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White Blood Cells

Salmonella Typhi Isolates

Highlights

Blood and Body Fluids

Escherichia Coli to Alkaloid

Discovering Thoughts, Inventing Future

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Effect of Exercise on the Interleukin-10, White Blood Cells and Creatine Kinase in Sportsmen and Sedentaries

By Aynur Otağ, Durmuş Deveci, İlhan Otağ & İlkay Koşar
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Abstract - Interleukin-10 (IL-10) is an important anti-inflammatory cytokine increasing with exercise. It is stated in the literature that the increase of IL-10 after exercise may be related to muscle damage. Creatine kinase (CK) is a dimeric protein whose plasma levels are increasing especially in muscle pathologies. At the same time, it is known that it increases relating to the muscle damage caused by exercise.

In this study planned, it shall be researched there is IL-10 and CK values before and immediately after the exercise according to Bruce protocol. Moreover, the changes in white blood cells shall also be examined.

Those who are convenient for the test were elected from 30 healthy voluntary males in the same age group who do sports amateurly and who don't do sport. The subjects were applied to exercise test on a treadmill according to Bruce protocol, and the blood samples were taken into heparinize tubes before and after the exercise. Evaluations were made in biochemistry and microbiology laboratories.

Keywords : *exercise, il-10, creatine kinase, sportsman, sedentary.*

GJMR-C Classification : *NLMC Code: WB 541*

Effect of Exercise on the Interleukin-10, White Blood Cells and Creatine Kinase in Sportsmen and Sedentaries

Aynur Otağ [α], Durmuş Deveci [σ], İlhan Otağ [ρ] & İlkay Koşar [ω]

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While IL-10 levels did not change in sedentaries after the exercise (*P>0,05*), it decreased in sportsmen (*P<0,05*). CK values increased in both of the groups after the (*P<0,01*). No regressional relation was found between IL-10 and CK values in both of the groups.

The Bruce protocol we applied increased CK and leukocyte levels in sportsmen but decreased plasma IL-10 level significantly.

Keywords : exercise, il-10, creatine kinase, sportsman, sedentary.

I. Introduction

In recent studies, it has been emphasized that exercise has a preventive effect on type II diabetes, cardiovascular diseases, cancer, psychological disorders and depression (1, 17). Interleukin-10 (IL-10) is an important anti-inflammatory cytokine increasing with exercise (15). IL-10 is produced not only by other cells in the body but also by the cells belonging to immune system, and the other cytokines affect the production. It is stated that the increase in IL-10 after exercise may be related to the muscle damage (17, 22). IL-10 in exercise; interleukin-1α (IL-1α) inhibits IL1β and TNF-α production (18). The muscle damage relating to intensity of the exercise increases release of pro-inflammatory cytokines; and furthermore, exercise induces release of anti-inflammatory cytokines (16).

Creatine kinase (CK) is a dimeric protein whose plasma level increases especially in muscle pathologies. At the same time, it is known that it increases relating to the muscle damage resulting from exercise (3, 4, 5, 6, 14, 22). Total CK level changes according to age, gender, muscle tissue, physical activity and kinetic state. Very high CK level is found after the activities such as marathon and triathlon. It reaches minimum 300-500 IU/l after the exercise. It was found higher in athletes that sedentaries during rest (2, 3).

In this study planned, it was researched whether there is any relation between IL-10 and CK values before and after the exercise according to Bruce protocol. Moreover, the changes in white blood cells were also examined. Thus IL-10 and muscle injury will be explain between the relationship.

II. Material and Methods

Those who are convenient for the test were elected from 30 healthy voluntary males in the same age group who do sports amateurly (sportsmen) and who don't do sport (sedentary). In the comparison group, the same subjects were used. The subjects were applied to exercise test on a treadmill according to Bruce protocol. Bruce protocol test is the general used incremental graded exercise test to evaluate cardiovascular function (10, 19). The test fatigue was determined when the subjects indicated their desire to stop. Blood samples (5ml) were taken antecubital vein into heparinize tubes before and immediately after the exercise. The plasma were kept at -80 0C until the analysis. IL-10 values were measured by means of ELISA using original kit (AviBion Human IL-6 ELISA Kit). The other samples were analyzed using appropriate procedures in biochemistry and microbiology laboratories. Body composition values of the subjects were determined by bioelectrical impedance analysis (BIA). In order to compare sportsmen and sedentary groups, t-test of differences between independent two groups was used. Matched t-test was used in dependent subjects in order to compare the groups within themselves. İf the values were $P<0.05$, they were regarded meaningful.

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Step	Speed (km/h)	Gradient
1	2.74	10
2	4.02	12
3	5.47	14
4	6.76	16
5	8.05	18
6	8.85	20
7	9.65	22

Table 1 : Bruce Protocol (19)

III. Results

Profile of sedentary and sportsmen subjects is shown in Table 2.

Table 2 : Profile of sedentary and sportsmen subjects

	Sedentary	*Sportsman*
Age (years)	22,40±2,66	23,06±1,98
Height (cm)	176,20±7,63	179,16±4,69
Weight (kg)	66,33±8,28	73,46±8,96
Fat free Mass (kg)	59,37±1,06	64,38±1,09

In sedentaries, while IL-10 values did not change after the exercise, CK values increased ($p<0,01$). Values of the white blood cells did similarly increase after the exercise ($p<0,01$) (Table 3)

Table 3 : IL-10, CK and white blood cell values of treadmill test applied to sedentaries

	Pre exercise	*Post exercise*	*P*
IL-10 (pg/ml)	1,33±0,34	1,35±0,45	$p>0,05$
CK (U/L)	158,53±14,19	194,96±19,26	$p<0,01$
WBC (10^3u/L)	7,46±0,29	11,98±0,49	$p<0,01$
Neutrophil (10^3u/L)	4,44±0,29	6,87±0,46	$p<0,01$
Lymphocyte (10^3L)	2,23±0,097	3,94±0,27	$p<0,01$
Monocyte (10^3u/L)	0,41±0,016	0,56±0,026	$p<0,01$
Eosinophils (10^3u/L)	0,16±0,020	0,20±0,023	$p<0,05$
Basophil (10^3u/L)	0,02±0,005	0,05±0,004	$p<0,01$

While IL-10 values decreased significantly after the exercise in sportsmen ($p<0,05$), CK values increased significantly ($p<0,01$). White blood cell (WBC) values increased significantly both in total and in all other various types ($p<0,01$) (Table 3).

When the values of IL-10, CK and white blood cells obtained after exercise were compared in sportsmen and sedentaries, IL-10 values decreased significantly in sportsmen (Table 3 and 4). However; CK values were significantly higher in sportsmen before and after the exercise (Table 2 and 3). When white blood cells of sportsmen and sedentaries were compared, it was observed that the values after the exercise were meaningfully higher in sedentaries statistically although no difference was observed before the exercise (Table 3 and 4). In regressional analysis, no meaningful relationship was found between IL-10 and CK and white blood cells.

Table 4 : IL-10, CK and white blood cell values of treadmill test applied to sportsmen

	Preexercise	*Post Exercise*	*P*
IL-10 (pg/ml)	1,60±0,74	1,48 ±0,68	$P<0,05$
CK (U/L)	378,56±57,85	416,56±60,40	$p<0,01$
WBC (10^3u/L)	7,44±0,26	9,19±0,39	$p<0,01$
Neutrophil (10^3u/L)	4,42±0,24	5,15±0,33	$p<0,01$
Lymphocyte (10^3L)	2,19±0,10	3,04±0,16	$p<0,01$
Monocyte (10^3u/L)	0,38±0,018	0,43±0,020	$p<0,01$
Eosinophils (10^3u/L)	0,22±0,05	0,26±0,031	$p<0,05$
Basophil (10^3u/L)	0,03±0,002	,0,04±0,003	$p<0,01$

IV. Conclusion

The muscle damage relating to the intensity of the exercise causes IL-10 to increase which is an anti-inflammatory cytokine (16). On the other hand, in lower intense exercises, plasma IL-10 rate decreased as there wasn't higher tiredness and metabolic consumption (11, 21). Nieman et al found an increase in cytokine values after a walking of 30 minutes (13). In our study, while IL-10 values did not change in sedentaries, it decreased significantly in sportsmen. These values showed that Bruce protocol we applied which is a submaximal exercise does not have an important effect on the increase of plasma IL-10 values.

Creatine kinase is an important enzyme which shows muscle damage. Many researchers stated that CK level increases after exercise (3, 22). In this study, plasma CK levels increased after the exercise in both of the groups. Sportsmen showed higher values than sedentaries before and after the exercise (12). This increase observed in sportsmen may have been caused by higher exercise intensity of the sportsmen and resulting muscle damages, and moreover, it may have also been caused by higher muscle volume of sportsmen than sedentaries. The more muscle mass increases, the more CK rate released from muscle increases (3). On the other hand, while CK rate increased 9% in sportsmen after the exercise, it increased 18% in sedentaries. This may have probably been caused by the adaptation at cellular level occurring due to exercise in sportsmen and lack of this adaptation in sedentaries. Furthermore, in order to meet the

increasing energy need, use of creatine stores to create ATP is faster and more efficient in sportsmen than sedentaries (3). CK may have been found higher in sportsmen due to this reason.

A parallel increase was observed in white blood cells together with exercise (7, 20). The reason for this increase may be the increase in leukocyte number attending to the circulation with exercise, and it may also be the damages in muscles resulting from exercise (9). The reason for higher increase in sedentaries than sportsmen may probably be more damages in ill-antrened tissue. This result may show that the responses of muscle damages resulting from exercise develop faster in sedentaries (16).

As a conclusion, the Bruce protocol we applied increased CK and leukocyte levels more in sedentaries, but no changed plasma IL-10 level. While IL-10 values decreased significantly after the exercise in sportsmen, CK and leukocyte values levels increased significantly. There aren't many publications on cytokine changes after short-term exercises. The studies do generally focus on the effect of long term and intense exercises on the cytokines. Our study seems to be important in terms of using Bruce test which is short termed and has high intensity between the steps. In our study, due to the change in IL-10 values after Bruce protocol, we can recommend this test protocol for other studies which will examine the cytokine changes after short term exercise.

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Prevalence of Resistance among *Salmonella Typhi* Isolates in Ekiti- State, Southwestern Nigeria 2009-2011

By Ajibade, V.A

Abstract - The prevalence of Sal typhi in the various locations in Ekiti state over the years of 2009 -2011 was investigated. The highest number of the isolates from 50 samples was found at Ikere-Ekiti, Ikole- Ekiti and Ifaki Ekiti at a total number of 131 respectively, this was followed by Ado- Ekiti with an isolate number of 130. The lowest number of isolate was recorded at Ido Ekiti with 78 isolates in the year 2009 and this increased in both 2010 and 2011. The susceptibility patterns reported in 2009 in the different locations showed that resistance was indicated in streptomycin, chloramphenicol, cefepime, nalidixic acid tetracycline and trimethoprim-sulfamethoxazole. This same trend of resistance was repeated in 2010 and 2011. The notable change was a significant increase in the resistance to ampicillin from 20% to 100% in Ado Ekiti and from 39% to 100% at Ikole Ekiti and also Gentamycin from 18% at Ikere-Ekiti in 2009, to 86% and 84% in 2010 and 2011 respectively. The difference in demographic factors in the different locations could be a convincing factor in the prevalence of Sal typhi. With PFGE, a total of thirty-five 35 (79%) patterns in the locations were observed and thirty-six 36(72%) of each isolates had the 3 most common patterns. The isolates with these patterns were found to show high resistance to Streptomycin, Chloramphenicol, Cefepime, Ampicilin and nalidixic acid (SCCAN). All isolates that are resistant to the antibiotics mentioned earlier contain qnrB2, 35(70%) isolates contained blaCMY-2'; 39 (78%) isolates contained blaCMY-23' the mechanism for extended-spectrum cephalosporin, Aminoglycosides and Quinolone resistance. The genes that code for this resistance have proven to be remarkably mobile and widely distributed within and between species.

Keywords : *ekiti state, prevalence, resistance, salmonella typhi.*

GJMR-C Classification : *NLMC Code: WC 269*

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Keywords : *ekiti state, prevalence, resistance, salmonella typhi.*

I. Introduction

During the past decade, multidrug-resistant (MDR) *Salmonella typhi* have increased substantially in most part of the world especially in developing countries. The prevalence of this bacterium increased from 1% of human isolates in 1998 to 21% in 2003. *S. typhi* is resistant to at least chloramphenicol, streptomycin, sulfamethoxazole, cefoxitin, tetracycline, amoxicillin-clavulanic acid, and ampicillin. This phenotype exhibits decreased susceptibility to Ceftriaxone (CDC, 2006), a critically important antimicrobial agent for treating invasive Salmonellosis in children (Guerrant *et al.,* 2001). Members of the bacterial genus *Salmonella* are among the major pathogens that cause Salmonellosis in humans. Most human *Salmonella* infections are thought to be associated with food borne transmission from contaminated animal-derived meat and dairy products (Voetsch *et al*., 2004). Human infections are commonly caused by ingestion of food that has been contaminated by animal feces (Herikstad *et al.,* 2002). Although >2,500 serovars of *S. typhi*havebeen identified, most human infections are caused by a limited number of serovars. *S.* serovar *typhimurium*and *enteritidis* are the most common causes of humanSalmonellosis worldwide, although other serovars havebeen reported to be more prevalent in some regions(Hald*et al.,* 2007).Adequate sanitary measures have led to a decrease in cases of typhoidal Salmonella infections in developedcountries such as the USA where the incidence is low. However, in these countries, non-typhoidal *Salmonellosis* is common, and most of these cases are associated with outbreaks from contaminated meat, dairy products or by cross-contamination from foods contaminated with *Salmonella* (,Glynn,*et al.,* 2005). In Nigeria especially in Ekiti state, morbidityassociated with illness due to *Salmonella* continues to be on the increase and in some cases, resulting in death (Ajibade *et al.*, 2010).

Although, the primary cause of Salmonellosis is consumption of contaminated foods, there is the potential for secondary spread, from person-to-personand also to other foods. Person- to-person spread with family groups is often associated with poor personal hygiene but there is the opportunity of air-borne andsurface–to-surface spread within the toilet and the bathroom, especially during the diarrhea phase (Ajibade*et al.,* 2010; Ajibade, 2012). Despite improved public health, serious infections with *Salmonella* remain a major clinical and public health concern in Nigeria and worldwide.*S. enterica* are a leading cause of food borne disease worldwide (Hald *et al.,* 2007). In the United States, as many as 1.4 million cases of *S.enterica*-associated disease occur annually (Humphery, 2000; Voetsch *et al*., 2004; Marie *et al.,* 2012) Whileusually self-limiting, Salmonellosis may require antimicrobial drug treatment in infants, the elderly or immunocompromised

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persons. However, antimicrobialdrug resistance has become increasingly common in *S. typhi*, which has complicated therapy (Herikstad *et al.,* 2002). Antimicrobial agents such as fluoroquinolone and third-generation cephalosporin's are commonly used to treat severe human *Salmonella* infections (Lee *et al.,* 2009). Resistance to these and other antimicrobial drugs, as well as multidrug resistance, has increased over the last several decades, probably as a consequence of antimicrobial agents in use at intensive animal husbandry and medicine (WHO, 2002). Antimicrobial resistant strains of *Salmonella species* are now widespread all over the world. In developed countries, it is becoming more and more accepted that a majority of resistant strains are of zoonotic origin and have acquired their resistance in an animal host before being transmitted to human through the food chain (Helms *et al*., 2004). The emergence of antimicrobial drug resistance is a matter of concern. This work intends to determine the prevalence of resistance of *Sal typhi* isolated from major towns in Ekiti Stateto conventional antimicrobial drugs.

II. Materials and Methods

a) Collection of Sample

Stool samples were collected from patients who visited hospital in Ado Ekiti, Ido Ekiti, Ikere Ekiti, Ikole Ekiti, Ifaki Ekiti between February 2009 and September 2011. Fifty (50) samples each were collected from the hospital.

b) Culture, Isolation and Identification of Samples

Samples were cultured on Salmonella-Shigella agar, incubated at 37°C for 24hrs. Smooth colonies withblack centre were sub cultured on to MacConkey agar (Oxoid), incubated at 37°C for 18-24hr. Pale colonieswere isolated and confirmatory biochemical test (Gram's reaction, urease reaction, citrate utilization andmotility) were conducted. *Salmonella enterica* was identified by using standard biochemical tests andcommercial typing antiserum (Statens Serum Institute, Copenhagen, Denmark) according to the manufacturer's instructions.

c) Antimicrobial Susceptibility Profile

The susceptibility profile of samples from which *Sal typhi* (44) was isolated was determined. Minimum inhibitory concentration (MIC's) of ten (10) antimicrobial drugs: **Phenols** (chloramphenicol), **Penicillins** (Ampicillin), **Cephalosporin (**cefepime, cefotaxime), **Tetracycline** (tetracycline), **Aminoglycosides** (Gentamycin, streptomycin), **Sulfonamides** (trimethoprin-sulfamethoxazole), **Quinolones and Fluoroquinolone** (nalixidic acid, ciprofloxacin) were determined by usingthe broth-microdilution method; susceptibility to streptomycin was measured by using the disk-diffusionmethod as recommended by the Clinical and Laboratory Standards Institute(CLSI,2012).

d) Pulsed – Field Gel Electrophoresis

All *Salmonellae* isolates were analyzed for genetic relatedness by PFGE by using *Xba*1 according to the CDC PulseNet protocol (Ribopt *et al.,* 2006). Electrophoresis was performed with a CHEF-DR111 system in the Science Technology Research Lab, Federal Polytechnic, Ado- Ekiti by using 1% Seakem agarose in 0.5x Tris-borate-EDTA at 180V. Running conditions consisted of 1 phase from 2.2 to 63.8s for a run of 22h.

e) PCR Detection of Antimicrobial Drug Resistance Genes

Presence of *qnr* genes was determined by using PCR with primers QP1 and QP2 for *qnrA,* FQ1 and FQ2 for *qnrB,* and 5'-ATGGAAACCTACAATCATAC-3' and 5'-AAAAACACCTCGACTTAAGT-3' for *qnrS*. The *qnrB* allele was determined by amplification and sequencing with primers FQ1 and FQ2. Screening for *aac(6')-1b-cr* was performed as as described in Park *et al.* (2006). Primer pairs used for amplification of **β-**lactamase genes were: bla_{cmy} (5'-ATGATGAAA AAATCGTTATGC-3') and (5'TTGCAGCTTTTCAAG AATGCGC-3'), bla_{OXA-1} (5'AATGGCACCAGATTCAACTT-3') and (5'-CTTGGCTTTTATGCTTGATG-3'); and bla_{SHV} (5'-GGTTATGCGTTATATTCGCC-3') and (5'-TTAGCGTT GCCAGTGCTC-3'). bla_{CTX-M} genes were screened by using a multiplex PCR assay (Woodford *et al.,*2006).

III. Results and Discussion

The prevalence of *Saltyphi* in the various locations in Ekiti state over the years of 2009 - 2011 is shown in table 1. The highestnumber of the isolates from 50 samples was found at Ikere-Ekiti, Ikole- Ekiti and Ifaki Ekiti at a total number of 131 respectively, this was followed by Ado- Ekiti with an isolate number of 130. The lowest number of isolate was recorded at Ido Ekiti with 78 isolates. The lowest number of isolates was recorded in the year 2009 and this increased in both 2010 and 2011.The susceptibility patterns reported in 2009 in the different locations are shown in table 2. It was observed that resistance was indicated in streptomycin, chloramphenicol, cefepime, nalidixic acid tetracycline and trimethoprim-sulfamethoxazole. This same trend of resistance was repeated in 2010 and 2011 (tables 3 and 4). The notable change was a significant increase in the resistance to ampicillin from 20% to 100% in Ado Ekiti and from 39% to 100% at Ikole Ekiti and also Gentamycin from 18% at Ikere –Ekiti in 2009, to 86% and 84% in 2010 and 2011 respectively.

The difference in demographic factors in the different locations could be a convincing factor in the prevalence of *Sal typhi* in the towns. Ado-Ekiti been a capital city with high urbanization is prone to a lot of cross contamination due to its congestion and over-crowding condition. Ido Ekiti for example, is not over crowded and has a medical center situated there-in. This has a meaningful influence on the environmental

condition and the inhabitants especially in the access to good medical condition.

The increasing prevalence of multidrug-resistant Salmonella *sp* is a global health concern. In Ekiti State, colonization of infection with *Saltyphi* has been a reportable condition for > 20 years. From 2009 to 2011, the agents for which a major change in susceptibility was observed were Tetracycline, Trimethoprim-sulfamethoxazole, nalidixic acid, ampicillin, cefotaxime and streptomycin. With PFGE, a total of thirty-five 35 (79%) patterns in the locations were observed and thirty-six 36(72%) of eachisolates had the 3 most common patterns. The isolates with these patterns were found to show high resistance to Streptomycin, Chloramphenicol, Cefepime, Ampicilin and nalidixic acid (SCCAN). All isolates that are resistant to the antibiotics mentioned earlier contain *qnrB2*, 35(70%) isolates contained $bla_{CMY-2'}$; 39 (78%) isolates contained $bla_{CMY-23'}$ the mechanism for extended-spectrum cephalosporin, Aminoglycosides and Quinolone resistance. The genes that code for this resistance have proven to be remarkably mobile and widely distributed within and between species. Integrons are widely distributed among *Saltyphi* and are potentially capable of transmitting drug resistance (Mary *et al.,* 2009). These findings correspond to that of Karen *et al.* (2010) where it was reported that most Salmonella isolates in South Africa are resistant to at least ampicillin (A), Chloramphenicol (C), Streptomycin(S), Sulfonamide (Su) and Tetracycline (T) (ACSSuT). They have been described as been found in a multidrug resistance (MDR) region that is located on the chromosome in a region termed *Salmonella* genome island 1 (SGI1) (Boyd *et al.,* 2001).The spread of resistance has repeatedly been shown to be associated with antimicrobial drug use, which stresses the importance of the prudent use of these drugs; a notion reinforced by the observation that resistance can be revised (Marie *et al.,* 2012). Plasmid mediated Fluoroquinolone resistance have been reported in South Africa to have implications for cons transference of resistance to the major antimicrobial agents used to treat typhoid fever (Karen *et* al.,2010). Multidrug resistance is evidenced in this study and this can be ascribed to the wide spread use of antibiotics both inside and outside of medicine, selling of the antibiotics over the counter without prescription, misuse and over use of antibiotics by doctors as well as patients and inappropriate prescription. The emergence of *Sal typhi* strains that are resistant to commonly used antibiotics is important to clinicians, microbiologists and those responsible for the control of communicable diseases.

Because use of antimicrobial agents contributes to increasing resistance and facilitates transmission of multidrug-resistant Salmonellae, promoting guidelines aimed at improving appropriate use of antimicrobial agents may help prevent transmission of multidrug-resistant *Sal typhi* infections in the environment. In addition, the avoidance of unnecessary antibiotics usage and good hygiene at all stages in food production chain and adequate treatment of sewage, supply and drinking of portable water is of paramount importance in reducing the incidence and preventing multi-drug resistance.

IV. Conclusion

In conclusion, this study underscores the magnitude of the problem of antimicrobial drug resistance in low resource settings and the urgent need for surveillance and control of this phenomenon is necessary. The state has seen a persistently high incidence of multidrug-resistant *Sal typhi* because ma vigilant screening and decolonization program has not been implemented. From this work it was observed that for the past three (3) years, growth of multidrug resistant strain of *Sal typhi* has been exponential, rural and metropolitan rates apparently stabilized. The findings of this study can be used to assist in the direction of policy and interventions; to conduct other analyzes like the evaluation of economic cost of these diseases while attributing it to various environmental conditions. Endemic persistence of the bacterium and the measures that should be undertaken to control or eradicate it from the state are likely to remain topical subjects.

Table 1 : Incidence of *Salmonella sp* in the different towns between the years; 2009-2011

Locations	2009	2010	2011	Total(no)
Ado- Ekiti	43(86)	41(82)	46(92)	130
Ikere- Ekiti	43(86)	47(94)	41(82)	131
Ido –Ekiti	30(60)	27(54)	21(42)	78
Ikole-Ekiti	40(80)	46(92)	45(90)	131
Ifaki -Ekiti	41(82)	45(90)	47(94)	131
Total	197	206	200	

Table 2 : Susceptibility pattern of *Salmonella sp* reported in different locations in 2009(% resistance)

Antibiotics	Ado 43	Ikere 43	Ido 34	Ikole 40	Ifaki 41
Streptomycin	100	100	100	100	100
Chloramphenicol	100	100	100	100	100
Cefepime	20	79	78	98	87
Cefotaxime	100	100	100	100	100
Ampicillin	20	79	94	39	94
Nalidixic acid	100	100	100	100	100
Gentamycin	81	18	76	94	78
Ciprofloxacin	35	15	93	90	78
Tetracycline	100	100	100	100	100
Trimeth/Sulfamethoxazole	100	100	100	100	100

Table 3 : Susceptibility pattern of *Salmonella sp* reported in different locations in 2010(% resistance)

Antibiotics	Ado 41	Ikere 47	Ido 27	Ikole 46	Ifaki 45
Streptomycin	100	100	100	100	100
Chloramphenicol	100	100	100	100	100
Cefepime	20	92	89	98	39
Cefotaxime	100	100	100	100	100
Ampicillin	99	93	99	76	90
Nalidixic acid	100	100	100	100	100
Gentamycin	99	68	86	74	78
Ciprofloxacin	33	15	73	70	88
Tetracycline	100	100	100	100	100
Trimeth/Sulfameth oxazole	100	100	100	100	100

Table 4 : Susceptibility pattern of *Salmonella sp* reported in different locations in 2011(% resistance)

Antibiotics	Ado 41	Ikere 47	Ido 27	Ikole 46	Ifaki 45
Streptomycin	100	100	100	100	100
Chloramphenicol	100	100	100	100	100
Cefepime	20	92	89	98	39
Cefotaxime	100	100	100	100	100
Ampicillin	100	100	100	100	100
Nalidixic acid	100	100	100	100	100
Gentamycin	96	86	84	70	76
Ciprofloxacin	43	54	67	69	67
Tetracycline	100	100	100	100	100
Trimeth/Sulfameth oxazole	100	100	100	100	100

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Antioxidant Capacity and Microbial Attributes of Raw Cow Milk Fortified with *Hypotrigona Squamuligera* Honey

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Abstract - Diseases such as diabetes, cancers, hypertension and obesity are problematic diseases in the adult population. The use of functional foods that consists of phytocompounds such as antioxidants that can act as prophylactics against such diseases has received considerable attention by the academia, food industry and the general public. The present study aimed at investigating antioxidant capacity and microbial attributes of cow milk fortified with Hypotrigona squamuligera honey. Antioxidant capacity was assayed using 2.2-diphenyl- 1-picrylhydrazyl (DPPH) antiradical activity and peroxide value method. Pour plate assay was used to investigate microbial attributes. The fortified milk revealed a significant DPPH antiradical activity than unfortified milk $p > 0.05$. A higher percentage inhibition of 98.38 ± 0.40 was achieved with fortified milk as compared to 47.27 ± 1.00. Fortified milk also exhibited significantly lower peroxide values and microbial attributes than unfortified milk. The present study results reveals that fortifying milk with H. squamuligera honey improves its antioxidant capacity and microbial inhibitory activity. This means that fortifying milk with H. squamulingera honey preserve milk and maintains its health promoting effects.

Keywords : *antioxidant capacity, microbial attributes, hypotrigona squamuligera, fortified milk, honey.*

GJMR-C Classification : *NLMC Code: QW 51, QW 85*

Strictly as per the compliance and regulations of:

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Abstract - Diseases such as diabetes, cancers, hypertension and obesity are problematic diseases in the adult population. The use of functional foods that consists of phytocompounds such as antioxidants that can act as prophylactics against such diseases has received considerable attention by the academia, food industry and the general public. The present study aimed at investigating antioxidant capacity and microbial attributes of cow milk fortified with *Hypotrigona squamuligera* honey. Antioxidant capacity was assayed using 2.2-diphenyl-1-picrylhydrazyl (DPPH) antiradical activity and peroxide value method. Pour plate assay was used to investigate microbial attributes. The fortified milk revealed a significant DPPH antiradical activity than unfortified milk $p > 0.05$. A higher percentage inhibition of 98.38 ± 0.40 was achieved with fortified milk as compared to 47.27 ± 1.00. Fortified milk also exhibited significantly lower peroxide values and microbial attributes than unfortified milk. The present study results reveals that fortifying milk with *H. squamuligera* honey improves its antioxidant capacity and microbial inhibitory activity. This means that fortifying milk with *H. squamulingera* honey preserve milk and maintains its health promoting effects.

Keywords : antioxidant capacity, microbial attributes, hypotrigona squamuligera, fortified milk, honey.

I. Introduction

Studies in alternative methods of counteracting adult long standing diseases such as type 2 diabetes, obesity, hypertension, and cancers has increased over the years (Ariyoshi et al., 2004). Diet management is one of the most effective ways of lowering the occurrence of such diseases in the general public (Praveeshi et al., 2011). Milk plays an important role in the diet (Chirlaque, 2011). Drinking raw milk is believed to have many advantages (Chirlaque, 2011). It can act as an immune system booster. This is because it consists of natural lymphocytes, antibodies, hormones and growth factors (Chirlaque, 2011). It can also help in fighting diseases such as gout, kidney stones, breast cancer, rheumatoid arthritis and migraine headaches. Raw milk has been used successfully to cure diseases in the past. Raw milk is a wholesome food source of many essential nutrients such as vitamin B, C and antioxidants (Krushna et al., 2007). Antioxidants inhibit the accumulation of free radicals in the body (Francois et al., 2010). Free radicals have been implicated in the pathology of diseases such as cancers, diabetes, obesity and cardiovascular diseases. Vitamins help in preventing breast cancer, colorectal cancer and pancreatic cancer. Although raw milk is beneficial unfortunately it is not free from microbial contamination (Tasci, 2011). Contaminating bacterial can be pathogenic and therefore resulting in serious illness. Heating and sealing is the widely used method. Although this is an effective method unfortunately it destroys useful milk components such as enzymes and antioxidants that are crucial in maintaining health in humans. Other methods of ensuring that milk is free from harmful microorganisms include the use of H_2O_2 and lactoperoxidase system however their acceptance by the public is low. *Apis mellifera* honey has also been tried and interesting results were reported (Krushna et al., 2006). The advantage of using honey is that it is a food substance and it consists of many other useful components such as antioxidants which have been reported to help in preventing current problematic adult diseases. This study aimed at investigating antioxidant capacity and microbial attributes of raw cow milk fortified with *Hypotrigona squamuligera honey. A study conducted by (Dzomba et al., 2012) revealed that H. squamuligera* Fig 1 honey Fig 1 consists of invitro antibacterial and antioxidant activity. *H. squamuligera* honey is used to treat various diseases in folk medicine therefore addition of its extract to milk may help in increasing the prophylactic activity of milk.

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II. Materials and Methods

a) Honey Collection

H. squamulingera honey used in this study was collected from Chinyani village forests in Murewa, Mashonaland East, Zimbabwe in November 2012. Samples were stored in a freezer before use.

b) Extraction

Two grams of honey was added to 20ml of absolute ethanol in a conical flask. The contents were mixed by vortexing and centrifuged at 3000rpm for 10 minutes. The supernatants were then collected, filtered using a Whatman filter paper No. 1 into stoppard test tubes and then dried under vacuum at 40oC. The yield was recorded. The extract was dissolved in ethanol/water (2:3) mixture and stored in a freezer at between 0-5oC.

c) Collection of Milk

Raw milk was collected from farms around Bindura. The milk was divided into two. One lot was quickly fortified with *H. squamuligera* honey extracts at different concentrations. Both the fortified and unfortified milk was placed in a cooler box and transported straight to the laboratory.

Fig. 1 : H. squamulingera insect and its honey

d) DPPH Antiradical Assay

A volume of 1 ml of fortified/unfortified milk was added to 2 ml of DPPH (100µM) dissolved in ethanol. Absorbance of the samples was measured at 517nm after standing for 30 minutes. The blank consisted of ethanol/water mixture. Percentage antiradical activity was calculated as follows;

$$\% \text{ antiradical activity} = \frac{Ac - AS}{Ac} X\ 100$$

Where A_c is the absorbance of DPPH solution without milk, A_s the absorbance of milk and DPPH solution.

e) Peroxide Value Determination

This was performed following a method reported by (Lafka et al., 2007) with some modifications. Two millimeters of milk (fortified or unfortified, 10ml of chloroform, 15ml of acetic acid and 1 ml potassium iodide (10%) were added to a stoppard conical flask. The flask was shaken for 2 minutes and left standing for 10 minutes in the dark. The contents were then titrated with 0.01M sodium thiosulphate and 1% starch as the indicator. A blank run was also performed. Peroxide value expressed as mmoles of active oxygen per Kilogram was calculated as follows.

$$\text{PV (mM/Kg)} = \frac{V - Vo\ X\ C\ X\ 1000}{m}$$

Where V is the volume of sodium thiosulphate for the sample, Vo is volume of sodium thiosulphate of blank, C is the concentration of sodium thiosulphate and m the mass of the sample in g, All assay were repeated five times.

f) Microbial attributes

Enumeration of total viable counts was performed by tenfold dilution of milk samples using ringer solution. Total Viable Count (TVC) was determined using the pour plate technique. Each dilution 0.1 was transferred using a sterile pipette into Petri dishes followed by nutrient agar. The plates were incubated at 37oC for 48 hours. Following incubation plates exhibiting 30-300 colonies were counted. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain TVC. TVC was expressed as the number of organisms of Colony Forming Units (CFU) of samples according to (ISO, 1995).

III. Statistical Analysis

The data is presented as mean ± Standard deviation of five determinations. T-test, $p = 0.05$ was used to compare results of fortified and unfortified milk.

IV. Results and Discussion

a) DPPH antiradical activity

Fortified milk exhibited the highest DPPH antiradical activity. Antioxidant activity remained constant from day 1 to day 4 Table 1. From day 4 percentage inhibitory activity decreased to 77.45 ± 0.80% Table 1. This shows that antioxidants were used to counteract free radicals. Unfortified milk antiradical activity was significantly lower than that for fortified milk in all days. Values started to decrease from day 1 Table 1. Milk consists of appreciable levels of lipids. During storage lipids are easily attacked by oxygen forming peroxyl radicals which are dangerous to health. Even though milk consists of natural antioxidants these are quickly depreciated. This is shown by a quick decrease in antiradical activity. Adding functional foods fortify the milk such that its integrity is maintained. The present results show that fortifying milk with *H. squamuligera* honey maintains its antioxidant properties for some time therefore its health promoting effect.

b) Peroxide Value Determination

The primary products of lipid oxidation are hydroperoxides. Determination of peroxide value gives an indication of how far the milk has gone bad. Peroxidation value for the fortified milk was significantly

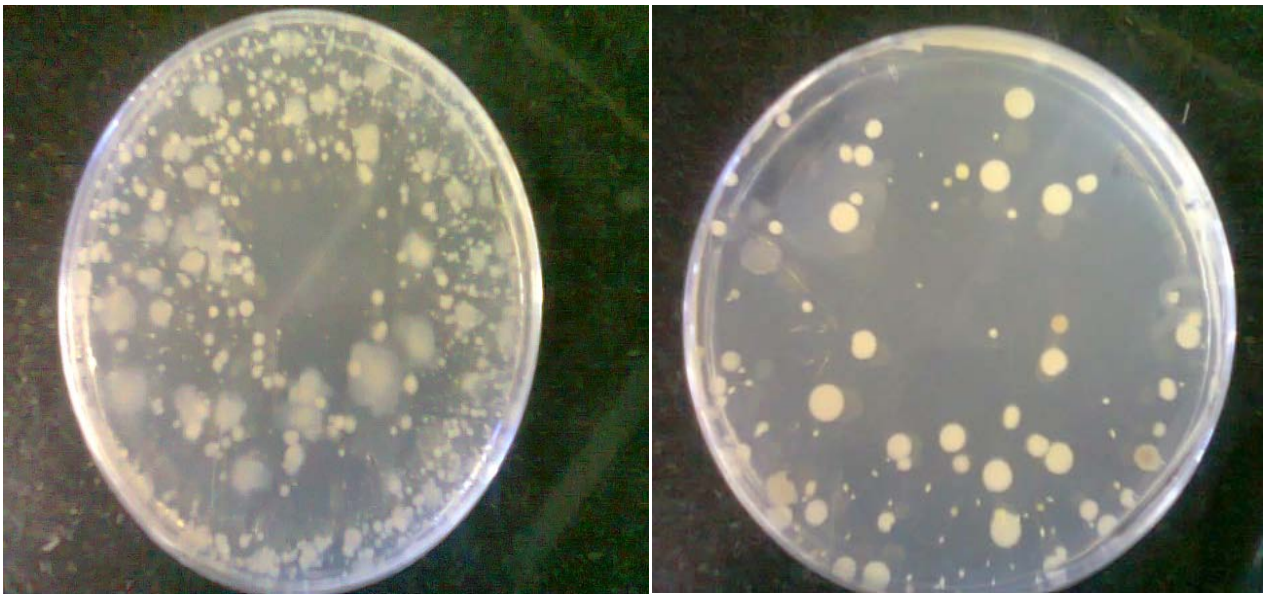
lower than that for the unfortified milk. Peroxidation values for the fortified milk were almost constant Table 2 revealing stabilization. Peroxidation values for unfortified milk increased from day 1 showing quick deterioration.

c) Microbial Attributes

Total Viable Count results reveal that fortify milk with H. squamuligera honey reduces multiplication of bacteria in milk Fig 2.

Table 1 : DPPH free radical scavenging assay

Percentage inhibition ± SD n = 5, All results significantly different T-test, p = 0.05					
	Day 1	Day 2	Day 3	Day 4	Day 5
Fortified	98.00 ± 0.26	97.50 ± 0.50	98.38 ± 0.40	77.45 ± 0.80	73.23 ± 0.50
Unfortified	47.27 ± 1.00	34 .50 ± 0.93	28.86±1.06	18.32 ± 1.30	2.00 ± 0.10



A. B.

Figure 2 : Antibacterial activity of (A) unfortified raw milk (B) raw milk fortified with ethanolic extract of *H. squamuligera* honey

Table 2 : Mean peroxide value for fortified and unfortified milk

% Peroxide value (mM/Kg) ± n = 5 All results significantly different T-test, p = 0.05					
	Day 1	Day 2	Day 3	Day 4	Day 5
Fortified	5.57 ± 0.18	7.05 ± 0.54	7.13 ± 0.15	8.40 ±1.15	8.30 ± 0.34
Unfortified	25.10 ± 0.72	38.20 ± 0.17	55.20 ± 0.07	56.2 ± 0.10	55.20 ± 1.31

Table 3 : Antibacterial activity of ethanolic extract of H. squamuligera honey for extract concentration 10, 20, 50, 100% and unfortified raw milk

Time	Bacterial count cfu/ml n =5, * = significantly different T-test, p = 0.05				
	10%	20%	50%	100%	Unfortied Milk
Day 1	4.5×10^{2}	4.2×10^{2}	3.8×10^{2}	3.2×10^{2}	5.5×10^{2}
Day 2	1.32×10^{6}	1.28×10^{6}	1.01×10^{6}	$4.5\times10^{2*}$	1.41×10^{7}*
Day 3	1.20×10^{7}	1.16×10^{6}	1.11×10^{6}	$8.7\times10^{2*}$	1.70×10^{7}*
Day 4	1.10×10^{7}	1.09×10^{7}	8.50×10^{6}	$1.67\times10^{2*}$	1.50×10^{7}*

Samples that were treated with H. squamuligera honey extracts significantly inhibited the bacteria growth, T-test p > 0.05 as compared to unfortified raw milk. The mean bacterial count of fortified milk was 7.13 X 105 at a concentration of 100% (v/v) and for unfortified was 1.17 X107. Bacterial multiplication inhibition was concentration dependent. Low inhibition was observed at 50% and less. According to (Chambers 2002) when raw milk is of good quality the bacterial load should be around 103/ml. Bacterial load of 107/ml show poor

quality milk. Present results Table 2 show that raw milk collected from the farms bacterial load falls within 103/ml showing quality milk. However milk quality deteriorated as days went by except that for 100% extract concentration. Even on the fourth day its bacterial load was far below 103/ml. The antibacterial activity of honey can be attributed to peroxide and non peroxide components. H2O2 is the major antibacterial factor in honey (Krushna et al., 2007). It is produced during oxidation of glucose and other monosaccharides by glucose oxidase. Non peroxide components include phenolic acids and flavonoids. Phenolic acids and flavonoids are important phytocompounds that have been shown to promote health in humans (Francois et al., 2010).

V. Conclusion

Results of the present study are interesting. Fortifying milk with H. squamuligera honey extracts improves its antioxidant capacity and its microbial attributes. Quality raw milk is favoured by the public because it consists of many nutrients that are believed to promote health. It consists of micronutrients that can act as prophylactics against most adult diseases such as middle age cancers and diabetes type 2. Honey is an attractive option for use in preserving milk because it is a food substance and has been shown to consist of antioxidants.

VI. Acknowledgement

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In-Vitro Susceptibility of Fluoroquinolone Resistance *Escherichia Coli* to Alkaloid Extracted from *Phyllanthusniruri*

By Ajibade, V. A. & Ajenifuja, O. A.

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Keywords : *In-vitro, fluoroquinolone, escherichia coli, alkaloid, phyllanthus niruri.*

GJMR-C Classification : *NLMC Code: QW 138.5.E8*

Strictly as per the compliance and regulations of:

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Abstract - The antibacterial potency of alkaloid extracted from *Phyllanthus niruri* was examined on Fluoroquinolone resistant *Escherichia coli* isolated from different clinical samples using disk diffusion method. Different concentrations (0.1 – 5mg/ml) of the alkaloid were used. It was observed that at 0.5mg/ml the extract showed more potency on *Escherichia coli* isolated from urine than from other samples with a diameter of zone of inhibition of 25.5mm. The percentage susceptibility of the isolated bacterium from urine, blood, semen, swab, and high vagina swab(HVS) to the alkaloid were 75%, 75%, 100%, 60% and 60% respectively. Thirty-seven (37) strains were tested for extended- spectrum beta- lactamase (ESBL) identification. They were all positive for bla_{CTX-M} in 37(100%) of the ESBL-carrying strains. CXT-M-14 was the most frequently isolated ESBL (n=15), followed by CTX-M-27 (n=12) and CTX-M-15(n=5), one strain (CEC7) was carrying both $bla_{CTX-M-14}$and $bla_{CTX-M-15}$. Strain CEC14 was carrying a $bla_{CTX-M-14}$ variant, which differed from the parental enzyme by a single transversion. Using PCR amplification,4 clusters containing 9, 8, 3, and 2 strains were identified. Pulsed-field gel electrophoresis of FQ-resistant *E.coli* identified clonal spread of 1(one) strain among 18 patients. It was concluded that all the bacterium resistant to fluoroquinolone were susceptible to the alkaloid extract.

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I. Introduction

The increasing prevalence of antimicrobial resistance affecting hospitalized populations has gained prominence. Recent investigations reported that among hospitalized patients, residence in a long-term care facility was a risk factor for colonization or infection with *Escherichia coli* that was resistant to higher generation cephalosporin and to the fluoroquinolone (FQ) antimicrobial agents (Lautenbach*et al*., 2001; Lautenbach*et al*., 2002). *Escherichia coli* are a common constituent of the gastro-intestinal flora of most vertebrates, including humans, and may be isolated from a variety of environmental sources. While most strains are nonpathogenic, certain ones can cause a variety of intestinal and extra intestinal infections. Pathogenicity is largely determined by gene-encoding virulence factors such as adhesions, toxins, and polysaccharide surface coatings (Johnson *et al.*, 2009). Phylogenetic analysis showed that most *E.coli* strains fall into 4 main phylogenetic groups, designated A, B1, B2 and D (Arpin *et al*., 2007). *E.coli* strains that cause extra intestinal infections derive predominantly from group B2 and, to a lesser extent, group D. Strains of group A and B1 represent most commensal strains and are largely devoid of virulence determinants (Johnson *et al*., 2009). Although strains harboring a robust extraintestinal virulence factors repertoire cluster predominantly in groups B2 and D, isolates within each phylogenetic group can be further classified as extraintestinal pathogenic *E.coli* (EXPEC) or non-EXPEC depending on whether specific virulence traits are present (Johnson *et al*., 2003; Calbo*et al*.,2005).

The fluoroquinolone (FQs) are potent antimicrobial agents used for the treatment and prophylaxis of infections caused by Gram-negative bacteria, including *E.coli*. FQ-resistant *E.coli* has been reported increasingly during the last decade in both the hospital environment and the community, which may ultimately limit the utility of these broad-spectrum agents (Calbo*et al.*, 2005). However, FQs are still the most frequently prescribed antimicrobial class in hospitals at Ado-Ekiti accounting for 25% of all antimicrobial prescriptions. While evidence suggests that the prevalence of FQ-resistant *E.coli* carriage among residents in Ado-Ekiti is increasing, the present level of risk factors for FQ-resistant *E coli* colonization has not been studied.

Before the advent of modern medicine of which many drugs were synthetically produced, extract of many plants were known to elicit certain reactions in human body when applied in a prescribed manner. Among such plant is *Phyllanthus niruri* L., (Syn. *P. fraternus.* Webster). It belongs to the Euphorbiaceae family and has been claimed to be an excellent remedy for jaundice and hepatitis (Qudhia and Tripathi, 2002; Tabasum*et al*., 2005). The plant is considered analgesic, digestive, emmanagogue, laxative stomachic tonic (Khanna*et al.*, 2002). It is also helpful in treating edema, anorexia and diabetes (George and Roger, 2002.). According to Ayurvedic system of medicine it is considered acrid, cooling, alexiopharmic and useful in thirst, bronchitis, leprosy, anemia, urinary discharge, anuria, biliousness, asthma, for hiccups, and as a diuretic. According to Unani system of medicine, the

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plant is stomachic and good for sores and useful in chronic dysentery (Unander, 1990; Raphael *et al*., 2000; Halim and Ali, 2002). A poultice of the leaves with salt cures scabby infection. The bark yields a bitter principle phyllanthin (Tabasum*et al.,* 2005). Many of the active constituents found in the plant are biologically active lignands, glycosides, flavonoids, saponins, alkaloids, ellagitannins and phenylpropanoids (Tiwalade*et al*.,2000; Dhir *et al.*,2002), common lipids sterols and flavonoids also occur in the plant (Barros *et al.*,2003). Alkaloids are organic nitrogen-containing compound found in 20%-30% of vascular plants ((Tabasum*et al*., 2005) and at lower doses they are useful pharmacologically. Some have important clinical use such as analgesics, antimalarial, antispasmodics, for pupil dilation, and treatment of hypertension, mental disorders and tumors. Morphine, codeine, atrophine and ephedrine are just a few of the plant alkaloid currently used in medicine (Naik and Juvekar, 2003). Other alkaloids, including cocaine, nicotine and caffeine, enjoy a widespread non-medical use as stimulants or sedatives (Naik and Juvekar, 2003). Some alkaloids are medically useful for the cure of human diseases e.g. atrophine in treatment of bronchial asthma (Tabasum*et al*., 2005); intestinal and biliary colic, and to dilate pupil of the eye (Naik and Juvekar, 2003).

The aim of this work is to study the potency of alkaloid extracted from *Phyllanthus niruri* on *Escherichia coli* found to be resistant to fluoroquinolone.

II. Materials and Methods

a) Collection of Plant Material

Phyllanthusniruri was collected from shrubs around the Federal Polytechnic compound, Ado- Ekiti, Nigeria between the months of July and September, 2008 and was identified at the Department of Plant Science, University of Ado-Ekiti, Nigeria. A voucher specimen was deposited at the herbarium of the Department of Science Technology, Federal Polytechnic, Ado-Ekiti. The sample used for the analysis were air-dried at room temperature of (28 ± 2°C) and pulverized.

b) Collection of Specimens and detection of FQ-resistant E.coli

FQ-resistant *E.coli*was detected by antimicrobial activity against Nalidixic acid multo-disk and a 1-step screening procedure (Maslow *et al*., 2004). Species identification and FQ resistance were confirmed by automated testing (Vitek, bioMerieux, USA). Urine, blood, High vaginal swab (HVS), semen and rectal swab samples were obtained from the University Teaching Hospital, Ado-Ekiti.

c) PCR Amplifications

Template DNA was prepared by boiling. Briefly, from 59 initial patient samples, 37(62%) colonies of *E. coli* were suspended thoroughly in 1mL DNase- and RNase-free water and boiled for 10minutes. After centrifugation, supernatant was used as template DNA.The*ampC* was amplified in theupstream region, bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{OXA-I}, and $bla_{CMY}$by PCR, using specific oligodeoxynucleotides. PCR was performed in a 25-µL mixture of 1 x buffer, 2.5 mmol/L $MgCl_2$2.5U of FIREPol DNA polymerase, 200µmol/L of each deoxynucleoside triphosphate, and 25 pmol of each primer. The PCR mixture was subjected to a 5min denaturation step at 94°C, followed by 30cycles of 45s at 94°C, 45s at 55°C, and 60s at 72°C, and a final elongation step of 5min at 72°C. PCR products were separated by 100V elkectrophoresis in a 2% agarose gel for 30min, after which they were stained with ethidium bromide (Maslow *et al.*, 1993;Tenover*et al.,*1995; Etienne *et al*., 2009)

d) Extraction of Crude Alkaloid

The method of Naik and Juvekar (2003) was employed for the extraction. The dried, coarsely powdered whole plant of *P. niruri* (200g) was moistened with 25% ammonium hydroxide, allowed overnight standing and then Soxhlet extracted with 95% ethanol. After concentration under vacuum, the syrup residue (30g) was treated with concentrated hydrochloric acid. The acidic filtrate was washed with benzene, made basic (pH 10) with 25% ammonium hydroxide and extracted with chloroform to afford the alkaloidal fraction.

e) Bacteriological Assay

Isolates were removed from stocks, streaked onto Nutrient agar (LAB) incubated overnight at 37°C to resuscitate the cultures. The organisms were identified by Gram's reaction, colony characteristics and biochemical reactions.

f) Determination of antibacterial potency

The disk Diffusion method described by Odetola and Okorosobo (1996) was employed. Various concentrations of the extracts (10-30mg/ml) were prepared and spread evenly on Nutrient agar. Nalidixic disc was placed on the dried agar and incubated for 24hrs at 37°CThe diameter of zones of inhibition was measured with a meter rule. The plates were examined in triplicate according and the average diameter recorded. Zone measuring≥5.0mm was recorded as susceptible and≤4.0 as resistant. The percentage resistance/sensitive was also calculated.

g) Statistical Analysis

Statistical analysis was performed by using SPSS software version 13.0. The Mantel-Haenszel x^2 test was used for trend analysis.

III. Results and Discussion

The results of the resistant pattern of *E. coli* to fluoroquinolone are shown in table 1. It was observed that all the isolates from urine, blood, semen, rectal

swab and HVS were resistant to fluoroquinolone. Table 2 represents the susceptibility pattern of *E. Colii* solated from different samples to different concentration of alkaloid. The highest susceptibility was seen in urine sample (18) while the lowest was seen in rectal swab and HVS. (3). the percentage susceptibility pattern of the isolates to different concentration of alkaloid is shown in table 3; it was observed that all the isolates were susceptible with the highest susceptibility showed in isolates from semen. Thirty-seven (37) strains were tested for extended- spectrum beta- lactamase (ESBL) identification. They were all positive for bla_{CTX-M} in 37(100%) of the ESBL-carrying strains. CXT-M-14 was the most frequently isolated ESBL (n=15), followed by CTX-M-27 (n=12) and CTX-M-15(n=5), one strain (CEC7) was carrying both $bla_{CTX-M-14}$ and $bla_{CTX-M-15.}$ Strain CEC14 was carrying a $bla_{CTX-M-14}$ variant, which differed from the parental enzyme by a single transversion

Table 1 : Resistant pattern of *E. coli* to fluoroquinolone

Samples	Sensitive (%)	Resistant (%)
Urine	0(0)	24(100)
Blood	0(0)	12(100)
Semen	0(0)	5(100)
Rectal Swab	0(0)	5(100)
HVS	0(0)	5(100)

Table 2 : Susceptibility pattern of different concentration of alkaloid on *E. coli* Isolated from different samples

Samples (No) Number of sensitivities	Concentration (mg/ml)				
	0.1	0.2	0.3	0.4	0.5
Urine (24)	9	10	14	17	18
Blood (12)	8	8	9	9	10
Semen (4)	3	3	3	3	4
Rectal swab (5)	2	2	3	3	3
HVS (5)	0	2	3	3	3

Table 3 : Percentage sensitivity pattern of *E. coli* to alkaloid at 0.5mg/ml

Samples	Sensitive (%)
Urine	18(75)
Blood	10(84)
Semen	4(100)
Rectal Swab	3(60)
HVS	3(60)

Although the patients included in this work came from the clinical, antimicrobial drug resistance was prevalent among UTI-causing strains and those isolated from blood, particularly to β-lactamase producing strains (including extended-spectrum cephalosporins). The findings of this work suggest that CTX-M type β-lactamase is widespread in Ado Ekiti (the area where the research was undertaking). CTX-M production was significantly associated with resistance to quinolones. The spread of CTX-M in the community has already been described through prospective studies in industrialized countries such as Canada (Etienne *et al*., 2009). The excessive use of β-lactam antimicrobial drugs has led to the emergence of resistant strains worldwide. B-lactam resistance is mostly mediated through acquisition of β-lactamase genes located on mobile genetic elements such as plasmids or transposons.

Epidemiologic surveillance of antimicrobial resistance is indispensible for empirically treating infections, implementing resistance control measures, preventing the spread of antimicrobial-resistant microorganisms and most significantly revisiting nature that is cheap and affordable for treating ailments from these pathogens.

The use of alkaloid extracted from the whole plant of *P. niruri* showed high veracity and potency on *E. coli* at the various concentration tested, and especially at 0.5 mg/ml. The data obtained in this study have led to the conclusion that the alkaloid extracted from *P. niruri* is potent on fluoroquinolone resistant *E. coli* and may be responsible for the significant antibacterial effect of this plant on a wide range of organisms. This mayexplain some of the ethno pharmacological claims thatthis plant, especially its application as poultice for the treatment of chronic dysentery is effective (George and Pamplona-Roger, 2002).

Because the resistance patterns are continually evolving and *E. coli* invasive isolates undergo progressive antimicrobial resistance, continuously updated data on antimicrobial susceptibility profiles will continue to be essential to ensure the provision of safe and effective empiric therapies by using herbs. Moreover, results obtained from these surveillance systems must be used to implement prevention programs and policy decisions to prevent emergence and spread of antimicrobial resistance and most importantly embrace phytotherapy.

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Occupational Exposures to Blood and Body Fluids (BBFS) among Health Care Workers and Medical Students in University of Gondar Hospital, Northwest of Ethiopia

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Abstract - Introduction: Occupational exposure to blood and body fluid is a serious concern for health care workers. The problem is more devastating in developing countries like Ethiopia with poor infrastructure and health setup. This study tried to assess the magnitudes of occupational exposure and its associated factors among HCWs and medical students to BBFs in University of Gondar Hospital.

Methods: A cross sectional survey was conducted from September 6 to October 2, 2012, in University of Gondar hospital. Two hundred eighty five participants (including health professionals, janitors and medical students) were participated in the study. Stratified simple random sampling technique was used to select the participants. Data was collected through Self-administered questionnaire and interview using structured questionnaire.

Result: The overall lifetime and one year prevalence's of occupational exposure to BBF during the study period were 177(70.2%) and158 (62.9%), respectively. The exposure rate of BBFs in the last-one year was highest among interns 29(90.6%), followed by health professionals 100(63.3%) and least among housekeeping staffs 28(45.2%).

Keywords : *occupational exposure, health care workers, blood and body fluid.*

GJMR-C Classification : *NLMC Code: QY 400, QY 450*

Strictly as per the compliance and regulations of:

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Conclusion: BBFs exposure among HCWs was a widespread occupational hazard. Occupation, work experience and training were predictors for BBFs exposure. Altering the behavior of HCWs to apply Safety Precautions and an establishment of a surveillance system for registering, reporting and early management of occupational exposure are required.

Keywords : occupational exposure, health care workers, blood and body fluid.

I. Introduction

Blood and body fluid (BBF) exposure to health care workers and the infectious complications associated with it, is a global issue (1, 2, 3).Each day thousands of healthcare workers (HCWs), around the world, suffer accidental occupational exposures during the course of their role of caring for patients. These injuries can result in a variety of serious and distressing consequences ranging from extreme anxiety to chronic illness and premature death for the individual involved (4, 5).

HCWs in developing countries are at serious risk of infection from blood-borne pathogens particularly hepatitis B virus (HBV), hepatitis C virus (HCV) and Human Immunodeficiency Virus (HIV) because of the high prevalence of such pathogens in many poorer regions of the world, especially they are endemic in sub-Saharan Africa (3,6).

In May 2007, the World Health Assembly endorsed the Global Plan of Action on Workers Health (GPA) for the period 2008-2017 with the aim to move from strategy to action and to provide new impetus for action by Member States. It calls on all countries to develop national plans and strategies for its implementation. There have been made countless interventions by employers and workers to attempt to make workplaces healthier, in many countries and many diverse settings (7, 8).

Ethiopia's Federal ministry of health (FMOH) has been leading reform in Hospitals throughout the country using EHRIG and IP is one of thematic area for the reform with aim of stopping the transmission of infectious agents is the only way to reduce the occurrence of Health care acquires infections (HCAIs) to ensure the safety of employees, patients and visitors (9).

Occupational hazards faced by healthcare personnel in University of Gondar hospital have received increasing attention but existing surveillance systems and HCWs responsiveness for Safety Precautions are inadequate to describe the scope and magnitude of occupational exposures to infectious agents that HCWs experience, the outcomes of these exposures and injuries, and the impact of preventive measures (10).

a) Study Setting

The study was conducted in University of Gondar Hospital; Gondar was one of the metropolitan cities of Ethiopia, founded in 1636 by Emperor Fasilades, 748 kms northwest from Addis Ababa.

b) Participants

Two hundred eighty five participants (including health professionals, janitors and medical students) of Gondar University Hospital were included in the study.

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c) *Study Population*

Sampled health professionals, janitors and medical students

i. *Inclusion and Exclusion Criteria*

Health Care Workers that are assigned for clinical services, academic staffs that had regular program for patient care and graduate bacth interns were included in the study.

Health Care Workers who were on leave (maternity, annual, sick, fieldwork) during the study period as well as HCWs who had not worked at least six months within the last one year , were excluded in this study.

d) *Sample size and sampling technique*

Sample size was calculated using a single population proportion. Considering 20.2% of one-year prevalence of at least one BBFs exposure in previous study in Ethiopia (13), a sample size of 285 was required with sampling error 5%, at a 5% c confidence level.

A stratified sampling technique was used to select the participants using lists of monthly payroll as a sampling framework and the number of participants selected from each stratum was determined by proportional to size.

e) *Study Variables*

Dependent:- Exposures of HCWs and medical students to blood and body fluids.

Independent:- Age, gender, marital status, religion, occupation, work experience in the area, uses of Personal Protective equipments, Infection Prevention trainings, educational status and working shift.

f) *Data collection procedures and quality assurance*

The data collection was done via self-administered questionnaire and interview using structured questionnaire. Self-administered question-naire was used for Health professionals and interns, and data from janitors was collected by interview using the structured questionnaire.

Quality of data was assured through intensive training for data collectors and supervisors, pre-testing the questionnaire on similar setting that was not included in the study, close supervision and assistance of data collectors, checking filled questionnaires on daily basis for completeness, clarity and accuracy of data. In addition data cleaning was also made before commencement of the analysis.

II. Data Processing and Analysis

Data was coded and entered into the SPSS version 20. Descriptive statistics was made using frequencies, tables, and figures and narrative explanations. Associations were examined using binary and multiple logistic regressions. P-value less than0.05 was taken as a cut-off point to say significant at 95% confidence level.

a) *Ethical Considerations*

Ethical approval was obtained from the Research and Ethics Review Committee at Bahir Dar University. Written consent was also obtained from Amhara-regional health Bureau and University of Gondar hospital to conduct the study. All the study participants were informed about the purpose of the study and finally verbal consent was obtained before data collection. The respondents had the right to refuse participation or terminate their involvement at any point during the study. Information provided by each respondent was kept confidential. Furthermore, report writing did not refer a specific respondent with identifiers.

III. Result

Out of the 285 selected HCWs and graduate batch interns, 252 responded giving response rate of 88.4%. Twelve incomplete questionnaires were discarded. Among the respondents, 127 (50.4%) were males. From the total participants 158 (62.7%) were health professionals, (24.6%) were housekeeping staffs, and 32 (12.7%) were graduated interns. Respondents age range from 19 to 54 years with a mean age of 29 years. Among participants in this study 113(44.5%) had less than two years of work experiences. HCWs who hand experiences two to four years were 62(24.6%) and only 45(17.7%) were five years and more experienced in their current position. Other demographic information is presented in Table 1.

The overall prevalence of lifetime and one-year prevalence of one occupational exposure to BBF at least once during the study period were 177(70.2%) and1587 (62.3%) respectively (Table 2). The exposure rate of BBFs in the last-one year was highest among interns 29(90.6%), followed by HP 100(63.3%) and least among housekeeping staffs 28(45.2%). Among exposed for NSI in the last twelve months, 50(61.0%) of them encountered once and 32 (39.0%) of faced two and more times.

Of those NSI exposed, 25 (30.5%) of respondents reported that the needle or sharp objects that cause the recent injury were visibly contaminated with blood prior to causing the injury, where as 38(46.3%) and 19(23.2%) were not contaminated and not sure respectively. Majority of the recent NSI, 58(70.7%) of were moderately penetrated skin while superficial skin scratch and deep punctures or wounds were 18(22.0%) and 6(2.7%) respectively. Hollow needles contributed to 45(54.9%) of the recent needle-stick injuries. Suture needles and other solid sharps contributed to 23 (28.0%) and glass and non-sharp safety devices causes 14 (17.1%) of the percutaneous exposures.

Of mucosal membranous exposure for blood and other body fluids in the last one-year, 46(34.3%) of

faced once, 60(44.8%) of two-three times and 28(20.9%) of four and more. Blood and blood product caused 86(64.2%) of splash exposures within the last one year. Body fluids and other body products like tissue accounts 32(23.9%) and 16(11.9%) respectively. Mucosal exposures to amniotic and vaginal fluid 28(43.1%), pleural and other organ fluid 19(29.2%), urine, saliva/sputum and stool 18 (27.7%) and were contributing factors to accidental exposure to body fluids. Of Mucosal exposed HCWs, 62(46.3%) faced small (<5cc) and 12(9.0) moderate (6-50cc) but 60(44.8%) did not know the amounts of BBF they faced. The most common exposed body parts were hand and finger 82(52.2%), followed by face, eye and mouth 43 (27.4%). Other less frequent sites were any two body parts at a time (e.g. hand and face) 19(12.1%) and leg and foot 13 (8.3%).

Overall, performing procedure for patients 78(52.2%) was the most hazardous procedure particularly among interns and staff health professionals. Disassembling, processing and cleaning used equipments 30(19.1%), phlebotomy, collecting and transporting blood sample 21 (13.4%) and assisting delivery 16(10.2%) were other hazardous procedures exposing the HCWs to potential infectious material in order of frequency. Major causes for occurrences BBFs exposure are presented in Figure 1.

Regarding the overall situation in which the recent exposures occurred, 49(31.2%) were occurred in the morning shift, while 40(25.5%) and 31 (19.7) occurred in the afternoon and in the night shifts respectively; but 37(23.6%) of them did not remember the shift that the exposure was happened.

IV. Discussion

Exposure to blood and potentially infectious body fluids has been recognized as a potential health hazard in HCWs (1, 4). In this study, 62.3% of the respondents were exposed to blood and contaminated body fluids at least once in the preceding year through NSI and splash .This was similar with studies in South Africa (62%) (11). There was a 32.5% of one year prevalence of needle sticks and sharps injuries identified in this study ,which was the same with previous study in Hawassa City(30.9%), but different from Harari Regional State and Dire Dawa Administrative Council (17.5%) and Addis Ababa (19%) (12-14). Although needle stick injuries are most common means of exposure for health care workers, blood borne pathogens can also be transmitted through contact with eyes, nose, and mouth or through broken skin (15, 16). The prevalence of one year splash exposure of BBFs was 53.2%, which was also different from Harari Regional State and Dire Dawa Administrative Council (20.2%) (12). This high prevalence of NSI and splash exposure in this study might be due to inclusion of medical students and housekeeping staffs which were not targeted in previous studies and might also be due to high work load on few professionals in the study area.

It has been reported that medical students experience the majority of needle-stick injuries in the world (3, 5). The results of this study showed that highest prevalence of percutaneous (68.8%) and mucosal (75%) exposure among medical students, which was almost comparable with other studies in South Africa (62%) and Uganda that reveal interns suffered more NSI than any occupational groups (17,18).

Eighty two percents of respondents had work experiences of less than five years. There was significant correlation between work experiences and the rate occupational exposure to BBFs. HCWs whose experience was two-four years were more exposed than HCWs who had experiences above four years (AOR=3.2,95% CI=1.4-7.5). Studies done in Iran showed that about 54% of the personnel with less than five years of working experience were exposed at least once during the previous year; however, this was 30.6% among the personnel with more than ten years of experience (19). Study in India identified that as experience was increasing, incidence of injury was decreasing (21). Moreover, HCWs in University of Gondar hospital have to deal with a high load of patients, this fact combined with urgency of some interventions and unavailability of PPEs might contributed to this high prevalence of BBFs among studied groups.

Healthcare workers have increased chance of acquiring blood borne pathogens through occupational exposure in developing countries due to a combination of increased risk and fewer safety precautions (3, 22). Most exposures are caused by a departure from standard precautions (23). One of the factors for occurrences of BBFs exposure in this finding was lacks of use of PPEs (39.5%), non-consistent use of PPEs was found to be associated with chance of sustaining NSI previous studies in Ethiopia (12,13,14,24). Wearing gloves may reduce (>50%) the volume of blood introduced through an injury (23). A major reason for not using all PPEs was inadequate supply (59.7%). Previous study of Ethiopia showed that Seventy-nine percent of HCWs reported that they did not wear any of the PPEs because of unavailability (24). Exacerbating the risk to health care workers in developing countries is a lack of gloves, gowns, masks, and goggles to protect them from contact with blood(3).

Needle-stick injuries to providers are usually attributable to the abrupt movement of patients during the procedure (25). Unexpected movement of patients during care was second major factor for BBFs exposures (23%); this was similar with report from West Africa (23%) (27). In previous studies in Ethiopia and abroad, needle recapping was major causes of NSI

(5,12-14,19), promisingly, it was of interest that needle recapping was not listed as a cause for accidental exposure in this study, suggesting adherence to the hospital's protocol and guidelines(9).

The risk of acquiring blood borne infections (BBI) from occupational exposures is dependent on the concentration of infectious virus in the implicated body fluid, the volume of infected material transferred, frequency of percutaneous, per mucosal exposure to blood or body fluid, device visibly contaminated with the source patient's blood, depth of injury, procedures involving a needle placed directly in the patient's vein or artery and type of needle involved (hollow needles contain more blood therefore there will be a higher risk of transmission) (3,26). In this survey, Hollow needles contributed to 55% of the needle-stick injuries which was the same with prior studies in Ethiopia and overseas (5, 6, 12-14, 19, 20, 24). Suture needles contributed to 28% of the percutaneous exposures, which was the same as South Africa (17).

Of the mucosal exposures within the last 12 months, blood and bloody products were involved in 64% of exposure. NaSH showed that blood and blood product involved in almost 79% of all reported splash exposures and it was 86% study in Iran (19, 20).

Study in India explored that exposure was inversely related to training, a sizeable number of those trained were subsequently exposed (21). In this study satisfactory training, but not training by itself, was highly associated with preventions of BBFs exposures. HCWs who was satisfied with trainings given was less likely exposed for BBFs than none trained and unsatisfied one (AOR=0.501, 95% CI=0.23-0.91).

a) Strength and Limitations of the Study

Including both health professionals and non health professionals like janitors/cleaners to asses occupational exposure to BBF could be taken as the strength of the study but not screening of viral infections like HIV and hepatitis for exposed HCWs and since the study was based on self report about previous one year and life time occupational exposure to BBF the result might be affected by recall bias.

V. Conclusion

Injuries from sharp objects and BBFs splash exposure among HCWs were a widespread occupational hazard in University of Gondar hospital and higher previous studies in Ethiopia. Occupation, work experience and satisfactory training were factors for occurrences occupational exposure. This study showed that medical students were more exposed to BBFs than health professionals and housekeeping staffs.

This loss of a wage-earning health care worker can be devastating to the financial security of the worker's family. The loss of HCWs can also have a disproportionate effect on the fragile health care infrastructure of Ethiopia, where trained health professionals are scarce in relation to the overall populations they serve.

VI. Recommendations

1. Initial effort is supposed to be focused on altering the behavior of HCWs to follow standard operating procedures during patient care and consistent availability of PPEs.
2. The ways of infection prevention training supposed to be revised and participant centered because the results of this study showed that satisfactory training, but not training by itself, has impacts on prevention of blood and body fluid exposures to healthcare workers.
3. Attending physicians and senior HCP must be made responsible for ensuring that students are capable of performing procedures safely before expecting them to do so without supervision. It is responsibility of medical educators to provide a safe learning environment for students before they face the risks of direct patient care.

VII. Acknowledgements

We sincerely thank the study participants for their participation in the study.

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Table 1 : Socio-demographic variables of healthcare workers and medical students in University of Gondar Hospital, 2012

Demographic Variables	Frequency(N=252)	(%)
Age of respondent		
19-24 years	106	42.1
25-34 years	118	46.8
35 and above	28	11.1
Marital status		
Single	161	63.9
Married	83	32.9
Divorced & widowed	8	3.2
Religion		
Orthodox	215	85.3
Muslim	18	7.1
Other Christians	19	7.5
Educational status		
Intern	32	12.7
12/10 not completed	5	2.0
12/10 completed	41	16.3
Diploma	56	22.2
1st degree and above	118	46.8

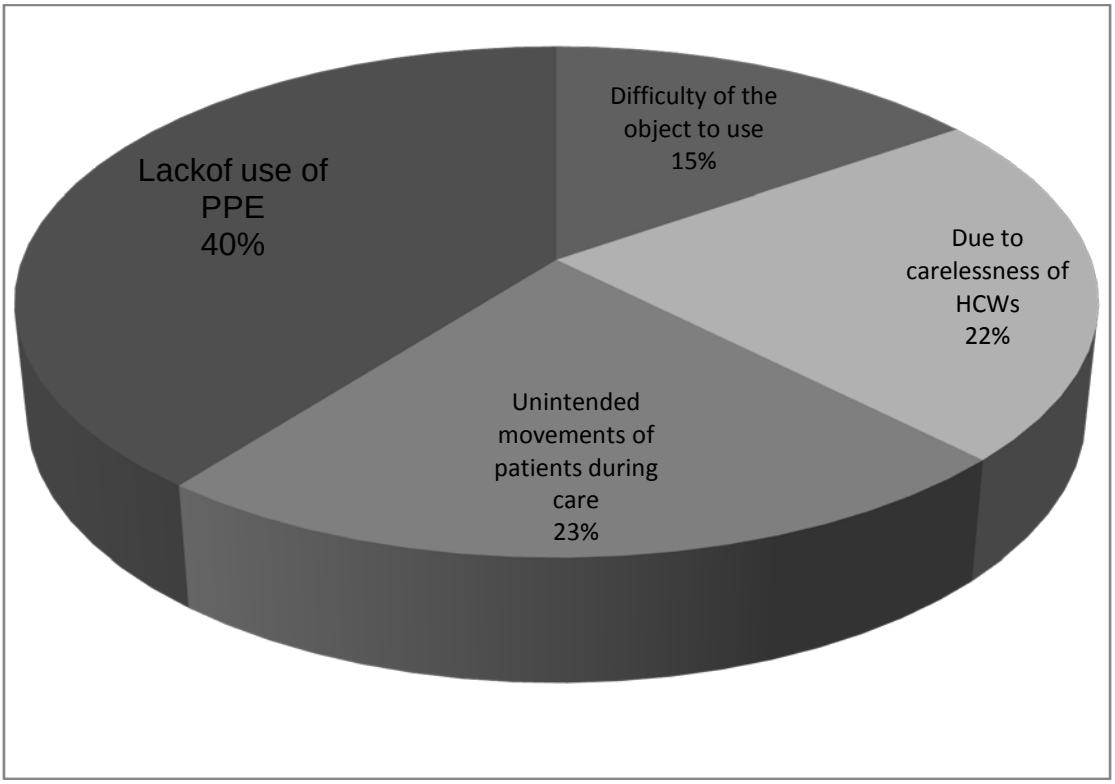
Table 2 : Prevalence's of blood and body fluid exposure to healthcare workers and medical students in University of Gondar hospital, Sep. 2012

Exposure	NSI N (%)	BBF splash N (%)	Total Exposure rate N (%)
Life time			
Health prof.	66(41)	108(68)	117(74)
Intern	22(69)	25(78)	29(91)
Housekeep	14(23)	23(37)	31(50)
Total	104(41)	156(62)	177(70)
Last 12 months			
Health prof.	49(31)	89(56)	100(63)
Intern	22(69)	25(78)	29(91)
Housekeep.	11(18)	21(34)	28(45)
Total	82(33)	134(53)	157(62)

Table 3 : Multivariate logistic regression results of blood and body fluids exposure within the previous one year in University of Gondar hospital, Sep.2012

Variable	Exposed (n=157)	Not Exposed (n=95)	AOR (95% CI)	P-Value
Occupation				
Intern	29(19)	3(3)	**9.4(1.8-49.9)***	0.009
Health Prof.	100(64)	58(61)	1.2(0.37-3.69)	0.799
Housekeep.	28(18)	34(36)	1	
Experience in years				
< two years	86(55)	59(62)	0.94(0.5-2)	0.863
2-4years	47(30)	15(16)	**3.2(1.4-7.5)***	0.008
Above four years	24(15)	21(22)	1	
IP training				
Satisfactory	49(31)	42(44)	**0.5(0.3-0.9)***	0.023
Not satisfactory	108(69)	53(56)	1	

Figure 1 : Major factors for occupational exposure to blood and body fluids among healthcare workers and medical students in University of Gondar hospital, Sep.2012



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The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also.Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

Papers: These are reports of significant research (typically less than 7000 words equivalent, including tables, figures, references), and comprise:

(a)Title should be relevant and commensurate with the theme of the paper.

(b) A brief Summary, "Abstract" (less than 150 words) containing the major results and conclusions.

(c) Up to ten keywords, that precisely identifies the paper's subject, purpose, and focus.

(d) An Introduction, giving necessary background excluding subheadings; objectives must be clearly declared.

(e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition; sources of information must be given and numerical methods must be specified by reference, unless non-standard.

(f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;

(g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.

(h) Brief Acknowledgements.

(i) References in the proper form.

Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and to make suggestions to improve briefness.

It is vital, that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

Format

Language: The language of publication is UK English. Authors, for whom English is a second language, must have their manuscript efficiently edited by an English-speaking person before submission to make sure that, the English is of high excellence. It is preferable, that manuscripts should be professionally edited.

Standard Usage, Abbreviations, and Units: Spelling and hyphenation should be conventional to The Concise Oxford English Dictionary. Statistics and measurements should at all times be given in figures, e.g. 16 min, except for when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelt in full unless, it is 160 or greater.

Abbreviations supposed to be used carefully. The abbreviated name or expression is supposed to be cited in full at first usage, followed by the conventional abbreviation in parentheses.

Metric SI units are supposed to generally be used excluding where they conflict with current practice or are confusing. For illustration, 1.4 l rather than 1.4 × 10-3 m3, or 4 mm somewhat than 4 × 10-3 m. Chemical formula and solutions must identify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by underlines at the first mention. For following use the generic name should be constricted to a single letter, if it is clear.

Structure

All manuscripts submitted to Global Journals Inc. (US), ought to include:

Title: The title page must carry an instructive title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) wherever the work was carried out. The full postal address in addition with the e-mail address of related author must be given. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining and indexing.

Abstract, used in Original Papers and Reviews:

Optimizing Abstract for Search Engines

Many researchers searching for information online will use search engines such as Google, Yahoo or similar. By optimizing your paper for search engines, you will amplify the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in a further work. Global Journals Inc. (US) have compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Key Words

A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy and planning a list of possible keywords and phrases to try.

Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art.A few tips for deciding as strategically as possible about keyword search:

- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals Inc. (US) homepage at the judgment of the Editorial Board.

The Editorial Board and Global Journals Inc. (US) recommend that, citation of online-published papers and other material should be done via a DOI (digital object identifier). If an author cites anything, which does not have a DOI, they run the risk of the cited material not being noticeable.

The Editorial Board and Global Journals Inc. (US) recommend the use of a tool such as Reference Manager for reference management and formatting.

Tables, Figures and Figure Legends

Tables: Tables should be few in number, cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g. Table 4, a self-explanatory caption and be on a separate sheet. Vertical lines should not be used.

Figures: Figures are supposed to be submitted as separate files. Always take in a citation in the text for each figure using Arabic numbers, e.g. Fig. 4. Artwork must be submitted online in electronic form by e-mailing them.

Preparation of Electronic Figures for Publication

Even though low quality images are sufficient for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit (or e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings) in relation to the imitation size. Please give the data for figures in black and white or submit a Color Work Agreement Form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution (at final image size) ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs) : >350 dpi; figures containing both halftone and line images: >650 dpi.

Figure Legends: Self-explanatory legends of all figures should be incorporated separately under the heading 'Legends to Figures'. In the full-text online edition of the journal, figure legends may possibly be truncated in abbreviated links to the full screen version. Therefore, the first 100 characters of any legend should notify the reader, about the key aspects of the figure.

6. AFTER ACCEPTANCE

Upon approval of a paper for publication, the manuscript will be forwarded to the dean, who is responsible for the publication of the Global Journals Inc. (US).

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The corresponding author will receive an e-mail alert containing a link to a website or will be attached. A working e-mail address must therefore be provided for the related author.

Acrobat Reader will be required in order to read this file. This software can be downloaded

(Free of charge) from the following website:

www.adobe.com/products/acrobat/readstep2.html. This will facilitate the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof.

Proofs must be returned to the dean at dean@globaljournals.org within three days of receipt.

As changes to proofs are costly, we inquire that you only correct typesetting errors. All illustrations are retained by the publisher. Please note that the authors are responsible for all statements made in their work, including changes made by the copy editor.

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Before start writing a good quality Computer Science Research Paper, let us first understand what is Computer Science Research Paper? So, Computer Science Research Paper is the paper which is written by professionals or scientists who are associated to Computer Science and Information Technology, or doing research study in these areas. If you are novel to this field then you can consult about this field from your supervisor or guide.

TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish 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.

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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
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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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