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Pharma, Drug Discovery, Toxicology & Medicine

Evaluation and Ranking of Drug

A Prospective Observational Study

Highlights

Antibiotic use During Pregnancy

Cross-Linkers in Submicron Particles

Discovering Thoughts, Inventing Future

VOLUME 22 ISSUE 3 VERSION 1.0

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Evaluation and Ranking of Drug Release from Different Grades of Guar Gum, Acacia Gum and Polyvinyl Pyrrolidone as Cross-Linkers in Submicron Particles

By Negla Abdulghani Elsayed Yagoub, Dr. Abubakar Osman Mohamed Nur, Fadilah Sfouq Aleanizy & Sarah Ahmed

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Abstract- Due to their unique properties, nanoparticles made of polysaccharides are promising carriers to deliver and protect the physiological properties of hydrophilic drugs. They have been successfully applied as drug delivery systems (83).

Objective: The main goal of this research is to Improve Carbamazepine water solubility and drug release properties by nano sizing, and using guar gum, Acacia Gum and poly-vinylpyrrolidone, each of two viscosity grades, as crosslinking agents. Moreover, the study is extrapolated, utilizing composite index (CI) design and mathematical modelling, in an attempt to locate the most suitable set of the factors that affect nanoparticles produced with optimum specifications.

Keywords: polymer, Guar gum, acacia gum, polyvinyl pyrrolidine, carbamazepine, drug release, composite index.

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Abstract- Due to their unique properties, nanoparticles made of polysaccharides are promising carriers to deliver and protect the physiological properties of hydrophilic drugs. They have been successfully applied as drug delivery systems (83).

Objective: The main goal of this research is to Improve Carbamazepine water solubility and drug release properties by nano sizing, and using guar gum, Acacia Gum and poly-vinylpyrrolidone, each of two viscosity grades, as crosslinking agents. Moreover, the study is extrapolated, utilizing composite index (CI) design and mathematical modelling, in an attempt to locate the most suitable set of the factors that affect nanoparticles produced with optimum specifications.

Methods: The method used nano and submicron particles that were produced in our previous study (Evaluation of different grades of guar gum, acacia gum and polyvinyl pyrrolidone as cross-linkers in producing submicron particles). All runs were subjected to drug release investigations according to which a weighted composite index was generated.

Results: Based on the obtained findings and the associated statistical analysis, particles of run8 were found to be the best ranked as they fulfilled all the constraints.

Conclusion: Acacia gum was found to have the most interesting properties in developing submicron particles with controlled drug release, accordingly the study recommends the need for further investigations.

Keywords: polymer, Guar gum, acacia gum, polyvinyl pyrrolidine, carbamazepine, drug release, composite index.

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I. Introduction

a) Drug Release

A central reason for pursuing nanotechnology is to deliver drugs, hence understanding the manner and extent to which the drug molecules are released is important. The drug loading of the nanoparticles is generally defined as the amount of drug bound per polymer mass (usual moles of drug per mg polymer or mg drug per mg polymer); it could also be given as a percentage relative to the polymer.

Nanoparticles made of polysaccharides, due to their unique properties, are promising carriers to deliver and protect the physiological properties of hydrophilic drugs and have been successfully applied as drug delivery systems (1) As natural biomaterials, polysaccharides are stable, safe, nontoxic, hydrophilic, and biodegradable.

b) Biological benefits of nanoparticles

The property of nanoparticle formulations that make this approach highly beneficial is related to the surface properties imparted on nanometer-sized entities (2). Applying Nano-crystal Technology or one of the alternate nanoparticle formulation approaches to the many formulation and performance issues associated with poorly water-soluble compounds in the pharmaceutical industry provides many benefits.

c) The Solubility Challenge

It is estimated that ~40% of active substances identified through combinatorial screening programs are difficult to formulate as a result of their lack of significant solubility in water (3, 4, and 5). In one sense, this is understandable. If a molecule must penetrate a biological membrane to be absorbed, the molecule generally must possess some hydrophobic or lipophilic characteristics. When these types of situations arise, a nanoparticle formulation approach has proven to be very useful and invaluable in all stages of drug development and has opened opportunities for revitalizing marketed products with suboptimal delivery.

d) Guar gum

Guar gum (GG) is galactomannan derived from Guar *Cyamopsis tetragonolobuskernels* which belong to family *Leguminosae.*

It is biocompatible, biodegradable, non-toxic, low-cost and amenable to chemical modifications, properties that make it an ideal material for developing drug delivery formulations (6). However, native guar gum has also shortcomings such as, uncontrolled rates of hydration, high swelling, thickening effect, instability upon storage, high susceptibility to microbial attack and the difficulty to control viscosity due to relative fast biodegradation (7).

Thermal treatment of guar gum at 70℃ for 10 minutes is an efficient tool to produce guar gum with desired properties for pharmaceutical processing and industries. The treatment has resulted in the production of treated guar gum with improved flowability, swellability, and compressibility. On the other hand, the method of drying seems to have a significant influence on the viscosity of the resultant treated guar powder and verification of such effect might necessitate a more collaborated extended study (8).

e) Acacia Gum

This is the dried exudate of the acacia tree (*Acacia senegal*or related species of *Acacia* Fam. *Leguminosae*. The gum is highly soluble in water. Physically, acacia is considered to be a complex, highly branched, globular molecule, which is closely packed rather than linear, thus accounting for its low viscosity. Rheologically, acacia gum solutions exhibit typical Newtonian behavior at concentrations up to 40%. Above 40%, solutions become pseudoplastic, as is shown by a decrease in viscosity with increasing shearing stress (9).

f) Povidone

PVP is a water-soluble pharmaceutically acceptable polymer. Due to its ability to improve solubility and wettability of poorly soluble drugs, it is frequently used in solid dispersions to enhance solubility and dissolution rate (10, 11), Due to its hydrophilicity and rapid dissolution in an aqueous medium, PVP is very frequently applied as a carrier in immediate release dosage forms. PVP has a long history of use in human drug products and high molecular weight PVPs generally do not get absorbed in the GI tract.

g) Carbamazepine (*CBZ*)

One of the bad soluble active drug substances. Although Carbamazepinehas a high intestinal permeability, its bioavailability is limited by its low water solubility (0.11mgmL−1) (2).

5H-ibenz[b,f]azepine-5-carboxamide A white or almost white crystalline powder. It exhibits polymorphism that is very slightly soluble in water; sparingly soluble in alcohol and in acetone, and freely soluble in dichloromethane.

Carbamazepine is widely distributed throughout the body and is about 70 to 80% bound to plasma proteins. It induces its own metabolism so that the plasma half-life may be considerably reduced after repeated dosage.

The mean plasma half-life of carbamazepine on repeated dosage is about 12 to 24 hours; it appears to be considerably shorter in children than in adults.

Carbamazepine is a dibenzazepine derivative with antiepileptic and psychotropic properties. It is used to control secondarily generalisedtonic-clonic seizures and partial seizures and in some primary generalized seizures.

h) Composite index

A composite index is a grouping of equities, indexes or other factors combined in a standardized way, providing a useful statistical measure of overall market or sector performance over time, and it is also known simply as a "composite." Usually, a composite index has a large number of factors that are averaged together to form a product representative of an overall market or sector (12).

II. Materials and Methods

Materials: The Nano and submicron particles produced in our previous study (Evaluation of different grades of guar gum, acacia gum and polyvinyl pyrrolidone as cross-linkers in producing submicron particles) as in Table1 are used in this study

Table I: Layout of formulation runs according to mixed 3-2 -levels factors and 1- 3-levels factor statistical design

Run	Stirring Rate	Polymer grade	Polymer load	Polymer type
R1	1000	G-non treated	1%	Guar gum
R2	1000	Acacia lower viscosity	1%	Acacia Gum
R3	1000	Povidone K90 higher viscosity	1%	Povidone
R4	1000	G-non treated	10%	Guar gum
R5	1000	Acacia lower viscosity	10%	Acacia Gum
R6	1000	Povidone K90 higher viscosity	10%	Povidone
R7	1000	G- treated	1%	Guar gum
R8	1000	Acacia higher viscosity	1%	Acacia Gum

R9	1000	PovidoneK30 lower viscosity	1%	Povidone
R10	1000	G- treated	10%	Guar gum
R11	1000	Acacia higher viscosity	10%	Acacia Gum
R12	1000	PovidoneK30 lower viscosity	10%	Povidone
R13	500	G-non treated	1%	Guar gum
R14	500	Acacia lower viscosity	1%	Acacia Gum
R15	500	PovidoneK30 lower viscosity	1%	Povidone
R16	500	G-non treated	10%	Guar gum
R17	500	Acacia lower viscosity	10%	Acacia Gum
R18	500	PovidoneK90 higher viscosity	-10%	Povidone
R19	500	G- treated	1%	Guar gum
R20	500	Acacia higher viscosity	1%	Acacia Gum
R21	500	PovidoneK30 lower viscosity	1%	Povidone
R22	500	G- treated	10%	Guar gum
R23	500	Acacia higher viscosity	10%	Acacia Gum
R24	500	PovidoneK30 lower viscosity	10%	Povidone

a) Apparatus

The following instruments were used in the experimental part of this study:

Instrument	Specification and Source
Analytical balance	Reblab ®, Germany
Zetasizer 90 plus	Malvern Panalytical Ltds
U.V. Spectrophotometer	double beam UV-1800, Shimadzu, Japan
Magnetic stirrer	Stuart, England
Scanning electron microscope	Zeiss EVO LS10; Cambridge, United Kingdom

b) Methods

Collected submicron particles from all runs were subjected to the following qualifications.

c) Particle size analysis

By using particle size analyser 90, measurements of polydispersity (PD %) were performed.

A specified amount of dry particles was completely dissolved in ethyl acetate, filtered and transferred to the instrument cell and subjected to the test.

d) Entrapments efficiency of nanoparticles

Dried nanoparticles were dissolved in ethyl acetate (a common solvent for polymers and drug samples). The amount of entrapped carbamazepine that was present in the solution was measured spectrophotometrically at 287 nm (USP, 13).

Drug incorporation efficiency was expressed both as Drug Content (% w/w), also referred to as drug loading in the literature, and Drug Entrapment (%); represented by Eqs. (1) and (2) respectively. The individual values for two replicate determinations and their mean values were reported

$$\text{Drug loading (\% w/w)} = \frac{Mass\ of\ drug\ in\ nanoparticles}{Mass\ of\ nanoparticle}\%100 \quad (1)$$

$$\text{Drug Entrapment (\%)} = \frac{Mass\ of\ drug\ in\ nanoparticles}{Mass\ of\ drug\ used\ in\ formulation}\%100 \quad (2)$$

e) Nanoparticle drug release assessment

All runs were subjected to drug release investigations where the amount of particles equivalent to 1 g of carbamazepine was weighed and transferred to a dissolution test beaker containing 1L of sodium lauryl sulphate. 3ml of each sample was filtered into 100 ml volumetric flask and the absorbance of the samples was determined at 287 nm against water as a blank (14). Making use of the drug calibration curve (as discussed next), the amount of carbamazepine was then estimated. The assay method was derived from the USP carbamazepine tablets dissolution test monograph (USP, 13).

f) Calibration curve

From the reference standard Carbamazepine, 40 mg was accurately weighed and dissolved in 8 ml absolute methanol, 1 ml of this solution was taken and diluted to 10 ml. Serial dilutions were then carried out to obtain solutions of different drug concentrations. The absorbance of each concentration at 287nm was determined spectrophotometrically and a calibration curve was thus generated (USP,13).

g) Composite index design

A weighted composite index was generated for the data to designate a single score utilizing three constraints (15). This was done in order to select the optimized factors setting (polydispersity, Entrapment Efficiency and nanoparticle drug release rate at 60 mints) that could possibly yield the most desired properties for drug granules and tablets. The process of statistical composite index application was aided by the computer Excels program.

III. Results

a) Characterization of produced particles

Table 2 summarizes the polydispersity index (PDI %) and entrapment efficiency (EE %) properties of produced particles within different formulation runs. The Carbamazepine calibration curve and drug release profiles of different formulation runs are depicted in figures 1 and 2, respectively.

Table 2: Polydisperse index (PDI %) and entrapment efficiency (EE %) of yielded particles within different formulation run

Run No.	EE	PDI
R1	52.3%	5.50%
R2	52.3%	0.52%
R3	52.3%	1.76%
R4	13.1%	0.37%
R5	13.1%	0.50%
R6	13.1%	0.43%
R7	52.3%	0.62%
R8	52.3%	0.30%
R9	52.3%	0.67%
R10	13.1%	0.39%
R11	12.5%	4.04%
R12	11.8%	0.40%
R13	39.6%	0.55%
R14	47.0%	0.44%
R15	45.0%	0.69%
R16	12.7%	0.38%
R17	13.1%	1.09%
R18	13.1%	1.04%
R19	52.3%	0.09%
R20	52.3%	0.38%
R21	52.3%	0.56%
R22	13.1%	1.74%
R23	13.1%	0.81%
R24	13.1%	16.64

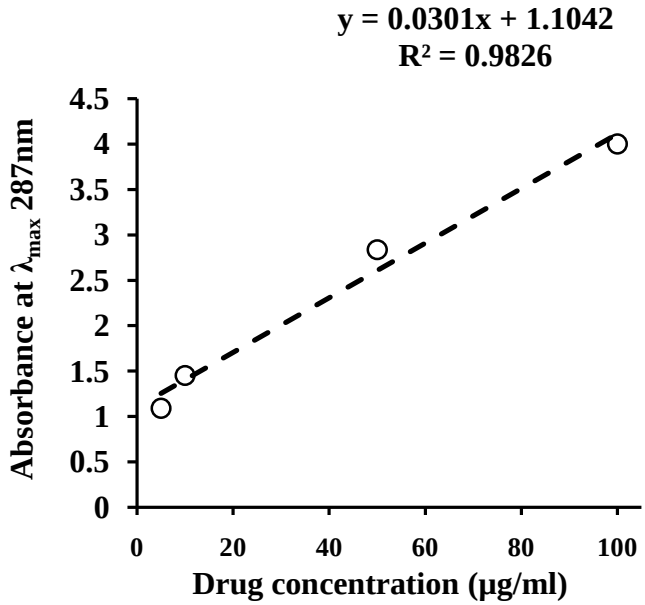

Fig. 1: Calibration plot for determination of Carbamzepine in solutions using UV method. Each data point is the average of 3 determinations, R^2: correlation coefficient

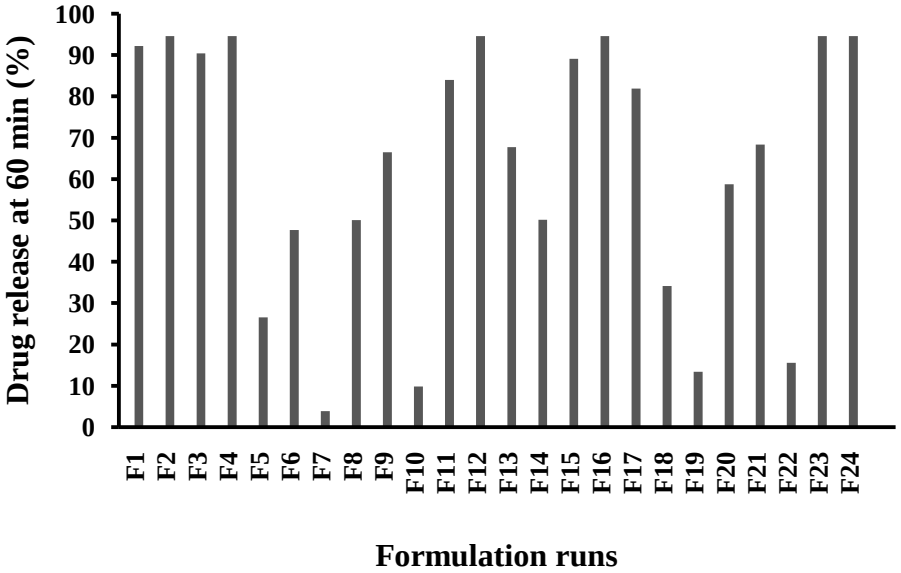

Fig. 2: Cumulative % drug released after 60 Min. of particles within different formulation runs

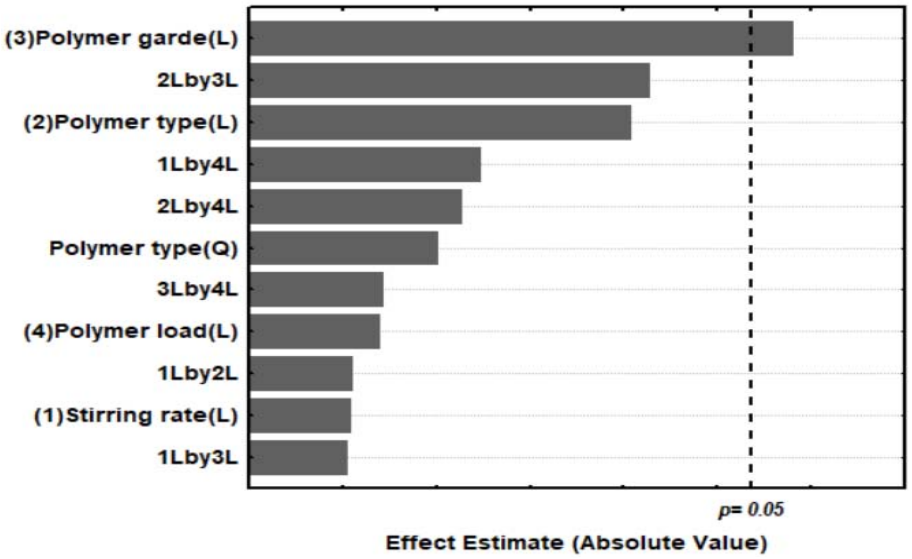

Fig. 3: Estimated effects of the linear (L) and quadratic (Q) and joined influences of the investigated variables on percent drug release at 60 min of different formulations within the experimental design where p= 0.05 denotes cut-off point for significant influences

Composite index scoring and ranking of different formulations of carbamazepine-loaded polymeric particles

Table 3 abridges the composite index scoring and the subsequent ranking of different formulations in the design based on preset of selected 3 constraints of polydispersity index (PDI), entrapment efficiency (EE %) and drug release at 60 min (% $Rel_{60\ min}$).

Table 3: Composite index (CI) and subsequent ranking order of different formulations in the design based on pre-set constraints for particles polydispersity index (PDI %), entrapment efficiency (EE %) and drug release at 60 min (%Rel_{60min})

Run No.	Responses values			Transformed responses			CI	Ranking
	PDI %	EE %	%Rel_{60min}	PDI %	EE %	%Rel_{60min}		
R1	5.50	52.3	70	0	0.14	0	0.14	7
R2	0.52	52.3	73	0	0.14	0.07	0.21	4
R3	1.76	52.3	68	0	0.14	0	0.14	7
R4	0.37	13.1	73	0.16	0	0.07	0.23	3
R5	0.50	13.1	15	0	0	0	0.00	13
R6	0.43	13.1	26	0.04	0	0	0.04	12
R7	0.62	52.3	5	0	0.14	0	0.14	7
R8	0.30	52.3	28	**0.29**	**0.14**	0	**0.43**	**1**
R9	0.67	52.3	45	0	0.14	0	0.14	7
R10	0.39	13.1	6	0.12	0	0	0.12	8
R11	4.04	12.5	62	0	0	0	0.00	13
R12	0.40	11.8	73	0.10	0	0.07	0.17	6
R13	0.55	39.6	46	0	0	0	0.00	13
R14	0.44	47.0	28	0.02	0.08	0	0.10	9
R15	0.69	45.0	67	0	0.06	0	0.06	11
R16	0.38	12.7	73	0.14	0	0.07	0.21	5
R17	1.09	13.1	60	0	0	0	0.00	13
R18	1.04	13.1	12	0	0	0	0.00	13
R19	0.09	52.3	8	0	0.14	0	0.14	7
R20	0.38	52.3	37	0.14	0.14	0	0.28	2
R21	0.56	52.3	46	0	0.14	0	0.14	7
R22	1.74	13.1	9	0	0	0	0.00	13
R23	0.81	13.1	73	0	0	0.07	0.07	10
R24	0.54	13.1	73	0	0	0.07	0.07	10

IV. Discussion

a) Drug release Studies

A central reason for pursuing nanotechnology is to enhance drug delivery, hence understanding the manner and extent to which the drug molecules are released is important. In order to obtain such information most release methods require that the drug and its delivery vehicle be separated (16, 17).

For the drug to be released from the Polymer particles, the Polymer undergoes degradation by hydrolysis or biodegradation through cleavage of its backbone ester linkage into oligomers and finally monomers (18).

b) Calibration curve of standard carbamazepine

The generated calibration curve for standard CBZ in solutions using the validated UV assay method shows high acceptable linear correlation regression between drug concentration and UV absorbance with a highly established correlation coefficient ($R^2 = 0.9826$) in the drug concentration range of 1—100μg/ml (Fig. 1).

c) Effects on drug release characteristics

The effect of different variables on drug release at 60min for different formulations has been studied. Fig 3, showed the linear, quadratic and joined influences of polymer type, polymer grade, polymer load and stirring rate. Among the different variables investigated, the polymer grade has the predominant and significant effect on drug release over the other variables, it has a linear effect with $p > 0.05$ which is the cutoff point. Polymer type (2) has less effect than the polymer grade(3) and when joining their linear effect (2 and3) it appears less than (2) and more than (3). Only the polymer type has a quadratic effect on drug release but it was a non-significant one.

d) Relation between polymer (type, grade) and drug release

Similar to what was found in a study done by Nur et al (19) considering Guar gum, Treated Guar Gum, and Xanthan Gum, as drug fabricating polymers, different drug release profiles were also present in this study. This might be related to their dissimilar hydration and swelling attributes that determine the rate at which the surface viscous barrier (controlling gel) is being formed. These findings along with the effect of particle size and EE% can explain the variation in CBZ release profile from the different gums. Moreover, the statistical work shown in fig 3 reveals the predominated effect of polymer grade (viscosity) as a significant effect over the other factors. Following is a discussion on the effect of different polymer grades on CBZ release.

Considering Native Guar gum, a fast release of 20% to 40% was observed immediately after the addition of loaded particles. This doesn't go along with Nur et al study and it's likely due to a fraction of CBZ present on the surface of the particles being immediately released upon coming in contact with the SLS medium.

However, native guar gum has also shortcomings such as uncontrolled rates of hydration, high swelling, thickening effect, instability upon storage, high susceptibility to microbial attack and the difficulty to control viscosity due to relative fast biodegradation (20). Various strategies were developed in order to overcome these issues, offering the opportunity to tailor the physical and chemical properties of guar gum thus yielding materials that may find a wide range of applications

Regarding Treated Guar gum, the CBZ release was found to be delayed. Less than 30% of the drug entrapped was released within 120 Min., This goes parallel with the results of Nur et al (21), which reported low hydration and swelling capabilities of the treated gum. Accordingly, this is reflected in the enhancement of drug release as a result of the delay in the formation of the gel layer that controls the drug release. Such a result is a good explanation of the poor release profile from the treated guar as for the particle in order to release the entrapped drug, the particle must be swollen to permit the drug release.

In Povidone K_{30} (Lower viscosity) the fastest and uniform release was shown with polymer concentration 1% (R 9 &R21) which has higher EE%. This can be explained by the lower viscosity of the prepared emulsion producing small particles and the high hydrophilicity of povidonek30. All these parameters can increase drug dissolution, which is reported by a study published in ISP Pharmaceuticals (11). The study used low molecular weight PVPs as carriers in solid dispersions due to their higher aqueous solubility, lower viscosity in the diffusion boundary layer, and faster dissolution rate. the study revealed that solid dispersions of indomethacin from co-precipitation and spray drying processes showed faster release from PVP with low molecular weight (PVP K30) than those with high molecular weight (PVP K90) (22).

In our study, the release of CBZ from PVP K30 was very fast in R24. This is can be due to the lower EE% which means that the drug is on the surface of particles not entrapped due to the emulsion's high viscosity as a consequence of increased polymer concentration (10%). This high viscosity renders the drug from diffusing into a polymer molecule and crosslinking with it.

Another study, done by Bharali et al (23), investigated the characteristics of in vitro release of entrapped PVP at low loadings of the compound, which remains in the form of a molecular dispersion inside PVP particles. It was found that when the concentration of dye inside the core of the particle is very high, a part of it is associated or clustered, which has to be dissolved and released more slowly out of the particles. These

phenomena appear clearly in our study in R 21 which has a higher EE% of 52% with a lower release rate.

Regarding Povidone k90 (High viscosity) High molecular weight grade PVP K90 dissolves in a large variety of organic solvents. However, due to its hydrophilicity, its moisture uptake level is high (24) which may result in difficulties in its physical stability leading to drug crystallization in the carrier polymer caused by the plasticizing effect of absorbed water.

The drug release profile of the four runs (3,6,15,18) is strongly linked with EE% as increase EE% increase drug release, R 3 and R15 reached 90% release in 60 minutes as shown in Table 4. The fastest one is in R15 (76% release at 30 mins) can be attributed to the amount of CBZ entrapped (less than 50%) and hence more drugs are on the particle surface leading to burst release (more than 30% in the first 10 mints) (25)

With respect to Lower viscosity, Acacia gum showed the slowest release rate among runs, higher viscosity of acacia, large particle and higher polydispersity as seen in Table2 are the responsible factors. R2 small particle and high EE% these results are not in accordance with relevant published work discussed above. As EE% is a result of how a drug is cross-linked with a polymer, a decreased viscosity will lead to an increase in EE% as less barrier is present, this was seen in R2 (1%polymer concentration produces a solution of lower viscosity) even with large particle sizeR5 with smaller particles (1433.38) than R2 though with lower EE% can be explained the same way.

Considering the higher viscosity of acacia gum runs, a fast release profile was observed which can be relied on for the burst release. More than 20% to 47% of drugs are released in the first 10 minutes with lower EE%, which means the drug is on the particle surface and not entrapped as seen in R 11 and R 23 with less EE%.

In R 8 and R 20 the EE% is high; it has a fast release of 20 % this can be explained by their small particle increasing drug solubility and accordingly enhancing drug release

e) Effect of particle size on drug release

Particle size distribution and morphology are the most important parameters of the characterization of particles. In a study done by (25), it has been found that particle size affects drug release. Smaller particles offer a larger surface area. As a result, most of the drug-loaded onto them will be exposed to the particle surface leading to fast drug release, despite these findings present study found that the smallest particle of R19 (131.72) and R22 (168.25) have the slowest drug release. This may be contributed to the nature of treated guar gum used, thermal treatment of guar gum lead to new gum with odd properties due to degradation of the polymer chain. On the contrary, R1 (native guar) which has a particle size (769.81) showed fast drug release (41.83%) in the first 10 minutes, which support the finding of Robinson (11). Such results can give us a good indication that drug release is mainly affected by polymer characteristics rather than particle size. When we go through the runs we find that R 5 & R 3 have almost the same particle size (1.43 & 1.45) but with different drug releases. R5 (lower viscosity Acacia gum) have 18.83% of drug released in the first 10 minutes while R 3 (povidone lower viscosity) has 45.48% of drug released in the first 10 minutes which support the above finding as seen in Table 4.

Polymer degradation can also be affected by particle size. For instance, the degradation rate of poly (lactic-co-glycolic acid) was found to increase with increasing particle size in vitro (26).

f) Relation between EE% and drug release

The fast drug release in first 10 minutes can be explained by the EE%, as the drug on surface of the particle is released before the entrapped one. This finding appear in R 16 and R 4 (native Guar) with large particle size (3,600.58 &26,450.88) and drug release 34.12% &50.20% respectively

It also ppear in povidone k90 R 12 and R 24 (release 33.66% & EE% 11.49%) (Maximum release 67.02% and EE% 12.88%), respectively (Table 4).

A fast release of 20% to 40% was observed for native guar run just after the addition of loaded particles, likely due to a fraction of CBZ present on the surface of the particles being immediately released in contact with the simulated fluids. The CBZ released in the SLS medium over the total duration of the experiment reached 85 %, indicating that the release of CBZ from the particles can also be controlled by pH.

g) Optimization by composite indexing

Using composite index design as ranking tool prove to be effective in evaluating each factor in an equal way that help in making decision with strong statistical view.

Since the relative contribution of each individual constraint to the true composite score within each step was unknown, the decision was made to assign an arbitrary value of 1/3 to each of the three factors and, accordingly, each test result was transformed to a value between 0 and 0.33. Within each separate step, multi-linear regression equations were applied for the three constraints in order to generate the composite index (CI) for each selected constraint including higher than and lower than ideal values. The run having the highest composite index would be considered as a batch fulfilling the constraints and consequently would be considered as an optimized one.

Table 3 abridged the composite index scoring and the subsequent ranking of the different 24 runs based on the previously mentioned preset 3 constraints of (EE%, PDI and R% at 60 mints) in composite index are summarized in Table 3,

The generated composite index scoring for Runs in this series has ranked R 8 as first run though it has R% 28 at 60 min with increased EE% and the smallest PDI(0.3) lead to increase its efficiency in rank

V. Conclusion

It was found that Acacia gum has the more interesting properties in developing submicron particles like controlling drug release, and hence need to be studied further, while polymer viscosity has large impact on particles behavior.

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What Caused Her Fall? A Clinical Case of Leg Swelling

By N. Stacy Amadife MD & Constance Mere MD

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Abstract- Minimal change disease (MCD) is typically not a disease seen in adults as it comprises only 10-15% of cases (1). Disease can be further characterized as primary/idiopathic or secondary. Typical secondary causes include drugs such as NSAIDs and Lithium and malignancies including Non-Hodgkin Lymphoma. Thus, secondary causes are often the culprit. We present a 47-year-old African-American female patient with a history of Multiple Sclerosis (MS) and HIV who presented with sudden onset worsening lower extremity edema and 6.6 grams (g) urine protein to creatinine ratio with primary MCD.

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WHATCAUSEDHERFALLACLINICALCASEOFLEGSWELLING

Strictly as per the compliance and regulations of:

What Caused Her Fall? A Clinical Case of Leg Swelling

N. Stacy Amadife MD α & Constance Mere MD σ

Abstract- **Minimal change disease (MCD) is typically not a disease seen in adults as it comprises only 10-15% of cases (1). Disease can be further characterized as primary/idiopathic or secondary. Typical secondary causes include drugs such as NSAIDs and Lithium and malignancies including Non-Hodgkin Lymphoma. Thus, secondary causes are often the culprit. We present a 47-year-old African-American female patient with a history of Multiple Sclerosis (MS) and HIV who presented with sudden onset worsening lower extremity edema and 6.6 grams (g) urine protein to creatinine ratio with primary MCD.**

I. Introduction

Minimal change disease (MCD) is a nephrotic syndrome primarily seen in children and early teens (1). In adults, the major nephrotic disease remain Focal Segmental Glomerulosclerosis (higher prevalence in people of African origin) and Membranous nephropathy (higher prevalence in people of European descent). It is rare to see MCD in adults as it comprises only 10-15% of cases (2). Patients usually present with sudden onset edema, proteinuric kidney injury, and hyperlipidemia. Disease can be further characterized as primary/idiopathic or secondary. Typical secondary causes include drugs such as non steroidal anti-inflammatory drugs (NSAID) and Lithium, infections such as Syphilis, Mycoplasma, allergens, autoimmune disorders like Systemic Lupus Erythematous (SLE), Celiac disease, diabetes, as well as malignancies including Non Hodgkin Lymphoma and bronchogenic carcinoma (1). The pathogenesis hypothesis states that disruption of actin cytoskeleton within the podocyte and basement membrane in conjunction with a disrupted immune system cause an increase in mediating factors leading to filtration of albumin into the urinary system (2).

II. Case Report

We present a case of a 47 -year old African-American woman with biopsy proven MCD.

The patient presented to the Emergency Department (ED) after sustaining a fall at home. She hit her head albeit did not lose consciousness. She reports myalgia, nausea, and acute worsening of paresthesia in her hands and lightheadedness over the past one month. In addition, she notes worsening leg swelling spanning three weeks and involuntary 30 pound weight gain over the past month. She denies any herbal medication use, illicit drug use, or recent illness. The last time she took NSAIDs was for menses four months prior to presentation and totaled no more than six doses.

Her past medical history is significant for Multiple Sclerosis (MS) diagnosed in 2005 and her last flare in 2008. Flares are characterized by fatigue, frequent fall, and dizziness. Her disease is managed with Glatramer injections three times weekly. She also has a history of HIV with undetectable viral load and takes Biktarvy daily. CD4 count at time of admission 976. Finally, patient has leiomyomas and follows with outpatient gynecology.

Her vitals: heart rate 101 beats per minute Blood pressure 150/90mm Hg, 16 Respirations per minute and oxygen saturation of 99% on room air.

Upon admission, lab investigations demonstrated:

C3, serum	95.62 (mg/dl) (79-152)
C4, serum	13.75 (mg/dl) (16-38)
Albumin ,serum	Less than 1.5 (g/dl)
Calcium, serum	7.3 (mg/dl)
Brain natriuretic peptide (BNP)	7.5 (pg/mL) (less than 100)
CPK	9 IU/L (35-230)
D dimer	2.58 (ug/ml) (0-0.48)
White blood cell count	4.36x10 ^ 9 per microliter (3.2-10.6)
Hemoglobin	12.5 (g/dl) (12.1-15.9)
Platelet	120x10 ^ 9 per microliter (177-406)

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Sodium	138 (meq/L)
Potassium	5.3(meq/L)
Chloride	109 (meq/L)
Bicarbonate	26 (meq/L)
BUN	29(mg/dl)
Creatinine	1.3 (mg/dl) (baseline 0.7-0.8)
Glucose	97(mg/dL)

Lipid panel

Cholesterol	341 (mg/dL)	(125-200)
HDL	35.6 (mg/dL)	(>47)
LDL	169.7 (mg/dL)	(less than 130)
Triglyceride	424 (mg/dL)	(less than 150)

Urine studies

Urinalysis:	Amber appearing urine, with greater than 500mg/dL protein with few bacteria, 16-25 WBC (normal 0-4 per high powered field). No nitrites, no leukocyte esterase, and no Redblood cell cast. Specific gravity: 1.032 (normal 1.01-1.03)
Urine protein	>1500 mg/dL
Urine Creatinine	225.66 mg/Dl
Urine BUN	1780 mg/dL
Urine Sodium	20 mg/dL

Imaging

Renal ultrasound	Patent renal veins and normal sized kidneys
Lower extremity Vein Doppler	NEGATIVE for deep vein thrombosis
CT Head and Cervical spine	No acute intracranial process and evidence of multi-level disk disease.

Exam notable for obese woman with generalized edema, normal heart sound intensity, no adventitious breath sounds, and no focal neurological deficits. Patient oriented to person, place, and situation.

Neurology initially consulted due to concern for MS flare and patient completed four day course of daily Solumedrol. Head imaging showed no evidence of acute flare.

Nephrology consulted due to concern for nephrotic syndrome. Urine studies, autoimmune workup including SPEP, UPEP, ANCA, RPR, serum free light chains recommended. Results all negative. ANA positive and reflex to titre pending. Double stranded DNA (dsDNA) quantified as indeterminate. Urine protein: creatinine ratio is 6.64g/day. Interventional Radiology (IR) consulted for kidney biopsy. Patient started on IV Furosemide , IV albumin, and anti hypertensives. Protein and sodium restriction intake enforced. Plan for biopsy of kidney.

Biopsy results on electron microscopy demonstrated effacement of podocytes and absence of tubule-reticular structures. On light microscopy normal appearing glomeruli seen with some evidence of interstitial edema. Immunofluorescence demonstrated no glomerular positivity with IgG, IgA, IgA, C3, C1q, kappa, lambda, or fibrinogen. Faint one plus glomerular positivity seen with IgM, however non specific. No specific tubulointerstitial or vascular positivity with any of the above mentioned immunoreactants.

Patient started on prednisone 80mg every morning. Testing for G6PD negative, and patient started on Dapsone 100mg day for Pneumocystis jiroveci pneumonia (PJP) prophylaxis.

At time of discharge, labs demonstrated

Sodium	138 (meq/L)
Potassium	3.6 (meq/L)
Chloride	99 (meq/L)
Bicarbonate	32 (meq/L)
BUN	17(mg/L)
Creatinine	0.8 (mg/L) (baseline 0.7-0.8)
Glucose	112 (mg/L)

White blood cell count	16.46x10^9 per microliter	(3.2-10.6)
Hemoglobin	10.5 (g/dl)	(12.1-15.9)
Platelet	179x10^9 per microliter	(177-406)
Glucose 6 phosphate dehydrogenase	9 u/g of Hemoglobin	(7-20)

III. Discussion

The incidence of primary MCD in adults is not well defined (1). The hallmark of biopsy results is absence of immunofluorescence staining for varying antigens/immunoreactant (IgG, IgM, IgA, C1, etc.) and effacement of podocytes (1) on electron microscopy. If other features are seen, it cannot be MCD (1). Nonetheless, low intensity staining of C3 and IgM can be normal (8). This was seen in our patient. Typically, this disease has a higher prevalence in children who are often steroid responsive. By two weeks, 50% of kids have responded, whereas the percentages are more sobering in adults. Here, 75% have responded by 13 weeks (8). Furthermore, adults have greater risk for progression to renal failure in adults. In study by Nolasco et. al, ten of nineteen patients progressed to renal failure, with eight of those eventually requiring dialysis (9).

There have been few reports of adults with MCD and even fewer in patients with comorbidities such as HIV and MS, as in our patient. However, given the biopsy results this remains a case of primary MCD. In spite of the patient's history of well controlled HIV, HIV Associated nephropathy (HIVAN) remained on the differential. It is important to recognize that anti retroviraltherapy (ART) does not protect against MCD. In fact, seven of eight patients were diagnosed with MCD while on ART. HIVAN detected in only one case (4). On the other hand, a viral load of greater than 400 was also not a good predictor of HIVAN, as only 37% of such patients diagnosed with HIVAN (6).

While the patient did have abrupt onset edema, hyoalbuminemia, and proteinuria, her serum creatinine was not greater than 2. Above 2 is more typical for HIVAN (5). Variability in labs and presentation echo the importance of biopsy. Biopsy will demonstrate tubular atrophy and dilation as well as flattened epithelial cells in setting of collapsing FSGS (due to podocyte proliferation). Furthermore, a large number of tubular and glomerular cells coated with HIV RNA (4). Important to note that low CD4 count and presence of proteinuria are not predictive of HIVAN. Furthermore, a viral load of greater than 400 was also not a good predictor of HIVAN, as only 37% of such patients diagnosed with HIVAN (6).

Our patient did not have HIVAN in spite of medical history. Similarly, one could postulate MCD secondary to MS drugs. While the patient was treated for presumed flare on admission, there are very little reports in the literature of Glatiramer induced nephrotic syndrome. On the other hand, Interferon gamma B (IFN B) has been linked to MCD after long time use. Kumasake et al. describe case of a woman with MS on IFN B who develops MCD after 21 months on MS treatment (7). Our patient was never treated with IFN B and no evidence seen on renal biopsy.

IV. Conclusion

MCD is a type of nephrotic syndrome, characterized by a urine protein/creatinine of 3500mg and greater. Patients usually present with sudden onset edema, proteinuric kidney injury, and hyperlipidemia. It is believed that disruption of actin cytoskeleton within the podocyte and basement membrane in conjunction with a disrupted immune system cause an increase in mediating factors leading to filtration of albumin into the urinary system and marked proteinuria. Patients need close follow up to ensure steroid responsiveness, as measured by reduction in proteinuria. Due to long duration of steroid therapy, patient's need PJP prophylaxis. This includes Atovaquone or Dapsone. It is prudent to be aware that adults have greater risk for progression to renal failure (than children). In a study by Nolasco et. al, ten of nineteen patients progressed to renal failure, with eight of those eventually requiring dialysis. If adults have truly failed steroid therapy, there will be no improvement after four months. The next step is to discuss the efficacy of second line non-steroidal therapies such as calcineurin inhibitors. This case highlights a case of primary MCD in a woman with HIV and MS, while illustrating that even when patients have other comorbidities or concern for secondary causes of MCD, it is imperative to obtain a renal biopsy to clarify the picture.

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Antibiotic use during Pregnancy: A Retrospective Study of Prescription at the District Health Center of Kangaba, Mali

By Karim Traoré, Seidina AS. Diakité, Mahamadou Ballo, Drissa Konaté, SoryI. Diawawa, Bourama Keita, Abdoulaye Maiga, Modibo Sangaré, Aiguérou A. Guindo, Fatoumata Daou, Moussa Soumana, Ibrahim Sanogo, Fousseyni S. Doucouré, Mahamadou Diakité & Sékou Bah

Abstract- *Background:* Pregnancy is a critical stage in a woman life, and the use of drugs, especially antibiotics calls for concern. The service and choice of antibiotics during pregnancy depends mainly on maternal factors such as health, nutrition, and socio-economic status, as well as the mode of delivery. This study was aimed to assess antibiotic use among pregnant women according to the Food and Drug Administration categorization of drugs based on their risk in pregnancy.

Methods: The study was a retrospective, cross-sectional survey. The sampling consisted of all prescriptions for pregnant women with at least one antibiotic drug and recorded in a registry.

Keywords: *antibiotics, prescription, pregnancy.*

GJMR-B Classification: *DDC Code: 618.2 LCC Code: RG525*

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Methods: The study was a retrospective, cross-sectional survey. The sampling consisted of all prescriptions for pregnant women with at least one antibiotic drug and recorded in a registry. Data included primary demographic data, the nature of the antibiotic medicines, their dosage, the duration of treatment, and the type of prescribed antibiotic combination, were analyzed based on the FDA classification guidelines; Data were analyzed using the statistical software Epi info.

Results: One thousand four hundred and ninety-nine (n=1,499) pregnant women received at least one prescription of antibiotics during pregnancy. The average age was 28 years old, and the most represented age group was 21-25(29.6%); Regarding drug delivery, amoxicillin (36.6%), erythromycin (31.7%), and azithromycin (15.6%) were the most prescribed drugs during the first trimester of pregnancy. Metronidazole (54.9% and 40.1%), erythromycin (29.9% and 20.7%), and azithromycin (9.9% and 29.5%) were the most prescribed molecules during the second and third trimesters of pregnancy, respectively. The frequently prescribed therapeutic class was macrolides, with 65.7%, followed by beta-lactams, with 15.1%. The dosage of the most prescribed drugs was 500mg, with 94.7%.The most used route of administration was oral (96.7%). The duration of treatment in most of the prescriptions was less than one week, with 99.2%. Antibiotics belonging to category B of the FDA classification were the most prescribed with 43.5%, followed by category A at 37.7%, category C at 10.8%, and category D at 8%.

Conclusion: The antibiotics prescribed for pregnant women fell within the FDA risk categories A and B, with rare cases of prescription occurring in categories C and D. The most frequently prescribed antibiotic class was the macrolides.

Keywords: antibiotics, prescription, pregnancy.

I. Background

Maternal mortality and morbidity are high in sub-Saharan Africa due to complications from microbial infections[1]. Managing of complications related to these infections during pregnancy requires the prescription of many drugs, including antibiotics. The best use of antibiotics to treat infectious diseases during the antenatal visits, in addition to iron administration and dietary supplements, could reduce maternal and baby mortality during pregnancy[2]. Reports suggest that antibiotics account for nearly 80% of all prescription medications during pregnancy, and approximately 20–25% of women receive an antibiotic during pregnancy [3-5]. Poor management of antibiotics is one of the leading causes of antibiotic resistance in microbial agents [6]. The use and choice of antibiotics during pregnancy depends on health resources, nutrition status, mode of delivery, and socio-economic factors. A better knowledge of the pharmacokinetics, potential toxicity, and teratogenic risks of these drugs is essential to optimize the efficacy and safety of antibiotic treatment [7]. The pharmacokinetics of antibiotics during pregnancy can be affected by multiple factors, including absorption, distribution, metabolism, and elimination [8]. Some antibiotics can potentially to affect embryo-fetal development at different stages of pregnancy. Teratogenic effects occur mainly during the embryonic period (first trimester of pregnancy) [9]. Prescribing in pregnancy always raises the issue of drug risks to the embryo or fetus, an additional pharmacokinetic compartment related to transplacental drug distribution. The use of medications during pregnancy is a significant concern for patients and prescribers. The incidence of

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thalidomide in the 1960s and the teratogenic effects discovered in 1971 with diethylstilbestrol are some examples of the hazards that prescription drugs may pose to pregnant patients [10, 11]. Pregnancy is associated with changes in the physiological, psychological, and psychosocial aspects of a woman life. Antibiotics are among the more frequently prescribed medicines in pregnant women, and the use of antibiotics is increasing. However, with limited studies available in this population, the safe use of antibiotics in pregnancy remains a concern.

The Food and Drug Administration (FDA) categorization of drugs based on their risk of pregnancy should be considered before prescribing a medication to pregnant women. The health center receives pregnant women for prenatal consultations and various types of care.

No study on antibiotics prescribed in pregnant women and their compliance with the FDA classification on drug safety during pregnancy has been done in this village. This study will contribute to the improvement of antibiotic prescription in pregnant women.

II. Methods

The study was carried out in the district health centers of Kangaba, a malaria-endemic area located 80 km southwest of Bamako. A cross-sectional study was carried out from January to March 2021 to collect data on the use and prescription of antibiotics during the antenatal visits. The sampling consisted of all prescriptions for pregnant women with at least one antibiotic drug and recorded in a registry. The nature of the antibiotic drugs, the dosage, the duration of treatment, and the type of prescribed antibiotic combination were analyzed based on the FDA classification guidelines. A non-compliant prescription was defined as any breach of one or more of the parameters listed above concerning, to the FDA classification guidelines. In the registries, we also collected information about the socio-demographic characteristics (age and sex of the patient). In addition, a report form was administered to all prescriber's Data focusing on their professional qualification and their level of knowledge of the FDA classification.

FDA classification of drug safety in pregnancy[12]

- Category A: No adverse effects in human pregnancies. Safety established using well controlled human studies.
- Category B: Presumed safety in human pregnancies. Limited human studies/no adverse effects in animal studies.
- Category C: Uncertain safety: Limited human studies/adverse effects in animal studies.
- Category D: Adverse effects in pregnancies. Benefits may outweigh associated risks.
- Category X: Adverse effects in pregnancies. Risksoutweigh possible benefit.

Anti-Microbials: D and X FDA drug categories[12]

- Category D: Aminoglycosides: Gentamycin, Streptomycin, Tobramycin, Tetracyclines, Doxycycline, Minocycline, Tetracycline, Voriconazole, Chloramphenicol, Antimycotics (Amphotericin B, 5-flucytosine, Griseofulvin).
- Category X: Quinine, Thalidomide, Ribavirin, Miltefosine, oral contraceptives, statins.

III. Statistical Analysis

Data were collected on a report form, entered into Excel, and analyzed using the statistical software Epi info 6.04.

a) Ethical considerations

Our study protocol was approved by the ethics committee of the Faculty of Medicine and Odontostomatology, and Pharmacy of the University of Sciences, Techniques, and Technologies of Bamako (USTTB). The health and administrative authorities of Kangaba were informed before the beginning of data collection.

The information found in the logs was kept entirely confidential and was not disclosed to anyone outside the study investigators. The personal information concerning each pregnant woman was coded. Only the principal investigator could identify the patients during the data analysis for publication of the results.

IV. Results

Table 1: Antibiotics prescribed during the antenatal visit to the district health center of Kangaba.

Antibiotics	*Age of the pregnancy*			Total n (%)
	First Trimester N (%)	Second trimester N (%)	Third Trimester N (%)	
Amoxicillin	225(36.6)	0(0)	0(0)	225(15)
Erythromycin	195(31.7)	355(54.9)	95(40.1)	645(43)
Azithromycin	96(15.6)	193(29.8)	49(20.7)	338(22.5)
Metronidazole	28(4.6)	64(9.9)	70(29.5)	162(10.8)
Ciprofloxacin	11(1.9)	19(2.9)	16(6.8)	46(3.1)

Doxycycline	0(0)	12(1.9)	7(2.9)	19(1.3)
Cefixime	4(0.7)	0(0)	0(0)	4(0.3)
Gentamycin	53(8.6)	0(0)	0(0)	53(3.5)
Lincomycin	2(0.3)	0(0)	0(0)	2(0.1)
Ceftriaxone Associated	1(0.2) 0(0)	0(0) 4(0.6)	0(0) 0(0)	1(0.1) 4(0.3)
Total	615(100)	647(100)	237(100)	1499(100)

Table 2: The distribution of prescriptions according to the therapeutic class of antibiotics and the age of the pregnancy.

Therapeutic class of antibiotics	Age of the pregnancy			Total n (%)
	First trimester N (%)	Second trimester N (%)	Third trimester N (%)	
Aminosides	53(8.6)	0(0)	0(0)	53(3.5)
Bêta-lactamines	226(36.7)	0(0)	0(0)	226(15.1)
Céphalosporines	4(0.7)	0(0)	0(0)	4(0.3)
Lincosamides	2(0.3)	0(0)	0(0)	2(0.13)
Macrolides	291(47.3)	550(85)	144(60.8)	985(65.7)
Macrolides+bêta-lactamines	0(0)	1(0.2)	0(0)	1(0.06)
Macrolides+ Fusidanines	0(0)	1(0.2)	0(0)	1(0.06)
Macrolides+ Nitroimidazoles	0(0)	1(0.2)	0(0)	1(0.06)
Nitroimidazoles	28(4.6)	63(9.7)	70(29.5)	161(10.7)
Quinolones	11(1.8)	19(2.9)	16(6.8)	46(3)
Tétracyclines	0(0)	12(1.8)	7(2.9)	19(1.39)
Total	615(100)	647(100)	237(100)	1499(100)

Table 3: Dosage frequency per day, dosage form, and duration of treatment of antibiotics prescribed to pregnant women.

Variables	Category	(%)
Dosage of antibiotic in mg	<500mg	77(5.1)
	500mg	1419(94.7)
	1000mg	3(0.2)
	>1000mg	0
Daily frequency of antibiotic use	Once	65(4.3)
	Twice	1216(81.2)
	Thrice	13(0.9)
	Four times	205(13.7)
Forms of antibiotics	Tablet	1450(96.7)
	Injection	49(3.3)
Duration of treatment	<7days	1487(99.2)
	7days	10(0.7)
	>7days	1(0.1)

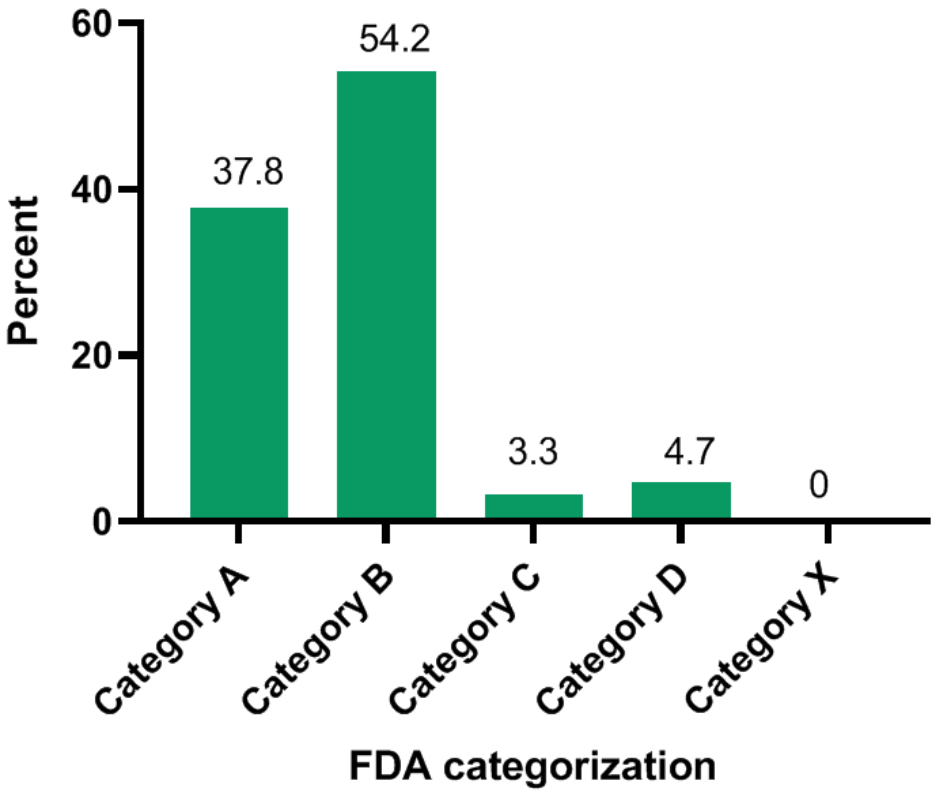

Figure 1: Antibiotics prescribed to pregnant women according to the FDA categorization

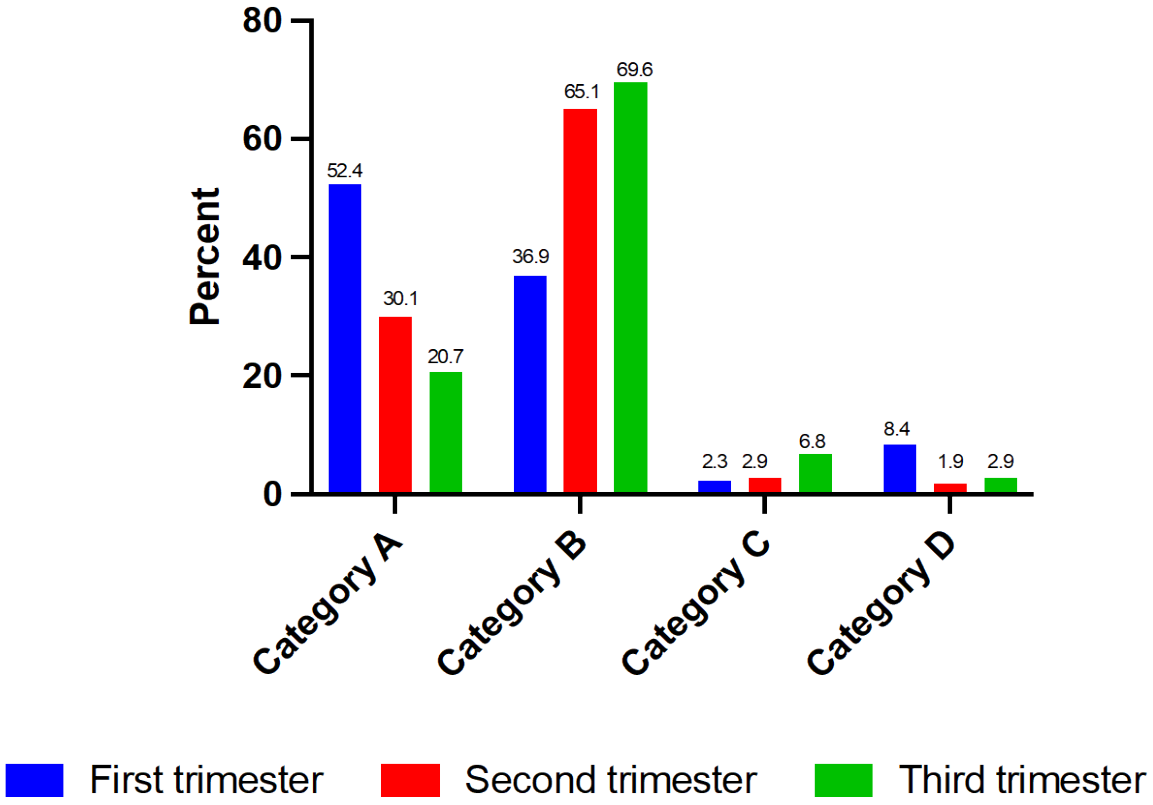

Figure 2: Antibiotic prescribed according to age of the pregnancy and FDA categorization

Table 4: Types of antibiotics prescribed to pregnant women according to FDA classification in the health center

Drug/ FDA recommendation	Age of the pregnancy		
	1st trimester	2nd trimester	3rd trimester
FDA recommended	Amoxicillin, Erythromycin, Azithromycin, Metronidazole, Ceftriaxone, Cefixime	Erythromycin, Azithromycin, Metronidazole, Ciprofloxacin	Erythromycin, Azithromycin, Metronidazole, Ciprofloxacin
Not FDA recommended	Ciprofloxacin, Gentamycin, Lincomycin	Doxycycline	Doxycycline

Pregnant women underwent an antibiogram before the prescription of the antibiotics in 0.5% (8/1,499).

V. Discussion

Most pregnant women are exposed to some type of medication during pregnancy. Drugs prescribed during pregnancy can exercise a teratogenic effect on fetuses, and those prescribed during breastfeeding can also impact on infant health. Antibiotics are among the more frequently prescribed types of medications during pregnancy and lactation [13].

The risk of antibiotic exposure was highest in the first and second trimesters but lowered in the third trimester. Mensah et al. 2017 in Ghana found that the risk of antibiotic exposure was highest in the last trimester. This is reassuring because the acquisition of specific fetal immunity begins in the third trimester, and is highly dependent on the microbiome, which can be altered by antibiotics [14].

Amoxicillin (category A) at 36.6%, erythromycin (category B) at 31.7%, and azithromycin (category A) at 15.6%, were the mainlydrugs prescribed during the first trimester of pregnancy (Table 1). Erythromycin (category B) at 54.9%, azithromycin (category A) at 29.8%, and metronidazole (category B) at 9.9%, were the mainly drugs prescribed during the second trimesters (Table 1). In the third trimesters, erythromycin (category B) at 40.1%, metronidazole (category B) at 29.5%, and azithromycin (category A) at 20.7%, were the mainly drugs prescribed (Table 1). A study carried out in northern Nigeria by Ogboma et al. in 2019 reported that ciprofloxacin (25.3%) and erythromycin (21.7%) were the mainly drugs prescribed during pregnancy[15].

In Kangaba health center, macrolides were the most prescribed antibioticsat 65.7%, followed bybeta-lactamsat15.1%, and nitroimidazoleat 10.7%.Ogboma et al. in 2019 in Nigeria, and Elizabeth C. Ailes et al. in 2018 in the USA reported that fluoroquinolones were the most prescribed class in pregnant women with 46.7% and 32%, respectively [15, 16]. A study carried out in Ghana between 2011 and 215 by Mensah et al. reported that 67% of prescriptions for antibiotics in pregnant women were beta-lactams [14].

Prescribing macrolides during pregnancy is common, as similar results have been reported in the literature[17-20]. The use of macrolides in pregnancy is, however, a growing concern [18]. Significantly, a recent study by Fan et al. followed 104,605 children from birth to 14 years old, and it was concluded that prescribing macrolides in any trimester was associated with an increased risk of genital malformation [18]. Whereas a previous cohort of 1,033 women exposed to macrolides (erythromycin, azithromycin, clarithromycin or roxithromycin) reported that there was no association between this drug and the development of significant abnormalities in the fetus [17].

The dosage in mg of most drugs prescribed was 500mg with 94.7% regardless of the age of pregnancy. This result is similar to that observed by Ogboma et al. in 2019 in Nigeria [15]. The dosage frequency per day of most drugs prescribed was twice with 81.2%. The most common route of administration was oral with, 96.7%. The dosage form of most prescribed drug was tablet (96.7%). The duration of treatment in most of the prescriptions was less than one week (99.2%). This does not appear to be in line with the management of antibiotic resistance, where a minimum of seven days and a maximum of twenty-one days is recommended to avoid resistance that could result from incomplete treatment. The duration of treatment depends mainly on the nature of the disease, the severity, the presentation of the drug (dosage in mg and dosage form), the age of the pregnancy, and the pharmacokinetic of medicarion.

Most drugs fell into category B at 54.2%, and category A at 37.8%. Mensah et al. 2017 in Ghana reported that most of the antibiotics prescribed were of category B at 96.6%, followed by C and D at 2.9% and 0.5%, respectively [14]. Drugs in categories C and D are toxic to the fetus but can be used during pregnancy if the benefits to the mother outweigh the risks to the fetus.

The prescription of, ciprofloxacin (1.85%), gentamycin (8.6%) and, lincomycin (0.3%) in the first trimester of pregnancy does not conform to FDA recommendations. According to the FDA, ciprofloxacin, gentamycin, and lincomycin should be prescribed in the second and third trimesters of pregnancy due to their potential embryotoxicity.

The prescription of, doxycycline (Category D) in second (1.2%) and third (2.9%) trimesters of pregnancy is not recommended by FDA, because doxycyclineis toxic on the fetus.

VI. Conclusion

The antibiotics prescribed for pregnant women fell within the FDA risk categories A and B, with rare cases of prescription occurring in categories C and D. The most frequently prescribed antibiotic in Kangaba was the macrolides.

Singles

FDA: Food and Drug Administration

MRTC: Malaria Research, and Training Center

USTTB: University of Sciences, Techniques, and Technologies of Bamako

Contribution

Karim Traoré, Seidina Diakité, Sékou Bah, and Mahamadou Diakité participated in the conception and design of the manuscript. Karim Traoré, Bourama Keita, Sory I Diawara, and Drissa Konaté performed the statistical analysis, and Karim Traoré Mahamadou Ballo, Modibo Sangaré drafted the manuscript. All authors read, and approved the final version of the manuscript.

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Conflict of interest: None

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PCO2 gap - As an Endpoint of Resuscitation and Predictor of Mortality in Patients with Shock: A Prospective Observational Study

By Dr. Prabhu S, Dr. Vimal Bhardwaj, Dr. V. Viju Wilben & Mr. Vinil Kumar

Abstract- *Introduction:* Endpoint of resuscitation is essential to be determined objectively as we get more substantial evidence supporting the fact that both under resuscitation and over resuscitation is detrimental to overall outcomes. Since carbon dioxide is more diffusible than oxygen it readily gets in to the blood in low perfusion states whereas oxygen doesn't. Hence widening the PCO2 gap. Since this PCO2 gap can be determined easily in the ICU we propose that PCO2 gap can be used as a reliable indicator of endpoint of resuscitation and predictor of mortality in patients with shock.

Aim: To evaluate the association between PCO2 gap and outcome of resuscitation in patients with shock. The Objectives of the project are to study the association between PCO2 difference and in-hospital mortality in patients admitted with shock and to study the correlation between PCO2 difference and lactate clearance.

GJMR-B Classification: *DDC Code: 617.044 LCC Code: RD156*

Strictly as per the compliance and regulations of:

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Dr. Prabhu S [α], Dr. Vimal Bhardwaj [σ], Dr. V. Viju Wilben [ρ] & Mr. Vinil Kumar [ω]

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Aim: To evaluate the association between PCO2 gap and outcome of resuscitation in patients with shock. The Objectives of the project are to study the association between PCO2 difference and in-hospital mortality in patients admitted with shock and to study the correlation between PCO2 difference and lactate clearance.

Materials and methods: 71 adult patients presenting with shock to our ER were enrolled in the study. They were resuscitated according to standard protocols. PCO2 gap was measured at presentation, then every 2 hours until the resolution of shock which were correlated to the lactate clearance, hemodynamics and the IVC index of the patient. The data was then analyzed using the R software and logistic regression was done to analyze various factors associated with mortality. P value less than 0.05 was considered statistically significant.

Results: The correlation between pCO2 gap and the in hospital mortality was statistically significant at 0,2,4,6 and 24 hours. The correlation between pCO2 gap and the end point of resuscitation was statistically significant at 2,4,6 and 24 hours implied by the pearson's correlation. We also found a positive correlation between PCO2 gap and lactate clearance which was statistically significant.

Conclusion: The PCO2 gap can be used a marker of the adequacy of the cardiac output in patients with shock. Using pCO2 gap has potential to avoid administration of unnecessary fluids and inotropes in patients, who have lactate elevated in the absence of tissue hypo perfusion. We suggest using pCO2 gap as a complementary tool to evaluate the adequacy of blood flow to global metabolic demand. A high pCO2 gap on initial presentation was associated with high mortality rates. So it can be used as a predictor of outcomes in patients with shock.

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I. Introduction

Shock is the clinical expression of circulatory failure that results in inadequate cellular oxygen utilization.[1] Shock is a common condition in critical care, affecting about one third of patients in the intensive care unit (ICU), both over resuscitation and under resuscitation can adversely impact the outcomes.[2,3,4] End point of resuscitation has always been a matter of debate, initially continuous SCvo2 monitoring as introduced by Rivers et al had the obvious limitation that normal/high values cannot discriminate whether delivery is adequate or in excess to demand[5,6,7]. High ScvO2 profiles have even been shown to be related to elevated blood lactate concentration and poor survival rates.[8]

Lactate cannot differentiate between different etiologies of shock and it can get elevated in various other conditions.[9] Carbon dioxide (Co2) is highly diffusible and can be a marker of adequacy of venous return, the central venous and arterial CO2 gap, as an easily available clinical monitoring tool. Observational study has shown that Persistence of such a large pCO2 gap after 24 hours of treatment was predictive of higher mortality.[10]

In conclusion, determining the PCo2 gap during resuscitation of critically ill patients is useful in deciding when to stop resuscitation.[11] Central venous-arterial carbon dioxide difference (PCO2 gap) can be a marker of cardiac output adequacy in global metabolic conditions that are less affected by the impairment of oxygen extraction capacity. Assessing the adequacy of oxygen delivery with oxygen requirements is one of the key-goal of hemodynamic resuscitation. Clinical examination, lactate and central or mixed venous oxygen saturation (SvO2 and ScvO2, respectively) all have their limitations. Many of them may be overcome by the use of the carbon dioxide (CO2)-derived variables. The venoarterial difference in CO2 tension ("ΔPCO2" or "PCO2 gap") is not an indicator of anaerobic metabolism since it is influenced by the oxygen consumption. By contrast, it reliably indicates whether blood flow is sufficient to carry CO2 from the peripheral tissue to the lungs in view of its clearance: it, thus, reflects the adequacy of cardiac output with the metabolic condition. We investigate the relation between

the PCO2 gap and serum lactate and its role in resuscitation of patients with septic shock.

II. Review of Literature

Shock is defined as inability to maintain MAP which is refractory to fluid resuscitation. It has a guarded prognosis, there are many upstream and downstream markers for resuscitation, septic shock guidelines endorses Lactate as a prognostic marker; has got its own limitations as it can be elevated in other clinical conditions9 and it cannot differentiate the cause of shock9. With enough evidence coming up about over resuscitation and positive balance being one of the predictor of mortality there is a need for ideal resuscitation marker which can be easily employed bedside with present day equipment used on day to day basis.

CO2 is the end product of aerobic metabolism, PCO2 in the venous blood reflects the global tissue blood flow relative to metabolic demand. CO2 is about 20 times more soluble than O2 so it more reliably diffuses out of ischemic tissues into the venous effluent making it a sensitive marker of hypoperfusion in situations where an O2 diffusion barrier exists (e.g. non-functional and obliterated capillaries), "masking" poor O2 extraction (O2ER) and increased tissue O2 debt, CO2 still diffuses to the venous effluent, "unmasking" the low perfusion state for the clinician when venous-to-arterial CO2 difference is evaluated the gap is a marker of adequacy of venous blood flow to remove CO2 produced rather than a marker of tissue hypoxia or dysoxia[11]

Table 1: PCO2 Gap in Different Shock States

Shock type	Lactate	O2ER	ScvO2	cvaCO2gap
Cardiogenic or hypovolemic	HIGH	HIGH	LOW	HIGH
Anemic or hypoxemic	HIGH	HIGH	LOW	LOW
Distributive	HIGH	LOW	HIGH	HIGH
Cytopathic	HIGH	LOW	HIGH	LOW

As illustrated in table 1, Lactate is high in all types of shock, PCO2 Gap is high in cardiogenic and distributive shock which is amenable to fluid resuscitation and inotropic support and low in hypoxemic and cytopathic shock where fluid resuscitation has no role thus it can be concluded that PCO2 gap is useful in determining when to start and stop fluid resuscitation.[11] Co2 gap is a marker of adequacy of venous blood rather than marker of tissue hypoxia or dysoxia as shown by Vallet et al in an experimental model of isolated limb in which ischemic hypoxia (IH) and hypoxic hypoxia (HH). The authors demonstrated that when DO2 was reduced beyond its critical threshold in IH (dysoxia), this was associated with an increased limb venous-to-arterial PCO2gap.[12]

Conversely, in HH, pCO2 gap did not increase in spite of a marked VO2 and VCO2 reduction.[12] There is a good correlation between Mixed CO2 and Central CO2 difference with Arterial CO2 as demonstrated by Van Beest et al in severe sepsis and septic shock patients, hence Central CO2 can be substituted for mixed CO2 for determining the CO2 gap which acts as surrogate marker for Cardiac Index.[10]

Cushieri J etal conducted study in ICU patients to see the correlation between Central Venous and Arterial CO2 gap and Cardiac index determined by thermodilution technique and showed statistically significant correlation.[13]

Hence CO2 gap can be used as a marker of Cardiac output.

a) Role in Sepsis

In sepsis although Cardiac output may be normal but regional compromise of circulation is well documented phenomenon which may lead to increase in CO2 secondary to micro-circulation compromise. P(cv-a)CO2 could be considered as a better indirect assessment of systemic blood flow than ScvO2 in resuscitated-septic shock patients.[14]

A cutoff value for pCO2 gap of 0.8 kPa (6mmHg) discriminated between high and low lactate clearance and CI.[15,16] In study done by Vallee et al done in septic shock patients compared When the 70% ScvO2 goal value is reached, the presence of a P(cv-a)CO2 larger than 6 mmHg shown to be an useful tool to identify patients who still remain inadequately resuscitated.[14]

We hypothesize that CO2 gap is non inferior to lactate clearance in resuscitation of critically ill patients.

III. Research Question

Would pCO2 gap serve as an ideal bedside marker to predict the outcome of resuscitation in a patient with shock?

IV. Aims and Objectives

Aim of the Project: To study the association between PCO2 gap and outcome of resuscitation in patients with shock.

Objectives of the Project: The Objectives of the project are as follows:

- Primary objectives- To study the association between PCO2 gap and in-hospital mortality in patients admitted with shock.
- Secondary objectives

➢ To study the correlation between PCO2 gap and lactate clearance.

➢ To study the role of PCO2 gap as a marker for endpoint of resuscitation in patients with shock.

V. Methods and Methodology

Study area: Emergency Department and medical intensive care unit, NH Health City, Bangalore

Study population:

- Inclusion Criteria

– All adult patients (more than 18 years of age) in shock requiring vasopressor to maintain MAP of 65mmHg, having a central venous access and arterial line.

- Exclusion Criteria

– Patient Refusal
– Pregnancy
– Advance directive with consensus against active resuscitation
– Disseminated Malignancy

Sample size: 71

Study design: Prospective observational study.

Study intervention: No interventions

Study duration: One Year

VI. Methodology

✧ All shock patients were resuscitated according to the standard protocol with fluid bolus of 30 ml/kg over 1 hour and guided therapy with fluid challenges targeting heart rate, base deficit, urine output and pulmonary congestion as per routine clinical practice.

✧ Lactate clearance was documented every 2nd hourly and VBG from Central line and ABG from Radial Line was analyzed at the same time and CO2 gap was checked every 2nd hourly.

✧ Screening 2D-echocardiography was done at the emergency department and inotropic agent was decided based on heart contractility.

✧ Patient demographic details, diagnosis, SOFA Score, was done in the first 6 hours of resuscitation (two hours apart) and the data was collected. Lactate and Co2 Gap were captured and documented after 24 hours of resuscitation.

✧ Aim of resuscitation was to target MAP of 65 mm Hg and two stable lactate values 2 hours apart. If lactates had not improved then further fluid boluses were decided upon reviewing pulmonary congestion in ultrasound (M mode of lung will be done and if B lines are more than 4 then it is indicative of pulmonary congestion). The corresponding CO2 Gap was noted.

✧ First choice of vasopressor was nor-adrenaline as per the standard infusion dose. If patient requires vasopressor support despite fluid boluses then steroid in the form of injection Hydrocortisone 50mg IV every 6th hourly was administered.

✧ Antimicrobial administration and further management was decided by clinical examination and supportive investigations as per clinician's judgement.

Data collection methods: Proforma

Data collection forms: Attached

VII. Statistical Methods

a) Sample Size Calculation

Sample size was calculated using nMaster software v2.0

In a study done by Beest PV et al, the mortality of patients with sepsis was 24.5% (13 out of 53) and risk of mortality for those with high PCO2 gap ranged from 1.6 to 5.3

Keeping a conservative value in odds ratio as 2.5, with power of 80% and 5% alpha error the minimum required sample size is 71.

b) Statistical Analysis Plan

Data was analyzed using R software. Continuous variable were described using mean and standard deviation. Categorical variables were described using frequency and percentage. Patients were categorized based on PCO2 difference and logistic regression was done to analyze various factors associated with mortality. Correlation between PCO2 difference and lactate was done using appropriate statistical methods. P value less than 0.05 was considered statistically significant.

c) Ethical consideration

Ethical clearance was obtained prior to the study from the ethics committee of the institution. Informed consent was obtained from the patient or guardian before the onset of study. Confidentiality of

patient details are and will be maintained. It was explained to the patient that the study is purely descriptive and merely for data collection. There is no intervention required specifically for the study. Management of these patients were along the standard international guidelines. As the study did not involve any extra procedure, no compensation was offered during and after the study.

VIII. Results

A total of 71 patients were enrolled in the study. 7 patients died from the 48 to 72 hours time period. Their samples were collected and analyzed till the 24th hour of admission. The mean age of the patients was 54 years (SD 16.2; range 18–81 years).

Table 2: Demographic and disease characteristics

Variable					
Age	Median 57	Mean 54	SD 16.2	Minimum 18	Maximum 81
Gender	Male - 24 Female- 47				
SOFA score at enrollment		Mean 9		Minimum 2	Maximum 19
Type of shock	Frequency				
Anemic	1				
Cardiogenic	14				
Distributive	50				
Hypovolemic	3				
Hypoxemic	2				
Neurogenic	1				
Fluid requirement In ml	Median 2000	Mean 2076	SD 998	Minimum 500	Maximum 4500

The primary outcome of the study was the correlation between the PCO2 gap and the in hospital mortality at each of the sampling time points. The correlation between the PCO2 gap and the in hospital mortality was positive at 0, 2, 4, 6 and 24hours. The correlation was statistically significant at 0 and 2 hours. (Table 2)

Table 3: Correlation between the PCO2 gap and the in hospital mortality

Time point	Point biserial correlation (rpb)	Probability (p) value
0 hour	0.309	0.009
2 hours	0.358	0.002
4 hours	0.200	0.108
6 hours	0.096	0.473
24 hours	0.170	0.207

There was a statistically significant negative correlation between end point of resuscitation and pCO2 gap at 2h,4h, 6h and 24 hours as implied by the Pearson's correlation in Table 3.

Table 4: Correlation between the PCO2 gap and end point of resuscitation

Time Point	Point Biserial Correlation (Rpb)	Probability (P) Value
0 hour	-0.206	0.121
2 hours	-0.206	0.011
4 hours	-0.350	0.010
6 hours	-0.380	0.007
24 hours	-0.398	0.007

It was also observed that the pco2 gap at 0h,2h,4h, 6hours had a statistically significant positive correlation with lactate clearance.(Table 4)

Table 5: Correlation between the PCO2 gap and lactate clearance

Time point	Point biserial correlation (rpb)	Probability (p) value
0 hour	0.390	0.001
2 hours	0.362	0.002
4 hours	0.318	0.009
6 hours	0.311	0.018
24 hours	0.311	0.068

IX. Discussion

The association of lactate accumulation and oxygen debt during shock states has been described for decades15. Throughout the years, there has been continued interest in refining resuscitation triggers, and response to therapy. Lactate clearance as an endpoint of resuscitation is supported by at least two multi-center studie[16,17]. However, lactate clearance has disadvantages as lactates can sometimes be normal in septic shock[18], lactate elevation not solely due to oxygen delivery- consumption mismatch and it has different prognostic implications based on the initial value.

It was recognized in sepsis that pCO2 gap (or its mathematical derivatives) outperformed other markers in detecting tissue hypoperfusion[13,19-21]. The arterial carbon dioxide is dependent on the pulmonary gas exchange and the venous carbon dioxide is dependent on the blood flow to the tissue[22]. So, when the flow reduces in low cardiac output states like shock, the difference between the venous and arterial carbon dioxide increases. It has been demonstrated that the pCO2 gap increases in various types of shock.[2]

In our study we found a statistically significant correlation of pCO2 gap at 0 hour and and 2nd hour of resuscitation and mortality in patients. It shows that high pCO2 gap on initial presentation can be used as a predictor of outcomes in patients with shock. Ospina-Tascón, G.A. et al.,[24] found that the persistence of high PCO2gap during the early resuscitation of septic shock was associated with higher 28 day mortality.

We also found that there was a statistically significant correlation between end point of resuscitation and pCO2 gap at 2h,4h, 6h and 24 hours. Hence, pco2 gap can be used as an endpoint of resuscitation in patients with shock. This was similar to the findings of Vallet B et al.,[11] who found that determining the gap during resuscitation of critically ill patients is useful when deciding when to stop resuscitation.

Our analysis also showed that PCO2 gap at various time points had positive correlation with lactate clearance. This was similar to a study done by Shyam M et al.,[25] who showed that the PcvCO2–PaCO2/CaO2–CcvO2 ratio and lactate are positively correlated during the first 24 hours of active resuscitation from sepsis-induced hypotension,

Pco2 gap is not inferior to lactate levels as a hemodynamic marker. It can be substituted in place of lactate levels to predict outcomes in patients presenting with shock. It can also be used as a guide for therapy to achieve endpoint of resuscitation.

X. Limitations

Our study has its limitations. It is a descriptive study without randomization of the patients. Also some technical aspects should be kept in mind when these indices are used in clinical practice. First, some errors in the PCO2 gap measurements may occur when sampling the venous blood: incorrect sample container, contaminated sample by air or venous blood or catheter fluid. Second, a too long delay of transport of blood sampling may significantly change the blood gas content at the venous and the arterial site.

XI. Summary and Conclusion

The PCO2 gap can be used a marker of the adequacy of the cardiac output in patients with shock. Using pCO2 gap has potential to avoid administration of unnecessary fluids and inotropes in patients, who have lactate elevated in the absence of tissue hypo perfusion. We suggest using pCO2 gap as a complementary tool to evaluate the adequacy of blood flow to global metabolic demand. A high pCO2 gap on initial presentation was associated with high mortality rates. So it can be used as a predictor of outcomes in patients with shock.

List of abbreviations

ICU - Intensive care unit

MAP- Mean arterial pressure

CO2- carbon dioxide

PCO2- Partial pressure of carbon dioxide

EtCO2- End tidal concentration of carbon dioxide

CVP- Central venous pressure

SCVO2- Central venous oxygen saturation

VO2- Oxygen consumption

VCO2- Carbon dioxide output

CaCO2- Carbon dioxide content in the blood

K pa- Kilo pascal

SOFA- Sequential organ failure assessment

Mm Hg- millimeters of mercury.

VBG- Venous blood gas

ABG- Arterial blood gas.

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Appendices

Proforma

PCO2 Gap – AS AN ENDPOINT OF RESUSCITATION AND PREDICTOR OF MORTALITY IN PATIENTS WITH SHOCK.

1. DATE OF ADMISSION :
2. AGE : ___ YEARS
3. SEX : MALE / FEMALE
4. COMORBIDITIES : DIABETES / HYPERTENSION/ IHD/ CKD/THYROID DISORDERS/ OTHERS __
5. PROVISIONAL DIAGNOSIS :
6. TYPE OF SHOCK : CARDIOGENIC/HYPOVOLEMIC/DISTRIBUTIVE/ANEMIC OR HYPOXEMIC/CYTOPATHIC
7. SOFA SCORE :
8. MEAN ARTERIAL PRESSURE (ON ARRIVAL TO ER) :
9. FLUID BOLUS : YES/ NO
   SPECIFY DETAILS –
10. VASOPRESSOR : YES/ NO, if YES specify the drug _____
11. DOBUTAMINE SUPPORT : YES/ NO
12. ENDPOINT OF RESUSCITATION:
13. FINAL OUTCOME OF PATIENT :

TIME	LACTATE mmol/L	P(cv-a)CO2 mmHg	SCVO 2 %	END POINT OF RESUSCITATION		
				MAP	IVC COLLAPSIBILITY	PULMONARY EDEMA
ARRIVAL						
2 HOURS						
4 HOURS						
6 HOURS						
24 HOURS						

Informed Consent and patient information sheet

Dr. Prabhu,
Emergency medicine department,
Narayana health.

This Informed Consent Form is for men and women who come to the emergency department in state of shock- with low blood pressure not responding to IV fluids, and who we are inviting to participate in research. The title of our research project is PCO2 Gap – AS AN ENDPOINT OF RESUSCITATION AND PREDICTOR OF MORTALITY IN PATIENTS WITH SHOCK: A PROSPECTIVE OBSERVATIONAL STUDY.

This Informed Consent Form has two parts:
Information Sheet (to share information about the research with you)
Certificate of Consent (for signatures if you agree to take part)

PART I: Information Sheet

Introduction

I am Dr. Prabhu. We are doing research on patients presenting with shock to the emergency room, which can occur due to various causes like blood loss, cardiac failure, infection, anemia. I am going to give you information and invite

you to be part of this research. Before you decide, you can talk to anyone you feel comfortable with about the research.

There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask them of me, or the staff.

pCO2 gap is the difference between the venous and arterial carbon dioxide. When a patient presents with shock, they will be treated with IV fluids or medication to increase blood pressure (inotropes) by constriction of blood vessels depending upon the cause of the shock. To know when the shock has resolved, we are going to compare pCO2 gap to other parameters which have been previously established.

Purpose of the research

To evaluate if pCO2 gap can be used to predict mortality and marker for end point of resuscitation

Participant selection

We are inviting all adults with shock to participate in the research on pCO2 gap.

Voluntary Participation

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at the hospital will continue and nothing will change. You may change your mind later and stop participating even if you agreed earlier.

Procedures and Protocol

Once you understand the study and give consent, your pCO2 gap will be measured on presentation, 2nd hour, 4th hour, 6th hour and at 24th hour. Patients presenting with shock will have an arterial line for invasive blood pressure measurement and a central line for administration of inotropes to treat the shock. Blood samples from these lines will help us to measure pCO2 gap. Treatment will be given for the shock as per standard guidelines and hospital protocol according to the patient's condition. Other parameters such as mean arterial pressure, IVC collapsibility, lactates will be compared to find out if pCO2 gap has a good correlation for endpoint of resuscitation (resolution of shock)

Duration

The research takes place over the course of 1 year. You will be followed up for12 to 24 hours depending upon your clinical condition.

Side Effects

No new intervention or procedure is done for the study. You will already have lines from which blood samples will be taken. Hence there are no side effects for the study.

Risks

No additional risks and discomfort will be caused during this study.

Benefits

The findings of this study can change the views of using pCO2 gap as an endpoint of resuscitation.

Confidentiality

The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no-one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up with a lock and key.

Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so and refusing to participate will not affect your treatment at this hospital in any way. You may stop participating in the research at any time that you wish without losing any of your rights as a patient here. Your treatment at this hospital will not be affected in any way.

Whom to contact

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the following:

Contact the principal investigator
Name: Dr. Prabhu
Address: Narayana Health City, Bangalore
Contact No. 7358248887
Email: prabhu.adms@gmail.com

This proposal has been reviewed and approved by Narayana Health Academic ethical committee, which is a committee whose task it is to make sure that research participants are protected from harm. If you wish to find about more about the IRB, contact Narayana Health Academic Ethics Committee. Name: Dr. Sanjay Rao
Designation: Member Secretary
Contact No. 9538008940;
Email: nhaec@narayanahealth.org

PART II: Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant_____

Signature of Participant _____

Date _____
Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands the objectives of the research.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____ Day/month/year

Print name of the impartial witness in capitals_____

Signature of impartial witness_____

Date _____ Day/month/year

Deferred Consent for Research Participation

Title of Project: PCO2 Gap – AS AN ENDPOINT OF RESUSCITATION AND PREDICTOR OF MORTALITY IN PATIENTS WITH SHOCK: A PROSPECTIVE OBSERVATIONAL STUDY.

Principal Investigator: Dr. Prabhu
Emergency medicine department,
Narayana health,Phone Number: 7358248887

The patient named below is being enrolled in this research study by deferred consent. The process of obtaining written informed consent will be deferred until after the patient is able to understand and has capacity to give consent. Written informed consent will be obtained to continue data collection after resuscitation from the patient or, if the patient lacked capacity, a legal representative.

Patient's Name: _____

Date/time assessed for enrolment: _____/_____/_____ (dd/mm/yyyy) at ____ : _____ (time)

Reason(s) deferred consent process is used (check all that apply):

____ The patient is unconscious or lacks capacity to understand the risks, methods and purposes of the research study.

____ No next of kin/substitute decision maker is available to provide consent, or attempts to contact them have been unsuccessful despite diligent and documented efforts.

____ A substitute decision maker _____ (name and relationship) has been contacted by telephone, and the purpose, methods and risks of participation in this study have been explained to the third party. While the substitute decision maker has given verbal consent for participation, written consent must be still be obtained.

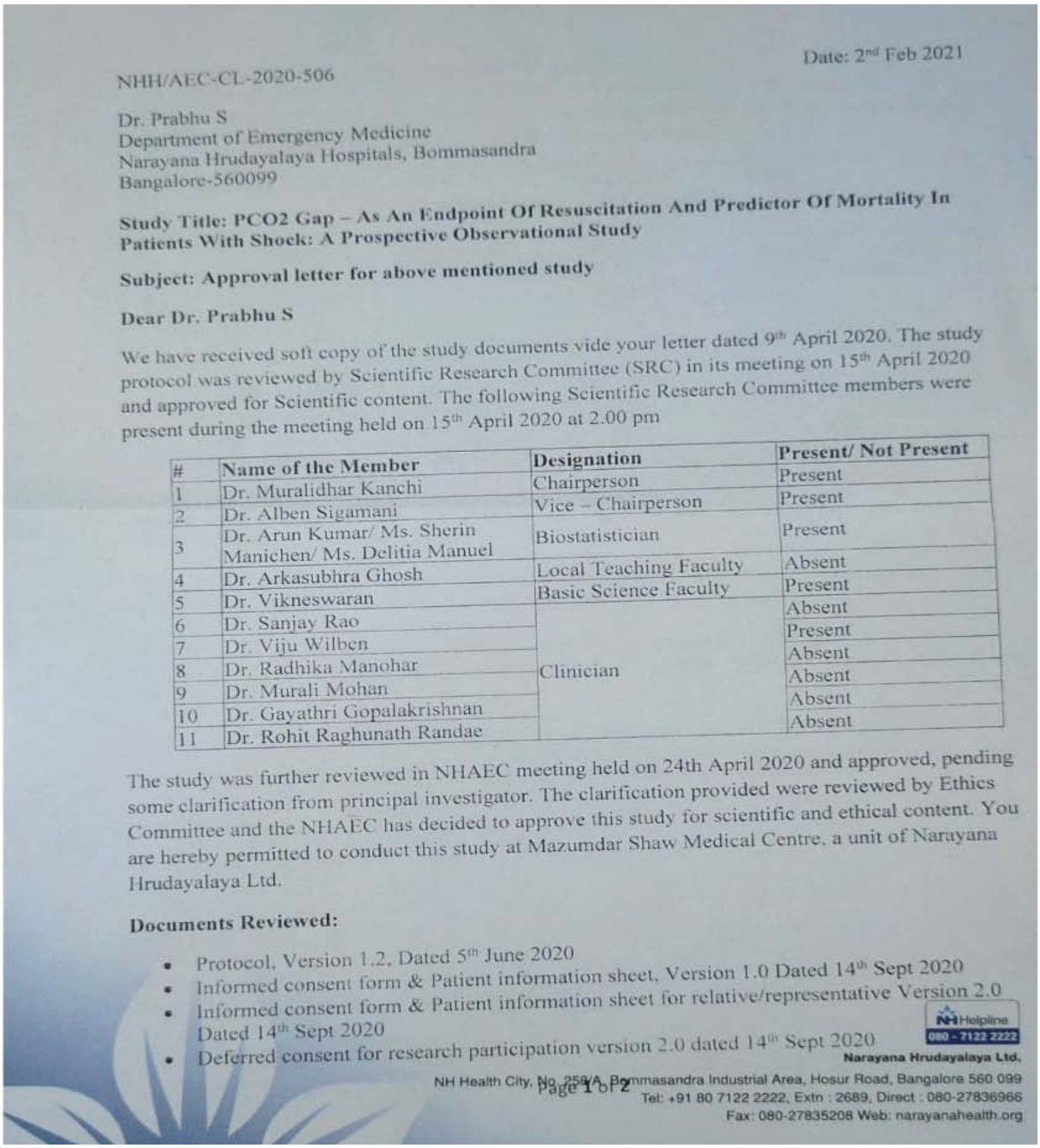
____ No relevant prior directive by the patient is known to exist.

____ Other: _____

_____ _____

Signature of investigator Date and Time

Annexure 1



Date: 2nd Feb 2021

NHH/AEC-CL-2020-506

Dr. Prabhu S
Department of Emergency Medicine
Narayana Hrudayalaya Hospitals, Bommasandra
Bangalore-560099

Study Title: PCO2 Gap – As An Endpoint Of Resuscitation And Predictor Of Mortality In Patients With Shock: A Prospective Observational Study

Subject: Approval letter for above mentioned study

Dear Dr. Prabhu S

We have received soft copy of the study documents vide your letter dated 9th April 2020. The study protocol was reviewed by Scientific Research Committee (SRC) in its meeting on 15th April 2020 and approved for Scientific content. The following Scientific Research Committee members were present during the meeting held on 15th April 2020 at 2.00 pm

#	Name of the Member	Designation	Present/ Not Present
1	Dr. Muralidhar Kanchi	Chairperson	Present
2	Dr. Alben Sigamani	Vice – Chairperson	Present
3	Dr. Arun Kumar/ Ms. Sherin Manichen/ Ms. Delitia Manuel	Biostatistician	Present
4	Dr. Arkasubhra Ghosh	Local Teaching Faculty	Absent
5	Dr. Vikneswaran	Basic Science Faculty	Present
6	Dr. Sanjay Rao	Clinician	Absent
7	Dr. Viju Wilben		Present
8	Dr. Radhika Manohar		Absent
9	Dr. Murali Mohan		Absent
10	Dr. Gayathri Gopalakrishnan		Absent
11	Dr. Rohit Raghunath Randae		Absent

The study was further reviewed in NHAEC meeting held on 24th April 2020 and approved, pending some clarification from principal investigator. The clarification provided were reviewed by Ethics Committee and the NHAEC has decided to approve this study for scientific and ethical content. You are hereby permitted to conduct this study at Mazumdar Shaw Medical Centre, a unit of Narayana Hrudayalaya Ltd.

Documents Reviewed:

- Protocol, Version 1.2, Dated 5th June 2020
- Informed consent form & Patient information sheet, Version 1.0 Dated 14th Sept 2020
- Informed consent form & Patient information sheet for relative/representative Version 2.0 Dated 14th Sept 2020
- Deferred consent for research participation version 2.0 dated 14th Sept 2020

NH Helpline 080 - 7122 2222

Narayana Hrudayalaya Ltd.
NH Health City, No. 258/A, Bommasandra Industrial Area, Hosur Road, Bangalore 560 099
Tel: +91 80 7122 2222, Extn : 2689, Direct : 080-27836966
Fax: 080-27835208 Web: narayanahealth.org

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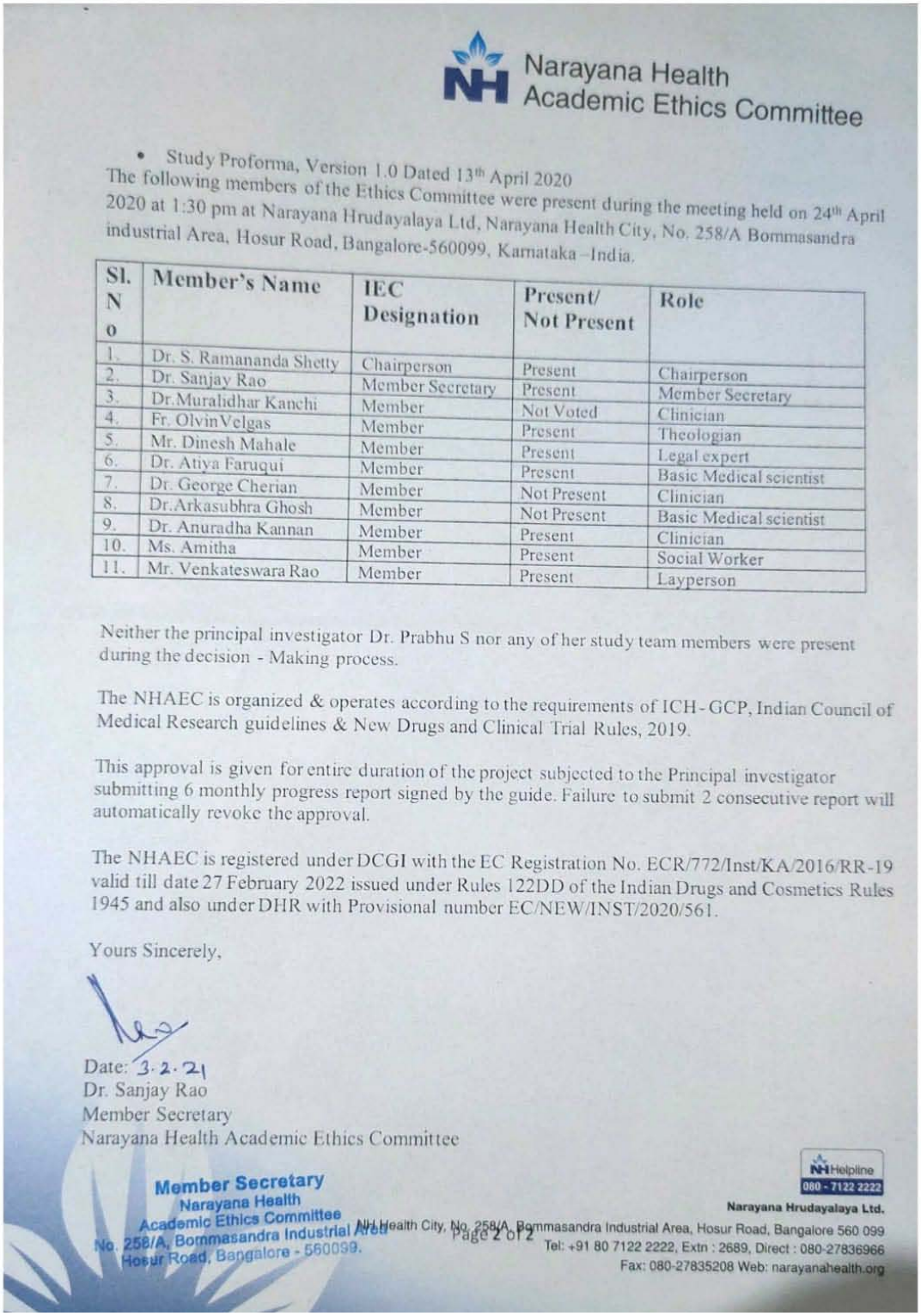
Annexure ii



Narayana Health
Academic Ethics Committee

- Study Proforma, Version 1.0 Dated 13th April 2020

The following members of the Ethics Committee were present during the meeting held on 24th April 2020 at 1:30 pm at Narayana Hrudayalaya Ltd, Narayana Health City, No. 258/A Bommasandra industrial Area, Hosur Road, Bangalore-560099, Karnataka –India.

Sl. No	Member's Name	IEC Designation	Present/ Not Present	Role
1.	Dr. S. Ramananda Shetty	Chairperson	Present	Chairperson
2.	Dr. Sanjay Rao	Member Secretary	Present	Member Secretary
3.	Dr.Muralidhar Kanchi	Member	Not Voted	Clinician
4.	Fr. Olvin Velgas	Member	Present	Theologian
5.	Mr. Dinesh Mahale	Member	Present	Legal expert
6.	Dr. Atiya Faruqui	Member	Present	Basic Medical scientist
7.	Dr. George Cherian	Member	Not Present	Clinician
8.	Dr.Arkasubhra Ghosh	Member	Not Present	Basic Medical scientist
9.	Dr. Anuradha Kannan	Member	Present	Clinician
10.	Ms. Amitha	Member	Present	Social Worker
11.	Mr. Venkateswara Rao	Member	Present	Layperson

Neither the principal investigator Dr. Prabhu S nor any of her study team members were present during the decision - Making process.

The NHAEC is organized & operates according to the requirements of ICH-GCP, Indian Council of Medical Research guidelines & New Drugs and Clinical Trial Rules, 2019.

This approval is given for entire duration of the project subjected to the Principal investigator submitting 6 monthly progress report signed by the guide. Failure to submit 2 consecutive report will automatically revoke the approval.

The NHAEC is registered under DCGI with the EC Registration No. ECR/772/Inst/KA/2016/RR-19 valid till date 27 February 2022 issued under Rules 122DD of the Indian Drugs and Cosmetics Rules 1945 and also under DHR with Provisional number EC/NEW/INST/2020/561.

Yours Sincerely,

Date: 3.2.21
Dr. Sanjay Rao
Member Secretary
Narayana Health Academic Ethics Committee

Member Secretary
Narayana Health
Academic Ethics Committee
No. 258/A, Bommasandra Industrial Area
Hosur Road, Bangalore - 560099.

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To the institution

Get letter of appreciation

Global Journals sends a letter of appreciation of author to the Dean or CEO of the University or Company of which author is a part, signed by editor in chief or chief author.

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Financial

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Associates are authorized to organize symposium/seminar/conference on behalf of Global Journal Incorporation (USA). They can also participate in the same organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent. Additionally, they get free research conferences (and others) alerts.

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Exclusive | Financial

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Associate	Fellow	Research Group	Basic
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Preferred Author Guidelines

We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

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Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at submit@globaljournals.org or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

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Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

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3. Ensure corresponding author's email address and postal address are accurate and reachable.
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Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

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Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
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.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

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

Format Structure

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

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative 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) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

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

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning 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 a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be 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. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). 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.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for writing a good quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. 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.

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

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

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

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

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. 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 for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

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

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

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

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon 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 for 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 of 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 criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to 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 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 avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

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

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite 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 approaches 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. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a 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 limit the initial two items to no more than one line each.

Reason for writing the article—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 for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity 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 of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets 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 for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory 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 soundly written procedures segment allows a capable scientist to replicate 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 to give the least amount of information that would permit another capable scientist to replicate 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 section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show 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:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the 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—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and 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 as 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. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give 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 manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit 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 section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an 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 implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully 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 the prospect, and let it drop at that. Make a decision as to whether each premise is supported or 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 an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of 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 other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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

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