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VOLUME 16 ISSUE 3 VERSION 1.0

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Assessment of Volume Status of Hemodialysis Patients using Sonographic Lung Comets

By Hala S El-Wakil, Iman E El-Gohary, Doaa M. Emara & Reham Abd El Wahab

Alexandria University

Abstract- *Background:* Fluid balance is important in patients with renal impairment and undergoing hemodialysis. ''Dry'' weight is usually assessed clinically, and also, bioimpedance is considered reliable. The use of chest ultrasound to detect lung water received growing attention in clinical research in intensive care patients and in patients with heart failure. Recently ultrasonographic lung comets (counting B-lines artifact) evaluates extravascular lung water while ultrasonography of inferior vena cava (IVC) estimates central venous pressure, so ultrasound is considered as a useful tool to evaluate the hydration status of hemodialysis patients.

Objectives: The study was designed to use lung ultrasound to assess lung congestion before and after a dialysis session in correlation to clinical signs and symptoms and the achieved dry weight in end stage renal disease patients on maintenance hemodialysis.

Keywords: *hypervolemia, hemodialysis, dry weight, ultrafiltrtaion, lung comets score.*

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Assessment of Volume Status of Hemodialysis Patients using Sonographic Lung Comets

Hala S El-Wakil [α], Iman E El-Gohary [σ], Doaa M. Emara [ρ] & Reham Abd El Wahab [ω]

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Objectives: The study was designed to use lung ultrasound to assess lung congestion before and after a dialysis session in correlation to clinical signs and symptoms and the achieved dry weight in end stage renal disease patients on maintenance hemodialysis.

Methods: The present study included 25 patients on maintenance hemodialysis in Alexandria University Hospitals. All the patients were subjected to thorough history taking with special concern on grade of dyspnea and ultrafiltration volume, as well as full clinical examination before and after dialysis including vital signs and signs of hypervolemia as congested neck veins, fine basal crepitations, congested liver and lower limb edema. Radiological examination including ultrasound lung comets score and diameter of hepatic portion of inferior vena cava (IVC) before and after dialysis session.

Results: The mean lung comets score before dialysis was high and decreased significantly after dialysis. There was a significant positive correlation between ultrafiltration volume and the absolute change of lung comets score while there was no correlation between the ultrafiltration volume and the absolute change of IVC diameter. There was a significant correlation between lung comets score and grade of dyspnea before dialysis as well as after dialysis. There was a significant positive correlation between the grade of lung comets and IVC diameter both before and after dialysis.

Conclusions: Ultrasound lung comets score is a promising sensitive tool for assessment of the degree of lung congestion and hence the dry weight achievement in in end stage renal disease patients on maintenance hemodialysis.

Keywords: hypervolemia, hemodialysis, dry weight, ultrafiltrtaion, lung comets score.

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I. Introduction

In patients with end-stage renal disease (ESRD) on intermittent hemodialysis (HD), it is vital to maintain fluid status within an optimal range to avoid circulatory complications. Clinical assessment of body weight change, Neck veins congestion, edema together with blood pressure and chest x-ray are usually used for evaluation of fluid status.[1] However, clinical evaluation alone is not accurate enough for evaluation of HD patients, so other methods such as biochemical markers, bio-impedance analysis and inferior vena cava diameter have been developed to assess the fluid status, yet no single method is considered a gold standard and combination of more than one method should be used for more accurate assessment.[1,2]

The main issue for the achievement of dry weight in HD patients is that ultrafiltration should be tailored to the individual patient's hemodynamic tolerance taking into account cardiac performance, which is very often compromised in ESRD patients.[3]

Lung ultrasound is simple, non-invasive, non-ionizing, available, and inexpensive which is suitable for the assessment of ideal body weight in maintenance hemodialysis (MHD) patients.[4-7] Moreover, lung comets can be used in association with IVC diameter for more accurate assessment of dry weight in HD patients.[8]

So, the aim of this work was to use the lung ultrasound to assess lung congestion before and after a dialysis session in correlation to clinical signs and symptoms and the achieved dry weight in end stage renal disease patients on maintenance hemodialysis.

II. Patients & Methods

The present study included 25 patients on maintenance hemodialysis in Alexandria University Hospitals.Patients with congestive heart failure, those with any problem in the right side of the heart and patients with interstitial lung fibrosis, lung malignancy or mediastinal syndrome and obese patients were excluded from the study. An informed consent was taken from all patients and the study was conducted according to the declaration of Helsinki.

All the patients were subjected to thorough history taking with special concern on grade of dyspnea (it is assessed by The New York Heart Association (NYHA) classification) and ultrafiltration volume, as well as full clinical examination before and after dialysis

including vital signs (Blood pressure; supine and standing position, respiratory rate, pulse, and temperature were measured before and after the dialysis session) and signs of hypervolemia as congested neck veins, fine basal crepitations, congested liver and lower limb oedema. Routine laboratory investigations were done once before dialysis. Radiological examination including ultrasound lung comets score and diameter of hepatic portion of inferior vena cava (IVC) before and after dialysis session. We were using a commercially available ultrasonograghic equipment (Siemens medical solution, with 5-10 MHz linear or 2–5 MHz convex probe).The time needed for the chest US ranged between 10 to 15 min. All patients were subjected to chest U/S examination for lung comets measurements before and within 6 hours after the dialysis session for the assessment of the lung congestion. Patients were in a supine position during the examination. Ultrasound examination of the anterolateral chest was carried out with longitudinal scan of the right and left hemi-thoraces, from the second to the fourth (on the right side to the fifth) intercostal space. In each intercostal space, the number of B-lines was counted at the parasternal, midclavicular, anterior axillary, and midaxillary lines for a total of 28 sectors examined. The total number of B-lines was the sum of the artefacts recorded in the 28 sectors explored yielding a score called lung comet score. The collected data were recorded in a table and the lung comets scores for each patient before and after dialysis, the absolute change of lung comets score and the percentage change of the lung comets score were calculated.[7]

III. Results

The patients were classified into three groups according to their lung comets grades (mild, moderate, severe) before and after dialysis:

a) Lunge comets grades before dialysis

The patients were classified as follow: only one patient had mild lung comets grade (4%), two patients had moderate lung comets grade (8%), 22 patients had severe lung comets grade (88%), Table (I)

b) Lung comets grades after dialysis

The patients were classified as follow: 6 patients had mild lung comets grade (24%), 9 patients had moderate lung comets grade (36%), 10 patients had severe lung comets grade (40%), Table (I).

The patients were classified before dialysis into groups according to presence of dyspnea (NYHA class II, III,VI) or absence of dyspnea (NYHA class I) and the grade of lung comets (mild, moderate, severe):

25 patients had dyspnea (NYHA class II, III, VI) (100%) before dialysis and they were classified as follow: one patients had mild lung comets grade and the remaining 24 patients had either moderate or severe lung comets grade, Table (II).

The patients were classified after dialysis into groups according to presence of dyspnea (NYHA class II, III,VI) or absence of dyspnea (NYHA class I) and the grade of lung comets (mild, moderate, severe):

Out of the 25 patients there were 6 patients that had no dyspnea (NYHA class I) after dialysis and they were classified as follow: 5 patients had mild lung comets grade (20%) and one patient had moderate lung comets grade (4%) while 19 patients had dyspnea (NYHA class II, III,VI) and they were classified as follow: 18 patients had either moderate or severe lung comets grade (72%) while only one patient had mild lung comets grade (4%). Table (II).

There was a significant correlation between lung comets score before dialysis and NYHA class of dyspnea before dialysis, Table (III).

There was a highly significant correlation between lung comets score after dialysis and NYHA class of dyspnea after dialysis, Table (III).

Table (IV) shows the correlation between lung comets score and grade before and after dialysis, their percent and absolute change and clinical data (blood pressure, pulse and respiratory rate).

There was a significant correlation between ultrafiltration volume and the lung comets score absolute change while there was no correlation between the ultrafiltration volume and the IVCD absolute change or IVCD percentage change or the lung comets percentage change Table (V).

Table (VI) shows the correlation between lung comets and IVC diameter before and after dialysis.

IV. Discussion

The mean age of the studied group was 47.39 years that is comparable with other studies in the developing countries in which the age of hemodialysis patients' age ranged between 32 – 42 years while the age of our patients were much lower than that in the developed world in which the hemodialysis patients' age ranged between 52 to 63 years.[9-11] Among the reasons for this difference are the delay in detecting renal disease and the failure to institute controlling and preventive measures in patients with progressive renal failure, both of which result in faster deterioration of renal function and progression to ESRD. Late referrals lead to a faster progression of co-morbid conditions, increase the cost of therapy, and worsen overall patient survival as mentioned in a study conducted by Kher[10] that studied the end stage renal disease in the developing countries.

In our study, patients with interstitial lung fibrosis were excluded because the thickened interlobular septae characterizing fibrosis may not be modified by the state of hydration or congestion.[7] We also excluded the presence of lung malignancy and mediastinal lesions to avoid their effect in development of dyspnea or orthopnea in the studied patients and to

avoid the pulmonary congestion resulted from pulmonary veins compression that may be encountered in case of mediastinal lesions.[12,13] We also excluded obesity as large body habitus also degrades image quality, making it difficult or impossible to obtain adequate images for clinical interpretation.[14]

In our patients three main underlying cause of chronic kidney disease were found to be the hypertension (28%) followed by chronic glomerulonephritis (20%) and diabetes (16%) and this quietly matches the result of a study conducted by Barsoum et al, about burden of chronic kidney disease in North Africa that showed hypertension, glomerulonephritis and diabetes as the major underlying cause of chronic kidney disease.[15]

In the present study hypertension was found in 80 % of our patients, which means that most of our cases suffer from high risk of developing cardiovascular complications.[16-18] Our result is relatively comparable with results found in several studies like that conducted by Portolés et al,[19]

In our study there was a significant reduction in both of systolic supine blood pressure, Diastolic supine blood pressure, the systolic standing blood pressure and the diastolic standing blood pressure after dialysis in comparison to predialysis values. The mean blood pressure before dialysis for the whole group ranged between 80-133.33 mmHg with a mean of 111.33±15.25 mmHg while the mean blood pressure after dialysis for the whole group ranged between 70-116.67mmHg with a mean of 90.93 ±14.58 mmHg with a significant change .The mean blood pressure significantly reduced towards normal range and this could be attributed to the underlying pathology of hypertension found among our cohort to be volume dependent. This matches a study conducted by Lazarus et al,[20] who confirmed that removing excess salt and water during maintenance hemodialysis normalizes BP in at least 70% of their cases and attributed to that extracellular volume expansion causes hypertension in approximately 75% of patients with chronic renal failure and therefore their cases were found to be responsive to hemodialysis.

In the present study 68% of the whole group were receiving calcium channel blockers as antihypertensive drugs which means that calcium channel blockers (CCBs) are widely used in this category of hypertensive patients on maintenance hemodialysis that matches a study conducted by Kestenbaum et al,[21] that showed that greater than half of the ESRD were receiving calcium channel blockers and a lower relative risk of mortality reported in patients taking a calcium channel blocker. The use of any calcium channel blockers was associated with a 21% lower risk of all cause mortality and a 26% lower risk of cardiovascular specific mortality.

The lung comets score before dialysis in our study ranged between 7-136 with a mean of 54.72± 28.47 while the lung comets score after dialysis for whole patients ranged between 3-74 with a mean of 28.52±19.88 with a significant change (p=0.00). That matches a study conducted by Noble et al.[22]

We also found a significant correlation between lung comets score before dialysis and NYHA class of dyspnea before dialysis and a highly significant correlation between lung comets score after dialysis and NYHA class of dyspnea after dialysis. This means that the lung comets score is a more sensitive tool in achieving dry weight rather than the clinical examination only and it reflects the state of the hypervolemia, especially in the extra vascular lung water (EVLW) compartment, that is considered an important factor related to the risk for the cardiac compromise.

Our results showed that 6 patients having pulmonary congestion, as evidenced by presence of mild lung comets in 5 patients out of them and moderate degree of lung comets in one patient of them after hemodialysis, however, they did not show any clinical manifestations and they had no dyspnea with their ordinary physical activity "NYHA class I" and this demonstrates the sensitivity of the lung comets as a marker for pulmonary congestion in asymptomatic patients, therefore the lung comets could be the only indicator for lung congestion in the preclinical phase in hemodialysis patients. This result matches a study conducted by Mallamaci et al.[5]

There was a significant positive correlation between lung comets grade and IVCD before and after dialysis and also between the lung comets score and the IVCD. This reflects the reliability of the lung comets score in assessment of the hydration state in relation to the other reliable tool like IVCD. It could be used as an easy tool for hydration state assessment in comparison to IVCD which is somewhat difficult maneuver needing professional skills. Our result matches a study conducted by Basso et al.[23]

In our study, there was a highly significant positive correlation between absolute change of lung comets after dialysis and body ultrafiltration volume during dialysis and this matches with the study done by Vitturi et al.[24]

In the present, we found that there was a significant reduction in IVCD after dialysis but there was no correlation between the ultrafiltration volume and the IVCD absolute change or IVCD percentage change, in contrast to the significant correlation found between the lung comets absolute score change and the ultrafiltration volume. This indicates the superiority of ULCs over IVCD as a marker to ultrafiltration volume.

V. Conclusion

Ultrasound lung comets score is highly correlated with the clinical signs and symptoms and even may precede the development of symptoms in hemodialysis patients. Moreover, lung comets score is highly correlated with ultrafiltration volume, thus, it could be used as a good marker for achieving dry weight in dialysis patients. Furthermore, ultrasound lung comets score is more superior to IVCD in assessing the volume status in hemodialysis patients and hence the target dry weight for those patients.

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Table (I) : Demonstrates patients' lung comets grades before and after dialysis

Lung comets grade	Before dialysis		After dialysis	
	Frequency	Percent	Frequency	Percent
Mild	1	4	6	24
Moderate	2	8	9	36
Severe	22	88	10	40
Total	25	100	25	100

Table (II) : Classification of the patients before and after dialysis according to presence or absence of dyspnea and the grade of lung comets (mild, moderate, severe)

Parameter	Before dialysis				After dialysis			
	Without dyspnea "NYHA class I"		With dyspnea "NYHA class II,III,VI"		Without dyspnea "NYHA class I"		With dyspnea "NYHA class II,III,VI"	
	Frequency	Percent	Frequency	Percent	Frequency	Percent	Frequency	Percent
Mild lung comets degree	0	0	1	4	5	20	1	4
Moderate or severe lung comets degree	0	0	24	96	1	4	18	72
Total number	0	0	25	100	6	24	19	76

Table (III) : Correlation between dyspnea (assessed by NYHA classification) and lung comets score before and after dialysis

		Lung comets score before dialysis	Lung comets score after dialysis
Dyspnea before dialysis	r	0.418*	0.463*
	p	0.037	0.020
Dyspnea after dialysis	r	0.496*	0.635**
	p	0.012	0.001

Pearson Correlation (r)
*Correlation is significant ≤ 0.05 level (2-tailed).
**Correlation is highly significant at ≤ 0.01 level (2-tailed).

Table (IV) : Correlation between ultrasound lung comets score and ultrasound lung comets grade before and after dialysis with clinical data

Parameters		ULCs score before dialysis	ULCs grade before dialysis	ULCs grade after dialysis	ULs score after dialysis	ULCs score percentage change	ULCs score Absolute Change
Mean supine BP before dialysis	r	0.222	0.281	0.358	0.286	0.406*	-0.049
	p	0.285	0.173	0.079	0.166	0.044	0.816
Mean Standing BP before dialysis	r	0.230	0.266	0.302	0.233	0.276	-0.144
	p	0.270	0.198	0.142	0.262	0.181	0.491
Mean supine BP after dialysis	r	0.266	0.305	0.427*	0.307	0.390	-0.111
	p	0.199	0.138	0.033	0.135	0.054	0.597
Mean standing BP after dialysis	r	0.319	0.356	0.363	0.286	0.204	0.257
	p	0.120	0.080	0.075	0.165	0.327	0.215
Pulse before dialysis	r	0.606**	0.192	0.639**	0.682**	0.543**	-0.281
	p	0.001	0.359	0.001	0.000	0.005	0.174
Pulse after dialysis	r	0.598**	0.225	0.719**	0.720**	0.698**	-0.206
	p	0.002	0.281	0.000	0.000	0.000	0.323
Pulse percentage change	r	-0.071	0.042	0.053	0.002	0.209	0.156

	p	0.737	0.841	0.800	0.992	0.317	0.457
RR before dialysis	r	0.485*	0.080	0.476*	0.482*	0.342	-0.321
	p	0.014	0.702	0.016	0.015	0.095	0.118
RR after dialysis	r	0.489*	0.004	0.561**	0.580**	0.554**	-0.180
	p	0.013	0.985	0.004	0.002	0.004	0.388
RR percentage change	r	0.039	-0.186	0.173	0.211	0.407*	0.234
	p	0.853	0.373	0.407	0.311	0.044	0.260

Pearson Correlation (r)
*Correlation is significant ≤ 0.05 level (2-tailed).
**Correlation is highly significant at ≤ 0.01 level (2-tailed).

Table (V) : Correlations between changes of lung comets score (absolute change, percentage changes) and IVCD changes (absolute change, percentage changes) and ultrafitration volume

		Lung comets score absolute change	Lung comets score percentage change	Ultrafiltration volume (UF)
IVCD Absolute change	r	0.228	-0.003	0.305
	p	0.362	0.990	0.219
IVCD percentage change	r	0.313	0.287	0.298
	p	0.207	0.248	0.230
Ultrafiltration volume (UF)	r	0.564**	-0.012	1
	p	0.003	0.955	

Pearson Correlation (r)
*Correlation is significant ≤ 0.05 level (2-tailed).
**Correlation is highly significant at ≤ 0.01 level (2-tailed).

Table (VI) : Correlation between ultrasound lung comets and inferior vena cava diameter

		ULCs score before dialysis	ULCs score After dialysis	ULCs grade before dialysis	ULCs grade after dialysis	ULCs score percentage change	ULCs Score absolute change
IVCD before dialysis	r	0.432	0.552*	0.650**	0.688**	0.496*	-.0164
	p	0.073	0.018	0.004	0.002	0.036	0.514
IVCD after dialysis	r	0.359	0.557*	0.559*	0.652**	0.628**	-0.013
	p	0.143	0.016	0.016	0.003	0.005	0.960
IVCD percentage change	r	-0.152	0.007	-0.174	0.010	0.287	0.313
	p	0.548	0.979	0.491	0.969	0.248	0.207
IVCD absolute change	r	-0.221	-0.169	-0.310-	-0.258	-0.003	0.228
	p	0.378	0.503	0.210	0.302	0.990	0.362

Pearson Correlation (r)
*Correlation is significant ≤ 0.05 level (2-tailed).
**Correlation is highly significant at ≤ 0.01 level (2-tailed).

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Copper (II) Oxide Nanoparticles Induce High Toxicity in Human Neuronal Cell

By Abudayyak Mahmoud, Guzel E. Elif & Özhan Gül
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Abstract- Copper (II) oxide nanoparticles (CuO-NPs) are widely used in industry, cosmetics and medicine. People have increasingly been exposed to these active materials. Several studies indicate that CuO-NPs could be taken up by different organs and cause toxicities. However, there is still a lack of data on the toxicological effects of CuO-NPs in neuronal system. In the present study, the toxic potentials of CuO-NPs were investigated in human SH-SY5Y neuroblastoma cells. After assessment of their cellular uptake potential, cytotoxicity by MTT and neutral red uptake (NRU) and genotoxicity by comet assay were evaluated. Enzyme-Linked Immune Sorbent Assays (ELISA) determination of malondialdehyde (MDA), 8-hydroxy-deoxyguanosine (8-OHdG), protein carbonyl (PC), and glutathione (GSH) levels for oxidative damage, and Annexin V-FITC with propidium iodide (PI) for apoptosis were used. In conclusion, CuO-NPs were found to accumulate in the cells and induced significant cytotoxic and genotoxic, and oxidative and apoptotic effects. CuO-NPs are hypothesized to dangerously affect human health, especially neuronal system.

Keywords: *copper oxide; nanoparticle; neurotoxicity; cellular uptake; genotoxicity; apoptosis.*

GJMR-B Classification : *NLMC Code: QV 600*

Strictly as per the compliance and regulations of:

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Abudayyak Mahmoud [α], Guzel E. Elif [σ] & Özhan Gül [ρ]

Abstract- Copper (II) oxide nanoparticles (CuO-NPs) are widely used in industry, cosmetics and medicine. People have increasingly been exposed to these active materials. Several studies indicate that CuO-NPs could be taken up by different organs and cause toxicities. However, there is still a lack of data on the toxicological effects of CuO-NPs in neuronal system. In the present study, the toxic potentials of CuO-NPs were investigated in human SH-SY5Y neuroblastoma cells. After assessment of their cellular uptake potential, cytotoxicity by MTT and neutral red uptake (NRU) and genotoxicity by comet assay were evaluated. Enzyme-Linked Immune Sorbent Assays (ELISA) determination of malondialdehyde (MDA), 8-hydroxy-deoxyguanosine (8-OHdG), protein carbonyl (PC), and glutathione (GSH) levels for oxidative damage, and Annexin V-FITC with propidium iodide (PI) for apoptosis were used. In conclusion, CuO-NPs were found to accumulate in the cells and induced significant cytotoxic and genotoxic, and oxidative and apoptotic effects. CuO-NPs are hypothesized to dangerously affect human health, especially neuronal system. However, further studies should be done to elucidate their toxic mechanism.

Keywords: copper oxide; nanoparticle; neurotoxicity; cellular uptake; genotoxicity; apoptosis.

I. Introduction

CuO-NPs are widely used in gas sensors, catalysts, high temperature conductors, solar energy converters and antimicrobial agents owing to their high temperature conductivity, electron correlation effects, antimicrobial activity and special physicochemical properties in various fields (Chang et al., 2012; Huang et al., 2010). Indeed, as it is well known, nanoparticles exist as contaminants in water, air and food products as outputs of natural phenomena or due to the high increase in the anthropogenic activity (Ahamed et al., 2013; Elsaesser et al., 2011; Kim et al., 2010). CuO-NPs caused changes in different organs like lung, kidney, renal tubular, liver, spleen, gastrointestinal tract and stomach tissue (Barceloux, 1999; Cho et al., 2012; Lei et al., 2008; Manna et al., 2012). Acute death, abnormalities in the embryo and gill damage were observed in Zebra fish exposed to CuO-NPs (Griffitt et al., 2007; Yeo et al., 2009). The toxicity studies of CuO-NPs have been focused more generally on the pulmonary system and to a lesser extent on skin, breast, intestine and liver (Ahamed et al., 2010; Akhtar et al., 2012; An et al., 2012; Cuillel et al., 2014; Laha et al., 2014; Piret et al., 2012; Siddiqui et al., 2013; Sun et al., 2011; Wang et al., 2011). However, there are few reports on the nervous system (An et al., 2012; Chen et al., 2008; Perreault et al., 2012). Therefore, it was aimed to evaluate the toxicity and possible mechanism of action of CuO-NPs in neuroblastoma cells following their cellular uptake potential.

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II. Materials and Methods

Chemicals: Eagle's minimum essential medium (EMEM), fetal bovine serum (FBS), phosphate buffered saline (PBS, 10X), antibiotic solutions and ethylene diamine tetraacetic acid (EDTA) were purchased from Multicell Wisent (Quebec, Canada). Triton X-100 and MTT(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) were purchased from Biomatik (Ontario, Canada). GSH, 8-OHdG, MDA and PC ELISA kits were purchased from Yehua Biological Technology Co., Ltd. (Shanghai, China). Annexin V-FITC apoptosis detection kit with PI and dye reagents for protein assay were obtained from Exbio (Vestec, Czech Republic) and Bio-rad (Munich, Germany), respectively. All other chemicals were obtained from Merck (NJ, USA).

CuO-NPs were obtained from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). The CuO-NPs suspensions in milli-Q water and cell culture medium with 10% FBS, were measured by Transmission Electron Microscopy (TEM) (Jem-2100 HR, Jeol, USA) (Abudayyak et al. 2016; 2016a). The average diameter was calculated by measuring over 100 particles in random fields of TEM view.

Copper release into cell medium: Copper release from CuO-NPs into the cell culture medium was determined using the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Thermo Elemental X series 2, USA) method (Abudayyak et al. 2016; 2016a). The released amount of copper was analyzed by ICP-MS. Cu content of the cell culture medium was also measured.

Cell culture conditions: Human neuronal cell line (SH-SY5Y) was obtained from the American Type Culture

Collection (CRL-2266™, ATCC, VA, USA). The cells were incubated in EMEM medium supplemented with FBS 10% and antibiotics at 5% CO_2, 90% humidity and 37°C for 24 h (60-80% confluence). Cell densities were in the range from 1×10^5 to 1×10^7 cells/mL for all assays (Abudayyak et al. 2016; 2016a). Exposure occurred for 24 h.

Cellular uptake and morphology examinations: It was evaluated by ICP-MS and TEM (Abudayyak et al. 2016; 2016a). The cells were washed several times with equal volumes of PBS and cell culture medium with 10% FBS and counted via Luna cell counter (Virginia, USA) following exposure to two different concentrations of the particle suspension (2.5 and 25 µg/mL). Ultra-thin sections (50-60 nm) were cut by an ultra-microtome (Reichert UM 3, Austria). Sections were analyzed and photographed using a TEM (Jeol-1011, Tokyo, Japan) with attached digital camera (Olympus-Veleta TEM Camera, Tokyo, Japan).

Cytotoxicity assays: Cytotoxic activities of CuO-NPs on SH-SY5Y cells were determined by MTT and NRU assays based on different cellular mechanisms (Abudayyak et al. 2016; 2016a; Repetto et al., 2008; Van Meerloo et al., 2011). Optical density (OD) values were read at 590 and 540 nm for MTT and NRU, respectively, using a microplate spectrophotometer system (Epoch, Germany). In every assay, unexposed cells were served as a negative control. The inhibition of enzyme activity was calculated as compared to a negative control. The half-maximal inhibitory concentration (IC_{50}) was then expressed as the concentration of the sample causing a 50% inhibition of enzyme activity in cells. The CuO-NP concentrations were 2.5-60 µg/mL in the cytotoxicity assays.

Genotoxicity assay: Genotoxic activities of CuO-NPs were determined by comet assay (Abudayyak et al. 2016; 2016a; Collins et al., 2004; Speit et al., 1999). Hydrogen peroxide (H_2O_2) (100 µM) and PBS were used as positive and negative controls, respectively. The number of DNA breaks was scored under a fluorescent microscope (Olympus BX53, Olympus, Tokyo, Japan) at 400X magnification using an automated image analysis system (Comet Assay IV, Perceptive Instruments, Suffolk, UK). DNA damage to individual cells was expressed as a percentage of DNA in the comet tail (tail intensity %). The CuO-NP concentrations were 5-50 µg/mL in the comet assay.

Oxidative damage assays: The oxidative damage potentials of CuO-NPs were measured by human GSH, MDA, 8-OHdG, or PC ELISA kits with different endpoints according to the manufacturer's instructions. The OD value was read at 450 nm using a microplate spectrophotometer system. In every assay, the unexposed cells served as a negative control. The protein amount in 10^6 cells was measured according to Bradford (1976). Results were expressed as µmol, µmol, µg, and µg per g protein for GSH, MDA, 8-OHdG, and PC, respectively, using a standard calibration curve. The CuO-NP concentrations were 5-25 µg/mL in the oxidative damage assays.

Apoptosis assay: The cellular apoptosis or necrosis was determined by Annexin V-FITC apoptosis detection kit with PI (Abudayyak et al. 2016; 2016a). In every assay, the untreated cells served as a negative control. The results were expressed as a percentage of the total cell amount. The CuO-NP concentrations were 10-80 µg/mL in the apoptosis assay.

Statistical analysis: The assays were done in triplicate and repeated four times. Data were expressed as mean±standard deviation (SD). Significant differences between untreated and treated cells were calculated by one-way ANOVA Dunnett t-test using SPSS version 17.0 for Windows. *p* values of less than 0.05 were considered significant.

III. Results and Discussion

Particle size and distribution: According to the X-ray diffraction results supplied by the manufacturer (Sigma Chemical Co. Ltd., USA), the surface area of CuO-NPs was 29 m^2/g (Figure 1). The average size was observed to be 34.9 nm with a narrow size distribution (ranging from 16.7-64.2 nm) after suspending in water. When suspending in the culture medium, the size of the particles was found to be slightly agglomerated and/or aggregated with 38.8 nm (ranging from 18.8-73.8 nm) (Figure 2). The copper ion release of CuO-NPs was evaluated in the cell culture medium. Although the concentration was 3.1 ± 0.322 µg/mL, which represented 15.5% of the nanoparticles, in the CuO-NPs cell culture suspension, there was no observed copper ions in the cell culture medium. Based on that, the observed toxicological endpoints and morphological changes were mainly due to CuO-NPs.

Cellular uptake: ICP-MS revealed that the particles were taken up by SH-SY5Y cells in the range of 0.390-0.917 $\mu g/10^5$ cells in concentration dependent manner following exposure to CuO-NPs at 5-25 µg/mL concentrations (Table 1). Some researchers reported iron oxide and two different types of titanium dioxide nanoparticles to enter SH-SY5Y cells in concentration dependent manner (Kilic et al., 2016; Valdiglesias et al., 2013).

Cellular morphology by TEM: The particles were observed in the cytoplasmic vacuoles. Mitochondria were visible in few of the cells exposed to both 2.5 and 10 µg/mL CuO-NPs. Some cells exposed to 2.5 µg/mL CuO-NPs revealed nuclear fragmentation. The electron-lucent cytoplasmic vacuoles lead to complete disruption of the cytoplasm in few of the cells (Figure 3).

Cytotoxicity: IC_{50} values of CuO-NPs were 25.49±2.06 and 7.27±0.843 µg/mL by MTT and NRU assay,

respectively. The reduction in cell viability was concentration-dependent (Figure 4). The CuO-NPs were found to cause cytotoxic effects to HaCaT keratinocytes, BALB3T3 embryonic fibroblasts (Akhtar et al., 2012; Kilic et al., 2016), HepG2 (Siddiqui et al., 2013; Wang et al., 2011), A549 lung epithelial (Karlsson et al., 2008; Wang et al 2012), HEp-2 airway epithelial (Wang et al 2012), Caco-2 intestinal (Piret et al., 2012), cardiac microvascular endothelial cells (Sun et al., 2011) and primary culture of channel catfish hepatocytes (Wang et al., 2011). Perreault et al. (2012) found mouse N2A neuroblastoma cell viability decreased to 63% at 400 μg/mL for 24 h. Chen et al. (2008) reported CuO-NPs showed the cytotoxic effect in SH-ST5Y neuroblastoma and H4 neuroglioma cells were dose-dependent. It caused a drop of 60 and 40% in live cell percentages in SH-ST5Y and H4 cells, respectively, at 100 μM concentration.

Genotoxicity: In positive controls (100 μM H_2O_2), the tail intensity was 21.43%. The results revealed that CuO-NPs significantly induced DNA damage in all exposure concentrations (2.57-7.09 fold) and generally in dose dependent manner ($p \leq 0.05$). The highest tail intensity was 24.09 observed at a concentration of (15 μg/mL). The cell death was ≤50% in all concentrations (Figure 5). CuO-NPs induced genotoxic responses in A549 (Ahamed et al., 2010; Akhtar et al., 2016; Cronholm et al., 2011; Wang et al., 2012) and BEAS-2B lung epithelial cells (Cronholm et al., 2011). Perreault et al. (2012) found CuO-NPs significantly induced DNA damage in mouse N2A neuroblastoma cells at 12.5 μg/mL. Researchers suggested CuO-NPs induced DNA damage significantly correlated with reactive oxygen species (ROS) (Akhtar et al., 2016). Also, it could be via disruption of cell membrane integrity (Cronholm et al., 2011). However, there was no study about genotoxicity on SH-SY5Y cells.

Oxidative damage: The oxidative damage potential of CuO-NPs was evaluated by measuring cellular levels of GSH, MDA, 8-OHdG, and PC (Table 2). CuO-NPs induced oxidative damage resulting in significant decrease in the GSH levels (≤46.1%). Although an increase on the levels of MDA (≤1.33 fold) was observed it was not significant. On the other hand, the levels of PC and 8-OHdG protein and DNA oxidative damage biomarkers did not change. In previous studies, it was observed that CuO-NPs induced oxidative damage in HaCaT keratinocytes (Alarifi et al., 2013), BALB3T3 fibroblasts (Akhtar et al., 2012), A549, (Ahamed et al., 2010; Akhtar et al., 2013; Karlsson et al., 2008; Kim et al., 2010), HEp-2 (Fahmy and Cormier, 2009), and HepG2 cells (Piret et al., 2012; Siddiqui et al., 2013). The reduction in cell viability observed could be due to an increase in oxidative stress after CuO-NPs exposure.

Apoptosis: Death in SH-SY5Y cells was significantly induced by CuO-NPs, with a maximum percentage of 73.4 and 40.0% for apoptosis and necrosis, respectively. According to our results, apoptosis was seen to be the main pathway for cell death in the SH-SY5Y cell line. At the highest exposure concentration (40 μg/mL), the apoptosis percentage was 79.2% of the dead cells (Figure 6). The previous studies showed CuO-NPs could induce apoptosis in the following cells: MCF7 breast cancer (Laha et al., 2014), HepG2 (Siddiqui et al., 2013), and Caco-2 cells (Piret et al., 2012). In rats, CuO-NPs induced apoptosis via increased cleaved caspase-3 levels (An et al., 2012). Siddiqui et al. (2013) observed CuO-NPs induced apoptosis via a decrease in mitochondrial membrane potential with a concomitant increase in the gene expression ratio of Bax/Bcl2, up-regulation of p53 tumour suppressor and caspase-3 apoptotic genes. Also, the researchers showed apoptosis could be induced by reduction of BAD phosphorylation and an increase in cleaved caspase-3 products (Laha et al., 2014). An et al. (2012) indicated that the apoptosis and cognitive impairment could be via increased cleaved caspase-3 levels on hippocampal CA1 neuron in rats.

IV. Conclusion

Generally, the studies about Cu based nanoparticles and CuO-NPs were focused on the pulmonary system. However, very few researchers were concerned about the possible toxicity over other systems. In the present study, it was observed that CuO-NPs taken up by the neuronal cells could produce cytotoxic, genotoxic, and apoptotic effects, as well as oxidative damage in the neuronal cells *in vitro*. Their commercial and industrial applications should be carefully evaluated because of their potential hazardous effects on human health. Further *in vivo* studies are needed to fully understand the toxicity mechanisms of CuO-NPs.

V. Acknowledgement

This work was supported by the Research Fund of Istanbul University (Project No: 52253). Dr. M. Abudayyak carried out cell culture and exposure conditions, the toxicological assays and the particle characterisation. Prof. Dr. G. Özhan participate the toxicological assays and carried out the evaluation of the results. Dr. E. Guzel carried out the uptake and morphological changes in the cells. All authors wrote, read and approved the manuscript. Also, the authors declare there is no conflict of interest.

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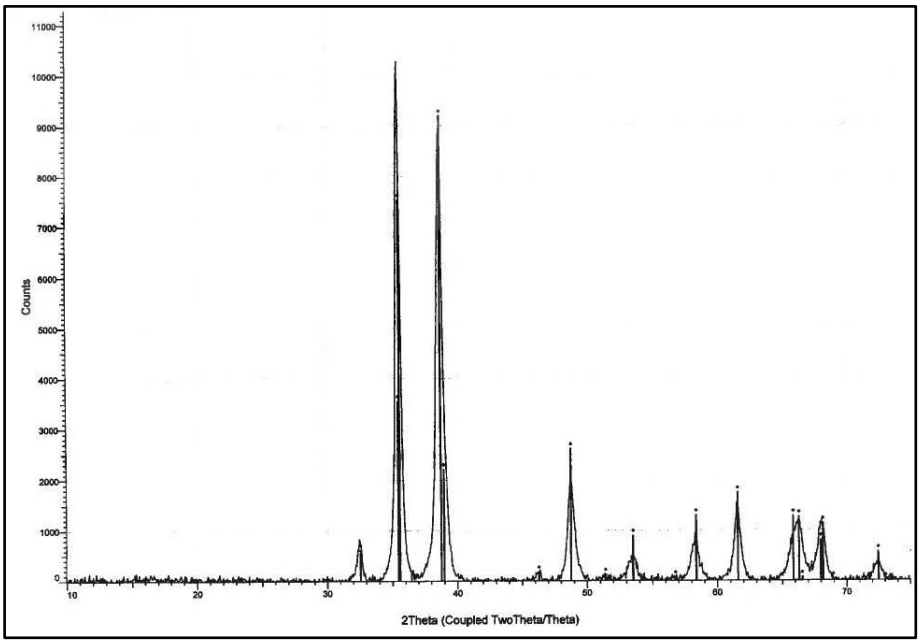


Figure 1 : The X-ray diffraction analysis of CuO-NPs.

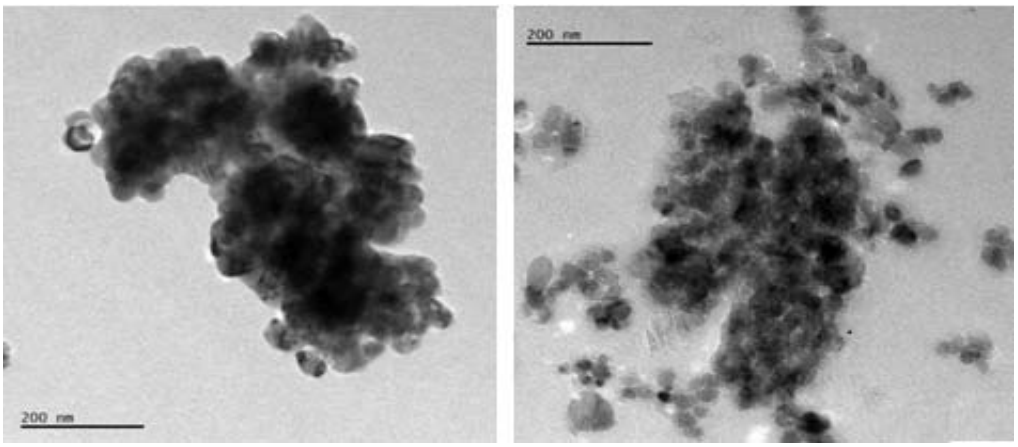


Figure 2 : The TEM images and size distributions of CuO-NPs after dissolution in water (a) and cell culture medium (b).

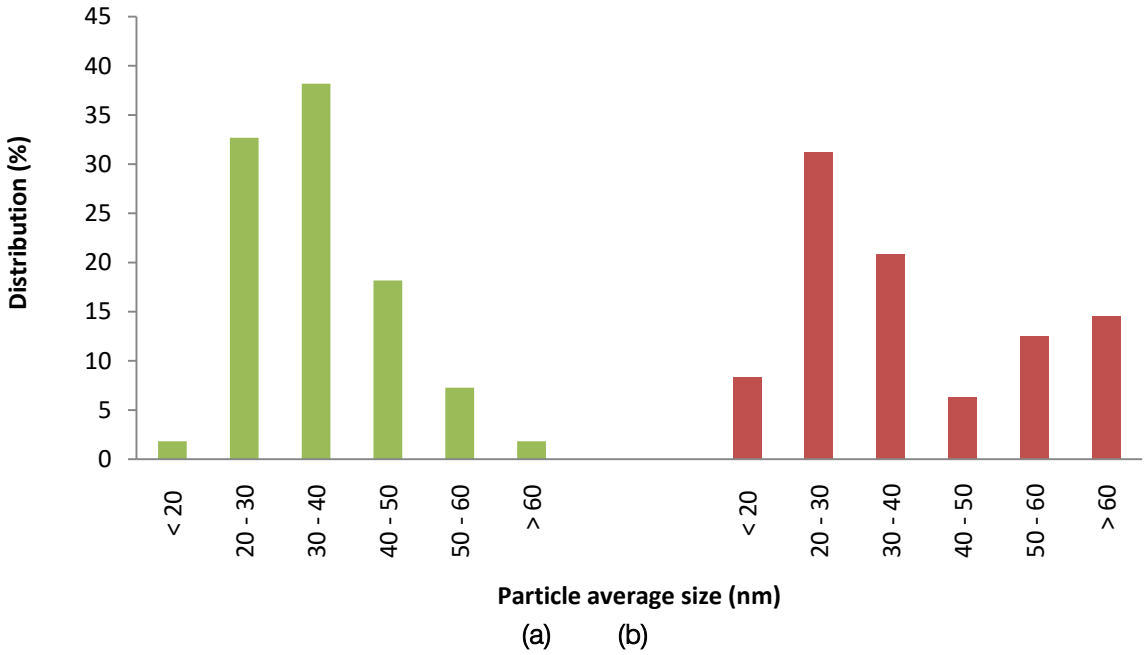


Figure 3 : TEM observations of SH-SY5Y cells after exposure to CuO-NPs. (a) cells exposed to CuO-NPs at 2.5 μg/mL; (b) cells exposed to CuO-NPs at 10 μg/mL; (c) unexposed cell (negative control).

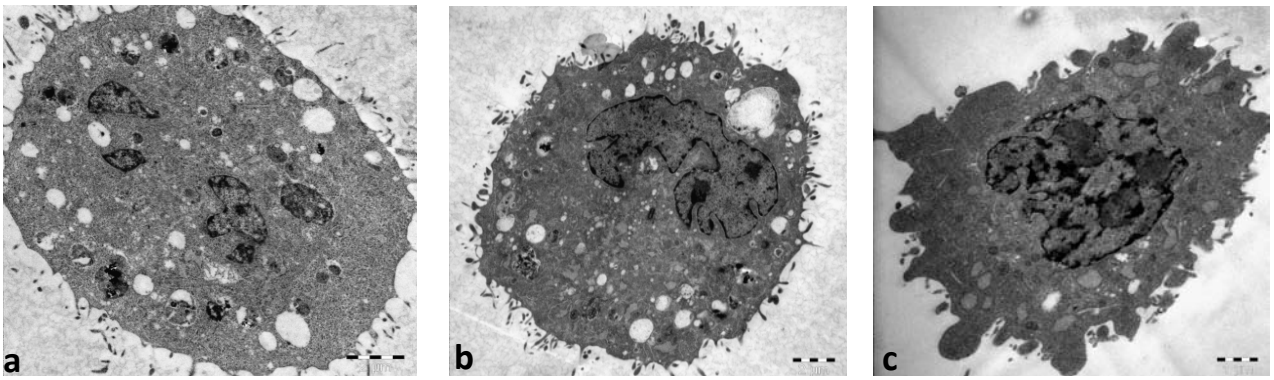


Figure 4 : Effects of CuO-NPs on SH-SY5Y cell viability.

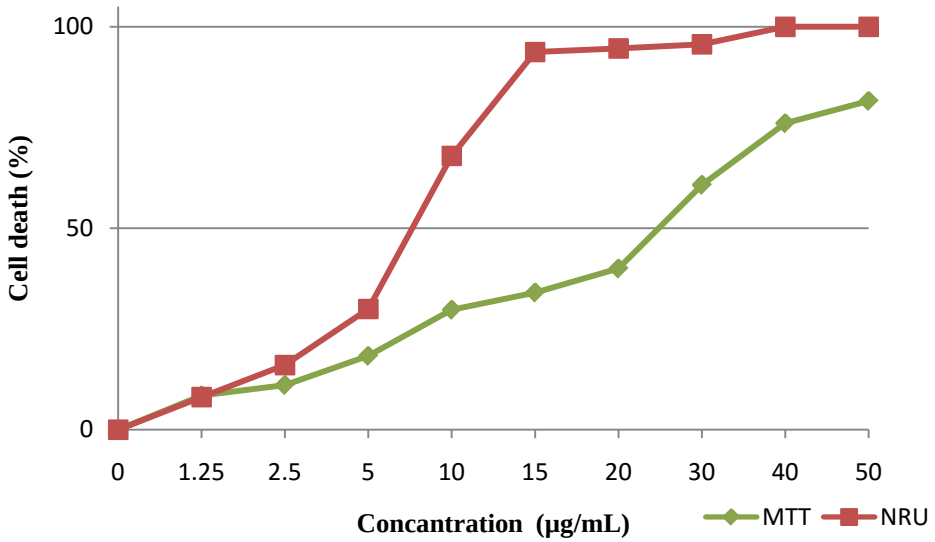


Figure 5 : Evaluation of DNA damage potential of CuO-NPs in SH-SY5Y cells.
All experiments were done in triplicate and each assay was repeated four times.
The results were expressed as the mean cell death (%) compared to negative control (unexposed cell).

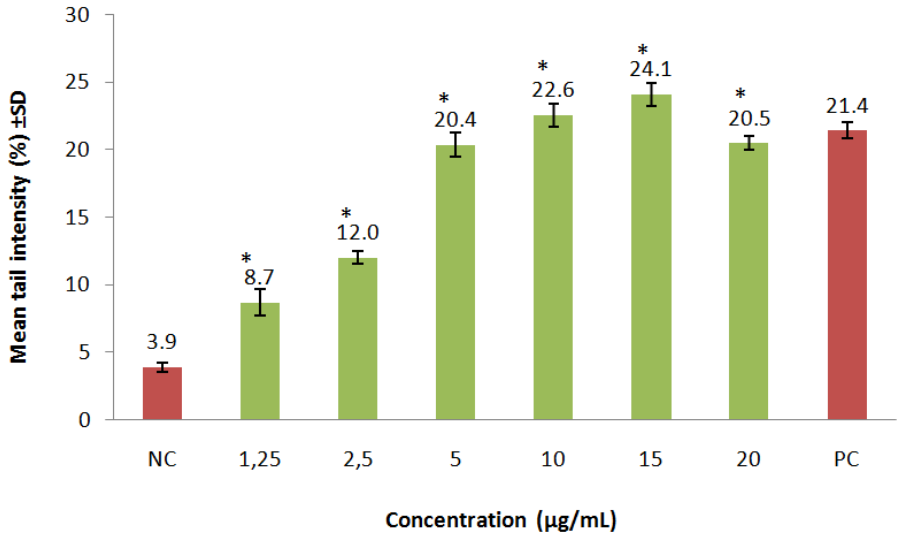
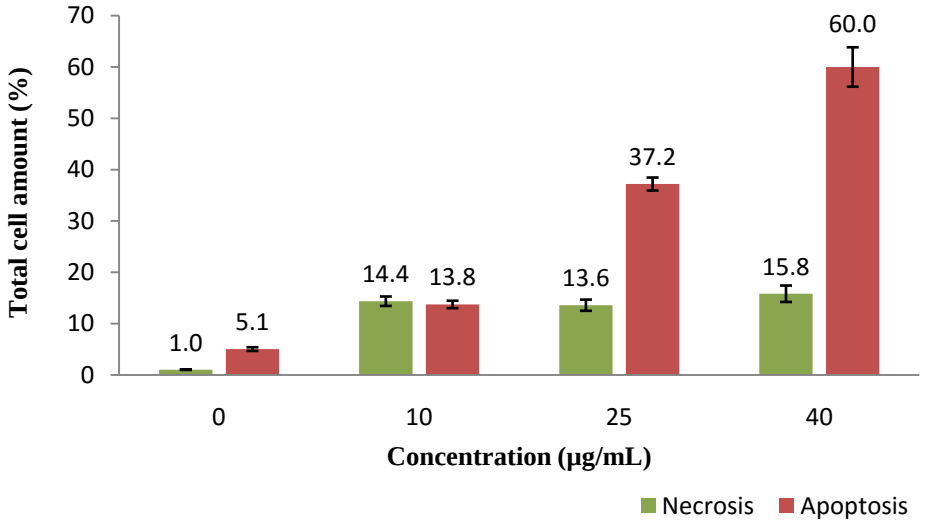


Figure 6 : Evaluation of the apoptosis- and necrosis-inducing potential of CuO-NPs in SH-SY5Y cells.

All experiments were done in triplicate and each assay was repeated four times.

The results were presented as mean tail intensity (%) with ± SD, NC and PC mean negative and positive controls, respectively.

*****$p \leq 0.05$ were selected as the levels of significance by one-way ANOVA Dunnett t-test.



All experiments were done in triplicate and each assay was repeated four times.

The results were presented as percentages of the total cell amount.

Table 1 : Evaluation of the cellular uptakes of CuO-NPs from SH-SY5Y cells.

Exposure concentration ($\mu g/mL/10^5$ cells)	Cu amount ($\mu g/10^5$ cells)
Negative control	0.0137±0.002
5	0.390±0.051
10	0.378±0.076
15	0.641±0.062
25	0.917±0.980

Cu content of the negative control (unexposed cell) was also measured. Every assay was repeated four times. The results were expressed as mean ± SD.

Table 2 : Evaluation of oxidative damage potentials of CuO-NPs in SH-SY5Y cells.

Exposure concentration (µg/mL)	GSH (µmol /g protein)	MDA (µmol/g protein)	8-OHdG (µg/g protein)	PC (µg/g protein)
0	46.796±0.952	0.320±0.086	6.777±0.0988	0.916±0.019
5	30.514±1.319*	0.280±0.092	5.870±0.529	0.747±0.057
10	25.247±1.072*	0.350±0.064	6.287±0.418	0.807±0.120
15	32.160±1.491*	0.416±0.108	6.576±0.246	0.866±0.109
25	34.884±1.220*	0.425±0.156	6.836±0.098	0.805±0.088

The protein amount calculated for $4x10^4$ cells in every assay according to Bradford (1976).

The results were expressed as µmol, µmol, µg and µg per g protein for GSH, MDA, 8-OHdG and PC, respectively, using standard calibration curve.

*$p \leq 0.05$ were selected as the levels of significance.

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The Relation of Serum High-Sensitive C- Reactive Protein to Serum Lipid Profile, Vitamin D and Other Variables in a Group of Hypertensive Patients in Erbil-Iraq

By Salam Naser Zangana
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Abstract- *Background and objectives:* Hypertension is an established risk factor for atherosclerosis. Elevated levels of high-sensitive C-reactive protein (hs-CRP) were detected in hypertensive patients. Recent studies suggest a link between high-sensitive C-reactive protein (hs-CRP) and atherosclerosis in hypertension. Growing evidence suggests that vitamin D affects the cardiovascular system The objective of this study was to assess the relationship of hs-CRP to lipid profile, vitamin D and other variables in hypertensive patients in Erbil-Iraq.

Subjects and Methods: This cross-sectional study was conducted on two-hundred adults (130 hypertensives and 70 normotensives). The participants were classified into three groups according to their BP measurements as normotensive (group I), stage I hypertension (group II) and stage II hypertension (group III). Serum hs-CRP, lipid profile, vitamin D levels, and other variables were evaluated in all studied groups.

Keywords: *Hs-CRP, hypertension, lipid profile, vitamin D.*

GJMR-B Classification : *NLMC Code: WG 340*

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Results: Hs-CRP level was significantly higher in hypertensives as compared to normotensives (P <0.001). The means of total cholesterol (TC), triglyceride (TG) and low-density lipoprotein (LDL) were significantly higher, while the mean of high-density lipoprotein (HDL) was significantly lower in hypertensives than in normotensives (P <0.001). The mean of vitamin D was significantly lower in hypertensives than in normotensives (P<0.001). Hs-CRP was positively correlated with TC, TG, and LDL but inversely correlated with HDL and vitamin D.

Conclusions: Higher levels of hs-CRP were detected in hypertensive patients than normotensives. The higher hs-CRP levels were significantly correlated with higher grades of hypertension. Hs-CRP was positively correlated with lipid profile and inversely correlated with vitamin D .Increased levels of hs-CRP in hypertension may suggest a role of inflammation in hypertension. Hs-CRP estimation may be recommended in evaluation of all hypertensive patients.

Keywords: Hs-CRP, hypertension, lipid profile, vitamin D.

I. Introduction

Hypertension is an established major independent risk factor for development of atherosclerosis and multiple cardiovascular diseases worldwide.[1] According to the 2006 Iraqi national survey for chronic disease risk factors, 40.4% of the Iraqi adult populations have elevated blood pressure.[2] Many recent studies correlate between hypertension and inflammation.[3] New proof indicates that vascular inflammation may have a role in the initiation and /or development of hypertension.[4] Several researchers have noticed higher high-sensitive C-reactive protein (hs-CRP) levels in patients with hypertension.[5] Vitamin D deficiency or insufficiency is a common condition that affects up to one-half of otherwise healthy middle aged to elderly population.[6] Although vitamin D deficiency involves mainly musculoskeletal system, growing evidence suggests that vitamin D affects the cardiovascular system also.[7]

High concentrations of CRP might reduce nitric oxide production in endothelial cells, leading to vasoconcentration and increase blood pressure. Endothelial dysfunction and inflammation were associated with arterial stiffness.[8] Hs-CRP ,an acute phase reactant protein, is a proinflammatory atherogenic marker which can be an early cardiac risk predictor.[9] A hs-CRP test measures low levels of CRP using laser nephelometry. The test gives a sensitivity results down to 0.04 mg/L. The American Heart Association and U.S. Centers for Disease Control and Prevention have defined risk groups as follows: low: hs-CRP level under 1.0 mg/L, average: between 1.0 and 3.0 mg/L, and high: above 3.0 mg/L.[10,11]

To date, and up to our knowledge, there was no previous study done regarding the same subject in Erbil city. The objective of this study was to assess the correlation between hs-CRP levels to serum lipid profile and other variables in a group of hypertensive patients in Erbil city-Iraq.

II. Patients and Methods

This cross-sectional study was conducted in Rizgary teaching hospital between July 2015 and July 2016 .A total of 200 participants (130 participants with essential hypertension and additional 70 normotensives, as control group) were enrolled in the study. According to blood pressure (BP) measurements, The participants were classified into three groups; Group I (normotensive participants, SBP ≤120 mmHg, and /or DBP ≤80

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mmHg, n=70), group II (stage 1 hypertension, SBP 140-159 mmHg, and /or BDP 90-99 mmHg, n=67) and group III (stage 2 hypertension, SBP≥160 mmHg, and /or DBP≥ 100 mmHg, n= 63). All participants were assessed by a detailed history, physical examination, echocardiographic evaluation and other investigational tools. Blood samples were drawn to measure the hs-CRP, serum lipid profile and vitamin D level for each participant.

The inclusion criteria were patients with essential hypertension, age≥ 18 years and of both genders.

The exclusion criteria were patients with secondary hypertension (diabetic nephropathy, polycystic kidney disease, renovascular hypertension), Cushing syndrome, thyroid disease, chronic renal failure, patients with primary hyperparathyroidism, malabsorption, osteomalcia or osteoporosis, patients on medications like anticonvulsants, glucocorticoids and vitamin D supplements.

BMI (Body Mass Index, weight/height^2) was calculated according to a standard definition.[12]

Based on recommendations of the Eighth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 8)[13], hypertension was defined as systolic blood pressure ≥140 mmhg and diastolic blood pressure ≥ 90 mmhg for adults aged 18 years and less than 60 years, and systolic blood pressure ≥ 150 or diastolic ≥ 90 in general population ≥ 60 years. Blood pressure measurements used in this study were taken with a mercury sphygmomanometer. Measurements were made to the nearest 2mmhg, in the sitting position with the arm supported, and repeated after 5 minutes' rest if the first recording is high. We will take 2 measurements at each visit.

Transthoracic echocardiographic examinations were performed in the left lateral position. Standard M-mode, 2-Dimensional and Doppler echocardiographies were performed using (Brand GE Vivid E9 -2009) echocardiography machine. LV end-diastolic diameter (LVDd), LV end-systolic diameter(LVSd), left atrial (LA) and all other diameters were measured according to established standards of the American Society of Echocardiography.[14] LV mass (LVM) were calculated according to the Devereux formula [15] : LVM=1.04[(LVDd + IVSth +PWT) ^{3} –(LVDd) ^{3}]-13.6. Thereafter, LV mass index(LVMI) was obtained by the following formula: LVM/body surface area (g/m^2)[16]. In the presence of LVH, the LVM exceeds 134 grams in men and 110 grams in women per meter square body surface area (m^2 BSA).

Although a consensus regarding the optimal level of serum 25(OH) D has not yet been established , most experts define vitamin D deficiency as a 25(OH) D level of <20 ng/ml , vitamin D insufficiency as 21 to 29 ng/ml and the optimal concentration of 25(OH) D is at least 30 ng/ml.[17]

Estimation of serum lipid profile was done by using automated biochemistry analyzer and according to standard methods.[18]

Pulse pressure (PP) is the difference between the systolic and diastolic pressure readings (PP= SBP-DBP). It is measured in millimeters of mercury (mmHg). It represents the force that the heart generates each time it contracts.[19]

The mean arterial pressure (MAP) is a term used to describe an average blood pressure in an individual. It is defined as the average arterial pressure during a single cardiac cycle.[19] MAP=DBP+1/3 PP.

The data were collected by interviewing the patients using a questionnaire designed by the researchers. The questionnaire included information about socio-demographic data (age, gender, marital status,…), hypertension, risk factors like hyperlipidemia, IHD, obesity, family history, others), and history of smoking and alcoholism.

Ethical considerations: The study protocol was approved by the ethics committee of the College of Medicine of Hawler Medical University. This study was conducted by using an informed verbal consent from the patients prior to participation in the study. The purpose of the study was carefully explained to each patient.

Statistical analysis of data: Data were analyzed using the statistical package for social sciences (SPSS, version 19). Student's t test for two independent samples was used to compare means. Correlation coefficient (r) was obtained to demonstrate the correlations between variables. A 'P' value of≤ 0.05 was considered as statistically significant.

III. Results

The age and BMI were matched in all three groups of the study (P =0.49 and 0.98, respectively). As expected, SBP, DBP, PP and MAP values were significantly higher in hypertensive groups as compared to normotensive group (P<0.001, for each). Statistically higher levels of TC, TG, LDL (P<0.001, for each) and lower level of HDL (P<0.001) were found in hypertensives than in normotensives. IVS, PW, LVM, LVMI and RWT levels were significantly higher (P <0.001, for each) in hypertensives as compared to normotensives. We found also that the mean of vitamin D level was significantly lower (7.61 ng/dl) and the mean of hs-CRP level was significantly higher (2.75 mg/dl) in hypertensives than normotensives (17.3 ng/dl and 0.74 mg/dl respectively) (P<0.001, for each), as shown in Table 1.

In Table 2, which compares between the two hypertensive groups and as expected, SBP , DBP , PP and MAP values were significantly higher (P<0.001, for each) in group III as compared to group II. There were no differences in both groups regarding serum lipid profile values (P=0.91, 0.87, 0.74 and 0.8 respectively),

the same applies to EF (P=0.85). IVS, PW, LVM, LVMI, RWT and left atrium values were significantly higher in group III patients than in group II patients. Group III patients had significantly higher hs-CRP values (3.67 mg/dl) than group II patients (1.83 mg/dl) (P<0.001). Although the mean value of vitamin D was lower (6.9 ng/dl) in group III patients than in group II patients (8.32 ng/dl), but it was not statistically significant (P=0.5).

Hs-CRP correlated positively with SBP, DBP, PP, MAP, TC, TG, LDL, LVM, LVMI, and correlated negatively with HDL and vitamin D, as shown in Table 3.

IV. Discussion

In the present study, hypertensive patients had higher hs-CRP levels than normotensives. This indicates that inflammation might be associated with hypertension. This is compatible with other studies. Ki Chul Sung et al[20] and Sesso et al[21] found a positive relation between increasing levels of hs-CRP and risk of developing hypertension. But Bautista et al[22] in 2003 didn't find such association.

CRP has been reported to decrease nitric oxide production[8] and increases endothelin-1 and plasminogen activator inhibitor-1 activity in endothelial cells[23] to induce vasoconstriction, platelet activation, and thrombosis. In addition, CRP has shown to up regulate angiotensin receptor-1 and thus enhancing angiotensin-II activity and this leads to rise in blood pressure.[24]

In our study, hypertensive patients had abnormal lipid profile and that was evident by the presence of high TC, TG and LDL levels and low HDL level. This is compatible with other studies. Rasouli M et al[25] found higher cholesterol and TG levels in hypertension. In the Strong Heart Study (2006) [26], an abnormal lipid profile was found in hypertensive American Indian population. Marco et al[27] found that participants who were prehypertensives and later developed hypertension had higher levels of TG and lower HDL levels. All these data suggest that vascular inflammation plays a role in pathophysiology of hypertension and may exacerbate the pro-atherogenic effects of hypertension.

In our study, elevated hs-CRP levels were associated with high PP. This result is compatible with Abramson et al[28] study which found such a positive association. Recent studies emphasize the possibility that arterial stiffening may precede the development of hypertension. Arterial stiffening was associated with many circulating inflammatory markers suggesting that inflammation may play a role in arterial stiffness.[29] If the blood vessel becomes rigid in conditions such as arteriosclerosis or atherosclerosis, the pulse pressure would be very high. Some evidence suggests that pulse pressure is a better predictor of clinical outcome than the systolic or diastolic blood pressure alone. Several studies have identified that high pulse pressure causes more artery damage compared to high blood pressure with normal pulse pressure.[30] Recent work suggests that a high pulse pressure is an important risk factor for heart disease. A meta-analysis in 2000, which combined the results of several studies of 8,000 elderly patients in all, found that a 10 mm Hg increase in pulse pressure increased the risk of major cardiovascular complications and mortality by nearly 20%.[31]

A positive association between high hs-CRP level and high MAP was also found in the present study. Many other studies found the same relationship.[32,33]

In the present study, hs-CRP was positively related to LVM and LVMI, an echocardiographic marker of left ventricular hypertrophy (LVH). This result is compatible with other previous studies[34], which found that patients with different involved target organ had different inflammatory degree, which hypertensive patients with LVH had the highest hs-CRP levels.

Finally, in the present study, hs-CRP was negatively related to vitamin D level. Although vitamin D deficiency involves mainly musculoskeletal system, growing evidence suggests that vitamin D affects the cardiovascular system also. [7] Recent clinical studies showed that low levels of vitamin D are associated with a higher prevalence of hypertension and LVH.[35] Elevated hs-CRP and vitamin D deficiency are associated with inflammatory changes that have been associated with cardiovascular events.[36]

V. Conclusions

Higher levels of hs-CRP were seen in hypertensive patients than normotensives. The higher hs-CRP levels were significantly correlated with higher grades of hypertension. Hs-CRP was positively correlated to lipid profile and inversely correlated to vitamin D. Increased levels of hs-CRP in hypertension implies a role of inflammation in hypertension. Hs-CRP estimation may be recommended in evaluation of all hypertensive patients.

Conflicts of interest:

The authors report no conflicts of interest

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Table 1 : Comparison of baseline, biochemical and echocardiographic characteristics between hypertensives and normotensive participants.

Variables	Group I Normotensive N=70		Group II and III hypertensive N=130		p
	Mean	SD	Mean	SD	
Age	49.9	9.3	52.24	9.15	0.49
BMI	26.77	6.8	27.60	7	0.98
SBP	118	8.8	157.26	7.39	<0.001
DBP	78	4.7	95.49	4.2	<0.001
PP	40	4.1	61.77	2.3	<0.001
MAP	91.3	5.6	116.04	4.1	<0.001
Cholesterol	145.1	29.3	200.83	19.5	<0.001
TG	99.75	32	180.29	19.3	<0.001
LDL	71.52	17.9	113.56	20.1	<0.001
HDL	41.5	4.6	37.05	3.6	<0.001
EF	61.7	3.6	62.1	3.7	0.58
IVS	8.25	0.5	12.46	0.7	<0.001
PW	7.86	0.3	11.86	0.6	<0.001
Left atrium	25.12	1.25	33.17	2.1	<0.001
LVM	102	9.18	223.79	15.6	<0.001
LVMI	60.66	5.3	121.14	7.5	<0.001
RWT	0.38	0.01	0.50	0.03	<0.001
Vit D	17.3	7.4	7.61	5.6	<0.001
Hs-CRP	0.74	0.8	2.75	1.5	<0.001

Table 2 : Comparison of baseline, biochemical and echocardiographic characteristics between two hypertensive groups.

Variables	Hypertensive group (II and III) N=130				p
	Stage I Group II (n=67)		Stage II Group III (n=63)		
	Mean	SD	Mean	SD	
Age	51.74	8.4	52.75	9.9	0.89
BMI	27.40	9	27.80	5	0.57
SBP	142.92	8.29	171.6	6.5	<0.001
DBP	90.22	2.8	100.75	5.6	<0.001
Cholesterol	199.44	20.5	202.22	18.6	0.91
TG	179.78	19.1	180.8	29.5	0.87
LDL	111.52	19.2	115.61	21.1	0.74
HDL	36.6	1.8	37.5	6.1	0.8
PP	52.7	3.5	70.85	1.1	<000.1
MAP	107.2	3	124	5.3	<000.1
EF	62.4	3.5	61.8	3.9	0.85
IVS	11.65	0.6	13.28	0.9	<0.001
PW	11.07	0.5	12.66	0.7	<0.001
Left atrium	32.20	2	34.15	2.2	0.066
LVM	203.89	11.2	242.69	20.4	<0.001
LVMI	111.35	6.1	130.93	9.4	<0.001
RWT	0.47	0.02	0.54	0.04	0.001
Vit D	8.32	7.22	6.9	6.5	0.5
Hs-CRP	1.83	1.1	3.67	2.1	<0.001

Table 3 : Correlation of hs-CRP with serum lipid profile and other variables in hypertensive patients.

Variables	r value	P value
SBP	0.75	<0.001
DBP	0.68	<0.001
PP	0.7	<0.001
MAP	0.72	<0.001
Cholesterol	0.63	<0.001
TG	0.32	0.001
LDL	0.6	<0.001
HDL	-0.35	0.001
LVM	0.58	<0.001
LVMI	0.58	<0.001
Vit D	-0.32	0.044

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Endocrine Disruptors in Endometriosis

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Abstract- Endometriosis is an estrogen-dependent disease, which involves the growth of endometrial tissue outside the uterine cavity, commonly in the pelvic region. The etiology of the disease is unclear, but multiple factors may contribute to its prognosis. Toxicological studies indicate that many chemicals are able to interfere with endocrine homeostasis, called endocrine disrupting chemicals (EDC) like Bisphenol A, Phtalate, Polychlorinated Biphenyls and Dioxins. As well documented, endometriosis is an estrogen-dependent disease; therefore, environmental toxicants that either mimic estrogen or enhance estrogenic exposure in the endometrium are thought to increase the risk of endometriosis. The purpose of this mini-review is to provide an overview of epidemiological studies, which have evaluated the relationship between endometriosis and exposure to endocrine disruptors.

Keywords: *endometriosis, endocrine disruptors, infertility, bisphenol-A, phthalate, PCBs, TCDD.*

GJMR-B Classification : *NLMC Code: WP 390*

Strictly as per the compliance and regulations of:

Endocrine Disruptors in Endometriosis

Mariana Antunes Ribeiro [α] & Wellerson Rodrigo Scarano [σ]

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Keywords: endometriosis, endocrine disruptors, infertility, bisphenol-A, phthalate, PCBs, TCDD.

I. Endometriosis

Endometriosis is an estrogen-dependent disease defined as the growth of endometrial glands and stroma at extra-uterine sites. Reports on the incidence of endometriosis vary widely, from approximately 10% of reproductive-aged women (Barbieri 1990) up to 30% of women with chronic pelvic pain (Howard 1993). These reports may underestimate the true prevalence of this disease, which may approach 45% of women in their reproductive years(Rawson 1991). Although retrograde menstruation occurs in 70-80% of women of reproductive age, not all develop endometriosis (Halme et al. 1984). Therefore, other factors must play a role in the pathogenesis of endometriosis, like genetic background, malfunctioning inflammatory/immunological mechanisms and potentially environmental factors (Bischoff & Leigh 2004).

Endometriosis is intimately associated with steroid metabolism and associated pathways, corresponding to the dominant roles estrogen receptors (ESRs) and progesterone receptors (PGRs) play in uterine biology. Both human and animal model studies show endometriosis is estrogen (E2) dependent and is regulated through the ESRs alpha and beta (*ESR1* and *ESR2*) (Burns et al. 2012; Han et al. 2015; Zhao et al. 2015). Toxicological studies indicate that many chemicals are able to interfere with endocrine homeostasis, called endocrine disrupting chemicals (EDC), may directly or indirectly impair female reproduction (Mantovani 2006). The definition of endocrine disruptor by European Union is an exogenous substance able to mime the hormones that can interfere with the production, release, transportation, metabolism, link, action or elimination of natural hormones, which are responsible of maintenance of homeostasis and regulation on development processes (Caserta et al. 2008). The main targets EDC are bisphenol A (BPA), di-(2-ethylhexyl) phthalate (DEHP), mono-ethyl-hexyl phthalate (MEHP) and polyhalogenated aromatic hydrocarbons that consists of dioxins, mainly, 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and poly-chlorinated biphenyls (PCB). Recently, they have gained special attention as emerged chemicals because of their persistence in the environment, potential for bioaccumulation and toxicity. Nuclear receptors pathways are the main cellular targets of the EDC under study, thus they are considered meaningful biomarkers of effective dose. The panel of nuclear receptors includes estrogen receptor alpha (ERα) and beta (ERβ), androgen receptor (AR) and aryl hydrocarbon receptor (AhR), it of these act in different pathways (Caserta et al. 2013).

As well documented, endometriosis is an estrogen-dependent disease; therefore, environmental toxicants that either mimic estrogen or enhance estrogenic exposure in the endometrium are thought to increase the risk of endometriosis. Therefore, this article aims to review the main endocrine disrupters that may be involved with endometriosis.

II. Bisphenol a (bpa)

BPA is a compound used in the production of polycarbonate plastics and epoxy resins. Given its similarity to endogenous estrogen, BPA has the ability to interact with estrogen receptors and stimulate estrogen production and also alter gonadotrophin hormone secretion (Buck Louis et al. 2013). Cobellis and co-workers correlated BPA and endometriosis (Cobellis et al. 2009). In this study, they found detectable BPA serum levels in more than half of patients with endometriosis, whereas it was absent in women without the disease. This data is still controversial once other studies could not observe a relation between BPA and endometriosis (Buck Louis et al. 2013; Itoh et al. 2007). More studies should be performed once it was reported that BPA causes subfertility in male rats that neonatally exposed to 2.4 μg of the compound per day for five

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days, by subcutaneous injection. This subfertility is manifested as embryo resorption, also known as post-implantation loss. In these resorbed embryos, the expression levels of three types of DNA methyl-transferases involved in CpG methylation were significantly decreased compared to viable embryos of neonatally BPA exposed males or control embryos. The authors suggested that BPA might have altered the epigenome. As suggested by Guo (2009), there is accumulating evidence supporting a concept that endometriosis is an epigenetic disease, therefore further studies should be performed to demonstrate the correlation between the epigenetics changes and BPA in endometriosis.

III. Phthalates

Phthalates are chemicals used in numerous industrial and consumer products and also exhibit endocrine disruptive properties or to mimic or alter endogenous hormone activity. Adult human exposure to phthalates is primarily through ingestion of contaminated food from food processing machines and packaging materials and dermal application of personal care and cosmetic products. Exposure is also possible through inhalation of indoor air contaminated from building materials, and parenteral exposure through medical equipment such as IV tubing and blood bags (Upson et al. 2013). Di-(2-ethylhexyl) phthalate (DEHP) is the most commonly used chemical additive to provide flexibility to polyvinylchloride and in humans, it is likely that the stomach acid lipases hydrolyze DEHP into mono-(2-ethylhexyl) phthalate (MEHP))(Albert & Jégou 2013). This compound is metabolized quickly and excreted in urine without evidence of accumulation within the body. Phthalates produce antiandrogenic effects largely through the reduction in testosterone production and, possibly, reduced estrogen production at high doses (Buck Louis et al. 2013). Results of investigations into the pathophysiology of endometriosis have suggested that disease onset and progression involve steroid-related mechanisms, including hormone-related changes of the endometrium and peritoneal cavity, excess estrogen production by ectopic endometriotic lesions, and alterations in ovarian steroidogenesis. Thus, it is plausible that endocrine-disrupting chemicals such as phthalates may affect endometriosis risk(Ulukus et al. 2006).

The in utero and neonatal exposure to low doses of bisphenol A (BPA) and/or phthalates (DEHP/MEHP and BBP/DBP/MBP) may cause DNA hypermethylation/hypomethylation at CpG islands near gene promoter regions, histone modifications (acetylation, methylation, phosphorylation, ubiquity-nation, sumoylation and ADP ribosylation), and expression of non-coding RNAs, including micro RNAs. These epigenetic marks can induce up/down alterations in gene expression that may persist throughout a lifetime (Singh & Li 2012).

IV. PCBS and TCDD

The main group of environmental pollutants that have been proposed to play a role in the pathogenesis of endometriosis includes polyhalogenated aromatic hydrocarbons, a class of widespread environmental contaminants consisting of polychlorinated dibenzo-*p*-dioxins (PCDD), dibenzofurans and 12 polychlorinated biphenyls (PCB) (Schecter et al. 2006).

Dioxins are byproducts of industrial processes such as bleaching of paper pulp and the manufacture of certain pesticides and incineration of plastic and medical waste (Foster et al. 2010). Dioxins are lipophilic substances that resist biological and environmental degradation, remaining in the environment. Studies in animals have shown that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is considered the environmental contaminant, within dioxin group, with the greatest toxicity and thus is also significant to human health (Schecter et al. 2006).

Seventy-five dioxin congeners and 135 furan congeners comprise the complex mixture of dioxins, 7:10 congeners which are respectively capable of binding to and activating the aryl hydrocarbon receptor (AhR) (Van den Berg et al. 2006). This binding induces the proliferation, differentiation and apoptosis, although the mechanism for this stimulation is not completely understood (Kogevinas 2001). Of the 209 congeners of polychlorinated biphenyls (PCBs), twelve have the potential to activate the AhR (Van den Berg et al. 2006). In normal physiological conditions, AhR resides in an inactive state in the cytoplasm. After association with TCDD, the AhR is activated by a change in conformation and translocates to the nucleus where it forms a heterodimer with ARNT (Aryl hydrocarbon receptor nuclear translocator). The heterodimer binds to the XRE (Xenobiotic Response Element) and alters the expression of genes controlled by the enhancer XRES. XRES, with the conserved sequences " GCGTG " are found in the promoter regions of various genes involved in the metabolism of xenobiotics, including CYP1A1 (Cytochrome P450 Family, subfamily a polypeptide 1a -1), CYP1A2 (Cytochrome P450 Family, 1, subfamily a polypeptide -2) CYP1B1 (Cytochrome P450 family, subfamily B, polypeptide 1 -1) and NAD(P)H quinone Oxidoreductase (Mimura & Fujii-Kuriyama 2003). In addition to the expression of various genes to CYP connection with TCDD because several toxicological effects such as teratogenesis, tumor promotion and immunosuppression (Shimizu et al. 2000).

Furthermore, it is reported that, in somatic cells, the gene expression of DNA methyltransferase 1 (Dnmt1) is controlled by the transcription factor Sp1 (Bigey et al. 2000) and the promoter region Dnmt 3B

also contains an Sp1 binding site (Ishida et al. 2003). The Sp1 is important for a number of physiological processes, including angiogenesis, cell cycle progression, inflammation and senescence (Chang & Hung 2012). Taking into account the involvement of Sp1 with DNMTs, the change of the activity of Sp1 may affect the level of expression of DNA methyltransferases and their activity. Lee et al (Lee et al. 2011) showed that exposure to TCDD causes Sp1 phosphorylation. Based on this evidence, the phosphorylated Sp1 would bind to receptors of DNMTs, thereby increasing its activity. Thus, changes in methylation status in the promoter region of some genes can cause alterations in gene expression and consequently contribute to endometriosis development.

Dioxins have also been postulated to stimulate the development of endometriosis via their immune-suppressive effects and their interference with the estrogensignaling pathway. The immunosuppressive effect of high doses of dioxins is well documented(Oh et al. 2005). Firstly, dioxin exposure may lead to inhibition of leukocyte phagocytic function, which is possibly important in the prevention of endometriosis by the elimination of menstrual debris (Levin et al. 2005). Additionally, dioxins can decrease immunological memory induce apoptosis in both T cells and B cells), inhibit T-lymphocyte function and decrease natural killer cell activity in plasma and peritoneal fluid (Puebla-Osorio et al. 2004; Ahmed et al. 2005). Furthermore, dioxin may stimulate the activity of peritoneal fluid macrophages and their local production of pro-angiogenic factors, cytokines (e.g. interleukin-1) and growth factors. The combined effect of immune dysfunction and peritoneal inflammation could favor the development of endometriosis. Furthermore, cellular changes or genetic predisposition may predestine an individual to the immunological modulation caused by dioxin exposure (Simsa et al. 2010).

Local estrogen production can be increased following dioxin exposure and facilitate development of endometriotic lesions by elevating mRNA expression of aromatase, the key catalytic enzyme in estrogen synthesis (Attar & Bulun 2006). Dioxins and PCB are known to interfere with estrogen concentrations. Both agonistic and antagonistic effects have been ascribed to dioxins and PCB by direct interference with the estrogen receptor or by the interaction between the activated aryl hydrocarbon receptor (AHR)/aryl hydrocarbon receptor nuclear translocator heterodimer and the estrogen receptor a and b, leading to estrogen-dependent gene activation (Mimura & Fujii-Kuriyama 2003).

V. Conclusion

Developing a better understanding the basic mechanisms that may allow environmental toxicants to promote endometriosis, will enable us to develop better strategies to reduce the potential toxic impact of these compounds to the future generation.

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Prevalence of Health Related Disability among Community Dwelling Urban Elderly from Middle Socio-Economic Strata in Serampore

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Abstract- The present study has been conducted in Serampore, West Bengal. The health of geriatric population is a present as well as future concern. This poses mounting pressures on various socio-economic fronts of the state, including pension outlays, health care expenditures, saving levels etc. This makes it necessary to look into the various aspects of their problems: Health, social rejection, economic, psychological and other allied aspects. In the traditional joint families, infirmities were taken care of by the individuals, immediate circle of relations and family members. Older people enjoyed a sense of honour and authority and had the responsibility in decision-making. However, in recent times, as a result of changing circumstances due to demographic transition, rapid pace of industrialization and urbanization, disintegration of joint family structures into unitary ones, the older people become more vulnerable to physical disabilities as a result of different morbidities and poor health seeking behaviour. This study will prove to be useful for the planners and policy makers in Government and private organizations and will help in enhancing the understanding of the problems of elderly people in the state.

Keywords: *morbidity, elderly population, ageing, physical disabilities.*

GJMR-B Classification : *NLMC Code: W 84*

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I. Introduction

The phenomenon of population ageing is becoming a major concern for the policy makers all over the world during the last two decades. Ageing of population is affected due to downward trends in fertility and mortality i.e. due to low birth rates with long life expectancies. Life expectancy at birth is projected to continue to rise in the coming years all over the world. The aged population has specific health problems that are basically different from those of adults or young persons. Most diseases in the aged are chronic in nature-cardiovascular, arthritis, stroke, cataract, deafness, chronic infections, cancer. Disease process is usually multiple. Availability and utilization of health services is an important determinant of the health status of population. The needs for health services tend to vary directly with the age of the individuals. The older the one gets, the more health care he needs. Although the aged people face multiple health problems, even then, they do not consider seeking medical aid and as a result, many conditions remain unreported and untreated till they become complicated. This emphasizes the need for strengthening of health care system for elderly population. According to Paul Wallace, all individuals should be prepared to face later years in life within their own limitation gloriously. Chhattisgarh is moving fast towards an 'aged society', with the aged population constituting 7.2 percent (India 8 percent) and in another 10 years, percentage of elderly is projected to be 10 percent. Though a large number of studies on various factors influencing the aged are available in western countries, not much data have been generated as applicable to the Indian scenario. Urban areas are expected to grow at higher rate as compared to non-urban. Consistent with these changes; there were health institutions both demographically and epidemiologically, hence associated with the changes in prevalence of chronic illnesses.

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II. Material and Methods

Serampore is an important city of Hooghly district, state of West Bengal, India. At the time of 2011 census, the population within the Municipal area of Serampore was 181,842.Study was conducted in randomly selected 32 areas distributed in Serampore city including Urban and Slum areas. List of zones and wards including Slum and Urban areas were obtained from Municipality of Serampore. From eight zones of Serampore city by simple random technique, four zones were selected. Out of the four zones, four wards were selected by simple random technique. From each ward, one slum area and one urban area were included in the study using simple random technique. A total of 32 areas were included in this study. Door to door survey was conducted. From each area, 20 elderly were included in study.

Sampling method: Multi stage simple random sampling technique.

Sample size: 640

Sample size was calculated by using statistical formula, n= Z2 I-a/2 P (I-P)/d

P = Morbidity Problems (50%),
d= Absolute Precision (4%), Confidence level= 95%

As there was no baseline study in Serampore, therefore it was not possible to estimate 'P', so a figure

of 0.5(50%) was used. This is the 'safest' choice for the population proportion, since the sample size required is largest when P = 0.5(50%) [128].

A total of 600 figures come using statistical formula. For making uniformity, 20 subjects from each of 32 areas were selected that comes 640. Therefore, a total 640 subjects were included in the study.

III. Objectives of the Study

1) To study morbidity pattern in elderly population of Serampore city.
2) To determine the pattern of morbidity in elderly population of Serampore city.
3) To study the health-care seeking behaviour of elderly population.
4) To make suitable recommendations on the basis of the study.

IV. Observations and Discussion

Table 1 : Sex wise distribution of level of cognition among studied elderly

Level of cognition	Male		Female		Total	
	No	%	No	%	No	%
Normal	89	47.34	99	52.65	188	29.37
Some degree of mental confusion	155	37.25	261	62.74	416	65
Severe confusion	23	63.88	13	36.11	36	5.62
Total	267	41.71	373	58.28	640	100

Chi-square = 13.123 (df = 2, $p < 0.001$)

Above table shows statistically significant relation between level of cognition and sex of study population. Cognition was normal in 29.37% elderly whereas 65% had some degree of mental confusion, 5.62% had severe confusion. Severe confusion was more among males(63.88%) than females (36.11%).In another study by Srinivasan Krishnamachari et al (2010), reported that cognitive impairment was shown to be positively associated with disability and was independent of age, gender and co-morbid medical condition. Present study shows sex differentiation among cognitive impairment. More males were severely confused than females.

Table 2 : Association of Morbidity with Socio-economic status in elderly population

SES	Morbid		Healthy		Total	
	No	%	No	%	No	%
Class I	68	(94.44)	4	(5.55)	72	11.25
Class II	158	(91.32)	15	(8.67)	173	27.03
Class III	120	(95.23)	6	(4.76)	126	19.68
Class IV	229	(98.28)	4	(1.71)	233	36.40
Class V	35	(97.22)	1	(2.77)	36	5.62
Total	610	(95.31)	30	(4.68)	640	100

Chi-square = 11.162 (df = 4, p = 0.024)

Above Table-2 shows that there is statistically significant association between morbidity and socio-economic status. Maximum morbidity(37.54%) was observed in Class IV Socioeconomic status(98.28%) followed by Class V (97.22%), Class III (95.23%), Class I (94.44%) and Class II (91.32%).In present study, maximum morbidity was in Class IV and V Socio-economic group and all belonged to slum areas and were vulnerable group related to both environmental factors and literacy status.

Table 3 : Age and sex wise distribution of illnesses in elderly

Age groups in yrs	No examined	Persons ill	Number of illness		Total illnesses	Mean no of illnesses
			Male	Female		
60-74	523	495(94.64)	652	1303	1955	3.94
75-84	114	112(98.24)	261	226	487	4.34
>85	03	3(100)	0	19	19	6.33
Total	640	610(95.31)	913	1548	2461	4.03

Above Table-3 shows that, out of 640 elderly included in the study, 610 (95.31%) were found to have one or more illnesses at the time of examination. There was 2461 illnesses in 610 persons, 913 in males and 1548 in females. Mean number of illness was 4.03. In males, 3.78 whereas in females, mean number of illness was 4.19. There was positive association between mean number of illness and advancement of age. Mean illness for young old was 3.94, for old was 4.34 and for very old was 6.33.

Prevalence of illness was 100% among very old, 98.24% among old and 94.64% among young old. Similar findings were observed in another study done by M Jamal et al (1977), observed that 88.66% in their study were found to be ill;86.67% males and 90.78% females. Illness was observed more frequently in older age group; 79.36% in young old to 100% in very old. Raj and Prasad (1970) observed that the brunt of illnesses fell on the persons who were 80 years and over.

Table 4 : Age wise distribution of total illnesses in study area

Age group	Slum		Urban		Total	
	No	%	No	%	No	%
60-74	950	(75.15)	1005	(83.95)	1955	(79.43)
75-84	295	(23.33)	192	(16.04)	487	(19.78)
>85	19	(1.50)	0	0	19	(0.77)
Total	1264	(51.36)	1197	(48.63)	2461	(100)

Chi-square = 40.538 (df = 2, $p < 0.0001$)

Above Table-4 shows statistically significant relation between age and illness of slum and urban elderly. Overall total illness was more in young old (79.43%), followed by old (19.78%) and very old (0.77%); but the mean was increasing with advancement of age. In urban areas, 83.95% of illnesses lying in young old whereas in slum areas, 75.15% illnesses were in young old. Young old in urban areas were more overweight and obese and physically less active, whereas young old in slum areas were more active and were heavy activity performer. In old and very old, illnesses were more in slum than urban dwellers.

Table 5 : Age sex wise distribution of spells of illness in morbid elderly (n=610)

Age groups in years	Persons ill		Spells of illnesses			
	Male	Female	Male	Mean Spells	Female	Mean Spells
60-74	176	319	773	4.39	1526	4.78
75-84	65	47	293	4.50	262	5.57
>85	0	3	0	0	22	7.33
Total	241	369	1066	4.42	1810	4.90

Chi-square = 83.484 (df = 2, $p < 0.0001$)

Above Table-5 shows statistically significant relation between mean of spells of illness and age. In both sexes, mean spell was increasing with advancement of age. In males, mean was more (4.42) in comparison to females (4.90).

Table 6 : Distribution of elderly as per subjective perception of health status

Perceived Health status	Number of elderly	Percentage (%)
Well	174	27.18
Ill	466	72.81
Total	640	100

Table-6 shows that 72.81% population perceived themselves ill, whereas 27.18% perceived well.

Table 7 : Sex wise distribution of subjective perception of health status in elderly

Health status	Male	Female	Total
Well	85(31.85%)	89(23.86%)	174(27.18%)
Ill	182(68.16%)	284(76.13%)	466(72.81%)
Total	267	373	640(100%)

Chi-square = 4.999 (df = 1 , p = 0.025).

Above table shows that72.81% population perceived themselves ill. Out of the total female population, 76.13% and out of the total male population, 68.16% perceived themselves ill.

Table 8 : Age wise Health seeking practice of elderly (n=466)

Age group (years)	Treatment taken	Treatment not taken	Total
60-74	361(97.30%)	10(2.69%)	371
75-84	92(100%)	0	92
>85	3(100%)	0	3
Total	456(97.85%)	10(2.14%)	466

Chi- square 2.617 (df 2, p = 0.270).

Above table shows that 97.85% of the elderly were observed to be receiving treatment where as 2.14% were not receiving treatment. With advancement of age, health care seeking was increased from 97.30% in young old to 100% in very old.

Table 9 : Sex wise Health seeking practice of elderly (n=466)

Sex	Treatment taken	Treatment not taken	Total
Male	180(98.90%	2(1.09%)	182
Female	276(97.18%)	8(2.81%)	284
Total	456(97.85%)	10(2.14%)	466

Chi-square 1.559 (df=1, p = 0.211).

Above table shows that, out of total 466 elderly who perceived themselves ill, 97.85% were taking treatment whereas 2.14% did not take any treatment. Among males who perceived themselves ill, 98.90% had taken treatment whereas among females 97.18% had taken treatment.

Table 10 : Health seeking as per agency of treatment in Urban and Slum elderly

Area	Government	Private	Quacks	Others	Total
Urban	72(29.26%)	127(51.62%)	38(15.44%)	9(3.65%)	246(53.94%)
Slum	55(26.19%)	35(16.66%)	82(39.04%)	38(18.09%)	210(46.05%)
Total	127(27.85%)	162(35.52%)	120(26.31%)	47(10.30%)	456(100%)

Chi-square = 86.24 (df = 3, p= 0.000).

Above Table-10 shows that, out of total 466 elderly who perceived themselves ill, 456 elderly were taking treatment. Out of 456 elderly who were taking treatment, 53.94% were residing in urban areas whereas 46.05% were residing in slum areas. Out of various agencies, maximum were utilizing private facility (35.52%) followed by Government agency (27.85%), quacks (26.31%) and 10.30% from other source. In urban elderly, maximum were utilizing private facility (51.62%), followed by Government (29.26%), quacks (15.44%) and others (3.65%). Among slum dwellers, maximum elderly went to quacks (39.04%) followed by Government facility (26.19%), others (18.09%) and private facility (16.66%).This may be due low socio-economic status of slum elderly and high socio-economic status among urban dwellers.

Table 11 : Distribution of reasons for not utilizing Government facility

Reasons	Persons	Percentage (%)
Health centre too far	16	4.86
Facility available but lack of faith	4	1.21
Long waiting time	147	44.68
Due to misconduct of staff	110	33.43
Others*	52	15.80
Total	329	100

*Others included OPD time not suitable.

Present study shows that, out of total 456 elderly seeking treatment from different agencies, only 127 elderly were taking treatment from Government facility ; rest 329 were not utilizing Government facility. Above

table shows that most common reasons for not utilizing Government facility were long waiting time (44.68%), due to misconduct of staff (33.43%) and others. Others included not suitable OPD time. The least common reasons were too far health centre (4.86%), lack of faith (1.21%).

Table 12 : Distribution of reasons for not utilizing Government facility in Urban and Slumelderly

Reasons	Urban		Slum		Total	
	No	%	No	%	No	%
Health centre too far	1	0.57	15	9.67	16	4.86
Facility available but lack of faith	3	1.72	1	0.64	4	1.21
Long waiting time	95	54.59	52	33.54	147	44.68
Due to misconduct of staff	23	13.21	87	56.12	110	33.43
Others*	52	29.88	0	0	52	15.80
Total	174	100	155	100	329	100

Chi-square = 114.34 (df= 4,p = 0.000)

Table-12 shows that total 329 elderly were not utilizing Government facility, out of which 174 belonged tourban areas and 155 belonged to slum areas. Most common reasons for not utilizing government facility were long waiting time in 44.68%, Misconduct of staff 33.43% and others 15.80%. Least common reasons were too far health centre (4.86%) and lack of faith (1.21%).Common reasons for not utilizing Government facility among urban elderly were long waiting time in 54.59%, misconduct of staff 13.21%; whereas in slum elderly, misconduct of staff was the major reason in 56.12% for not seeking care from Government facility, followed by long waiting time (33.54%).

Table 13 : Health seeking as per system of medicine (n=456)

Age group (years)	Allopathic	Ayurveda	Homeopathy	Others	Total
60-74	308(85.31)	14(3.87)	18(4.98)	21(5.81)	361
75-84	86(93.47)	1(1.08)	0	5(5.43)	92
>85	3(100)	0	0	0	3
Total	397(87.06)	15 (3.28)	18(3.94)	26(5.70)	456

Chi-square = 7.382 (df = 6, p = 0.286) Figure in parenthesis denote percentages.

Above table shows that majority of the elderly availed modern allopathic system of therapy (87.06%). Homeopathy was also used by a substantial percentage of elderly (3.94%). Advancement of age had positive association with allopathic system of therapy from 85.31% in young old to100% in very old age groups.

Table 14 : Distribution of reasons for not seeking health care (n=10)

Reasons for not seeking health care	Persons	Percentage
Financial reasons	1	10
Considered disease due to age	1	10
Nobody to take me to hospital	5	50
Health services too far	3	30
Total	10	100

Out of 466 who perceived themselves ill, only 10 did not take any treatment. Above table shows that 50% were not seeking health-care due to nobody was available to take them to hospital, 30% were not seeking health-care due to too far health services, where as 10% shows financial reasons and disease due to old age were observed in 10%.

Table-14 : Distribution of elderly spending on health as percent of per capita income in Urban and Slum areas

% of Per capita income	Urban		Slum		Total	
	No	%	No	%	No	%
<10%	161	50.94	155	49.05	316	62.94
10-20%	56	56	44	44	100	19.92
20-30%	29	80.55	7	19.44	36	7.17
>30%	0	0	4	100	4	0.79
Total	246	49.00	210	41.83	456	100

Chi-square = 16.258 (df = 3, p = 0.001)

Above table shows statistically significant relation between urban and slum elderly on health spending. Table-14 shows that, expenditure on health was more in urban than slum elderly. This is similar to trend at national and international level. Those who are more developed and economically more sound are spending more on health than developing countries. In slum areas, maximum of their income is spent on food. In another study by Srinivasan Krishnamachari et al (2010), reported that majority of the elderly spent less than 10% of their monthly income on medication and health related issues.

Table 15 : Distribution of diseases of genitourinary system

Diseases	Male(n=241)	%	Female(n=369)	%	Total(n=610)	%
Urinary Incontinence	6	2.48	1	0.27	7	1.14
BPH	19	7.88	0	0	19	3.11
UTI	14	5.80	10	2.71	24	3.93
Stress Incontinence	0	0	1	0.27	1	0.16
Trichomonas vaginitis	0	0	3	0.81	3	0.49
Carcinoma Cervix	0	0	1	0.27	1	0.16
Prolapsed Uterus	0	0	2	0.54	2	0.32
Total	39	-	18	-	57	-

Note: Multiple disorders have been seen in many subjects.

The study shows, prevalence of Genitor urinary system disorders was 7.37%; among males prevalence was 12.03% whereas in females 4.33%. Above table shows, out of all disorders of Genitor- urinary system, common disorders were Urinary tract infection (UTI) (3.77%), Benign Prostatic Hypertrophy (BPH) (3.44%), Urinary Incontinence (1.14%). The least common condition was Trichomonas vaginitis (0.49%), Prolapsed Uterus (0.32%), Stress Incontinence (0.16%), and Carcinoma Cervix (0.16%). Among males, the commonest condition observed was Benign Prostatic Hypertrophy followed by UTI, whereas among females, Urinary Tract Infection was the commonest illness. In other study done by Shradha K et al (2012) reported prevalence of Genitourinary disorders as only 1.7%. The commonest condition was Renal calculi (1.4%), Urinary Incontinence (0.9%), Urinary frequency (0.9%) and Urinary Tract Infection (UTI) (0.9%). Renal Calculi and Urinary Incontinence was almost equally distributed in both genders, while Urinary frequency and UTI was reported by only female respondents. Present study was different from Shradha K et al (2012), UTI were distributed in both genders. P Ray Karmakar et al (2012), in their study showed that 9.8% elderly had Genito urinary system disorders. Male suffered more (10.3%) than females (9.3%), which is comparable to 9.35% observed by Purohit and Sharma (1976). In present study almost similar feature has been reported. A study from Israel by Polliack and Bialik (1975) revealed very high prevalence (over 33.0%) of Benign Prostatic Hypertrophy, which might be due to older study population (65 years and above) and possibly better cooperation in conducting internal examination, on account of greater awareness and health consciousness. In the present study, the elderly population is 60 years and above thereby diluting the percentage of BPH cases found, as this is a disease more common in higher age groups. In present study, there was limitation for internal examination of female and male genital organ. Diagnosis was made on the basis of history, presenting symptoms and available medical records and medicines if possible.

V. Conclusion

The present study is an endeavour to find out the morbidity pattern among elderly in Serampore city on a small scale of young growing state of West Bengal, along with the existing health practices and finally to suggest a pattern of health services suitable for the elderly population in the city. The study was conducted in 640 elderly subjects selected randomly from 32 areas

including urban and slum areas from 8 zones and 77 wards of Serampore city. Elderly persons in the age group, 60 years and above were 63635 (6.3% of total population in Serampore city), out of which only 640 persons (267 males and 373 females) were included in the study. Elderly females 373 (58.28%) out-numbered elderly males 267 (41.71%). Majority of the elderly persons (81.71%) belonged to "young old" age group. Bulk 40.15% of the elderly persons received education upto higher secondary. Graduates and above was only 15.78%, out of which 83.16% were in urban whereas 16.83% were from slum areas.

36.40% of the elderly population belonged to socio-economic Class IV, followed by Class II. A large proportion (84.07%) was living in joint families and 15.93% in nuclear family settings. Only 5.93% were living alone. 51.09% of the elderly were themselves heading the family with males predominating. A large proportion 42.03% of elderly population was unemployed. The principle occupation of the persons who were currently employed in some gainful occupation was agriculture/ shop owner/clerical 11.25%, while 18.12% were professional including retired persons. A large proportion 48.28% was financially dependent on others. Only 14.84% were receiving old age pension. Out of total dependent, 66.66% were dependent on their children, 13.26% on grand children and 1.29% on spouse, 14.56% on others. A small proportion 33.59% was aware about various Government welfare schemes for the elderly. The geriatric population is a dependent population. Hence, health care delivery system should reorganize their timing other than routine schedule. Periodic comprehensive health check up, preferably twice a year must be carried out and primary health care delivery must be ensured to geriatric population.

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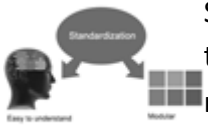

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- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

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
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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
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- 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."
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- 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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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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