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A Study on Hypertension in School Children of Chitradurga District, Karnataka

By Yuvaraj. B.Y, Nagendra Gowda M. R, Rajeev. K.H, Prashanth Kumar. J. H, Santhosh Ujjanappa & Shreyas. M

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Abstract- *Background:* The hypertension in children need not be secondary. The essential hypertension is also increasing in trend. The available literature suggests that there is wide variation in the prevalence of hypertension. This study was taken up with the aim of studying the prevalence and factors responsible for hypertension in school children.

Material and Methods: A cross sectional study was conducted to study the prevalence of hypertension in 1,734 school children in Chitradurga district from June 2011 to August 2011. A detailed history was taken in a predesigned proforma. The data thus obtained was compiled and analyzed.

Results: The prevalence of hypertension in children in this study was 2.5% and more in females compared to males. The prevalence was higher after 10 years of age. Family history was one of the important risk factor for hypertension in children. The prevalence was also higher in children with higher BMI percentiles.

Conclusion: Hypertension is not only common in children but also a neglected entity in children. This calls for urgent attention for the policy makers to begin the preventive measures as early as possible in the life to prevent the co morbidities.

GJMR-K Classification : *FOR Code: WC 750, QW 4*

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Strictly as per the compliance and regulations of:

A Study on Hypertension in School Children of Chitradurga District, Karnataka

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I. Introduction

Being an interesting disease entity of its own, hypertension remains silent, generally asymptomatic during its natural course. Since most of hypertensives remain asymptomatic, the disease does immense harm to the body in the form of "Target Organ" damage; hence, the WHO has named it after 'Silent Killer'[1].

The belief that the essential hypertension is rare compared to secondary hypertension in children is obsolete now. Due to epidemiological transition, hypertension is also increasing in trend in pediatric population[2]. Evidence of progression of childhood hypertension to adult hypertension is documented by some studies in the western world[3]. It has been also shown that the familial aggregation of blood pressure starts from first week of life[4]. The childhood hypertension is amenable to pharmacological or non pharmacological intervention at earliest possible point of time. Such treatment can prevent the development of adult hypertension and its sequel.

By 2025, the number of people with hypertension will increase by about 60% to a total of 1.56 billion as the proportion of elderly people will increase significantly[4]. This prediction serves as the most important matter of concern for the early intervention in the childhood to tackle this fatal disease. Other reasons are the continuing population increase and changes in lifestyle, which includes a diet rich in sugar and high-fat processed foods and sedentary behavior, mediated by televisions, computers and cars.

There is a wide variation in the prevalence of hypertension in Childern[5, 6, 7]. The prevalence of Childhood hypertension varies from 1% to 16.2%. The diversity in prevalence of Childhood hypertension may be due to varying cut off blood pressure levels by various authors. Hypertension in children is influenced by various risk factors, both modifiable and non modifiable. i.e., Age, Ethnicity, Family history of hypertension and childhood obesity. Some studies have also shown that hypertension tends to increase with increase in age and with no sexual predilection.

The prevalence of hypertension in children amongst Indian studies varies between 6.6 %[8] to less than 1 %[9]. The prevalence may be high in South India, because of increased genetic inheritance secondary to more number of consanguineous marriages in south India, and also due to altered dietary habits and life style[10,11].

The present study was undertaken with the aim to find out the prevalence of hypertension amongst apparently healthy, asymptomatic school children of 9 – 16 years age from few government and private schools of Chitradurga district.

II. Materials and Methods

A cross sectional study was undertaken in the children of ten schools of Chitradurga district. A total of 1,734 students were included as samples in a period from June 2011 to August 2011. A detailed history with particulars of age, sex, history of illness suggestive of

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renal diseases, family history of hypertension i.e., hypertensive status of father, mother and grandparents if they are alive, was taken in a predesigned proforma.

Height and weight was recorded in all children by standard techniques as described by Indian Council of Medical Research and the percentile between weight and height was calculated.

Blood pressure was recorded using a standard mercury Sphygmomanometer (Diamond deluxe). In children above 7 years of age standard adult size cuff (12.5 cm) was used, except in a few children who requires 7.5 cm cuff. This was conducted according to the recommendations made by Moss that a cuff which covers approximately two-thirds of upper arm with enough space left over the cubital fossa to place the diaphragm of stethoscope to be employed in the measurement of blood pressure in children[9].

Before recording the blood pressure, children in groups of 10 were taken to a separate room away from noise, and they were explained in detail, the procedure of pressure recording and were reassured that the procedure was not painful. All children were made to void urine before blood pressure recording, as a full bladder is a source of strong sensory stimuli, which may increase the blood pressure. All efforts were made to eliminate factors which might affect the blood pressure such as anxiety, fear, crying, laughing, recent activities in order to facilitate the blood pressure recording under simulated "basal" or "near basal" conditions. Blood pressure was recorded only when the child had become accustomed to the observer, instrument and surroundings.

Blood pressure recording were taken as recommended by 1987 Second Task Force on blood pressure control in children. Blood pressure was recorded in sitting position and right arm. After applying appropriate size cuff, it was inflated to about 30 mm Hg above the point at which the radial pulse disappears. The pressure within the cuff was then released at a rate of about 2 to 3 mm Hg per second, while the auscultation was done over brachial artery. The onset of sound was taken as the systolic pressure (Korotkoff Phase I) and the absence of all sounds (Korotkoff Phase V) was taken as diastolic blood pressure. Three readings were taken in succession with an interval of 1½ to 2 minutes and the cuff was completely deflated between the readings. In those cases where the difference between first reading and third reading was more than 10 mm Hg, the first reading was omitted and another recording was obtained. The average of the three readings was calculated and entered in the proforma. Those children in whom blood pressure could not be recorded satisfactorily were omitted from the study. Those children in whom blood pressure was found to be abnormal for his/her age, were re-examined on two different occasions at an interval of 1 to 2 weeks and blood pressure was recorded in lying posture also.

All blood pressure recordings were taken on the same time of the day, i.e. during afternoon hours and recorded by the same person. Blood pressure was recorded after completing all other procedures.

The children were labelled as hypertensive, if the blood pressure was above the 95th percentile for that age, sex and height. In the hypertensive children, the readings were confirmed by recording blood pressure in both upper limbs[8].

The data thus collected was tabulated according to various epidemiological parameters like age, sex, anthropometry etc. Appropriate statistical tests were used to refute the statistical evidence by using Statistical Package for Social Services (vs 18).

III. Results

Table 1 : Prevalence of hypertension according to age

Age in years	Non hypertensives n (%)	Hypertensives n (%)	Total n (%)
9	2 (100)	0	2 (0.1)
10	55 (94.8)	3 (5.2)	58 (11.8)
11	201 (98.5)	3 (1.5)	204 (11.8)
12	322 (97.3)	9 (2.7)	331 (19.1)
13	456 (98.5)	7 (1.5)	463 (26.7)
14	381 (98.2)	7 (1.8)	388 (22.4)
15	153 (95.0)	8 (5.0)	161 (9.3)
16	120 (94.5)	7 (5.5)	127 (7.3)
Total	1690 (97.5)	44 (2.5)	1734 (100)

Above table shows the age and sex-wise distribution of childhood hypertension. A total of 1734 children were surveyed for hypertension. Among the surveyed children 2.5% had hypertension. The prevalence of hypertension was high in 10 years, 15 and 16 years children.

Table 2 : Age and Sex wise prevalence of Childhood hypertension

Age in years	Female			Male		
	Non Hypertensives n (%)	Hypertensives n (%)	Total n (%)	Non Hypertensives n (%)	Hypertensives n (%)	Total n(%)
9	2 (100)	0	2 (0.3)	0	0	0
10	42 (93.3)	3 (6.7)	45 (9.7)	13 (100)	0	13 (1.3)
11	69 (95.8)	3 (4.2)	72 (18.4)	132 (100)	0	132 (13.3)
12	128 (93.4)	9 (6.6)	137 (27.1)	194 (100)	0	194 (19.6)
13	197 (97.5)	5 (2.5)	202 (27.1)	259 (99.2)	2 (0.8)	261 (26.4)
14	148 (98.0)	3 (2.0)	151 (20.3)	233 (98.3)	4 (1.7)	237 (24.0)
15	76 (93.8)	5 (6.2)	81 (10.9)	77 (96.3)	3 (3.8)	80 (8.1)
16	52 (94.5)	3 (5.5)	55 (7.4)	68 (94.4)	4 (5.6)	72 (7.3)
Total	349 (95.8)	31 (4.2)	745 (100)	976 (98.7)	13 (1.3)	989 (100)

Females were more hypertensives than males. About 4.2% of the females and 1.3% of the male children were hypertensives. Female children of 15 years and more had higher prevalence compared to children of less than 14 years.

Table 3 : Prevalence of hypertension by BMI percentile

BMI Percentile	Female		Male	
	Non Hypertensives n (%)	Hypertensives n (%)	Non Hypertensives n (%)	Hypertensives n (%)
< 5th	79 (96.3)	3 (3.7)	92 (100)	0
5th	73 (97.3)	2 (2.7)	93 (98.9)	1 (1.1)
10th	151 (97.4)	4 (2.6)	207 (100)	0
25th	188 (94.0)	12 (6.0)	232 (98.7)	2 (1.3)
50th	115 (97.5)	3 (2.5)	93 (98.9)	3 (1.7)
75th	39 (90.7)	4 (9.3)	83 (95.4)	4 (4.6)
90th	41 (97.6)	1 (2.4)	68 (97.1)	2 (2.9)
> 95th	28 (93.3)	2 (6.7)	27 (100)	0
Total	714 (95.8)	31 (4.2)	976 (98.7)	13 (1.3)

The prevalence of hypertension was high for female children who had 25th, 75th and more than 95th BMI percentiles. In male children the prevalence was high for those children with 75th and 90th BMI percentiles.

Table 4 : Prevalence of hypertension according to family history

Family history	Non Hypertensives n (%)	Hypertensives n (%)	Total n (%)
No family history	1405 (97.8)	31 (2.2)	1436 (82.8)
Hypertensive grand parents	4 (3.1)	4 (3.1)	128 (7.4)
Hypertensive parents	161 (94.7)	9 (5.3)	170 (9.8)

Family history of hypertension is one of the important risk factor for hypertension. Table no 5 shows the family history of hypertension. About 82.8% of the children had no family history of hypertension. About 7.4% of the children had history of hypertension in grandparents and in 9.8%, the parents had history of family history of hypertension.

Table 5 : Regression analysis of the risk factors of hypertension in children

Coefficients

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	-.100	.038		-2.629	.009
	Age	.004	.003	.037	1.526	.127
	Sex	.027	.008	.085	3.541	.000
	BMI	.002	.001	.043	1.771	.077
	Family history	.014	.006	.054	2.275	.023

a. Dependent Variable: Hypertension

The regression analysis of some risk factors of hypertension had shown that, Sex and Family history of hypertension were significant at 0.05 levels.

IV. Discussion

The determination of prevalence of hypertension helps not in study of variation of blood pressure form one community to other but also helps in delineation of population at risk. Early identification of hypertension helps in translation into early intervention and thus modification of the natural course of it to decrease the mortality and morbidity.[12]

The prevalence of hypertension in study population was 2.5%. The prevalence was high in children of 10, 15 and 16 years. Female children of 15 years and more had higher prevalence compared to their counterparts. The prevalence of hypertension in our study is in accordance with other studies ranging from 0.41 to 5.9 %[7, 8, 11, 12, 13, 14]. The prevalence was much lesser than a large sample survey in Pakistan which was reported to be 12.2%. However different studies used different criteria for defining the hypertension. Few studies were available for the comparison as suggested by Joint National Committee criteria for evaluation of blood pressure in children. The regional differences in prevalence of hypertension cannot be ruled out as Krishna et al reported higher systolic blood pressure in North Indian girls.[16]

Girls were more hypertensives than boys. About 4.2% of the females had hypertension in comparison with 1.3% of the male children. Similar to these findings in a study in Shimla, Sharma et al also observed a more prevalence in girls than boys.[12]

The association of obesity with hypertension in children has been demonstrated by many studies across India and world.[2, 5, 12, 17] In this study the prevalence of hypertension was high in female children of 25th, 75th and more than 95th BMI percentiles. In male children the prevalence was high for those children with 75th and 90th BMI percentiles. But the regression analysis in this study had shown no association with BMI. In a study by Sharma et al had found that the hypertension was common in the children who had high BMI and Pre hypertension in the children with low BMI.[12]

Family history of hypertension is one of the important risk factor for hypertension. About 82.8% of the children had no family history of hypertension. About 7.4% of the children had history of hypertension in grandparents and in 9.8%, the parents had history of family history of hypertension. The regression analysis also had shown an association between the hypertension and Family history.

Hypertension in children is influenced by various risk factors, both modifiable and non modifiable. i.e., Age, Ethnicity, Family history of hypertension and childhood obesity. Some studies have also shown that hypertension tends to increase with increase in age and with no sexual predilection[5]. A study by Larioia has shown that there will be spurts in blood pressure levels in 11-13 year boys and 12-14 year girls[6].

To conclude, this study has shown that the hypertension is not only the disease entity of adults but also children. This calls for urgent attention for the policy makers that the co morbidities of the hypertension can be prevented from childhood itself. But the hypertension can be essential or also secondary to a physical entity in the body. Hence all the hypertensive children should be followed thereon to prevent the progression. The limitations of this study are worth to mention. This study was not conducted systematically or adjusted for factors such as salt intake, physical activity and dietary habits. Findings of the present study suggest a need for larger population based studies to accurately estimate the prevalence hypertension among children in our country.

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Differently Abled Children Striving to Lead a Normal Life - What Program Managers Can Do?

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Abstract- As per World Health Organization estimates more than a billion people live with some form of disability worldwide. Disability casts a significant impact in the development of a disabled child and a constant struggle for the families as well. Multiple political, health care delivery system and social determinants have been identified which have limited the scope of benefit to disabled children. A disabled child represents a vulnerable section of the society because of the socio-psychological restrictions due to disability. The need is to have a comprehensive program for the welfare of the disabled child and their family members to enable them to lead a normal life. To conclude, for doing adequate justice to the differently-abled child, ample scope exists. Political commitment, multi-sectoral involvement and collaboration with international agencies are the main pillars for extending the benefit of welfare measures to the disabled child.

Keywords: disabled, rehabilitation, differently-abled, developing countries.

GJMR-K Classification : NLMC Code: WS 22.1

DIFFERENTLYABLEDCHILDRENSTRIVINGTOLEADANORMALLIFE-WHATPROGRAMMANAGERSCANDO?

Strictly as per the compliance and regulations of:

Differently Abled Children Striving to Lead a Normal Life - What Program Managers Can Do?

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Abstract- As per World Health Organization estimates more than a billion people live with some form of disability worldwide. Disability casts a significant impact in the development of a disabled child and a constant struggle for the families as well. Multiple political, health care delivery system and social determinants have been identified which have limited the scope of benefit to disabled children. A disabled child represents a vulnerable section of the society because of the socio-psychological restrictions due to disability. The need is to have a comprehensive program for the welfare of the disabled child and their family members to enable them to lead a normal life. To conclude, for doing adequate justice to the differently-abled child, ample scope exists. Political commitment, multi-sectoral involvement and collaboration with international agencies are the main pillars for extending the benefit of welfare measures to the disabled child.

Keywords: disabled, rehabilitation, differently-abled, developing countries.

I. Introduction

As per World Health Organization estimates more than a billion people (15% of world's population) live with some form of disability. Almost 95 million children in the age group of 0-14 years have some form of disability, of which approximately 13 million suffer from "severe disability"(World Health Organization 2011). An increasing number of disabled infants are surviving into childhood and adulthood, presenting a unique challenge to country's health, education and social care services (Sen & Yurtsever 2007).

II. Impact of Disability on Individual, Family & Society

Disability casts a significant impact in the development of a disabled child and a constant struggle for the families as well (Sen & Yurtsever 2007). A disabled child tends to have poorer health related outcomes – being more vulnerable to preventable conditions like obesity / dental caries / intestinal parasitic infestations (Reinehr et al. 2010 and Nahar et al. 2010 and Tappeh et al. 2010); lower educational opportunities and achievements (World Health Organization 2011); unemployment (World Health Organization 2011); risk of exposure to violence (World Health Organization 2011); numerous types of deprivations – food, housing, access to safe water, sanitation, and health care services (Sen & Yurtsever 2007 and Yousafzai et al. 2011); and increased dependency on others for their development and survival (World Health Organization 2011 and Yousafzai et al. 2011). At the same time, family members have to face multiple challenges such as poor awareness about the child's condition (World Health Organization 2011); adverse impact on social life, working life and family relationships (Sen & Yurtsever 2007); financial constraints (Beecham et al. 2007 and Tolou-Ghamari et al. 2013); and stigma / anxiety / stress / depression (Yousafzai et al. 2011).

III. Determinants for Poor Health Care Delivery to the Disabled Children Proposed Measures

Multiple political, health care delivery system and social determinants such as inadequate and incomplete policies (viz. lack of financial and other targeted incentives for children with disabilities to attend school or lack of social protection and support services) (World Health Organization 2011); dearth in the provision of health care, support and rehabilitation services (Sen & Yurtsever 2007); shortcomings in the service delivery system (viz. lack of coordination, deficient staffing, and incompetent staff) (Greco et al. 2006); inadequate funding (World Health Organization 2011 and Sen & Yurtsever 2007); negative attitudes / beliefs / prejudices among the stakeholders such as political leaders and employers (World Health Organization 2011 and Sen & Yurtsever 2007); scarce number of institutes for differently-abled thus limiting accessibility (Sen & Yurtsever 2007 and Yousafzai et al. 2011); no involvement of disabled persons in decision-making pertaining to matters directly influencing their lives (World Health Organization 2011); and inadequate data & evidence (Read et al. 2010); have been identified which have limited the scope of benefit to disabled children.

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IV. PROPOSED MEASURES

A disabled child represents a vulnerable section of the society because of the socio-psychological restrictions due to disability. The need is to have a comprehensive program for the welfare of the disabled child and their family members to enable them to lead a normal life. Diversified measures should be implemented to exhaustively address the multiple concerns of a disabled child as discussed in Table I. All these measures should be effectively supported with robust childhood disability data collection system and adequate support for encouraging research activities in the field of disabled child.

Table I : Potential measures for addressing the concerns of a disabled child

Concerns of a disabled child	Potential measures
Accessibility to health care institutions	1. Increasing number of centers offering services to different types of disability 2. Provision of integrated package of services under the same institute 3. Ensuring uniform geographical distribution of the hospitals / centers 4. Advocating structural modifications in facilities to make them user-friendly
Attitude of health care provider	Involving influential people with disabilities as trainers to improve the attitude and behavior of health care professionals
Creating enabling environment	1. Development of specific programs and services for children with disabilities 2. Removing barriers in public accommodations, transport, information, and communication to enable children with disabilities to participate in education, employment, and social life, reducing their isolation and dependency 3. Involvement of non-governmental organizations and international funding agencies for provision of adequate funding support 4. Creation of awareness among the general population using mass media 5. Facilitating early support to disabled children
Rehabilitation of the disabled child	Capacity building and human resource development measures: 1. By ensuring training of rehabilitation professionals or community-based workers to address geographical access 2. Fostering community based rehabilitation services 3. Involvement of nursing staff or key workers in the process of rehabilitation
Education opportunity	1. Encouraging inclusion of children with disabilities in mainstream schools 2. Provision of financial support to schools for facilitating such inclusion and bringing about the desired structural renovations 3. Appropriate training of mainstream teachers to deal with disabled children can improve teacher's confidence and skills 4. Advocating establishment of special schools for those disabled children who cannot be integrated in mainstream schools 5. Reservation of seats in professional courses 6. Provision of scholarships
Employment options	1. Vocational rehabilitation 2. Formulation and enforcement of antidiscrimination laws at workplace 3. Application of principles of ergonomics for enhancing their involvement and contribution to the national economy
Lack of self-belief	1. Empowering children to manage their own health through self-management courses 2. Psychological rehabilitation
Social and financial aspects	A range of financial measures, such as tax incentives or funding for reasonable accommodation, etc
Support to family members	1. Trained nurses can be utilized in managing the disabled child in early stages 2. Assisting family in developing good coherence

V. CONCLUSION

To conclude, for doing adequate justice to the differently abled (previously disabled) child, ample scope exists. Political commitment, multi-sectoral involvement and collaboration with international agencies are the main pillars for extending the benefit of welfare measures to the disabled child.

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Induction of CDC2 Phosphorylation in Skin Biopsies from Patients with Solid Tumors Undergoing DNA-Damaging Chemotherapy

By Amy Sun, Raymond L. Lam, Amy Harman, Anna C. Pavlick, Gary A. Lisa M. Dauffenbach, Christopher A. Kerfoot, Pearl Huang, Jonathan Cheng, Tim Demuth & Robert Iannone

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Abstract- This study was to clinically validate phosphorylated CDC2(pCDC2) as a biomarker for Wee1 kinase inhibitors by measuring pCDC2 in skin biopsies from patients receiving DNA damaging chemotherapy. Skin biopsies were performed at scheduled times after chemotherapy. Total CDC2 and pCDC2 in epidermal cells, hair follicle and bulb from skin biopsies were determined using chromogenic multiplex immunohis-tochemistry with multispectral image analysis. Statistical analyses were performed for each cell type after log-transformation of data. Thirty-one patients (29-88 years) completed the study. Significant induction of pCDC2 in response to chemotherapy was detected. Epidermis was most consistently evaluable across skin biopsies, demonstrating strong induction of pCDC2. The percentage of cells positive for total CDC2 and pCDC2 showed a 1.40-fold induction from baseline to 24h post-infusion (p=0.012) and a 2.05-fold increase from baseline to 48h (p<0.001).

Keywords: Cell cycle proteins, phosphorylated CDC2, biological markers, biomarkers, proteins, Cells, chemotherapy, cancers.

GJMR-K Classification : NLMC Code: QZ 310

Induction of CDC2 Phosphorylation in Skin Biopsies from Patients with Solid Tumors Undergoing DNA-Damaging Chemotherapy

Amy Sun [α], Raymond L. Lam [σ], Amy Harman [ρ], Anna C. Pavlick [ω], Gary A. Herman [¥], Lisa M. Dauffenbach [§], Christopher A. Kerfoot [χ], Pearl Huang [ν], Jonathan Cheng [ε], Tim Demuth [ζ], & Robert Iannone [π]

Abstract- This study was to clinically validate phosphorylated CDC2(pCDC2) as a biomarker for Wee1 kinase inhibitors by measuring pCDC2 in skin biopsies from patients receiving DNA damaging chemotherapy. Skin biopsies were performed at scheduled times after chemotherapy. Total CDC2 and pCDC2 in epidermal cells, hair follicle and bulb from skin biopsies were determined using chromogenic multiplex immunohis-tochemistry with multispectral image analysis. Statistical analyses were performed for each cell type after log-transformation of data. Thirty-one patients (29-88 years) completed the study. Significant induction of pCDC2 in response to chemotherapy was detected. Epidermis was most consistently evaluable across skin biopsies, demonstrating strong induction of pCDC2. The percentage of cells positive for total CDC2 and pCDC2 showed a 1.40-fold induction from baseline to 24h post-infusion (p=0.012) and a 2.05-fold increase from baseline to 48h (p<0.001). The results suggest that pCDC2 may be used to assess the degree of Wee1 kinase inhibition in the chemotherapy setting.
[Clinicaltrials.gov; NCT00800865]

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I. Introduction

There remains a significant unmet need for more effective cancer ther apies that can be developed quickly and safely. The identification of biomarkers that can be used in early clinical trials of potential anti-cancer agents remains a critical component of cancer drug development, so that doses used in therapeutic trials are maximally engaging the therapeutic target and provide a robust evaluation of the mechanism. A series of checkpoints exist within the cell cycle to prevent damaged cells from undergoing mitosis. Promoting checkpoint escape when used in combination with cytotoxic standard-of-care regimens is one potential mechanism under investigation in evaluating potential anti-cancer agents. Pathways within G2 arrest are logical therapeutic targets, as cytotoxicity may be improved by permitting DNA-damaged cells to undergo mitosis (Shapiro & Harper, 1999). Wee1 kinase, a regulator of the G2/M checkpoint (Kawabe, 2004; O'Connell, Walworth, & Carr, 2000), phosphorylates CDC2 in response to genotoxic injury, leading to cell arrest and damage repair (Mizuarai et al., 2009). Agents that inhibit Wee1 allow progression of damaged cells to mitosis, progressing to cell death via apoptosis (Mizuarai et al., 2009). Wee1 inhibitors would augment current chemotherapeutic regimens, and Wee1 inhibitors, such as PD0166283 (Hashimoto et al., 2006; Li, Wang, Sun, & Lawrence, 2006; Wang et al. 2001) and MK-1775, a direct substrate of Wee1 kinase in cells (Hirai et al., 2010; Leijen, Beijnen, & Schellens, 2010) have been shown to sensitize cancer cells to cytotoxic agents.

Therapies, such as chemotherapeutic agents that cause damage to DNA, engage the G2/M checkpoint in the cell cycle leading to increased phosphorylation of CDC2 (pCDC2). MK-1775 inhibits phosphorylation of CDC2 at Tyr15 (CDC2Y15). *In vivo*, MK-1775 potentiates tumor growth inhibition by DNA-damaging chemotherapeutic agents such as gemci-tabine, carboplatin, and cisplatin. The enhancement of anti-tumor effect by MK-1775 was well correlated with inhibition of CDC2Y15 phosphorylation in tumor tissues and skin hair follicles (Hirai *et al., 2009*). pCDC2 is normally measured by Western blot (Hirai *et al.*, 2009), gene expression (Mizuarai *et al.*, 2009; Yun *et al.*, 1999), or via qualitative immunohistochemistry methods. Since pCDC2 is a substrate of Wee1 kinase, it is a logical target engagement biomarker to use in the development of Wee1 kinase inhibitors (Mizuarai *et al.*, 2009), particularly if the investigational agents will be used

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concomitantly with DNA-damaging chemotherapy. In normal skin, the measurement of Wee1 inhibitor-mediated decreases in pCDC2 is therefore most accurately assessed in the context of the expected pCDC2 induction by DNA damage from chemotherapy. The pCDC2 biomarker has been studied in preclinical animal models, but because of the species differences, biomarker qualification studies such as this must be conducted in patient populations undergoing various chemotherapies.

This study was designed to quantify pCDC2 in skin punch biopsies from patients receiving DNA-damaging chemotherapy. If a large increase was observed with chemotherapy, then abrogation of the expected increase from Wee1 inhibition could be used to assess target engagement. Immunohistochemistry staining is routinely used in the clinical diagnosis of cancer and is typically a qualitative method. This study employed a quantitative immunohistochemistry assay that was developed to measure skin pCDC2 in patients with solid tumor(s) after they received a single dose of standard-of-care mono- or combination cytotoxic agents.

II.   Methods

a)   Patients

Patients over 18 years of age were eligible to participate in the study if they had a solid tumor and were being treated with one of the following agents: gemcitabine, cisplatin or carboplatin monotherapy or gemcitabine/cisplatin, gemcitabine/erlotinib, gemcitabine/carboplatin, cisplatin/vinorelbine, cisplatin/pemetrexed, carboplatin/vinorelbine, or carboplatin/pemetrexed combination therapies. The chemotherapy regimen was determined by the investigator. Patients were to have a performance status of ≤2 on the Eastern Clinical Oncology Group Performance Scale (Oken et al., 1982) at the first visit to enroll in the study. Patients were excluded if they had undergone chemotherapy or radiotherapy within 2 weeks or biological therapy within 4 weeks prior to study entry, had not recovered from adverse events due to agents administered more than 4 weeks earlier, or were currently participating or had participated in a study with an investigational compound or device within 30 days or 5 half-lives of signing informed consent, whichever was longer. Any patient with a history, or current evidence of any condition, prior or current therapy, psychiatric disorder, or lab abnormality that could confound the results of the study, interfere with participation, or was not in the best interest of the patient was also excluded.

b)   Study design

This Phase 1b, open-label, 2-part study was conducted at 4 sites in the United States and Europe. Institutional review board or ethics committee approval and informed consent were obtained prior to the initiation of study procedures. Each part of the study was to enroll approximately 15 patients; Part 2 commenced upon full enrollment of Part 1. The study duration was approximately 23 days from the first visit to the last visit for a total of 5 to 6 study visits.

c)   Collection of skin biopsy samples

Each patient underwent 3 biopsies. In Part 1, patients underwent skin biopsies at baseline and at 24 and 48 hours post-chemotherapy. Skin biopsies were obtained from patients in Part 2 at 24, 32, and 48 hours post-chemotherapy. Punch skin biopsies were to be at least 3 mm and were obtained per institutional standards from hair rich areas behind the ears. The administration of cytotoxic agents followed standard-of-care dosing, route of administration, and duration. Patients in both parts returned to the clinic or to their own physician for a follow-up safety visit 7 days after the final biopsy to assess the biopsy site and remove any sutures. A follow-up phone call occurred approximately 14 days after the last visit. Safety assessments included procedurerelated adverse event collection, physical exams, electrocardiograms, vital signs, and laboratory evaluations.

d)   Immunohistochemistry methods

Representative images of epidermis, hair follicle, and hair bulb samples from a patient (Part 1, number 23) are illustrated in Figure 1. A multiplex immunohistochemistry assay for total CDC2 (sc-54 antibody, Santa Cruz Biotechnology, Inc., Santa Cruz CA) and pCDC2 [Y15] (AF888 antibody, R&D Systems, Inc., Minneapolis MN) was used to evaluate formalin-fixed, paraffinembedded skin samples at Mosaic Laboratories (Lake Forest CA). Samples were stained using proprietary multiplex chromogenic immunohistochemistry methods. Vulcan Red Chromogen (Biocare Medical Inc., Concord CA) was used to stain CDC2, 3, 3' diaminobenzidine tetrahydrochloride (DAB, Dako North America Inc., Carpinteria CA) was used to stain pCDC2, and hematoxylin functioned as a counterstain. Regions of epidermis, follicles, and hair bulbs were imaged at 20 times using the Nuance FX2 Multispectral Imaging System (Cambridge Research & Instrumentation, Inc., Woburn MA) attached to a Leica DMLS2 brightfield microscope. Multispectral imaging was performed from 420 to 720 nm in 20 nm increments, and the image stack was quantitatively unmixed using spectral absorption patterns for each chromogen to produce quantitative grayscale images.

Evaluation of multiplex staining results was performed by two methods, manual review and histogram analysis. Manual review of re-colored images was performed to identify the frequency of CDC2 and pCDC2 in the cell type of interest (epidermis, hair follicles, or hair bulbs). This method also allowed the reviewer to exclude melanin or off-target membrane

staining in some specimens, which was observed with the pCDC2 antibody. Using the Nuance software, CDC2, pCDC2, and hematoxylin images were used to create a pseudo-colored darkfield composite image with CDC2 colored red, pCDC2 colored green, and hematoxylin colored blue (Figure 1B). Cells within the cell type of interest were enumerated as positive for CDC2, pCDC2, both, or neither. The percentage of cells positive for CDC2, pCDC2, and both epitopes was enumerated by a trained technician followed by secondary quality control review. Pixel-based image analysis was performed on 8-bit images using ImageJ version 1.38w (National Institutes of Health, Bethesda MD). Histogram analysis of staining intensity in the region of interest was determined for CDC2 and pCDC2, and co-localization analysis was performed to determine the percentage of the CDC2-positive area that was positive for pCDC2 at three staining intensity thresholds. All low, medium and high optical density (OD) staining thresholds were set for both CDC2 (>20, 20-70, 70-90, >90 OD units) and pCDC2 (>20, 20-40, 40-60, >60 OD units). For co-localization analysis, the percentages of CDC2-positive (>20 OD units) pixels that demonstrated all, low, moderate and high pCDC2 staining were determined.

e) *Primary endpoints*

A total of 16 immunohistochemistry parameters were identified in the protocol (Table 1) with two being defined for analysis in the primary objective: Parameters 3 (% CDC2 + pCDC2 [manual enumeration]) and 13 (%CDC2 [all] with pCDC2 [all] [histogram analysis]). These two parameters were chosen because they trended well with preclinical Western blot data (correlation coefficients 0.85 and 0.91, respectively). Prior to performing the statistical analysis and upon visual assessment of the raw images for Part 1 patients, Parameter 4 (proportion of total CDC2 positive cells that are positive for pCDC2 from manual enumeration) was chosen as an additional primary parameter as this parameter corrects for variability in the frequency of CDC2 positive cells across samples. The manual review of images allowed the reviewer to exclude melanin or off-target membrane staining in some specimens, which was observed with the pCDC2 antibody.

From the skin biopsy samples, several subtypes of skin tissues were identified, including, hair follicles and bulbs, cells lining the sebaceous glands, and epidermis. Distinct tissue types for analysis were not prespecified in the protocol. Epidermis was chosen as the primary tissue for analysis due to a consistent presence of sufficient material for analysis in samples before and after treatment.

Part 1 data were analyzed prior to Part 2 patients completing enrollment. We recommended (and documented in the Part 1 results memo) Parameter 4 in epidermis tissue as the preferred endpoint. Consequently, Part 2 data were analyzed based on the preferred endpoint (Parameter 4 in epidermis tissue), consistent with the Part 1 recommendations. For completeness, the results of Parameters 3, 4 and 13 are also reported.

f) *Statistical methods*

Statistical analyses were performed separately for each tissue type and each part of the study after log-transformation of the data. The longitudinal analysis of variance (ANOVA) model included patient and time. An unstructured covariance matrix was used to model the correlation among repeated measurements over time. As the majority of patients received gemcitabine, sensitivity analyses for patients with or without gemcitabine therapy were also performed. The sensitivity analysis model included patient, time, chemotherapy (with or without gemcitabine), and time-by-chemotherapy. Geometric means for each time point, geometric mean ratios between two time points, 90% confidence intervals, and nominal 1-sided P-values were calculated. Safety data were summarized for all patients who received at least one dose of standard chemotherapy during the study. Statistical analyses were performed using SAS v 9.1 (SAS Institute Inc., Cary NC). The sample size of 15 patients per study segment was to allow a precise estimate of the within patient standard deviation.

For Part 1 analysis on all patients, the multiplicity adjustment for the 3 key parameters was based on the Hochberg procedure and those significant results before adjustment ($P<0.05$) remained significant after adjustment (adjusted $P<0.05$). The adjustment was made across the parameters but separately for each of the three comparisons (24 hours versus baseline, 48 hours versus baseline, and 48 hours versus 24 hours). Part 2 analysis was based on Parameter 4 in epidermis tissue and no multiplicity adjustment was required. Only the raw P-values are reported here.

III. RESULTS

a) *Patient characteristics*

A total of 31 patients aged 29 to 88 years were enrolled and completed the study; no patients discontinued prematurely. Nineteen (61.3%) were males and 12 (38.7%) were females. Gemcitabine monotherapy or combination therapy was administered to 12/15 patients (80.0%) in Part 1 and 11/16 patients (68.8%) in Part 2. The treatment regimens for the three patients in Part 1 that did not include gemcitabine were comprised of carboplatin monotherapy and cisplatin/pemetrexed combination therapy. The treatment regimens for the five patients in Part 2 that did not include gemcitabine consisted of carboplatin monotherapy. Patients tolerated the study well and no serious

adverse reactions were reported. Six patients (19.4%) reported a total of ten procedure-related adverse experiences including pain, bleeding, and swelling at the biopsy site. All events were of mild intensity and all but one had resolved at the end of the trial.

b) pCDC2 characterization and changes

Expression of CDC2 and pCDC2 was observed in subtypes of skin tissues including hair follicles and bulbs, cells lining the sebaceous glands, and epidermis. However, many skin specimens contained mostly epidermis but few other skin structures leading to insufficient measurements of tissue types (other than epidermis) from each patient over time. For example, patient number 23 was deficient in hair bulbs at the 24-hour time point (Figure 1A). Although induction in pCDC2 was observed in all tissue types, epidermis was the only tissue structure that had sufficient measurements across baseline and post-chemotherapy time points in the majority of the patients. For this reason, epidermis was selected as the key tissue component and results presented are from epidermis only.

Results are presented for Parameters 3, 4 and 13 in epidermis tissue. Consistent results were obtained among the primary parameters. Sample photomicrographs of CDC2/pCDC2 staining are presented in Figure 1B. Results for all three parameters in the epidermis by time point for Part 1 and Part 2 patients are presented in Table 2 and Table 3, respectively. In Part 1, Parameters 4 and 13 both showed a 1.4 fold induction from baseline to 24 hours postchemotherapy ($P = 0.012$, both parameters), and a 2.05 and 1.47 fold increase from baseline to 48 hours ($P < 0.001$, $P = 0.013$), respectively. Parameter 3 showed a 1.45 fold increase from baseline to 24 hours post-chemotherapy ($P = 0.070$) and a 4.01 fold increase from baseline to 48 hours ($P < 0.001$). Parameter 4 was significantly higher at 48 hours than that at 24 hours in both Part 1 ($P = 0.012$) and Part 2 ($P = 0.046$) of the study, suggesting pCDC2 induction continued to increase between 24 and 48 hours post-chemotherapy. Similar results were observed for the gemcitabine-treated subgroup.

In the 8 patients not receiving gemcitabine, no significant increases in pCDC2 were observed in Parameter 4: 1.06 fold increase from baseline to 24 hours ($P = 0.412$) in Part 1; 1.68 and 1.00 fold increases from 24 to 48 hours in Part 1 ($P = 0.068$) and Part 2 ($P = 0.499$). Parameter 4 results in scalp punch biopsy epidermis from all patients and the gemcitabine subgroup in both parts of the study are displayed in Figure 2, adjusted to the common 24-hour time point.

IV. Discussion

This is the first clinical study to evaluate pCDC2 with quantitative multiplex immunohistochemistry methods at multiple time points in patients with solid tumors receiving DNA-damaging chemotherapy. Immunohistochemistry is a subjective, semi-quantitative assay scored on a discrete scale. We had no previous knowledge regarding the lower limit of detection and quantitation. No reports regarding the expected magnitude or time course of pCDC2 induction in patients with or without chemotherapy existed. By developing a parameter analysis strategy, we were able to gain experience in quantitatively evaluating the pCDC2 signal in skin samples in this study.

Greater expression of both CDC2 and pCDC2 was typically observed in the hair follicles, cells lining the sebaceous glands, and hair bulbs. Ideally, there would be sufficient measurements of the same tissue types from the same patients across different time points for the assessment of changes induced by treatment. However, due to the scarce presentation of hair bulbs, very few hair bulbs in each specimen from each patient were identified. Epidermis was the only tissue type that, by itself, had sufficient measurements across baseline and postchemotherapy time points in the majority of the patients. The epidermis therefore became the tissue of choice. This determination was made based solely on evaluable tissue without any knowledge of pCDC2 results.

Whether one should combine different tissue types for the assessment of changes may depend on 1) whether or not there are sufficient measurements of the same tissue types and 2) how the measurements are combined (equal or unequal weights). In this analysis, similar conclusions from both epidermis and 'all tissue type combined' were obtained (data not shown). Restricting analysis to epidermis does not require assumptions to inform weighing of the various tissues, another reason that epidermis was the tissue type selected for analysis of pCDC2 levels. In the clinical setting where hair follicles could be reliably sampled, the sensitivity of this measure could be increased.

In preclinical studies, pCDC2 levels peaked at about 32 hours after chemotherapy administration (Hirai et al., 2010). In this study, cytotoxic chemotherapy significantly induced the epidermal pCDC2 level up to 48 hours; statistically significant increases from baseline were noted at 24 hours with levels continuing to increase between 24 to 48 hours. In a preclinical study, the inhibition of CDC2 phosphorylation by MK-1775 correlated with antitumor efficacy. Although MK-1775 was not used in this study, given the magnitude of pCDC2 induction after chemotherapy administration, we believe relative decreases of pCDC2 in the presence of MK- 1775 could be measured as a tool for assessing the degree of target engagement.

In the subgroup analysis, the gemcitabine-treated group showed significant induction of pCDC2 24 and 48 hours after cytotoxic chemotherapy. In the subgroup of eight patients receiving therapeutic

regimens that did not contain gemcitabine, no significant increase in pCDC2 was observed. An increase from baseline was observed in Part 1 but no change was observed in Part 2. The reason for this observation is unknown; it may be related to the time course, sample size, or sensitivity to cisplatin/carboplatin.

In a preclinical study, MK-1775 inhibited phosphorylation of CDC2 with an EC50 value of 85 nmol/L in cells pretreated with gemcitabine, whereas EC50 values of pCDC2 inhibition for carboplatin- and cisplatin-treated cells were 180 and 159 nmol/L, respectively (Hirai *et al.*, 2009). This suggests that a higher concentration of non-gemcitabine treatment is required to achieve comparable pCDC2 inhibition. Alternatively, gemcitabine is a deoxycytidine analogue with a mechanism of action distinct from other cytotoxics, and the differential observed here may be due to its unique action on cellular regulatory processes (Plunkett *et al*., 1995).

When examining pharmaceutical R&D productivity, attrition in phase 2/3 of compound development cycles is a key cause in productivity decreases. Finding ways to reduce this attrition is a cornerstone of effective R&D planning (Paul *et al.,* 2010). The careful use of biomarkers in proof-of-concept trials augments target selection and increases the probability of success (Tan *et al.,* 2009). The methodology reported here allows for the quantitative measurement of pCDC2 and has provided recommended analysis parameters and tissue types for future studies. The study procedures were minimally invasive and were well tolerated by study participants. Our data indicate that pCDC2 is an appropriate target engagement biomarker for assessing pCDC2 inhibition in early clinical evaluations of Wee1 kinase inhibitors such as MK-1775.

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Figure 1

Figure Legends
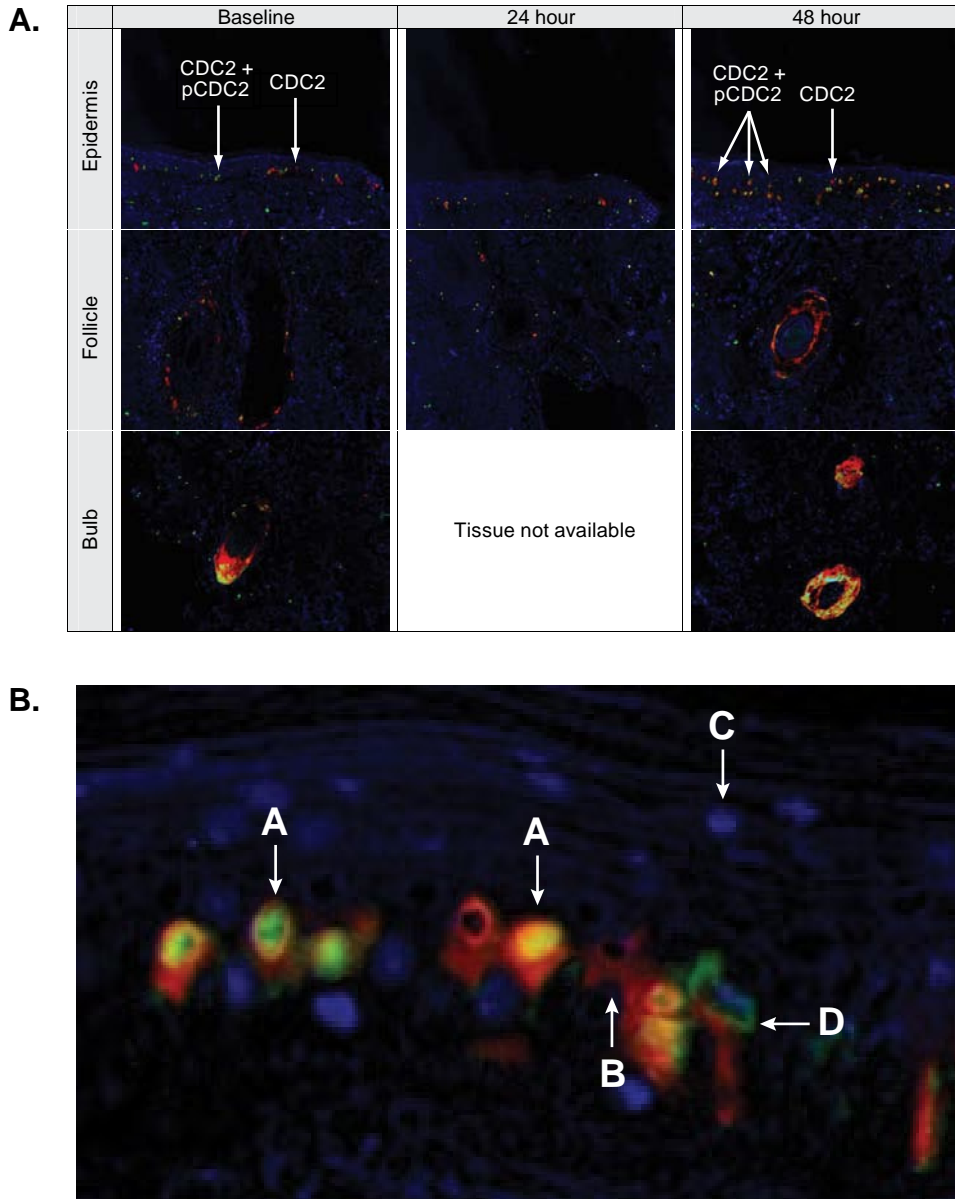
Fig. 1 : Representative images of epidermis, hair follicle, and hair bulb samples from a patien (Part 1, number 23). Panel A: Samples collected at Baseline, 24 hours and 48 hours after chemotherapy administration. Panel B: Recolored image for manual enumeration of an epidermis sample at 48 hours. A: CDC2 + pCDC2 (yellow); B: CDC2 only (red); C: negative cells (blue); D: melanin (green). For this sample, 74% of the CDC2 positive cells expressed pCDC2 (parameter 4 shown).

Figure 2

A.

All chemotherapies

B.

Patients with Gemcitabine
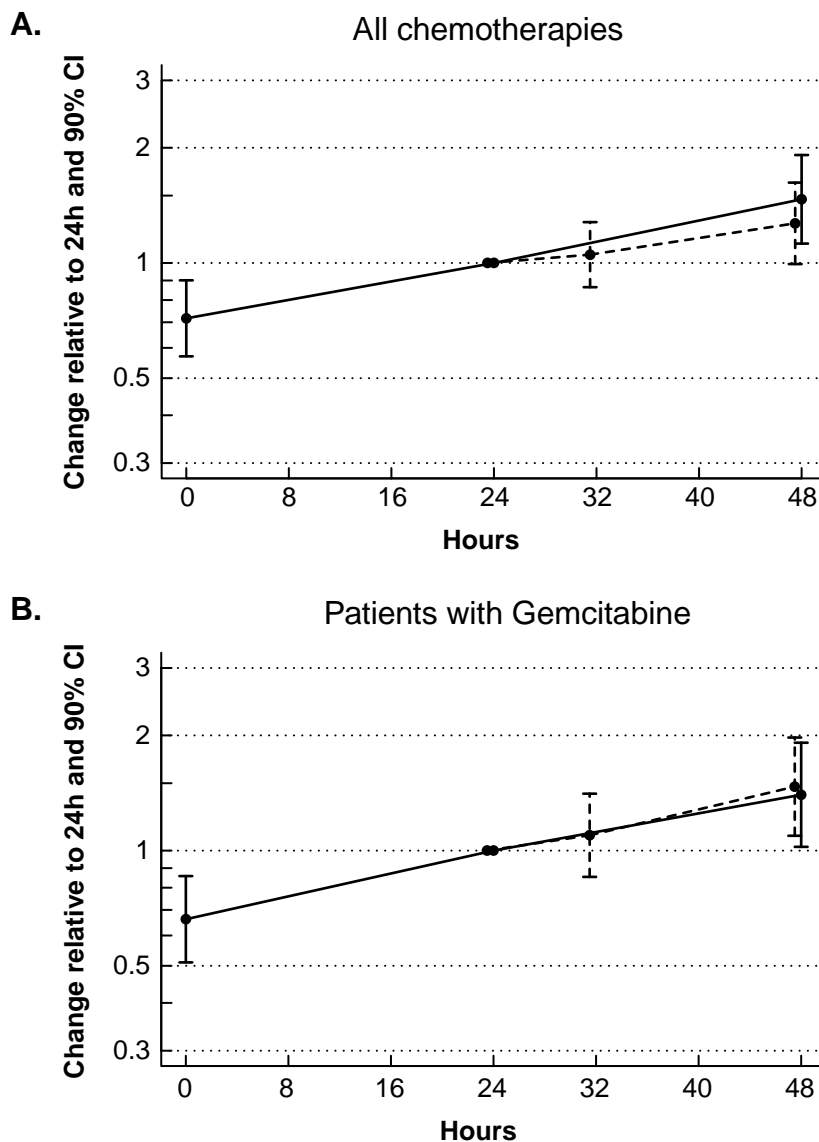
Fig. 2 : Expression of pCDC2 measured by the percentage of CDC2 positive cells that are pCDC2 positive in epidermis scalp punch biopsies for Parts 1 and 2 of the study, adjusted to the common 24-hour time point. Panel A: All patients (n=31); Panel B: Subgroup of patients taking gemcitabine (n=23). The overlap of the two lines indicates strong agreement between Part 1 and 2 results.

Table 1 : Immunohistochemistry parameters

	Description	Methodology
1	Percentage of all cells CDC2 positive	
2	Percentage of cells pCDC2 positive	
3[a]	Percentage of cells CDC2 positive co-localized with pCDC2	Manual Enumeration
4[a]	Percentage of CDC2-positive cells that express pCDC2	
5	Percentage of all positive pixels for CDC2 > 20 OD	
6	Percentage of positive pixels for CDC2 > 20 OD and < 70 OD (weak)	
7	Percentage of positive pixels for CDC2 > 70 OD and < 90 OD (moderate)	

8	**Percentage of positive pixels for CDC2 > 90 OD (strong)**	**Histogram Analysis**
9	**Percentage of all positive pixels for pCDC2 > 20 OD**	
10	**Percentage of positive pixels for pCDC2 > 20 OD and < 40 OD (weak)**	
11	**Percentage of positive pixels for pCDC2 > 40 OD and < 60 OD (moderate)**	
12	**Percentage of positive pixels for pCDC2 > 60 OD (strong)**	
13[a]	**Percentage of all cells CDC2 positive co-localized with all cells pCDC2 positive**	**Histogram and Co-Localization Analyses**
14	**Percentage of all cells CDC2 positive co-localized with weak pCDC2**	
15	**Percentage of all cells CDC2 positive co-localized with moderate pCDC2**	
16	**Percentage of all cells CDC2 positive co-localized with strong pCDC2**	

[a]Primary endpoint parameter

OD=optical density; pCDC2=phosphorylated CDC2

Table 2 : pCDC2 changes post-chemotherapy, Part 1, epidermis tissue

Parameters	n	Geometric mean or ratio[a]	90% CI	*P* value[b]	SD	Median
Parameter 3: % CDC2 + pCDC2 (manual enumeration)						
All patients in Part 1						
Baseline	15	2.2	(1.3, 3.8)		3.2	3.4
24h post-chemotherapy	14	3.3	(1.9, 5.5)		2.8	5.2
48h post-chemotherapy	15	9.0	(6.3, 12.9)		2.2	9.0
24h post-chemotherapy/baseline	14	1.45	(0.95, 2.22)	0.070	2.45	1.50
48h post-chemotherapy/baseline	15	4.01	(2.40, 6.70)	<0.001	3.09	4.89
48h/24h post-chemotherapy	14	2.76	(1.79, 4.24)	<0.001	2.49	3.26
Gemcitabine subgroup						
Baseline	12	1.9	(1.0, 3.4)		3.5	2.5
24h post-chemotherapy	11	2.7	(1.5, 4.9)		3.1	4.6
48h post-chemotherapy	12	8.5	(5.6, 12.9)		2.1	7.7
24h post-chemotherapy/baseline	11	1.46	(0.89, 2.40)	0.100	2.78	1.48
48h post-chemotherapy/baseline	12	4.52	(2.52, 8.11)	<0.001	2.93	4.72
48h/24h post-chemotherapy	11	3.10	(1.91, 5.04)	<0.001	2.25	3.21
Parameter 4: % of CDC2 that are pCDC2+ (manual enumeration)						
All patients in Part 1						
Baseline	13	24.6	(19.0, 31.8)		1.7	26.3
24h post-chemotherapy	13	34.3	(25.6, 46.0)		1.9	31.1

48h post-chemotherapy	15	50.3	(39.9, 63.5)		1.7	59.4
24h post-chemotherapy/baseline	13	1.40	(1.11, 1.75)	0.012	1.59	1.37
48h post-chemotherapy/baseline	13	2.05	(1.56, 2.69)	<0.001	1.78	2.47
48h/24h post-chemotherapy	13	1.47	(1.12, 1.91)	0.012	1.73	1.38
Gemcitabine subgroup						
Baseline	10	25.4	(18.7, 34.5)		1.8	27.4
24h post-chemotherapy	10	38.4	(27.7, 53.2)		1.9	47.1
48h post-chemotherapy	12	53.8	(41.4, 69.8)		1.5	62.8
24h post-chemotherapy/baseline	10	1.51	(1.17, 1.96)	0.008	1.64	1.56
48h post-chemotherapy/baseline	10	2.11	(1.53, 2.93)	<0.001	1.48	2.34
48h/24h post-chemotherapy	10	1.40	(1.02, 1.91)	0.040	1.59	1.36

Parameter 13: %CDC2 (all) with pCDC2 (all) (histogram analysis)

All patients in Part 1

Baseline	15	37.6	(26.3, 53.7)		2.2	47.5
24h post-chemotherapy	14	52.6	(42.2, 65.5)		1.6	60.5
48h post-chemotherapy	15	55.2	(42.0, 72.6)		1.8	76.7
24h post-chemotherapy/baseline	14	1.40	(1.11, 1.77)	0.012	1.68	1.24
48h post-chemotherapy/baseline	15	1.47	(1.12, 1.93)	0.013	1.83	1.60
48h/24h post-chemotherapy	14	1.05	(0.79, 1.39)	0.382	1.86	1.21
Gemcitabine subgroup						
Baseline	12	39.8	(26.4, 60.1)		2.4	51.4
24h post-chemotherapy	11	58.8	(46.8, 73.8)		1.6	63.0
48h post-chemotherapy	12	55.5	(40.3, 76.4)		1.9	78.5
24h post-chemotherapy/baseline	11	1.48	(1.13, 1.92)	0.011	1.75	1.26
48h post-chemotherapy/baseline	12	1.39	(1.02, 1.91)	0.042	1.79	1.43
48h/24h post-chemotherapy	11	0.94	(0.70, 1.28)	0.628	1.80	1.10

[a] Back-transformed least squares mean from log scale: Geometric mean for individual time points and mean ratio between two time points

[b] 1-sided *P* value

CI = confidence interval; h=hours; pCDC2=phosphorylated CDC2; SD=geometric (between-patient) standard deviation

Table 3 : pCDC2 changes post-chemotherapy, Part 2, epidermis tissue

Parameters	n	Geometric mean or ratio[a]	90% CI	P value[b]	SD	Median
Parameter 3: % CDC2 + pCDC2 (manual enumeration)						
All patients in Part 2						
24h post-chemotherapy	16	5.1	(3.5, 7.3)		2.3	5.6
32h post-chemotherapy	16	4.6	(3.6, 5.9)		1.7	4.2
48h post-chemotherapy	16	6.6	(4.6, 9.5)		2.3	7.4
32h/24h post-chemotherapy	16	0.92	(0.62, 1.35)	0.651	2.42	1.02
48h/24h post-chemotherapy	16	1.31	(0.91, 1.90)	0.109	2.33	1.10
48h/32h post-chemotherapy	16	1.43	(1.01, 2.03)	0.045	2.21	1.25
Gemcitabine subgroup						
24h post-chemotherapy	11	5.6	(3.6, 8.9)		2.7	5.8
32h post-chemotherapy	11	5.1	(3.8, 6.8)		1.9	4.2
48h post-chemotherapy	11	9.2	(6.4, 13.2)		2.1	9.9
32h/24h post-chemotherapy	11	0.91	(0.56, 1.47)	0.635	2.74	0.75
48h/24h post-chemotherapy	11	1.64	(1.07, 2.50)	0.031	2.48	1.69
48h/32h post-chemotherapy	11	1.80	(1.22, 2.66)	0.009	2.29	2.12
Parameter 4: % of CDC2 that are pCDC2+ (manual enumeration)						
All patients in Part 2						
24h post-chemotherapy	16	23.0	(17.4, 30.3)		1.9	28.8
32h post-chemotherapy	16	23.7	(17.5, 32.1)		2.0	22.4
48h post-chemotherapy	16	29.9	(23.2, 38.6)		1.8	30.7
32h/24h post-chemotherapy	16	1.03	(0.84, 1.27)	0.399	1.60	0.97
48h/24h post-chemotherapy	16	1.30	(1.01, 1.69)	0.046	1.80	1.31
48h/32h post-chemotherapy	16	1.26	(0.99, 1.62)	0.058	1.75	1.15
Gemcitabine subgroup						
24h post-chemotherapy	11	26.1	(18.8, 36.3)		2.0	34.0
32h post-chemotherapy	11	28.6	(20.3, 40.4)		2.1	37.4
48h post-chemotherapy	11	38.4	(30.2, 48.8)		1.6	45.5

32h/24h post-chemotherapy	11	1.10	(0.85, 1.41)	0.266	1.62	0.98
48h/24h post-chemotherapy	11	1.47	(1.08, 2.00)	0.022	1.75	1.36
48h/32h post-chemotherapy	11	1.34	(0.99, 1.82)	0.056	1.81	1.21

Parameter 13: %CDC2 (all) with pCDC2 (all) (histogram analysis)

All patients in Part 2

24h post-chemotherapy	16	18.7	(9.8, 35.6)		4.4	29.2
32h post-chemotherapy	16	20.3	(13.4, 30.8)		2.6	19.2
48h post-chemotherapy	16	21.4	(12.2, 37.3)		3.6	30.0
32h/24h post-chemotherapy	16	1.09	(0.69, 1.70)	0.376	2.78	0.79
48h/24h post-chemotherapy	16	1.14	(0.54, 2.40)	0.379	5.43	1.16
48h/32h post-chemotherapy	16	1.05	(0.61, 1.82)	0.437	3.52	1.03
Gemcitabine subgroup						
24h post-chemotherapy	11	31.6	(16.1, 62.3)		2.4	36.6
32h post-chemotherapy	11	29.3	(19.2, 44.7)		2.1	26.0
48h post-chemotherapy	11	30.8	(16.5, 57.6)		2.1	32.9
32h/24h post-chemotherapy	11	0.93	(0.54, 1.60)	0.595	2.22	0.67
48h/24h post-chemotherapy	11	0.97	(0.39, 2.44)	0.520	2.65	1.06
48h/32h post-chemotherapy	11	1.05	(0.53, 2.10)	0.451	1.96	1.00

[a] Back-transformed least squares mean from log scale: Geometric mean for individual time points and mean ratio between two time points

[b] 1-sided *P* value

CI=confidence interval; h=hours; pCDC2=phosphorylated CDC2; SD=geometric (between-patient) standard deviation

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Plasma Total Amino Acids, Plasma Glutamate & Alanine Levels in Diabetic Subjects

By Kashinath R. T., Nagendra. S., Rudrappa. G. & Srinivas. S.

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Abstract- Generally, in fasting state, gluconeogenesis accounts for about 30% of overall hepatic glucose output, the increase in type 2 diabetic subjects may be much more than this level. There is a close relationship between glucose metabolism and amino acid metabolism which is established through transamination reactions. The key amino acid -keto acid pairs involved in transamination reactions are glutamate – **α** ketoglutarate, aspartate – oxaloacetate and pyruvate - alanine. A study was undertaken to assess the plasma levels of total amino acids, glutamate and alanine in type 2 diabetic subjects. A blood sample (5ml) with heparin as an anticoagulant was collected in the fasting state, from each of the selected normal as well as diabetic subjects. The separated plasma samples were employed for the estimation of total amino acid nitrogen levels, as well as for the estimation of plasma alanineand glutamate levels. The results indicate a significant rise in the plasma levels of total amino acids, alanine & glutamate in type-2 diabetic subjects as well as a significant increase in plasma alanine levels in diabetics of 30- 40 yrs age as compared to diabetics of 41-60yrs of age. These findings suggest an increased availability of glucogenic amino acid precursors for glucose formation may be due to lack of proteolytic suppression of insulin.

Keywords: amino acids, gluconeogenesis, glutamate, alanine.

GJMR-K Classification : NLMC Code: WK 830, QU 55

PLASMATOTALAMINOACIDSPLASMAGLUTAMATEALANINELEVELSINDIABETICSUBJECTS

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Plasma Total Amino Acids, Plasma Glutamate & Alanine Levels in Diabetic Subjects

Kashinath R.T. [α], Nagendra. S. [σ], Rudrappa. G. [ρ] & Srinivas.S. [ω]

Abstract- Generally, in fasting state, gluconeogenesis accounts for about 30% of overall hepatic glucose output, the increase in type 2 diabetic subjects may be much more than this level. There is a close relationship between glucose metabolism and amino acid metabolism which is established through transamination reactions. The key amino acid -keto acid pairs involved in transamination reactions are glutamate – α ketoglutarate, aspartate – oxaloacetate and pyruvate - alanine. A study was undertaken to assess the plasma levels of total amino acids, glutamate and alanine in type 2 diabetic subjects. A blood sample (5ml) with heparin as an anticoagulant was collected in the fasting state, from each of the selected normal as well as diabetic subjects. The separated plasma samples were employed for the estimation of total amino acid nitrogen levels, as well as for the estimation of plasma alanineand glutamate levels. The results indicate a significant rise in the plasma levels of total amino acids, alanine & glutamate in type-2 diabetic subjects as well as a significant increase in plasma alanine levels in diabetics of 30-40 yrs age as compared to diabetics of 41-60yrs of age. These findings suggest an increased availability of glucogenic amino acid precursors for glucose formation may be due to lack of proteolytic suppression of insulin.

Keywords: amino acids, gluconeogenesis, glutamate, alanine.

I. INTRODUCTION

Gluconeogenesis, the process of formation of glucose from non-Carbohydrate metabolites is generally increased in Diabetes mellitus mainly due to deficiency of insulin as insulin has a gluconeogenic suppression effect (13). Among the various Glucogenic metabolites, glucogenic amino acids alanine, aspartate and glutamate are significant. The transamination of these amino acids through respective transaminases yield the ketoacids: α-ketaglutarate, oxaloacetate and pyruvate, which are readily convertible to glucose through gluconeogenesis. Insulin apart from its hypoglycemic action also has a

tissue proteolysis suppression effect thereby decreasing the availability of amino acids. Hence a deficiency of insulin as observed in diabetes mellitus may lead to increased gluconeogenesis due to the lack of suppression effect of gluconeogenesis on one hand and the lack of suppression effect of tissue proteolysis on the other hand. There are no much studies available regarding the plasma levels of glucogenic amino acids in particular glutamate and alanine levels in diabetic subjects. Gercia etal reported that increased glucose recovery was observed in insulin induced hyperglycemia in rats upon oral or intraperitonial glutamine or alanine administration to these rats (14) suggesting a close relationship between plasma amino acid and plasma glucose levels. The reports of Agustino consoli etal suggest that increased conversion of alanine to glucose in NIDDM subjects. Further it has been proposed that increased muscle glycolysis might provide additional alanine and lactate to sustain gluconeogenesis in type 2 diabetic subjects (1).

Hence a study was undertaken to assess the plasma levels of total amino acids, glutamate and alanine in type 2 diabetic subjects.

II. MATERIALS & METHODS

The type 2 diabetic subjects in the age group of 30-60 years visiting the medical OPD of SUBBAIAH INSTITUTE OF MEDICAL SCIENCES & RESEARCH CENTER, PURLE, SHIMOGA, were randomly selected. Age matched normal subjects were randomly selected from the employees of the college and hospital. A blood sample (5ml) with heparin as an anticoagulant was collected in the fasting state, from each of the selected normal as well as diabetic subjects after obtaining an informed consent from them. The blood samples were centrifuged at 3600rpm for 8 minutes to separate plasma. The separated plasma samples were employed for the estimation of total amino acid nitrogen (AAN) levels (9), as well as for the estimation of plasma alanine (AL) and glutamate (GM) levels using quantitative paper chromatographic procedure (18). Ethical clearance from the institutional research council, for the present work was taken.

III. RESULTS

The study included 80 normal subjects of both sexes in the age group of 30-50yrs and 103 type 2 diabetic subjects of both sexes in the age group of 30-

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60yrs. The normal subjects included equal numbers of male and female subjects. The type 2 diabetic subjects included 85 male diabetics and 38 female diabetics. Among the diabetic subjects 31 were with family history of diabetes. A split in the diabetics as per the age, there were 36 in the age group of 30-40yrs, 67 in the age group of 41-60yrs. These groupings or division of the normal subjects and diabetic subjects are given in chart 1.

Table-1 shows the levels of total amino acid nitrogen (AAN) alanine (AL) and glutamate (GM) levels in plasma in normal and type 2 diabetic subjects. It is seen from the taste that the plasma levels of AAN, AL and GM are significantly elevated in type 2 diabetic subjects as compared to normal subjects may be due to lack of insulin.

Table 2 and table 3 narrate the plasma levels of AAN, AL and GM in diabetic male subjects and diabetic female subjects as compared to their normal counter parts respectively. It is evident from the tables that in both male as well as in female diabetics the plasma levels of AAN, AL and GM are significantly raised as compared to their normal counter parts showing that the diabetics induced alteration in these parameters is common to both sexes.

Table 4 gives the plasma levels of AAN, AL and GM in male diabetic subjects as compared to female diabetic subjects. It is seen from the table that there is a significant rise in GM levels in female diabetics as compared to male diabetic subjects where as there is no significant change observed in AAN and AL levels among these two groups.

Table 5 gives the plasma levels of AAN, AL & GM in diabetic subjects of different age groups i.e. diabetics in the age group of 30-40yrs and diabetics in the age group of 41-60yrs age group. It is evident from the table that a significant rise in plasma AL in diabetics of 30-40 yrs age as compared to diabetics of 41-60yrs of age. Whereas no significant changes observed between these two groups in plasma AAN and plasma GM levels

IV. Discussion

Generally, in fasting state, gluconeogenesis accounts for about 30% of overall hepatic glucose output (1 6), the increase in type 2 diabetic subjects may be much more than this level (2-6, 8, 12). The majority of gluconeogenic precursors may originate from muscle glycolysis and in the form of alanine (7). In the present study it has been observed that there is a significant rise in plasma AL and GM levels in type 2 diabetic subjects which is in line with the observation made by augustino consoli etal (1). There is a close relationship between glucose metabolism and amino acid metabolism which is established through transamination reactions. The key amino acid -keto acid pairs involved in transamination reactions are glutamate

α- ketoglutarate, aspartate-oxaloacetate and pyruvate - alanine. Insulin, the sole hypoglycemic hormone, not only decreases the formation of glucose through gluconeogenesis by suppressing the activities of key gluconeogenic enzymes but also suppresses the tissue proteolysis (11, 15, 17) thereby decreasing the availability of amino acids for gluconeogenesis. The results of present study with type 2 diabetic subjects clearly establishes an elevated plasma levels of AAN,AL, and GM in type 2 diabetic subjects (refer table 1) which may be due to decreased insulin levels or due to decreased insulin action. This decreased insulin levels in these subjects might have caused a rise in the plasma levels of AAN, AL and GM as insulin is known to possess a suppression effect on tissue proteolysis (13). A parallel increase in plasma GM and AL levels along with AAN levels in type 2 diabetic subjects as compared to their normal counter parts(ref table1,2 and 3) suggests that the increase in plasma AAN in these subjects may be partly due to an increase in these two amino acids (plasma AL and GM levels). The increase in plasma and probably tissue GM levels in type 2 diabetic subjects (male and female diabetic subjects), as compared to their normal counterparts may further suppresses the utilization of glucose adding to hyperglycemia as it is shown by Visweswaran and Subramanyan (20) that added glutamic acid could reduce the glucose utilization.

Further a little elevation in the plasma levels of AAN, AL and GM observed in the present study in female diabetic subjects as compared to male diabetic subjects which may be due to hormone prolactin which has a diabetogenic effect (10) and to hormone estrogen, which acts through releasing biogenic amines (19).

When the plasma levels of AAN, GM and AL in diabetic subjects of different age group (diabetes of 30-40 yrs and diabetic of 41-60yrs age group are compared a significant rise is seen in the present studies in plasma AL levels in diabetics of 30-40yrs age group as compared to diabetics of 41-60yrs age group, which may probably be due to decreased glucose utilization with increasing age as well as due to increased tissue response to decreased insulin levels as there may be an increased tissue proteolysis in the early stages of diabetic disease, as much tissue wastage and weight loss is seen in early stage of diabetic disease. It can be concluded by the present studies that there is a significant rise in plasma levels of AL and GM in type 2 diabetic subjects of both sexes which may be due to increased proteolysis because of insulin deficiency, also a significant rise observed in plasma AL level in diabetics of 30-40yrs age group as compared to other groups.

CHART—1

Chart showing the division of normal and diabetic subjects according to different parameters.

Total number of subjects in the present study   ----183
Total type 2 diabetic subjects   ---- 103
Total normal subjects   -----80
     Normal male subjects   ---- 40
     Normal female subjects   ----40

Male diabetic subjects   ------65
Female diabetic subjects   ------38
Diabetic with family history   ------31
Diabetic without family history   -----67
Diabetic subjects in the age group of 30--40yrs   ----36
     41—60yrs   -----67

Table 1 : Table showing the levels of Total Amino acid nitrogen, Glutamic acid and Alanine in whole Blood of Normal and Diabetic Subjects

Parameter	Normal Subjects (n=80)	Diabetic Subjects (N=103)
Total Amino acid nitrogen (mg/dl)	6.26 ± 0.71	17.55*** ± 4.43
Glutamic acid (mg/dl)	3.75 ± 0.68	9.86*** ± 2.43
Alanine (mg/dl)	2.95 ± 0.53	5.55*** ± 1.40

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

Table 2 : Table showing the levels of Total Amino acid nitrogen, Glutamic acid and Alanine in Blood of Normal Male and Diabetic Male Subjects

Parameter	Normal Male Subjects (n=40)	Diabetic Male Subjects (N=65)
Total Amino acid nitrogen (mg/dl)	8.26 ± 0.72	15.17*** ± 4.38
Glutamic acid (mg/dl)	3.60 ± 0.72	8.92*** ± 2.38
Alanine (mg/dl)	2.94 ± 0.51	5.30** ± 1.33

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

Table 3 : Table showing the levels of Total Amino acid nitrogen, Glutamic acid and Alanine in Blood of Normal Females and Diabetic Females Subjects

Parameter	Normal Female Subjects (n=40)	Diabetic Female Subjects (N=38)
Total Amino acid nitrogen (mg/dl)	8.60 ± 0.71	17.70*** ± 3.92
Glutamic acid (mg/dl)	3.80 ± 0.72	11.53*** ± 2.61
Alanine (mg/dl)	3.12 ± 0.54	6.24** ± 1.47

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

Table 4 : Table showing the levels of Total Amino acid nitrogen, Glutamic acid and Alanine in Blood of Diabetic Male and Diabetic Female Subjects

Parameter	Diabetic Male Subjects (n=40)	Diabetic Female Subjects (N=65)
Total Amino acid nitrogen (mg/dl)	15.17 ± 4.38	17.70 ± 3.92
Glutamic acid (mg/dl)	8.92 ± 2.38	11.53 ± 2.61
Alanine (mg/dl)	5.30 1.33	6.24 ± 1.47

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

Table 5 : Table showing the levels of Total Amino acid nitrogen, Glutamic acid and Alanine in Whole Blood Diabetic Subjects with different age-group

Parameter Age Group	Total Amino acid nitrogen (mg/dl)	Glutamic acid (mg/dl)	Alanine (mg/dl)
30-40 years (N=49)	15.75 ± 4.11	9.6 ± 2.09	8.44** ± 2.09
41-60 years (N=35)	15.04 ± 2.60	8.68 ± 0.30	4.03 ± 0.49

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

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We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The MARSM member can apply for approval, grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A.

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Institutional Fellow of Open Association of Research Society (USA) - OARS (USA)

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The "FARSC" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., FARSC or William Walldroff, M.S., FARSC.

The IFOARS institution is entitled to form a Board comprised of one Chairperson and three to five board members preferably from different streams. The Board will be recognized as "Institutional Board of Open Association of Research Society"-(IBOARS).

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The author fees of such paper may be waived off up to 40%.

The Global Journals Incorporation (USA) at its discretion can also refer double blind peer reviewed paper at their end to the board for the verification and to get recommendation for final stage of acceptance of publication.

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After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.

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- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

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> "
> - In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
>
> - In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
>
> - In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.
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2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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Complete support for both authors and co-author is provided.

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Review papers: These are concise, significant but helpful and decisive topics for young researchers.

Research articles: These are handled with small investigation and applications

Research letters: The letters are small and concise comments on previously published matters.

5.STRUCTURE AND FORMAT OF MANUSCRIPT

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

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

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

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

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

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

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

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

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

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

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

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

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

Acknowledgements: Please make these as concise as possible.

References

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

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1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

INFORMAL GUIDELINES OF RESEARCH PAPER WRITING

Key points to remember:

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

Final Points:

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

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

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

General style:

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

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

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

In every sections of your document

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

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

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

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

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

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

Title Page:

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

Abstract:

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

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

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

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

Approach:

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

Introduction:

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

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

Approach:

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

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

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

Materials:

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

Methods:

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

Approach:

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

What to keep away from

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

Results:

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

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

Content

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

What to stay away from

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

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

Approach

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

Figures and tables

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

Discussion:

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

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

Approach:

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

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

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