[60]

V. Conclusions

Because of the complex pathogenicity of diabetic nephropathy, new therapeutic interventions targeting primary mechanisms contributing to renal damage are critical for the future treatment of diabetic nephropathy. The use of phosphodiesterase (PDE) inhibitors, tadalafil, known to restrain the degradation of the second messenger cyclic GMP, could offers great opportunities in the treatment of kidney dysfunction. As presented this study by improving serum levels of IL-6, and PAI-1, indicating a possible anti-inflammatory effect by tadalafil therapy, that might be mediated through its vascular effects, also presented by improving serum levels of prorenin and the important indicator of nephropathy (nephrin). Generally, PDE inhibitors are regarded as efficacious therapy, have a rapid onset of action and favorable effect-to-side effect ratio.

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Effect of Membrane Cholesterol on Glucose Uptake in Diabetic Erythrocytes

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Abstract - The generally observed common phenomenon of decreased utilization of glucose by tissue cells in type 2 diabetes mellitus is attributed to either lack of insulin or due to non availability of functioning insulin. Some of the recent studies indicate the decreased glucose utilization may be due to variations in the membrane lipid composition, there by altering glucose transport across the membrane possibly by disorienting the membrane transport molecules. Such a membrane lipid alteration may be due to diabetes induced dyslipidemia.

In order to check this hypothesis, we studied the effect of media cholesterol concentration on the erythrocyte membrane cholesterol levels as well as the effect of such an altered membrane cholesterol level, if any on glucose uptake in diabetic erythrocytes. Erythrocytes derived from type 2 diabetic subjects were incubated in cholesterol rich albumin medium for a period of 2 hours and amount of cholesterol included on the erythrocyte membrane was estimated in washed incubated erythrocytes along with glucose uptake, lactic acid production and glycolytic index were studied.

Keywords : *Type 2 diabetes, membrane cholesterol, Glucose uptake.*

GJMR-B Classification : *NLMC Code: WK 825, WK 840, WK 870*

Strictly as per the compliance and regulations of :

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Abstract - The generally observed common phenomenon of decreased utilization of glucose by tissue cells in type 2 diabetes mellitus is attributed to either lack of insulin or due to non availability of functioning insulin. Some of the recent studies indicate the decreased glucose utilization may be due to variations in the membrane lipid composition, there by altering glucose transport across the membrane possibly by disorienting the membrane transport molecules. Such a membrane lipid alteration may be due to diabetes induced dyslipidemia.

In order to check this hypothesis, we studied the effect of media cholesterol concentration on the erythrocyte membrane cholesterol levels as well as the effect of such an altered membrane cholesterol level, if any on glucose uptake in diabetic erythrocytes. Erythrocytes derived from type 2 diabetic subjects were incubated in cholesterol rich albumin medium for a period of 2 hours and amount of cholesterol included on the erythrocyte membrane was estimated in washed incubated erythrocytes along with glucose uptake, lactic acid production and glycolytic index were studied. The results suggests that there is a significant increase in cholesterol inclusion (N=3.78 ± 0.38, T2DM= 4.13±0.09, $p<0.001$), a significant decrease in glucose uptake (N=2.12±0.96,T2DM=0.79±0.28, $p<0.001$), lactic acid production(N=0.24 ± 0.10, T2DM= 0.16±0.07 $p<0.001$), percentage of glucose uptake(N=18.97 ± 7.20, T2DM= 7.07±2.80, $p<0.001$),and glycolytic index(N=11.04 ± 1.04, T2DM= 4.24±2.05, $p<0.001$) in erythrocytes of type 2 diabetic subjects. Suggesting a positive effect of media cholesterol on erythrocyte membrane cholesterol in diabetic erythrocytes.

Keywords : Type 2 diabetes, membrane cholesterol, Glucose uptake.

I. Introduction

The most common biochemical alterations observed in type 2 diabetes mellitus is decreased utilization of glucose, which may be due to subnormal insulin amount or suboptimal function of insulin. The most relevant findings are hyperglycemia and glucosuria with changes in lipid as well as protein metabolism.

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Cholesterol is essential for maintenance of the structural and functional integrity of all biological membranes, including erythrocytes membrane and it plays a key role in maintenance of the bilayer matrix in an intermediate fluid state. (1). The decreased utilization of glucose by tissue cells as well as by the erythrocytes seen in diabetes mellitus may be due to decreased transport of glucose into the cells which is purely a function of erythrocyte membrane. Though the glucose transport is facilitated by glucose transporter (GLUT) presents in membrane, whose action may be influenced by insulin, the role of membrane lipids specifically phospholipids and cholesterol cannot be ignored.

The relative amounts of phospholipids and cholesterol are responsible for the fluid properties of the erythrocyte membrane (11) and for the shape as well as basic structural integrity of erythrocyte. An alteration in membrane lipid composition may bring about certain changes in glucose transport. The increased membrane cholesterol content, increased saturated fatty acid content was observed in diabetic erythrocyte membrane (8). The diabetes induced hyperglycation of membrane proteins including related GLUT particles may induce changes in distribution of membrane lipid components as well as may induce certain changes in membrane transport activity (2) possibly including glucose transport.

Present study was undertaken to establish the effect of incubation media cholesterol concentration on erythrocyte membrane cholesterol content as well as to establish the effect of such included cholesterol, if any, on the glucose transport in type 2 diabetic erythrocytes.

II. Materials and Methods

Diabetic type 2 subjects (male and female) in the age group of 30-60 years attending Medical OPD of Basaveshwara Medical College Hospital and Research Center, Chitradurga, were randomly selected.

The normal subjects (male and female) were randomly picked among house surgeons and employees of the college as well as Hospital, who were in the age group of 30-60 years.

Blood samples (6-7ml) from the selected normal subjects and type 2 diabetic subjects were collected, in the fasting state, with heparin as an anticoagulant after obtaining informed consent. Plasma was separated by centrifugation at 3500 rpm, for 10

minutes. Erythrocytes were washed three times with an aliquot of 5 ml normal saline and then were mixed with equal volume of normal saline so as to give 50% saturated erythrocyte suspension. This erythrocyte suspension was used in the present studies.

III. Cholesterol Inclusion Studies

Cholesterol- enriched- albumin solution was used as a cholesterol donor in the present study. (1 gram of fine powered cholesterol in 100 ml 1% albumin solution). Cholesterol content of this media was determined by triplicate estimation of cholesterol (7).

1ml of 50% saturated erythrocytes both normal/ diabetic were separately incubated with 0.6 ml of cholesterol rich albumin medium at 37°C in a temperature controlled water bath for 2 hours. After stipulated incubation period, the erythrocytes were washed with 3 times with 3ml aliquot of normal saline. One part of washed erythrocytes was mixed with 4 ml distilled water, the mixture stirred vigorously with a clean glass rod to lyse the erythrocytes. This was centrifuged at 3500 rpm for 5 minutes. Supernatant was discarded. The sedimented membranes were washed 3 times with 3 ml aliquot of normal saline. The resultant membranes were mixed with 9 parts of chloroform: methanol mixture (1:1 v/v) and homogenized for 8 minutes in a Potter-Elvejham tissue homogenizer. The extracts were used for estimation of membrane cholesterol (7).

The rest of the erythrocytes incubated with cholesterol-rich-albumin medium, were employed for glucose uptake studies.

IV. Studies on Glucose Uptake by Erythrocytes and Lactic Acid Production

To 0.5 ml of cholesterol-rich-albumin medium was incubated erythrocyte of both normal and diabetic subjects were separately mixed with 0.5 ml of normal saline, 1 ml of 0.1% freshly prepared aqueous glucose solution was added to both. An aliquot of 0.5 ml mixture was immediately pipette out into a tube marked N_o and D_o containing 4 ml of 10% TCA, the contents were mixed and centrifuged at 3500 rpm for 5 minutes and the supernatants were employed for estimation N_0 and D_0 minute glucose content (10) and lactic acid contents (3). The rest of the erythrocyte mixture was incubated in temperature controlled water bath at 37°C for 1 hour. At the end of the incubation time another aliquot of 0.5 ml mixture was pipette out into a tube marked N_{60} and D_{60} and proceeded as above. The supernatants were used for 60 minutes glucose and lactic acid estimation in normal and diabetic erythrocytes.

The data obtained was statistically evaluated using students't 'test.

V. Results

In the present study, a total number of 192 subjects were employed, which include 52 normal subjects and 140 diabetic subjects. The normal subjects were consisted of 44 male subjects and 08 female subjects. Further diabetic group consisted of 85 male diabetic subjects and 55 female diabetic subjects. The results of the present study are narrated in table 1and 2. Table 1 gives, glucose uptake, percentage of glucose uptake, lactic acid production, as well as glycolytic index in erythrocytes of normal subjects and in erythrocytes of diabetic subjects. As seen from the table there is a significant decrease observed in glucose uptake ($p<0.001$), percentage of glucose uptake ($p<0.001$), lactic acid production ($p<0.001$), as well as glycolytic index ($p<0.001$) in erythrocytes of diabetic subjects as compared to normal subjects, indicating there is a decrease in glucose uptake and utilization in diabetic erythrocytes.

Table 2 depicts erythrocyte membrane cholesterol prior to the incubation and post incubation with cholesterol rich albumin medium, as well as glucose uptake by these erythrocytes. It is evident from the table that there is a significant elevation in cholesterol inclusion on both normal as well as diabetic erythrocytes which are exposed to cholesterol rich medium, as compared to non- exposed erythrocytes ($p<0.001$). Further it is evident from the table that, the glucose uptake is decreased ($p<0.001$) in cholesterol rich albumin medium exposed erythrocytes (normal/diabetic) as compared to non-exposed counter parts. This decrease in glucose uptake may probably due to extra cholesterol included onto the membrane.

VI. Discussion

The membrane surrounding the erythrocyte serving as a barrier, the membrane contains pumps and channels for the movements of sodium, potassium and calcium and it facilitates the transport of glucose and other small molecules. It is also responsible for the basic structural integrity of the erythrocytes. A decreased utilization of glucose by the tissue cells in type 2 diabetes mellitus is attributed to either lack of insulin or due to non-availability of functioning insulin (13).

Increased cholesterol and phospholipid contents in erythrocyte have been correlated with decrease in erythrocyte membrane fluidity in diabetes mellitus and these parameters identified as contributing factors for decrease in membrane fluidity (5). This erythrocytes membrane lipid alteration may be due to diabetes induced dyslipidemia. An increase in cholesterol may induce rigidity into the membrane, whereas increase phospholipid induces more flexibility. In addition probably the glycation of membrane proteins including related GLUT particles may induce changes in distribution of membrane lipid components as well as

may induce certain changes in membrane transport activity (4).

In the present study, a significant raise in the inclusion of cholesterol ($p<0.001$), onto the erythrocyte membrane as been observed in erythrocytes which are incubated with cholesterol rich albumin medium compared to non-exposed erythrocytes. This is in agreement with the reports of Christopher (6) and Steven (12). When the erythrocyte which are incubated with cholesterol rich albumin medium were used for glucose uptake studies, it was found that there is a significant decrease in glucose uptake ($p<0.001$), percentage of glucose uptake ($p<0.001$), lactic acid production ($p<0.001$), as well as glycolytic index($p<0.001$), in erythrocyte of type 2 diabetic subjects. This suggests that an increase in cholesterol content of erythrocyte membrane may result in decreased glucose uptake, which may partly due to an alteration in membrane lipid composition, leading to altered membrane proteins orientation, possibly GLUT particles, which may cause a decrease in glucose uptake in these erythrocytes.

In conclusion it can stated that, when erythrocytes (normal/diabetic) exposed to cholesterol rich albumin medium, an extra cholesterol migrate onto the membrane (9), resulting in increase of membrane cholesterol level. Further such an increase in membrane cholesterol level decrease significantly glucose uptake by these erythrocytes.

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Table 1 : Showing glucose uptake, percentage of glucose uptake, lactic acid production and glycolytic Index in normal erythrocytes as well as in diabetic erythrocytes.

Groups → / Parameter ↓	Erythrocyte of normal subjects (n = 36)	Erythrocyte of diabetic subjects (n = 90)
Glucose uptake by erythrocyte mg/cc	2.12 ± 0.96	0.79*** ± 0.28
Percentage of glucose Uptake	18.97 ± 7.20	7.07*** ± 2.80
Lactic acid production mg/cc	0.24 ± 0.10	0.16*** ± 0.07
Glycolytic index	11.04 ± 1.04	4.24*** ± 2.05

Note : 1.The number in parenthesis shows the number of samples.
2. Values are expressed as their Mean ± SD.
3. p- value * $p<0.05$, ** $p<0.01$, *** $p<0.001$.
4. Glycolytic index = $\frac{\text{Glucose uptake mg/cc erythrocytes}}{\text{Lactic acid production mg/cc erythrocytes}}$

Table 2 : Showing erythrocyte membrane cholesterol and glucose uptake in normal erythrocytes as well as in diabetic erythrocytes both prior and post incubation in cholesterol rich albumin media.

Groups → / Parameter ↓	Erythrocyte of normal subjects (n = 16)	Erythrocyte of diabetic subjects (n = 50)
Erythrocyte membrane cholesterol prior to the incubation mg/cc	1.25 ± 0.31	1.52*** ± 0.13
Erythrocyte membrane cholesterol after incubation mg/cc	3.78 ± 0.38	4.13*** ± 0.09
Glucose uptake by erythrocytes prior to the incubation mg/cc	2.12 ± 0.38	0.79*** ± 0.26
Glucose uptake by erythrocytes after incubation mg/cc	1.66 ± 0.47	0.38*** ± 0.15

Note: 1.The number in parenthesis shows the number of samples.
2. Values are expressed as their Mean ± SD.
3. p- value * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

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A Proof of Principle Study of a Novel Silicon based Retina Sensor for Patients with Macula Degeneration

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Abstract - Recent advances in semiconductor technology have made it possible to achieve imaging devices that can serve as bionic retinas when implanted within the human eye. Traditional concepts for bionic retina prosthesis involve implantation of a CMOS CCD array in place of the dysfunctional retina of the patient [Ref. 2]. This concept suffers from a limitation of a finite battery life, which leads to frequent replacement of batteries. Secondly, the need to bias each pixel makes the number of electrodes large enough to occupy a large portion of active area on the chip. Moreover, more number of electrodes means greater data bandwidth required for restoring vision. It is proposed to use passive devices like solid state photo-voltaic Cells, which instead of consuming external power would in fact generate signals to stimulate the nerve fibers of the optic nerve. The need for digital data processing can be circumvented as the visual information (photo-generated analog signal) is directly coupled to the ganglion fibers of the macula region. The use of silicon as sensor material makes the device sensitive to infrared wavelengths making it possible for the recipient to have good visibility even at night.

Keywords : *Technology Computer Aided Design, Silicon & solid state photo-voltaic Cells.*

GJMR-B Classification : *NLMC Code: WW 270*

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A Proof of Principle Study of a Novel Silicon based Retina Sensor for Patients with Macula Degeneration

Pourus Mehta

Abstract - Recent advances in semiconductor technology have made it possible to achieve imaging devices that can serve as bionic retinas when implanted within the human eye. Traditional concepts for bionic retina prosthesis involve implantation of a CMOS CCD array in place of the dysfunctional retina of the patient [Ref. 2]. This concept suffers from a limitation of a finite battery life, which leads to frequent replacement of batteries. Secondly, the need to bias each pixel makes the number of electrodes large enough to occupy a large portion of active area on the chip. Moreover, more number of electrodes means greater data bandwidth required for restoring vision. It is proposed to use passive devices like solid state photo-voltaic Cells, which instead of consuming external power would in fact generate signals to stimulate the nerve fibers of the optic nerve. The need for digital data processing can be circumvented as the visual information (photo-generated analog signal) is directly coupled to the ganglion fibers of the macula region. The use of silicon as sensor material makes the device sensitive to infrared wavelengths making it possible for the recipient to have good visibility even at night.

This paper presents a detailed illustrated summary on the design aspects of the sensor, which is essentially an array of p-n junctions. It also presents a detailed overview on the device physics aspects of the proposed Solid-State (Silicon) retinal sensor. The design of this sensor was evaluated analytically through extensive physics based device simulations using a commercial Technology computer aided design (TCAD) tool.

The mask layout consisting many variants of this sensor has been designed for fabrication in BiCMOS technology. The device physics and biological compatibility aspects of the individual pixel of the sensory array have been addressed with possible solutions to be implemented in future.

Keywords : Technology Computer Aided Design, Silicon & solid state photo-voltaic Cells.

PACS: 85.60.-q , 42.66.-p

I. Introduction

Restoration of sight in the human eye is the subject of cutting-edge research worldwide. The most prevalent concept of restoration of sight for patients with corneal damage is a corneal replacement. But corneal damage is not the only malaise, which inhibits vision in patients throughout the world. Retinal dysfunction or retinopathy accounts for vision loss a significant percentage of patients. The only artificial method to help patients with retinal damage is by way of implanting a sensor in place of the retina and hard wiring the sensor to various points in the cerebral cortex. The problem with implants of electrical nature lays with the life of the power source in this case a battery. This makes it highly impractical to use when considering the implant would be sitting in the eye. The extremely delicate nature of tissue in the human eye makes the job of realizing a viable semiconductor device a very big challenge. To avoid these problems, it is proposed to use a pixilated solid state (silicon) photo-voltaic cell array [Fig. 2] having a pixel size of the order of 100 µm x 100 µm (minimum resolution of the human eye). The concept is such that the image will be focused upon the sensor array and the subsequently the individual pixels would be activated and a potential difference would be generated at the electrodes of these individual pixels and which will in turn be coupled to the ganglion fibers in the Macula Fovea region of the eye cavity. Thus establishing an electrical communication between the optic nerve and the artificial retina.

II. Sensor Design

Starting with a p-type, low resistivity (100 Ohm-cm), <111>, 300 µm thick Silicon wafer, a phosphorus implant on the front side formed the n+ field shaping electrodes (anodes/strips) whose widths in this design were 40 µm with a 30 µm interstrip gap between adjacent n+ strips (Pitch = Strip Width + Gap = 70µm) [Refer Fig. 1 (a)]. The peak phosphorus concentration in n+ anode region was approximated around 1 x 10^{20} cm^{-3} with a Gaussian distribution along depth. Next, a boron implant on the backside formed the p+ cathode region (substrate contact) having a 40µm width. The next step was to open contact windows for both n+ and p+ regions on front and backsides respectively. The last step being metal deposition and patterning for creation of electrode regions over n+ and p+ regions of the p-n junction. The complete two dimensional layout of the sensor consists of 900 pixels with a total active area of (2mm x 2mm).

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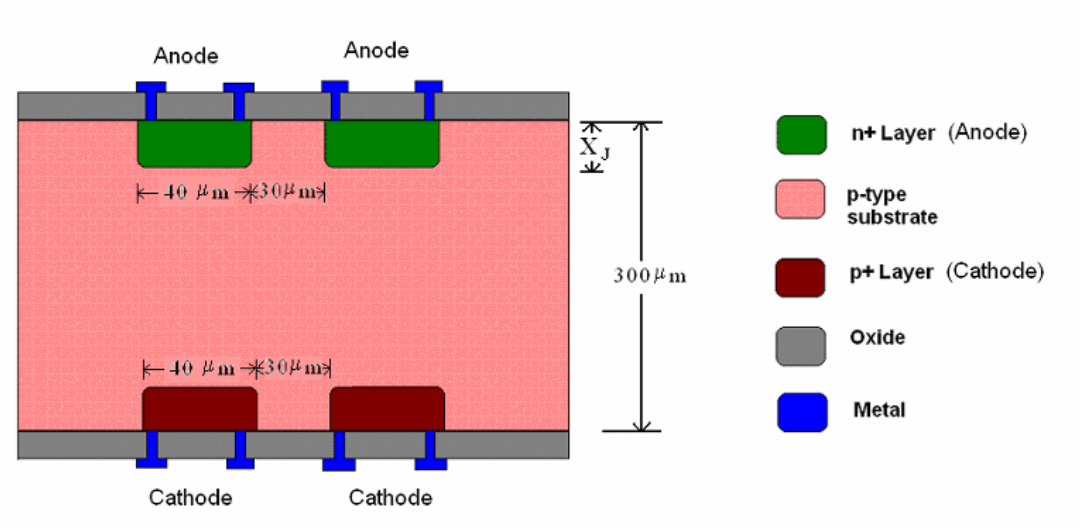

Figure 1 (a) : 2-Dimensional cross section of individual pixels of the photo-voltaic device.

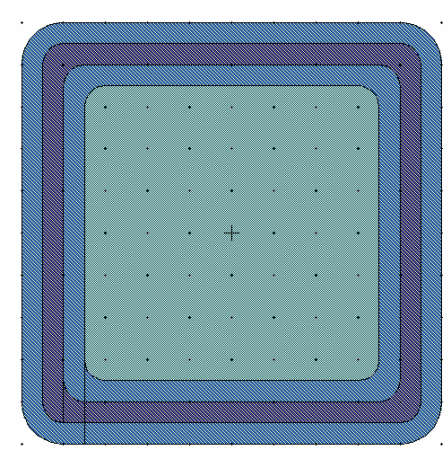

Figure 1 (b) : Composite layout of a single pixel of the retinal sensor.

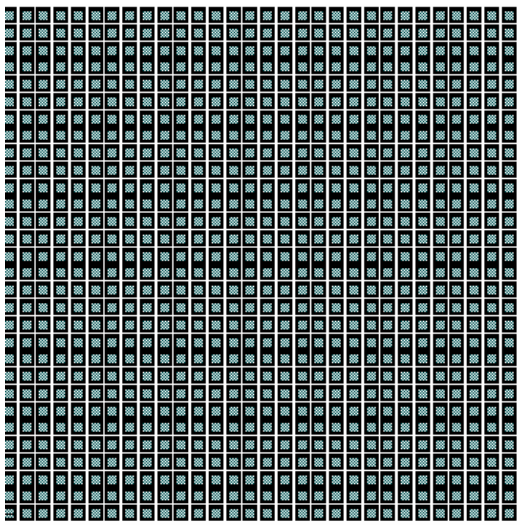

Figure 2 : Layout of the silicon photovoltaic array consisting of 900 pixels.

III. Physics Based Device Simulations

a) Objectives and Methodology

The hypothetical 2-dimensional cross-section was then exposed to an incident optical photon flux and wavelength was varied from 400 nm to 1.5 microns. The optical beam intensity was kept at a level equivalent to the incident normal photon intensity (10 micro-Watts) on a human eye for co-relation. Moreover, the simulation was done only for normal incidence of photons on the sensor. The simulation was performed to derive the relation between the terminal anode current (amperes / micron) with the optical photon wavelength [Fig. 3]. Additionally, the Quantum efficiency (extrinsic & Intrinsic) was also extracted for the simulated 2-D cross-section of the device.

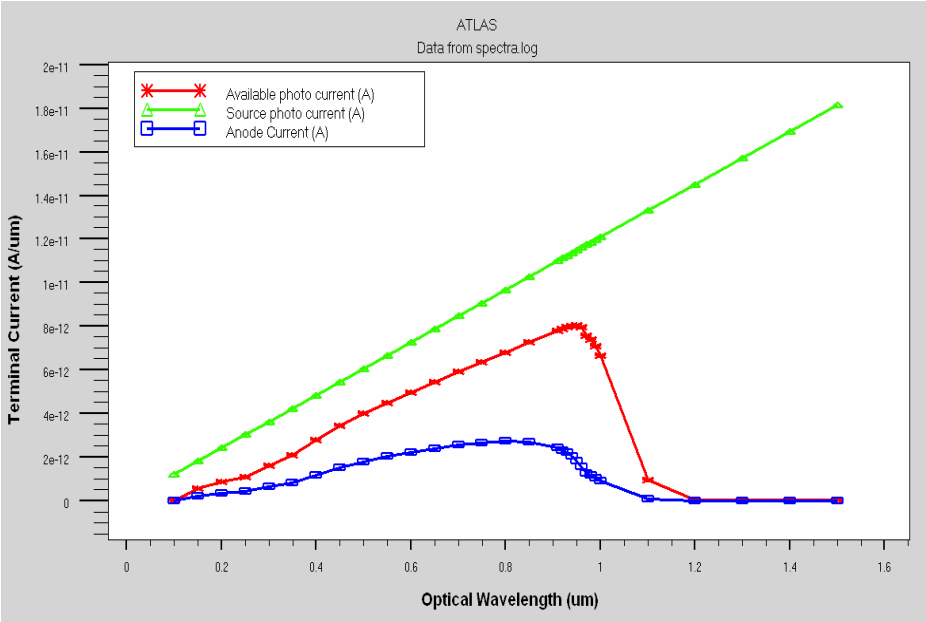

Figure 3 : Anode current versus Optical Photon wavelength.

b) Results and Discussions

As seen from the plot of Terminal current versus optical wavelength, the terminal current shows maxima at an optical wavelength of 900 nm. This is typical of property of the substrate material used, in this case silicon. The source photo-current which is a linear function of the wavelength increases with increase in wavelength. The available photo-current is a measure of the amount of current that would result if all the incident photons were converted to photo generated carriers in the device. The optical photonic radiation was made incident at the closest approach to the top surface of the device. Inspite of that, a significant portion photon flux was lost due to reflection from the specular silicon surface and the metal contacts. Hence this shows in the vast difference in maximum values of available photo current and the terminal current appearing at the device. The plot of quantum efficiency versus wavelength also shows a maximum at 900 nm thereby verifying the earlier results [Fig. 4]. The difference in between the extrinsic and intrinsic quantum efficiency also shows evidence of certain amount of reflection losses. These losses can be substantially minimized using Anti-Reflection Coating over the front surface of the sensor. Alternatively, the simulation for the effect of a variation in optical beam intensity on the terminal voltage (Open circuit voltage) shows a linear increase in voltage with an increase in beam intensity [Fig. 5]. This is analogous to a similar effect of incident light on the human eye.

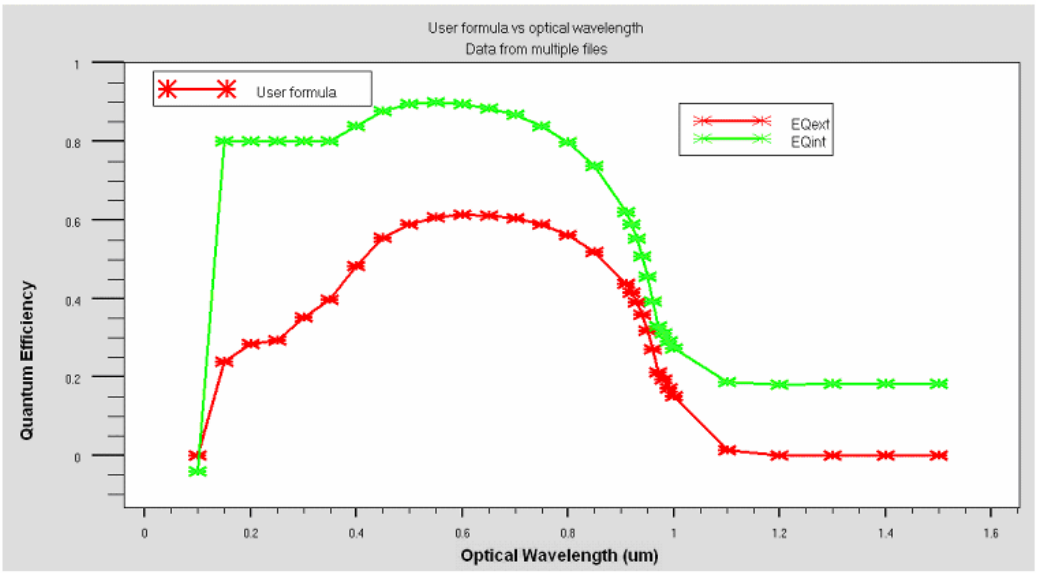

Figure 4 : Quantum Efficiency (External & Internal) versus Optical Photon wavelength.

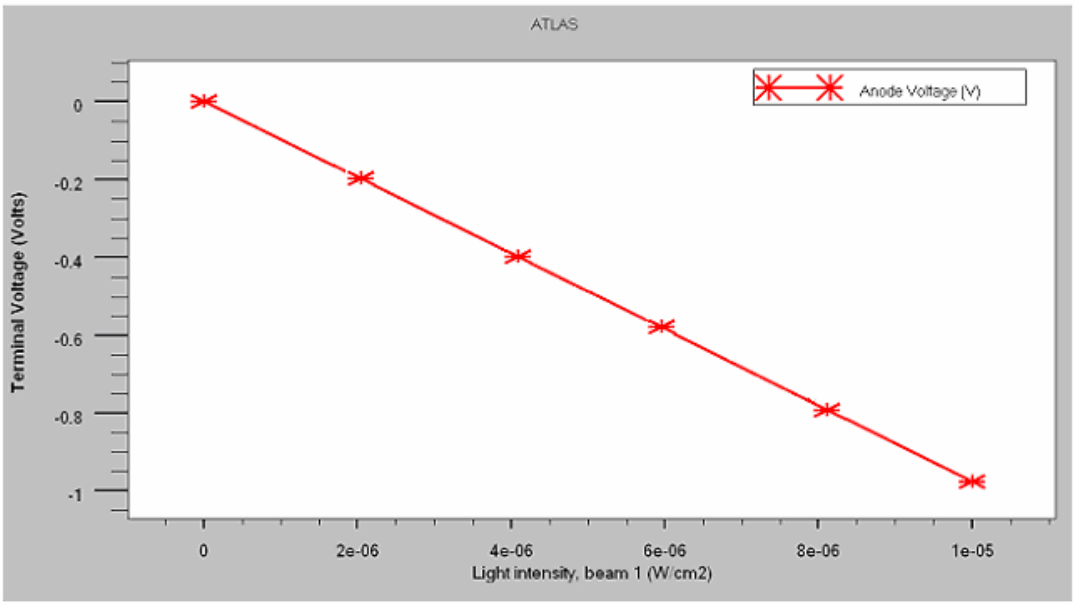

Figure 5 : Anode Voltage versus Light Beam Intensity (Watt/cm^2).

IV. Bio-Compatibility Issues

A bio-compatible conducting glue will be used to attach the sensor over the damaged retina in the Macula Fovea region (Fig. 6). The glue layer will be patterned by photo lithography such that the glue only remains in the region over the electrode surface and nowhere else. Before attachment of the sensor certain bio-compatibility issues need to be addressed. Firstly, the problem of damage to the inner walls of the eye caused by sharp edges of the scribed sensor die needs utmost attention. This problem can be subverted by introduction of a bio-compatible polymer over the side-wall regions along the thickness region of the sensor die. Secondly, the problem of dead volume occurring due to the curved nature of inner wall of the eye and planar nature of the silicon die. This problem may lead to improper electrical contact between the ganglion fibers in the macula and the sensor. This problem can only be subverted by designing solar cell based sensors over polymer substrates employing an organic electronics regime.

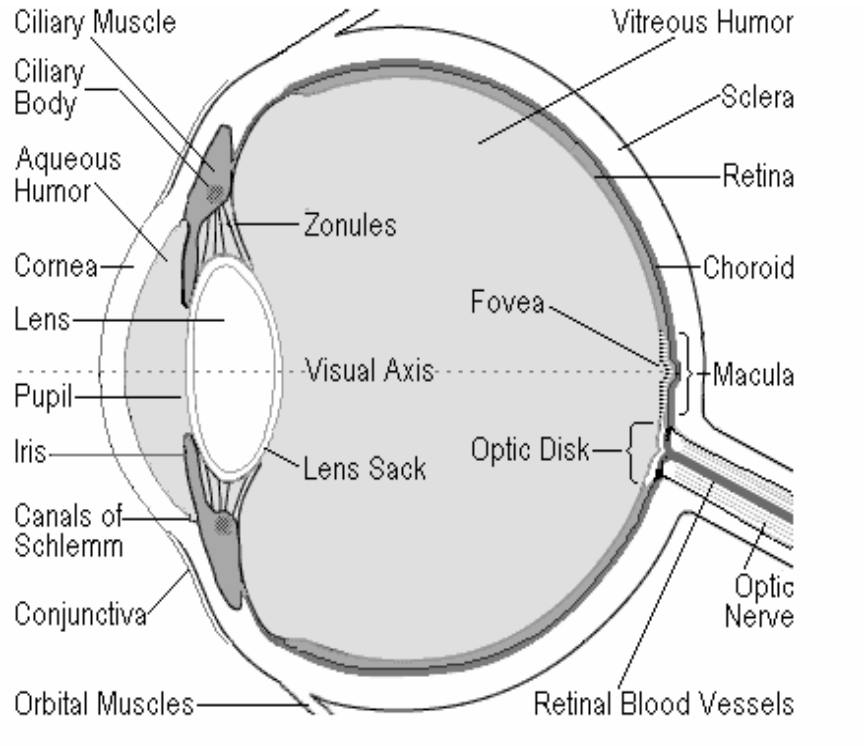

Figure 6 : Lateral cross-section of the human eye.

V. Conclusions

The retinal sensor array has been designed for fabrication in BiCMOS technology. A thorough device physics based analytical study has been carried out to extract terminal electrical parameters of the individual pixel in the array. Biological compatibility issues relating to the implantation of the silicon sensor in the human have also been addressed. The forthcoming stages are the actual fabrication and electrical (dc & optical) characterization of the sensor array.

VI. Acknowledgements

The author expresses a deep sense of gratitude for Late Dr. S. K. Kataria for his guidance and leadership. The author would like to especially thank Mr. Shekhar Basu and Dr. Sinha for their support, in addition to Mr. G. P. Srivastava, Mr. C. K. Pithawa & Mr. V.B. Chandratre. The author also profoundly expresses his sincere gratitude for the unknown blind man who evoked a sense of inspiration in him to pursue this project.

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Attitudes of Patients at Disclosure of Their HIV Sero-Positive Status During Post-Test Counselling in a Tiartiary Institution in Northeastern Nigeria

By Dr. Ballah Akawu Denue, Dr. Stella Jacks, Dr. Suleiman Bello, Mrs. Cecilia Akawu, Mal Anas Yusuf Hussaini, & Dr. Oladimeji Adebayo

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Abstract - Aim : To document the attitudes of patients at disclosure of their HIV sero-positive status during post-test counselling in a Tertiary institution in North eastern Nigeria. Methods : This cross-sectional descriptive study was carried out among clients that presented for VCT at the HIV clinic at the University of Teaching Hospital. Questionnaire administration took place at the clinic; those that consented to participate in the study were subjected to elaborate pre-test counselling. Blood sample was obtained from each participant. The result of each participant was revealed to them confidentially at post-test counselling in the presence of a trained counsellor and social worker. The attitudes, behaviours and concerns of the patients at post test counselling were observed and documented.

GJMR-J Classification : NLMC Code: QW 168.5.H6, WC 503.7, WD 308

Strictly as per the compliance and regulations of :

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Dr. Ballah Akawu Denue [α], Dr. Stella Jacks [ρ], Dr. Suleiman Bello [ω], Mrs. Cecilia Akawu[¥], Mal Anas Yusuf Hussaini [§] & Dr. Oladimeji Adebayo [χ]

Abstract – Aim : To document the attitudes of patients at disclosure of their HIV sero-positive status during post-test counselling in a Tertiary institution in North eastern Nigeria.

Methods : This cross-sectional descriptive study was carried out among clients that presented for VCT at the HIV clinic at the University of Teaching Hospital. Questionnaire administration took place at the clinic; those that consented to participate in the study were subjected to elaborate pre-test counselling. Blood sample was obtained from each participant. The result of each participant was revealed to them confidentially at post-test counselling in the presence of a trained counsellor and social worker. The attitudes, behaviours and concerns of the patients at post test counselling were observed and documented.

Results : A total of 130 participants were consecutively recruited into this study, with male to female ratio of 1:1.9. The mean age of the participants was 35.72±9.43, the mean age of males was significantly higher than females (40-40±10.58 vs 33.34±7.80; p<0.05). Majority of the participants (69.2%) were married and 43.9% had no formal education. It was observed that 51.5% of the cohort were non-challant at disclosure of their HIV sero-positive status during post- test counselling, 34.6% were worried, 10.0% were sad and 3.9% denied the outcome of their results. Significantly more males (47.7%) were non-challant than females (27.9%), more females were sad at disclosure of their HIV sero-positive status but fails to reach a significant level (0.095).

Conclusion : This study highlights the need to anticipate actual outcomes of disclosure and concerns of newly diagnosed HIV patients. It is evident from this report that disclosure of HIV status is associated with varying reactions and psychological attitude that need to be addressed for successful patient management and HIV/AIDS preventive interventions.

I. Introduction

The burden of HIV/AIDS on the health sector and the health professionals that work in it is enormous[1]. Researches conducted in developed and developing countries has shown that Voluntary counselling and testing (VCT) can reduce high risk sexual practices and can decrease rates of sexually transmitted infections[1-4]. In addition, VCT is necessary for directing HIV infected people to antiretroviral therapy, with is becoming increasingly available in Nigeria[5,6]. Voluntary counselling and testing for HIV entails confidential counselling with patients/clients that help them make informed decisions related to HIV testing and risk reduction, it consist of two sessions; one prior to taking the test known as pre-test counselling and one following the HIV test results are given known as post test and follow up counselling. The need to expand HIV voluntary counselling and testing as an integral part of preventive strategies has been advocated[7,8]. Expansion of HIV screening services is a cost effective way of increasing life expectancy and decrease disease transmission especially in sub Saharan Africa [4,9]. Expert attributes delay in seeking medical attention and continued risky behaviour partly due to lack of knowledge of HIV status. Awareness and acceptance of HIV sero-positive status by patients is an important step in both its management and prevention. Effective care and preventive strategy could help overcome the devastating outcome of this disease. Through appropriate response and support to those affected by HIV/AIDS, everyone gains; the family remain intact with steady income or at least no economic loss, the state gains as production by its productive youths targeted by HIV scourge is not disrupted in key sectors[1,3,7-10]. Against this background, this cross sectional study was undertaken to document the attitude of newly diagnosed HIV sero-positive patients at disclosure of their status. Understanding of the attitude of patients will help the caregiver to appropriately respond to the challenges that may hinder effective management during follow up counselling.

II. Materials and Methods

This cross-sectional descriptive study was carried out among clients that presented for VCT at the HIV clinic at the university of Maiduguri Teaching Hospital after obtaining an ethical clearance from the

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Institutions research and ethics committee. Eligibility criteria were; 18 years and above and willingness to participate in the study. Subjects were recruited into the study after given oral/written consent at presentation at the HIV clinic.

Questionnaire administration took place at the clinic, it was administered in English and local languages(Hausa, Kanuri, Bura and Marghi) and translated into English language by trained interviewers/counsellors. The questionnaire covered a range of social issues including marital status, educational background, sexuality and sexual relationships, educational background, social network, past history suggestive of sexually transmitted infections or HIV /AIDS, intravenous drug use and blood transfusion. After the administration of the questionnaire, those that indicated interest to participate in the study were subjected to elaborate pre-test counselling.

Blood samples from the participants that fulfilled the inclusion criteria were collected by venipuncture after scrubbing the area with sterile cotton soaked in methylated spirit from the antecubital vein into ten millilitres of ethylene di tetra acetic acid (EDTA) tubes bottles. Participants were asked to present after 48 hours for the outcome of their result. The result of each participant was revealed to them confidentially at post-test counselling in the presence of a trained counsellor and social worker. Married couples were encouraged to disclose their status to their spouses. The attitudes, behaviours and concerns of the patients at post test counselling were observed and documented. Patients that had concerns were recommended follow up counselling.

Data analysis was carried out using Statistical package for social sciences (SPSS) for window, Chi square test was used to test level of significance. Statistical significance was considered present when the p-value was <0.05.

III. Results

Distribution of the participants by age group and marital status

A total of 130 participants were consecutively recruited into this study, with 86 (66.2%) females and 44 males (33.8%) and male to female ratio of 1:1.9. The mean age of the participants was 35.72 ± 9.43, the mean age of males was significantly higher than females (40-40 ± 10.58 vs 33.34 ± 7.80; $p<0.05$). However the distribution of the participants within the defined age group based on gender was similar ($p>0.05$). Majority of the participants (69.2%) were married followed by widows/widowers (20.0%), with female preponderances in comparison to their male counterpart ($p<0.05$) as shown in Table 1.

Stratification of participants by risky behaviour, reason for VCT and source of support

Of the 130 participants evaluated, 40(30.8%) engaged in extra marital marital or in multiple heterosexual relationship, this trend was similar in both gender. A total of 25 (19.2%) had past History suggestive of sexually transmitted infections(STIs), with similar propenderance in both males and females ($p>0.05$). Majority of the participants presented at the clinic with symptoms suggestive of HIV infection, VCT was suggested to them as an essential part of their management, 19 (14.6%) presented at the clinic as a result of a sick spause/ died of HIV/AIDS complications, with significantly higher females than males ($p<0.05$). Significantly more females received support and encouragement from their families and friends when asked at presentation. Males supported themselves more than females, though this fail to reach a significant level ($p=0.078$), at disclosure all participants agreed to access the available Government HIV Program services as depicted in Table 2.

Categorization of the participants based on educational status

As shown in figure 1, one out of two study participants had no formal education; this was followed by secondary education that had prevalence of 21.5%, then tertiary and primary education with 21.5% and 14.6% respectively.

Classification based on their attitude at disclosure of their sero-positive HIV status

It was observed that 51.5% of the cohort was non-challant at disclosure of their HIV sero-positive status during pos test counselling, 34.6% were worried, 10.0% were sad and 3.9% denied the outcome of their results as shown in figure 2. Significantly more males (47.7%) were non-challant than females (27.9%), while more females were sad at disclosure of their HIV sero-positive status but fails to reach a significant level (0.095) as shown in Table 3.

Table 1 : Distribution of the participants by age group and marital status.

Age (mean±SD,95%CI		males	females	p-value
35.72±9.43(34.0-37.36)		40.40±10.58(37.19-43.63)	33.34±7.80(31.65-35.0)	0.000
Age Group,	no (%)			
18-25	13	01(2.30)	12(14.0)	0.073
26-35	65	18(40.9)	47(54.7)	0.193
36-45	33	14(31.8)	19(22.1)	0.322
46-55	14	08(18.2)	06(8.80)	0.202
56-65	05	04(9.10)	01(1.1)	0.070
66-75	01	01	00	0
Marital status,	no (%)			
Married	90	40(86.4)	48(55.8)	0.001
Single	12	02(4.60)	10(11.6)	0.325
Widowed	26	03(6.80)	23(26.7)	0.014
Divorced	06	01(2.30)	05(5.80)	0.647

Table 2 : Stratification by risky behaviour, reason for VCT and source of support

	Overall	Males (44)	Females (86)	p-value
Risky HIV behaviour				
Multiple sex partners	40	13 (29.6)	27 (31.4)	0.992
Hx of STDS	25	10 (22.7)	15 29.0)	0.569
IV drug use	0	00	00	-
Blood transfusion	0	00	00	-
Reason for VCT				
Symptomatic of HIV	99	30(90.9)	69(80.2)	0.188
Spouse sick/died of HIV	19	02(4.60)	17(19.8)	0.040
Child died of HIV	03	00	07	
Premarital counselling HCT	09	02(4.60)	03(3.60)	0.999
Source of support				
Self	52	31(59.6)	21(40.4)	0.078
Family/friends	66	20(30.3)	46(69.7)	0.000
Willingness to access support from Government HIV program	130	44(33.9)	86(66.1)	0.000
none	0	0	0	-

Table 3 : Attitude of participants at disclosure of their HIV status.

	Males (44)	Females (86)	p-value
Non challant, no(%)	21(47.7)	24(27.9)	0.040
Worried, no(%)	17(38.6)	48(55.8)	0.095
Sad, no(%)	02(04.6)	12(14.0)	0.182
Denial, no(%)	04(09.1)	02(02.3)	0.193

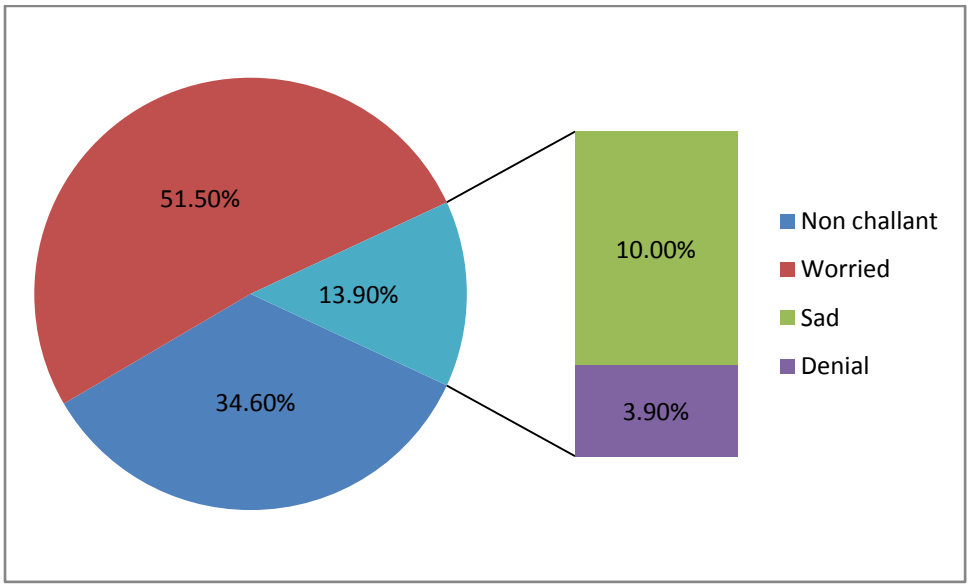

Figure 1 : Attitude of participants at disclosure of their HIV status.

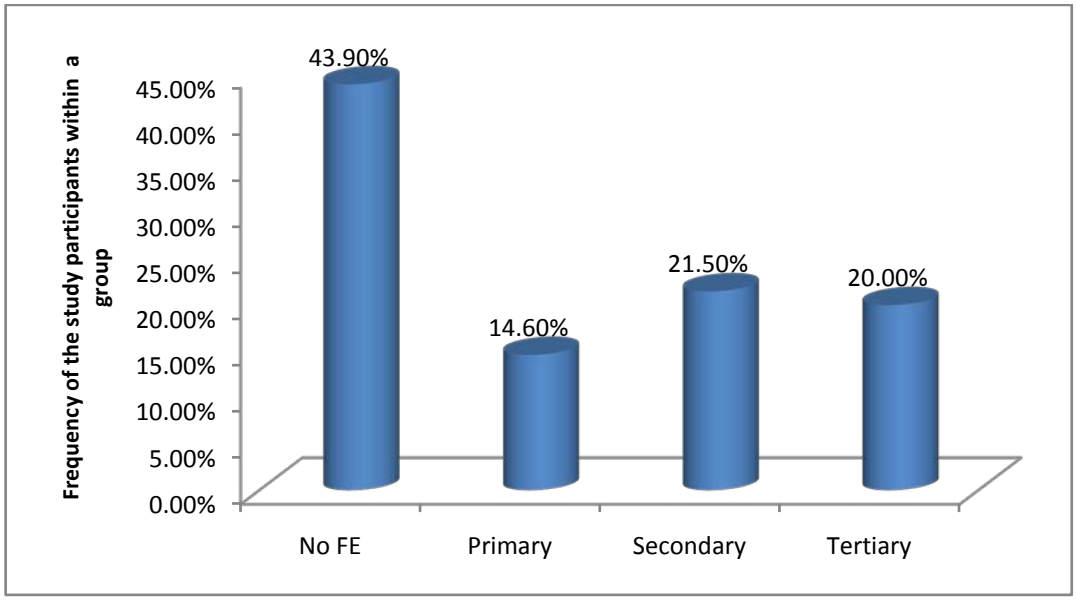

Figure 2 : Classification of participants based on their educational attainment.

IV. Discussion

The prevention and control of human immunodeficiency virus (HIV) infection depends on the success of strategies to prevent new infections and to treat currently infected individuals. Voluntary HIV testing and counselling is an essential step in achieving this goals. It provide the needed information and support to individuals at risk for contracting HIV, enabling uninfected individuals to remain uninfected and those infected to plan for future and prevent HIV transmission to others [11,12]. Knowledge of HIV status may be critical to decision making around both health and sexual risk behaviours, as it will enable HIV-infected individuals to access timely and appropriate treatment, care and support programmes.

However, despite awareness campaign on the need for Voluntary HIV testing and counselling by National agency for the control of AIDS (NACA) and related implementing partners in Nigeria, majority of our cohort 99 (76.2%) were either referred or presented with features suggestive of HIV/AIDS, and VCT was suggested based on their clinical presentation, this trend was similar in both males and females.

Forty (30.8%) and 25 (19.2%) of the study participants had multiple sexual partners outside marriage and past history of sexual transmitted infections (STIs) respectively, with similar frequency in both gender, this observation is a public health concern. Although 50% of our participants had no formal education, almost all of them were aware of HIV infection and its routes of transmission, however their knowledge of HIV/AIDS may have been driven by the suspicion that they may be HIV positive from their illness. Voluntary HIV services place emphasis on HIV status disclosure among HIV-infected clients, particularly to their sexual partners. Many international organizations including UNAIDS, WHO and CDC emphasize the importance of HIV status disclosure [13,14]. Self-disclosure of sensitive information is generally thought to have beneficial effects on an individual's health, lower stress, and lead to better psychological health [15]. Disclosure offers a number of benefits to the infected individuals, partners and the general public [13-15].

Along with these benefits, however, there are a number of potential risks, disclosure is a sensitive issue, often causing psychological distress due to the uncertainty of how people will react [16]. They may experience stigma and rejection related to their HIV status and be less open about their status [17]. Conversely lack of disclosure of HIV status has been associated with personal distress and loneliness [18]

Existing research regarding HIV disclosure has primarily focused on documenting rates of disclosure,[21] predictors of disclosure,[22] issues regarding disclosure to children,[23–25] and individuals to whom PLWHA disclose their serostatus.[24,26] Research on the relationship between disclosure and psychological attitude has produced mixed findings and has been conducted largely in HIV-positive populations from developed countries. Some studies have found an association between disclosure and lower levels of depression,[22,27,28] while other studies have reported higher levels of depression being associated with

disclosure of HIV serostatus, [29] and others have reported no association between depression and disclosure.[26,30]

However, disclosure does not always mean, individuals will use the information to protect themselves or others; in fact, some will knowingly place themselves at risk of infection [31]. Other studies have found no association between disclosure and safer sex [32,33].

In this report it was observed that 51.5% of the cohort were non-challant at disclosure of their HIV sero-positive status during pos test counselling, 34.6% were worried, 10.0% were sad and 3.9% denied the outcome of their test results. Significantly more males (47.7%) were non-challant than females (27.9%), while more females were sad at disclosure of their HIV sero-positive status but fails to reach a significant level. Our study is in agreement with a study conducted in South Africa that revealed males were more casual and complacent about the outcome of their HIV results, disclosed their result more often to partner than females [34]. Indeed, expecting rejection due to stigma especially in females is associated with denial, deception, and social withdrawal, which leads to more constricted social networks and low self-esteem [19] The fear of being found out by the community, of disgracing one's self and family, and of mistreatment by health care workers are related indirectly to health seeking intentions and behaviours. Furthermore, social avoidance or rejection can then, of course, hinder peoples' psychological and physical wellbeing [20].

Although, there were few individuals who regretted their decision to consent for Voluntary HIV testing and counselling at disclosure of their HIV status by becoming sad, we were limited by time to determine rates of depression among the study population as most of them returned for their result within 72 hours. Negative consequences or psychological adjustment to learning one is HIV-positive likely takes time.

These findings affirm that disclosure is a complex process with widely varying consequences. Although it may result in greater social support, negative consequences such as feelings of regret and undermining reactions from close friends, intimate partners, and parents have been associated with depression and anxiety

This study highlights the need to anticipate actual outcomes of disclosure and concerns. It is evident from this report that disclosure of HIV status is associated with varying reactions and psychological attitude that need to be addressed for successful patient management and HIV/AIDS prevention interventions.

V. Limitations

However, results from this study must be viewed within the context of the following limitations. Firstly, the cross sectional nature of this study limits our ability to interpret causal direction in the findings. secondly, the impact of disclosure may not be apparent immediately following the disclosure. It is possible that the Negative consequences or psychological adjustment to learning one is HIV-positive likely takes time. Furthermore positive gains of disclosure may diminish over time and other factors such as stressful life events, medication adverse effects, and symptomatic disease may mitigate the initial effect of disclosure. These factors were not controlled for in this study.

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the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

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3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

5. Ask your Guides: If you are having any difficulty in your research, then do not hesitate to share your difficulty to your guide (if you have any). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work then ask the supervisor to help you with the alternative. He might also provide you the list of essential readings.

6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. Use the Internet for help: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

10. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right! It is a good habit, which helps to not to lose your continuity. You should always use bookmarks while searching on Internet also, which will make your search easier.

11. Revise what you wrote: When you write anything, always read it, summarize it and then finalize it.

12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. **Pick a good study spot:** To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. **Think technically:** Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

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

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be

sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page

- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to

shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.
- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw th

principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

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

What to keep away from

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

Results:

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

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

Content

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

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.

- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

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

Figures and tables

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

Discussion:

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

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

Approach:

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

Administration Rules Listed Before Submitting Your Research Paper to Global Journals Inc. (US)

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.

- Do not give permission to anyone else to "PROOFREAD" your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

Criterion for Grading a Research Paper (Compilation) by Global Journals Inc. (US)

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals Inc. (US).

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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