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In Vitro Sperm Preparation by Progesterone, Pentoxifylline and *Glycyrrhiza Glabra* for Asthenozoospermic Men

By Saad S. Al-Dujaily & Khitam Malik

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Abstract – Background: Results of artificial insemination (AI) still does not exceed 20-25% because of different factors one of them is male factor infertility. Therefore, the global companies concern on the culture media that used for activation of semen samples of infertile men. However, till now, there are many semen samples did not response to these media leading to failure in AI. The objective of this study is to improve certain sperm function parameters *in vitro* of asthenoteratospermic patients by using novel combination motility stimulants substances that improved medium to be used for assisted reproductive technologies (such as artificial insemination) in future.

One hundred asthenozoospermic patients are involved in this study. They are divided randomly into equal four groups. Each semen sample had been divided into two parts. One part was considered as a control by using Ham's F10 medium and the other part is considered as treated group by adding the following substances in combination; Pentoxifylline (PF) 1mg/ml, Progesterone (P) 0.409 mg/ml and *Glycyrrhiza glabra* (Gg) 1mg/ml in the following groups. Group 1:PF and Gg, Group2: PF and P, Group3: Gg and P, and Group4:PF,Gg and P. Sperm function parameters are examined before and following *in vitro* preparation using wash and spin technique.

Keywords : *pentoxifylline, glycyrrhiza glabra, progeste-rone, in vitro sperm preparation.*

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In Vitro Sperm Preparation by Progesterone, Pentoxifylline and *Glycyrrhiza Glabra* for Asthenozoospermic Men

Saad S. Al-Dujaily^α & Khitam Malik^ο

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Keywords : pentoxifylline, glycyrrhiza glabra, progesterone, *in vitro* sperm preparation.

I. INTRODUCTION

Since about half of the infertility cases are due to male factors, a detailed sperm analysis became the most important examination to be performed in the approach to the infertile couples (Karpuz *et al.*, 2007). Basically, sperm count, motility and percentage of normal sperms are conventional criteria for semen quality (Check *et al.*, 1992). Among these parameters, sperm morphology and motility are the best criteria for demonstrating the fertilization capacity of a male (Bonde *et al.*, 1998). Sperm motility gives a measure of the integrity of the sperm axoneme and tail structures as well as the metabolic machinery of the mitochondria, and sperm morphology is a surrogate measure of the integrity of DNA packaging and the quality of spermatogenesis (Pacey, 2006).

One of the major causes of male factor infertility is related to asthenozoospermia which is characterized by reduced forward motility (WHO grade a+b sperm motility $< 50\%$ or a $< 25\%$) or absent sperm motility in fresh ejaculate (Mehranian *et al.*, 2009). Although there is a general consensus that ultrastructural anomalies underlie severe asthenozoospermia (Chemes *et al.*, 1998), the etiology and pathogenesis of temporary and/or mild asthenozoospermia remains, for the most part, undefined. (Yunes *et al.*, 2003).

Sperm motility is an important factor to consider in couples pursuing assisted reproductive technologies (ART). Several studies have demonstrated the influence of sperm motility on the outcomes of various ARTs, including intrauterine insemination (IUI) (Yalti *et al.*, 2004), conventional *in vitro* fertilization (IVF) (Repping *et al.*, 2002), and IVF with intracytoplasmic sperm injection (ICSI) (Stalf *et al.*, 2005).

Over the last decade, it have been noticed the increasing use of assisted reproductive technologies (ARTs) to overcome the problems of infertile couple (Katz *et al.*, 2002). ARTs are now routinely proposed in the cases of male partner of infertile couple who wish to have a child (Kaewnoonual *et al.*, 2008). Even that, the result of AI still do not exceed 20% as a result of different factors one of them is male factor through activation technique. Therefore, the objective of the present study is to investigate the possibility of using different

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components to enhance the certain sperm functions *in vitro*. The study will be achieved by selection of certain substances such as Pentoxifylline, Progesterone and medical plants as *Glycyrrhiza glabra* for *in vitro* activation of infertile human semen.

II. MATERIALS AND METHODS

a) Patients

The study was carried out in the High Institute for Embryo Researches and Infertility Treatment, AL-Nahrain University. One hundred with mild asthenozoospermia and/or other male factor infertility were involved in this research. The patient's age ranged between 24-50 years. The clinical examination performed by a consultant urologist in the charge of male infertility Unit in the Institute including presence or absence varicocele, cryptorchidism, hydrocele, hernia and others.

b) Seminal Fluid Analysis

Semen samples were collected by masturbation into wide-mouth glass or plastic containers, supplied by the laboratory, after 3–7 days of sexual abstinence. The sample was transported to the laboratory immediately and placed in an incubator at 37 °C till complete liquefaction, then semen samples were analyzed by a macroscopic and microscopic examination using standardization of WHO (1999).

c) Preparation of *Glycyrrhiza Glabra* Extract

This extract was provided in a powder from which is water –soluble. The extract was obtained by crushing the root of plant and extracted as described by Chakravarty, (1976). Storage of *Glycyrrhiza* was done in well –closed container protected from light and moisture (Maisonneave, 1971).

d) Preparation of *Glycyrrhiza glabra* for Human Sperm Activation In Vitro

The concentration of *Glycyrrhiza glabra* of 0.1% was prepared by adding 10 mg from Gg extract to 10 ml PBS in plastic test tubes contained broad spectrum antibiotic (Ampicillin 0.004g) to prevent bacterial growth, (Al-Dujaily and Al-Shammary, 2008). The solution was filtered using Millipore (0.45 µm).

e) Preparation of Pentoxifylline Stock Solution

Pentoxifylline powder (Sigma, Germany) 10 mg was dissolved in 10 ml of Phosphate buffered saline (PBS). These concentrations prepared daily under sterile condition using Millipore filter, 0.45 µm.

f) Preparation of Progesterone

The medroxyprogesterone acetate (150 mg/mL vial, Depo Provera®, Pharmacia) was used in a dose of 0.409 mg/ml and mixed with one ml of PBS. These concentrations prepared daily under sterile condition using Millipore filter, 0.45µm.

All the media were fixed at a pH 7.5-8, temperature 22-25 °C and Osmolarity 285-300 mOsm/L.

g) In Vitro Activation Technique

After liquefaction of human semen using wash and spin method (Avery and Elder, 1992).

Each semen sample had been divided into two parts. One part was considered as a control by using Ham's F10 medium(Sigma,Germany) and the other part is considered as treated group by adding the following substances in combination; Pentoxifylline (PF) 1mg/ml, Progesterone (P) 0.409 mg/ml and *Glycyrrhiza glabra* (Gg) 1mg/ml in the following groups. Group 1:PF and Gg ,Group2: PF and P,Group3: Gg and P, and Group4:PF, Gg and P. Certain sperm function parameters are examined following *in vitro* activation as described by WHO (1999) too.

h) Statistical Analysis

Data from treatment and control groups were expressed as mean ±SEM and using Student's t-test to compare values from experimental and control groups. Differences between values were considered significant at P<0.05. Analysis of variance (ANOVA) was used to compare the differences between the four prepared media. When F values reach the significant level at 5%, least significant difference (LSD) test was used, (Sorile, 1995).

III. RESULTS

a) Effect of pentoxifylline glycyrrhiza glabra and progesterone on human sperm in vitro

Tables 1, 2, 3 and 4 shows that the activation of human sperm *in vitro* with both control (Hams F-10 medium) and treated (PF and Gg medium, Table -1; PF and P medium, Table -2; Gg and P medium, Table-3 and PF, Gg and P medium Table-4) groups caused a significant (P<0.05/P<0.01) decrease in sperm concentration compared to before activation. There is no significant (P>0.05) difference in the mean of sperm concentration between control and treated groups. Regarding sperm motility, although there is highly significant (P<0.01) increase in sperm motility (grade a) in both control and treated groups compared to before activation, there is highly significant (P<0.01) increase in sperm motility (grade a) in treated group when compared to control. A highly significant (P<0.01) improvement in sperm motility (grade b) in treated semen samples is recorded compared to before activation, while there is a significant (P<0.05) increase in sperm motility (grade b) in control when compared to before activation. Activation of human sperm caused a highly significant (P<0.01) increase in the percentage of morphologically normal sperm in both control and treated group when compared to before activation, while there is a significant (P<0.05) increment in morphologically normal sperm of treated semen samples when compared to control semen samples.

Table 1 : Effect of pentoxifylline and *glycyrrhiza glabra* on certain sperm function character of asthenospermic patient following *in vitro* activation technique

Certain Sperm Function Characters	<i>In vitro</i> activation		
	Before Activation	After Activation Control	After Activation PF+Gg
	Mean± SEM	Mean ±SEM	Mean±SEM
Sperm Concentration (million/ml)	43.12± 3.69	^A 20.28± 2.77	^A 22.64 ±2.55
Sperm Motility Grade a(%)	2.8 ±0.752	^b 16.72 ±1.21	^a 27.08±1.10 ^c
Sperm Motility Grade b(%)	31.48 ±1.99	^B 40.0 ±0.913	^a 50.80 ±1.72 ^c
Sperm Motility Grades a+b(%)	34.28 ±2.55	^B 56.72 ± 3.88	^a 7.08 ±3.72 ^c
Morphologically Normal Sperm (%)	47.64 ±1.62	^b 78.0 ±1.66	^a 84.60 ±1.98 ^c

Different small letters indicate a highly significance P<0.01

Different capital letters indicate a significance P<0.05

No. of sample used = 25

Ham's F-10 *medium* used as control

Table 2 : Effect of Pentoxifylline and Progesterone on certain sperm function characters of asthenospermic patient following *in vitro* activation

Certain Sperm Function Characters	<i>In vitro</i> activation		
	Before Activation	After Activation Control	After Activation PF+P
	Mean ± SEM	Mean ± SEM	Mean ± SEM
Sperm Concentration (million/ml)	43.24±+2.98	^a 19.84±1.94	^a 20.52±1.60
Motility Grade a(%)	3.96±0.524	^B 13.6±1.02 ^B	^a 21.40±1.40 ^c
Motility Grade b(%)	31.2±1.54	^B 49.2±0.85	^a 48.20±1.25
Motility Grades a+b(%)	35.16±2.97	^B 52.8±2.77 ^B	^a 69.6±3.55 ^c
Morphologically Normal Sperm (%)	44.84±1.75	^B 79.60±1.47	^a 80.80±1.57

Different small letters indicate a highly significance P<0.01

Different capital letters indicate a significance P<0.05

No. of sample used = 25 Ham's F-10 *medium* used as control

Table 3 : Effect of glycyrrhiza glabra and progesterone on certain sperm function characters of asthenospermic patient following *in vitro* activation

Certain Sperm function Characters	<i>In vitro</i> activation		
	Before Activation	After Activation Control	After Activation Gg+ P
	Mean ±SEM	Mean ±SEM	Mean ±SEM
Sperm Concentration (million/ml)	41.64+ ±2.5	^a 16.24±1.77	^a 13.4±1.37 ^c
Motility Grade a(%)	2.2±0.551	^B 13.4±0.945	^a 15.88±1.39 ^c
Motility Grade b(%)	37.36±0.9	^B 49.4±0.781	^a 46.12±1.38
Motility Grades a+b(%)	39.56±2.44	^B 62.8±4.25	^a 62.0±3.98
Morphologically Normal Sperm (%)	44.56±1.21	^B 78.6±1.62	^a 84.4±1.83 ^c

Different small letters indicate a highly significance P<0.01

Different capital letters indicate a significance P<0.05

No. of sample used = 25

Ham's F-10 *medium* used as control

Table 4 : Effect of *Glycyrrhiza glabra*, Progesterone and Pentoxifylline on certain sperm function character of asthenospermic patient following *in vitro* activation

Certain Sperm Function Characters	<i>In vitro</i> activation		
	Before Activation	After Activation Control	After Activation PF+Gg+P
	Mean±SEM	Mean ±SEM	Mean ±SEM
Sperm Concentration (million/ml)	41.64±2.2	^a 18.4±1.37	^a 16.08±1.28
Sperm Motility Grade a (%)	1.6±0.523	^B 12.8±0.961	^a 18.04±1.12 ^C
Sperm Motility Grade b (%)	36.64±0.991	^B 49.4±0.666	^A 47.4±0.918
Sperm Motility Grade a+b (%)	38.24±3.65	^b 61.12±3.88	^a 65.44±4.11
Morphologically Normal Sperm (%)	47.12±1.25	^B 79.8±2.88	^a 84.4±1.27 ^C

Different small letters indicate a highly significance $P<0.01$

Different capital letters indicate a significance $P<0.05$

No. of sample used = 25

Ham's F-10 *medium* used as control

b) *Comparison the Results of Certain Sperm Function Characters following in vitro Activation between PF +GG, PF+P, GG+P and PF+P+GG Media*

The sperm concentration following *in vitro* activation with PF and Gg medium is highly significant ($P<0.01$) increase compared with other media (Table-5). A significant ($P<0.05$) increase in sperm concentration is observed following *in vitro* activation with PF+P than that of Gg+P and PF+P+Gg media. The results

revealed highly significant increment ($P<0.01$) in the percentage of total active sperm motility grades (A+B) and forward progressive sperm motility grade (A) with a significant improvement ($P<0.05$) in the percentage of progressive sperm motility grade (B) and morphologically normal sperm when using PF and Gg medium in comparison with control medium and other combination medium following 10, 30 and 60 minutes incubation.

Table 5 : Comparison results of certain sperm function characters following *in vitro* activation between PF +Gg, PF+P, Gg+P and PF+P+Gg media

Sperm Function Characters	<i>In vitro</i> Activation			
	After Activation PF+Gg	After Activation PF+P	After Activation Gg+P	After Activation PF+Gg+P
	Mean± SEM	Mean± SEM	Mean± SEM	Mean± SEM
Sperm Concentration (million/ml)	25.64 ±2.55**	20.52±1.60 ⁺	13.4±1.37	16.08±1.28
Active Motility Grade a (%)	27.28±1.10**	21.40±1.40	15.88±1.39	18.04±1.12
Active Motility Grade b(%)	50.80 ± 1.72	48.20±1.25	46.12±1.38	47.4±0.918
Active Motility Grades a+b (%)	77.08 ±3.72*	69.6±3.55	62.0±3.98	65.44±4.11
Morphologically Normal Sperm (%)	84.60 ± 1.98	80.80±1.57	84.4±1.83	84.4±1.27

No. Samples per group=25

* $P<0.05$ significantly different from other media

** $P<0.001$ significantly different from other media

+P <0.05 significantly different from Gg+P and PF+P+Gg media.

IV. DISCUSSION

There was a significant reduction in the concentration of spermatozoa in both control and treated groups following *in vitro* activation. This is due to the inability of the dead and abnormal sperms with

poor motility spermatozoa to swim up and migrate from sperm pellet to the upper layer of culture medium. These results were in agreement with other studies using culture for the separation and activation of sperm *in vitro* (Check *et al.*, 1993 and Allaw, 1999).

Regarding sperm motility, there was highly significant increase in sperm motility (grade a and grades a+b), while there is a significant increase in sperm motility (grade b) in treated group. This in agreement with studies that revealed a significant improvement in the sperm motility percentage with forward progressive movement (grade a) and hyperactivation, following activation by PF and Gg, independently (Al-Dujaily *et al.*, 2006 and Al-Dujaily *et al.*, 2007). Adding PF at a concentration of 1mg/ ml usually results in the stimulation of various aspects of sperm functions ,including motility, acrosomal reaction ,penetration of zona free hamster oocyte and the binding of the zona pellucida in hemizona assay (Tesarik *et al.*, 1992a and Nassar *et al.* ,1998). Thus, these observations were consistent with Al-Dujaily *et al.*, (2007) study that show the sperm motility percentage of mild asthenospermic semen was significantly improved by the addition of PF at a concentration of 1mg/ ml, at the same time Al-Dujaily and Al-Shammary, (2008) noticed that addition of Gg 1 mg/ml revealed a significant improvement in active sperm motility (grade a+b).

This study believed that the medium contains PF and Gg gave excellent improvement in sperm motility because PF may increase curvi-linear Velocity (VCL) , straight line velocity (VSL), ALH (lateral head displacement) , BCF (beat cross frequency) and sperm hyperactivation in both normospermic and asthenospermic specimen (Tesarik *et al.* , 1992b). In addition, an increased ALH may improve VCL by moving sperm head further from side to side. An increase in ALH and VCL may reflect an increase in energy consumption facilitated by pentoxifylline (Ward and Clissod, 1987) .On the other hand, Gg contents (glucose and fructose) are considered to be a source of energy of sperm motility (Al-Dujaily and Alsaadi, 2009). The sum of these may make the penetration power of sperm greater leading to improving fertilization rate (Sheena, *et al.*, 1993; Al-Dujaily and Al-saadi, 2009). Furthermore, Gg contain proteins, vitamins (E, C, folic acid and others) and sugars like: glucose, sucrose, fructose and maltose (Taylor, 2004), all of these substances stimulate sperm motility and the grade activity of forward movement. Protein and amino acids, which maintain sperm osmolarity and in turn integrity of sperm cell membrane (AL- Dujaily *et al.*, 2006). *In vitro* studies showed that vitamins E and C are major chain – breaking antioxidants in sperm membranes and appears to have a dose dependent protective effect (Agarwal and Prabakaran, 2005).

It is concluded that the best combination could be added as sperm motility stimulants for the culture medium used for *in vitro* activation of asthenoteratospermic patients alone or with other male infertility factors is PF and Gg. More researches are recommended to utilize this medium in the ART centers.

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Respiration Rate Diagnosis Using Single Lead ECG in Real Time

By K.Ramya & K.Rajkumar

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Abstract – Respiration rate is the respiratory signal or the respiratory knowledge. Numeral methods can be applied to derive a respiratory signal from the ECG. The efficacy of this monitoring can be improved by deriving respiration, which previously has been based on overnight polysomnography studies where patients are stationary or the use of multi lead ECG systems. In this paper, ECG features of Heart rate variability (HRV) and ECG-derived respiration (EDR) including ECG filtering methods are examined. This ECG features are compared with the simultaneously recorded respiratory signal, it is estimate from RR-interval, R-wave time duration and R-wave amplitude. These values are evaluated using discrete wavelet transform. Based on the respiratory signal, time domain measures are MeanRR, SDRR, Maxrate, Minrate, RMSDD, SDEDR, MeanEDR, pNN50, NN50 are calculated, that reflect the Respiration rate variability (RV). Those Respiration variability measures have been established their use, by the ability to distinguish between periods of rest and during respiratory rate testing time. Moreover, these RV measures are able to differentiate between the first resting period and the periods following the respiration rate.

Keywords : *discrete wavelet transform, ECG-derived respiration, heart rate variability, Respiration rate variability.*

GJMR Classification : *NLMC Code: WV 410, WB 284*



Strictly as per the compliance and regulations of:



Respiration Rate Diagnosis Using Single Lead ECG in Real Time

K.Ramya^a & K.Rajkumar^a

Abstract - Respiration rate is the respiratory signal or the respiratory knowledge. Numeral methods can be applied to derive a respiratory signal from the ECG. The efficacy of this monitoring can be improved by deriving respiration, which previously has been based on overnight polysomnography studies where patients are stationary or the use of multi lead ECG systems. In this paper, ECG features of Heart rate variability (HRV) and ECG-derived respiration (EDR) including ECG filtering methods are examined. This ECG features are compared with the simultaneously recorded respiratory signal, it is estimate from RR-interval, R-wave time duration and R-wave amplitude. These values are evaluated using discrete wavelet transform. Based on the respiratory signal, time domain measures are MeanRR, SDRR, Maxrate, Minrate, RMSDD, SDEDR, MeanEDR, pNN50, NN50 are calculated, that reflect the Respiration rate variability (RV). Those Respiration variability measures have been established their use, by the ability to distinguish between periods of rest and during respiratory rate testing time. Moreover, these RV measures are able to differentiate between the first resting period and the periods following the respiration rate.

Keywords : discrete wavelet transform, ECG-derived respiration, heart rate variability, respiration rate variability.

I. INTRODUCTION

Respiration rate is an interesting physiological process. Numeral scheme to obtain a respiratory signal include impedance sensors, pressure sensors and a thermistor in the nose. However, there are 2 common disadvantages of using these devices: 1) these complex devices involved might interfere with natural physiological breathing. 2) Such devices cannot be used for certain clinical purposes, for example, ambulatory or long-term monitoring in naturalistic settings. Therefore, the development of a convenient method to record or estimate respiratory signals is important from a clinical perspective. ECG is the most well-known, feasible, and an accessible tool in diagnostic respiration rate, during ECG recording no extra equipment is needed. The respiration rate calculation based on these ECG two facts 1) the positions of ECG electrodes on the chest surface move relative to the heart. 2) transthoracic impedance varies at the lungs fill and empty, during the recording of the ECG. The modified V2 lead based ECG signal gives the acceptable result for the respiration rate. The respiration rate calculation mainly based on the two ECG features that are Heart rate variability (HRV) and ECG-derived

respiration (EDR). These ECG features are two prominent physiological functions which are both modulated by fluctuations of the autonomic nervous system (ANS). These information's are may be used to gain insight into the autonomic control of the heart or the lung. Amendment of these modulations may appear during ageing or during the progress of a disease (e.g. congestive heart failure or sleep-disordered breathing). Based on an electrocardiogram (ECG) recording the heart rate time series may be derived with high accuracy and provides a basis to analyze the heart rate variability (HRV). Accordingly, HRV has been used extensively as a non-invasive tool to investigate the autonomic control of the cardiovascular system and many characteristic features of heart rate variations in health and disease have been explored in the past and will further be explored. The 'Breathing rate variability' or 'respiration rate variability' (in analogy to HRV) has not been established yet because it is more difficult to determine the instantaneous respiratory rate unambiguously. Throughout, everyday conditions the influence of voluntary activities like speaking or eating on respiration is more pronounced compared to that on the heart and agitate the determination of respiratory oscillations. Consequently, even if a respiratory trace is available, the determination of inspiration and expiration is less precise compared to the determination of the instantaneous heart rate. The RR-interval based HRV analysis is best compared to others. The ECG-derived Respiration may utilize the R-peak amplitude and time duration.

II. PREVIOUS WORK

A constructive appraisal of ECG-derived respiration techniques is provides the groups algorithms [9] into categories based on beat morphology, heart rate, or a combination of both. This sector is included on algorithm evaluation, which stresses the importance of comparing the derived respiratory information with a simultaneous recording of the respiration signal. The Fourier transforms and spectral features based EDR values [12] analyzed. Kernel principal component analysis based algorithm is developed [11] which derive the EDR value and this value analyzed using Eigen and entropy value. Principal Component Analysis technique based three different algorithms derived [10]. These algorithms are mainly used to analysis the ECG-derived

respiration. The automatic respiration rate analysis using ambulatory based single lead ECG signal [7], this ECG signal recording mainly based on the holter monitor, it describes the HRV based analysis of respiration rate. The HRV and ECG amplitude values based respiration rate is calculated [6]. The HRV features based the respiration rate is calculated [8].

III. ECG DATA PROCESSING

a) ECG Data Collection

The database collection is the one of the most important part in signal processing. In this paper, we have tested using Physionet Apnea-ECG Database [4]. This database contains totally 35 subjects sleep studies. The ECG signal recordings were visually scored by an expert for sleep apnea/hypopnea events on the basis of respiration on a per minute basis. This 35 subjects recordings (30 men, 5 women) were arranged in three groups: Group A recordings the 20 subjects with clear occurrence of sleep apnea (100 min or more), Group B (borderline) recordings (five subjects) with some degree of sleep apnea (between 5 and 99 min) and Group C (control) recordings 10 recordings of healthy subjects with no sleep apnea (fewer than 5 min).

Each apnea scoring record was divided into 1-min nonoverlapping segments. The each minute was classified as either a "nonapnea minute" or an "apnea minute" [5]. This minutes containing either apnea or hypopnea were classified as apnea minutes and also called as Apnea Index (AI) or Hypopnea Index (HI). AI is the number of apneas observed per hour and the HI is the number of hypopneas observed per hour. Apnea-hypopnea index (AHI) is defined as the sum of AI and HI. The single channel of ECG was extracted from all polysomnographic recordings, sampled at 100Hz with 16-bit resolution and 5- μ V A-D converter (ADC) gain per step. The standard sleep laboratory, modified lead V2 position ECG is used.

b) Preprocessing of ECG Signals

The ECG signal mainly contains different types of noise, like frequency interference, Baseline drift, electrode contact noise, polarization noise, muscle noise, the internal amplifier noise and motor artifacts. ECG artifacts are the noise induced to ECG signals that result from movements of the real time electrodes. The most common problems in ECG signal processing is baseline wander removal. The removal of baseline wander is required in the analysis of the real time ECG signal to minimize the changes in beat morphology. The respiration and electrode impedance changes due to respiration are important sources of baseline wander in most types of ECG recordings. The baseline wander of frequency content is usually in a range well below 0.5Hz. The baseline drift can be eliminated without changing or disturbing the characteristics of the waveform. Use the median filters (200-ms and 600-ms) to eliminate

baseline drift of ECG signal [2]. The process is as follows:

- The real time ECG signal is processed with a median filter of 200-ms width to remove QRS complexes and P waves
- Resulting signal is then processed with a median filter of 600-ms width to remove T waves. ECG signal resulting from the second filter operation contains the baseline of the ECG signal.
- Subtracting the filtered signal from the original signal and a signal with baseline drift can be obtained.

The before and after removal of baseline wander signal sample beats from record No. a01 of Apnea-ECG Database are shown in Fig.1 where X-axis represents number of samples and Y-axis indicates the amplitude of signal in mV.

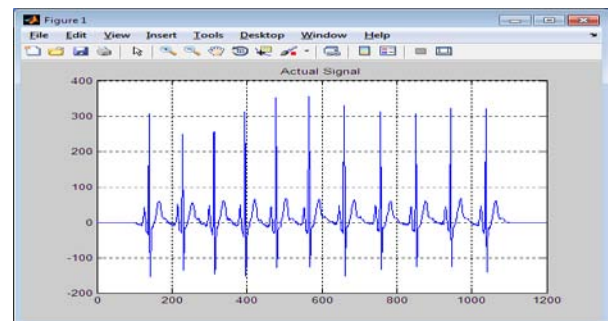


Figure 1(a) : Samples beats from record No.a01

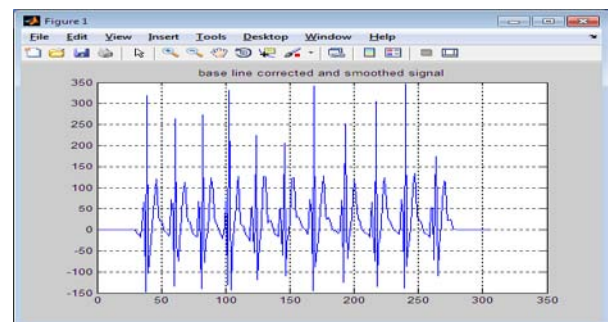


Figure 1(b) : Beats after removal of baseline wander

IV. WAVELET TECHNIQUE

The wavelet transform (WT) provides a new dimension to signal processing and event detection. The time-frequency localization properties, the wavelet transform is an efficient technique tool for analyzing non-stationary ECG signals. It provides a description of a signal in a timescale domain, analogous to a time-frequency domain, allowing the representation of temporal features at multiple resolutions. The WT is achieved by the decomposition of the signal over dilated (scale) and translated (time) versions of a prototype wavelet. The prototype wavelet function is Mother Wavelet function and it is used for the analytical

requirements. The Discrete Wavelet Transform (DWT) Mother wavelet function defined as:

$$\varphi_{a,b}(x) = \frac{1}{\sqrt{a}} \varphi\left(\frac{x-b}{a}\right) \quad a, b \in R, a > 0, \quad (1)$$

Where

a=coefficient of time translation,

b=coefficient of scale (compression),

X= It is the baseline wander noise removal ECG signal,

R= It is the wavelet space,

The DWT contains number of families like Haar, Daubechies, Biorthogonal, Coiflets, Symlets, Morlet, and Mexican Hat. The Daubechies (DB4) filter has been found to give details more accurately signal features [3]. This wavelet shows similarity with QRS complexes and energy spectrum is concentrated around low frequency noises. The DB4 filter contains number of levels based coefficient values, each level contain the wavelet function. The mother wavelet applied into the four levels of DB4 filter, it is used to calculate the features of the ECG signal. The Fig 2 has shown the coefficient levels of the DB4 filter. This coefficient level split into two type's approximation coefficient and detail coefficient. The detail level coefficient values are used to calculate the ECG feature value.

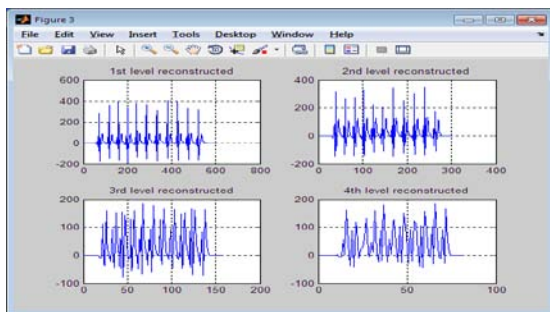


Figure 2 : The DB4 filter coefficient levels

a) ECG-Derived Respiration (EDR)

We evaluated EDR by two methods:

1. Based on the extraction of the R wave amplitude (RWA) after the subtraction of the ECG baseline.
2. Based on the calculation of the R wave duration (RWD)

b) R-Wave Amplitude (RWA)

The R-wave amplitude is calculated by using the five point derivative function. It gives the QRS-complex slope information [1]. The five point derivative with the transfer function

$$H(x) = (1/8T)(-x^{-2} - 2x^{-1} + 2x^1 + x^2) \quad (2)$$

The amplitude response is

$$|H(\omega T)| = (1/4T)[\sin(2\omega T) + 2\sin(\omega T)] \quad (3)$$

The equation (2) difference response is

$$y(nT) = (1/8T)[-z(nT - 2T) - 2z(nT - T) + 2z(nT + T) + z(nT + 2T)] \quad (4)$$

The frequency response of this derivative (4) gives the slope value of QRS-complex in linear format. The after differentiation process the signal is squared point by point. The equation of this operation is

$$y(nT) = [z(nT)]^2 \quad (5)$$

This function makes all data points positive and does nonlinear amplification of the output of the derivative emphasizing the higher frequencies.

c) R-wave time duration (RWD)

The R-peak time duration calculation based on the Moving window integration. It is to obtain wave from feature information in addition to the slope of the R wave, it is calculated from

$$y(nT) = (1/N)[z(nT - (N - 1)T) + z(nT - (N - 2)T) + \dots + z(nT)] \quad (6)$$

Where, N is the number of samples in the width of the integration window. The number of samples N is the important in the moving window. The width of the window should be approximately the same as the widest possible QRS complex. The window is too wide, the integration waveform will merge the QRS and T complexes several peaks in the integration waveform. This can cause difficulty in subsequent QRS detection processes. The width of the window is determined empirically. The QRS complex corresponds to the rising edge of the integration waveform. The duration of the rising edge is equal to the width of the QRS complex. The temporal location of the QRS complex can be determined from this rising edge according to the desired waveform feature to be marked such as the maximal slope or the peak of the R wave. Fig 3 shown the result of the RWD and RWA

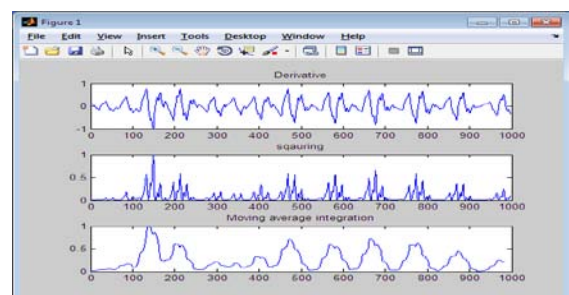
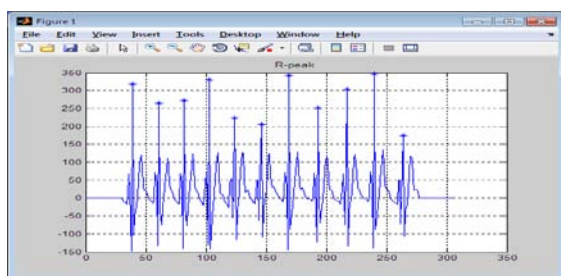


Figure 3(a) : Result of the derivative function, squaring function and moving window integration



Figur 3(b) : Thre R-peak marked value

d) *Heart rate variability (HRV)*

The Heart rate variability is calculated based on the RR-intervals. The RR-intervals RR_i were calculated as the difference between the times of successive R-peaks R_i . Next, the time series RR_i at the times R_i was transformed into an equidistant time series at a sampling rate of 10 Hz using discrete wavelet transform.

$$RR_i = RR_i + RR_{i+1} \quad (7)$$

Where $i=1 \dots N$, N is number of R-peak value.

e) *Features derived from HRV and EDR*

RR-interval is a distance of two successive top R-waves. The EDR is the combination of both Ramp and R-peak values. These values based number of feature parameters are calculated:

- Maximum respiration rate per minute of RR-interval and EDR
- Minimum respiration rate per minute of RR-interval and EDR
- Standard deviation for respiration rate per minute of RR-interval and EDR
- Mean for respiration rate per minute of RR-interval and EDR
- Root of mean of sum of squared difference of consecutive RR-intervals (RMSSD),
- Two pNN50 measures is defined as each NN50 measure divided by the total number of NN value
- The NN50 measure is defined as the number of pairs of adjacent NN where the second respiration rate exceeds the first respiration rate by more than 50 ms

V. RESULT

Figure 1(a) shows the 10-sec time duration of the ECG signal recording, it is acquire from the polysomnogram studies. The ECG signal common noise is removed from the median filter. The ECG signal features are HRV and EDR values are derived from the DWT function. The DB4 filter based decomposition levels remove other low frequency noise in the ECG signal (Figure 2). The ECG signal RWA calculate from the derivative and squaring function and the RWD values are analyzed using Moving widow integration. This R-peak value based RR-interval (HRV) value analyzed. This HRV and EDR values based obtain the respiration variability features are RMSSD, MeanRR,

MeanEDR, SDEDR, SDRR, MaxrateRR, MinrateRR, MaxrateEDR, MinrateEDR, pNN50 and NN50. Table 1 gives the Heart rate variability based Respiration Variability analysis features and Table 2 gives the EDR based Respiration variability examines features. This respiratory variability features gives input to the classification process, it provides the acceptable result. This RV values based sleep apnea disease is diagnosed.

VI. CONCLUSION

The information about HRV and EDR obtained is very useful for ECG classification, analysis, diagnosis, authentication and identification performance. The HRV can also serve as an input to a system that allows automatic cardiac diagnosis. The main advantage of this kind of detection is less time consumption compared to Holter monitor based ECG signal. Several techniques are used for respiration calculation. The discrete wavelet transform based respiration rate computation gives the precise values. This respiration variability is mainly used to diagnosis the sleep apnea disease. The future enhancement process we have apply this respiration variability features analysis the stress testing and Atrial fibrillation disease diagnosis process.

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Evaluation of Therapeutic Effect of a Polyherbal Formulation in Patients of Asthma with Different kind of Temperament

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Abstract – Asthma or Rabu (Bronchial asthma) is common in those people who have Balghami Mizaj (Phlegmatic temperament) as it is being described by unani physicians. It is treated by several herbo-mineral drugs, which have bronchodilator, mucolytic and expectorant properties. In a clinical trial carried out in Ajmal Khan Tibbiya College hospital during 2000 to 2003, the therapeutic response of a polyherbal formulation of Barg-e-aroosa, Gul-e- Zoofah and Aslus-soos, was found encouraging in patients having Balghami Mizaj(Phlegmatic temperament) while least response was observed in patients with saudavi Mizaj (Melancholic temperament).

The study shows that the concept of ancient Unani scholars about asthma is true and scientific.

Keywords : *asthma, balghami, temperament, thera-peutic and saudavi.*

GJMR Classification : *NLMC Code: WF 553*



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Evaluation of Therapeutic Effect of a Polyherbal Formulation in Patients of Asthma with Different kind of Temperament

Shoaib Ahmad^a, Abdul Mannan^σ & Izharul Hasan^p

Abstract - Asthma or Rabu (Bronchial asthma) is common in those people who have Balghami Mizaj (Phlegmatic temperament) as it is being described by unani physicians. It is treated by several herbo-mineral drugs, which have bronchodilator, mucolytic and expectorant properties. In a clinical trial carried out in Ajmal Khan Tibbiya College hospital during 2000 to 2003, the therapeutic response of a polyherbal formulation of Barg-e-aroosa, Gul-e- Zoofah and Aslus-soos, was found encouraging in patients having Balghami Mizaj(Phlegmatic temperament) while least response was observed in patients with saudavi Mizaj (Melancholic temperament).

The study shows that the concept of ancient Unani scholars about asthma is true and scientific.

Keywords : asthma, balghami, temperament, therapeutic and saudavi.

I. INTRODUCTION

Asthma is not a new disease. Mankind knows it since last 3500 years, where asthma like symptoms were recorded in an Egyptian Manuscript called Eberus Paperus. At present asthma has spread globally. The affected people belong to various age groups, but children and elders are more prone. An increasing prevalence and severity of asthma has been reported worldwide.^[1] Asthma affects 4% of the total world population.^[2] In 2003; 2.8 million people had been diagnosed with asthma in USA, and 11 million people experienced an attack of asthma in the previous year.^[3] In 2005; 32.4 million people had been diagnosed with asthma. About 4000- 5000 people die each year in America alone.^[3] Prevalence of asthma in Wales is among the highest in the World, with 260,000 people living with Asthma.^[4]

In India, recent report shows wide variation (4-19 %) in the prevalence of asthma in School going children from different geographic areas of India.^[5] There has been an increase in mortality as well, particularly in younger age groups.^[6] In the city of Bangalore the prevalence of asthma in 1979 and 1999 was 9 % and

29.5 % respectively.^[7] In Lucknow, prevalence of asthma is 2.3 % and 3.3 % in the children of age groups, 6-7 and 13- 14 years, respectively.^[8] In urban Delhi, prevalence of asthma in children is 11.6 %.^[9] Prevalence of asthma in rural children from Ludhiana^[10] was 2.6 % and from Punjab^[11] was 1%.

II. UNANI CONCEPT OF ASTHMA

Asthma is known as Rabu, Buhar, Zeeq-un-nafas and Intesabun-nafas.^[12-14] Asthma is defined in the books of almost every renowned unani scholars like Hippocrates (460 BC), Galen/ Jalinoos (130-200 AD), Rabban Tabri (810-875 AD), Razi (860- 932 AD), Ibne-sina (980-1037), and Ismail Jurjani (death 1140 AD). They believed that asthma is caused by Thick Phlegm (Ghaleez khilt or ghaleez Balgham) which is adhered on the bronchial mucosa to develop narrowing. They described that due to narrowing of bronchial lumen, air becomes incapable to enter into the lungs during inspiration, and to fulfill the deficit of air (O₂) the subject is compelled to breathe rapidly. The rapidity of the breathing depends upon the severity of the disease.^[15-19] Some Unani Scholars like, Majoosi, Jurjani and Rabban Tabri, described that asthma is caused by Barid and Raqeeq khilt (cold and dilute fluid). They believed that such fluid develops more severe form of breathlessness.^[18, 20, 21] Tabri AABM, stated that asthma is a name of thick fluid (Ghaleez Balgham) which is adhered on the inner layer of bronchioles and develops narrowing of the airways, resulting in hypoventilation of the lungs and ultimately breathlessness.^[22] Hussain MA, stated in his book, Moaleja-e-Nafissi that asthma is caused by concentrated and unhealthy cold fluids (Ghaliz wa barid ratoobat). He emphasized that fluids may be Balgham or sauda or both.^[23] The most scientific description regarding to etiology of asthma was given by Ali bin Abbas Majoosi(930- 994) and stated that asthma is caused by bronchospasm.^[18]

In Unani system of medicine treatment of the diseases of known etiology is based on administration of drugs having actions contrary to etiological changes i.e. known as Ilaj-Bil- Zid. According to this principal of treatment, unani scholars have designed various formulations comprising of drugs with hot and dry temperament for asthma which temperament is cold and moist.

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III. MATERIAL AND METHODS

Twenty five patients of asthma were selected from the OPD of Moalejat, Ajmal khan Tibbia College Hospital during 2000-2003. Patients with history of atopic, episodic asthma and additional allergic conditions such as rhinitis, dermatitis were included in this study. Patients having chronic bronchitis, bronchiectasis, tuberculosis, emphysema, pneumonia with asthma, pregnancy, patients with oxygen therapy receiving corticosteroids and patients with cor-pulmonale were not included in the study. Peak expiratory flow rate (PEFR) was recorded under dynamic condition before the start of the Study and at an interval of 2 weeks for six weeks. PEFR was measured using Vitilograph peak flow meter in sitting position. The data were tabulated and statistically analysed by applying paired t-test for the observations recorded during the study for evaluation of the significance of differences. For data analysis SPSS statistical software was used.

Determination of temperament is one of the most important aspects of Unani System of Medicine.

Each subject possesses a specific temperament. Occurrence of disease and effect of drugs used for treatment depend upon temperament of the patient. Temperament of the 25 patients was assessed on the basis of Proforma given in *table no. 1*.^[24]

In present study, Arusa (*Adhatoda vasica* Nees.), Zoofa (*Hyssopus officinalis* Linn.) and Aslus-soos (*Glycyrrhiza glabra* Linn.) were given @ 6 gram each 12 hourly in the form of decoction. *Adhatoda vasica* Nees., is a potent bronchodilator due to presence of vasicine and vasicinone alkaloids.^[25-30] It has anti-histaminic activity also and the leaves of Arusa are extensively used in whopping cough, bronchitis, asthma and urticaria.^[31-33] *Glycyrrhiza glabra* Linn., has strong anti-histaminic, bronchodilating and expectorant properties.^[32-34] *Hyssopus officinalis* Linn., is an excellent anti-inflammatory and expectorating agent.^[35,36] Temperament of Arusa (*Adhatoda vasica* Nees), Aslus-soos (*Glycyrrhiza glabra* Linn.) and Zoofa (*Hyssopus officinalis* Linn.) is Hot and Dry.^[37,38]

Table 1 : Showing parameters for determination of temperament

Parameters for temperament	Damvi (Hot & Moist)	Balghami (Cold & Moist)	Safravi (Hot & Dry)	Saudavi (Cold & Dry)
1. Body appearance	Muscular, Robust veins are prominent	Lose musculature, fatty/obese look excess fat ,not prominent	Not thick, average/less musculature	Thin, emaciated weak musculature
2. Feel of body	Tough glazed & hot	Flabby ,soft loose & cold	Dry hard and hot	Hard & rough and Cold
3. Complexion of body	Brown, Reddish	Dusky	Pale	Dark, Purple
4. Type & colour of hair on the body	Thick dense but slow growth, black & Glazed	Thin & less but slow growth Brownish thick	Thick & dense but fast growth thin black and curly	Thick & scanty but fast growth brownish & thin
5. pulse	Full & strong	Slow, feeble, sluggish	Rapid regular	Wide & soft
6. Functions of the body	Reaction time fast	Reaction time slow, Slow movement	Excessive movement	Sense of anxiety, wake fullness
7. Mental alertness.	Enthusiastic and prepared	Dull, sluggish	Short tempered	Deep thinking
8. Behavior	Angry soon	Cool, calm	Irritative	Different from fellow beings
9. Thirst.	Normal	Less	Increase	False
10. Appetite	Normal	Less	Increase	Decrease
11. Sleep	Sound	Excessive	Less	Less & Disturbed
12. Urine colour	Conc. & reddish	Colourless white	Dilute pale	Smoky dark
13. Dreams	Occasional red article	White article, lake, water	Fire, Red & yellow articles	Fearful
14. Sexual desire.	Excessive but controlled	Occasional but controlled	Uncontrolled	False & confused
15. Additional.	Epistaxis, like cold climate	Like hot climate	Dry & rough tongue, nauseating feeling, likes cold climate	Heart burn, dark circle around eyes likes hot climate and things

IV. RESULTS

In present study, 25 patients were selected. Their mean age (year) was 30 (SD= 10) and mean weight (kg) was 49 (SD =12). Out of 25 patients, there were 17 (68 %) male and 8 (32 %) female (*Graph- 1*). Out of 25 patients, 24 (96 %) patients had positive history of allergy and only 16 (64 %) patient had positive family history of asthma (*Graph-2*). Patients were also categorized according to temperament. The 25 patients who were categorized in this manner included Balghami (Phlegmatic) 16 (64 %), Damvi (Sanguine) 4 (16 %), safravi (Bilious) 3 (12 %) and of Saudavi (Melancholic) 2 (8 %) temperaments (*Graph- 3*).

During the follow ups PEFR was performed and change in parameter was assessed. Increase in the value of PEFR on 15th day, 30th day, and 45th day as compared to 0 day and symptomatic relief in the condition of patient was taken as improvement.

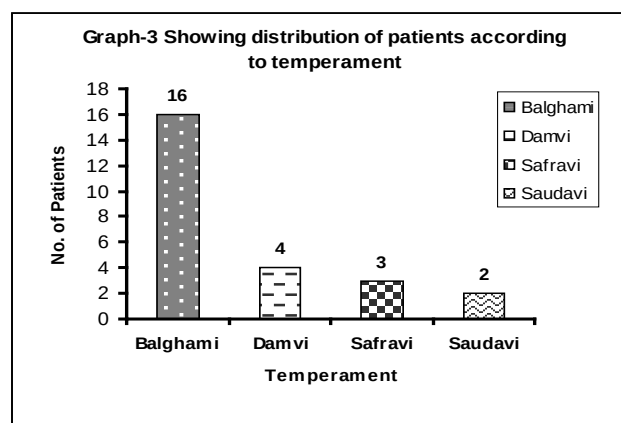
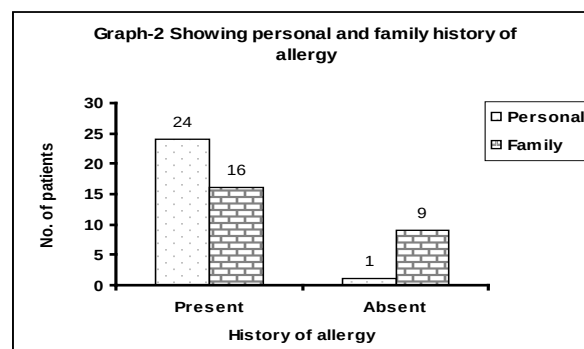
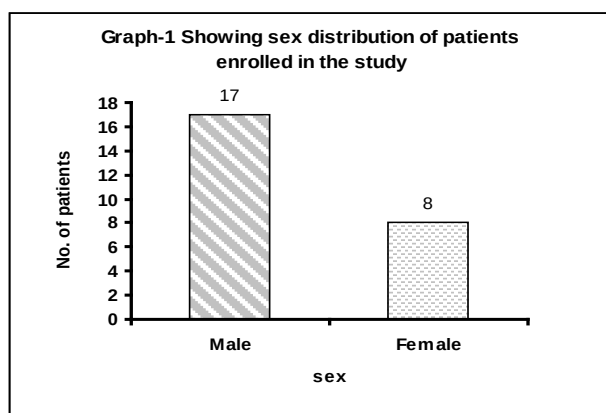


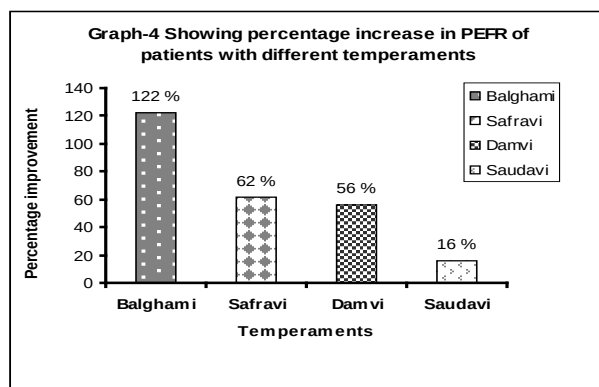
Table 2 : Effect of Drugs on Peak Expiratory Flow Rate (lt/min) in whole study and in patients with different temperaments

Effect on	0-Day	15th Day	30th Day	45th Day	t-value and p-value
Whole study	205 ± 73	257 ± 79	309 ± 76	378 ± 70	t=15.1 p=0.000
Balghami	181± 69	236 ± 76	300 ± 75	380 ± 69	t=23.1 p=0.000
Damvi	250 ± 61	297 ± 68	350 ± 63	391 ± 52	t=6.6 p=0.007
Safravi	257 ± 84	332 ± 84	363 ± 55	418 ± 51	t= 8.4 p=0.14
Saudavi	235 ±71	245 ± 64	215 ± 35	268 ± 53	t= 3 p= 0.02

To assess the percent increase in PEFR, first percent increase was calculated for individual patients on 15th, 30th and 45th day, then mean percent increase was calculated by dividing the sum by number of patients on 15th, 30th and 45th day respectively. It was found that maximum increase in PEFR on 45th day in all patients was 85 %. When paired t-test was applied to PEFR recorded during the study. It was found to be highly significant (p= 0.000) throughout.

Percent increase in PEFR according to temperament was also calculated. It was found that maximum percent improvement in PEFR was in Balghami (Phlegmatic) temperament i.e. 122 % and lowest was in Saudavi (Melancholic) temperament i.e. 16 % as shown in *graph 4*. When paired t-test was applied to PEFR according to temperament, it was found to be highly significant (p=0.000) in Balghami

(Phlegmatic), significant in Damvi (Sanguine, $P=0.007$) and insignificant in Safravi (Bilious, $P=0.14$) and Saudavi (Melancholic, $p=0.20$) temperaments (table-2).



Patients were also assessed for any adverse effect of test drugs. For that purpose, liver function test, renal function test and haemoglobin percentage were measured before and after the study. After study it was found that there was no significant change in these parameters which indicates that the test drugs are safe.

V. DISCUSSION

Asthma is a global phenomenon. In children even though genetic predisposition is one of the factors responsible for increased prevalence, urbanization, air

pollution and environmental tobacco smoke contribute more significantly. Our results show significant improvement in PEFR as a whole and in patients with different temperament. As the temperament classification is not scientifically proved, it is difficult to determine accurate temperament of subjects. At present, it is also difficult to explain pharmacological intervention leading to unequal improvement in illness of patients with different temperament. The medicinal plants, used in this study, have hot and dry temperament of first degree except Zoofa (*H. officinalis* Linn.) which has third degree hot and dry temperament. According to basic principal of management of illness in Unani System of Medicine, only patients with Balghami (Phlegmatic) temperament should respond well. It is because; their temperament is cold and moist, which is similar to the temperament of illness and absolutely contrary to the temperament of drugs.

Our study also indicates that certain medicinal plants used in unani medicine could be better alternative of conventional drugs. As well as significant improvement in PEFR, present treatment was also associated with significant improvement in anxiety, insomnia, frequency of dyspnoea, rate of respiration and rhonchi (tab-3). So the quality of life also shows improvement with present treatment.

Table 3 : Showing improvement in different parameters

	Before Treatment	After Treatment	
Parameters	Present in No. of Patients/Frequency	Present in No. of Patients/Frequency	Percentage Improvement
1. Anxiety	21	04	81 %
2. Rhonchi	25	22	88 %
3. Insomnia	18	6	67 %
4. Rate of respiration	25 ± 3	19 ± 01	24 %
5. Frequency of dyspnoea	6 ± 3	1 ± 1	83 %

VI. CONCLUSION

The study concludes that polyherbal formulation used in present study, exhibits significant effect on patients with asthma and may be a better alternative to present conventional drugs used for the treatment of all kinds of asthma. The study also concludes that, to establish scientific correlation between temperaments of the disease the subjects and also that of the drugs, a multidisciplinary clinical trial should be initiated.

VII. ACKNOWLEDGEMENT

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Immunization Status among Government Urdu Higher Primary Schools of Azad Nagar and its Surrounding Area, Bangalore

By Izhar-UI Hasan, Mohd Zulkifle & A.H Ansari

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Abstract – Background: Children are the future of the country. In India, children under 15 years of age constitute about 40 percent of the population. School children constitute a large pool of children of this age group. Immunization forms a critical component of primary health care, and ensures nation's health security. Although international agencies such as the World Health Organization (WHO), the United Nations Children's Fund (UNICEF) and now the Global Alliance for Vaccines and Immunization (GAVI) provide extensive support for immunization activities, the success of an immunization program in any country depends more upon local realities and national policies. **Objectives:** To assess the immunization status of government Urdu higher primary schools in Azad Nagar and its surrounding area, Bangalore. **Methodology:** The present study was one time cross sectional, conducted in the three government Urdu higher primary schools of Azad Nagar and its surrounding area. Total 500 children were included in the study. **Results:** In the present study, distribution of school children according to immunization status revealed that out of 500 children 386(77.20%) were completely immunized, 32(6.40%) were incompletely immunized and 82(16.40%) children had no information about the immunization. **Interpretation and Conclusion:** In the present study, immunization status was found highly related to socio economic status and parent's educational status. Main emphasis may be given towards immunization education, personal hygiene education, health education apart from the regular educational activities in the community.

Keywords : immunization status; school children; health education.

GJMR Classification : NLMC Code: WA 115



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Izhar-UI Hasan^α, Mohd Zulkifle^σ & A.H Ansari^σ

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I. INTRODUCTION

Disease prevention is the key to public health. It is always better to prevent a disease than to treat it. Vaccines prevent disease in the people who receive them and protect those who come into contact with unvaccinated individuals. Vaccines help prevent infectious diseases and save lives. Parents are constantly concerned about the health and safety of their children and take many steps to protect them. These steps range from child-proof door latches to child safety seats. In the same way, vaccines work to protect infants, children, and adults from illnesses and death caused by infectious diseases. Even diseases that have been eliminated in this country, such as polio, are only a

plane ride away. Polio, and other infectious diseases, can be passed on to people who are not protected by vaccines. If a child is not vaccinated and is exposed to a disease germ, the child's body may not be strong enough to fight the disease. Before vaccines, many children died from diseases that vaccines now prevent, such as whooping cough, measles, and polio (Education.com/immunization).

At national level, 61% of the children aged 12-23 months have received full immunization. The coverage of immunization was higher in urban areas (67.4%) compared to that in the rural areas (58.5%). It is a matter of concern that, nearly 8% children did not receive even a single vaccine. Nearly 62% of the male children aged 12- 23 months have received full immunization, while among the females it was nearly 60%. It is shocking to note that, the birth order of the child still continues to affect the immunization coverage (Coverage Evaluation Survey 2009).

Of all the things protect children; immunization may be the most important. Explore all the wonderful reasons why choosing to immunization of children is the best choice for their health and the health of our community.

II. METHODOLOGY

One time observational cross sectional study of Urdu higher primary school children was conducted to know the immunization status in Azad Nagar govt. school children, Bangalore, India. Universal immunization policy in India includes BCG vaccination at the time of birth or in case of deliveries at home, at first contact with a health professional. Previous studies have reported moderate coverage of BCG vaccination in the Indian population (Bhatia V 2004 and Balraj V 1993). Total strength of children 500 were included for the study.

Study Period

January 2010- December 2010

a) Immunization Status

The immunization status of children was assessed by examining the immunizations cards or receipts of the vaccination done. BCG scar was specially examined. The children were instructed to

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bring the immunization records before the day of examination. The children were classified into three categories based on immunization.

- Completely immunized children:** The children who have received BCG (1), DPT (3), OPV (3), measles (1), OPV and DPT booster vaccinations were defined as complete immunized children.
- Incompletely immunized children:** The children who have missed any one or more of the BCG (1), DPT (3), OPV (3), measles (1), OPV and DPT booster vaccinations were defined as incompletely immunized children.
- No information:** The children who failed to bring the immunization cards or receipts were included in this category.

b) *Personal hygiene status*

The hygiene status was assessed by interviewing and observing the children. The children were grouped under three categories; good, fair and poor.

c) *Literacy of the parents*

The data related to the literacy of both the parents was assessed to know the educational status and the socioeconomic status of the family.

d) *Socioeconomic status (SES)*

The SES was assessed by using the Kuppuswami's SES Scale for Urban population, 1976. Due to changes in the economy to year, the classification or scale was modified accordingly (Kumar N *et al* 2007).

e) *Data Analysis*

Data were tabulated in a systematic way for presentation and analysis on the basis of recorded

parameter regarding demographic and immunization profiles. Studied children were classified into different relevant categories. Then data was entered in windows excel and analysis was done for mean and standard deviations. Statistical tests like t test and chi square test were applied.

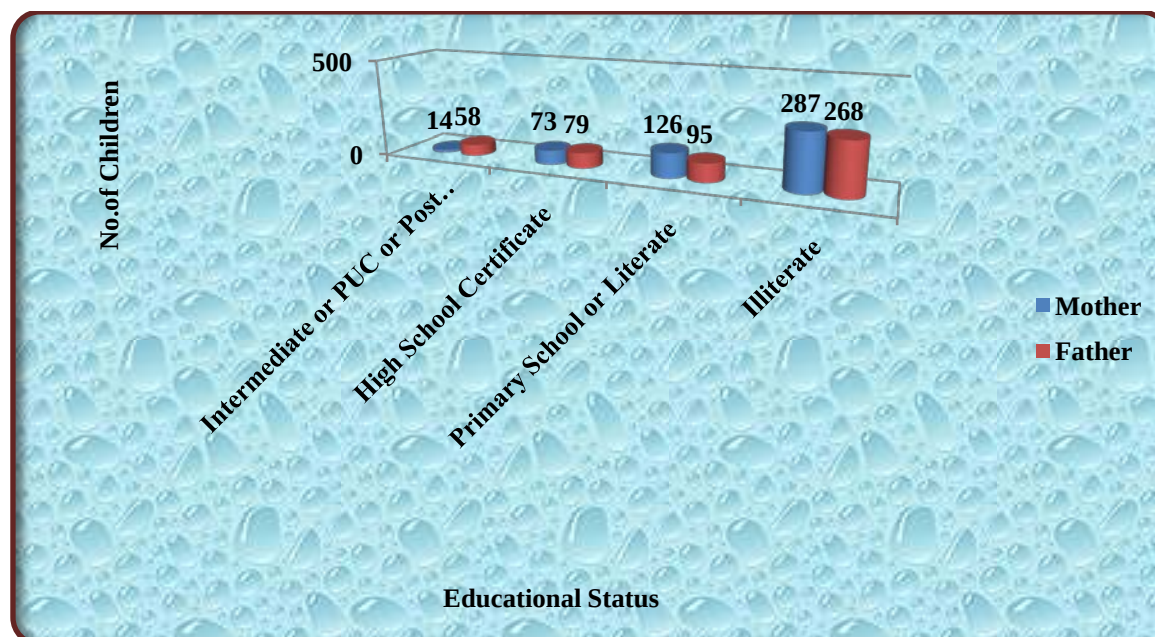
III. RESULTS AND DISCUSSION

a) *Parent's educational status*

In the present study 73(14.60%) children had their mother education up to High School, 14(2.80%) children had up to Intermediate, 126(25.20%) had up to primary school, and 287(57.40%) had illiterate mothers. In the study 58(11.60%) children had their father education up to Intermediate or PUC or Post High School diploma, 79(15.80%) had up to High School, 95(19.00%) had up to primary school and 268(53.60%) had illiterate fathers.

The number of children who had illiterate mother was more as compared to children having illiterate fathers (57.40% Vs 53.60%). The illiteracy in the mothers was more than illiteracy in fathers. This may be due to the population in the study area showing less interest in female education in comparison to male education. As per the report of the Directorate of Economics and Statistics, Bangalore (2000-2001), the female literacy rate was less as compared to male literacy rate in Bangalore (77% Vs 88%). In present study literacy rate was lower than reported. This may be because of lower, middle socioeconomic status of the under study population area Azad Nagar. The results are summarized in Figure N0.1.

Figure 1 : School Children Parent's Educational Status



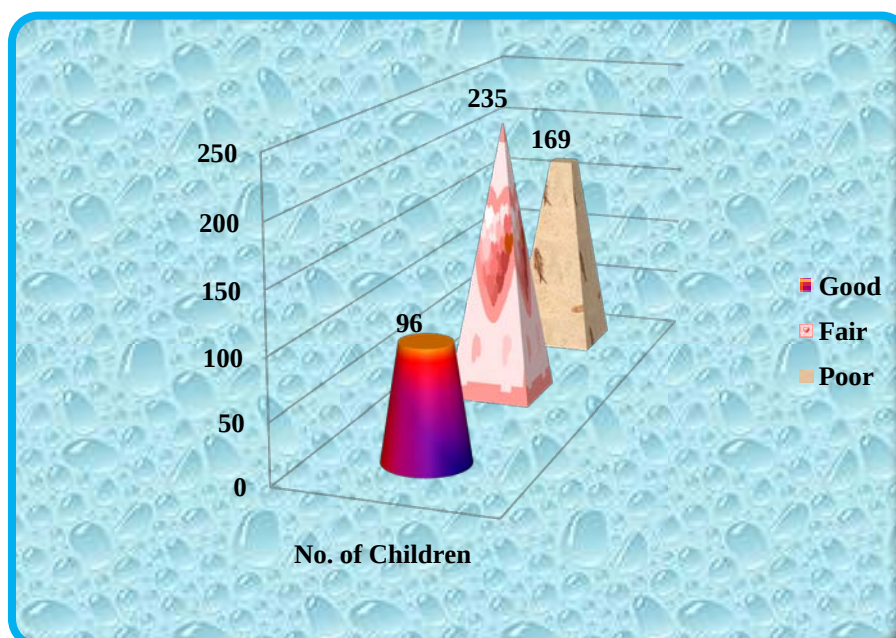
b) Personal hygiene status

Out of total 500 children in the study 96(19.20%) had good personal hygiene status, 235(47.00%) children had fair personal hygiene status and 169(33.80%) children had poor personal hygiene status. Maximum number, 235(47.00%) of children had fair personal hygiene status and minimum number, 96(19.20%) of children had good personal hygiene status.

A study conducted by Sundaram MV *et al* in Madras city reported that 28.7% school children had poor personal hygiene status (Sundaram MV *et al* 1978).

The percentage of the children with poor personal hygiene status in the present study was more in comparison to the above study. The difference may be because the present study included only the children of Government schools whereas the above study included both Government and Corporate/ Private schools children. The SES of the parents in both studies may vary widely or SES gap of the parents may be wide. SES of parents is closely related to the personal hygiene status of children, as this is more related to sensitization towards personal cleanliness. The results are summarized in Figure N0.2.

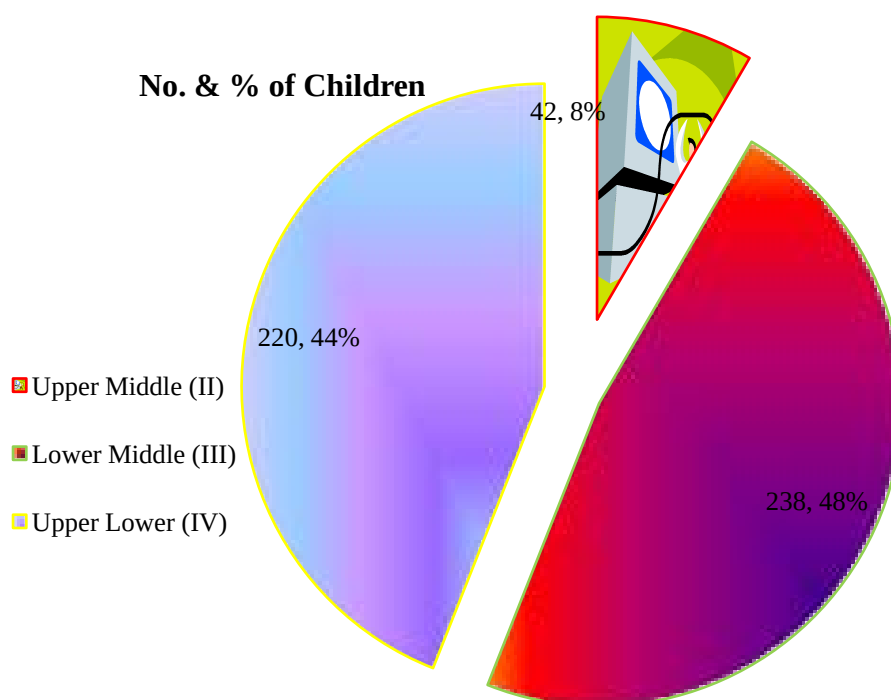
Figure 2 : Personal Hygiene Status among School Children



c) Socioeconomic Status (SES)

Out of total 500 children in the study 42(8.40%) children were belonging to upper middle (2) SES, 238(47.60%) children were belonging to lower middle (3) SES, and 220(44.00%) children were belonging to upper lower (4) SES. Maximum numbers, 238(47.60%) children were belonging to lower middle (3) SES. This can be attributed to lower educational and occupational status of parents, especially of fathers' of under study children. The results are summarized in Figure N0.3.

Figure 3 : School Children parent's Socioeconomic Status



d) Immunization Status

In the present study, distribution of school children according to immunization status revealed that out of 500 children 386(77.20%) were completely immunized, 32(6.40%) were incompletely immunized and 82(16.40%) children had no information about the immunization.

Chowti JV *et al* in their study in school children in Hubli and Dharwad cities reported that 72.24% children were completely immunized (Chowti JV *et al* 1995).

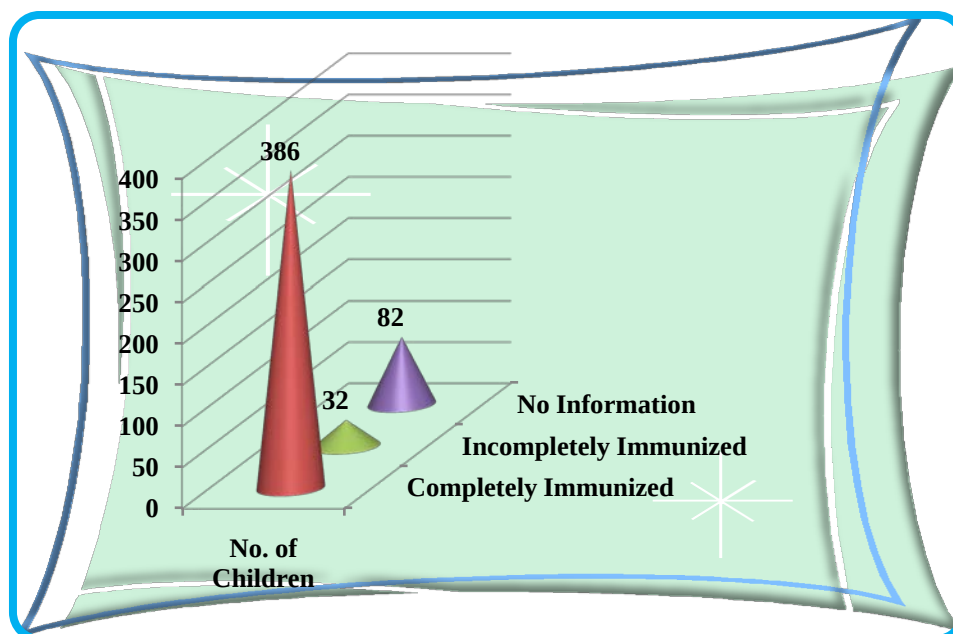
As per Coverage Evaluation Survey, 2009, at national level, 61% of the children aged 12-23 months have received full immunization. The coverage of immunization was higher in urban areas (67.4%) compared to that in the rural areas (58.5%). In 2009, nearly 8% children of the children aged 12-23 months did not receive even a single vaccine. Nearly 62% of the male children aged 12-23 months have received full immunization, while among the females it was nearly 60%.

The full immunization coverage of children age 12- 23 months of mother's education with 12 or more years is 76.6% whereas for mothers who had no education only 45.3% of children got full immunization.

The percentage of completely immunized children in present study was more as compared to the children of the above study. This may be due to awareness about the importance of the immunization and better

immunization facilities in Bangalore as compared to Hubli and Dharwad cities. The results are summarized in Figure N0.4.

Figure 4 : Immunization Status among School Children



IV. CONCLUSION

Parent's education and socio economic status has a significant role in ensuring full immunization coverage to their children. All children should be immunized at regular health care visits, beginning at birth immunizations are very important in keeping children healthy. The recommended childhood and adolescent schedule urges shots starting at birth and going through 24 months of age, with boosters and catch-up vaccines continuing through the teenage years. By immunizing, we safeguard our children against the potentially devastating effects of vaccine preventable diseases.

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



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

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

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

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

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

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

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

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

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

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

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

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

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

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

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



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

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

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

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

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

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

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

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

INFORMAL GUIDELINES OF RESEARCH PAPER WRITING

Key points to remember:

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

Final Points:

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

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



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

General style:

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

To make a paper clear

- Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

- Use standard writing style including articles ("a", "the," etc.)
- Keep on paying attention on the research topic of the paper
- Use paragraphs to split each significant point (excluding for the abstract)
- Align the primary line of each section
- Present your points in sound order
- Use present tense to report well accepted
- Use past tense to describe specific results
- Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives
- Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

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



Abstract:

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

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

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

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

Approach:

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

Introduction:

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

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

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.



- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
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Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

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

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

Approach:

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

What to keep away from

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

Results:

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

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



Content

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

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

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

Figures and tables

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

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

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

Approach:

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



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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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