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# Angiotensin Converting Enzyme Gene I/D polymorphism correlates with complications in HCV infected Egyptian Patients

By Mohamed Y Elsammak M, Hisham S El Banawy, Manal Mahmoud
*Alexandria University, Egypt*

*Abstract –* Hepatitis C (HCV) infection represents a major health problem in Egypt with a reported prevalence of more than 20%. About 60 to 80% of patients develop chronic infection, which may progress to complications; others may have HCV latent infection for years or may have an eventual recovery. Different factors may affect the outcome of HCV (e.g. age, other virus infections). Different studies have illustrated a genetic predisposition for viral infections and development of complications. The Angiotensin Converting Enzyme (ACE) gene I/D polymorphism has been associated with the development of different diseases, however few data are available about the association if any with HCV infection and development of complications. Aims: The current study aimed at investigating whether there is a difference in I/D ACE genotypes distribution in a cohort of HCV Egyptian patients compared to their healthy counterparts and whether there is a significant association between different I/D genotypes and markers of HCV disease severity.

*Keywords :* *Angiotenisn Converting enzyme gene polymorphism, I/D polymorphism, HCV, PCR.*

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# Angiotensin Converting Enzyme Gene I/D polymorphism correlates with complications in HCV infected Egyptian Patients

Mohamed Y Elsammak M[α], Hisham S El Banawy[Ω], Manal Mahmoud[β]

*Abstract* - Hepatitis C (HCV) infection represents a major health problem in Egypt with a reported prevalence of more than 20%. About 60 to 80% of patients develop chronic infection, which may progress to complications; others may have HCV latent infection for years or may have an eventual recovery. Different factors may affect the outcome of HCV (e.g. age, other virus infections). Different studies have illustrated a genetic predisposition for viral infections and development of complications. The Angiotensin Converting Enzyme (ACE) gene I/D polymorphism has been associated with the development of different diseases, however few data are available about the association if any with HCV infection and development of complications.

Aims: The current study aimed at investigating whether there is a difference in I/D ACE genotypes distribution in a cohort of HCV Egyptian patients compared to their healthy counterparts and whether there is a significant association between different I/D genotypes and markers of HCV disease severity.

Subjects and methods: The current study included 2 groups: Hepatitis C (HCV) patients' group comprised of 78 patients (56 men and 22 women) aged (Mean ☐SD) 47.5 ± 7.0 years and a sex and aged matched control group comprised of 42 control subjects (30 men and 12 women) aged 45.2±7.5 years.

Results: Data showed a significant diference in the distribution of Angiotensin converting enzyme between HCV patients and healthy controls (p: 0.021). The percentage of the I/I, D/I and D/D in the patients and controls were: 57.1 %, 33.3%, 9.5% and 23.1%, 46.2%, 30.8% in controls and HCV patients respectively. The D allele was associated with increased lecuocytic count, wider portal vein diameter, higher Child Pugh score, increased ALT and glucose levels.

Conclusion: Our data suggest a possible role for the D allele in the progression and development of complications in Egyptian HCV patients. Larger studies are needed to confirm this hypothesis.

*Keywords : Angiotenisn Converting enzyme gene polymorphism, I/D polymorphism, HCV, PCR.*

## I. Introduction

Hepatitis C virus (HCV) infection is the leading cause of chronic liver disease worldwide[1]. HCV infection represents a major health problem in Egypt[2]. About 60 to 80% of patients develop chronic infection, which may progress to complications (e.g. cirrhosis, variceal bleeding and hepatocellular carcinoma)[3]. On the other hand some patients had HCV latent infection for years and others may have an eventual recovery with sero-positivity as the only indication of their past HCV infection[4]. Many factors, including age, gender, alcohol consumption[5], body mass index, steatosis[6], and concomitant other viral infections (e.g. human immunodeficiency virus (HIV), hepatitis B virus)[7] affect disease outcome but are insufficient to explain it. Immunologic and genetic factors may also play an important role and are believed to have an impact on the outcome of HCV infection. Studies among monozygotic twins suggest that host genetic factors may account for 50% or more of the variability in the major outcomes in infectious diseases,[8] [9]. Different studies have illustrated a genetic predisposition for viral infections [10, 11, 12].

The ACE gene insertion/deletion (I/D) polymorphism was first identified in 1990. The gene-encoding ACE (or dipeptidyl carboxy peptidase1: DCP1) is located on chromosome 17q35 and consists of 26 exons. A 250-bp deletion/insertion polymorphism exists in intron 16 of the ACE gene and the deletion variant is associated with higher serum levels of the enzyme. The ACE gene insertion/deletion (I/D) polymorphism has been investigated in several diseases [13,14,15]. The angiotensin converting enzyme (ACE) gene I/D polymorphism influences the production of angiotensin II (ANG II), whose role in the regulation of fibrosis in the liver and other organs is increasingly recognized[16]. Recently, an inflammatory role for ACE gene has been suggested[17]. A Finnish study revealed an association between the deletion variant (D) and certain granulomatous disease "sarcoidosis[18] with a possible role in altering the cytokines level during the inflammatory process. This is alteration and suseptability of disease progression is mainly evident in certain genotypes of the angiotensin converting enzyme gene[18]. Up to our knowledge, scanty data area available about the distribution of I/D ACE gene polymorphism in patients affected with hepatitis C. The current study aimed at investigating whether there is a difference in I/D ACE genotypes distribution in a cohort of HCV Egyptian patients compared to their healthy counterparts and whether there is a significant

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association between different I/D genotypes and the HCV disease severity.

## II. Patients and Methods

The study was approved by Alexandria University Ethical committee. Patients included in this study were seen in the Internal Medicine Department of the Medical Research Institute Teaching Hospital of Alexandria University, Egypt. All patients and control subjects gave their written informed consent before participating in the study. The current study included 2 groups: Hepatitis C (HCV) patients' group comprised of 78 patients (56 men and 22 women) aged (Mean ±SD) 47.5 ± 7.0 years and a sex and aged matched control group comprised of 42 control subjects (30 men and 12 women) aged 45.2±7.5 years.

Exclusion criteria included cases of hepatitis B infection, autoimmune hepatitis, metabolic liver diseases (haemochromatosis, Wilson's disease, non alcoholic steatohepatitis), history of alcohol consumption or malignancy.

The followings were done for the patients and control subjects: full clinical examination including history taking, blood pressure measurement and abdominal ultrasound examination with evaluation of different hepatobillry parameters including portal, hepatic and mesenteric veins diameters. Complete urine and stool examination[19]. Venous blood samples were taken from each subject after an over night fast. Blood samples were collected in plain tubes, centrifuged and analyzed for fasting serum glucose, urea, creatinine, total serum protein, albumin, liver enzymes (Aspartate aminotransferase and alanine aminotransferase), gamma glutareyl transferase, alkaline phosphastase, total and direct bilirubin. These were measured using a Konelab Chemistry analyzer[20] (Thermo Electron Oy, Vantaa, Finland. https://www.thermo.com). Citrated and EDTA samples were taken for prothrombin activity and full blood count. The remaining serum was used for testing HCV antibodies. The presence of anti-HCV antibodies was determined in serum samples by enzyme linked immunosorbent assay (ELISA-II; Ortho Diagnostic Test Systems, Raritan, NJ, USA).

Genomic DNA was isolated from nucleated blood cells (separated from EDTA blood sample) using standard technique[21]. DNA samples were kept at -80 $^0$C till analysed. The I/D polymorphism of the ACE gene was determined according to the method of Rigat et al[22]. Briefly, about 50 to 80 ng DNA samples were amplified in a final volume of 25 µL containing 1×PCR buffer with 1.5 mmol/L MgCl2, 2 unit Taq DNA polymerase, 100 µmol/L dNTP,and 0.5 µmol/L of each primer and 5% DMSO (dimethyl sulphoxide). The sequences of the sense and antisense primers were 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3', respectively. DMSO was included in the PCR to prevent underestimation of heterozygotes and overestimation of D/D genotype[23]

PCR was performed in a GeneAmp, thermocycler (Biorad, USA). Samples were denatured for 1 minute at 94°C and then cycled 30 times through the following steps: 45 seconds at 94°C, 1 minute at 62°C, and 1 minute at 72°C. PCR products were electrophoresed in 1.6% agarose gel and visualized directly with ethidium bromide staining. The insertion allele (I) was detected as a 490-bp band, and the deletion allele (D) was detected as a 190-bp band. While The I/I genotype was detected as a single band of 490 Bp, the D/D genotype was detected as a single band of 190-bp while the I/D was detected by the presence of two bands a 490-bp and 190 –bp. To ensure quality control, genotyping was performed with blinding to case/control status, and random samples of cases and controls were tested twice by different persons, and the results were concordant for all masked cases.

## III. Statistical Analysis

Prevalence of alleles and genotype among cases and control subjects were counted and compared with Hardy–Weinberg predictions[24]. Chi-square test (Fisher's exact test) was used to test the distribution of the different genotypes in the different groups. P value of $< 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS 11.5 statistical Package.

## IV. Results

Clinical and biochemical data of the studied groups are illustrated in table1. There was no significant age difference between the different groups enrolled in this study. Ultrasound findings showed a significantly enlargement of the liver right lobe and spleen diameters and portal vein diameters in HCV patients compared to the health controls ($P<0.05$) Spleenic vein diameter correlated positively with portal vein diameter and longitudinal spleen length (r: 0.649 P: 0001 & r:0.37 &P:0.02 ).

The different genotypes were in agreement with Hardy–Weinberg equilibrium. Analysis of the I/D angiotensin gene polymorphism revealed a significant difference for the different genotypes between the different groups. ($P<0.021$). Figure 1 shows an illustration of the different genotypes of I/D Ace gene polymorphism. HCV patients group showed a higher percentage of D/I and DD genotypes than the control group. Table II shows the frequency of each genotype in the different groups. Multivariate analysis did not show a significant confounding effect of age, sex, history of schistosmoal infection on the ACE genotyping results.

HCV patients were stratified according to the different I/D genotype and the different parameters were analyzed (table III). There was a significant difference

within the three groups, namely I/I, D/I and those with D/D genotype for total leucocytic count, Child Pugh score, portal vein diameter, ALT and plasma glucose glucose($P<0.05$).

Patients with D/I and those with D/D had significantly higher total leucocytic counts, Child Pugh scoring, portal vein diameter, ALT (alanine amino transferase) and plasma glucose (P values: 0.032, 0.027, 0.0495, 0.029 and 0.043 respectively).

## V. Discussion

HCV infection is characterized by continuous inflammation that slowly results in liver fibrosis that eventually may result in the development of hepatocellular carcinoma. Hepatic fibrosis in HCV affected patients has been attributed to increased cytokines production as a result of HCV infection and uncontrolled activation of the immune system. Other factors that may contribute in the progression of hepatic fibrosis, include male sex, older age, longer duration of HCV infection, high levels of alcohol consumption and HIV co-infection. These factors have been associated with more severe liver damage in patients with chronic hepatitis C and accelerated HCV-related liver fibrosis.

Recent reports have revealed that the renin-angiotensin system (RAS) plays an important role in the liver fibrosis development with RAS components significantly up-regulated during the liver fibrosis development. Furthermore, it has been recently reported that the combination treatment with IFN and ACE-blockers exerted a more potent inhibitory effect on murine liver fibrosis development than either single agent, . Collectively these reports point to an important role that RAS system plays in the development of HCV complications.

The current study centered on exploring the possibility of the presence of genetic factors affecting the RAS specially the I/D polymorphism of the Angiotensin Converting Enzyme gene, that might influence the susceptibility to HCV infection and development of complications. The study evaluated the distribution of I/D polymorphism of the angiotensin converting enzyme gene in HCV patients and an age and sex matched control group. There was a significantly higher percentage of HCV Egyptian patients having the I/D and DD genotypes than the healthy controls. In HCV patients, the D allele carriers had a significantly higher total leucocytic counts, alanine aminotransferase, plasma glucose and had a higher Child Pugh scoring. Our results are in agreement with Fabris et al who found a carriers of the D allele especially female patients have a poor outcome with increased complication post hepatic transplantation[32].

The current study also showed an association between the D allele and increased plasma glucose level in HCV positive patients. Insulin resistance is a known complication of HCV patients. Previously, increased insulin resistance has been documented in HCV positive patients that correlated with the HCV infectivity and was attributed to increase cytokines production in chronic HCV patients.[33] Recently, tudies have also demonstrated that ACE insertion/deletion (I/D) polymorphism is associated with development of insulin resistance and eventually diabetes mellitus complications in a different ethnic populations.[34]

The findings of increased blood glucose in D allele carriers of HCV patients is in keeping with the findings of Mittal et al who clearly demonstrated an association of the components of metabolic syndrome especially fasting glucose and the D allele of ACE gene polymorphism[35]. Similarly in Iranians, the D allele of the angiotensin converting enzyme gene seemed to be associated with Diabetes mellitus and poor glycemic control.[36]Thus the association found in the current study between elevated blood glucose and the D allele may offer an important rationale for the increased insulin resistance commonly seen in chronic HCV patients. The adverse effect on glycemic control that is seen in our HCV patients may possibly be through end organ damage, fibrosis, and poor inflammatory response36 and control of microvascular blood flow[37]and free radical levels[38]secondary to modulated ACE gene expression.

In summary, in Egyptians our results shows an association between D allele of the Angiotenisn Converting Enzyme gene and the different complications of HCV infections (namely; higher Child Pugh scoring, portal vein diameter and poor glycemic control. Our findings may be important in detecting HCV who may need more intensive treatment to prevent complications.

Future work may concentrate on evaluating whether Angiotensin gene polymorphism may be a factor in determining the response of different antiviral therapies used in HCV infection.

## VI. Limitation of the Current Study

This is a pilot study involving only 78 HCV positive Egyptian patients an 42 controls. The small sample size may have been a limiting factor in the detection of other possible association between ACE gene polymorphism and other clinical variables. ACE activity was not performed as the study aimed mainly at evaluation I/D ACE genotype distribution in the studied group of Egyptians.

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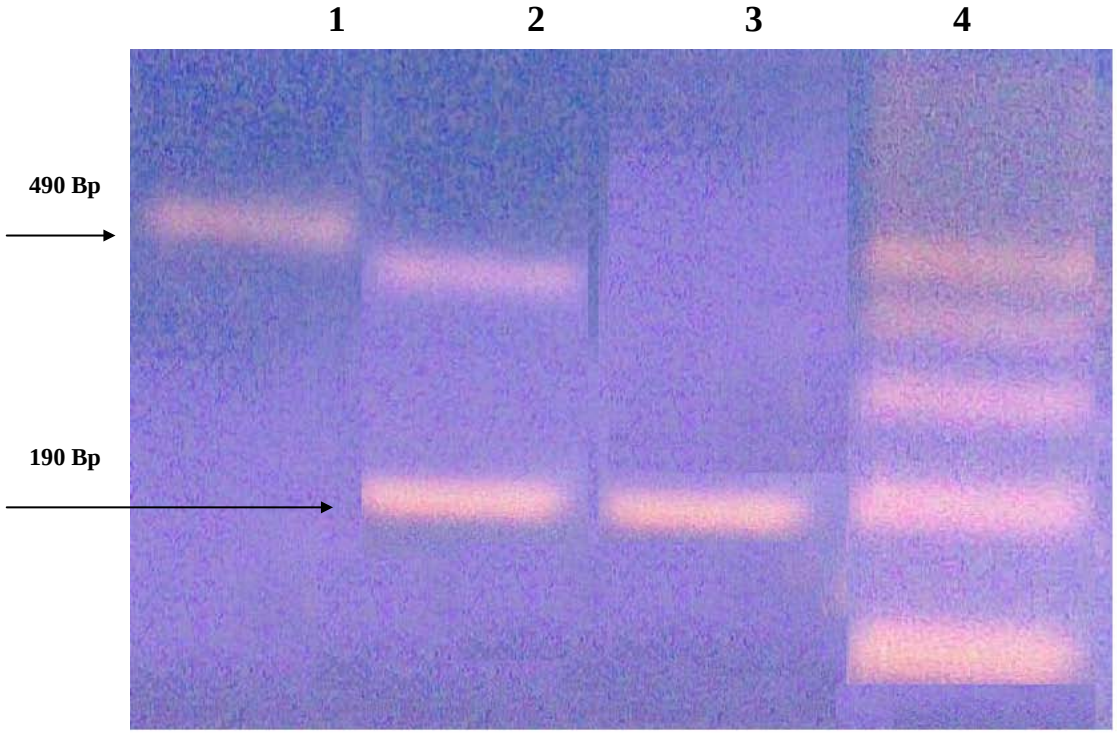


*Figure 1 :* Products of PCR amplification of the I/D polymorphism in the Angiotensin converting enzyme gene.

PCR products were electrophoresed in 1.6% agarose gel and visualized directly with ethidium bromide staining. The insertion allele (*I*) was detected as a 490-bp band, and the deletion allele (*D*) was detected as a 190-bp band. DNA samples were amplified using PCR and digested with *Taq1b* restriction enzyme. The presence of the Taq1B restriction site produces the B1 allele pattern in electrophoresis with 2 DNA fragments at 361 and 174 Bp respectively. The lack of Taq1B restriction site results in the B2B2 pattern with only one band at 535 Bp. The B1B2 genotypes resulted in 3 bands at 535, 361 and 174 Bp.

Lane 1: I/I genotype showing as one DNA band at 490 Bp
Lane 2: I/D genotype with 2 DNA bands at 490 and 190 Bp
Lane 3: D/D genotype showing as one band at 190 Bp
Lane 4: Ladder marker.100 base pairs (Bp)

| Parameter | Controls (n: 42) | HCV patients (n:78) |
|---|---|---|
| AGE (years) | 45.2±7.5 | 47.5 ± 7.0 |
| Males/females | 30/12 | 56/22 |
| History of Encephalopathy | 0 | 20 % (16cases)* * |
| Liver Right lobe (cm) | 12.9±0.22 | 14.8±1.9** |
| Spleen (Cm) | 10.8 ± 0.92 | 15.7 ± 2.4** |
| History of Ascites | No | 46 No ascites<br>22 Mild<br>4 Moderate<br>6 Severe |
| Spleenic vein Diameter (mm) | 7.6±0.8 | 10.2 ± 2.8** |
| Superior Mesenteric vein Diameter (mm) | 7.6 ± 0 .71 | 10.6 ± 2.5** |
| Portal Vein Diameter (mm) | 10.92 ± 0.7 | 15.65 ± 2.3** |
| Prothrombin activity (%) | 92.9±6.8 | 61.9 ±16.5** |
| Haemoglobin (gm/dl) | 14.3 ± 0.5 | 11.8 ± 1.5 |
| Leucocytic count $X10^3$ | 4. 2 (2.3 -14.9.) | 6.2 ( 4.5 – 9.0) * |
| Platelets $X10^6$ | 286.0(174.0-432.0) | 115.0(110.0- 303.0)* * |
| Child Pugh score | 0 | 7.1± 2.6<br>10 cases score 5<br>18 cases sscore:6<br>8 Cases scores 7<br>14 Cases scores 8<br>6 Cases scores 9<br>4 Cases scores 10<br>8 Cases scores 11<br>4 Cases scores 12<br>4 Cases scores 13<br>2 Cases scores 14 |
| ALT (U/L) | 23.0(6.0- 41.( | 49.0(12.0-122.0)* |
| AST (U/L) | 21.08.0-38.( | 47.0(21.0- 157.0)* |

| | | |
|---|---|---|
| GGT( U/L) | 21.0(16.5-61.0 | 77.0(20- 625.0)* |
| ALP (U/L) | 78.0(47.0-117.( | 112.0(57.0- 589.0)* |
| Serum Protein (g/dl) | 7.9 ± 0.7 | 7.03 ± 0.7* |
| Serum Albumin (g/dl) | 4.4 ± 0.5 | 3.5 ± 0.7* |
| Total bilirubin (umol/L) | 13.0 ± 4.0 | 32.0 ± 21.0* |
| Direct bilirubin (umol/L) | 4.0 ± 2.0 | 15.0 ± 5.0* |
| Urea ( mmol/L) | 4.5 (2.0- 8.2) | 4.3 (2.7 – 31.3) |
| Creatinine (umol/L) | 76.9 ± 20.3 | 97.2 ± 34.4* |
| FPG (mmol/L) | 4.8 (3.7-6.3) | 6.2 (3.6- 14.7)* |

*Table1 :* Clinical and biochemical criteria of the studied groups:

Data are presented as Mean ±SD for normally distributed variables for non-normally distributed variables results are presented as Median (range)

ALT: Alanine amino transferase

AST: Aspartate amino transferase

ALP: Alkaline phosphatase

GGT: Gamma Glutaryl transferase

FPG: Fasting plasma glucose

* = Significant difference versus the control groups (P<0.05)

** = Significant difference versus the control groups (P<0.05)

| Group | | | Gene | | | Total | P value |
|---|---|---|---|---|---|---|---|
| | | | D / D | D / I | I / I | | |
| | Controls | Number | 4 | 14 | 24 | 42 | |
| | | % within Controls | 9.5% | 33.3% | 57.1% | 100.0% | |
| | | % of Total | 3.3% | 11.7% | 20.0% | 35.0% | |
| | HCV (Patients) | Number | 24 | 36 | 18 | 78 | 0.021* |
| | | % within patients | 30.8% | 46.2% | 23.1% | 100.0% | |
| | | % of Total | 20.0% | 30.0% | 15.0% | 65.0% | |
| Total | | Count | 28 | 50 | 42 | 120 | |
| | | % of Total | 23.3% | 41.7% | 35.0% | 100.0% | |

*Table II :* Frequency of the different D/I genotypes in HCV patients and healthy controls and the result of the Chi square testing in the different studied groups. Table shows the frequency and the results of the cross tabulation of the different genotypes in the studied groups. The number in each subgroup is shown and the (percentage). Chi square test was used in calculation.

* : Significant P with a value <0.05

| Parameter | I /I (n:18) | D /I (n:36) | D /D (n:24) | *P* value |
|---|---|---|---|---|
| Liver Right lobe (cm) | 15.3 ±1.4 | 14.4 ±1.99 | 14.9 ± 2.0 | 0.227 |
| Spleem (Cm) | 14.4 ±1.82 | 16.01 ± 2.55 | 16.2 ± 2.4 | 0.097 |
| Spleenic vein Diameter (mm) | 9.16 ± 1.6 | 10.57 ± 3.21 | 10.48 ± 2.98 | 0.74 |
| Superior Mesenteric vein Diameter(mm) | 10.1 ± 2.45 | 10.33 ± 2.62 | 11.45 ± 2.3 | 0.32 |
| Portal Vein Diameter (mm) | 14.67 ± 0.68 | 15.95 ± 2.51 | 15.91 ± 2.83 | 0.038* |
| Prothrombin activity (%) | 70.0 ± 17.6 | 60.76 ± 15.84 | 53.68 ± 12.78 | 0.049 |
| Haemoglobin (gm/dl) | 12.7 ± 1.6 | 11.4 ±1.4 | 11.5 ±1.2 | 0.35 |
| Leucocytic count X$10^3$ | 3.4 (2.3 -7.6) | 4.3 (3.0 .- 14.90.0) | 4.3 (3.0 .- 9.0) | 0.032* |
| Platelets count X $10^6$ | 115.0 (75.0 -176.0) | 102.5 ( 110.0 – 303.0) | 148.0 (77.0 – 212.0) | 0.62 |
| Child Pugh score | 6.75 ±1.8 | 8.9 ± 2.6 | 8.6 ± 2.8 | 0.04* |
| ALT (U/L) | 34.0(12-76) | 45.0(16-92) | 57.50( 17.0-122.0) | 0.029 |
| AST (U/L) | 40.0(21-72) | 51.5(26-112) | 49.50(25-157.0) | 0.81 |
| GGT( U/L) | 77.0(35.0-457) | 68.0(20-625) | 116.0(29.0-248.0) | 0.05 |
| ALP (U/L) | 112.0(88.0-589) | 112.0(66-226) | 105.0(57.0- 188.0) | 0.459 |
| Serum Protein (g/dl) | 6.9 ± 0.8 | 7.2 ± 0.7 | 7.05 ± 0.5 | 0.245 |
| Serum Albumin (g/dl) | 3.41 ± 0.9 | 3.27± 0.73 | 3.73 ± 0.5 | 0.211 |
| Total bilirubin (umol/L) | 22.0 ±19.0 | 40.0 ±26.0 | 24.0 ± 9.0 | 0.332 |
| Urea ( mmol/L) | 4.5(3.5-18.8) | 4.2(3.2-19.6) | 4.3(2.7-15.6) | 0.561 |
| Creatinine (umol/L) | 105.2 ± 40.7 | 91.1 ± 30.9 | 86.6 ± 33.6 | 0.446 |
| Glucose (mmol/L) | 4.22.(2.85-12.9) | 5.4(4.1-14.7) | 5.6(4.5- 14.1) | 0.04* |

*Table III :* Clinical and biochemical parameters in HCV patients included in the study according to their ACE genotype. Data are presented as Mean ±SD for normally distributed variables for non-normally distributed variables results are presented as Median (range)

ALT: Alanine amino transferase

AST: Aspartate amino transferase

ALP: Alkaline phosphatase

GGT: Gamma Glutaryl transferase

FPG: Fasting plasma glucose

* = Significant difference versus the control groups ($P<0.05$)

$P<0.05$= Significant difference

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# A Diagnosis of the gastroschisis in the first trimester of pregnancy in Serbia - a case report

By Lončar Dragan
*Clinical Center Kragujevac, Vojislava Kalanovića , Serbia*

*Abstract –* Gastroschisis (gastroshisis) represents evisceration of the abdominal organs, most commonly small bowels, stomach and gonads through the front abdominal wall defect, almost always to the right of the umbilicus (90%) from which it is separated by thin skin bridge. The incidence of this anomaly is 0.5 to 4 in 10.000 liveborn babies. We presented a patient, age 27, who had the gastroschisis of the fetus in the 13th week of gestation diagnosed by ultrasound. Ultrasound examination is the method of choice for prenatal detection of fetal anomalies. By differential diagnosis, the possible existence of omphalocele should be eliminated using (2D, 3D) and power Doppler technology which significantly makes the assessment of gynecologist easier during establishment of the final diagnosis.

*Keywords : prenatal diagnosis; gastroschisis; ultra-sonography; fetal anomalies.*

*GJMR-B Classification: NLMC Code: WN 208,WP 150, QS 645, QS 621*


*Strictly as per the compliance and regulations of:*

# A Diagnosis of the gastroschisis in the first trimester of pregnancy in Serbia - a case report

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*Abstract-* Gastroschisis ( gastroshisis) represents evisceration of the abdominal organs, most commonly small bowels, stomach and gonads through the front abdominal wall defect, almost always to the right of the umbilicus (90%) from which it is separated by thin skin bridge. The incidence of this anomaly is 0.5 to 4 in 10.000 liveborn babies. We presented a patient, age 27, who had the gastroschisis of the fetus in the 13th week of gestation diagnosed by ultrasound. Ultrasound examination is the method of choice for prenatal detection of fetal anomalies. By differential diagnosis, the possible existence of omphalocele should be eliminated using (2D, 3D) and power Doppler technology which significantly makes the assessment of gynecologist easier during establishment of the final diagnosis.

*Keywords : prenatal diagnosis; gastroschisis; ultrasonography; fetal anomalies.*

## I. Introduction

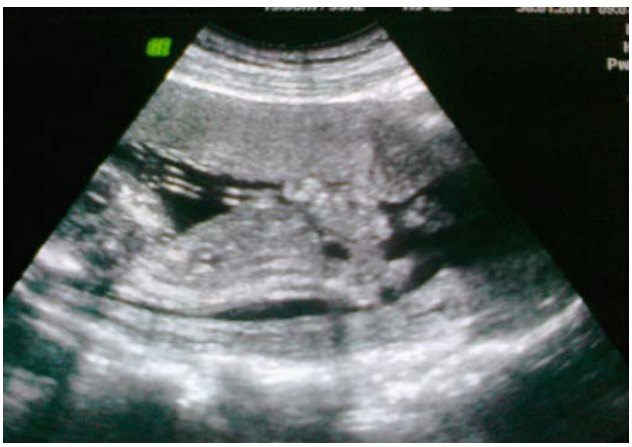
Gastroschisis (gastroshisis) represents evisceration of the abdominal organs, most commonly small bowels, stomach and gonads through the front abdominal wall defect, almost always to the right of the umbilicus (90%) from which it is separated by thin skin bridge. Eviscerated intestines are thickened, edematous, sticky, aperistaltic as a consequence of influence of the amniotic fluid on the serosa of intestines (1). The incidence of this anomaly is 0.5 to 4 in 10.000 liveborn babies (2). In about 60% of the cases it is about prematurely born children. This anomaly is more common in male children. Gastroschisis is rare with associated anomalies, although malrotation and malfixation are always present. There are several theories concerning the cause of this anomaly. According to one of them the interruption in development of omphalomesenteric artery occurs, and according to the other pathological involution of the right umbilical vein, it leads to a weakening of the anterior abdominal wall and consequent protrusion of the intestine through a weakened part. Teratogenic agents for occurence of this anomaly are smoking and vasoactive medications. Reference is to the aspirin, ibuprofen, alcohol and cocaine abuse and malnutrition. Seasonal occurrence of gastroschisis is associated with teratogenic influence of pesticide and herbicides (2).Ultrasonography is the dominant method in the diagnosis of this fetal anomaly. Ultrasonographic features of gastroschisis are clear and allow, in most cases, the exact prenatal diagnosis in the first trimester of pregnancy. In the ultrasound examination, the gastroschisis is shown as a mass resembling the cauliflower (small intestines), which floats freely in the amniotic fluid, close to the anterior abdominal wall. Ultrasound examination remains the method of choice in the diagnosis of fetal anomalies, although the application of magnetic resonance imaging (MRI) can provide a more detailed examination of fetus with anomaly of the anterior abdominal wall. The amniotic fluid contains the elevated concentrations of alpha-fetoprotein and acetylcholinesterase (2, 3).

Treatment is strictly operative after a good preoperative preparation.

## II. Case Report

The patient I.J., aged 27, worker by vocation, was hospitalized at the Department of Fertility Control in CC Kragujevac with the diagnosis: Graviditas ml III. Gastroshisis foetii, due to pregnancy termination after the decision of Second Instance Commission of Department of Obstetrics and Gynecology in CC Kragujevac that approved pregnancy termination for medical indications. The Commission was in session at the request of I.J, after the report of the Consilium for Fetal Anomalies of CC Kragujevac reaching the following conclusion Dg Gastroshisis, suggestion: Perform CVS. Ultrasound finding: fetal pelvis leading, BPD 25mm, AC 89mm, 12mm FL, fetal heart rate recorded, normal amniotic fluid, placenta at left lateral side. Gestation week by ultrasonographic findings is 13.5. In front of the anterior abdominal wall the convoluted intestines are observed 20x11mm in size. Stomach is in the abdomen (Figure 1).

*Figure 1 :* Gastroshisis



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Ultrasound examination was performed on the machine Aloka Pro Sound 3 500, by multifrequency abdominal sector probe of 3.5 to 5 MHz. Cytogenetic finding after chorionic villi biopsy performed on a date 18. 01. 2011. states: 46, XY, five metaphases were analyzed, by G strip technique. Cytogenetic analysis is done in the Genetic laboratory of Department of Obstetrics and Gynecology in Kragujevac, protocol number 8-2011.

A thorough informative conversation with the family was performed and once again the options and procedures in the following course of pregnancy observation were presented, the procedure for surgical treatment of the baby after birth was explained and the success of such treatment and the possibility of any possible complications were reported.

The attitude of the family to perform pregnancy termination was explicit after all performed consultation of which there are adequate medical records in the medical history of a patient.

*a) Preparation and procedure for termination of pregnancy*

Laboratory processing of pregnant women:

Blood type: 0 Rh D + (positive).

Hematological analyses: WBC 5.4 x 109 / L, Neutrophil granulocytes 58.1%, 29.8% Lymphocytes, Erythrocytes 3.74 x 1012 / L, Hemoglobin 118 g / L, Hematocrit 0.34 L / L, Thrombocites 204 x109 / L.

Biochemical analysis: Glucose 3.8 mmol / L, Urea 2.1 mmol / L, Creatinine 55µmol / L, Uric acid 182µmol / L, C-reactive protein (CRP) 5.1 mg / L, Fibrinogen 3.6 g / L.

Coagulation status factors: Prothrombin time (PT) - 10.3 sec., International Normalized Ratio (INR) 0.91, Activated partial thromboplastin time (Activated partial thromboplastin time-APTT) 26.0 sec.

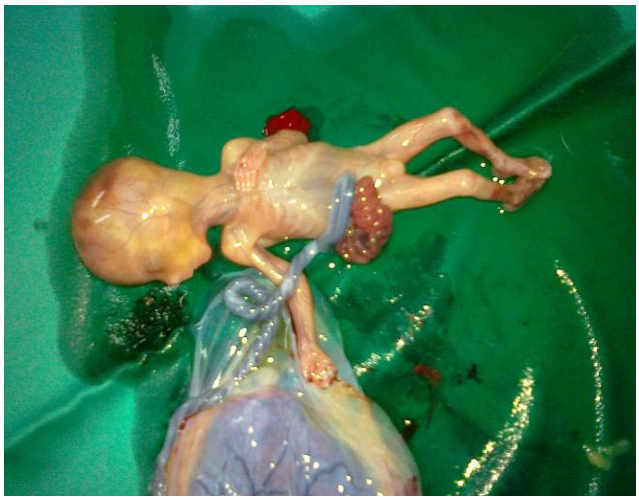
Jonogram: Potassium 4.0 mmol / L, Sodium 137 mmol / L, Calcium 2.14 mmol / L, Magnesium 0.88 mmol / L, Iron 19.1 mmol / L.

Alpha-fetoprotein (AFP) 55.74 IU / mL

Urine analysis: Finding is normal.

Laboratory analyses were conducted in Central Biochemical Laboratory of CC Kragujevac and the Blood Transfusion Centre in CC Kragujevac. Intracervical misoprostol was applicated in two individual doses of 400 mg, at intervals of 12 h. An antibiotic cefuroxime was prescribed at a dosage of 1.5 g/12 h intravenously as well as anxiolytic diazepam, tablets of 5 mg per os. It was decided at the Consilium that the induced abortion should continue with prostaglandin E2 (PGE2) dinoprostone intracervically in the interval of 12 hours. After 24 hours the patient had an abortion of the male fetus, with a clearly visible evisceration of intestinal loops, which was sent for pathohistological analysis along with the placenta (Figure 2).

*Figure 2 :* The fetus and the placenta after abortion

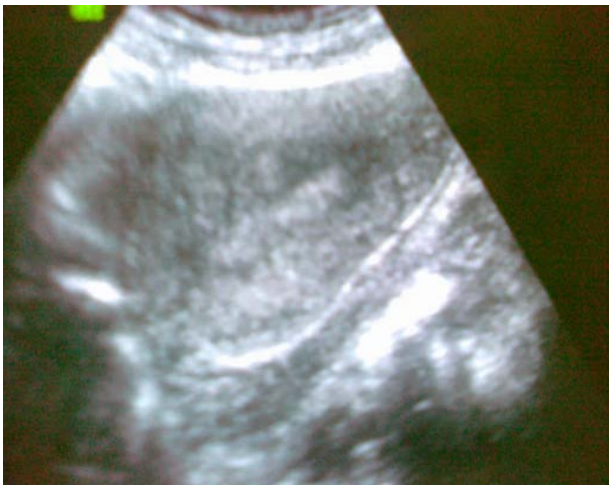


Instrumental revision of uterine cavity was carried out and it was continued with the aforementioned intravenous antibiotic therapy with intramuscular application of uterotonic during three days. By control ultrasound examination after two days the following finding was stated: Uterine anteversion / anteflexion (AVF), measures 79x56x45mm, with emphasized horns. Right horn without content, with decidual reaction of 6mm. The left horn with no content, with decidual reaction to 11mm. Right ovary measures 36x27mm with cystic formation that measures 23x25mm. The left ovary measures 40x29mm, and it is of cystic structure. Empty pouch of Douglas (Figure 3). By bimanual gynecological examination the following finding is stated: under the speculum vagina is of normal depth, portio vaginalis uteri (PVU) cylindrical, 2.5 cm long, the orificium externum uteri is transversally placed, sparse bleeding ex utero. PVU insensitive, mobile, can be inserted with a finger tip. Uterus in the AVF, firm, mobile and in good involution, insensitive, size of women's fist. Adnexa free on both sides with, no pathological changes, insensitive to palpation. Pouch of Douglas insensitive.

*b) Pathohistological finding*

810 - Foetus maceratus in utero. Infarctus anaemicus recens in texti stromae placentae Chorioamnionitis chronica, light to moderate degree. Gastroshisis.

*Figure 3 :* Ultrasonographic view of the uterus at the patient's discharge from hospital.



The patient was discharged three days after an abortion in good condition.

## III. Discussion

The significant survival of patients with gastroschisis was noted since the introduction of early diagnosis of this anomaly, best prenatally (4). Good preoperative preparation and appropriate postoperative treatment are also necessary parameters in the final outcome of treatment of these patients (5). Great progress in prenatal medicine was enabled by the rapid development of ultrasonography (6). Ultrasonography improves the prognosis by enabling control during pregnancy, planned delivery by Caesarean section in an appropriate institution and the surgical team ready for the gastroschisis treatment (7.8). Treatment of gastroschisis begins in maternity ward with high umbilical cord ligation and adequate surgical treatment that follows (9). Early diagnosis, good preoperative preparation, adequate anesthesia, gentle handling during surgical intervention, as well as good postoperative care, contribute considerably to better treatment outcomes of gastroschisis which increases the survival rate above 80% (10,11). We didn't have dilemma at any time about the need for additional diagnostic methods such as computed tomography (CT) or magnetic resonance (MR) in order to establish the final diagnosis as used by many perinatologists according to the literature data. (12, 13, 14).

By differential diagnosis, we eliminated the possible omphalocele in the fetus. Gastroschisis occurs later, because the hole (abdominal front wall defect) before 16th week is very small and because the abdominal front wall muscles and peristaltic waves are visible only in 12ththat is 14th week (15). Omphalocele (omphalocoela) is a herniation of abdominal cavity contents into the cord base. This occurs because of lack of fusion of lateral ectomesodermal folds. Small intestines are always the content of the hernia bag, and liver, stomach, spleen, colon, and gonads can be found. It is covered by amnioperitoneal membrane and umbilical cord is located at its top (16). In gastroschisis, intestinal convolutions pass through a small defect (<1 cm), which is still localized to the right of the normal umbilical cord insertion, float freely in the amniotic fluid. There is no membrane covering the content as with the omphalocele. With omphaloceles in the syndrome formation, there is a strong genetic component (17). The incidence of this disorder is 1-3 per 5000 liveborn babies. By careful ultrasonographic evaluation, which is facilitated by (2D, 3D) and power Doppler technology, it is detected that fetal end of the umbilical cord ends on the apex of the mass and that it is covered with membrane (18). In 80% of the cases, the liver and small intestines are in a bag and in 20% of the cases there is only the small intestine. Polyhydramnion is not a rare finding, and in 40% there is an elevated level of alpha-fetoprotein in maternal serum. Searching for the associated anomalies is the mandatory part of the fetal examination due to is frequency (50-70%) (19). The prevalence is 30% in the second trimester, and only 15% at birth, which indicates a high mortality rate during pregnancy. Before 12$^{th}$ week, omphalocele should be suspected only if the bag is greater than 7 mm, irregular and /or inhomogeneous. Several of the above stated sentences represent a brief summary of our attempt to inform the parents in the mentioned case about the possibility of healing their baby. We have not met with approval; on the contrary, they were categorical in insisting that the pregnancy termination should be performed.

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# Analysis of the Bacterial Vaginosis Predictive Significance in the Diagnosis of Inflammatory Processes in Female Pelvic Minor

By Dragan Lončar
*Clinical Center Kragujevac, Vojislava Kalanovića , Serbia*

*Abstract –* Pelvic inflammatory disease (PID) occurs with the incidence of 100 - 200/ 100 000. The aim of this study was to determine whether there is a correlation between serum proinflammatory cytokines IL-1**β** and IFN-**γ** and the presence of bacterial vaginosis (BV) or Chlamydia infections (Chl) in women with symptoms of inflammatory processes in the pelvic minor. The study included fifty patients diagnosed with PID with the average age of 32 years. The results of this study reveal that women with bacterial vaginoses and PID level of IL- 1**β** in serum is increased, whereas in women with Chlamydial infection and PID serum level of IFN-**γ** is increased. The study showed that in patients with PID, in whom there was no diagnosis of BV and infection with Chlamydia trachomatis, the levels of IL-1**β** and IFN-**γ** are increased. The conclusion of this research points out to the importance of monitoring levels of cytokines in patients with homeostasis of vaginal flora disorders in the prevention of PID.

*Keywords : Bacterial vaginosis, Chlamydia trachomatis, interleukins, pelvic minor infection.*

*GJMR-H Classification: NLMC Code: WP 15*


*Strictly as per the compliance and regulations of :*

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## I. Introduction

Bacterial vaginosis (BV) is a disorder of normal vaginal flora, characterized by reduction of the number of lactobacilli (*Lactobacillus* $H_2O_2$ spp) and an increase in the number of anaerobic microorganisms (*Mobiluncus spp, Bacteroides spp, Fusobacterium spp, Prevotella spp, and Peptostreptococcus spp and Prophiromanas spp*), gram-variable coccobacilli (*Gardnerella vaginalis*), and genital mycoplasmas (*Micoplasma hominis*) (Hillier et al., 1993). These changes in vaginal flora were associated with an increase in vaginal pH and changes in vaginal secretion. Chlamydia trachomatis (Chl) is the carrier of sexually transmitted diseases, which often manifest as asymptomatic infection of the lower genital tract. In the early phase of the local immune response to infection, activated macrophages produce large amounts of cytokines, which activate prostaglandin F2-α and E2(Pickering et al., 2006; Jerant-Patić, 2000). The spectrum of genital infections in women includes, beside the vaginal inflammation (colpitis or vaginitis) or vulva (vulvitis), a number of diseases, which beside their separate occurrence, they also occur in causal connection in various combinations. Inflammation of the cervix (*cervicitis),* inflammation of the mucous membrane of the uterus (*endometritis*), and inflammation of the oviducts and ovaries (*salpingitis /adnexitis*) are in fact very often inherent in both the etiology and in the clinical and therapeutic terms, and are referred to the term pelvic inflammatory disease (PID). PID occurs with an incidence of 100-200 /100 000 women, that is in the age of adolescence: one of 8 girls (Soper & Mead, 2005) . The aim of this study was to determine whether there is a correlation between serum pro-inflammatory cytokines IL-1β and IFN-γ and the presence of bacterial vaginosis or Chlamydial infections in women with symptoms of inflammatory processes in the pelvis minor (pelvic inflammatory disease-PID).

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## II. Materials and Methods

The research was conducted, as a prospective study, at the Department of Gynecology and Obstetrics, Clinical Center in Kragujevac. The protocol was approved by the Ethics Committee Institution of the Clinical Center in Kragujevac. The study included fifty women diagnosed with PID. The subjects were divided into groups according to the following criteria:

1) PID patients with bacterial vaginosis - BV (N = 18) and
2) PID patients with Chlamydia trachomatis infection – Chl (N = 10);

The women that were classified as a PID category, had to meet the following criteria:

1) present pelvic pain
2) positive bimanual gynecological finding
3) elevated body temperature> 38.5 ° C measured rectally
4) positive laboratory finding for the presence of infection as follows:
   - the number of leukocytes ≥ 10.0 x$10^9$ / L
   - neutrophilic granulocytes ≥ 75%
   - sedimentation ≥ 30 mm / h
   - C-reactive protein ≥ 30.0 mg / L
   - fibrinogen ≥ 6.0 g / L
5) a positive ultrasound finding of pelvic
6) normal findings of colposcopic examination and Papanicolau test

In addition, factors that may affect the level of interleukin in serum, such as autoimmune diseases, hormonal disorders, particularly complications of

hypersensitivity and infectious diseases were also excluded in the selection of patients. A sample of vaginal secretion was taken from the vaginal side walls and was used for the diagnosis of BV by Amsel and Nugent methods (Amsel, 1983; Nugent, 1991). In one step, an immunochromatographic test was used for selective identification of LPS antigen for Chlamydia trachomatis (*Biorapid Chlamidia AG kit for 20 tests, BIOKIT SA, Barcelona, Spain*) form endocervical samples of all subjects. Sample preparation for determination of cytokines was performed as follows: 5 ml of blood was collected from the patient's cubital veins. Blood was placed into test tubes to separate the serum, and after half an hour, the sample was centrifuged for 30 minutes at 1000 rpm per minute. Furthermore, serum samples were immediately frozen and stored at -20 ° C until use. In the serum samples the levels of IL-1β and IFN-γ were determined by ELISA kit (I & R systems, UK). Sensitivity of the test for IL-1β was 1.0 pg/L, and for IFN-γ was 8.0 pg/ml. The results were statistically analyzed using the nonparametric Mann-Whitney test, a p-value less than 0.05 was considered statistically significant.

## III. Results

The average age of women who participated in this study was 32 years and ranged between 22 and 40. The presence of BV was found in 18 patients with PID, Chlamydial infection (Chl) in 10 women with PID, while 6 patients with PID had BV and Chlamydial infection as well. Sixteen patients with inflammatory syndrome in the pelvis minor had neither BV nor Chlamydial infection. The calculated values of parameters are shown in tables 1, 2 and 3 depending on the criteria used to divide patients into groups. It can be seen that the lowest detectable value was found for IL-1β in the PID group with BV (14.6%) (table 1) and highest for IFN-γ in the PID group with BV (42.2%) (table 1). In patients with PID divided into two groups according to the first criterion (table 1), there were no statistically significant differences between the levels of interleukins in the serum of women from BV group and the group without BV. However, in the patients group, according to the second criterion (table 2), it can be seen that women with Chlamydial infection and PID (10 patients) had increased level of IFN-γ in relation to the group with BV ($p < 0.010$), while for other interleukins, there were no significant differences. On the other hand, when we compared the levels of interleukins obtained from the blood of PID patients with Chlamydial infection (10 women) with the values of the PID patients without Chlamydial infection (40 women), it is obvious that the average value of IFN-γ was significantly higher in the group with Chlamydial infection ($p < 0.010$). The table 3. shows the levels of interleukins in the group of patients with PID in whom we have not found vaginal flora disorder, where we showed a significant increase in both types of parameters.

Table 1. >here
Table 2. > here
Table 3. > here

## IV. Discussion

Many clinical studies have shown that with women with PID and bacterial infection, intrauterine endo and exotoxin are the cause of hyperproduction of pro-inflammatory IL (IL-1β and IFN-γ (Curry et al., 2007; Basso et al., 2005; Hedges et al., 2006) . Cytokines can induce the synthesis of prostaglandins and metalloproteinases, which may increase the inflammatory processes in the pelvic minor. Studies are published showing that the level of pro-inflammatory cytokines IL-1 β in vaginal secretions of women with PID and BV (table 1) compared to the healthy population is significantly higher (about 10 times) than the control group (Cauci et al., 2002; Alvarez-Olmos et al., 2004; Imseis et al., 1997; Sturm-Ramirez et al., 2000; Spandorfer et al., 2001) , and that these levels decrease after treatment of BV with metronidazole (Yudin et al., 2000) . In addition, several studies have confirmed that level of IL-8 in vaginal secretion in women with BV is elevated (Yudin et al., 2000; Zariffard et al., 2005) , although this increase is generally less than twofold compared to the control group. In addition, it was found that in *in vitro* conditions, vaginal discharge collected from women with BV strongly induces IFN-γ secretion from immune cells (Zariffard et al., 2005) . Levels of IL-6 and TNF-α in vaginal secretion of patients with BV were not increased compared to controls. There is no much data on the level of interleukin in serum with women with BV in prediction of PID. In this study, we found increased levels of IL-1β in serum of women with bacterial vaginosis compared with the controls, which is consistent with recent results obtained for the levels of interleukins in vaginal secretions of women with BV and PID (Wennerholm et al., 1999; Gupta et al., 2009; Ondondo et al., 2009). In addition, in previous studies it was reported that cells infected with Chlamydia trachomatis produce high levels of IFN-γ (table 2) and small amounts of IL-10, IL-12, IL-23 and TNF-α (Srivastava et al., 2008; Golden, 2003) . This is consistent with the results of our study, where the level of IFN-γ in serum of women with Chlamydial infection and PID is significantly higher than in the control group. The results of our study indicate that bacterial vaginosis and Chlamydial infections can cause systemic, partially immune response of the woman, which may cause further boost of the inflammatory reaction. Modulation of the immune response during inflammatory process may be an explanation of our contradictory results in the group of patients with PID, in which we have not demonstrated vaginal flora disorder (table 3). Due to the fact that the PID pathophysiology is not yet known, the results of this study may contribute to its explanation.

Determination of levels of interleukins in women with PID in the presence of vaginal flora disorders is still based on a small number of cases for the standardization of methods and possibilities of using interleukin as a marker of this pathological condition, which requires further investigation in resolving the problem (Ness, 2004). Results of this study demonstrate that in women with bacterial vaginosis and PID, level of IL-1β in serum is increased, whereas in women with Chlamydial infection and PID, serum level of IFN-γ is increased. In addition, the study showed that in patients with PID, in whom there was no diagnosis of BV and Chlamydial trachomatis infection levels of IL-1β and IFN-γ are also increased. The conclusion of this research points out to the importance of monitoring levels of cytokines in patients with homeostasis of vaginal flora disorders in the prevention of PID.

## Acknowledgement / Disclosures

Competing interests: none declared

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*Table 1 :* Sensitivity and statistical analysis of cytokine results in the patient group with PID BV

| Cytokine | PID group with BV (N = 18) | | | | | PID group without BV (N=32) | | | | | P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Detectability | Max | Min | X sr | SD | Detectability | Max | Min | X sr | SD | |
| IFN-ϒ | 42.2% | 41.3 | 5.4 | 22.4 | 12.1 | 30.4% | 80.9 | 10.0 | 30.3 | 36.0 | 0.993 |
| IL-1β | 14.6% | 1.6 | 0.8 | 16.2 | 2.6 | 48.0% | 2.4 | 1.5 | 1.6 | 0.41 | 0.092 |

*Table 2 :* Sensitivity and statistical analysis of cytokine results in the patient group with PID Chl

| Cytokine | PID group with Chl (N = 10) | | | | | PID group without Chl (N=40) | | | | | P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Detectability | Max | Min | X sr | SD | Detectability | Max | Min | X sr | SD | |
| IFN-ϒ | 32.2% | 117.4 | 16.4 | 52.4 | 47.1 | 32.4% | 22.0 | 9.1 | 14.0 | 5.0 | 0.010 |
| IL- 1β | 28.6% | 2.6 | 1.8 | 1.62 | 0.42 | 43.8% | 3.9 | 1.9 | 1.7 | 0.40 | 0.617 |

*Table 3 :* Sensitivity and statistical analysis of cytokine results in the patient group with PID and negative finding of BV and Chl

| Cytokine | PID group (N = 16) | | | | | PID group without BV - Chl (N=34) | | | | | P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Detectability | Max | Min | X sr | SD | Detectability | Max | Min | X sr | SD | |
| IFN-ϒ | 37.2% | 135.4 | 26.4 | 62.4 | 58.1 | 29.4% | 29.0 | 8.1 | 14.0 | 5.0 | 0.012 |
| IL- 1β | 16.3% | 1.55 | 0.75 | 15.8 | 2.65 | 46.1% | 2.38 | 1.34 | 1.65 | 0.61 | 0.091 |

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# Evaluation of Roasting and Brewing effect on Antinutritional Diterpenes-Cafestol and Kahweol in Coffee

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*Abstract –* Coffee brew prepared from roasted coffee beans contains quite a lot of compounds which are known to influence consumers health. Among these, cafestol and kahweol are associated with lipid fraction of coffee and reported to be responsible for elevated serum cholesterol levels in people who drink more coffee. Aim : A study has been taken up to find out the influence of roasting and brewing methods on antinutritional diterpenes, in coffee brew. Methodology : Coffee bean samples were roasted at different temperatures and the brew prepared from these beans was analyzed for cafestol and kahweol profiles by using HPLC. Similarly coffee brew was prepared by mocha, filter, espresso, french press etc., and their diterpene profiles were analyzed by HPLC. Results: There was a substantial difference in cafestol and kahweol profiles in brews with highest content of cafestol and kahweol in Turkish-style and French press coffee. Similarly higher roasting temperatures and prolonged roasting times had significant influence on diterpenes profiles in roasted beans.

*Keywords :* *Arabica, Brew, Cafestol, Coffee ground, Kahweol, Robusta.*

*GJMR-J Classification:* *NLMC Code: WB 438, WT 115*


*Strictly as per the compliance and regulations of:*

# Evaluation of Roasting and Brewing effect on Antinutritional Diterpenes-Cafestol and Kahweol in Coffee

V.Sridevi [α], P.Giridhar [Ω], G.A. Ravishankar [β]

*Abstract -* Coffee brew prepared from roasted coffee beans contains quite a lot of compounds which are known to influence consumers health. Among these, cafestol and kahweol are associated with lipid fraction of coffee and reported to be responsible for elevated serum cholesterol levels in people who drink more coffee.

Aim : A study has been taken up to find out the influence of roasting and brewing methods on antinutritional diterpenes, in coffee brew.

Methodology : Coffee bean samples were roasted at different temperatures and the brew prepared from these beans was analyzed for cafestol and kahweol profiles by using HPLC. Similarly coffee brew was prepared by mocha, filter, espresso, french press etc., and their diterpene profiles were analyzed by HPLC.

Results: There was a substantial difference in cafestol and kahweol profiles in brews with highest content of cafestol and kahweol in Turkish-style and French press coffee. Similarly higher roasting temperatures and prolonged roasting times had significant influence on diterpenes profiles in roasted beans.

Conclusion : The method of coffee brew preparation had significant influence on cafestol and kahweol content with maximum and minimum in french press and filter paper method respectively.

*Keywords : Arabica, Brew, Cafestol, Coffee ground, Kahweol, Robusta.*

## I. Introduction

Coffee is a non-alcoholic refreshing beverage that mainly keeps us awake and is reported to be good when used in moderate levels. Throughout the world it is consumed by up to 80% of the adult population. Earlier surveys reported consumers preference for espresso [1] over other types of coffee. The coffee bean mainly contains two major metabolites i.e. alkaloid caffeine and phenolic chlorogenic acids. It is a source of dietary minerals (such as magnesium) along with antioxidant polyphenols and in some countries coffee is the source of two-thirds of the population's antioxidant nutrient intake[2,3,4]. Drinking coffee has to be looked more from health point of view than as a just refreshing drink in view of researchers consensus on coffee drinking - longer term effect is that can raise LDL and total cholesterol [5]. The reason is likely to be the presence of two cholesterol-elevating diterpenes called cafestol and kahweol in coffee [6,7,8]. In view of their impact on lipid profiles of *coffea* consumers both cafestol and kahweol are considered as anti-nutritional factors which are unique to coffee. Cafestol concentrations range between 0.15 -0.37% d.m. of beans in robusta and between 0.27% to 0.67% d.m. of beans in Arabica. Similarly kahweol levels are of 0.11-0.35% d.m. and < 0.1% d.m. in Arabia and robusta beans respectively [9]. Variation in the content of these two diterpenes in different Coffee species was documented [10] along with influence of geographical distribution [9]. Researchers have attempted to find out the changes in cafesol and kahweol content in coffee brew prepared by various methods [11, 12, 13, and 14]. In general brewing releases oil droplets (lipid fraction of beans) containing diterpenes from ground coffee beans which are either retained by paper filter paper or directly passes to the brew depending on method of brew preparation. The chemical composition of the roasted beans is very essential as it is one of the major factor related to quality of coffee, which, in turn, is affected by the chemical composition of the green beans and by post-harvesting processing conditions. The influence of such an important step in Coffee processing i.e. roasting on caffeine profiles, coffee flavor and aroma along with biogenic amines have been well documented [15, 16], But similar studies pertaining to roasting influence on diterpenes are not available. In view of the above a holistic approach on coffee diterpenes- cafestol and kahweol presence in coffee beans is warranted mainly under the influence of various roasting methods and brewing methods.

## II. Aim

A study has been taken up to find out the influence of roasting and brewing on cafestol and kahweol profiles in coffee beans.

## III. Material and Methods

### a) Collection of Coffee beans

Freshly harvested coffee beans of both Arabica (parchment) and Robusta (cherry) were purchased from

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Mudramane coffee curing works, Mudramane, Chickamagalur District, Karnataka. The Roaster model used for performing roasting was Neuhaus Neotec (Germany, Year 2005. Maschinen- und Anlagenball GMBH, Type-RIB-L Signum) and Drum Roaster (Probat-Werke, Type-BRZ6, Emmerich- Rheii, Germany). The roasting of seeds were performed at Coffee Board, Bangalore by setting different temperature and time intervals.

*b) Roasting of coffee beans*

*C. arabica and C. canephora* seed samples (1 kg each) were weighed and roasted. After roasting the weight loss observed was 2%. Roasting of seeds was performed at different temperatures as prescribed for different types of roast, i.e., light roast (220˚C – 150 sec), medium roast (228˚C – 660 to 720 sec), high roast (260˚C – 160 to 170 sec), normal roast (240˚C – 600 sec), city Roast (400˚C – 90 sec), full city Roast 445˚C – 60 sec). To get coarsely ground powder these roasted samples were ground into fine powder by Ditting grinder, machine and known quantity of ground coffee (Arabica or Robusta) was used for brewing methods and extraction of diterpenes.

*c) Coffee Brew preparation*

Various methods of coffee brew preparation that pertaining to India and to other countries viz., Espresso, drip, Mocha, Indian Filter method, Turkish-style, French press, Drip filter method, have been selected. As per International Agency for Research on Cancer (IARC) [17] guide lines, the prescribed quantity of ground coffee was taken and accordingly the brew was prepared with known quantity of demineralised water. Later respective brews were cooled to room temperature in an ice bath and stored at 50° C until required for pH determination and analysis for cafestol and kahweol. For this experiment, the coffee ground was brewed in triplicates. The amount of ground coffee taken and the quantity of water used for obtaining known volume of brew varies with brewing method (Table 3). In brief the methods used for brew preparation are: Espresso method uses 30 pounds of pressure for tapping 9 bars of decoction. The flow rate through the reservoir for every 10 grams of powder taken will be 60 ml per 30 sec. Crema formed for Robusta coffee was observed to be Reddish brown which is its characteristic feature,(The espresso machine Model Astoria CC, A 240 N° 40039s Mod- SAE.I2-4L, HZ 50/60. Italy N 4200). The standard volume according IARC [17] is 30 ml per cup. In percolation method Coffee grounds were made by passing the grounds through a ditting grinder-6.0, with a sieve size of 700 $\mu$m and in Mocha method Ditting grinder-2.5, with sieve size of 800 $\mu$m were used. The standard volume for mocha method according to IARC is 60 ml per cup. Similarly for filter (used commonly in India) method coffee beans were ground with Ditting grinder -1.0, with 300 $\mu$m sieve. The brew was prepared by adding 30g of ground coffee powder to the 150ml boiling water and kept aside for 15min and slowly brew is collected in the vessel. To prepare brew by French press, the fine grounds were made by passing beans through a Ditting grinder-6.0, with 800 $\mu$m sieve. 150ml of water for every two table spoons of coffee powder (16g) was used in any event, coffee was measured after it was heated to the boiling. In Electric Drip Filter method, grounds were made through ditting grinder- 3.5 with a sieve size of 600 $\mu$m. Amount of water and ground coffee must be measured carefully to get. The standard volume 150ml per cup [17]. The Manual filter involves pouring hot water into a filter containing the coffee ground, which then drips into the cup or carafe. Filtered water at about 95°c was used to have fast dripping to get a standard volume of 150ml per cup. For this purpose paper filter of prescribed grade was (No.4, Filter a cafe blanc, Grand Jury, France). To obtain brew by Turkish method coffee was prepared by boiling a mixture of 5g roast and ground coffee, 10 g sugar and 60ml cold water. The grounds were prepared by passing the appropriate beans through Ditting grinder-1 with a sieve size of 250 $\mu$m. The brew collected in this method was a standard volume of 30 ml per cup. The pH of respective brewed coffee sample (prepared by different methods) was measured with a pH meter (Control Dynamics, Digital pH meter, APX 17, 175). The total solids content of prepared brew samples was analysed by using refractometer. A known quantity of each brew sample in quadruplicates was used and the ˚Brix value was recorded.

*d) Extraction and HPLC analysis of cafestol and kahweol*

Powdered samples of 10g each was transferred into the individual thimbles and extracted with tert-but-methyl ether as a solvent for 6h by using a soxhlet. The solvent was evaporated from extract and residue was dried in oven to get constant weight. Then the residue was unsaponified after extraction [18]. HPLC analysis was performed on Shimadzu LC 20 A (Shimadzu Corp., Kyoto, Japan) equipped with CLASS-VP integrator software for data processing. HPLC Separation was performed on Nucleosil column 120-3 C18, 250/4 (Macherey – Nagel, Gmbh, Germany) with UV absorbance at 230nm for cafestol and 290nm for kahweol. The mobile phase used was Acetonitrile: water: glacial acetic acid (70/29.5/0.5 v/v) with a ~ pH 3.1 and flow rate of 0.6 ml/min for 30 min [18]. The standards and their dilutions used for HPLC analysis were same as used for spectrophotometer analysis. The identification of compound was based on peak elution of compound i.e. retention time (RT) comparison and co-elution with authentic standards (Sigma-Aldrich, USA). Five replicates for each brew were analysed.

## IV. Results and Discussion

*a) Influence of roasting on cafestol and kahweol*

A perusal of table 1 indicates the significant influence of roasting methods on diterpene profiles in coffee beans. In Arabica coffee the highest concentrations free form of cafestol and kahweol were observed in light roast followed by medium and high roasts (Table 1). In light roast 622±5.29mg of cafestol and 453±8.62mg of kahweol per 100gms were found. As the roasting temperature increases there was a significant fall in both cafestol and kahweol profiles. Accordingly in full city roast there was ~56% reduction in cafestol and 61% reduction in kahweol compared to normal roasted beans. A similar trend was noticed in robusta beans though the diterpene profiles were less compared to Arabica. The highest percentage of cafestol (363.3±8.0mg per 100g) and kahweol (313±4.93mg per 100mg) respectively were found in light roast. But the difference in cafestol concentrations among medium, high and normal roasting was insignificant. In robusta full city roast the cafestol and kahweol profiles showed ~44% and 10% increase respectively compared to full city roast of Arabica beans (Table 1).

*b) Influence of brewing method on free form of cafestol and kahweol*

The method of coffee brewing had significant influence on total solid content in coffee brew of both Arabica and Robusta coffee (Table 2). The total solid content given as ◦brix in Table 2 for different volumes of brews in various methods of brew preparation. Highest solid content was evident in espresso for both Arabica **(5.4±0.4)** and Robusta **(4.5±0.26),** followed by brew prepared by Turkish-s style, mocha as per weight to brew obtained. In all other methods viz., filter paper, drip method the solid content was less. Analysis of cafestol and kahweol profiles in brewed samples (Table 3) indicates significant variation in their content, with highest levels of cafestol **(19.7±1.6mg)** in French press coffee followed by Turkish style **(7.3±0.72mg)** per cup. The kahweol levels are more or less same in both French press and Turkish style coffee which were maximum compared to brew prepared by other methods. In filter paper, Indian filter and drip method based brews, the cafestol and kahweol levels were found to be very less.

The absorption maximum for cafestol and kahweol were 220 and 280 nm respectively in spectrophotometric method and the sensitivity of detection of respective standard samples was good. In the present study we have used this only for screening the samples for the presence of cafestol and kahweol. The levels of free diterpenes cafestol and kahweol were analysed and quantified by HPLC in both Arabica and Robusta coffee beans. The elution of kahweol was good at 290 nm with a retention time of 7.5 min and for cafestol at 230nm,RT was 11.97 min. Diterpene extracts from beans and brew when subjected to HPLC analysis, in all the samples cafestol and kahweol (free forms) were detected at respective retention times in accordance with reference standards. Initially in order to standardize the cafestol and kahweol identification, the resolution of both these compounds in all extracts was evaluated as well as their simultaneous determination. The maximum resolution was evident at 230 nm for cafestol and 290 nm for kahweol, as both these compounds are having maximum structural similarity except the presence of one double bond in the kaurene ring of kahweol.

Both cafestol and kahweol profiles varied in different coffee brews. Similarly reduction in free diterpenes profiles in both Arabica and Robusta ground coffee that prepared by roasting at various temperatures was observed in our study, and the increase in roasting temperatures might be a reason. Because changes in roasting time and temperatures seem to effect CGA and caffeine contents significantly in the final coffee products [19,20 ] especially at higher roasting temperatures. Such variations may explain the major differences in pH and CGA content found among the commercial coffee tested. According to earlier report [21] there was a significant reduction in CGA levels by ~ 60% for light, 67% for medium, 88% for dark and 96.5% for very dark roast in Arabica coffee. A similar trend was also noticed in our study wherein, both cafestol and kahweol were found to be reduced by 56% and 61% in Arabica full city roast and 44% and 10% in Robusta full city roast respectively. Though there were any substantial evidences and reports available for this reduction at higher roasting temperatures, the same reasons for a similar observations for caffeine and CGA [21] might also be a reason for cafestol and kahweol in our study. The total solids content ($^0$brix) showed variation in different brews. In our study, the variation in total solids content of espresso and other methods is evident and also there was a difference in total solids content between Arabica and Robusta coffee. This may be attributed to the coffee ground coarse. A similar observations in total solids content in different brews was reported while analysing caffeine content in Coffee brew [22, 23]. Moreover, the extraction of coffee metabolites such as caffeine was dependent upon the time of brewing. The longer brew time implies longer contact time between the water and coffee grounds leading to more complete caffeine extraction of compounds and more solids, though the more solid matter pertains to fine ground coffee compared to coarse ground coffee [24]. The initial moisture content of the green coffee beans prior to roasting was ~12.5% and the pH of the coffee brew prepared from roasted seeds in our study was in the range of 5.5-5.65 which is in concomitant with earlier reports (23). In general the minor change in pH of the brew happens due to change in roasting time. In the present study, for preparing

brews we have followed normal method of roasting (240˚C, 600sec) due to this there was no significant change in pH of the brew [23]. In general several differences exist in the preparation of coffee which may influence consumption of different metabolites of coffee such as caffeine and CGA [20]. In addition the prepared coffee brew volume, ground coffee to water ratio also would influence the coffee metabolites in brew [24]. A similar effect on diterpenes profiles is the reason for variation of cafestol and kahweol profiles in different coffee brews in our study. Apart from this, increase in the amount of coffee ground taken for preparing brew certainly responsible for quantitative variation of cafestol and kahweol in respective brew samples in our study. The method of preparation of the brew is a critical determining factor in determining the daily intake of these diterpenes from coffee consumption [7]. Higher concentrations of cafestol and kahweol in coffee brews prepared by French press and Turkish method in our study is further supported by a similar studies with reference to caffeine and CGA in Coffee [20,24] wherein, the levels of caffeine are more in boiled coffee than filtered coffee though again the particle size of ground coffee also maters for this. Total diterpene content of brewed coffee was reported earlier, but the methods used were of inadequate sensitivity or specificity for application to measurement of individual diterpenes in brews [12, 24]. Urgert et al. [13] developed a simple and sensitive method of reverse phase HPLC method using solid-phase extraction procedures for cafestol and kahweol analysis. The higher levels of cafestol and kahweol in Turkish and French press coffee in our study was in accordance with earlier report [7] wherein, both the diterpenes per cup were at higher level in boiled Scandinavian coffee (7.2 mg each of cafestol & kahweol ) and Turkish coffee (5.3 mg of cafestol & 5.4 mg of kahweol) respectively. This may be due to higher amount of fine particles (solids) present in Turkish-style coffee, compared with boiled coffee and other brews [7]. Similarly insignificant levels of cafestol and kahweol were detected in our study in filter paper method, which is in accordance with earlier observations [25]. Thus the method by which coffee brew is prepared and decanted may have a great influence on its diterpene content [13]. The levels of cafestol and kahweol in espresso were more in our study compared to that of gross et al [7], but in concomitant with the study of Ratnayake et al [12] The reasons for this not clear, but might be due to the influence of steam pressure, contact of steam with ground coffee and its contact timing, efficacy of filter used. Though the values of cafestol and kahweol in our study in different brews were slightly more than that of earlier report [7] the trend was same. This variation may be attributed to coffee ground particle size, water content used for making brew, the roasting temperature used for roasting etc. The preference for specific type of brew varies with individual cultural preferences. Especially the pure coffee brew is a choice of many consumers in the World, but in India majority prefer coffee blended with chicory, hence, the levels of cafestol and kahweol in filter coffee brew would be obviously less. In mocha brew which is favored for its taste, contains more levels of these two diterpenes due to the presence of crema (rich in lipid fraction). So the influence of cafestol and kahweol on consumers health depends on the type of brew and the quantity of brew consumed.

## V. Conclusion

The method of coffee brew preparation had significant influence on cafestol and kahweol content with maximum and minimum in french press and filter paper method.

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*Table 1 :* Diterpenes profiles in various roasted coffee samples

| Roasting Type | Amount of Powder (gm) | Diterpene Levels (mg/100 g) | | | |
|---|---|---|---|---|---|
| | | Arabica | | Robusta | |
| | | Cafestol | Kahweol | Cafestol | Kahweol |
| Light Roast | 10 | 622± 5.29 | 453.3±8.62 | 363.3±8 | 313±4.93 |
| Medium roast | 10 | 559 ± 4.5 | 363±8.54 | 352.6±8.7 | 264±6.5 |
| High roast | 10 | 519± 9 | 354.3±12.34 | 354.3±8 | 210±5.8 |
| Normal roasting | 10 | 421± 4.16 | 336.3±4.72 | 361±4.35 | 144±6.14 |
| City Roast | 10 | 226 ± 8.0 | 209±6.55 | 290.6±7 | 183.6±9 |
| Full city Roast | 10 | 186.6± 8.02 | 132±6.55 | 281±6.5 | 146±5.56 |

Values are mean± S.D. of three samples

*Table 2 :* The total solid content and pH of different coffee brews

| Brewing method | Volume of brew (ml) | Refractometer (total solids content) $^0$Brix | | pH |
|---|---|---|---|---|
| | | Arabica | Robusta | |
| Turkish-style | 60 | 2.3±0.01 | 2.69±0.07 | 5.57 |
| Indian Filter | 150 | 2.37±0.02 | 3.27±0.03 | 5.6 |
| French press | 60 | 2.93±0.02 | 2.8±0.06 | 5.65 |
| Espresso | 60 | 5.4±0.4 | 4.53±0.06 | 5.62 |
| Mocha | 60 | 4.2±0.2 | 4.4±0.02 | 5.68 |
| Filter Paper | 150 | 3.23±0.25 | 2.41±0.07 | 5.4 |
| Electrical drip machine | 150 | 4.26±0.25 | 4.0±0.4 | 5.6 |

Values are mean± S.D. of three samples

*Table 3 :* Diterpene profiles in various brewed coffee samples

| Brewing method | Brew obtained (ml) | Cafestol (mg/cup) | % cafestol | Kahweol (mg/cup) | % kahweol |
|---|---|---|---|---|---|
| Turkish | 60 | 7.3 ±0.72 | 0.14 | 8.3±0.30 | 0.16 |
| Espresso | 60 | 6.0±0.8 | 0.06 | 5.1±0.45 | 0.051 |
| Mocha | 60 | 6.86±0.45 | 0.034 | 4.6±0.87 | 0.023 |
| Indian filter | 150 | 1.18±0.42 | 0.034 | 0.86±0.35 | 0.008 |
| French Press | 150 | 19.7±1.6 | 0.12 | 17.2±0.40 | 0.11 |
| Filter paper | 150 | 1.33±0.94 | 0.005 | 0.37±0.03 | 0.001 |
| Electrical drip | 150 | 1.76±0.65 | 0.005 | 0.62±0.09 | 0.007 |

Values are mean± S.D. of three samples

*Coffee cup sizes : 150 ml for filtered, electrical dip, 60 ml for Turkish, Espresso, Mocha coffees
Sample weight in g : Turkish (5), Espresso and Indian Filter (10), Mocha (20), French press (16), Filer paper (25), Electrical drip (30). % values means g/ 100 g coffee powder

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# Photodynamic therapy and Green Laser blood Therapy

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*Abstract –* Background: In vitro irradiation of human blood with laser light is under investigation for years, to study the biostimulatory effects on various blood cells. However, any positive effects of this light on the rheology of platelets have not been documented with authenticity due to lack of research. Methods: In our present study, we investigated the influence of different levels of laser on the damage threshold of blood cells .Laser diodes were used as a source of radiation in different levels of irradiation protocol. Blood was taken from one hundred adult patients. After adding anticoagulant (EDTA), the samples were divided into four groups for irradiating with different laser intensities. And each sample was subdivided into two, so that one was irradiated and the other considered as control sample. The samples were made to stand for 30 minutes before determining the change in rheological properties of blood cells. Results: It was established that low level laser therapy when used on human blood in vitro, affects the rheology of erythrocytes and leucocytes. It was observed that it changes the erytherocytatory, leucocytatory, BSR, aggregability indices of blood .Conclusions Thus it was concluded that low level laser therapy can affect the physical as well as chemical properties of blood cells which is not only helpful in preservation of blood but also in revitalizing the physically and chemically stressed erytherocytatory membranes. It was determined that the laser therapy decreases the viscosity of blood thus increasing the electrophoretic mobility of erythrocytes.

*Keywords :* *Erytherocytatory, Aggregability, leucocytatory, biostimulatory , Laser blood Therapy.*

*GJMR-B Classification:* *NLMC Code: WO 511*


*Strictly as per the compliance and regulations of:*

# Photodynamic therapy and Green Laser blood Therapy

Zahra Al Timimi[α], M.S. Jaafar[Ω], Mohd Zubir Mat Jafri[β]

*Abstract* - Background: In vitro irradiation of human blood with laser light is under investigation for years, to study the biostimulatory effects on various blood cells. However, any positive effects of this light on the rheology of platelets have not been documented with authenticity due to lack of research. Methods: In our present study, we investigated the influence of different levels of laser on the damage threshold of blood cells .Laser diodes were used as a source of radiation in different levels of irradiation protocol. Blood was taken from one hundred adult patients. After adding anticoagulant (EDTA), the samples were divided into four groups for irradiating with different laser intensities. And each sample was subdivided into two, so that one was irradiated and the other considered as control sample. The samples were made to stand for 30 minutes before determining the change in rheological properties of blood cells. Results: It was established that low level laser therapy when used on human blood in vitro, affects the rheology of erythrocytes and leucocytes. It was observed that it changes the erytherocytatory, leucocytatory, BSR, aggregability indices of blood .Conclusions Thus it was concluded that low level laser therapy can affect the physical as well as chemical properties of blood cells which is not only helpful in preservation of blood but also in revitalizing the physically and chemically stressed erytherocytatory membranes. It was determined that the laser therapy decreases the viscosity of blood thus increasing the electrophoretic mobility of erythrocytes.

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## I. Introduction

The objective of my study is to determine the effects and advantages of green laser pointer 532nm on the rheological properties of human blood in vitro. Researching the bio stimulatory effects of Low level laser therapy on rheological properties of blood cells is an area of great interest for hematologists. Four important effects of low level laser light have already been reported in the scientific literatures which are tissue regeneration, reduction of inflammation, pain relief and immune system enhancement.

The term Photodynamic therapy denotes the in vitro therapy of blood cells which is done to change the rheological properties of blood cells, when preserved for transfusion purposes. The underlying mechanism is that when blood is irradiated with low level laser in an oxygen rich environment, porphyrins absorb energy from photons and transfer this energy to the surrounding oxygen molecules.

Porphyrins are a component of hemoglobin which carries oxygen to various tissues of the body. When porphyrins are not a component of hemoglobin anymore, as in preserved blood, they absorb light.

Photodynamic therapy involves the use of photoactive drug (photosensitizer) and light which is typically visible or infrared light. When light is absorbed by porphyrin molecules, a chemical reaction is initiated which leads to direct and indirect production of cytotoxic radicals and singlet oxygen (Maiya 2000; Brancaleon and Moseley 2002) . These toxic chemicals once formed, damage the proteins, lipids, nucleic acids and many other particles of blood without causing any damage to the surrounding irradiated blood components which are PS-free. For example viruses can be killed in whole blood without destroying blood components. (Henderson and Dougherty 1992; Sitnik, Hampton et al. 1998; Maiya 2000; Castano, Mroz et al. 2006; Morton, McKenna et al. 2008; Wilson and Patterson 2008) .

Weber in 2005 used a green laser light for the first time for intravascular blood treatment. The basic idea was to increase the energy assimilation of blood by the absorption of green laser light as a complementary color to red light (and red color of erythrocytes). With intravascular positioning of the red light catheter, it was observed that a red spot shines spontaneously through the skin, when the red light was switched on, due to the light reflecting property of hemoglobin. Whereas, no green spot appeared on the skin by switching on a green laser light with a wavelength of 532 nm, as the laser light of this wavelength is almost completely absorbed by hemoglobin. This laser irradiation therapy was introduced for the first time by Weber for the treatment of many diseases. A comparative study between red and green laser light was also conducted, by treating those patients with green laser irradiation who had already been treated with red laser previously.

After this development in the field of low level laser therapy, 20 liver patients and 20 lip metabolism patients were treated with mere green laser light successfully, demonstrating more acceptable results than red light therapy. At that time the effects of green laser on the rheological properties of blood were discovered which were more beneficial than red light.(Weber, Fu ganger -May 2007)

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Following effects of green laser irradiation on blood cells have been observed;

- Absorption of the green light quants by haemoglobin,
- Absorption of the green light by different Cytochromes, Katalases und Peroxidases,
- Stimulation of electric activity of the erythrocyte membrane potential
- Activation of the membrane potential of the mitochondria

There are many different views about the intensity of laser light that is used to treat blood in vitro. The effluence rate of laser which is used to activate the toxic radicals in the blood should certainly be lower than the damage threshold of surrounding vital tissue components. Whereas according to Fischer and Aulmann (1998) most of the time it is desirable to use the highest possible effluence rates in order to achieve maximum effects of photodynamic therapy.

## II. Material and Methods

### a) Materials

During this research diode laser pointer 532 was used as the irradiation source with a wavelength of 532nm and a low power of 100mw. Unlike ordinary light, laser is a high energy device and emits photons on only one direction.

The apparatus used to measure values of the irradiated and non-irradiated blood samples was automate hematology analyzer machine (Sysmex XE - 2100). It is a machine that is used for measuring various chemicals and other properties in many biological samples. It is a quick method and requires almost no individual assistance. This method has many advantages. For example the blood samples can be read in batches or otherwise solely if needed. Thus it assists in research sample readings where a large number of samples are to be read. In blood analysis, the automate hematology analyzer machine is used to measure complete blood count, erythrocyte sedimentation rate and or coagulation profile.

For measurement, dilute samples of blood were passed through an aperture. Electric current was also passing through it. The flow of current brought a variation in the impedance between the ends. Then a lytic reagent for breaking red blood cells was added in the solution. It did not affect the white blood cells and platelets leaving them intact.

### b) Blood collection

This research was conducted on one hundred blood samples which were collected under the guidelines of National Medical Research from pathology lab in PULAUPINANG GERERAL HOSPITAL .This study was approved by the national institute of health for conducting research in the Ministry of Health Malaysia and also by the Committee of Medical Research and Ethics. Hundred pathological samples, 5ml each, were obtained from healthy and non healthy adults (all above 18 years age) with different medical histories. The samples were divided into four groups to determine the effect of different levels of laser therapy. After collection of the blood samples, an anti-coagulant potassium ethylenediaminetetreacetic acid (K2/EDTA) (Vacationer, BD Franklin Lakes NJ USA), was added to prevent coagulation. It is a poly amino carboxylic acid which has both in vivo and in vitro applications. It is the most widely used anticoagulant for complete blood count. Each blood sample was further divided into two halves (2.5ml each) and one of them was irradiated whereas the other was kept as control. This control was done to check for blood damage due to the irradiation system (Vacationers, etc.)

### c) Laser Irradiation

All the four major groups were irradiated with Green diode laser with a wavelength of 532 nm at 100mw in a continuous wave mode, with divergence < 1.5mRad, Beam Mode (TEMoo), Beam diameter at aperture ~1.5, Crystal type Nd:VYO4:KTP, Power Source 1 x 3V CR2 Alkaline batteries. The power density was 509.55mW /cm2 at a Distance of 6.5 cm from the laser device from blood inside the tube, and diameter of the laser spot was set 0.5 cm. Samples were irradiated in different time periods at energy effluence of 0.5j/ cm2, 1.5j/ cm2, 3j/ cm2 and 5j/cm2 for the first, second, third and fourth groups at 1, 3, 6, and 10 sec. respectively.

### d) Method

A diode laser pointer which was used during research was a laser pointer. All the irradiated and non-irradiated samples of blood were allowed to stand for about 30 minutes at room temperature, before counting was done. Blood counts were then performed both before and after the irradiation.

The method used to measure values of the irradiated and non-irradiated blood samples was automating hematology analyzer machine. It is a machine that is used for measuring various chemicals and other properties in many biological samples. It is a quick method and requires almost no individual assistance. This method has many advantages. For example the blood samples can be read in batches or otherwise solely if needed. Thus it assists in research sample readings where a large number of samples are to be read.

In blood analysis, the automate hematology analyzer machine is used to measure complete blood count, erythrocyte sedimentation rate and or coagulation profile.

### e) Complete blood cell measurements

For measurement, dilute samples of blood were passed through an aperture. Electric current was also passing through it. The flow of current brought a variation in the impedance between the ends. Then a

lytic reagent for breaking red blood cells was added in the solution. It did not affect the white blood cells and platelets leaving them intact. Then these solutions were passed through another detector this getting the measurements of red blood cells, white blood cells and platelets.

The counter was designed for measuring white blood cells (WBC), red blood cells (RBC), and hemoglobin content (HGB), hematocrit (HCT); mean (red) cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and platelets (PLT), Neutrophil (NEUT), Lymphocytes (LYMPH) and Monocytes (MONO).

- WBC white blood was analyzed by the flow cytometry method using semiconductor laser.
- Red Blood Cell count was analyzed by the RBC detector by Hydro Dynamic Focusing method (DC Detection)
- Hemoglobin (HGB) by the HGB detector based on the SLS hemoglobin detection method
- Hematocrit (HCT) by the RBC cumulative pulse height detection method,
- MCHC was calculated with RBC, HGB and PLT by the Hydro Dynamic Focusing method (DC Detection) or flow cytometry method using semiconductor laser.
- Blood was kept on a shaking device at room temperature 25 c during a sequence of measurements.

## III. Statistical Analysis

Statistical analysis was accomplished by using a paired test to analyze the mean and standard deviations of different experimental groups. The null hypothesis was for no statistical difference between the means of the different groups. (H0: M1=M2=M3 where M1, M2, M3 are the mean of the experimental groups). A significant difference was accepted between the means when the P value was less than 5 % (P< 0.05)

## IV. Results and Formats

The results of our research showed the effect of low level laser light on the rheology of different blood cells as well as a change in the number of cells as below:

*a) In Irradiated groups*

i. *Red blood cells*

3, 5 j/cm2 irradiation group showed a significant increase in the red blood cells of male patients ($p<0.05$).

0.5j/cm2 irradiation group showed p= 0.00

ii. *Hemoglobin*

3, 5j/cm2 irradiation group showed an increase in hemoglobin.

0.5j/cm2 irradiation group showed p=0.00

iii. *Hematocrit*

Only 0.5j/cm2 irradiation group showed a significant increase in hematocrit ($p<0.05$)

Thus it is evident from the above results at very low effluence of 0.5j /cm2, only a change in hematocrit is possible while 3 and 5j /cm2 increase the red blood cells and hemoglobin.

The test results showed the following changes in irradiated groups:

The increase in white blood cells and red blood cells seen with 3 and 5 j /cm2 was two times the increase seen with 0.5 and 1.5j/cm2 groups. Similarly HGB increased with 0.5, 3 and 5j /cm2 but decreased two times in 1.5j /cm2 group and the same change was seen in non-irradiation group.

HCT increased to double in 0.5 and 5j /cm2 group where decreased in 3j /cm2 group and even more in 1.5j /cm2 group.

Neutrophils increased to double in 0.5, 3 and 5j /cm2 group but decreased in 1.5j /cm2 group.

Lymphocytes increased in 0.5, 3 and 5j /cm2 group but double increased in 1.5j /cm2 group.

MCV and MCHC increased to double in all the groups.

Platelets double decreased in all groups except in 5j /cm2 in which no change was observed.

These results show a positive effect of 5j /cm2 effluence power on almost all the cells under investigation, whereas the other three intensities show a variation their effects on different indices.

Irradiation groups with different laser effluence showed the following results with gender differences:

*b) In males*

In 0.5 j/cm2 group HGB, RBC's and HCT increased significantly
In 1.5 j/cm2 group HGB, RBC's and HCT increased significantly
In 3j/cm2 group HGB and RBC's increased significantly
In 5j/cm2 group HGB and RBC's increased significantly.

*c) In females*

In 0.5 j/cm2 group RBC's and HGB increased significantly whereas HCT decreased non-significantly.

In 1.5, 3, 5 j/cm2 groups RBC's, HGB and HCT decreased non-significantly.

When the above information was extracted from the results on gender basis, it became evident that some gender difference is also an important factor in determining the efficacy of low level laser therapy on blood.

## V. Discussion

The main objective of this study is to explore the bio stimulatory effect of low level laser on human blood

samples. We conducted this research to determine the effect of low-level laser therapy (LLLT) on some rheological properties of human blood in vitro by using laser pointer 532 nm, low power 100mw.It also aims to evaluate the effect of this therapy on reducing inflammation be demonstrating the transformations of blood cells, the effect of LLLT dose response of blood cell and the changes in blood cell counts.

Laser therapy is applied on body tissues which may be cells or culture to bring a change in tissue functions and properties. More than 130 double blinded studies have confirmed the therapeutic benefit of low level laser therapy. Laser therapy is a matter of dose and treatment technology as it is with any other therapy. The power output of laser is important especially for dose calculation. The depth of penetration is dependent on wavelength of light.

The objective of our research was to determine the effects of green laser light on the rheology of different blood cells, in vitro. We evaluated the counts of red and white blood cells, HGB, HCT, MCV, MCHC, PLT and neutrophils.

We demonstrated many beneficial effects of green laser light irradiation on erytherocytatory, leucocytatory, aggregability indices. The bio stimulatory effect of Low level laser therapy on red cells was seen with changes in cell membranes, thus increasing the red cell functionality.The physically and chemically stressed erythrocyte membranes can be revitalized and brought back to functionality for performing its oxophoric function in transfusion reactions.

From the results of our research, we can say that low level laser therapy affects various rheological properties of different blood cells for example red cell deformability, aggregation of cells, critical stress on the cells during preservation time, leucocytarory, erytherocytatory indices, ESR etc.

## VI. Conclusion

The best effluence power that has a positive effect on almost all blood cells and indices is 5j /cm2. It increased white blood cells, red blood cells, hemoglobin with a non-significant decrease in hematocrit. Thus from our research it is proved that low level laser therapy with diode laser 532nm and a high power of 100 mw is advantageous for revitalizing the functional capability of preserved blood and also increases the number of blood cells, thus increasing the function of this blood when injected to recipient.

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# Primary Patency Rate of Native AV Fistula: Long Term Follow up Primary Failure / Vascular Access

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*Abstract –* The number of end stage of renal disease patients that need dialysis or renal transplantation increased in the world. Insertion and maintenance functional vascular access remain the challenging problem. Arteriovenous fistula is the common access for dialysis but complication and its failure is the main problem. The aim of this study is to evaluate patients with arteriovenous fistula during 4 years and describe the probable influenced factors on fistula patency. In this analytical descriptive study, we fallowed 245 patients during 4 years and evaluated them for primary failures and effective factors on vascular patency. The patients were asked about demographic data, how to caring condition arteriovenous fistula, dialysis and complications. The mean age of the patients was 47.77 years. The underline diseases were hypertension (43.3%), hypertension and diabetes mellitus (21.2%) and diabetes mellitus (4.5%). According Log rank test there were meaningful results between arteriovenous patency with sex and dialysis ($P<0.05$). Our result of primary patency at 6 months, 1, 2, 3 and 4 years for all patients were 79.5%, 70%, 65%, 60.5% and 48%. Our study showed dialysis could increase the fistulapatency rate. Other factors were not associated with primary patency. It seems ESRD patients undergoing dialysis have better fistula patency, may be due to homeostasis abnormalities induced by their particular conditions.

*Keywords :* *Dialysis, Arteriovenous fistula, primary failure, End stage renal disease, patency.*

*GJMR-B Classification:* *NLMC Code: WG 590*


*Strictly as per the compliance and regulations of :*

# Primary Patency Rate of Native AV Fistula: Long Term Follow up Primary Failure / Vascular Access

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*Abstract -* The number of end stage of renal disease patients that need dialysis or renal transplantation increased in the world. Insertion and maintenance functional vascular access remain the challenging problem. Arteriovenous fistula is the common access for dialysis but complication and its failure is the main problem. The aim of this study is to evaluate patients with arteriovenous fistula during 4 years and describe the probable influenced factors on fistula patency. In this analytical descriptive study, we fallowed 245 patients during 4 years and evaluated them for primary failures and effective factors on vascular patency. The patients were asked about demographic data, how to caring condition arteriovenous fistula, dialysis and complications. The mean age of the patients was 47.77 years. The underline diseases were hypertension (43.3%), hypertension and diabetes mellitus (21.2%) and diabetes mellitus (4.5%). According Log rank test there were meaningful results between arteriovenous patency with sex and dialysis ($P<0.05$). Our result of primary patency at 6 months, 1, 2, 3 and 4 years for all patients were 79.5%, 70%, 65%, 60.5% and 48%. Our study showed dialysis could increase the fistulapatency rate. Other factors were not associated with primary patency. It seems ESRD patients undergoing dialysis have better fistula patency, may be due to homeostasis abnormalities induced by their particular conditions.

*Keywords : Dialysis, Arteriovenous fistula, primary failure, End stage renal disease, patency.*

## I. Introduction

In patients suffering from renal failure, we can use hemodialysis or transplantation. This disorder has significantly increased from 209,000 in 1991 to 472,000 cases in 2004 in the USA [1]. The incidence of end stage renal disease (ESRD) has been increased 43 percent based on age, gender, and race around the world since 1991 [2]. The patient physical state and other factors determine choice treatment. Although, creation of vascular access is a necessary maneuver for hemodialysis, creation and maintenance of a well-functioning vascular access are remained the most challenging problems for hemodialysis therapy [3]. The first access method was Brescia-cimino fistula which was introduced in 1966. In the first years, only young and healthy patients were candidates for AVF creation [4]. Nowadays, creation of arteriovenous fistula (AVF) is feasible in most cases including diabetics and old patients. Thrombosis and/or lack of maturation are the reasons of primary failure [5], but the risk factor for primary failures is not limited to these like the site and diameter of vessels are thought to fulfill an important role [6]. One study which was done from 1997 to 1999 showed the primary failure rate between 10- 20% [7]. A recent meta-analysis has demonstrated 15.3% primary failure rate for native AVF [8]. Proper access-site selection, improved surgical techniques and appropriate management of complications are the main factors for long-term success. However, other factors such as regular vascular laboratory surveillance, post-operative and central coordination by a dedicated access coordinator are equally important in ensuring a functional and cost-effective access [9]. There are different reports for the radiocephalic AV fistula patency in previous reported article: 85% survival in the first year and 80% in the second year [10]. Kalman and his colleagues showed a total patency including primary, assisted primary and secondary success rate of 66% for 2 years and primary patency rate of 36% for 2 years [9]. The aim of this study was to finding the possible factors influencing on the fistula patency.

## II. Methods

### a) Study design

This study is a descriptive-analytic single-center prospective study based on referral patients to vascular surgery clinic of a University Hospital, whom underwent primary arteriovenous fistula.

### b) Patient's selection

All of 245 patients were included in a program for the first time AVF access from 22 November 2005 to 22 November 2006. Exact analysis of the type and reason of the previously reported complications was used to design a new questionnaire for this study.

The necessary information including demographic data, the primary renal disease, type and length of anastomosis, surgical technique, type and diameter of the artery and vein, the immediate result of surgery and complementary supports gathered from patients' recorded data. All patients were followed by face to face visit or phone after 1 week, 1 month, 3

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months, 6 months and then 1, 2, 3 and 4 years of surgery. The patients were asked about functional AVF, complications, AVF caring, the time they started dialysis, the number of dialysis per week and dialysis center. In addition, personal factors such as smoking and training about AVF caring were questioned.

Any complications or AVF loss was recorded and finally the questionnaire was filled in to define the reasons. Questionnaire included questions about hard jobs, wearing tight clothes, blood Pressure of the same limb, sleeping on anastomosed arm, frequency of puncture, and using garo. Additional complications such as Steal syndrome, venous hypertension (HTN), infection, aneurism, heart failure and neuropathy were recorded. In this study from the total number of 245 patients, 197 ones remained available until the end of the study.

*c) Statistical Analysis*

The collected data entered into SPSS software version 11.5. Cox models and Kaplan-Meier curves were used to analyze the primary patency of vascular access.

## III. Results

The mean age of patients was 47.77 years (6 to 85 years). Considering gender, 148 (60.7%) were males and 96 (39.3%) were females (Table 1).

Most of our patients were under-educated (36.5%) and was non smokers (81.25%). The underling diseases in our patients included 43.3% HTN, 21.2% HTN and diabetes mellitus and 4.5% only diabetes mellitus. Other diseases detected in 10.6% of patients were included glumrolonephritis, polycystic kidney, uropathy, pyelonephritis and lupus erithematous.

Frequency of AVF creation of in non-dominant, dominant, right, upper and left limbs was 63.3%, 14.7%, 13.1% and 82.4%, respectively. The AVF creation sites were snuff box, wrist, forearm and elbow: 36.3 %, 6.5%, 9.8% and 38.8%, respectively.

*Table 1 :* Demographic data and history of 245 patients.

| Variables | | Value | % |
|---|---|---|---|
| Men/women | | 148/96 | 60.7/39.3 |
| Mean age (year) | | 47.77 | |
| Smoking | | 36 | 18.75 |
| Underline disease | HTN | 106 | 43.3 |
| | HTN + DM | 52 | 21.2 |
| | DM | 11 | 4.5 |
| | No disease | 26 | 10.6 |
| | missing | 16 | 6.5 |
| Limb of AVF | Dominant | 36 | 14.7 |
| | Not dominant | 155 | 63.3 |
| | missing | 54 | 22 |
| Limb of AVF | Right | 202 | 82.4 |
| | Left | 32 | 13.1 |
| | missing | 11 | 4.5 |
| Place of AVF | Elbow | 95 | 38.8 |
| | Snuff box Forearm | 89 | 36.3 |
| | Wrist | 24 | 9.8 |
| | missing | 16 | 6.5 |
| | | 21 | 8.6 |
| Kind of anastomosis | S-S | 131 | 53.6 |
| | E-S | 92 | 37.6 |
| | E-E | 3 | 1.2 |
| | missing | 19 | 7.8 |
| Meand length of anastomosis (mm) | | 9.99(4-18) | |
| Vein | Cephalic | 145 | 59.2 |
| | Cubital | 75 | 30.6 |
| | Basilic | 1 | 0.4 |
| | missing | 24 | 9.8 |
| Atherosclerosis | No | 182 | 74.3 |
| | Mild | 30 | 12.2 |
| | Severe | 8 | 3.3 |
| | missing | 25 | 10.2 |
| AVF function | Good | 205 | 83.7 |
| | Poor | 14 | 5.7 |
| | No | 3 | 1.2 |
| | missing | 23 | 9.4 |

*Table 1 :* Demographic data and history of 245 patients. AVF: Arteriovenous fistula, HTN: Hypertension, DM: Diabetes Mellitus, S-S: side to side, E-S: End to Side, E-E: End to End.

Anastomosis type included side to side fistulas (53.6%), end to side (37.6%) and end to end (1.2%). The mean length of the used anastomosis was 9.9 mm (with minimum and maximum length of 4 mm and 18 mm, respectively).

Cephalic vein was in 59.2% of cases whereas antecubital vein and Basilic vein was in 30.6% and 0.4% of cases respectively. Radial, Brachial and Ulnar arteries used in 53.1%, 35.9% and 1.2% of patients, respectively. Patients with mild atherosclerotic arteries (12.2% of cases) encountered 18% loss of fistula, and severe atherosclerotic arteries (3.3% of cases) suffered 50% loss in 6 months. Reversely, in non-atherosclerotic arteries (74.3% of cases), 17% of AVF break down reported in 6 months.

Most of fistulas had appropriate functions immediately after the operation (83.7%), 5.7% had poor function and 1.2% showed no function. Patients monitoring revealed that 134 cases (69.8%) required immediate dialysis. Among the other 55 patients (28.1%), 20 of the live cases were not dialyzed till the end of follow up period. Thirty five of cases were not dialyzed at all due to death or kidney transplantation.

The minimum time starting to use access after surgery was 2 weeks. Large group of the patients (31.1%) had 4 weeks interval, while 14% after 6 and 9.8% after 8 weeks began dialysis. Long interval rate in starting dialysis (between 13 to 22 weeks after fistula creation) was 1.5%. Most of the patients were dialyzed 4 hours per day and 3 times a week. Considering the patients follow up, 28(11.5%) died, 7(2.9%) were transplanted and 31 (12.7%) encountered fistula loss.

*a) Access survival*

Primary patency for AVF is illustrated in Figure 1. Our result of primary patency at 6 months, 1, 2, 3 and 4 years for 245 patients were 79.5%, 70%, 65%, 60.5% and 48% respectively. We evaluated variables data by Log rank test, as well. According to the analysis, just significant relation between arteriovenous patency and sex and conduction of dialysis was found. As table 2 shows, there is no other significant relationship between access survival and the other variables including: education, underlying disease, smoking, location of AVF, surgical teqniques, surgeon experience, complications, type of vein and artery, diameter of vein and artery, degree of arteriosclerosis and age (P>0.05).

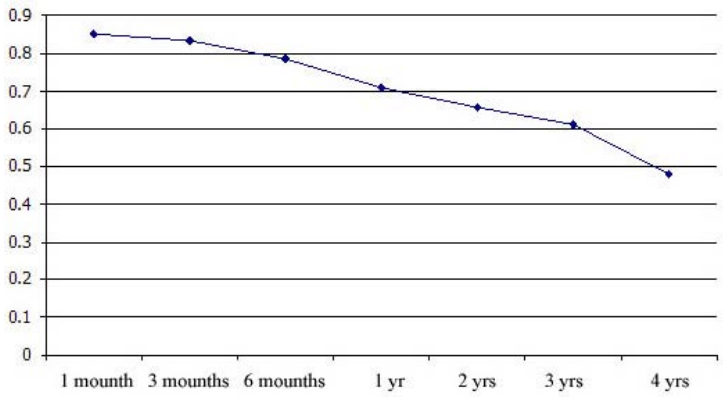


*Fig 1 :* Cumulative primary patency for AVF (4 years).

| Variable | | Access survival | | | Average of survival (days) | Log rank test result |
|---|---|---|---|---|---|---|
| | | 6 months | 1 yr | 3 yrs | | |
| Sex | Male | %87 | %77 | %66 | 140.98 | 0.023 |
| | Female | %72 | %63 | %55 | 116.71 | |
| Underline disease | No | %85 | %85 | %85 | 166.04 | 0.3 |
| | Yes | %81 | %70 | %59 | 126.32 | |
| Place of AVF | Snuff box | %82 | %78 | %62 | 130.91 | 0.273 |
| | Wrist | %58 | %46 | %35 | 91.217 | |
| | Forearm | %75 | %75 | %60 | 121.85 | |
| | Cubital | %84 | %67 | %67 | 131.48 | |
| Limb of AVF | Dominant | %88 | %82 | %70 | 158.07 | 0.122 |
| | Non dominant | %81 | %70 | %60 | 124.28 | |
| Kind of anastomosis | S-S | %82 | %76 | %64 | 135.9 | 0.463 |
| | E-S | %84 | %66 | %59 | 128.7 | |
| | E-E | %33 | %33 | %33 | 68.66 | |
| Surgeon | A | %79 | %71 | %56 | 120.05 | 0.7 |
| | B | %84 | %71 | %63 | 136.52 | |
| | C | %83 | %83 | %83 | 131.16 | |
| Dialysis | Yes | %93 | %82 | %70 | 147.05 | < 0.001 |
| | No | %48 | %43 | %43 | 86.97 | |
| Vein | Cephalic | %85 | %73 | %59 | 130.21 | 0.584 |
| | Cubital | %81 | %67 | %67 | 130.70 | |
| Artery | Radial | %80 | %72 | %59 | 129.52 | 0.19 |
| | Ulnar | %66 | | | 44.33 | |
| | Brachial | %86 | %79 | %69 | 134.98 | |
| Degree of AS | Nothing | %83 | %69 | %61 | 134.39 | 0.115 |
| | Mild | %82 | %82 | %57 | 119.75 | |
| | Severe | %50 | | | 20.33 | |
| Quality of surgery | Easy | %84 | %73 | %62 | 134.84 | 0.154 |
| | Difficult | %67 | %53 | %53 | 105.47 | |

*Table 2 :* Analytic results of access survival.
AVF: Arteriovenous fistula, S-S: side to side, E-S: End to Side, E-E: End to End.
Multivariate model (Cox regression) shows use of Av fistula by dialysis had meaningful affect on access survival.

## IV. Discussion

In current study, the patients were followed up for 4 years and the primary patency of AVF was estimated. In Kalman et al [11] study the primary success rate of 466 patients for 2 years was about 54% ± 4, while in our study the primary patency rate for two and four years were 65% and 48%. In our study investigators concerned about finding predisposing factor of primary patency of AVF. Monroy-Cuadros and colleagues studied the associating factors in the first 6 months of using AVF and the primary failure. They measured the primary failure rate 10% in the initial 6 months. In their study, the initial flow of each access measured lower than 500 ml/min, reported as a risk factor. The other risk factors like age, history of smoking, diabetes mellitus and forearm site fistula did not change the patency rate [12].

Diehm et al studied on the access outcome on diabetic female patients, subsequently they found out that being female gender and diabetic are risk factors in patency outcome [13]. However, Huijbregts et al showed that decrement in the primary patency and function of AVF in diabetic patients was as long as non-diabetic patients [14]. Wand and colleagues expressed that co-morbidities did not have meaningful association with primary failure. The primary success rate was 64% in 2 years [15]. Our study showed that the underlying diseases, like diabetes mellitus, hypertension, glumrolonephritis, uropathy and lupus had no considerable statistical relationship with primary patency. We could not show that smoking could affect on the AVF outcome. Similar results were revealed in atherosclerosis: the degree of atherosclerosis had no considerable role on outcome. Nguyen and colleagues worked on type of AV fistula and examined the outcome of brachicephalic and radiocephalic fistula. Their results showed primary patency and maturity was higher in brachiocephalic than radiocephalic [16] but in other study the outcome of this two type of access were

similar [15]. In radiocephalic we can made fistula by two procedures that include proximal and distal radiocephalic fistula (pRCF and dRCF). Bhalodia et al reported pRCF had a lower primary failure than dRCF [17]. In current study examined the patency of location (Dominant or non dominant limb and right or left limb) and site (elbow, forearm, snuff box and wrist) and our result showed no meaningful statistical relationship between site or type of fistula and primary patency ($P>0.05$).

Saran et al worked on surgeons' skills and outcome of their fistula patency [18]. They reported when fistula is placed by surgeons who tried equal or more than 25 fistulas during training courses, had lower primary failure than others [18]. Our Hospital is referral center and annually 1100 AVF surgeries are conducted by surgeons collaborated in current trial and we didn't find any relation between surgeon and primary patency. Moreover, we examined the different techniques used for anastomosis (side to side, end to side and end to end) and these variables were not important on outcome rate.

Our result showed the primary patency rate was 70% in hemodialysis patients in comparison with 43% in non-dialysis patients. Most of the patients were dialyzing 3 times a week. The mechanism of hemodialysis affect on AVF patency is unknown. But it seems that hemostasis and blood flow changes in hemodialytic patient are role-players. Platelet dysfunction is one of the hemostasis changes in ESRD patients [19]. Several factors influence the platelet dysfunction including impaired function of platelet glycoproteins, changes in ADP and serotonin from platelet granules, arachidonic acid and prostaglandin [19]. Also several factors such as increased tissue factors, protein C, factor XIIa, factor VIIa and platelet hyperactivity lead to thrombosis and increase the atherosclerosis risk [20-24]. So several changes in hemostasis, among mild stage of renal failure, cause cardiovascular complications and vascular access thrombosis, but the main changes in severe stage of renal disease is bleeding and coagulopathy. It is likely to happen due to platelet dysfunction and uremic condition in these patients. On the other hand, hemodialysis and using the access could advocate some hemodynamic changes on the access site. This condition may prevent thrombosis in vulnerable fistulas. So it seems lower risk of thrombosis and access loss in severe stage of disease, may be the consequence of these changes.

## V. Conclusion

Several factors could affect the AVF patency. Our study showed hemodialysis may increase fistula patency while the other mentioned factors do not have any significant association with primary patency of AVFs. Maybe hemostasis abnormalities in ESRD patients and blood flow changes in hemodialytic patients maybe the causes of theses results, so more controlled studies is recommended.

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# Effectiveness of Health Education in Improving HIV/AIDS Knowledge and Risk Behaviours of Commercial Vehicle Drivers in Ilorin, Nigeria

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*Abstract –* Objective: Prevention programmes at the work environment have been said to be a feasible strategy which allows a better understanding of the workers' setting and development of customized educational intervention. This study was therefore carried out to evaluate the effectiveness of health education in improving HIV knowledge and high risk behaviours of commercial drivers in Ilorin, Nigeria. Materials and Methods: This is an intervention study carried out in four motor parks in Ilorin which were mainly for inter-state routes. The sample size used for each of the study and the control group was 140. The study and control groups were matched for socio-demographic characteristics. The study was in three stages-: pre-intervention, intervention and post intervention. A pre-tested, semi-structured questionnaire was used for data collection after which analysis of data was done using SPSS version 15. Results: In the pre-intervention phase, no significant difference was found in the socio-demographic characteristics, knowledge about HIV/AIDS, high risk behaviours and HIV preventive practices of the respondents in both study and control groups.

*Keywords :* *HIV/AIDS; Knowledge; Risk Behaviours; Intervention; Drivers; Effectiveness; Health education.*

*GJMR-L Classification:* *NLMC Code: WC 503*


*Strictly as per the compliance and regulations of :*

# Effectiveness of Health Education in Improving HIV/AIDS Knowledge and Risk Behaviours of Commercial Vehicle Drivers in Ilorin, Nigeria

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*Abstract* - Objective: Prevention programmes at the work environment have been said to be a feasible strategy which allows a better understanding of the workers' setting and development of customized educational intervention. This study was therefore carried out to evaluate the effectiveness of health education in improving HIV knowledge and high risk behaviours of commercial drivers in Ilorin, Nigeria.

Materials and Methods: This is an intervention study carried out in four motor parks in Ilorin which were mainly for inter-state routes. The sample size used for each of the study and the control group was 140. The study and control groups were matched for socio-demographic characteristics. The study was in three stages-: pre-intervention, intervention and post intervention. A pre-tested, semi-structured questionnaire was used for data collection after which analysis of data was done using SPSS version 15.

Results: In the pre-intervention phase, no significant difference was found in the socio-demographic characteristics, knowledge about HIV/AIDS, high risk behaviours and HIV preventive practices of the respondents in both study and control groups. Post-intervention, there was significant difference in knowledge of means of HIV transmission between the study and control groups. Also, significant reduction in the practice of some risky sexual behaviours were noted in the study group; practice of extra-marital relationships reduced from 57.1% to 37.0% , p = 0.001) and patronage of CSWs had reduced to 19.3% from 33.6%, p = 0.01) 21. Furthermore, uptake of preventive practices increased significantly among the study group; proportion of drivers in the study group "currently" using condoms increased from 34.3% pre-intervention to 51.9% post-intervention (p=0.0033).

Conclusion: This study demonstrated that health education is effective in improving HIV knowledge and changing from practice of high risk behaviors. It is recommended that continuous health education programs and seminars on HIV prevention practices be organized by NGOs, and ministries of health within the motor parks for the drivers, to inform the drivers and equip them with skill to protect them from infection.

*Keywords : HIV/AIDS; Knowledge; Risk Behaviours; Intervention; Drivers; Effectiveness; Health education.*

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## I. Introduction

Nigeria has one of the largest HIV/AIDS epidemics in the world, fourth only to India, Ethiopia and South Africa (FMoH, 2002; UNAIDS factsheet) and second to South Africa within sub-Saharan Africa. (FMoH, 1999) The first case of HIV/AIDS seen in Nigeria was reported in 1986, (FMoH, 2002) and since then the statistics are indicative of a spreading epidemic. The high risk groups are the other Sexually Transmitted Infections (STI) patients, commercial sex workers (CSWs), injection drug users, long distance drivers, miners and other itinerant workers. (Peter, 2002; UNAIDS, 2000)

Long distance travelling has been implicated to be a risk factor in HIV infections. The drivers are more at risk because of the need to leave their families frequently and satisfy their sexual need by patronizing CSW and engaging in casual relationship with female hawkers in stop stations. The sexual risk behaviours that lead to increased incidence of HIV and STIs include unprotected sexual intercourse, premarital sex, extramarital and commercial sex, multiple sexual partners and extra-vaginal sex as in homosexuals. (Peter, 2002) Studies have however shown that despite high risk behaviours, long distance drivers consider themselves at low risk and so are not taking preventive measures in protecting themselves. (Stratford et al, 2000)

AIDS is the fourth leading cause of death in the world and the leading cause of death in sub-Saharan Africa. (UNAIDS factsheet; UNAIDS 2001) In countries most affected in Africa, life expectancy has declined by 10 years and infant death rates have doubled. (CDC, 2001) In Nigeria, it is estimated that one person dies of AIDS every other minute i.e. 800 Nigerians/day. (FMoH, 2002) By the end of 2002, about 1.3 million Nigerians had died of AIDS since the epidemics started and by 2005, it is estimated that an additional 1 million people may die if prevention and control measures are not seriously pursued. (FMoH, 2002) The growing numbers of AIDS orphans are now recognised as a major long-term threat to the stability of hardest-hit nations. According to USAID (Melvin & Chinua, 2000) by 2010 one in seven children less than 15 years in sub-Saharan

Africa would have lost a parent to AIDS and AIDS will account for 40 million orphans.

The AIDS epidemic has created an unparalleled complex medical and social challenge in Nigeria. The magnitude of this epidemic is not only in the loss in human lives, but also in the enormous financial burdens to health care systems and loss of productivity to countries. HIV could be an occupational hazard and commercial drivers have been identified as a high risk group. (Pison, 1993; Araoye, Onile & Jolayemi, 1996) This group has a high-risk sexual behaviour which includes casual and multiple sexual partners, (Orobuloye, Caldwell P & Caldwell C, 1993) patronage of CSW (Dallabetta, 1994; Araoye et al, 1999) and inconsistent or non-use of latex condom during unsafe sexual intercourse. (Araoye et al, 1996) Other characteristics that make commercial drivers a priority group include high mobility, low literacy level and poor utilization of STI treatment facility.

Apart from dissemination of health messages by the National AIDS and STI control programmes (NASCP) of the Federal Ministry of Health and other Non-governmental organizations (NGOs), there is need for more intervention programmes on prevention among this professional group. Prevention programmes at the work environment is a feasible strategy, it allows a better understanding of the workers' setting and development of customized educational intervention. This study was therefore carried out to document the sexual risk behaviour, the risk perception, knowledge of preventive measures of HIV infection, conduct a health education intervention programme and evaluate its effectiveness in improving HIV risk perception and sexual risk behaviour among the long distance commercial drivers in Ilorin, Nigeria.

## II. Materials and Methods

The study area was Ilorin town, the capital of Kwara state in Nigeria, which is in the North-Central zone of Nigeria. The projected population for Ilorin in 2000 was about 1.5 million. (Adeyemi & Parakoyi, 2000) Ilorin serves as a major stop for drivers travelling from the northern region of the country to the southern and western regions and vice versa. The population of inter-state commercial drivers in Ilorin is 2,096. (RTEA/ NURTW, 2004)

This is an intervention study in which 140 respondents were selected for each of the study and the control group using the multi-stage sampling technique. The study was in three stages: pre-intervention, intervention and post-intervention. A pre-tested semi-structured questionnaire was used for data collection. Data were collected before and after intervention, and the responses were analysed using Statistical Package for Social Sciences (SPSS) version 15.

At the intervention stage, health education focused on methods of transmission of HIV infection, risky behaviours, preventive measures against HIV infection, symptoms of STI and their prevention. The participants who received the intervention were divided into four groups and each group had a series of 2-3 hours of health education per day for three days; twelve days of intervention in all for the four groups. The health education was in form of lectures, motivational talks and demonstrations using audio-visuals, posters, role plays and practical demonstration. The materials used include public address system, television set and video player, pamphlets and posters on STI and HIV and condoms.

Ethical clearance was obtained from the ethical committee of University of Ilorin Teaching Hospital and permission was also obtained from the two union bodies concerned i.e. Road Transport Employers Association of Nigeria (RTEAN) and National Union of Road Transport Workers (NURTW). Individual written informed consent was also obtained before commencing the interviews. After the study, drivers in the control group also received the intervention (i.e. health education) exactly the same way it was given to those in the study group.

## III. Results

One hundred and forty questionnaires were administered to each of the study and control groups at both the pre-intervention and post-intervention stages of the survey. In both groups, all the questionnaires were completed giving a response rate of 100% in both groups.

The study and control groups were properly matched, such that there was no significant difference in the socio-demographic characteristics of respondents (Table 1). Also, pre-intervention, there was no statistical difference in the knowledge of respondents about HIV/AIDS (Table 2), their high risk behaviours (Table 3) and their HIV preventive practices (Table 4).

Post-intervention, there was a significant difference in awareness of HIV/AIDS among study group ($p=0.0001$) from 89.3% to 100%, but there was no significant difference in awareness among the control group ($p=0.414$). Furthermore, the knowledge of respondents in the study group on the modes of transmission of HIV significantly increased after intervention ($p<0.05$), but no statistically significant increase occurred among the control group ($p>0.05$). The knowledge of respondents in the study group about the cure of HIV improved post-intervention as more people (70% to 94.1%) knew that HIV has no cure. This difference in knowledge was significant statistically ($p=0.00001$), however there was no significant difference in knowledge among the control group ($p=0.863$).

There was no significant difference in alcohol intake among respondents in both the study and control groups pre and post-intervention ($p=0.730$ and 0.805 respectively). There was, however, a statistically significant difference in extramarital relationships in the study group (p-value=0.001). Eighty (57.1%) of the study

group had extramarital relationships pre-intervention, but this dropped to 50(37%) post-intervention. There was no significant difference in this practice among the control group pre- and post- intervention (0.802). Among the respondents in the study group, none had extramarital sexual activity within a month post-intervention evaluation, compared to 45(32.1%) reported pre-intervention. This difference was statistically significant (p=0.0001), but there was no difference in practice among the control group pre- and post-intervention (p=0.404). The number of respondents who patronised CSW significantly decreased from 47(33.6%) pre-intervention to 26(19.3%) post-intervention (p-value=0.01). There was no significant difference in practice among those in the control group, before and after intervention (p=0.779).

Condom awareness among respondents in the study group significantly increased from 81.4% pre-intervention to 100% post-intervention (p-value=0.0001), but there was no significant change about condom awareness among respondents in the control group (p-value=0.663). The use of condom also significantly increased among respondents in the study group from 34.3% before the health education intervention to 51.9% after the intervention (p-value=0.003). There was no significant difference in condom use in the control group, p-value=0.734.

## IV. Discussion

At the pre-intervention stage of the survey, about 9 out of 10 respondents in both groups were aware of HIV/AIDS, and this level of awareness was better than what was reported in a similar study carried out in India in 1999 (Singh & Malaviya, 1994) where only 56% of the drivers were aware of HIV infection. This increased awareness might be due to the increased health education about the infection on mass media over the years. The high level of awareness of HIV/AIDS in Nigeria was corroborated by a study (Odeyemi & Osibogun, 2003) done in Lagos, Nigeria among drivers and conductors, in which 97.2% of the respondents had heard of HIV infection. Post-intervention, the awareness of the study group about HIV/AIDS increased as all the respondents were then aware of HIV/AIDS. The observed increase in awareness shows the effectiveness of the education programme in increasing awareness about HIV/AIDS.

Heterosexual intercourse is a major mode of HIV transmission in Africa. (Peter, 2002) The pre-intervention knowledge of respondents about this route of transmission was very high, with almost all the respondents knowing that HIV could be transmitted through sexual intercourse. This is similar to the findings of a study carried out in Lagos, Nigeria (Odeyemi & Osibogun, 2003) but different from an Indian study (Singh & Malaviya, 1994) where only 25% were aware of sexual transmission of HIV. Also, most of the respondents knew that HIV can be transmitted through blood transfusion. Despite the good level of knowledge on certain modes of HIV transmission, most of the respondents still had misconceptions that HIV can be transmitted through kissing, hugging, insect bites and that it can be contracted from aero-drops. A lower percentage of respondents were observed in Lagos (Odeyemi & Osibogun, 2003) where only 17.2% had such misconceptions. These misconceptions need to be corrected as it could determine respondents' attitudes to people living with HIV/AIDS (PLWHA), their acceptance and interaction with this group of people as well as their care for PLWHA. Post-intervention, among the study group, the knowledge of all the modes of transmission of the infection increased significantly ($p<0.05$) while there was no significant difference observed among the control group. This shows that health education programme can be effectively used to increase respondents' knowledge of HIV transmission. The inference is that increased knowledge about HIV infection especially comprehensive knowledge could lead to better and improved attitude and preventive practices.

Most of the respondents knew before intervention that HIV/AIDS has no cure, and only about 1 out of 10 believed HIV/AIDS has a cure. Out of this few, some believed sleeping with a virgin was a means of curing HIV/AIDS. This believe is a dangerous one, because rather than curing the disease it would help in further spread of the infection. However, post-intervention, the knowledge of respondents in the study group about the cure of HIV changed as more people (70% to 94.1%) knew that HIV has no cure. It is hoped that this would influence the behaviour of the drivers.

Almost half of the respondents admitted to taking alcohol, and this has been similarly reported by another study among drivers in India. (Manjunath, Thappa & Jaisankar, 2002) There was a statistically significant association between alcohol intake and the patronage of CSWs, and this is similar to the finding of a study done in Bangladesh, (Gibney, Saquib & Metzger, 2003) where multiple logistic regression analysis done showed that subjects who ingest alcohol, were more likely to have had sex with a CSW. There was no significant difference in alcohol intake among the respondents in the study group post-intervention, although lesser respondents now take alcohol compared to the percentage of respondents pre-intervention.

Less than half of the respondents had multiple sexual partners. A higher percentage was observed from the study done in India (78%) (Singh & Malaviya, 1994) and in Ilorin (Araoye et al, 1996) where 91% of the drivers who were still single had multiple extramarital sex partners. Lower percentages were observed among commercial drivers in Beijing (13.6%) (Zhang X, Luo & Zhang K, 1994) and Brazil (19%). (Lacerda et al, 1997)

This might suggest that Nigerian commercial drivers are more promiscuous than those in Beijing and Brazil. Thirty- three point six percent of the study group and 32.1% of the control group had more than one sex partners. Similar value was observed in the study from Thailand (35%). (Podhisita, 1996) Post-intervention, there was a statistically significant difference in the number of those who had extramarital affair among the study group showing the effectiveness of the health education intervention programme.

Before the intervention, significant percentage of the respondents engaged in casual sex with CSWs. This practice puts the drivers at high risk of contracting HIV infection, considering the high prevalence of HIV among the CSWs (30%-40%). (Dallabetta, 1994) Similar result (30.6%) was observed in the previous study done among commercial drivers in Ilorin. Post-intervention, the number of respondents in the study group patronizing CSW decreased significantly. This study shows that health education is an effective tool in behavioural intervention in the control of HIV/AIDS. This result is supported by a study done in South India, (Ubaidullah, 2004) where an action research study was conducted in Chittoor District of Andhra Pradesh (India) among truck drivers. As part of the study, different strategies, namely mass media, personal contact, group discussion, folk media, and counseling, were adopted to provide AIDS education, to promote condom use for safer sex, and change sexual behaviour. The strategies adopted greatly enhanced the knowledge of the truck drivers on AIDS, changed their attitudes on sex, increased the use of condoms, and modified their sexual behaviour. (Ubaidullah, 2004)

Appreciable number of the respondents (39.3% and 37.2% respectively) in both the study group and the control groups had history of STI. These proportions were similar to that observed in Delhi India, where 35% of commercial drivers had history of urethral discharge. This study also showed that high percentage of respondents had previous history of STD treatment. This is due to high patronage of CSWs and involvement in extramarital affair and poor use of condom observed in this study. Various studies among commercial drivers have shown similar proportions. In South India, (Manjunath et al, 2002) 38.7% of commercial drivers had history of STDs, 32.7% of drivers in Burundi, (Buzingo et al, 1997) and 34.4% was observed among the Bangladesh (Gibney et al, 2002) truck drivers, while in Rajasthan, India, 75% had at least one form of STD a year before the study was carried out. (Mishra, 1998) A previous study among commercial drivers in Ilorin, Nigeria, found high prevalence of STIs among the respondents. (Araoye et al, 1999) STDs have been found to facilitate both the transmission and susceptibility to HIV infection.(Thior et al, 1997) In a study in South Africa, ( Cohen, Merle & Paul, 1994) it was found that genital ulcer increased the risk of HIV ten folds, while the presence of urethral or vaginal discharge increased the HIV risk five folds. (Cohen et al, 1994)

Pre-intervention condom awareness of respondents was high, however, many had never used the condom, and some used condom occasionally. High level of awareness was also observed among long-distance truck drivers in major truck stops along Nigerian highways, (Sunmola, 2005) where 70% knew about condom but only 9% were consistently using condom. Similar finding was observed in Mombasa, (Bwayo et al, 1991) where it was observed that though high percentage of drivers (76%) knew condom can prevent the transmission of STDs, only 32% had ever used condom. An Indian study (Singh & Malaviya, 1994) showed that 28% of respondents were using condom, while a previous study done in Ilorin showed that 40% of respondents were willing to use condom. However, in Uganda (Ntozi, 2003) which is the first country in Sub- Saharan Africa to reverse its HIV/AIDS epidemics, a study conducted among high risk groups including drivers showed that condom use was responsible for the reversal in HIV/AIDS epidemic, though majority of respondents still continue with multiple sexual partners. In this study, condom awareness among respondents in the study group increased significantly post-intervention, it is hoped that with better awareness, more drivers will use the condom to protect themselves when having casual sex.

The major reason given by respondents for not using the condom was reduction in sexual pleasure; some believed they had self-in-built immunity against HIV and STIs, while a few do not have any reason for not using the condom. However the finding in this study is in contrast with the previous study done in Ilorin (Araoye et al, 1996) where reason given for unwillingness to use condom was general dislike and lack of knowledge. This study shows better awareness about the condom use and improved use though the drivers find it uncomfortable. The problem of reduced sexual pleasure might be improved upon as technology improves. For respondent with the false sense of security of "in-built immunity", more education programs needs to be organized, so as to enhance an attitudinal change. The finding of the study is similar to that of the Beijing study (Odeyemi & Osibogun, 2003) where most of the respondents (56.2%) gave reduction in sexual pleasure as their reason for not using condom and that of the Brazilian study (Guerriero, Ayres & Hearst, 2002) where refusal to use condom was related to fear of failed erection and loss of sensitivity for both men and women. Also similar reasons such as reduced sexual satisfaction and hindrance of sexual interest were given by the truck drivers along major truck stops in Nigeria. (Sunmola, 2005) The post-intervention evaluation showed about 17.6% increase in condom use among the study group ($p=0.003$), but there was no significant difference in the

condom use among respondents in the control group. In a study on HIV prevention through peer education and condom promotion among truck drivers and their sexual partners in Tanzania, (Laukamm-Josten et al, 2000) similar magnitude of increase (56% to 74%) in condom use was observed. It is hoped that with more education programs among this occupational group, there will be increase in the use of the condom as a measure of controlling HIV spread among them.

## V. Conclusion and Recommendation

This study demonstrated that health education is effective in improving HIV knowledge and reducing high risk behaviours. Following the health education intervention, there was significant improvement in HIV knowledge and some risky practices such as multiple sexual partners, patronage of CSWs, and unprotected sex with an increase in condom usage. It is recommended that continuous health education programs and seminars on HIV prevention practices be organized by NGOs, and Ministries of Health within the motor parks for the drivers, to inform the drivers and equip them with skill to protect them from the infection.

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Tables

*Table 1 :* Socio-Demographic Characteristics of Respondents

| VARIABLES | FREQUENCY(PERCENTAGE) | | |
|---|---|---|---|
| | Study (n=140) | Control (n=140) | Total(n=280) |
| **Age group (years)** | | | |
| 15-24 | 20(14.3) | 14(10.0) | 34(12.1) |
| 25-34 | 35(25.0) | 37(26.4) | 72(25.7) |
| 35-44 | 51(36.4) | 51(36.4) | 102(36.4) |
| 45-54 | 26(18.6) | 28(20.0) | 54(19.3) |
| >=55 | 8(5.7) | 10(7.1) | 18(6.4) |
| **Ethnicity** | | | |
| Yoruba | 136(97.2) | 137(97.5) | 273(97.5) |
| Hausa | 2(1.4) | 3(2.1) | 5(1.8) |
| Others | 2(1.4) | 0(0.0) | 2(0.7) |
| **Religion** | | | |
| Islam | 127(90.7) | 123(87.9) | 250(89.3) |
| Christianity | 13(9.3) | 17(12.1) | 30(10.7) |
| **Educational level** | | | |
| Primary Educ. | 54(38.6) | 55(39.3) | 109(38.9) |
| Secondary Educ. | 29(20.7) | 30(21.4) | 59(21.1) |
| Tertiary Educ. | 11(7.9) | 12(8.6) | 23(8.2) |
| None | 26(18.6) | 24(17.1) | 50(17.9) |
| Quranic Educ. | 20(14.2) | 19(13.6) | 39(13.9) |
| **Marital status** | | | |
| Married: Monogamy | 44(31.4) | 56(40.0) | 100(35.7) |
| Polygamy | 78(55.7) | 62(44.3) | 140(50.0) |
| Single | 13(9.4) | 17(12.1) | 30(10.7) |
| Widowed | 3(2.1) | 4(2.9) | 7(2.5) |
| Separated | 2(1.4) | 1(0.7) | 3(1.1) |
| **Number of wives** | | | |
| 0 | 18(12.9) | 22(15.7) | 40(14.3) |
| 1 | 44(31.4) | 56(40.0) | 100(35.7) |
| 2 | 66(47.1) | 53(37.9) | 119(42.5) |
| 3 | 6(4.3) | 6(4.3) | 12(4.3) |
| >=4 | 6(4.3) | 3(2.1) | 9(3.2) |

*Table 2 :* Pre-interventional Knowledge of Respondents about HIV/AIDS

| Variables | Frequency(Percentage) | | p-value |
|---|---|---|---|
| | **Study(n=140)** | **Control(n=140)** | |
| **Awareness about HIV/AIDS** | | | |
| Yes | 125(89.3) | 126(90.0) | 0.845 |
| No | 15(10.7) | 14(10.0) | |
| **Presence of HIV in Nigeria** | | | |
| Yes | 134(95.7) | 135(96.4) | 0.758 |
| No | 6(4.3) | 5(3.6) | |
| **Modes of transmission** | | | |
| Blood Transfusion | 126(90.0) | 132(94.3) | 0.400 |
| Mother to unborn child | 110(78.6) | 123(88.5) | 0.820 |
| Sexual Intercourse | 136(97.1) | 133(95.0) | 0.124 |
| Sharing of sharp object | 129(92.1) | 130(92.9) | 0.903 |
| Through Insect Bite | 28(20.1) | 24(17.1) | 0.756 |
| Surgical instrument | 128(94.1) | 133(95.0) | 0.703 |
| Through casual contact | 27(19.3) | 22(16.0) | 0.717 |
| Through Breast Milk | 105(77.2) | 114(84.4) | 0.294 |
| Through the environment | 30(21.4) | 28(20.0) | 0.891 |
| **Mode of cure known** | | | |
| Knowledgeable | 98(70.0) | 101(72.1) | 0.572 |
| Not-knowledgeable | 42(30.0) | 39(27.9) | |

*Table 3 :* Pre-interventional High Risk Behaviours among Respondents

| Variables | Frequency(Percentage) | | p-value |
|---|---|---|---|
| | Study(n=140) | Control(n=140) | |
| **Alcohol Intake** | | | |
| Yes | 62(44.3) | 67(47.9) | 0.549 |
| No | 78(55.7) | 73(52.1) | |
| **Smoking Habit** | | | |
| Yes : Cigarette | 33(23.6) | 35(25.0) | |
| Indian Hemp | 6(4.3) | 5(3.6) | 0.926 |
| No | 101(72.1) | 100(71.4) | |
| **Regular Sexual Partner** | | | |
| Yes | 80(57.1) | 75(53.6) | 0.548 |
| No | 60(42.9) | 65(46.4) | |
| **Number of Girlfriends in the last 6 months** | | | |
| 0 | 60(42.9) | 65(46.4) | |
| 1 | 33(23.6) | 30(21.6) | 0.733 |
| More than 1 | 47(33.5) | 45(32.0) | |
| **Patronage of Commercial Sex Workers** | | | |
| Yes | 47(33.6) | 53(37.9) | 0.454 |
| No | 93(66.4) | 87(62.1) | |
| **Blood Transfusion Status** | | | |
| Yes: Treatment of Illness | 5(3.6) | 6(4.3) | |
| Complications of RTA | 9(6.4) | 6(4.3) | 0.681 |
| No | 126(90.0) | 128(91.4) | |
| **History Scarification Marks** | | | |
| Yes: Tribal Marks | 57(40.7) | 60(42.9) | |
| Spiritual Protection | 35(25.0) | 29(20.7) | |
| Blood Letting | 4(2.9) | 2(1.4) | 0.665 |
| No | 44(31.4) | 49(35.0) | |
| **History of STI symptoms** | | | |
| Yes | 55(39.3) | 52(37.2) | 0.712 |
| No | 85(60.7) | 88(62.8) | |

*Table 4 :* Pre-interventional HIV Preventive Practices among Respondents

| Variables | Frequency(Percentage) | | p-value |
|---|---|---|---|
| | Study(n=140) | Control(n=140) | |
| **Awareness of Condom Usage as a Preventive Practice** | | | |
| Yes | 114(81.4) | 116(82.9) | 0.755 |
| No | 26(18.6) | 24(17.1) | |
| Total | 140(100.0) | 140(100.0) | |
| **Current Condom-Use Status** | | | |
| Yes | 48(34.3) | 44(31.4) | |
| No: Reduced sexual pleasure | 64(45.7) | 61(43.6) | 0.535 |
| Have self-inbuilt immunity | 20(14.3) | 29(20.7) | |
| No reason | 8(5.7) | 6(4.3) | |
| Total | 140(100.0) | 140(100.0) | |
| **Frequency of Condom Usage** | | | |
| Always | 16(33.3) | 15(34.1) | |
| Occasionally | 32(66.7) | 29(65.9) | 0.939 |
| Total | 48(100.0) | 44(100.0) | |

*Table 5 :* Comparison of Pre- and Post-interventional Knowledge and Risk Behaviours about HIV/AIDS among the Respondents

| Variables | Study(Percentage) n=140 | | | Control(Percentage) n=140 | | |
|---|---|---|---|---|---|---|
| | Pre- | Post- | p-value | Pre- | Post- | p-value |
| **Awareness about HIV/AIDS** | | | | | | |
| Yes | 125(89.3) | 135(100) | 0.0001 | 126(90.0) | 128(92.8) | 0.414 |
| No | 15(10.7) | 0(0.0) | | 14(10.0) | 10(7.2) | |
| **Modes of transmission** | | | | | | |
| Blood Transfusion | 126(90.0) | 133(98.6) | 0.00001 | 132(94.3) | 128(92.8) | 0.827 |
| Mother to unborn child | 110(78.6) | 131(97.0) | 0.00002 | 123(88.5) | 121(87.7) | 0.999 |
| Sexual Intercourse | 136(97.1) | 135(100.0) | 0.00001 | 133(95.0) | 131(94.9) | 0.867 |
| Sharing of sharp object | 129(92.1) | 134(99.3) | 0.004 | 130(92.9) | 129(93.5) | 0.70 |
| Through Insect Bite | 28(20.1) | 132(97.8) | 0.00001 | 24(17.1) | 129(93.5) | 0.789 |
| Surgical instrument | 128(94.1) | 90(66.7) | 0.00001 | 133(95.0) | 24(17.4) | 0.102 |
| Through casual contact | 27(19.3) | 86(63.7) | 0.00001 | 22(16.0) | 27(19.6) | 0.592 |
| Through Breast Milk | 105(77.2) | 86(63.7) | 0.00001 | 114(84.4) | 30(21.7) | 0.903 |
| Through the environment | 30(21.4) | 129(95.6) | 0.00001 | 28(20.0) | 110(81.5) | 0.709 |
| **Mode of cure known** | | | | | | |
| Knowledgeable | 98(70.0) | 132(94.1) | 0.00001 | 101(72.1) | 100(71.7) | 0.86307 |
| Not-knowledgeable | 42(30.0) | 8 (5.9) | | 39(27.9) | 40(28.3) | |
| **Alcohol Intake** | | | | | | |
| Yes | 62(44.3) | 59(42.2) | 0.730 | 67(47.9) | 65(46.4) | 0.805 |
| No | 78(55.7) | 81(57.8) | | 73(52.1) | 75(53.6) | |
| **Patronage of Commercial Sex Workers** | | | | | | |
| Yes | 47(33.6) | 27(19.3) | 0.007 | 53(37.9) | 51(36.2) | 0.779 |
| No | 93(66.4) | 113(80.7) | | 87(62.1) | 89(63.8) | |
| **Extramarital relationships** | | | | | | |
| Yes | 80(57.1) | 52(37.0) | 0.001 | 75(53.6) | 77(55.1) | 0.802 |
| No | 60(42.9) | 88(63.0) | | 65(46.4) | 63(44.9) | |
| No | 85(60.7) | | | 88(62.8) | | |
| **Awareness of Condom Usage as a Preventive Practice** | | | | | | |
| Yes | 114(81.4) | 140(100.0) | 0.0001 | 116(82.9) | 119(84.8) | 0.663 |
| No | 26(18.6) | 0(0.0) | | 24(17.1) | 21(15.2) | |
| Total | 140(100.0) | 140(100.0) | | | | |
| **Current Condom-Use Status** | | | | | | |
| Yes | 48(34.3) | 73(51.9) | 0.003 | 44(31.4) | 47(33.3) | 0.734 |
| No | 92(65.7) | 67(48.1) | | 96(68.6) | 93(66.7) | |

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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.

**2. Evaluators are human:** First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

**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 the basic

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)

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**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.

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| 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<br><br>Above 200 words | No specific data with ambiguous information<br><br>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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