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INFORMATION

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MELATONIN MITIGATES RENOLIPOTOXICITY IN HIGH-FAT DIET-INDUCED OBESE RAT MODEL

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ABSTRACT:
Obesity, a widespread global health issue affecting both adults and young individuals, has raised concerns due to its strong correlation with chronic kidney disease and end-stage renal disease, significantly contributing to the burden of kidney disorders. Renal lipotoxicity, driven by disrupted lipid metabolism in obesity, results in intracellular lipid accumulation in the kidneys, leading to cellular dysfunction. This study investigated how melatonin affects renal function in Wistar rats with diet-induced obesity. Twenty-four animals were separated into four groups: Control (CTL) group, which received a standard diet and distilled water; Melatonin group (MEL), received melatonin at a dose of 4 mg/kg of body weight; the Obese group (OBS), which was fed a 40% high-fat diet (HFD); and Obese treated with melatonin group (OBS + MEL), which received both the high-fat diet and melatonin daily for 12 weeks. The findings revealed that the high-fat diet led to increased food consumption and body weight, and melatonin supplementation helped mitigate these effects. Notably, there was no significant increase in renal mass. The results also revealed increased fasting insulin, fasting glucose levels and insulin resistance. Total cholesterol and triglyceride concentrations were significantly increased, while HDL was decreased in HFD group. However, melatonin ameliorated the altered lipid profile. Melatonin also attenuated increased MDA and GGT concentrations and restored decreased GSH and NO levels affected by HFD. The present study therefore indicates that melatonin alleviates oxidative stress, inflammation, lipid deposition and renal injury induced by high fat diet, suggesting a potential reno-protective property.

Key words: High fat diet; Kidney; Lipid; Melatonin; Obesity.

INTRODUCTION:

Obesity has emerged as a global epidemic, affecting a staggering number of adults and youth, with projections indicating a significant increase in the coming years. Its association with chronic kidney disease (CKD) and end-stage renal disease (ESRD) has raised concerns, with a substantial portion of kidney disease cases attributed to obesity [1,2]. This escalating obesity-related kidney disease has prompted a closer examination of the interplay between obesity, kidney dysfunction, and the underlying mechanisms driving this correlation. The surge in both obesity and kidney disease from 1980 to 2000 underscores the intricate relationship between the two conditions, as

ESRD incidence nearly quadrupled over a decade later [3]. Although conditions such as diabetes mellitus and hypertension have traditionally been linked to kidney disease, emerging evidence indicates that obesity and its associated metabolic syndrome play a more pivotal role in this epidemic [4]. The influence of obesity extends beyond its coexisting conditions, as epidemiological studies demonstrate its autonomous association with CKD and ESRD [5,6,7].

The mechanisms by which obesity engenders kidney disease remain elusive, but one compelling pathway is the concept of lipotoxicity. As obesity ensues, an imbalance between energy intake and expenditure triggers adipose tissue expansion, which, when limited, leads to ectopic lipid accumulation in organs, culminating in lipotoxicity [8,9]. Dysregulated lipid metabolism in obesity fosters the intracellular buildup of lipids and lipoproteins, inducing cellular dysfunction across tissues, from cardiomyocytes to hepatocytes [10,11,12]. In the kidney, this abnormal lipid metabolism manifests as renal lipotoxicity, characterized by the accumulation of triglycerides and cholesterol in glomerular and tubulointerstitial cells [13]. Excessive lipid accumulation within the renal tissue can precipitate injury to renal tubular cells [14], structural changes in glomeruli and lead to kidney dysfunctions [15,16,17]. Renal lipotoxicity is also significantly linked to the onset of conditions such as excess protein in the urine, inflammation of the glomeruli, and CKD [18].

The kidneys are particularly vulnerable to oxidative stress due to their susceptibility to lipid peroxidation when subjected to a diet rich in fat. Reactive oxygen species (ROS) production leads to the oxidative degradation of biological molecules such as lipids, resulting in the initiation of lipid peroxidation, which results in the production of malondiadehyde (MDA), within renal tissues. This process subsequently disrupts the endogenous antioxidant defense system [19,20]. Additionally, this process sets off a sequence of interconnected reactions within renal tissues that culminate in reno-toxicity [21,22]. The repercussions of renal lipotoxicity are multifaceted, triggering the upregulation of proinflammatory cytokines [23]. Notably, this cascade parallels the association between ectopic lipid deposition and decreased insulin sensitivity, contributing to the development of type 2 diabetes [2]. Furthermore, the kidney, like other affected organs, becomes a target for detrimental lipid accumulation, fostering a deleterious environment.

Melatonin (MEL), a hormone produced by the pineal gland in response to darkness, is a versatile compound with potent antioxidant properties and various endocrine functions [24]. MEL is particularly abundant in mitochondria, where its well-characterized antioxidant actions prevail [25]. Upon release into the cerebrospinal fluid and bloodstream, MEL becomes widely available throughout the central nervous system (CNS) and peripheral tissues respectively,

holding potential clinical relevance in various diseases [26]. One of MEL's primary roles lies in maintaining proper energy balance by regulating energy flow to and from storage and directly influencing energy expenditure, primarily through the activation of brown adipose tissue. Additionally, MEL promotes the transformation of white adipose tissue into brown, aiding in the regulation of body weight [27]. Furthermore, MEL serves multiple biological functions, including anti-inflammatory and antioxidant effects, suppression of the sympathetic nervous system, and the maintenance of endothelial cell function. It is well-documented that MEL administration lowers blood pressure (BP), and endogenous MEL secretion levels diminish with declining renal function [28, 29, 30]. Moreover, MEL plays a pivotal role in renal physiology and has been associated with the pathophysiology of the kidneys. Some animal models have been utilized in experimental settings to investigate the effects of MEL on renal function and structure [31,32]. Through these models, it was shown that MEL plays a crucial role in preserving renal function and ameliorating renal structure. Despite this wealth of knowledge, the role of MEL in obesity-associated renolipotoxicity remains inadequately understood. Therefore, this current study investigated the influence of MEL on the intricate interplay between obesity, renal lipotoxicity, and kidney dysfunction.

METHODOLOGY:

Experimental Animals and grouping:

All experimental procedures in this study adhered to the guidelines set forth in the National Institutes of Health Guide for the Care and Use of Laboratory Animals [33]. The Institutional Ethical Review Board of Afe Babalola University, Nigeria, granted approval for this study (ABUADERC/10/2020). The ethical considerations of the 3 Rs, namely replacement, reduction, and refinement, were duly considered.

A total of twenty-four male Wistar rats, 170 – 200 grams, were sourced from the animal facility at the College of Health Sciences, Afe Babalola University, Nigeria. These rats were provided unrestricted access to standard rat chow and tap water. Following a 2-week acclimatization period, the rats were randomly divided into four groups, with six rats in each group. The rats were housed in a controlled environment with standard conditions, including a temperature range of 22–26°C, relative humidity between 50–60%, and a 12-hour light/dark cycle.

Treatment:

The study included four groups of animals with different treatments: the control group (CTL) received regular rat chow and distilled water; the melatonin-treated group (MEL) received melatonin at a dose of 4 mg/kg body weight; the obese group (OBS) was given a high-fat diet (HFD) consisting of 40% fat; and the obese group with melatonin treatment (OBS + MEL-

treated) received both the 40% high-fat diet and melatonin at a dose of 4 mg/kg daily.

Melatonin administration for groups 2 and 4 took place between 8:00 and 10:00 am daily. Obesity was induced in groups 3 and 4 by providing them with unrestricted access to the 40% HFD, as previously described [34,35]. This treatment regimen continued for 12 weeks. At the beginning and end of the 12-week period, the animals' initial and final body weights were measured, and the amount of weight gained was calculated. Furthermore, daily food and water consumption were carefully monitored during week 0 (initial) and week 12 (final) by subtracting any remaining food and water after 24 hours from the initial quantities provided to the animals. Changes in food and water consumption were determined by subtracting the initial consumption from the final consumption. All the results were recorded appropriately.

Assay of metabolic parameters:

Fasting blood glucose was determined with a hand-held glucometer (ONETOUCH1-LifeScan, Inc., Milpitas, CA, USA). The insulin concentration in the plasma was determined using Rat ELISA kits obtained from Calbiotech Inc. (El Cajon, USA). This study followed the manufacturer's instructions and used the direct sandwich approach, which involves using two monoclonal antibodies that target different antigenic regions on the insulin molecule. Insulin resistance was determined using the homeostatic model assessment of insulin resistance (HOMA-IR), calculated as fasting glucose (mmol/l) multiplied by fasting insulin (μU/l) divided by 22.5, following the methods described in previous studies [36].

Sample Preparation:

Following 12 weeks of treatment, the animals underwent a 12-hour overnight fast. Subsequently, they were anesthetized via intraperitoneal injection of 50 mg/kg body weight of sodium pentobarbital. Blood was collected through cardiac puncture into heparinized tubes and then centrifuged at room temperature for 5 minutes at 3000 rpm. The plasma was separated and stored frozen until it was required for biochemical assay.

Preparation of kidney tissue homogenates:

Following the kidney's weight measurement, a 100 mg tissue section was meticulously excised and homogenized using a glass homogenizer in a phosphate buffer solution. The homogenate was then subjected to centrifugation at 10,000 rpm for 10 minutes at 4°C.

Biochemical assays:

Lipid profile:

Concentrations of triglycerides (TG), total cholesterol (TC) and high -density lipoprotein (HDL) were estimated in the kidney tissue homogenates by standardized colorimetric methods using reagents obtained from Fortress Diagnostics Ltd. (Antrim, UK) [37,38].

Oxidative stress markers:

Malondialdehyde (MDA) was determined from the kidney tissue homogenate by standard non-enzymatic spectrophotometric method using assay kits from Randox Laboratory Ltd. (Co. Antrim, UK). Whereas Reduced Glutathione (GSH) was determined using non-enzymatic spectrophotometric method with assay kits obtained from Oxford Biomedical Research Inc. (Oxford, USA). Glutathione was determined by spectrophotometric method based on the oxidation of GSH in the sample by the sulfhydryl reagent 5,50 -dithio-bis (2-nitrobenzoic acid) (DTNB) to form the yellow derivative 50 -thio-2-nitrobenzoic acid (TNB), measured at 412 nm. These assays were carried out as previously described [35,38].

Renal Nitric Oxide (NO), Gamma-glutamyl transferase (GGT), Nitric oxide levels were determined spectrophotometrically by quantifying the stable degradation products, nitrate and nitrite, utilizing kits from Oxford Biomedical Research Inc. in Oxford, UK [35]. This assay kit employs the NADH-dependent enzyme Nitrate reductase to convert nitrate to nitrite before quantifying nitrite with the Griess reagent, ensuring precise measurement of total NO production. Renal GGT was assessed using a conventional enzymatic colorimetric method, and assay kits for this purpose were procured from Fortress Diagnostics Ltd. in Antrim, UK [37].

Plasma Urea and Creatinine concentration:

Plasma urea levels were assessed using a standard spectrophotometric method with kits from Oxford Biomedical Research Inc., Oxford, UK. Plasma creatinine concentrations were measured via a non-enzymatic colorimetric method using assay kits from Randox Laboratory Ltd., Co. Antrim, UK, following the manufacturer's assay protocols [38].

Statistical analysis:

The results were presented as mean values with standard error of the mean (SEM). Statistical group analysis was conducted using GraphPad Prism 9.5.1. One-way ANOVA was employed to compare the variable means among the groups. Subsequently, Bonferroni's test was applied for post hoc analysis. Statistically significant differences were considered when p-values were less than 0.05.

RESULTS:

Effect of melatonin on food and water intake, body weight, and renal mass in high-fat diet-induced obese Wistar rats:

The results showed a significant ($p<0.05$) increase in food consumption in the obese group compared to the control group, which was mitigated with concurrent melatonin treatment. Water intake exhibited no significant variation when compared to the control group. However, body weight significantly ($p<0.05$) increased in the obese group in comparison to the control. Nevertheless, supplementation with melatonin

significantly ($p<0.05$) reduced body weight when compared to the untreated obese group. Furthermore, renal mass remained largely unchanged across all the experimental groups in comparison to the control (Table 1).

Table 1: Effect of Melatonin on Food and Water Intake and Body Weight Gain in High-Fat Diet-Induced Obese Wistar Rats.

GROUPS	CTL	MEL	OBS	OBS+MEL
Food intake (g/day)				
Initial	25.22 ± 0.81	33.15 ± 2.30	30.59 ± 4.24	31.85 ± 2.27
Change	8.01 ± 2.71	5.33 ± 1.79	19.21 ± 3.77*	4.42 ± 0.35#
Water intake (mL/day)				
Initial	32.62 ± 1.47	27.79 ± 3.16	26.63 ± 3.43	35.63 ± 3.43
Change	7.34 ± 2.52	5.44 ± 10.19	6.18 ± 5.44	5.86 ± 3.88
Body weight (g)				
Initial	172.71 ± 6.41	174.93 ± 8.12	171.00 ± 6.65	171.43 ± 5.70
Gain	44.40 ± 6.70	36.67 ± 9.30	75.87 ± 4.72*	26.69 ± 3.62#
Renal mass (g/kg)	6.59 ± 0.40	5.49 ± 0.84	7.42 ± 1.02	7.16 ± 0.82

Data are expressed as mean ± SEM. n=7. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc test*. (*p<0.05 VS. CTL; #p<0.05 VS. OBS). Control (CTL), Melatonin (MEL), Obesity (OBS).

Effect of melatonin on metabolic indices in high-fat diet induced obese Wistar rats:

There was a significant ($p<0.05$) elevation in fasting insulin levels in obese animals when compared to the control group, although fasting blood glucose remained unaltered. However, the supplementation of melatonin led to a reduction in fasting insulin in the OBS+MEL group in comparison to OBS, which did not receive melatonin supplementation. Exposure to a high-fat diet induced insulin resistance, as indicated by a concurrent increase in HOMA-IR in obese animals in contrast to the control group. Nevertheless, melatonin supplementation resulted in a decrease in HOMA-IR, thereby reducing insulin resistance in the OBS+MEL group in comparison to OBS without melatonin supplementation (Table 2).

Table 2: Effect of melatonin on fasting blood glucose, fasting insulin and HOMA-IR in high-fat diet-induced obese Wistar rats

GROUPS	CTL	MEL	OBS	OBS+MEL
Fasting blood glucose (mmol/L)	4.97 ± 0.21	5.42 ± 0.36	5.08 ± 0.36	5.02 ± 0.30
Fasting insulin (µIU/mL)	1.77 ± 0.03	1.95 ± 0.08	2.98 ± 0.09*	1.82 ± 0.04#
HOMA-IR	0.39 ± 0.02	0.47 ± 0.04	0.67 ± 0.05*	0.40 ± 0.02#

Data are expressed as mean ± S.E.M. n=7. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc test*. (*p<0.05 VS. CTL; #p<0.05 VS. OBS). Control (CTL), Melatonin (MEL), Obesity (OBS)

Effect of melatonin on lipid profile in the kidney in high-fat diet-induced obese Wistar rats:

A significant ($p<0.05$) rise in the levels of TC and TG was observed in the kidney tissue of the obese group when compared to the control group. Nevertheless, melatonin treatment led to a significant decrease in TC and TG concentrations in the kidney. In addition, a significant drop in HDL levels was noted in the kidney tissue of the obese group in comparison to the control group. Conversely, the kidney tissue of the OBS+MEL group displayed an increase in HDL concentrations (Fig 1).

Effect of melatonin on MDA levels in high-fat diet-induced obese Wistar rats:

A significant increase ($p<0.05$) in kidney tissue MDA levels was evident in the obese group when compared to the control group. Nonetheless, melatonin supplementation led to a significant reduction in MDA concentration in the OBS+MEL group when compared to the untreated obese group (Fig 2).

Effect of melatonin on GSH level in high-fat diet-induced obese Wistar rats:

A significant reduction ($p<0.05$) in kidney tissue GSH levels was observed in the obese group when compared to the control group. Nevertheless, melatonin supplementation resulted in a significant increase in kidney GSH concentration in the OBS+MEL group when compared to the untreated obese group (Fig 3).

Effect of melatonin on Nitric oxide concentration in high-fat diet-induced obese Wistar rats:

A significant reduction ($p<0.05$) in kidney tissue NO levels was observed in the obese group when compared to the control group. Nevertheless, melatonin treatment led to a significant increase in kidney Nitric oxide concentration in the OBS+MEL group when compared to the untreated obese group (Fig 4).

Effect of melatonin on gamma-glutamyl transferase concentration [GGT] in HFD-induced obese rats:

A significant increase ($p<0.05$) in the level of GGT in kidney tissue was observed in the obese group compared to the control group. On the other hand, treatment with melatonin resulted in a significant decrease in GGT levels in obese animals (Fig 5).

Effect of melatonin on urea and creatinine concentrations in high-fat diet-induced obese Wistar rats:

No significant changes ($p<0.05$) in plasma urea and creatinine levels in the OBS group when compared to the control group, as well as in the OBS+MEL group when compared to the OBS group (Fig 6).

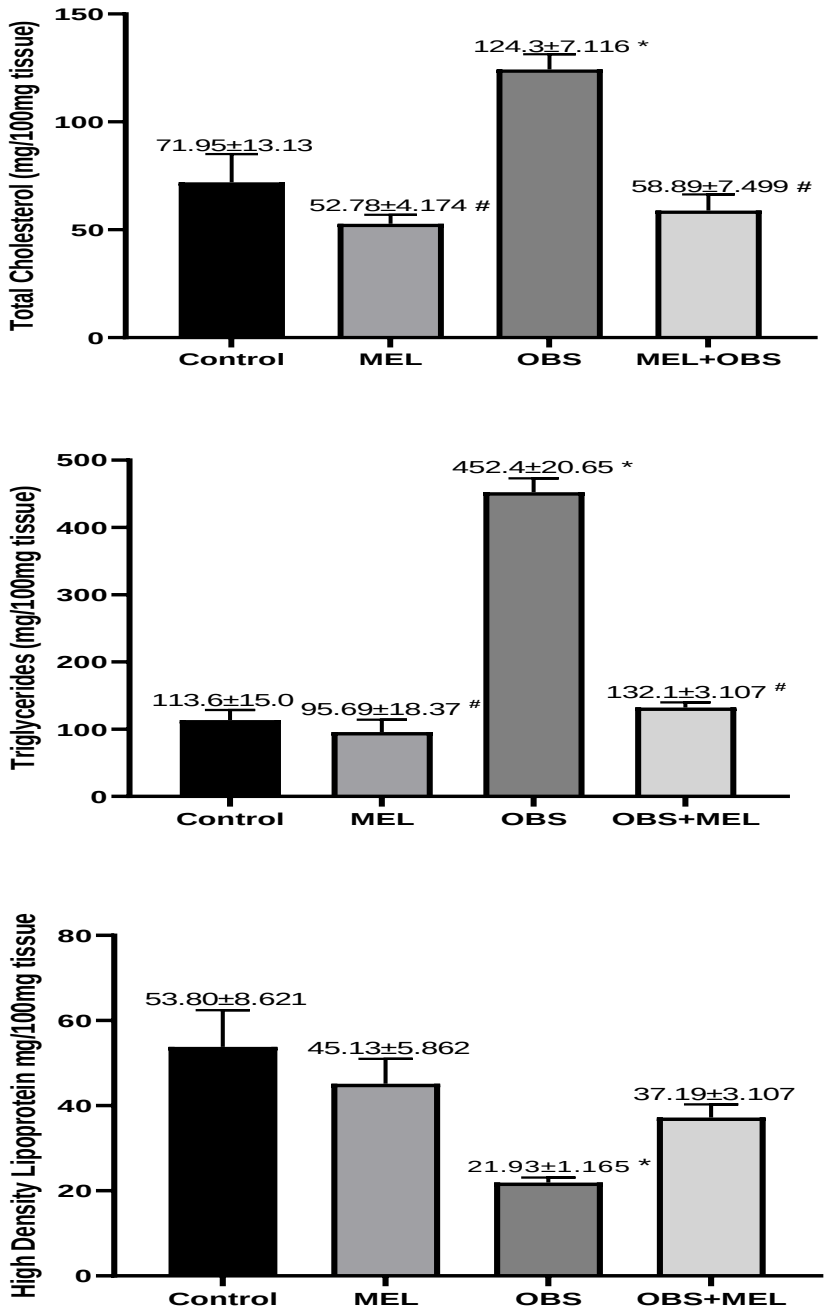

Figure 1: Effect of melatonin on total cholesterol, triglyceride and high density lipoprotein (HDL) levels in the kidney in high-fat diet-induced obese Wistar rat. Data are expressed as mean ± S.E.M. n=7. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. (*p<0.05 VS. CTL; #p<0.05 VS. OBS). Control (CTL), Melatonin (MEL), Obesity (OBS).

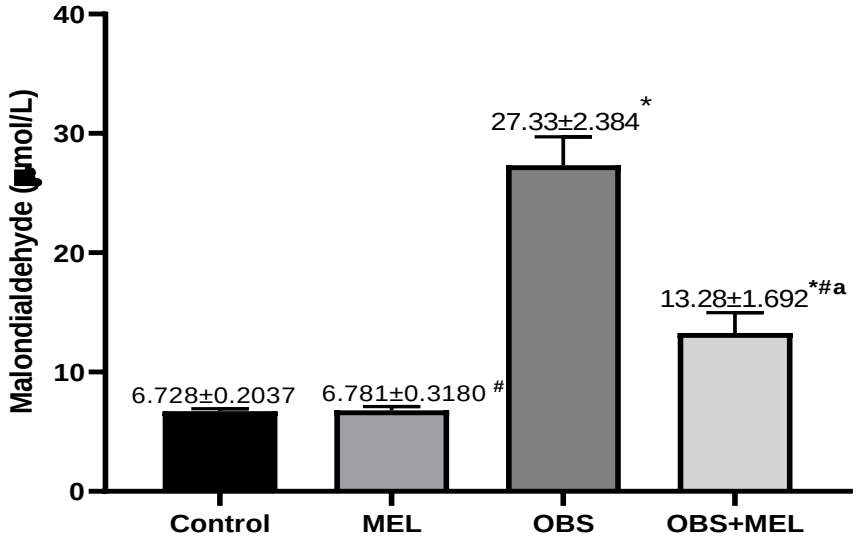

Figure 2: Effect of melatonin on malondialdehyde (MDA) in high-fat diet-induced obese Wistar rats.

Data are expressed as mean ± S.E.M. n=7. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc test*. (**p*<0.05 VS. CTL; #*p*<0.05 VS. OBS, ap<0.05 VS. MEL). Control (CTL), Melatonin (MEL), Obesity (OBS).

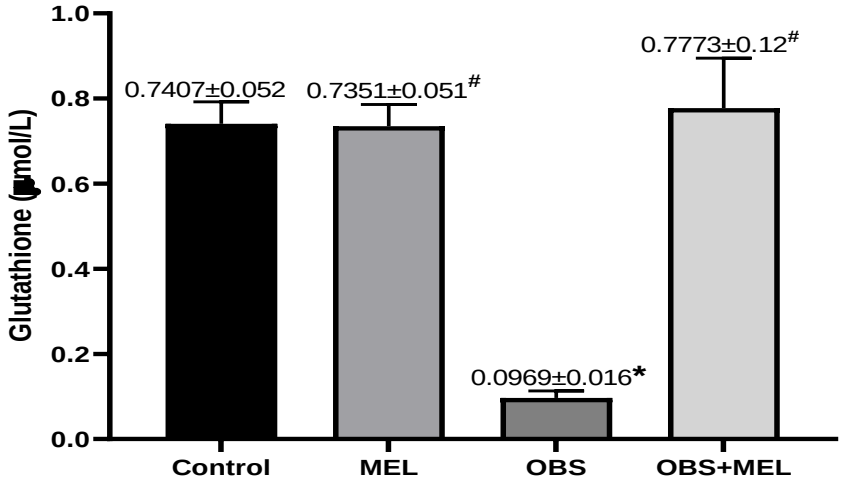

Figure 3: Effect of melatonin on glutathione (GSH) levels in high-fat diet-induced obese Wistar rats.

Data are expressed as mean ± S.E.M. n=7. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. (*p<0.05 VS. CTL; #p<0.05 VS. OBS). Control (CTL), Melatonin (MEL), Obesity (OBS).

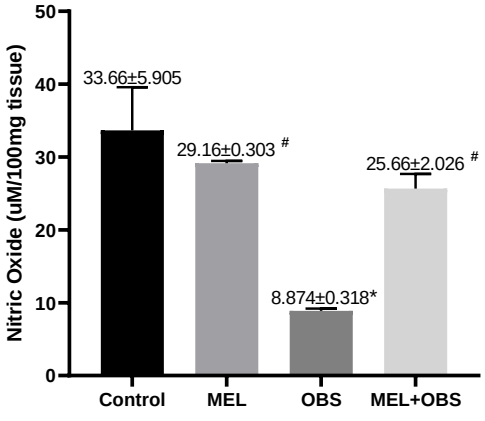

Figure 4: Effect of melatonin on nitric oxide concentration [NO] in high-fat diet-induced obese Wistar rats.
Data are expressed as mean ± S.E.M. n=7. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. (*p<0.05 VS. CTL; #p<0.05 VS. OBS). Control (CTL), Melatonin (MEL), Obesity (OBS).

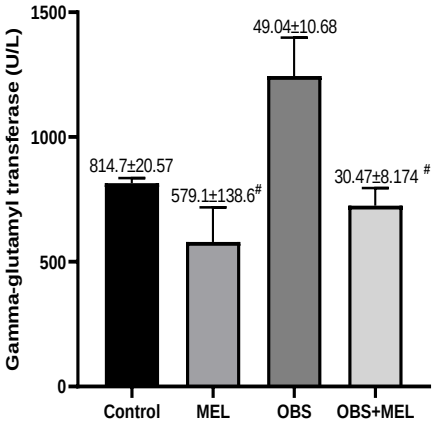

Figure 5: Effect of melatonin on gamma-glutamyl transferase concentration [GGT] in HFD-induced obese rats.
Data are expressed as mean ± S.E.M. n=7. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. (#p<0.05 VS. OBS). Control (CTL), Melatonin (MEL), Obesity (OBS).

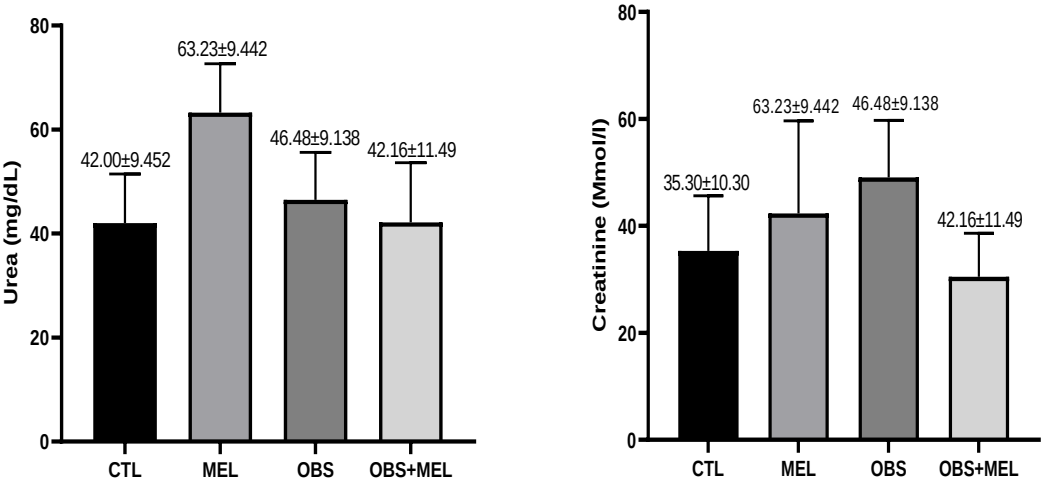

Figure 6: Effect of melatonin on plasma urea and plasma creatinine in high-fat diet-induced obese Wistar rats.
Data are expressed as mean ± S.E.M. n=7. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Control (CTL), Melatonin (MEL), Obesity (OBS).

DISCUSSION:

A high-fat diet (HFD) characterizes a significant risk factor for the onset of metabolic disorders, including hyperlipidemia, hypertension, diabetes mellitus, obesity, insulin dysfunction, and oxidative stress [39]. Among vital organs, the

kidneys are particularly susceptible to functional alterations induced by the influence of a high-fat diet [40]. This dietary pattern disrupts the delicate balance of energy metabolism, leading to the accumulation of lipids in unconventional anatomical locations and intracellular compartments [41]. The ectopic deposition of lipids within the renal tissue, in association with insulin resistance, has been linked to a gradual deterioration in renal function, [42, 43]. This harmful impact exerted by lipids on cells and tissues is referred to as lipotoxicity [2,44]. The present findings seek to assess the effect of melatonin on high-fat diet-induced obesity on kidney parameters in male Wistar rats. The results provide evidence that high-fat diet leads to decreased insulin sensitivity, disturbances in lipid profiles, increased body weight, oxidative stress, elevated concentrations of nitric oxide (NO) and gamma-glutamyl transferase (GGT) which were ameliorated upon melatonin treatment.

In a study reported by Charradi et al., HFD led to evident obesity, characterized by notable increases in body weight, abdominal fat accumulation, and elevated cholesterol, triglyceride, and phospholipid levels [45]. Notably, animals subjected to this HFD regimen did not exhibit a rise in fasting blood glucose levels. However, their area under the curve (AUC) increased, and their Kitt decreased, signifying glucose dysregulation and impaired insulin sensitivity [45].

These findings align with those reported by Higa et al. who observed a similar response in mice subjected to a cafeteria diet [46]. Furthermore, in a separate HFD investigation documented by Charradi et al., kidney mass remained unaffected, but there was a notable deposition of triglycerides without concurrent changes in cholesterol or phospholipid levels [47].

These outcomes parallel this current study, with increasing food consumption, body weight, fasting blood insulin levels, and insulin resistance, without a corresponding alteration in fasting blood glucose levels or renal mass. However, when melatonin was administered, the HFD group exhibited reduced food intake, body weight, insulin levels, and improved insulin sensitivity. The findings are consistent with a study of high-fat-fed mice treated with melatonin, which shows that there was an improvement in glucose tolerance and insulin sensitivity [48]. Furthermore, another investigation revealed that daily administration of melatonin led to a 54% reduction in body weight in high-fat-fed rats [49]. These reports indicated that melatonin mitigates the metabolic consequences of diet-induced obesity.

It has been reported that melatonin receptor 1 (MT1) signaling may regulate several protective metabolic responses, thus presenting the possibility that melatonin through MT1 may counter the metabolic effects of diet-induced obesity [50].

Numerous mice studies have provided evidence linking disrupted lipid metabolism to the onset of

kidney damage to high-fat diets [43,51,52]. Another animal model study depicting metabolic syndrome reported elevated leptin, triglyceride, reduced adiponectin levels and normal concentration of insulin [53]. Furthermore, these observations are in accordance with epidemiological studies that establish a direct relationship between elevated triglyceride levels and the predisposition to CKD [54], as well as the impact of altered lipid profile on the advancement of CKD [55].

In the HFD group in the current study, there was a significant increase in TC and TG levels, while the level of HDL was notably decreased. However, melatonin administration mitigated the alterations in lipid profiles induced by the HFD, restoring kidney lipid metabolism.

Previously, a study revealed that melatonin reduced body weight and low-density-lipoprotein (LDL) cholesterol in young Zucker diabetic fatty rats [56]. Additionally, another report conducted by Chen et al. demonstrated that melatonin enhances accumulation and metabolism of lipid in the liver [57]. It has also been observed that melatonin alleviates impaired insulin sensitivity, inflammation, and liver steatosis in HFD induced obese mice [58]. Clinical studies have also corroborated the positive impact of melatonin on lipid metabolism [59,60,61].

Lipotoxicity-induced oxidative stress has been associated with the development of various renal disorders [18,62]. Extended consumption of HFD leads to an overproduction of reactive oxygen species (ROS) within the kidneys, compounding the risk of renal injury due to ROS-induced oxidative stress [63]. Hyperlipidemia can result in the suppression of tissue and cellular antioxidant capacity, resulting in elevated levels of free radicals, along with decreased activities of antioxidant enzymes in both tissues and plasma [64,65]. Moreover, a study reported elevation in renal lipoperoxidation and carbonylation and as well as reduced levels of sulfhydryl radicals and the antioxidant enzymes functions after the administration of HFD confirming the induction of oxidative stress [48]. Similarly, other HFD studies also observed significantly elevated oxidative stress marker and decreased concentration of antioxidant markers [19, 66,67]. The present study, however, demonstrates that melatonin treatment effectively lowers MDA levels and elevates GSH levels, both of which were disrupted by the HFD. These findings support the scavenging role of melatonin as it serves as a protective agent against oxidative stress, safeguarding cells from its detrimental effects. Furthermore, additional studies reinforce this inquiry, indicating that melatonin has the potential to mitigate kidney injury by reducing free radicals, oxidative stress, and enhancing the activity of antioxidant enzymes in renal tissues [68,69,70].

Recent research has illuminated the role of dyslipidemia, a critical element within the multi-hit mechanism, in causing harm to healthy kidneys through lipotoxicity, oxidative stress, and inflammation [71]. This suggests that

disorders stemming from lipid accumulation may trigger oxidative stress or inflammation, both of which are essential factors in lipid-induced kidney damage. Oxidative stress and inflammation share a close relationship, jointly contributing to renal dysfunctions [72]. Hyperlipidemia can greatly increase ROS production in monocytes, acting as ROS activators in the kidneys. These activators stimulate macrophages to produce excessive oxygen radicals, potentially causing tissue dysfunction. Inflammation is a key player in most CKDs, with inflammatory factors being pivotal contributors to this stress. Inflammatory stress fosters kidney lipid accumulation, resulting in glomerular lesions, and kidney injury is positively linked to elevated serum proinflammatory cytokine levels [67,73,74]. Previous research has highlighted that dyslipidemia, systemic oxidative stress, and inflammation are resultant effects of prolonged exposure to HFD and ultimately contributes to CKD [75, 76]. A previous study has linked chronic renal diseases to reduced levels of Nitric oxide (NO) [77]. Another study indicated that HFD provokes renal injury, evidenced by increased plasma urea and uric acid levels, alterations in creatinine clearance, and the presence of protein in urine [47]. Consumption of a high-fat diet led to significant alterations in renal function, demonstrated by a reduction in glomerular filtration rate (GFR) and an increase in serum creatinine levels [78,79].

In the present study, HFD significantly reduced NO levels and increased gamma-glutamyl transferase (GGT) concentrations, which were restored upon melatonin treatment. However, there were no significant alterations in urea and creatinine levels. Melatonin is known for its potent anti-inflammatory properties, demonstrating remarkable efficacy in ameliorating a range of conditions associated with oxidative stress and inflammation in animal experiments [80,81].

The administration of melatonin has shown promise in mitigating oxidative stress, inflammation, and hypertension, while also slowing the deterioration of kidney function and structure in rats with renal ablation [82]. Melatonin exerts a direct scavenging effect against oxidative stress, leading to reduced levels of plasma nitric oxide, lipid peroxidation, and renal MDA and enhances glutathione level [83,84]. Moreover, melatonin has been reported to ameliorate oxidative stress in obesity and diabetes [85,86]. Given that obesity and diabetes often induce oxidative stress, which can detrimentally affect kidney function, melatonin's antioxidative effects hold the potential to mitigate oxidative stress-induced kidney disorders in individuals with metabolic disorders [87].

CONCLUSION:

These current findings suggest that high-fat diet leads to kidney lipotoxicity, characterized by oxidative stress, inflammation, altered lipid

profile, and metabolic disruptions that contribute to kidney damage. Furthermore, these results indicate that melatonin supplementation mitigates renal dysfunctions associated with obesity by reducing oxidative stress and inflammation while improving metabolic parameters.

Conflict of Interests: The authors declare no conflict of interest.

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PREVALENCE OF ANAEMIA AND ASSESSMENT OF KNOWLEDGE, ATTITUDES, PRACTICES AND CONCERNS ABOUT ANAEMIA AMONG FEMALE STUDENTS IN UNIVERSITY OF PAPUA NEW GUINEA

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ABSTRACT:
Anaemia is among the major public health problems in most resource limited countries. It occurs when the haemoglobin (Hb) concentration in the blood is lower than the recommended WHO cut-off values. Some recent studies revealed that most female university students had an appropriate level of knowledge about anaemia, but showed a lack of positive attitude and good practices towards preventing it. The aims of this study were to determine the prevalence of anaemia and to evaluate the Knowledge, Attitudes, Practices and Concerns (KAPC) about anaemia among female students in the University of Papua New Guinea (UPNG). In this institution-based cross-sectional observational descriptive study, 217 female students consented to participate. The Point-of-Care HemoCue® Hb 301 was used to determine the haemoglobin levels of the consented female students. A self-designed pretested questionnaire was used for data collection. The self-administered questionnaire was designed to assess the Knowledge, Attitudes, Practices, and Concerns (KAPC) of female students about anaemia. Of the 217 students only 169 (77.9%) gave consent for measurement of their Hb level. The mean Hb level for all the students was 11.7 ± 1.92 g/dL, the range was 8.3 to 15.4 g/dL. Mild to moderate status of anaemia was prevalent among 45.6% (77/169) of the students. This indicates severe public health significance. The knowledge, attitudes, practices and concerns scores were 74.4%, 85.0%, 77.8% and 81.4% respectively. The scores indicated fair knowledge, good attitudes, practices and concerns about anaemia. A statistically significant relationship was found between Knowledge and Attitudes (p-value= 0.000); Knowledge and Practices (p-value= 0.000); Attitudes and Practices (p-value= 0.000); Attitudes and Concerns (p-value= 0.000). There were no statistically significant differences between the KAPC scores for the anaemic and non-anaemic students.

Keywords: Anaemia, university, female students, knowledge, attitudes, practices, concerns

INTRODUCTION:

According to the World Health Organization (WHO), United Nations Children's Fund (UNICEF), Centres for Disease Control and Prevention (CDC) and other international agencies, anaemia is a condition in which the number of red blood cells (consequently their oxygen-carrying capacity) is insufficient to meet the daily physiologic needs of the body [1, 2]. This occurs when the haemoglobin (Hb)

concentration in the blood is lower than the recommended WHO cut-off values [1, 2]. It is a significant aspect of malnutrition that affects women of reproductive age (15-49 years). Anaemia has multiple causes, both nutritional (vitamin and mineral deficiencies) and non-nutritional (infections, infestations and genetic) that may coexist. Anaemia is associated with poor cognitive and motor development, and work capacity [1-4]. It is considered to be among the top ten contributors to the global burden of diseases [1 – 4].

Recent reports by WHO indicated that in 2019, the global anemia prevalence was increased to 29.9% (95% uncertainty interval (UI) was 27.0% to 32.8%) in women of reproductive age, equivalent to over half a billion women aged 15-49 years. The prevalence was 29.6% (95% UI was 26.6% to 32.5%) in non-pregnant women of reproductive age, and 36.5% (95% UI was 34.0% to 39.1%) in pregnant women [5 – 8]. Reducing the prevalence of anaemia is a crucial part of achieving the Sustainable Development Goals (SDG) Target 2.2 as it directly impacts the health and well-being of women of reproductive age and their children [5].

Several authors have indicated that anaemia can have a significant impact on the health, academic performances, work productivity and physical activity levels among female university or adolescent students [9 – 15]. Findings in a recent study revealed that most female university students had an appropriate level of knowledge about anaemia, but showed a lack of positive attitude and good practices towards preventing it [12]. Other studies reported variation in the attitudes among female students towards anaemia [9 – 15]. They reported that most female students had appropriate knowledge but lacked positive attitudes and good practices towards preventing anaemia.

These reports underscore the importance of implementing awareness and educational programs to improve the knowledge of female students regarding consumption of adequate nutrition including micronutrients to enhance their haemoglobin level and reduce the prevalence of anaemia [13 – 15].

Recent findings indicated that poor knowledge, attitude and practices (KAP) regarding anaemia prevention can be classified as risk factors, because they contribute toward poor dietary habits and negligence in prevention activities [14, 15].

Available data on the prevalence of anaemia in Papua New Guinea (PNG) is very scanty. The limited data on anaemia among women were mainly from studies on women attending clinics [16 – 22]. One of the major reasons was because of the difficulty of obtaining blood from “apparently healthy” women in the households. Reports from most of the studies indicated a high prevalence of anaemia among women.

The report from the 2005 National Nutrition Survey (NNS 2005) indicated that the national mean Hb level was 12.6 ± 1.9 g/dL. Just over one-third of the women were anaemic [23]. Age and education were not significantly associated with anaemia prevalence or mean haemoglobin levels among non-pregnant women of childbearing age. Furthermore, using the WHO criteria for defining anaemia as a public health problem, the public health significance of the prevalence of anaemia among non-pregnant women of child bearing age was regarded as moderate [23]. The report also stated that more research is needed to assess the prevalence of anaemia among non-pregnant women of childbearing in PNG.

In a recent study carried out in the National Capital District (NCD) PNG, the authors reported that mild to moderate anaemia was prevalent among 28% of the non-pregnant women. This indicates moderate public health significance among the non-pregnant women at the time of the study [24].

Currently, there are no published data on the prevalence of anaemia among female university students in Papua New Guinea. The current proposal of the WHO is to significantly reduce the prevalence of anaemia among women and children by 2025 [25]. The PNG National Health Plan also indicated the need to reduce the prevalence of anaemia among vulnerable groups in the population [26]. Therefore, the need to assess the prevalence of anaemia among the women of childbearing age cannot be overemphasized.

This study was prompted by the apparent lack of data on the prevalence of anaemia and the knowledge, attitude, practices and concerns (KAPC) about anaemia among female students in the University of Papua New Guinea (UPNG).

Aims and Objectives:

The aims of this study were to determine the prevalence of anaemia and to evaluate the Knowledge, Attitudes, Practices and Concerns (KAPC) about anaemia among female students in the University of PNG (UPNG). The objectives were to use the data obtained to answer the questions: What is the level of knowledge and awareness about anaemia as a public health problem among female students in UPNG? What are the attitudes and practices of these students towards anaemia and how concerned are they about the consequences of anaemia?

METHODOLOGY:

Study sites: This study was carried out in the University of Papua New Guinea (UPNG), which is the premier university in Papua New Guinea (PNG). The UPNG is made up of five schools. Four of the five schools (School of Natural and Physical Sciences, School of Humanities and Social Sciences, School of Business and Public Policy and School of Law) are located in the Waigani campus; the School of Medicine and Health Sciences is located in the Taurama

campus. Students in the Taurama campus complete their foundation year in the Waigani campus before moving over to Taurama to pursue the degree in medicine, dentistry or the health sciences. Post-basic nurses from various hospitals around the country are also enrolled in the Taurama campus to complete the nursing degree.

The study subjects were consented female students registered in both UPNG campuses. The subjects were randomly selected among participants that volunteered. The study was conducted from May to September 2023.

Study design and sampling: This was an institution-based cross-sectional observational descriptive study. The target population consisted of registered female students, whether residential or non-residential, in both UPNG campuses. All subjects were selected randomly through a convenient selection of participants that volunteered.

Sample size: The sample size was calculated based on the assumption that the probability of having a good knowledge, positive attitudes, good practices and concerns about anaemia was about 50% at confidence level of 95%, with 5% precision and predicted non-response rate of 15%. The calculated sample size obtained was 300 female students. This sample size was considered adequate for a mini-survey with limited resources.

Inclusion criteria: The students were informed about the nature of the study and that their participation was entirely voluntary. All consenting female students were eligible to participate in the study.

Determination of Hb level: The Point-of-Care HemoCue® Hb 301 was used to determine the haemoglobin levels of consented female students. The standardized procedure involved ensuring warm and relaxed hands, selecting appropriate fingers for sampling, cleaning and puncturing the fingertip with a single use disposable lancet, then filling the HemoCue 301 microcuvette with a drop of whole blood. The process, which adhered to stringent hygiene and quality control was used to determine the Hb level for each of the consented participants.

Exclusion criteria for assay of Hb level: Students that do not give consent and those that have been sick with malaria or having high fever in the last five days were excluded from the study.

Data collection using a questionnaire: A self-designed pretested questionnaire was used for data collection. The self-administered questionnaire was designed to assess the Knowledge, Attitude, Practices, and Concerns (KAPC) of female students about anaemia. The questionnaire contained two sections (A and B). Section-A contained the respondents' socio-demographic profile. Section-B was to elicit

information about the respondent's Knowledge of anaemia and explored their Attitude, Practices and Concerns regarding anaemia.
The questionnaire was pre-tested among 20 randomly selected students. Feedback and suggested changes were provided orally and in writing. The feedback was used to improve the questionnaire.

Ethical Clearance: Approval for this project was obtained from the Ethical and Research Grant Committee of the SMHS, UPNG. Hb level was obtained by finger stick of participants after obtaining their informed consent. Informed consent was obtained from each of the female students. Participant consent was documented on each participant interview form. This consent procedure was approved by the ethics committees.

Statistical analysis and interpretation of the data: Before the statistical analysis, completeness of the data was evaluated. The completed questionnaires were collected, coded and entered into Microsoft Excel Spreadsheets (version 2016) for analysis. Further statistical data analysis was performed using IBM Statistical Package for the Social Sciences (IBM SPSS) version 22. Descriptive and inferential statistics were used to analyse the data obtained and make inferences about the study population. Descriptive statistics were used to analyse demographic and anthropometric characteristics such as age, height and weight. The mean, standard deviation (SD), range and median, were calculated for quantitative parameters.

Assessment of Anaemia: Anaemia was indicated when the Hb concentration falls below the WHO recommended cut-off values for Hb concentration for non-pregnant women of child-bearing age. Thus, the WHO guidelines and cut-off points were used for the interpretation of the data [1 – 3]. For non-pregnant women, Hb levels below 12.0 g/dl indicate anaemia. Hb levels of 11.0 to 11.9 g/dl, 8.0 to 10.9 g/dl and below 8.0 g/dl indicate mild, moderate and severe anaemia, respectively. Anaemia was classified as a mild, moderate or severe public health problem when the prevalence was 5.0 to 19.9%, 20.0 to 39.9% and greater than or equal to 40.0%, respectively [1 – 3].

Interpretation of the KAPC scores: The number of correct answers for Q 6 to Q 15, Q 16 to Q 25, Q 26 to Q 35, and Q 36 to Q 45 were used to calculate the Knowledge, Attitude, Practices and Concerns scores, respectively. The knowledge score for each participant was determined by allotting a score of '1' to correct responses and '0' (zero) to incorrect and 'don't know' responses. The attitude, practices, and concern scores were obtained by assigning points to responses on the modified 3-point Likert scale (Agree, Disagree, Neither agree nor disagree).

To obtain the score for each of the sections (KAPC), the number of correct answers obtained was expressed as percentage of the total number of correct answers for the section. The questions in each of the sections (KAPC) used for calculating the scores are highlighted on the questionnaires.

The criteria proposed by Hasan et al [27] were used for the categorization of the KAPC levels of the respondents. Score ≥ 75% was categorized as Good. Scores between 74 – 65% were categorized as Fair. Scores below 64% were categorized as Poor.

RESULTS:

Demographic characteristic of the female students:

Of the 250 questionnaires distributed among the female students, 217 were completed and used for analysis (response rate of 86.8%). They were all full time students resident in the National Capital District (NCD) at the time of this study. The mean age (Mean ± STD) of all the students was 23.5 ± 4.45 years, age range was 18.0 to 45.0 years and the 95% confidence interval (95% CI) was 23.0 to 25.0 years and their median age was 22.0 years.

Distribution of the students according to age groups shows that 6.9% (15/217) were in the 15 to 19.9 years age group, 76.0% (165/217) were in the 20 to 24.9 years age group, 8.3% (18/217) in the 25 to 29.9 years age group and 8.8% (19/217) in the over 30 years age group.

The mean height of the students was 1.60 ± 0.07 meters, range was 1.46 to 1.80 meters and median height was 1.60 meters. The mean weight was 62.0 ± 11.3 kg, range was 40.0 to 105.0 kg and median weight was 62.0 kg. The mean BMI was 24.2 ± 4.3 kg/m2, the range was 18.8 to 32.4 kg/m2 and the median was 25.6 kg/m2.

Haemoglobin (Hb) levels of female students:

Of the 217 students only 169 (77.9%) gave consent for measurement of their Hb level. The mean Hb level for the consented students was 11.7 ± 1.92 g/dL, the range was 8.3 to 15.4 g/dL, the 95% CI was 11.3 to 12.1 g/dL, and the median was 12.0 g/dL.

According to the WHO guidelines and cut-off points for interpretation of the Hb data, the results show that Hb level was normal (Hb ≥ 12.0 g/dL) among 54.4% (92/169) of students, mild anaemia (Hb: 11.0 to 11.9 g/dL) was prevalent among 16.0% (27/169), moderate anaemia (Hb: 8.0 to 10.9 g/dL) was prevalent among 24.9% (42/169) and severe anaemia (Hb < 8.0 g/dL) was prevalent among 4.7% (8/169) of the students that participated in this study.

Thus, of the 169 female students that participated in this study 45.6% (77/169) were anaemic. This indicates severe public health significance.

For further analysis of the data, the 169 students were separated into two groups. Those that were anaemic (45.6%) and those that were not anaemic (54.4%) at the time of the study. The

mean height of the anaemic group was 1.59 ± 0.08 meters and median height was 1.6 meters. The mean weight was 61.1 ± 12.21 kg and the median weight was 63.0 kg. For those in the non-anaemic group, the mean height was 1.59 ± 0.06 meters and median height was 1.58 meters. The mean weight was 64.54 ± 12.42 kg and median weight was 62.35 kg. There was not statistically significant difference between the corresponding heights and weights of the students in both groups.

Analysis of the questionnaires:

In response to the question 4 (Q 4) "*Have you been ever diagnosed with anaemia?*" A total of 81.1% (176/217) of all the students said "No". The students were then asked Q 5 "*Does anyone in your family have anaemia?* 60.4% (131/217) of the students responded in the negative and 32.2% (70/217) said "don't know". The results indicate low prevalence of anaemia among the family members of the students that participated in this study.

Knowledge (K): The questions in this section (Q 6 to Q 15) were to assess the knowledge and awareness of the students about anaemia and the associated problems. The questions and responses are presented in Table 1. The modified 3-point Likert Scale was used to interpret the responses: Agree, Disagree, Neither agree nor disagree.

When asked if they know about anaemia (Q 6), 84.8% of the students answered in the affirmative. In response to the next question (Q 7) "*Is anaemia a serious health problem?*" 77.4% responded in the affirmative.

In Q 8, the students were asked to indicate if they agree or disagree with each of the five options listed that represents the common meanings of anaemia. A total of 92.6% agreed with "decrease of iron in blood"; 86.2% agreed with "lack of red blood cells"; 67.7% agreed with "Hb levels below 12 g/L".

The next question (Q 9) listed some of the common causes of anaemia. The students were requested to indicate if they agree or disagree with each of the options listed. A total of 93.5% agreed with "Iron deficiency"; 90.8% agreed with "Decreased dietary Iron intake"; 70.1% agreed with "Folic acid deficiency"; 77.4% agreed with "Heavy menstrual bleeding". The other results are presented in Table 1.

The follow up question (Q 10) listed the common types of anaemia. Students were asked to indicate if they agree or disagree with each of the options. 88.9% agreed with "Iron deficiency anaemia"; 59.9% agreed with "Vitamin deficiency anaemia"; 60.8% agreed with "Megaloblastic anaemia"; 74.6% agreed with "Sickle cell anaemia'; 57.1% agreed with "Thalassemia". The other results are presented in Table 1.

Some common symptoms of anaemia were listed in Q 11 and students were asked to indicate if they agree or disagree with each of the symptoms. 94.0% agree that "Pale skin colour" was a symptom; 95.4% agreed that "Fatigue or Weakness" was a symptom; 58.5%

agreed that "Decreased appetite" was a symptom. Q 12 listed some of the effects of anaemia. Students were requested to state if they agree or disagree with each of the options listed. The responses are presented in Table 1. 72.4% agreed that anaemia can cause "Decrease in growth and development". 90.3% agreed that anaemia can cause "Decrease in working capacity". 76.5% agreed that anaemia causes "Poor cognitive and motor development outcomes in children". The responses to the other knowledge questions (Q 13 to Q 15) are presented in Table 1.

Table 1: Knowledge (K): Responses to knowledge questions.

				RESPONDENTS (N = 217)
Q 6	Do you know about anaemia?		Yes	84.8% (184)
			No	6.9% (15)
			Don't know	8.3% (18)
Q 7	Is anaemia a serious health problem?		Yes	77.4% (168)
			No	3.7% (8)
			Don't know	18.9% (41)
Q 8	**We have listed some of the common meanings of anaemia. Please indicate, in each case, if you agree or disagree with the statement below about anaemia.**			
	i.	Decrease of Iron in blood	Agree	92.6% (201)
			Disagree	1.4% (3)
			Neither agree nor disagree	6.0% (13)
	ii.	Increase of Iron in blood	Agree	1.8% (4)
			Disagree	83.4% (181)
			Neither agree nor disagree	14.7% (32)
	iii.	Lack of red blood cells	Agree	86.2% (187)
			Disagree	6.9% (15)
			Neither agree nor disagree	6.9% (15)
	iv.	Hb levels <12 g/dL	Agree	67.7% (147)
			Disagree	8.3% (18)
			Neither agree nor disagree	24.0% (52)
	v.	Hb level below WHO recommended cut-off point	Agree	67.7% (147)
			Disagree	2.8% (6)
			Neither agree nor disagree	29.5% (64)
Q 9	**We have listed some of the common causes of anaemia. Please indicate, in each case, if you agree or disagree.**			
	(i) Iron deficiency		Agree	93.5% (203)
			Disagree	1.8% (4)
			Neither agree nor disagree	4.6% (10)
	(ii) Decreased dietary iron intake		Agree	90.8% (197)

		Disagree	2.3% (5)
		Neither agree nor disagree	6.9% (15)
	(iii) Folic acid deficiency	Agree	70.1% (152)
		Disagree	5.5% (12)
		Neither agree nor disagree	24.4% (53)
	(iv) Infections	Agree	67.3% (146)
		Disagree	12.9% (28)
		Neither agree nor disagree	19.8% (43)
	(v) Heavy menstrual bleeding	Agree	77.4% (168)
		Disagree	10.1% (22)
		Neither agree nor disagree	12.4% (27)
	(vi) Worm infestation	Agree	49.3% (107)
		Disagree	16.6% (36)
		Neither agree nor disagree	34.1% (74)
	(vii) Genetic	Agree	67.3% (146)
		Disagree	10.1% (22)
		Neither agree nor disagree	22.6% (49)
	(viii) Toxic effects of some heavy metals like lead in the diet	Agree	51.1% (111)
		Disagree	9.7% (21)
		Neither agree nor disagree	39.2% (85)
	(ix) Poor knowledge about the causes of anaemia	Agree	62.7% (136)
		Disagree	13.8% (30)
		Neither agree nor disagree	23.5% (51)
	(x) Lack of proper healthcare	Agree	63.6% (138)
		Disagree	10.1% (22)
		Neither agree nor disagree	26.3% (57)
Q 10	**We have listed the common types of anaemia. Please indicate in each case, if you agree or disagree.**		
	(i) Iron deficiency anaemia	Agree	88.9% (193)
		Disagree	0.5% (1)
		Neither agree nor disagree	10.6% (23)
	(ii) Vitamin deficiency anaemia	Agree	59.9% (130)
		Disagree	14.3% (31)
		Neither agree nor disagree	25.8% (56)
	(iii) Aplastic anaemia	Agree	57.1% (124)
		Disagree	3.2% (7)
		Neither agree nor disagree	39.6% (86)
	(iv) Non-responsive iron deficiency anaemia	Agree	39.6% (86)
		Disagree	12.4% (27)
		Neither agree nor disagree	47.9% (104)

	(v) Megaloblastic anaemia	Agree	60.8% (132)
		Disagree	2.8% (6)
		Neither agree nor disagree	36.4% (79)
	(vi) Sickle cell anaemia	Agree	74.6% (162)
		Disagree	2.8% (6)
		Neither agree nor disagree	22.6% (49)
	(vii) Thalassemia	Agree	57.1% (124)
		Disagree	5.1% (11)
		Neither agree nor disagree	37.8% (82)
	(viii) Pernicious anaemia	Agree	54.3% (118)
		Disagree	2.8% (6)
		Neither agree nor disagree	42.9% (93)
	(ix) Haemolytic anaemia	Agree	71.9% (156)
		Disagree	1.4% (3)
		Neither agree nor disagree	26.7% (58)
	(x) Anaemia of chronic disease	Agree	68.6% (149)
		Disagree	0.5% (1)
		Neither agree nor disagree	30.9% (67)
Q 11	**We have listed some common symptoms of anaemia. Please indicate if you agree or disagree.**		
	(i) Pallor	Agree	73.3% (159)
		Disagree	1.8% (4)
		Neither agree nor disagree	24.9% (54)
	(ii) Pale skin color	Agree	94.0% (204)
		Disagree	1.8% (4)
		Neither agree nor disagree	4.2% (9)
	(iii) Fatigue or Weakness	Agree	95.4% (207)
		Disagree	1.4% (3)
		Neither agree nor disagree	3.2% (7)
	(iv) Irritability	Agree	52.5% (114)
		Disagree	13.4% (29)
		Neither agree nor disagree	34.1% (74)
	(v) Shortness of breath	Agree	74.2% (161)
		Disagree	9.2% (20)
		Neither agree nor disagree	16.6% (36)
	(vi) Decreased appetite	Agree	58.5% (127)
		Disagree	11.5% (25)
		Neither agree nor disagree	30.0% (65)
Q 12	**We have listed some of the effects of anaemia. Please indicate if you agree or disagree.**		
	(i) Decreases growth and development	Agree	72.4% (157)
		Disagree	6.9% (15)

		Neither agree nor disagree	20.7% (45)
	(ii) Decreases working capacity	Agree	90.3% (196)
		Disagree	1.8% (4)
		Neither agree nor disagree	7.8% (17)
	(iii) Decreases learning abilities	Agree	72.3% (157)
		Disagree	9.7% (21)
		Neither agree nor disagree	18.0% (39)
	(iv) Poor cognitive and motor development outcomes in children	Agree	76.5% (166)
		Disagree	4.1% (9)
		Neither agree nor disagree	19.4% (42)
	(v) Causes fatigue and low productivity	Agree	91.7% (199)
		Disagree	2.3% (5)
		Neither agree nor disagree	6.0% (13)
	(vi) Shortness of breath	Agree	74.7% (162)
		Disagree	6.0% (13)
		Neither agree nor disagree	19.3% (42)
	(vii) Poor birth outcomes (including low birth weight and prematurity)	Agree	77.9% (169)
		Disagree	1.8% (4)
		Neither agree nor disagree	20.3% (44)
	(viii) Increased maternal and perinatal mortality	Agree	72.4% (157)
		Disagree	3.2% (7)
		Neither agree nor disagree	24.4% (53)
Q 13	**Some ways of preventing / reducing anaemia are listed below. Please indicate if you agree or disagree**		
	(i) Eat iron-rich foods / Increase intake of dietary iron	Agree	97.7% (212)
		Disagree	0
		Neither agree nor disagree	2.3% (5)
	(ii) Consume vitamin C-rich fruits or drinks during/after meals	Agree	71.4% (155)
		Disagree	7.4% (16)
		Neither agree nor disagree	21.2% (46)
	(iii) Use appropriate supplementation regularly	Agree	77.4% (168)
		Disagree	1.8% (4)
		Neither agree nor disagree	20.7% (45)
	(iv) Give proper attention to other causes of anaemia	Agree	85.7% (186)
		Disagree	2.8% (6)
		Neither agree nor disagree	11.5% (25)
	(v) Avoid drinking strong tea or coffee after meals	Agree	45.6% (99)
		Disagree	14.3% (31)
		Neither agree nor disagree	40.1% (87)
	(vi) Pay more attention to personal hygiene	Agree	58.0% (126)

		Disagree	13.4% (29)
		Neither agree nor disagree	28.6% (62)
	(vii) Reduce intake of vegetables with high fibre content	Agree	30.4% (66)
		Disagree	33.2% (72)
		Neither agree nor disagree	36.4% (79)
	(viii) Treat parasitic infections (particularly helminths)	Agree	74.2% (161)
		Disagree	1.8% (4)
		Neither agree nor disagree	24.0% (52)
	(ix) Avoid cigarette smoking	Agree	77.0% (167)
		Disagree	3.7% (8)
		Neither agree nor disagree	19.3% (42)
	(x) Avoid excessive consumption of alcohol	Agree	77.9% (169)
		Disagree	2.8% (6)
		Neither agree nor disagree	19.3% (42)
Q 14	Adolescent girls are at high risk of anaemia because of their rapid growth and increased loss of iron during menstruation	Agree	74.7% (162)
		Disagree	4.1% (9)
		Neither agree nor disagree	21.2% (46)
Q 15	**There are many sources of information on all issues. Please indicate if you agree or disagree that the following sources of information may influence your opinion about anaemia.**		
	(i) News on National TV / Radio	Agree	71.9% (156)
		Disagree	10.1% (22)
		Neither agree nor disagree	18.0% (39)
	(ii) Government agencies	Agree	72.3% (157)
		Disagree	10.6% (23)
		Neither agree nor disagree	17.1% (37)
	(iii) Social media (Facebook, Instagram, WhatsApp)	Agree	62.2% (135)
		Disagree	17.1% (37)
		Neither agree nor disagree	20.7% (45)
	(iv) Friends and family	Agree	75.6% (164)
		Disagree	6.4% (14)
		Neither agree nor disagree	18.0% (39)
	(v) Health care providers (Doctors, Nurses, others)	Agree	96.3% (209)
		Disagree	0.5% (1)
		Neither agree nor disagree	3.2% (7)

Attitude (A): A total of 10 questions / statements (Q 16 to Q 25) were used to assess the attitude of the students towards anaemia. The modified 3-point Likert Scale was used to interpret the responses. In response to Q 16 "*Do you agree it is likely that you are anemic*?" 34.1% of the

students said "Agree", compared to 39.6% that "Disagree". The following up statement (Q 17) was "*Females are at greater risk of developing anaemia than males*", 84.3% "agreed". The other responses in this section are presented in Table 2. Notably, 93.1% agreed with the statement "*Awareness campaigns about anaemia should be carried out among female students regularly*".

Table 2: Attitude (A): This section is designed to assess the attitude / feelings of the students towards anaemia.

			RESPONDENTS (N = 217)
Q 16	Do you agree it is likely that you are anaemic?	Agree	34.1% (74)
		Disagree	39.6% (86)
		Neither agree nor disagree	26.3% (57)
Q 17	Females are at greater risk of developing anaemia than males	Agree	84.3% (183)
		Disagree	3.2% (7)
		Neither agree nor disagree	12.4% (27)
Q 18	UPNG should give you iron and folate supplements daily	Agree	66.8% (145)
		Disagree	8.3% (18)
		Neither agree nor disagree	24.9% (54)
Q 19	UPNG should give iron and folate to female students	Agree	68.7% (149)
		Disagree	6.9% (15)
		Neither agree nor disagree	24.4% (53)
Q 20	Anaemia is a serious public health problem among female students	Agree	69.1% (150)
		Disagree	3.2% (7)
		Neither agree nor disagree	27.7% (60)
Q 21	It is beneficial to prepare meals for students with iron-rich products	Agree	94.5% (205)
		Disagree	0
		Neither agree nor disagree	5.5% (12)
Q 22	Female students should receive fresh citrus fruits with meals daily	Agree	83.4% (181)
		Disagree	0.9% (2)
		Neither agree nor disagree	15.7% (34)
Q 23	Iron fortified rice should be used to prepare meals for students	Agree	77.0% (167)
		Disagree	0.5% (1)
		Neither agree nor disagree	22.5% (49)
Q 24	University clinic should test Hb level of female students regularly	Agree	92.2% (200)
		Disagree	1.8% (4)
		Neither agree nor disagree	6.0% (13)
Q 25	Awareness campaigns about anaemia should be carried out among female students regularly	Agree	93.1% (202)
		Disagree	0.9% (2)
		Neither agree nor disagree	6.0% (13)

Practices (P): In this section a total of 10 statements /questions (Q 26 to Q 35) were used to assess the dietary habits of the students. The results are presented in Table 3.

In response to the first statement (Q 26) "*Female students should consume food containing Heam Iron daily*", 84.8% of the students agreed. 73.7% of the students agreed with the statement (Q 27) that "*Female students should avoid skipping meals*". The next statement (Q 27) was "*It is important for a female student to seek medical attention as early as possible if she suspects that she has anaemia*". A total of 95.4% of the students agreed. The responses to the other statements (Q 28 to Q 35) are presented in Table 3.

Table 3: Practices (P): This section is to assess the dietary habits of female students

			RESPONDENTS (N = 217)
Q 26	Female students should consume food containing haem iron daily	Agree	84.8% (184)
		Disagree	1.4% (3)
		Neither agree nor disagree	13.8% (30)
Q 27	Female students should avoid skipping meals	Agree	73.7% (160)
		Disagree	5.1% (11)
		Neither agree nor disagree	21.2% (46)
Q 28	It is important for a female student to seek medical attention as early as possible if she suspects that she has anaemia	Agree	95.4% (207)
		Disagree	0
		Neither agree nor disagree	4.6% (10)
Q 29	It is important to check your Hb level once or twice a year	Agree	89.4% (194)
		Disagree	4.1% (9)
		Neither agree nor disagree	6.5% (14)
Q 30	Female students should take vitamin supplements regularly	Agree	69.1% (150)
		Disagree	2.8% (6)
		Neither agree nor disagree	28.1% (61)
Q 31	Female students should take deworming tablets once or twice a year.	Agree	64.1% (139)
		Disagree	3.2% (7)
		Neither agree nor disagree	32.7% (71)
Q 32	Female students should avoid drinking strong tea / coffee regularly.	Agree	54.8% (119)
		Disagree	12.0% (26)
		Neither agree nor disagree	33.2% (72)
Q 33		Agree	87.1% (189)

	You need to minimize exposure to tobacco smoking and alcohol;	Disagree	1.8% (4)
		Neither agree nor disagree	11.1% (24)
Q 34	It is very important to maintain good personal hygiene	Agree	91.7% (199)
		Disagree	1.4% (3)
		Neither agree nor disagree	6.9% (15)
Q 35	Inappropriate lifestyle can cause anaemia	Agree	75.6% (164)
		Disagree	2.8% (6)
		Neither agree nor disagree	21.6% (47)

Concerns (C): A total of 10 questions / statements (Q 36 to Q 45) were used to assess the concerns of the students about anaemia. The responses are presented in Table 4. The students were asked to state if they agree or disagree to each of the 10 statements in this section. A total of 85.7% of the students agreed with the statement (Q 36) "*You need to always wash your hands with soap after defecation*". Most of the students (92.2%) agreed to Q 38, that "*You need to wash fruits and vegetables before eating them*". Furthermore, 83.4% agreed to Q 39, that "*You need to trim your fingernails regularly*". In response to the statement (Q 45) "*You need to do regular exercise and ensure adequate nutrition*", 93.1% said that they agree. The responses to the other statements are presented in Table 4.

Table 4: Concerns (C): There are still some general concerns people have regarding anaemia that may creating doubt in your mind about the consequences of anaemia. Do you agree/disagree that the statements below may influence your efforts to avoid developing anaemia?

			RESPONDENTS (N = 217)
Q 36	You need to always wash your hands with soap after defecation;	Agree	85.7% (186)
		Disagree	4.6% (10)
		Neither agree nor disagree	9.7% (21)
Q 37	You need to wash your hands with soap before eating	Agree	90.3% (196)
		Disagree	2.8% (6)
		Neither agree nor disagree	6.9% (15)
Q 38	You need to wash fruits and vegetables before eating them;	Agree	92.2% (200)
		Disagree	2.3% (5)
		Neither agree nor disagree	5.5% (12)
Q 39	You need to trim your fingernails regularly;	Agree	83.4% (181)
		Disagree	5.1% (11)

		Neither agree nor disagree	11.5% (25)
Q 40	You should not walk barefoot outside the home;	Agree	73.7% (160)
		Disagree	10.6% (23)
		Neither agree nor disagree	15.7% (34)
Q 41	You need to take Iron-Folic acid (IFA) tablets regularly	Agree	55.8% (121)
		Disagree	8.3% (18)
		Neither agree nor disagree	35.9% (78)
Q 42	You must take deworming medications once or twice a year	Agree	74.7% (162)
		Disagree	1.8% (4)
		Neither agree nor disagree	23.5% (51)
Q 43	You should check your Hb level regularly	Agree	85.3% (185)
		Disagree	4.6% (10)
		Neither agree nor disagree	10.1% (22)
Q 44	Taking the Iron-Folic acid tablets and other supplements does not prevent one from developing anaemia.	Agree	42.4% (92)
		Disagree	22.1% (48)
		Neither agree nor disagree	35.5% (77)
Q 45	You need to do regular exercise and ensure adequate nutrition	Agree	93.1% (202)
		Disagree	0.9% (2)
		Neither agree nor disagree	6.0% (13)

Interpretation of KAPC scores using the Hasan et al. criteria [27]:

The Knowledge score was 74.4%, which indicated Fair knowledge about anaemia. The Attitude score was 85.0%, which indicated good attitude towards anaemia. The Practice score was 77.8%, indicating good practices, which implies low risk of developing anaemia among the female students that participated in this study. The Concern score was 81.4%, indicating good level of concern regarding anaemia which creates less doubt in their minds about the negative consequences of anaemia. The results indicated that their fair Knowledge about anaemia might be one of the main causes of anaemia among some of the female students. The fair knowledge does not seem to have affected the good attitudes and practices of the students, as well as their concerns in the present study.

Further analysis of the data shows positive correlation between Knowledge and Attitude (spearman's rho=0.402, p<0.001). Positive correlation between Knowledge and Practice (spearman's rho=0.537, p<0.001). Positive correlation between Knowledge and Concern (spearman's rho=0.525, p<0.001). Positive correlation between Attitude and Practice (spearman's rho=0.522, p<0.001). Positive

correlation between Attitude and Concern (spearman's rho=0.411, p<0.001). Positive correlation between Practice and Concern (spearman's rho=0.605, p<0.001).

Table 5: Calculated KAPC scores in percent for all the students and for students with and without anaemia

	Total number of students (n = 169)			
	All the students (n = 217)	Non-anaemic students (n = 92)	Anaemic (n = 77)	
Knowledge scores	74.4	74.6	73.7	Fair
Attitude scores	85.0	84.4	87.9	Good
Practices scores	77.8	79.3	79.2	Good
Concerns scores	81.4	82.1	82.6	Good

Most students correctly answered questions about the general characteristics of anaemia, such as its symptoms, causes, and effects. There was a statistically significant (p-value <0.05) association between Knowledge and Attitude; Knowledge and Concerns; Attitude and Practices and also Practices and Concerns. There was also a statistical significance (p = 0.003) difference between levels of Knowledge and the Hb status among the students showing a negative (R = 0.001) coefficient of correlation. However, there was no statistical significant association between Attitude and Hb (p-value 0.186) and between Practice and Hb level (p-value 0.163).

The KAPC scores for the students in the anaemic and non-anaemic groups were calculated. The results are presented in Table 5. No statistically significant differences were observed when the corresponding KAPC scores for both groups were compared. The trend in the KAPC scores was similar to that observed for the KAPC scores for all the students.

DISCUSSION:

A total of 217 female students consented to participate in this study. However, consent for the measurement of Hb level was obtained from 169 students. This gave a consent rate of 77.9% (169/217). The non-consent rate of 22% obtained in the present study may be due to the reluctance of apparently healthy individuals to donate blood for research purposes. This has been reported by other researchers in PNG [22, 24].

The mean haemoglobin level of 11.7 ± 1.92 g/dL for the 169 consented students was lower than the 12.4 ± 1.1 g/dL reported for non-pregnant women in the National Capital District in PNG [24], mean Hb level of 12.19 ± 2.1 g/dL for female students in Sri Lanka [10], mean Hb level of 19.7 ± 3.01 g/dL for female students in India

[29], and mean hemoglobin (Hb) level of 11.97 g/dL for female students in Indonesia [29].

The 45.6% prevalence of anaemia in the present study was higher than the 28.0% obtained for non-pregnant women in NCD [24], the 44.2% prevalence among non-pregnant women in the Southern Region of PNG [23], the 44.0% for adolescent girls in Indonesia [29], the 38.9% for non-pregnant women in the Solomon Islands, 31.3% for non-pregnant women in Samoa, and 31.0% in Fiji, reported in the WHO global health observatory repository [28].

The 16.0%, 24.9% and 4.7% mild, moderate and severe anaemia status among the students in the present study are similar to the values reported for female students in some universities by other authors [10, 11, 29]. A study conducted among female students in Sri Lanka, found that 17.5% had mild anaemia and 7.9% had moderate anaemia [10]. Another study by Agusina et al. [29] found that nearly half of the female students in the study were anaemic, with 23% having mild anemia, 19.4% having moderate anemia, and 1.8% having severe anemia [29].

The authors indicated that one of the major factors for the cause of anaemia among the female students was lack of awareness and poor practices. In a recent study, Waghray et al [11] reported that 86.5% of female students were anaemic. Mild, moderate, and severe anaemia were observed in 22%, 55%, and 10% of the students, respectively [11]. The authors reported that lack of knowledge about nutrition and long menstrual duration were significant reasons for the high prevalence of anaemia among the female students [11].

Huong et al [12] reported high prevalence of anaemia among female university students in Malaysia. According to the authors less iron-rich foods were consumed by 52.9% of the female students and meals were skipped by 81.7% of them [12]. The same study also noted poor nutritional status, extended menstrual duration, and vegetarianism among the female students [12]. In a similar study, Ganasegeran et al. [14], reported that poor eating habits, such as skipping meals and consuming fast food, are a major public health concern among female university students. These habits make them vulnerable to nutritional deficiencies leading to development of anaemia [14]. The authors observed that most of the female students seem to have appropriate level of knowledge about anaemia, but lack of positive attitude and good practices towards preventing it was evident [14].

KAPC scores:

In our present study, the Knowledge, Attitudes, Practices and Concerns (KAPC) scores were calculated and used for the categorization of the students. The criteria proposed by Hasan et al [27] were used for the categorization of the KAPC scores. Score ≥ 75% was categorized as Good. Scores between 74 – 65% were

categorized as Fair. Scores below 64% were categorized as Poor.

The Knowledge score of 74.4% was higher than the 54.0% in a study conducted among female students in a college in Pakistan [33]. This shows a reasonable level of understanding about anaemia among the students in the present study.

When asked if they knew about anaemia, 84.8% of the students answered affirmatively. Similarly, most of students (77.4%) agreed that anaemia is a serious health problem. When asked about the common meanings of anaemia, most students agreed with "*decrease of iron in blood*", "*lack of red blood cells*", but only 67.7% agreed with the response "*Hb levels below 12.0 g/dL*". When asked about the common causes of anaemia, over 70% of the students agreed with "*Iron deficiency*", "*Decreased dietary Iron intake*", "*Heavy menstrual bleeding*". These findings are consistent with other studies in different countries [12, 29]. One such study found that 60% of the female students had an appropriate level of knowledge about anaemia [12].

The findings about Attitude show a good level of understanding about anaemia among the students. Most students agreed with the statements that "*Females are at greater risk of developing anaemia than males*" and that "*Awareness campaigns about anaemia should be carried out among female students regularly*". However, only around a third (34.1%) of the students agreed with the question "*Do you agree it is likely that you are anemic?*". These findings are similar to a study conducted among female university students in Malaysia, which showed that the participants had an appropriate level of attitude about anaemia [12]. On the contrary, female students' attitudes towards anaemia varies across several studies [13]. A study by Koeryaman et al. [13] reported that a significant proportion of female students had suboptimal attitudes, with 47.2 % exhibiting a low attitude towards anaemia. The authors concluded that most of the students showed a lack of positive attitude but good practices towards preventing anaemia.

In the section on Practice in the present study, dietary habits of the students were assessed. The findings revealed that 84.8% of the students agreed with the statement that "*Female students should consume food containing Heam Iron daily*". In response to the statement "*Female students should avoid skipping meals*", 73.7% of the students agreed. Furthermore, 95.4% of the students supported the statement that "*It is important for a female student to seek medical attention as early as possible if she suspects that she has anaemia*".

There are similar findings in other studies from different countries that show similar results [12, 13, 14, 30]. A study in Indonesia highlighted the importance of iron supplementation in reducing anaemia and improving the overall health of

adolescent girls [30]. These studies also reported that students' poor eating behaviour is strongly associated with stress and low self-esteem [12, 13, 14].

One such study found that 52.9% of the participants consumed less iron-rich foods and 81.7% reported skipping meals [12].

The tendency to skip meals was found in 60% of female student in Bangladesh [34]. Skipping meals and having an imbalanced diet is common among college students, as observed in Bangladesh, and this may contribute to the development of anaemia. Low nutritional status is a significant risk factor for anaemia, especially in underweight and overweight populations [34].

A study conducted at the Winneba Campus of University of Education in Ghana found that students moderately consumed fish, meat, eggs, and dairy products but had a low intake of fruits and vegetables. They also had a high consumption of energy-dense foods and fast foods [30]. Another study conducted in Ranchi, India, found that anemia was more common among female students that were vegetarians than non-vegetarians and among vegetarians that consumed predominantly rice-based diet [31]. There was increased anaemia among female students consuming tea and coffee post meals [31].

The authors reported that the female university students had an appropriate level of knowledge about anaemia, but showed a lack of positive attitude and good practices towards preventing it [12, 30, 31].

In the section of Concerns, an exploration of feelings and issues of importance regarding anaemia was conducted. A total of 10 questions were utilized to assess the Concerns of the students. The results found that 85.7% of the students concurred with the statement "*You need to always wash your hands with soap after defecation*". The results also found that 90.3% agreed with the statement "*You need to wash your hands with soap before eating*". The assertion "*You need to do regular exercise and ensure adequate nutrition*" was supported by 93.1% of the students.

The results obtained in some of the other studies seems to align with the findings in our present study, highlighting the awareness and understanding of some of the practices and concerns related to maintaining good health and preventing anaemia. It is important, however, to note that lack of appropriate practices towards preventing anaemia and the lack of attitude regardless of good knowledge may lead to high prevalence in anaemia among female students.

The knowledge scores for the students in the anaemia (73.7%) and non-anaemic (74.6%) groups indicated fair awareness of some of the issues related to anaemia. The attitude scores for both groups (87.9% and 84.4% respectively) indicate good attitude towards some of the risk

factors related to anaemia. The practice scores (79.2% and 79.3%) obtained for the anaemic and non-anaemic groups were also similar. Our results show that the female students that participated in this study have reasonable knowledge, good attitude, practices and concerns to reduce the prevalence of anaemia. The current analysis did not show any significant difference between KAPC of the students with anaemia and those without anaemia. This suggests that education alone did not guarantee a direct association with lower anemia prevalence. Thus, education and improved nutrition, such as micronutrient supplementation should be considered along with education related to anemia. The trends in the results were similar to those reported by some authors for female university students in Malaysia [12]. The authors concluded that both anemic and non-anemic students were knowledgeable and aware of the risk factors, symptoms, causes, and prevention of anaemia. The findings in our present study support the conclusion of these authors.

CONCLUSION:

The mean Hb level of the 169 female students that participated in the study was 11.7 ± 1.92 g/dL, the range was 8.3 to 15.4 g/dL. Mild to moderate status of anaemia was prevalent among 45.6% of the students. This indicates severe public health significance. The knowledge, attitudes, practices and concerns scores for all the 217 students were 74.4%, 85.0%, 77.8% and 81.4% respectively. The scores indicated fair knowledge, good attitudes, practices and concerns about anaemia. The knowledge scores for the students in the anaemia (73.7%) and non-anaemic (74.6%) groups indicated fair awareness of some of the issues related to anaemia. The attitude scores for both groups (87.9% and 84.4% respectively) indicate good attitude towards some of the risk factors related to anaemia. The practice scores (79.2% and 79.3%) obtained for the anaemic and non-anaemic groups were also similar. Our results show that the female students that participated in this study have reasonable knowledge, good attitude, practices and concerns to reduce the prevalence of anaemia. Improving their knowledge, attitude and practices, may result in reducing the prevalence of anaemia among the students. More research should be conducted to raise awareness that will promote changes in the KAPC among the female students to combat anaemia in the University of Papua New Guinea.

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CHITOSAN-BASED NANOPARTICLES TO BYPASS THE BLOOD-BRAIN BARRIER FOR THE TREATMENT OF NEUROLOGICAL DISEASES: A REVIEW

Running title: Chitosan Based Nanoparticles

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ABSTRACT:
Neurological disorders are increasing exponentially and at an alarming rate, affecting great number of people globally. Normal functioning of the central nervous system (CNS) depends on the blood-brain barrier's (BBB) integrity. Therapeutic amount of some drugs cannot reach the brain; therefore majority of effective pharmaceuticals that have been produced for the treatment of neurological illnesses have subpar therapeutic results. Due to lack of targeted drug delivery mechanism, there is a large concentration of these drugs in the body's essential organs, and this might be harmful to the body. To surmount this challenge, patients are given high doses of medication in an effort to reach the brain more quickly, which ultimately causes off-target organ toxicity. Therefore, there is a pressing need to develop effective treatments for neurological disorders. Nano systems for drug delivery have been investigated because of their targeting capabilities. Chitosan is a natural polymer that is frequently used to create drug delivery nano-systems. Because of its special qualities, including biocompatibility, biodegradability, and mucoadhesive properties, it enables targeted therapy without posing any hazardous risks. Recently, drug delivery nano-systems, hydrogels, and scaffolds made of chitosan have been employed to treat several neurological conditions. This review will concentrate on brain-targeting nanoparticles made of chitosan.

Key words: Chitosan; neurological disorders; blood brain barrier; nanoparticles; drugs

INTRODUCTION:

Chitosan – Properties and Uses:

Chitosan (CS), an amino polysaccharide that occurs naturally, is among the widely used polymers in biological and medical sciences and it comes after cellulose in terms of prevalence [1] Chitin, which is found in the water-inhabited animals, outer skeleton of insects, fungus, yeast, and microalgae, is converted into chitosan through partial deacetylation in an alkaline medium [2]. Chitosan is a linear polysaccharide with a random distribution of N-acetyl D-glucosamine and β-(1→4)-linked D-glucosamine. Its molecular weight (MW) varies between 300 and 1000 kD with a deacetylation degree (DD) of 30–90% depending on the source and methods of production [2]. With a pKa value of approximately 6.5, the amino group in CS undergoes considerable protonation in neutral solutions. These fundamental variables—MW, DD, and pKa—determine the characteristics and biological effects of chitosan. In terms of use and distribution, it is regarded as the second-largest renewable biomaterial after cellulose [3]. Because of chitosan's special qualities, including its high biodegradability, lack of toxicity, biocompatibility, and bio-adhesiveness, it can be used to create systems that target the brain. In situ gel, nanoparticles, liposomes and nano-emulsions are a few of the reported chitosan nanocarriers that have been developed for targeting the brain [4]. Chitosan's natural origin and a number of biological characteristics, such as lack of toxicity, lack of allergenicity, biocompatibility, and biodegradability, coupled with its ability to fight bacteria, fungi, oxidative stress, tumor and inflammation, have led to an increase in consideration of chitosan as a substance that is suitable for introduction in to the living tissues. Moreover, it has been known to help the immune system, fight thrombosis, and fight accumulation of cholesterol in the body [5, 6]. Also, due to its tremendous adaptability, it can be used to create a variety of physical objects, including membranes, films, fibers, nanoparticles, beads, sponges and gels [7]. Chitosan is very useful in various biological and medical fields, including regenerative therapies, gene delivery, drug delivery and tissue engineering, and many more, thanks to all these qualities [4].

Chitosan's Functional Groups:

The reactive functional group attached to C-2 location is amino group, C-3 location is primary hydroxyl and C-6 location is secondary hydroxyl. The functional groups in chitosan enable many alterations to be made to its structure, including O-carboxymethylation, tosylation and acylation, and lipids and antibodies functionalization [8]. Chitosan is converted into 3N-trimethyl chitosan (N-TMLCs) via amino functionalization with methyl iodide at high temperatures and alkaline conditions. Nevertheless, the typical method of modifying chitosan to produce N-TMLCs uses hazardous solvents such N-methyl

pyrrolidinone. By employing batches with varying degrees of deacetylation or by increasing the number of reaction steps or the reaction period, it generates N-TMLCs with a quaternization degree (QD) that can be increased. When increasing the DQ, O-methylation on the hydroxyl groups can occur, leading to a less soluble compound. But even N-TMLCs with a QD of 10% had a number of benefits because of its persistent positive (+ve) charges. The benefits entail enhanced porosity, strength, absorption effectiveness, and muco-adhesivity as well as better solubility in a wide pH range [9]. Moreover, a low QD can increase the loading efficiency of drugs [10].

The permanent positive charges can facilitate absorptive mediated transcytosis (AMT) of Nanoparticles (NPs) and, because of this, N-TMCs can be used to increase the transport of actives to the brain [11]. However, by raising the chitosan QD that consequently results in a bigger diameter of particle, more positive charges can be achieved [10].

Central Nervous System (CNS) and Blood-Brain Barrier (BBB):

The brain is a part of the CNS, which is regarded as one of the most significant component of the whole body. The neurons of the CNS are essential parts, and these neural networks are in charge of controlling neuronic signaling by using a variety of neurotransmitters and electrical signals to control the synapses and axons' ionic surrounding [12]. The BBB controls the ideal neural environment, hormonal system and preserves homeostasis [13, 14]. The BBB is a complex, dynamic network of blood vessels and brain tissues that serves as a great selective semi-permeable interconnection between the brain and blood. It blocks the influx of toxic materials like particles made of cells, blood and other microbial organisms that are toxic to the neurons [15]. To stop the onset of neurodegenerative conditions, it is crucial to effectively block this route of these blood-borne substances entering the CNS. This barrier is made up of several neurotransmitters that support efficient communication with other CNS cells in order to control important events and provide maintenance of homeostatic mechanism. For example, they respond to pathological situations, both at the start of a disease and as it progresses [15].

The BBB is made up of greatly specialized cells of the endothelial which are bound to one another tightly by uninterrupted tight junctions (TJ), including claudins, zonula occludens junctions and adherens junctions (AJ). TJs contribute to the BBB's tightness [16]. Other cellular substances, such as neurons, microglial cells, pericytes, and astrocytes which endothelial cells communicate with to form the neurovascular unit, also contribute to the BBB's tightness [17]. Pericytes which is one of the components of BBB consumed substantial amounts of lactate, suggesting that they are predestined to utilise this remnant of glycolytic metabolism. Lee *et al.* [18] also performed

metabolomic analyses to gain insight into pericytes' lactate usage. These metabolic labelling studies indicated that lactate is a readily available carbon source for pericytes, fuelling tri-carboxylic acid cycle (TCA) activity, ATP production, and amino acid biosynthesis. The BBB keeps the CNS in homeostasis and ensures that the brain is supplied with nutrients by preventing hazardous chemicals from entering it. The BBB highly expresses transporters and receptors to guarantee that the brain receives nutrients at an appropriate amount because it is greatly needed by the brain [19]. The BBB is a highly constrictive physical barrier that protects the brain's tissue's homeostasis [20, 21]. For every gram of brain tissue, the BBB's surface area is roughly 150 - 200 cm^2 or 12-18m 2 gross areas for an adult person [22]. Microvascular endothelial cells are mostly present in it [22], and this result in the creation of walls of blood vessel connected by tight joints [23].

Neurological Disorders:

Neurological illnesses that affect the CNS are the main global causes of morbidity and disability [24]. Parkinson's disease, Huntington's disease, brain tumors, stroke, multiple sclerosis, traumatic brain injury, Alzheimer's disease and motor neuron disease are examples of common neurological illnesses. According to a thorough analysis of disorders, injuries, and predisposing factors conducted in the year 2016, 276 million people worldwide have neurological disabilities, and around 9 million people die from neurological ailments each year [24]. There will be a greater need for more effective treatment and management of neurological illnesses due to an aging and expanding global population.

The brain and spinal cord, which are the components of the CNS, are the primary sites of neurological diseases. The CNS, which is crucial to the body's ability to operate and be regulated, contains three barriers: the choroid plexus epithelium, the cerebral microvascular endothelium (also known as the blood-brain barrier, or BBB), and the avascular arachnoid epithelium. Transporting drugs into the CNS can be quite challenging because of these natural barriers, especially the BBB. Additionally, important component of the CNS include neurons that have considerable cell-cell communication capabilities. Because of how sensitive neurons are to changes in temperature, infections, and toxins, neurodegenerative illnesses including Alzheimer's, Parkinson's, and Huntington's are frequently connected with irreversible neuronal cell death [25].

The progressive and slow loss of functions by the neuron caused by ischemia or hypoxic situations such as traumatic brain traumas, stroke and birth asphyxia can also lead to the irreversible process of neurodegeneration. Additionally, oncogenic alteration of cellular and genetic components in glial cells and neurons is what causes brain malignancies like glioblastoma. In addition to primary tumors, 9–

17% of adult cancer patients also develop secondary brain tumors involving brain metastases [26].

Alzheimer's disease (AD):

AD is a neurodegenerative condition that leads to increased deterioration and loss of neurons. It causes a steady loss in behavioural, cognitive, and other social abilities, interfering with the individual's capacity for independence. Dementia, anxiety, memory loss, restlessness, exhaustion, and vertigo are a few symptoms of AD [27]. AD is also linked to a number of genes, including presenilin-1 (PSEN1), apolipoprotein (APOE4), amyloid-beta precursor protein (APP), microtubule-associated protein tau (MAPT), and presenilin-2 (PSEN2) [28]. Neuronal loss, Aβ plaques, hyperphosphorylated tau neurofbrillary tangles (NFTs), and cerebrovascular dysfunction are all key factors in the etiology of cognitive impairment and AD [29].

NFTs can also result in AD, which is linked to chromosome 17q21, if phosphorylated tau protein builds up in the brain cell. In addition, tau is a protein connected to the microtubules that promotes transport of axons necessary for trafficking and signaling of neurones [30]. Every molecule of tau normally consists of phosphates which could be two or three; however, in tauopathy patients, the phosphoryl concentration is multiplied by many times. When tau protein is hyperphosphorylated, it separates from the microtubule, causing unbound microtubules to proliferate and phosphorylated tau protein to gradually build up until NFTs are formed [31]. PSEN (1 and 2) are the enzyme γ - secretase's catalytic components [32].

It has been demonstrated that human PSEN1 mutations encourage BBB disintegration and cerebrovascular impairment [33]. 5% of all cases of AD have PSEN2 mutations [34]. Additionally, among the main genetic predisposing factors for late-onset AD is APOE4. APOE4 alleles greatly increase the susceptibility of having AD more than APOE3 alleles. Because of APOE, the neurons, the brain, and the vascular system experience harmful consequences [35]. Human APOE4 carriers may experience early neurovascular dysfunction, pericyte degeneration, progressive BBB breakdown, and impaired BBB glucose absorption [36, 37]. Fibrinogen, IgG, albumin, hemosiderin and thrombin have been shown to leak from vascular capillaries inside the entorhinal cortex, hippocampus, and prefrontal cortex of a person suffering from AD. It has been determined that AD is caused by co-localization of proteins with Aβ and mutations of about 40 APP [38]. This results in BBB disintegration, cerebral amyloid angiopathy (CAA), and cerebrovascular disease [39].

Parkinson's disease (PD):

The build-up of α-synuclein (α-syn) and the subsequent degeneration of neurons that produce dopamine in the substantia nigra pars compacta (SNpc) are hallmarks of PD [29]. This eventually leads to a locomotory problem,

because the SNpc is connected to a nigrostriatal circuit which helps to stimulate the cerebral cortex and also initiate locomotion. Patients with PD struggle to do daily activities like walking, running, etc. because of muscular rigidity in their limbs. Cognitive issues, dementia, dizziness, diminished facial expression and a loss of postural reflexes are further symptoms.

In PD patients, basal ganglia's vascular dysfunction results in BBB degradation and malfunction. Neurotoxic fibrinogen, thrombin, plasminogen, and RBC extravasation build up as a result of BBB degradation. ROS are produced by the release of Hb and Fe^{2+} that damage dopaminergic neurons. During neurodegeneration, pro-inflammatory cytokines such as TNF, IFN- γ, and IL-1 β, are produced. Recently, few findings have shown the capacity of α-syn to pass through the BBB as well as its role to build-up of α-syn pools in the CNS. LRP1-mediated transcytosis then facilitates additional clearance of the brain through the BBB. Leucine-rich repeat kinase 2 (LRRK2) missense mutations have been connected with late-onset PD (>50 years) [40].

Epilepsy:

The erratic and uncontrolled activity of either the entire CNS or just a portion of it is what defines epilepsy. When CNS excitability exceeds a specific critical threshold, an epilepsy patient will experience attacks [41]. Seizure is different from epilepsy because it occurs only once, whereas epilepsy involved two or more seizures [42]. Seizures can also be localized, for example when scar from the brain tissue draws nearby tissue from neuron, specific area of the brain compressed by an abnormal growth, or when near-by brain's neural network is congenitally dysregulated [43].

Strong emotional stimulation, traumatic injuries, and over breathing-induced alkalosis can all lead to epilepsy. Epileptic person also manifest symptoms like loss of TJs, disrupted GABAergic processes, rise in microvascular density, leakage of IgG in hippocampal regions, a brief moment of memory loss [44], jerks, breathing problem, shock movements, lack of comfort, sudden anxiety, and rage [45]. The frequency of seizures and BBB dysfunction are categorically unrelated, and neither is neuronal loss.

Proinflammatory molecules like IL-1β, TNF-α and High Mobility Group Box 1 (HMGB1) are produced and discharged by epileptogenic injuries as well as seizures, which lower the threshold for seizures. This later leads to formation and recurrence of seizure due to swift transformation in phosphorylation of γ-aminobutyric acid (GABA) receptor and glutamate. Additionally, it results in channelopathies that alter the innate brain ability to excite [46]. BBB breakdown can result from seizures, and artificially opening the BBB causes rat neuronal activity to synchronize, causing neuropil, immunoglobulin G (IgG), and albumin eruption. Neuronal hyperexcitability is caused by albumin altering astrocytes' ability to buffer K+ [47]. The integrity of the BBB is also

affected by transforming growth factor- β (TGF-β) produced by other types of cell. When tissue plasminogen activator (tPA) is suppressed by plasminogen activator inhibitor-1 (PAI-1), astrocytes release TGF, which causes the BBB to close [48].

Cerebral ischemia:

Brain disorder known as cerebral ischemia is brought on by a transient or unending reduction in the blood flow of cerebral artery. One of the major causes of disability and mortality, cerebral ischemia's clinical feature is partial neurological dysfunction [49]. Neurons in the frontal sensorimotor cortices and caudate-putamen in particular may eventually die from cerebral ischemia, which can also cause a variety of motor and sensory-motor abnormalities which include loss of coordination, dyskinesia, and partial paralysis occasionally.

Multiple Sclerosis:

A neurodegenerative condition called multiple sclerosis (MS) causes the BBB to become damaged, eventually allowing B cells, peripheral macrophages, and CD4+ T cells, to move in to the central nervous system (CNS). This sets off sequence of inflammatory reactions that causes demyelination and loss of axon [50]. The optic nerves, brain, and spinal cord are all impacted by MS's demyelination and inflammation of the nerves. The Major Histocompatibility Complex (MHC) contains several genes that increase a person's susceptibility to MS. Leukocytes, especially T-cells, have been thought to move to the BBB [50]. The symptoms include tingling, numbness, and vision issues, changes in the optic nerve, sensory, bladder, and bowel functions, as well as cognitive deficits. The hallmarks of MS include an early BBB collapse, fibrinogen build-up, deterioration of endothelium, and decreased TJs' expression. Among the early cerebrovascular abnormalities seen in MS are movement of stimulated leukocytes from one endothelium to another, and dysregulation of BBB which results in the release of chemokines and inflammatory cytokines [51].

Chitosan Nanoparticles:

AS a result of their positive charge, this improved cell absorption and made them amenable for loading with negatively charged therapies, chitosan nanoparticles (CS-NPs) is promising for brain delivery. Chemotherapeutic medicines, siRNAs, and natural products can all be successfully delivered to the brain via chitosan nanoparticles. The chitosan utilized in different nanoparticles has varied molecular weights and is combined with other components. The primary method for creating nanoparticles is by crosslinking of ion, and this produces particles which is roughly 100 nm in size. Chitosan nanoparticles are often administered intravenously, while intranasal delivery is also widely employed.

The characteristic properties of the produced chitosan or chitosan-coated nanoparticles will

depend on the co-used material, chitosan's molecular weight, method of preparation, and modifications of various targets, which will then depend on the effects on the treatment of brain diseases. Chitosan is typically combined with other materials in intravenous delivery systems to enhance loading of drug, sustained release, drug uptake by the cell, drug delivery, and drug targeting. Additionally, chitosan can serve as a carrier to encapsulate siRNA, peptides, and proteins, by moderate interactions of ion because it is a linear polyamine that contains many groups of cationic amine.

In addition to the benefits mentioned for intravenous administration, the muco-adhesiveness and the positive charge of chitosan can enhance the time of retention and movement of CNps into the nasal mucosa after intranasal administration. Depending on the design, chitosan and its derivative coating can exhibit various chitosan properties.

Chitosan has a good biodegradation rate and is biocompatible, making it a viable excipient in pharmaceutics. Chitosan can be used to create nanoparticles that act as an excellent carrier for chemotherapy agents with low bioavailability and stability in gliomas.

Due to chitosan's inherent beneficial properties, including biodegradability, biocompatibility, bioactivity, ease of preparation, non-toxicity, and to a certain extent, target specificity as a result of its positive charge, chitosan nanoparticles are used as a great and efficient carrier of drugs [52]. CS-NPs have improved bioavailability, a high rate of hydrophobic drug dispersion, and good mucoadhesive properties. Additionally, CS NPs can be loaded with both water-soluble and water-insoluble medications.

Methods of Preparation and Mechanisms of Chitosan Nanoparticles:

The enhancement or addition of physicochemical and biological properties is possible by attaching chitosan to the surface of nanoparticles. Chitosan, for instance, can boost or flip the zeta potential of a nanoparticle from anion to cation that can result in a great biological association with negative cellular obstruction or barriers and a higher rate of cellular internalization. Furthermore, the use of chitosan in the decoration increases affinity for water, making it stable in water environment and enhancing the ability to experiment with additional administrative strategies [53]. The creation of both covalent and non-covalent associations between the molecular chains of chitosan and the nanoparticle materials' chemical groups are two ways that chitosan can perform the superficial modification [54].

The optimal process will produce superior outcomes since the architecture of chitosan coating is determined by the production technique, the chitosan type, and the nanoparticle's molecular characteristics. Notably, covalent pathways result in connections that are more persistent and are easily distinguishable by analytical methods like NMR, HPLC or infrared [55]. The process can,

however, become convoluted, rarely scalable, and difficult to duplicate. The non-covalent mechanism, on the other hand, exhibits weak interactions that are effectively stabilized by counter-ions and other polymers. In contrast to covalent bonds, which require strong chemical reagents or extremely harsh processing conditions, non-covalent bonds exhibit lower toxicity, are scalable, and simple to validate.

Non-Covalent Mechanism:

This process is dependent on connections created at the molecular and supramolecular levels by hydrogen bonds and coulomb attraction forces. The association between nanoparticles material's functional group and the chain of chitosan is responsible for the stable presence of chitosan on the nanoparticle. By soaking both the nanoparticles and a specified amount of chitosan in a solution, an adsorption process controlled by a mechanism of interaction of charge is often used to coat them with chitosan. Nevertheless, this idea is workable if the nanoparticles' chemical and physical characteristics are sufficiently stable, and if they already have a mostly anionic charge. Amine group's cation and the backbone of chitosan then encourage the particles' coating over when it interacts with chitosan. As long as they maintain a sufficient level of resistance to change in aqueous dispersion at the early phase, nanoparticles based on lipids, inorganic materials, polymers, and proteins are excellently suitable for the adsorption technique. By incubating nanoparticles in solutions containing chitosan with various amounts the two materials at various durations, the adsorption process may be improved in order to preserve a particular bio adhesion's level and size of particle.

Nanoparticles can be added either by resuspending it in the solution of chitosan or by adding drop wise of nanoparticle solutions (to distribute the particles) [56, 57]. The non-covalent method's main benefits are its low cost, hospitable chemical environment, and simplicity of application. As a result, before attempting another strategy, it is likely the first option that the majority of research organizations have examined for the chitosan-coating of the nanoparticles.

The Covalent Mechanism:

The covalent processes for chitosan coating on nanoparticle surfaces are concentrated on using chemical processes and reactions that need gentle circumstances, such as no severe pH levels, ambient temperature, less difficult purification process, and reagents that are low in toxicity. One of the most popular techniques is the crosslinking of chitosan via the carbodiimide reaction. The activation of carboxyl groups in this process results in the creation of a carbocation, which is then attacked by primary amino groups nucleophilically. One of the most popular chemicals used for this is 1-Ethyl, 3-(3-dimethyl aminopropyl) carbodiimide (EDC), which is the proper chemical. The new covalent

link is formed when chitosan's amino group readily displace the intermediate product of the EDC process, which is O-acylisourea. The reaction of EDC can be carried out before, pre-, during or post-synthesis of nanoparticles [58]. Some processes use chemicals like 1, 4-dioxane triethylamine and dimethyl aminopyridine to apply the carboxyl activation, and in a subsequent stage, EDC catalysed the production of amide bond. During the activeness of carboxyl group, the chitosan addition produces covalent bonding most effectively.

Chitosan Nanoparticles Routes for Passing through the Blood Brain Barrier:

Brain's drug delivery is complicatedly hampered by the BBB's highly selective and controlled molecular transport [59, 60]. In this regard, it has been demonstrated that both chitosan and its coated nanoparticles increase the effectiveness of targeting the brain, increasing the effectiveness and efficiency of medications. As a result, various scientists have examined the routes taken by CS-NPs to pass through the BBB, identifying a number of mechanisms that give rise to a variety of ideas [61, 62]. There has been an extensive research on how CS-NPs can carry medications to the brain. Example of such is Trapani *et al.* [63] who assessed the movement of CS-NPs that have been loaded with dopamine (DA/CS-NPs). The Madin-Darby canine kidney (MDCKII-MDR1) cell line was used in those tests to measure the internalization of the five mg of dopamine that was put into the nanocarrier. The Fluorescein Isothiocyanate (FITC)-labelled nanoparticles were incubated for duration of 3 hours at the apex after the cells were implanted in Transwell filter inserts. Nanoparticle-free media was used for the control studies. Apical media samples were taken at various intervals, and fluorescence microscopy was used to gauge the FITC concentration. According to their findings, the scientists hypothesized that the nanoparticles' internalization was caused by a transcytosis process that is mediated by adsorption, with the main contact being between the charges of the chitosan and the cell monolayer [64].

Similar to this, it was claimed that absorption-mediated transcytosis might be used to transport anti-neuroexcitation peptide-loaded N-trimethyl chitosan nanoparticles (ANEP/TMCNPs) over the BBB [9]. The ANEP/TMCNPs produced a robust signal in the brain, according to the data based on fluorescence. The controls, on the other hand, showed a meager fluorescence. The positive charge of the ANEP/TMC NPs and their connection with the anionic plasma membrane present on the endothelium of brain capillary were cited by Wang *et al*. [9] as the causes of this behaviour. On the other hand, a number of publications also noted that chitosan can allow epithelial cells' tight junctions to open. MDCK-C7 cells' tight junctions (an in vitro BBB model) and capsaicin-loaded chitosan-coated nano-

formulations interaction was examined by Kaiser *et al*. [65].

Chitosan opened the tight connections, according to analysis using digital holographic microscopy. Alkyl glyceryl chitosan nanoparticles' permeability and effect on bEnd3 cells layer which is another BBB model were also evaluated by Lien *et al*. [66] in 2012. Their findings showed that the electrical resistance was reduced as a result of the nanoparticles, suggesting a tight connection effect. The scientists noted that there were no changes in electrical resistance seen in glial due to nano-formulations, proving that cells of the endothelia are the only cells that can vary in this attribute. Additionally, the FITC-dextran translocation through the barrier was stimulated by the nanoparticles' incubation in the layer of aforementioned BBB model. Together, these findings suggest that chitosan and its coated nanoparticles may be able to pass through the BBB; as a result, those materials may be helpful for the brain's drug administration. It is also thought that the cation given to the nanoparticles' surface by chitosan may interact with the anionic places on tight junctions and cell membrane, enabling them to pass across the BBB.

Chitosan Applications in Brain Delivery System: Parkinson's, Alzheimer's, gliomas, and other brain illnesses affect about 1.5 billion people worldwide today [67]. Among the most significant and intricate organs in the human body is the brain. The BBB, a monolayer of polarized endothelial cells, is what gives it its special qualities. The microvasculature of the spinal cord and the brain has this monolayer that is a selective semipermeable boarder. Chemicals, neurotoxins, and microorganism transport from blood to the CNS are constrained by TJs among BBB endothelial cells. Further entrance barrier to drugs is efflux transporters, which are found in the brain microvasculature [68]. However, BBB also makes it challenging for drugs to reach the brain, which would be helpful in the treatment of CNS illnesses [67].

The need to reassess the brain medication delivery concept is highlighted by the increase occurrence of brain illnesses and the poor delivery of medications to the brain, but it also highlights the enormous room for innovation. Numerous novel strategies are being used in conjunction with recent developments in BBB research [69]. Chitosan has played a very intriguing role in the creation of these novel techniques, whether it is through the use of hydrogels, hydrophilic nanoparticles, and microparticles, or serving as a grafting for other medications' delivery methods. Among the most effective and efficient approaches of delivering drugs to the CNS is nanoparticulate drug systems, which can transport both hydrophilic and hydrophobic medicines as well as macromolecules [70, 71]. Nanoparticles' dimensions play a role in their ability to move through the blood-brain barrier, but also a balance between size, surface properties, and

form [69]. Chitosan-based nanoparticles are a promising method for delivering medications to the brain as a result of their positive charge, biocompatibility, and capacity to pass the blood-brain barrier through opening of TJs (a paracellular route) [70, 71].

Chitosan Nanoparticles Applications in Nose to Brain Drug Delivery System:

Current treatments for brain diseases face significant challenges with brain medication delivery. Regarding new tactics created in the nanotechnology industry, traditional administration routes like intravenous and oral still represent intrusive methods or are connected to adverse effects [72]. There is now a lot of keen interest in intranasal medicine delivery to the brain after evaluating the beneficial characteristics and adequate properties of the nasal cavity [73]. The intranasal path links the brain to the nasal cavity via the trigeminal nerve and the brain to the neuroepithelium via the olfactory system [74].

This makes the olfactory pathway the most auspicious non-invasive entrance into the brain since as soon as the formulated drug connects with the nasal mucosa; it is immediately transported there, skipping the BBB [73]. In addition, pharmaceuticals that are absorbed after nasal delivery don't go through hepatic or gastrointestinal pre-systemic metabolism, resulting in a greater bioavailability of drug than that attained following drug oral administration.

An important and frequently employed permeation promoter agent is chitosan. Chitosan's functional groups enable electrostatic interactions with the sialic acid found in nasal mucosa. It causes the TJs to open, which improves medication absorption via the nasal epithelium. Chitosan's role as a mucoadhesive agent is supported by evidence that it increases drug residential of the nasal cavity, which enhances absorption and bioavailability [75]. Some studies show that the inclusion of chitosan in nasal formulations increases medication bioavailability. CS-NPs made for the brain via intranasal pathway were able to improve nasal residence in recent in vivo trials, and they also demonstrated a delayed and steady drug release to the brain [76].The majority of the medications under study are not recently synthesized compounds. Researchers have been using well-known drugs, such as nicardipine, ibuprofen, or olanzapine, which are already used in therapy, to investigate their potential healing effects in the brain if taken orally [75]. These studies may also mark a significant advance, particularly in gene therapy. Intranasal gene-silencing agent injection into the brain using nanocarriers is a promising non-invasive method [77]. Additionally, a gel formulation appears to be far more effective than others at extending the duration of drug residence in nasal mucosa among the various intranasal dose forms [78].

Male Wistar rats were given nasal administration of polar pharmaceuticals with low nasal

membrane penetration using chitosan and methyl-cyclodextrin microparticles. For the first time, it was demonstrated that adjusting the quantity of the penetration promoters resulted in an optimum medication distribution between cerebrospinal fluid (CSF) and bloodstream [79]. Chitosan-glutamate microparticle zolmitriptan nasal delivery encourages CNS targeting with less side effects in the periphery. These days, it is impossible to discuss chitosan-based hydrogels without mentioning their usage in the delivery of medication to the brain. They can transport elements for cell development or differentiation, growth factors, or small molecules medications in this context. They can also raise the concentration of a drug at a specific spot while reducing side effects that are not intended to be there [80]. Chitosan hydrogels is created for intranasal administration system, for example, to administer Parkinson's disease's medications because of their muco-adhesiveness and thermo-sensitivity [81]. Poloxamer, carbopol, and chitosan were utilized to create an intranasal thermo-sensitive gel coated with rasagiline mesylate, a medication for treating Parkinson's disease. When compared to oral medication solution, intranasal gels have a much higher drug bioavailability, according to pharmacokinetic studies done in vivo. Furthermore, biological investigations revealed that the nasal formulations had no adverse effects on the animal nasal mucosa and were not irritating [82].

Chitosan-Based Nanoparticles to bypass Blood-Brain Barrier for the Treatment of Neurological Disorders:

The difficulty of drug accessibility makes it difficult to provide effective and efficient treatment for many diseases; including Parkinson's, gliomas, stroke, epilepsy, migraine, Alzheimer's, meningitis, and schizophrenia. Therefore, it has been suggested to use a variety of natural nanocarriers with chitosan-coated surfaces to facilitate their movement across the BBB and provide medications straight way to the brain. Galantamine, donepezil, and tacrine are the three acetylcholinesterase inhibitors that the FDA has approved recently [83]. The first acetylcholinesterase inhibitors authorized for the treatment of Alzheimer's disease is tacrine [84]. Nonetheless, tacrine's absolute bioavailability is too low because of the first pass effect. Wilson *et al*. [85] created tacrine-loaded chitosan nanoparticles to produce prolonged tacrine's release in order to address this issue. The chitosan nanoparticles considerably extended tacrine's half-life after intravenous administration and enhanced the effectiveness of brain delivery. In a different study, Hanafy *et al.* [86] delivered galantamine hydrobromide through the intranasal route using chitosan nanoparticles to treat AD. The findings showed that as compared to nasal and oral administration of the galantamine hydrobromide solution, the complexation of chitosan with

AChE protein considerably decreased its level and activity in the rat brain. Furthermore, the galantamine hydrobromide-loaded chitosan nanoparticles had no harmful effects, demonstrating their excellent biocompatibility [87]. According to reports, the phyto-pharmaceutical piperine (PIP) has the potential to be neuroprotective in AD. PIP chitosan nanoparticles (PIP-NPs) were created by Elnaggar *et al*. [88] for the management of AD. According to an in vitro research on drug release, there is a continuous and steady release of PIP from nanoparticles because only 10% of PIP is released from PIP-NPs in comparison to 82% of free PIP after two hours in phosphate buffer solution. The main characteristic of AD and other serious and prolonged neurodegenerative disorders is neuroinflammation. This finding demonstrated the mechanisms behind PIP's ability to fight apoptosis and inflammation in order to cure AD. In clinics, levodopa is the medication that best treats Parkinson's disease. However, because of its poor oral absorption and erratic plasma levels, levodopa's clinical reaction is unpredictable and inconsistent. In the management of Parkinson's disease, the ergot derivative bromocriptine (BRC), which shows the activity of dopamine receptor agonist, is frequently utilised clinically to postpone and reduce undesirable motor changes brought on by prolonged administration of dopamine. Md *et al*. [89] looked at the impact of intranasally administered CS-NPs on the effectiveness of BRC's brain-targeting. Due to inadequate absorption and substantial metabolism, only a tiny amount of BRC can reach the target region after oral delivery. BRC had a lower striatum-plasma ratio than pituitary-plasma ratio, which means that the BBB was limiting the drug's ability to travel outside of brain tissue. The produced chitosan nanoparticles with BRC can transport the medication across the nasal mucosa while also preventing it from deteriorating inside the nasal cavity. Additionally, by delaying mucociliary clearance, chitosan's mucosa adhesion characteristic could lengthen its stay in the nasal cavity, improving the nasal mucosa’s absorption. Exercise capacity and systemic stiffness were both demonstrated to be improved in the BRC loaded CS-NPs-treated groups and BRC solution, particularly in mouse model [89]. In addition to chemotherapy, neurotrophic substances like glial cell-derived neurotrophic factor (GDNF) could be utilized to protect dopaminergic neurons. Nevertheless, the use of GDNF in clinical settings is not properly imbibed because of its brief half-life and quick break down when injected in vivo, as well as challenges with passing through the BBB brought on by its high molecular weight, charge and innate hydrophilicity [90]. In a 6-OHDA-partly-lesioned rat model, Gartziandia *et al*. [74] investigated the in vivo neuroprotective impact of GDNF enclosed in chitosan coated lipid carrier (CS-NLC-GDNF). There was a considerable drop in the amount of rotations per 60 seconds after 49

days of continuous CS-NLC-GDNF administration; the drop was 80% till the completion of the trial. Additionally, the CS-NLC-GDNF group showed considerably higher tyrosine hydroxylase fiber density in substantia nigra and striatum, suggesting a more effective protective effect than free GDNF.

PD, MS, cerebral ischemia, and other conditions have all been treated with riluzole as a strong neuroprotective drug. Riluzole is the only available medication for the management of motor neuron disease. Nevertheless, due to brief half-life, poor water solubility, and adverse effects toxicity of the lung when given above normal dosage, its utilization has been restricted [91]. Verma *et al*. [92] found that even at very low drug concentrations, chitosan linked N-isopropyl acrylamide nanoparticles could transport riluzole through the BBB and display pronounced protective impact on the neurons. It significantly reduced the needed concentration while also lowering riluzole side effects.

Limitations and Challenges:

The low stability of CS-NPs is one of its main drawbacks. Controlling environmental variables, temperature, introducing the right stabilizing component, mixing CS with another polymer, and modifying the structure of CS using ionic/chemical materials can all increase stability. The poor solubility of CS-NPs is a second significant drawback. Only some medications that have affinity for water can be included in unaltered CSNPs. CS-NPs with modifications could, nevertheless, enclose non-hydrophilic medicines. As a result, some medications' poor solubility poses a serious challenge to the development of CSNPs. To fully understand why NPs are biocompatible with people, toxicology research and legislation are necessary. In vitro research typically yields positive outcomes. Unfortunately, the reality in vivo is frequently isolated from these outcomes. In the end, it is important to consider the financial implications of commercializing a new pharmaceutical medication delivery method for both patients and the pharmaceutical business.

Conclusion and Future Perspectives:

It has been observed that chitosan-based carriers are efficient at delivering medications in vivo for the treatment of a few different brain illnesses. When compared to chitosan nanoparticles lacking antibodies, which were characterized by aggregation, those containing antibodies on the surface were able to pass through the brain barrier and also offer protection to the neurons. Viscosity, size of the particle, charge of the particle, and the addition of antibodies to the nanocarriers all contributed to the significant transport of nanocarrier formulations to the brain by providing neuroprotection, preventing aggregation, extending the nanocarriers' residence time with the nasal mucosa, as well as inducing tight junctions' stretching. According to reports, the nanoparticles were either above or below 200 nm in size. More study is required to determine

the consistency of the range of nanoparticle sizes that are most beneficial for good brain absorption. More in vivo research on coated NPs and NPs that have been conjugated with antibodies are urgently needed. It is also crucial to conduct more in vivo research on these nanoparticles' toxicity over an extended period of time. More research must be done in order to improve and lessen the general problems experienced in treating brain illnesses, despite the unique and encouraging outcomes obtained employing chitosan drug delivery methods. It is crucial to do a long-term investigation on the nanocarriers' possible harm to humans. Additionally, a comprehensive analysis of these carriers' costs should be conducted.

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CASE REPORT

CEREBRAL AMYLOID ANGIOPATHY (CAA) AND DEMENTIA: CASE REPORT

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ABSTRACT

Cerebral amyloid angiopathy (CAA) is a cerebrovascular disorder caused by the accumulation of amyloid-beta peptides in the cerebral cortical and leptomeningeal vessels. These vascular changes can lead to micro-haemorrhages and lobar intracerebral haemorrhages. CAA becomes more prevalent as age increases. According to autopsy studies, CAA tends to be associated with Alzheimer's disease in most cases. Currently, there is no disease-modifying treatment available. Despite that, early identification may assist clinicians to guide management requiring utilization of antiplatelet, anticoagulant, or thrombolytic drugs in patients with CAA. A case of a patient with cognitive impairment and suspected CAA is described.

Keywords

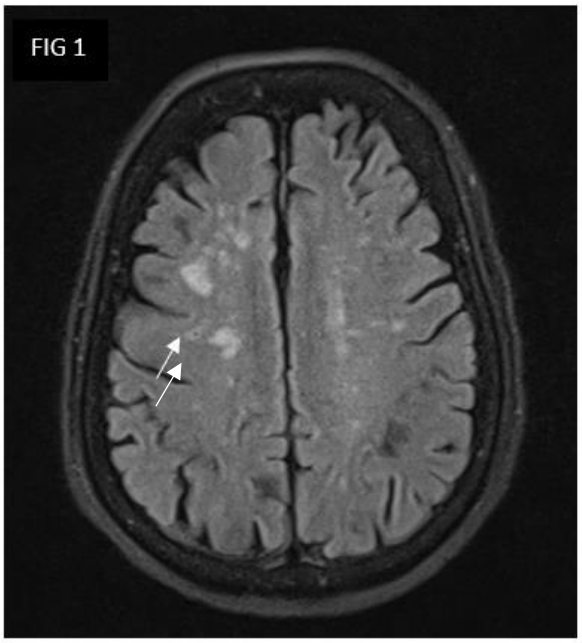
Alzheimer's Disease, Cerebral Amyloid Angiopathy, Cerebrovascular disorders, Dementia

CASE REPORT

A 72-year-old woman was referred to the Geriatric Outpatient Clinic for a 3-year history of progressive memory loss. This was associated with agitation and safety concerns from forgetting to switch the stove off after cooking and wandering onto the roads. Her abilities in activities of daily living (ADLs) were intact but she required supervision, assistance, and reminders for meals and medication administration. The past medical history includes hypertension, hyperlipidemia, and

Type 2 diabetes mellitus (DM). Physical examination including neurological examination was unremarkable. Laboratory tests and an outpatient magnetic resonance imaging (MRI) of the brain was requested. A working diagnosis of Alzheimer's or mixed dementia was given, and a trial of donepezil 5 mg daily was prescribed, in addition to up-titration of amlodipine, atorvastatin and metformin, as her blood pressure, lipid profile and glycaemic control were suboptimal [1].

The blood tests did not reveal any reversible causes of cognitive impairment biochemically or serologically. However, the MRI brain demonstrated chronic small vessel ischemic changes based on extensive white matter T2/ Fluid-attenuated inversion recovery (FLAIR) hyperintensity seen throughout both cerebral hemispheres. There were signs of diffuse punctuate foci of susceptibility induced signal dropout within both cerebrum and cerebellum hemispheres, highly suggestive of microhemorrhages. The main differentials of these multiple micro-haemorrhages were chronic hypertensive angiopathy or cerebral amyloid angiopathy. At the six-month follow-up clinic, donepezil was increased to 10mg with associated improvement in her global cognitive and physical functioning as reported by family, with a reduction in repetitive conversations, less agitation and wandering, as well as less dependence on caregivers for activities of daily living.



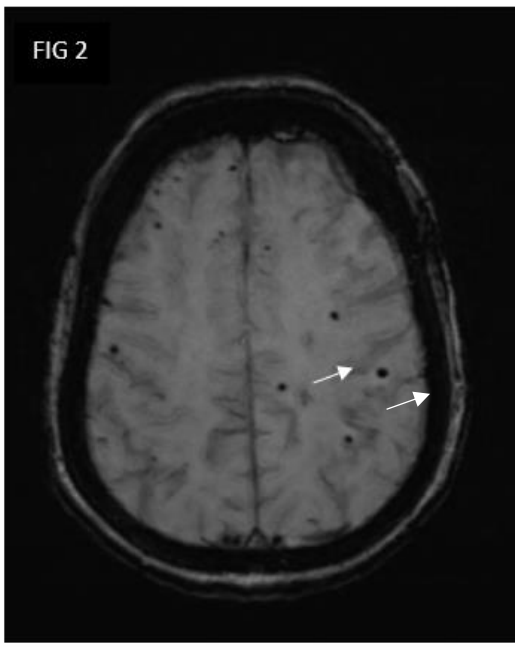


Fig 1 (Axial FLAIR) and Fig 2 (Axial SWI) demonstrated lobar-distributed multiple cortical-subcortical microhemorrhages.

DISCUSSION

Cerebral amyloid angiopathy (CAA) is characterized by amyloid beta-peptide deposits within small- to medium-sized blood vessels of the brain and leptomeninges, which can cause lobar intracerebral hemorrhage and microbleeds in older adults. This may present with transient neurological symptoms, inflammatory encephalopathy, incidental microbleeds/hemosiderosis on MRI or like in this case, contribute to cognitive impairment [2].

The incidence of CAA has a strong relation with age. Based on autopsy cases, the incidence is 2.3% for those aged 65 to 74 years compared to 12.1% in those over the age of 85 years. Most patients diagnosed with CAA appear to have cognitive impairment in at least one domain on neuropsychological testing. An autopsy series demonstrated one-third of the study population presented with moderate to severe CAA, which was associated with a rapid decline in global cognition, perceptual speed, episodic memory, and sematic memory. These were independent of age, sex, education, Alzheimer's disease pathology and other potential covariates [3].

The prevalence of CAA among older patients with dementia is higher than those without dementia. It is estimated about 60% of patients with dementia demonstrated CAA pathology compared with less than 40% among non-dementia patients [4]. Among patients with Alzheimer's disease, more than 80% had pathologic evidence of CAA, with 26% appearing in a moderate to severe form [5]. Another autopsy study found that patients with both CAA accompanied by Alzheimer's dementia had more severe cognitive dysfunction than patients with Alzheimer's disease alone [6]. Similarly, an MRI study of Alzheimer's disease patients found that the presence of multiple microbleeds was associated with worse cognitive performance. However, only about 25% of CAA patients appear to have a clinical history of dementia prior to their first haemorrhage [7].

With regards to vascular dementia, cerebrovascular disease may contribute to cognitive impairment in patients with CAA. Studies in population- and hospital-based subjects have correlated the number and presence of microbleeds with cognitive impairment and dementia, raising the possibility that these lesions contribute to the neurologic dysfunction and are markers of small-vessel disease [8]. Additionally, clinically silent, or subacute cerebral infarcts on diffusion weighted imaging have been detected in 15 to 23% of patients with CAA and cerebral microinfarcts on T1 and fluid-attenuated inversion recovery (FLAIR) imaging have been found in 35 to 39%. This data is consistent with autopsy and imaging studies showing an association between CAA

severity and volume of white matter hyperintensity and/or microinfarct burden [9].

The pathology of CAA involves deposition of amyloid beta-peptide within the cerebral vasculature. Vascular amyloid deposits in CAA are biochemically similar to the material comprising senile plaques in Alzheimer dementia. Despite these shared pathologic features, the pathophysiology of CAA and Alzheimer disease appear to be distinct [10].

Risk factors for CAA deposition of amyloid-beta peptide are not well-understood but genetic factors may play a big role in increasing the risk of sporadic CAA, especially autosomal dominant or carrier of amyloid precursor protein (APP) variant or Apolipoprotein E (APO-E) [11]. The deposition of amyloid-beta peptide into the media or adventitia of cerebral arteries can potentially lead to the destruction of smooth muscle, vascular wall thickening, vessel fragility and concentric splitting of vascular wall, leading to vascular rupture and bleeding. The relationship between CAA and hypertension is debatable. While most patients with CAA-related hemorrhage are normotensive, having high blood pressure will contribute to the risk of hemorrhage recurrence [12].

The commonest clinical manifestation of CAA is an acute lobar intracerebral hemorrhage (ICH). The term “lobar” refers to the location of the cortex and subcortical white matter of a hemispheric lobe of the brain, frequently in temporal and occipital lobes. This is in contrast to the deep locations such as the putamen or pons, which are characteristic of hypertensive hemorrhage. The lobar location of hemorrhages reflects the underlying distribution of vascular amyloid deposits favoring cortical vessels and largely sparing white matter, deep gray matter, and brainstem [13]. The clinical presentation of CAA-related hemorrhage varies with lesion size and the region of the brain affected, which ranges from headache to hemiparesis and depressed consciousness [14].

Unfortunately, CAA can only be definitively diagnosed at postmortem. The next best diagnosis of probable CAA is made with clinical evaluation and weighted brain MRI. This diagnosis should be considered in clinically suspected patients aged 50 years or more, with characteristics of acute or chronic hemorrhagic findings in lobar regions, entirely sparing typical regions of hypertension hemorrhage (basal ganglia, thalamus, or pons) and/or white matter features on MRI brain in the absence of alternative causes. The Boston Criteria initially proposed in 1990 and revised in 2022 helped standardize the CAA definition through the utilization of clinical, imaging, and pathological criteria [15].

Acute CAA-related hemorrhage is treated similar to other non-traumatic intracerebral hemorrhages. Survivors of lobar hemorrhage

and patients with other clinical manifestations of CAA are at risk of hemorrhagic complications in the future. These risks need to be factored into shared decision-making when discussing the risks and benefits of medications such as antiplatelets and anticoagulants [16]. The management of patients with cognitive impairment due to CAA does not differ from other causes; the mainstay of treatment is supportive care. Older age and larger size haematomas are associated with a worse prognosis [17].

Although vascular pathology does not appear to be primarily driven by hypertension and hyperlipidemia, blood pressure and cholesterol control within normal limits is advisable. In this patient with suboptimal management of her vascular risk factors, the doses of medications for hypertension, hyperlipidaemia and diabetes mellitus were increased. A systematic review showed that donepezil in combination with nimodipine was efficacious in improving cognitive testing scores, ability to carry out activities of daily living and reduced symptom severity in patients with vascular dementia [18]. Thus, this patient was also prescribed donepezil, which was uptitrated with improvement in her symptoms.

CONCLUSION

CAA has a strong association with progressive cognitive impairment, especially with Alzheimer's disease or vascular dementia. Early identifications of patients with dementia together with radiological evidence of CAA is important to decide the risk versus benefit of antiplatelets and anticoagulants, emphasize the importance of blood pressure control and affects their prognosis due to its high risk of recurrent spontaneous brain hemorrhages.

DECLARATIONS

Author's Contributions

All authors contributed to conception, drafting and finalizing the manuscript

Conflicts of interest

All authors declared that there are no conflicts of interests

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SHORT COMMUNICATION:

ROLE OF CONE BEAM COMPUTED TOMOGRAPHY IN IMPLANTOLOGY

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ABSTRACT:
Imaging of the dental implant site has become a mandatory protocol, to determine whether the patient can tolerate the surgical procedure. Prior to the invention of Cone Beam Computed Tomography (CBCT), dentists used orthopantomogram (OPG), but it had its limitations. CBCT offers improved accuracy and reduced distortion. The identification of underlying bony pathologies, assessment of bone density, proximity of vital anatomical structures, and prognosis of the implant to be inserted became easier with CBCT.

Keywords: implant, bone density, radiology, tomography, cone beam computed tomography

Diagnostic imaging plays a crucial role in formulating a suitable and precise treatment plan for patients receiving dental implants. The anatomical aspects of the implant placement site should determine the choice of radiological techniques. To achieve the most comprehensive presurgical assessment of the implant site, the use of Cone Beam Computed Tomography (CBCT) imaging is highly recommended [1]. CBCT scanners offer user-friendly operation and generate a three-dimensional image volume that can be customized for anatomical visualization using software. Specific protocols have been established to enhance the quality of images for evaluating the implant site [2]. This review aims to emphasize the nature of CBCT usage in imaging for placement of dental implants.

CBCT scanners vary in capabilities, and achieving high-quality diagnostic information depends on patient-specific factors and the operator's skills. Oral radiologist selects the scanner, field of view, and voxel parameters based on clinical indications for individual

patients and optimizing exposure for maximum diagnostic value. CBCT's multiplanar reconstruction capability has revolutionized implant dentistry by enabling clear visualization of structures without superimposition.

This ability to view structures from multiple angles enhances the precise evaluation of bone architecture, dimensions, contour, visual density, cortex, trabeculae pattern, and adjacent anatomical structures [3].

Imaging modalities for various treatment stages are presented in Table 1 [4].

Table 1: Imaging modalities used for various treatment stages [4].

Stage of treatment	Time (months)	Radiographic procedures
Treatment planning	-1	Periapical, Orthopantograph, tomo, CT, ceph
Surgery (placement)	0	Periapical, Orthopantograph, tomo, CT, ceph for correction of problems
Healing	0 to 3	Periapical, Orthopantograph, tomo, CT, ceph for correction of problems
Remodelling	4 to 12	Periapical, Orthopantograph
Maintenance	13+	Periapical, Orthopantograph
Complications	anytime	Periapical, Orthopantograph, CT (as indicated)

Abbreviations: tomo= conventional tomography; CT=reformatted computed tomography; Ceph= lateral cephalometric radiograph

IMAGING PROTOCOLS FOR IMPLANT PLACEMENT: CBCT imaging protocols for implant placement includes:

- Imaging the region of interest (ROI) and selecting the field of view (FOV),
- View the ROI at least in two planes right angle to each other,
- Evaluate bone height and width (bone dimensions),
- Determine quality of bone (Table 2),
- Determine long axis of alveolar bone,
- Identify and localize internal anatomy,
- Detection of bony pathology.

Table 2: Misch bone density classification [1]

Bone Density	Description	Tactile analogue	Typical anatomic location	Hounsfield units
D1	Dense cortical	Oak/maple	Anterior mandible	>1250
D2	Porous cortical and coarse trabecular	White pine/spruce	Anterior and posterior mandible, anterior maxilla	850-1250
D3	Porous cortical (thin) and fine trabecular	Balsa wood	Posterior mandible, anterior and posterior maxilla	350-850
D4	Fine trabecular	Styrofoam	Posterior maxilla	150-350

Anatomical structures and boundaries of those structures that are directly relevant to the area in which the implants are to be placed need to be identified and evaluated (Table 3) [5].

Table 3: Anatomical structures that needs to be considered prior to implant placement [5]

Anterior maxilla: • Nasal floor • Naso-palatine canal • Anterior superior alveolar canal	Posterior maxilla: • Maxillary sinus and related structures. • Posterior superior alveolar canal • Maxillary tuberosity • Pterygoid plates
Anterior mandible: • Lingual foramen • Incisive canal • Genial tubercles	Posterior mandible: • Inferior alveolar nerve canal • Mental foramina • Retromolar foramen • Sublingual fossa (lingual undercut) • Mylohyoid undercut • Lingula of ascending ramus
Zygomatic region: • Orbital floor • Infraorbital foramen • Zygomatic bone	

DATA TRANSFER

Stereolithographic models, which are computer-generated surgical guides, can be produced from Digital Imaging and Communications in Medicine (DICOM) data, effectively eliminating potential inaccuracies associated with conventional guide stent fabrication. The pre-implantation software planning aids surgeons in achieving more precise and safer implant placements. This technology enables minimally invasive surgery without the need to raise a flap, resulting in reduced surgical time, postoperative discomfort, swelling, and recovery period. The data obtained from the scan can be used in advance to create a master cast, and provisional restorations can be immediately placed following surgery (e.g., Teeth-in-an-Hour™ by Nobel Biocare in Kloten, Switzerland) [6 – 9].

POST SURGICAL APPLICATIONS OF CBCT

There are various indications described in guidelines and other scientific reports [5,10–12].

Indications for postsurgical use of CBCT in literature	Needed 3D info	Drawback CBCT
Postsurgical complications (e.g. neurovascular trauma)	Evaluate location and severity of problem and how to approach	Artefact by implant may mask neurovascular bundle CBCT fails to visualize neurovascular bundle
Healing follow-up of complex surgical procedures	Check bone healing and volumetric outcome	Detrimental artefacts of implants in borderline case (pneumatized maxillary sinus with inadequate bone)
Maxillofacial trauma with suspected complications at the implant level	Check mechanical failure implant or superstructure	Diagnostic failure to spot trauma caused by metal artefacts
Retrieval of Osseo integrated implants (infectious or mechanical failure etiology)		Blooming of implant masking neurovascular structures

ARTIFACTS

CBCT images often suffer from artifacts, especially when dense materials like metals are present, resulting in various artifact types. The most common artifacts among them are beam hardening, extinction, and exponential edge gradient effects [1].

These artifacts impact image quality in several ways, including bright streaks emanating from the metallic object, dark areas nearby, and even complete information loss between adjacent dense objects, collectively referred to as "metal artifacts." The presence of such artifacts in CBCT images compromises diagnostic accuracy and surgical planning. Material density and exposure parameters significantly influence artifact manifestation. Pauwels et al. quantified the impact of different CBCT devices and exposure protocols on the expression of metal artifacts caused by titanium implants, offering guidance on the development of optimized exposure protocols for effective metal artifact reduction [13]. Due to the clinical relevance of this matter, several efforts were made to reduce metal artefacts in CBCT images. A recent study conducted by Kuusisto et al. [14] demonstrated that composite materials give less artefacts, finding the cut-off point of artefacts at 20% radio-opaque filling material in composite implants.

CONCLUSION

In conclusion, the role of Cone Beam Computed Tomography (CBCT) in implantology is undeniably transformative and indispensable. CBCT technology has ushered in a new era of precision and efficiency in implant planning and placement, offering clinicians an unprecedented level of insight into the patient's anatomy. The ability to visualize critical structures, assess bone quality, and plan with meticulous detail has revolutionized the field, enhanced the success rates of implant procedures while minimizing risks. As we move forward in implantology, the significance of CBCT in optimizing patient outcomes cannot be overstated. However, it is essential that clinicians continue to stay updated

on the latest developments in CBCT technology and best practices to ensure its effective utilization in dental implant procedures. With its promising future and the potential for further advancements, CBCT stands as a cornerstone in the evolution of implantology, empowering professionals to provide the highest standard of care to their patients.

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AVAILABILITY AND IODINE CONTENT OF SALT AND SALTY CONDIMENTS IN HOUSEHOLDS AND MARKETS IN REMOTE COMMUNITIES IN JIMI, KEROWAGI, SINA-SINA AND OKAPA DISTRICTS, PAPUA NEW GUINEA

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ABSTRACT:
Iodine deficiency (ID) is recognized as the world's greatest single cause of preventable mental retardation in communities with low dietary consumption of iodine. Universal salt iodization (USI), is the universally agreed strategy for the control of ID. Communities in remote mountainous regions in Papua New Guinea (PNG) are considered to be most at risk of developing ID. This project rapidly assesses the status of salt iodization in the remote communities in three provinces in PNG. There are three separate analytical sections in this technical report. The first section presents the data and interpretations of the rapid assessment of the use of salt, salty condiments, and flavourings in households in remote communities in three provinces. The second section presents the data and interpretation of the rapid market survey that assessed the availability of staple foods and condiments in markets in these remote communities. The third section presents the quantitative analysis of iodine content in the salt samples collected from the markets. Each study was a prospective community based cross-sectional survey conducted by certified interviewers. The study sites were Jimi district in Jiwaka province, Kerowagi and Sina-Sina districts in Simbu province, and Okapa district in Eastern Highlands Province in PNG. Two different self-designed pretested questionnaires were used. One for the households and the other for the markets. Convenience sampling technique was used for selection of participants. The interviewers visited randomly selected households and markets in the villages in selected Local Level Government (LLG) areas and administered the appropriate questionnaires. The response rate was based on the willingness of a respondent to participate in the study. Both English and Tok-Pisin versions of the two sets of questionnaires were presented to each of the respondents. The completed questionnaires were checked, sorted and coded. During the distribution of the questionnaires to the respondents in the markets, the interviewers checked and recorded the various items available for sale in the market stalls. They purchased samples of each of the items. The questionnaires and the items purchased from the markets were transported to the Micronutrient Research Laboratory (MRL). In the MRL, the questionnaires were recorded and the data entered into Excel Spreadsheets. The data were analysed using the Excel Data Pack. Quantitative assay of iodine content in each of the samples was carried out using the WYD Iodine Checker. The criteria used for interpretation of the salt iodine results were based on the PNG Salt Legislation. In each of the four districts that participated in this study, the findings show that commercial packaged salt was available in more that 85% of the households. Similarly, over 85% of respondents in the households were aware of the importance of iodized salt. Salt was available in over 95% of the

market stalls in all the districts. In addition most of the salt available in the market stalls were adequately iodized according to the PNG salt legislation. More detailed survey is required to confirm the findings of this rapid survey.

Keywords: Salt Iodization, Iodine content, Remote communities, Sample, Papua New Guinea

INTRODUCTION:

The trace element Iodine is required for the biosynthesis of thyroid hormones, which are essential for growth and development. Low bioavailability or deficiency of this trace element usually leads to a spectrum of disorders called iodine deficiency disorders (IDD) [1, 2]

Iodine deficiency (ID) is considered as the world's greatest single cause of preventable mental retardation in communities with low dietary intake of iodine [1, 2, 3]. The recommended first-line strategy for the control and elimination of ID is Universal Salt Iodisation (USI). This is the policy of iodising all salts used in households, food processing, catering and agriculture [1, 2]. The USI was implemented in Papua New Guinea (PNG) in June 1995 following promulgation of the PNG Salt Legislation, banning the importation and sale of non-iodised salt [4]. It was incorporated in the PNG Food Sanitation Regulation issued in 2007 [5]. Systemic monitoring is required for effective implementation of the USI policy [1].

An assessment of some of the recent data on iodine status and availability of iodised salt among the population in some districts in PNG seems to indicate that the commercial salt sold in retail shops are adequately iodised and iodine status of the general population in major cities is adequate [6 – 11]. The results also indicated that there are remote communities that have very low access to commercial salt, and indications of prevalence of mild to moderate iodine deficiency, especially among school-age children [6 – 10]. It therefore became necessary to assess the awareness and availability of commercial salt in the households and markets, and whether the salt is adequately iodised or not, and also the availability of other foodstuffs and condiments that might be used as carriers of iodine to meet the dietary needs of residents in the remote communities. The decision was made to carry out rapid survey to assess the availability and use of commercial salt in selected remote communities in three provinces in PNG.

The three major objectives were:

Rapid assessment (appraisal) of the use of salt, salty condiments and flavourings in households in remote communities in three provinces.

Rapid market survey to assess the availability of staple foods and condiments in some markets in remote communities in the three provinces.

Quantitative assay of Iodine content in brands of salt collected from market stalls during the rapid market survey in the three provinces.

METHODOLOGY:

This prospective community based cross-sectional surveys were conducted by selected interviewers trained by the National Department of Health (NDoH) in PNG. They visited the various households and markets in the villages in selected Local Level Government (LLG) areas in the three provinces and administered the two sets of questionnaires. Convenience sampling technique was used for selection of participants. Each of the completed questionnaires were rechecked by the interviewers.

Survey sites:

The survey sites were villages in Local Level Government (LLG) areas in Jimi district Jiwaka province, Kerowagi district Simbu province, Sina-Sina Yonggomugl districts in Simbu province and Okapa district Eastern Highlands province (EHP). Some of the LLG sites were as follows.

LLG in Jiwaka province: Kol, Tapibuga, Koma, Koinambe.

LLG in Simbu province: Suai, Tabare, Yongomulg, Upper Koronigle, Lower Koronigle, Kup, Gena Waugla. LLG in EHP: East Okapa, Mage, Kasogu, Siru-Haga, Yagusa, Pusarasa, East Okapa

Survey instrument:

Two separate pre-designed semi-structured questionnaires were used. Each of the questionnaires, in both English and Tok-Pisin, were pre-tested in a rural LLG among selected households and markets in two different villages in Okapa district EHP. Feedback and suggested changes were provided orally and in writing. The feedback was used to adapt and improve the two versions of the questionnaires that were used in the survey.

Data collection:

Data was collected for the three separate analytical sections in this report. The first section presents the data for the rapid assessment of the use of salt, salty condiments and flavourings in households in remote communities in three provinces. The completed questionnaires were collected from households in Jimi district in Jiwaka province, Kerowagi and Sina-Sina districts in Simbu province and Okapa district in EHP. All the questionnaires were clearly labelled.

The second section was the rapid market survey to assess the availability of staple

foods and condiments in markets in the remote communities in the three provinces. The questionnaires completed by the stall-owners in the markets in Jimi district and Okapa district were clearly labelled. However, the questionnaires from stall-owners in markets in Kerowagi district and Sina-Sina Yonggomugl district were packed together in a single box. The questionnaires were not clearly labelled. Thus, in order to avoid errors in separating the questionnaires into the two districts (Kerowagi and Sina-Sina), they were coded and entered in the Excel Spreadsheet as Simbu province. This same problem was repeated in the third section. The salt samples from markets in Kerowagi and Sina-Sina districts were packeted together in three boxes but they were not clearly labelled to indicate the districts. Thus, the samples were analysed and presented for market stalls in Kerowagi & Sina-Sina in Simbu province. The iodine content in all the salt and other samples collected from the markets were quantitatively determined.

The survey was carried out between July and September 2022. The questionnaires and samples were delivered to the Micronutrient Research Laboratory (MRL) in the Division of Basic Medical Sciences (BMS) School of Medicine and Health Sciences (SMHS) University of Papua New Guinea (UPNG) for analyses. The questionnaires were sorted recoded and the data entered into Excel Spreadsheets for calculations and interpretation.

RESULTS AND INTERPRETATION:

Rapid assessment (appraisal) of the use of salt, salty condiments and flavourings in households in remote communities in three provinces: This section of the technical report presents the data obtained from the questionnaires completed by the respondents in the households in Jimi district in Jiwaka province, Kerowagi and Sina-Sina districts in Simbu province, and Okapa district in Eastern Highlands Province in PNG.

In Jimi district a total of 450 questionnaires were randomly distributed among the households (HH), but only 417 agreed to participate. This gave a response rate of 97.0% (417/430). In Kerowagi district 300 questionnaires were distributed, but 287 were completed and found suitable for analysis, the response rate was 95.7% (287/300). The response rate in Sina-Sina district was 95.4% (310/325). In Okapa district the response rate was 96.9% (252/260).

Socio-demographic characteristics of the respondents:

Table 1 summaries the socio-demographic characteristics of the respondents in each of

the four districts. The age range for respondents in Jimi, Kerowagi, Sina-Sina and Okapa were 17 – 92 years, 16 – 68 years, 18 – 85 years and 19 – 72 years respectively.

The gender distribution and mean age of the respondents in each of the four districts are also presented in Table 1. The number of females were significantly higher than that of males in Jimi district 58.8% (245/417) and Kerowagi district 62.0% (178/287).

The respondents were asked if they had a salaried job or if they work for money. Most of the respondents in Jimi (89.2%), in Kerowagi (90.2%), Sina-Sina (71.9%) and Okapa (88.9%) said that they do not have a job with salary. This was because most of them are subsistence farmers. They do not consider the amount of money that they receive after selling their products as salaries.

Availability and awareness of Iodized Salt and use of Salty Condiments, Flavourings, and Fortification Food Vehicles: This section of the questionnaire contains a total of 13 questions. The summary of the results obtained for each of the four districts are presented in Table 2.

The respondents were asked the question (Q1) "*Does your household use anything to give food a salty taste?*" A total of 99.3% of respondents in Jimi, 92.0% in Kerowagi, 97.1% in Sina-Sina and 92.5% in Okapa said "*YES*".

Those respondents were then asked the second question (Q2) *"If you responded "YES" to Q1, please tell me which of these products you use in your household (select the different products)"* Five groups of products were listed for selection.

Commercial packaged salt bought in the markets or shops was selected by 100% of the respondents in both Jimi and Kerowagi, 94.7% respondents in Sina-Sina and 86.3% in Okapa districts.

Stock / bouillon cubes was selected by 50.7%, 87.9%, 30.9% and 16.3% of respondents in Jimi, Kerowagi, Sina-Sina and Okapa respectively.

The results for *Seasoning salt/powder* and *Seasoning sauce* are presented in Table 2.

Traditional salt/local salt was selected by 27.0% of respondents in Okapa and 12.9% in Kerowagi compared to 0.2% in Jimi and 0.7% in Sina-Sina districts.

The respondents were then asked Q3: "*For the product you use in your household, please tell me how often you use them*".

Commercial salt was used daily by 89.1%, 92.0%, 85.3% and 93.5% of respondents in Jimi, Kerowagi, Sina-Sina and Okapa districts respectively. The other frequency of

uses of commercial salt are presented in Table 2.

Stock Bouillon cubes were used several times a week by 55.2% and 49.6% of respondents in Jimi and Kerowagi respectively, compared to 33.3% of respondents in Sina-Sina and 47.4% in Okapa that used them once a week.

The third product, *Seasoning salt /powder* was used daily by 35.4% of respondents in Jimi, 20.9% in Kerowagi and 47.1% in Sina-Sina.

Seasoning sauce was used several time a week by 61.1% respondents in Jimi, once a week by 42.1% in Okapa, once a month by 50.1% in Kerowagi and 67.8% in Sina-Sina.

Traditional / local salt was used by 69.8% in Okapa several times a week compared to 44.1% that used it once a month in Kerowagi. The other results are presented in Table 2.

In order to assess the availability of commercial salt in the HH, the respondents were asked (Q4) "*Does your family have commercial packaged salt bought in market/shop in the household today?*"

In Jimi 73.9%, in Kerowagi 91.7%, in Sina-Sina 81.1% and in Okapa 83.3% of the respondents answered in the "*affirmative"*.

Those that answered in the *negative* were asked (Q5), *if the HH had commercial packaged salt bought in the market/shop on any day in the last 7 days*. The response was "*Yes*" by 88.0%, 90.9%, 100% and 74.4% of respondents in Jimi, Kerowagi, Sina-Sina and Okapa respectively.

The results indicated that within the last seven days, 96.9% (404/414) of HH in Jimi, 98.2% (262/264) of HH in Kerowagi, 100% (301/301) of HH in Sina-Sina and 95.7% (223/233) of HH in Okapa had commercial packaged salt bought in the market/salt.

The respondents were then asked (Q6) "*What do you do with the salt bought at market/shop (select as many as apply)?*" Four options were provided; they were asked to select as many as possible.

In response to (Q6), 90.8% in Jimi, 80.9% in Kerowagi, 97.7% in Sina-Sina and 77.6% in Okapa stated that they use salt for cooking and add to food before eating. This was the major use of salt indicated by respondents in the four districts. The precent frequency of the other options used are presented in Table 2.

The next seven questions (Q7 to Q13) were to assess the availability in HH of some commercial products that can be fortified.

To obtain information about the availability of *wheat flour* and *wheat flour products* in the HH the respondents were asked Q7, Q8, Q9.

Question 7 (*Q7*) was "*Does your household have wheat flour or wheat flour foods such as dried noodles, pasta, macaroni, instant*

noodles; 2-minute noodles, bread, buns, rolls, cake, crackers, biscuits, scones, donuts today?"

The responses were "*Yes*" by 30.7%, 40.1%, 41.6% and 64.3% of respondents in Jimi, Kerowagi, Sina-Sina and Okapa respectively.

The follow up question (*Q8*) to those that said "*No*" was "*If No, did your household have wheat flour or wheat flour foods such as dried noodles, pasta, macaroni, instant noodles, 2-minute noodles, bread, buns, rolls, cake, crackers, biscuits, scones, donuts on any day in last 7 days?*"

The responses were "*Yes*" by 74.0%, 65.7%, 59.7% and 45.6% of respondents in Jimi, Kerowagi, Sina-Sina and Okapa respectively.

The results indicated that within the last seven days, 82.0% (342/417), 79.4% (228/287), 76.5% (237/310) and 80.6% (203/252) of HH in Jimi, Kerowagi, Sina-Sina and Okapa respectively had some wheat flour products in the HH.

The next question (*Q9*) was to those respondents that said "*Yes*" to Q7 or Q8. "*If you responded "Yes" to Q7 or Q8, which food do you have in your HH (tick any that apply)?"*

The respondents were asked to choose as many as necessary from the options given.

The most popular wheat flour product selected by respondents in the four districts was "Instant noodles/2-minute noodles". Jimi 95.3%, Kerowagi 90.8%, Sina-Sina 69.2% and Okapa 77.3%.

Wheat flour was selected by 31.7% in Jimi, 35.5% in Kerowagi, 44.3% in Sina-Sina and 14.3% in Okapa.

Q10 & Q11 were about the availability of "*Rice*" in the HH.

All the respondents were asked *Q10*: "*Does you HH have rice today?*" On the day of the survey rice was available in 33.6% of the HH in Jimi, 36.2% in Kerowagi, 48.7% in Sina-Sina and 55.6% in Okapa.

Q11 was directed to those respondents that did not have rice in the house on the day of the survey.

The question was, "*If "No" did your HH have rice any day in last 7 days?*" The responses are presented in Table 2. The results show that within the last seven day, rice was available in 82.0% (343/417) of HH in Jimi, 77.8% (206/287) in Kerowagi, 92.9% (288/310) in Sina-Sina and 84.1% (212/252) in Okapa.

All the respondents were also asked (*Q12 & Q13*) about the availability of "*Cooking oil*" in their HH.

On the day of the survey the question (*Q12*) was "*Does your HH have cooking oil today"?*

Cooking oil was available in 85.1% of HH in Jimi, 73.5% in Kerowagi, 81.9% in Sina-Sina and 81.3% in Okapa.

The follow up question (*Q13*) to those without cooking oil was, "*If No, did your household have cooking oil on any day in last 7 days?"* The responses are presented in Table 2. The results show that in the last seven days, cooking oil was available in 86.3% (360/417) of HH in Jimi, 96.9% (278/287) of HH in Kerowagi, 97.4% (302/310) HH in Sina-Sina and 91.3% (230/252) HH in Okapa.

TABLE 1: Socio-demographic characteristics of respondents in the four districts

Socio-demographic characteristics of respondents in					
	DISTRICT / PROVINCE	Jimi / JIWAKA	KEROWAGI / SIMBU	SINA-SINA SIMBU	OKAPA EHP
	Number of respondents	417	287	310	252
	Gender				
	Females	245 (58.8%)	178 (62.0%)	138 (44.5%)	127 (50.4%)
	Males	172 (41.2%)	109 (38.0%)	172 (55.5%)	125 (49.6%)
	Age in years				
	Mean age of females (years)	38	35	42	40
	Mean age of males (years)	42	43	43	40
	Mean age of respondents (years)	40	39	42	40
	Age range of respondents (years)	17 – 92	16 – 68	18 – 85	19 – 72
	Do you have a job with salary (pay?) or do you work for money?				
	Yes	10.8% (45/417)	9.8% (28/287)	28.1% (87/310)	11.1% (28/252)
	No	89.2% (372/417)	90.2% (259/287)	71.9% (223/310)	88.9% (224/252)

Table 2: Availability and awareness of iodised salt, and use of salty condiments, flavourings, and fortification food vehicles

	DISTRICT / PROVINCE	Jimi / JIWAKA	KEROWAGI / SIMBU	SINA-SINA SIMBU	OKAPA EHP
	Number of respondents	417	287	310	252
Q1	Q 1: Does your household use anything to give food a salty taste?				
	1. Yes	99.3% (414/417)	92.0% (264/287)	97.1% (301/310)	92.5% (233/252)
	2. No	0.7% (3/417)	8.0% (23/287)	2.9% (9/310)	7.5% (19/252)
	3. Not sure	0	0	0	0
Q2	Q 2: If you responded YES to Q 1, please tell me which of these products you use in your household (select all the products that applies)				
	1. Commercial packaged salt bought at market / shop:	100% (414/414)	100% (264/264)	94.7% (285/301)	86.3% (201/233)
	2. Stock / bouillon cubes:	50.7% (210/414)	87.9% (232/264)	30.9% (93/301)	16.3% (38/233)
	3. Seasoning salt / powder:	31.4% (130/414)	32.6% (86/264)	23.3% (70/301)	1.7% (4/233)

	4. Seasoning sauce:		16.9% (70/414)	15.9% (42/264)	9.3% (28/301)	3.0% (7/233)
	5. Traditional / local salt		0.2% (1/414)	12.9% (34/264)	0.7% (2/301)	27.0% (63/233)
Q3:	For the product you use in your household, please tell me how often you use them.					
Commercial package salt bought in market / salt:						
		1. Everyday	89.1% (369/414)	92.0% (243/264)	85.3% (243/285)	93.5% (188/201)
		2. Several times a week	5.8% (24/414)	6.8% (18/264)	11.9% (34/285)	6.5% (13/201)
		3. Once a week	2.9% (12/414)	0.8% (2/264)	2.8% (8/285)	0
		4. Once a month or less	2.2% (9/414)	0.4% (1/264)	0	0
Stock bouillon cubes:						
		1. Everyday	17.1% (36/210)	19.8% (46/232)	12.9% (12/93)	7.9% (3/38)
		2. Several times a week	55.2% (116/210)	49.6% (115/232)	32.3% (30/93)	34.2% (13/38)
		3. Once a week	11.9% (25/210)	12.1% (28/232)	33.3% (31/93)	47.4% (18/38)
		4. Once a month or less	15.7% (33/210)	18.5% (43/232)	21.5% (20/93)	10.5% (4/38)
Seasoning salt / powder:						
		1.Everyday	35.4% (46/130)	20.9% (18/86)	47.1% (33/70)	0
		2. Several times a week	33.8% (44/130)	25.6% (22/86)	21.4% (15/70)	75.0% (3/4)
		3. Once a week	24.6% (32/130)	23.3% (20/86)	22.9% (16/70)	25.0% (1/4)
		4. Once a month or less	6.2% (8/130)	30.2% (26/86)	8.6% (6/70)	0
Seasoning sauce:						
		1. Every day	4.4% (3/68)	4.8% (2/42)	3.6% (1/28)	14.3% (1/7)
		2. Several times a week	69.1% (47/68)	11.9% (5/42)	3.6% (1/28)	14.3% (1/7)
		3. Once a week	20.6% (14/68)	33.3% (14/42)	25.0% (7/28)	42.9% (3/7)
		4. Once a week or less	5.9% (4/68)	50.0% (21/42)	67.8% (19/28)	28.5% (2/7)
Traditional / local salt:						
		1. Every day	0	29.4% (10/34)	0	22.2% (14/63)
		2. Several times a week	0	11.8% (4/34)	(2/2)	69.8% (44/63)
		3. Once a week	(1/1)	14.7% (5/34)	0	6.4% (4/63)
		4. Once a month or less	0	44.1% (15/34)	0	1.6% (1/63)
Q 4	Does your family have commercial packaged salt bought in market/shop in the household today?"					
	1. Yes		73.9% (306/414)	91.7% (242/264)	81.1% (244/301)	83.3% (194/233)
	2. No		26.1% (108/414)	8.3% (22/264)	18.9% (57/301)	16.7% (39/233)
	3. Not sure		0	0	0	0
Q 5	If No, did your household have commercial packaged salt bought at market / shop any day in the last 7 days?					

	1. Yes	88.0% 95/108	90.9% (20/22)	100.0% (57/57)	74.4% (29/39)
	2. No	12.0% (13/108)	9.1% (2/22)	0	25.6% (10/39)
Q6	What do you do with the commercial packaged salt bought at market / shop? (Select as many as possible) (*Respondents gave multiple answers*)				
	1. Use for cooking and add to food before eating	90.8% (364/401)	80.9% (212/262)	97.7% (294/301)	77.6% (173/223)
	2. Use for cooking only	16.0% (64/401)	34.7% (91/262)	21.6% (65/301)	15.2% (34/223)
	3. Add to food before eating only	49.6% (199/401)	35.5% (93/262)	24.3% (73/301)	7.2% (16/223)
	4. Other uses (Specify)	29.4% (118/401)	13.7% (36/262)	14.3% (43/301)	0
Q7	Does your household have wheat flour or wheat flour foods such as dried noodles, pasta, macaroni, instant noodles; 2-minute noodles, bread, buns, rolls, cake, crackers, biscuits, scones, donuts today?				
	1. Yes	30.7% (128/417)	40.1% (115/287)	41.6% (129/310)	64.3% (162/252)
	2. No	69.3% (289/417)	59.9% (172/287)	58.4% (181/310)	35.7% (90/252)
Q8	If No, did your household have wheat flour or wheat flour foods such as dried noodles, pasta, macaroni, instant noodles, 2-minute noodles, bread, buns, rolls, cake, crackers, biscuits, scones, donuts on any day in last 7 days?				
	1. Yes	74.0% (214/289)	65.7% (113/172)	59.7% (108/181)	45.6% (41/90)
	2. No	26.0% (75/289)	34.3% (59/172)	40.3% (73/181)	54.4% (49/90)
Q9	If you responded Yes to question 7 or 8, which food did you have in your household (tick all that apply)? *{Values do not add up to 100%}*				
	1. wheat flour	31.7% (129/342)	35.5% (81/228)	44.3% (105/237)	14.3% (29/203)
	2. dried noodles/macaroni/pasta	3.2% (11/342)	17.5% (40/228)	4.6% (11/237)	11.3% (23/203)
	3. Instant noodles/2-minute noodles	95.3% (326/342)	90.8% (207/228)	69.2% (164/237)	77.3% (157/203)
	4. Bread/buns/rolls/	0.3% (1/342)	5.7% (13/228)	6.3% (15/237)	0
	5. Crackers/biscuits	25.1% (86/342)	21.1% (48/228)	8.9% (21/237)	2.0% (4/203)
	6. Cake/scones/donuts	2.0% (7/342)	19.3% (44/228)	0.8% (2/237)	0
	7. Other wheat flour food (Specify)	36.8% (126/342)	8.3% (19/228)	6.8% (16/237)	2.0% (4/203)
Q10	Does your household have rice today?				
	1. Yes	33.6% (140/417)	36.2% (104/287)	48.7% (151/310)	55.6% (140/252)
	2. No	66.4% (277/417)	63.8% (183/287)	51.3% (159/310)	44.4% (112/252)
Q11	If No, did your household have rice any day in the last 7 days?				
	1. Yes	72.9% (202/277)	55.7% (102/183)	86.2% (137/159)	64.3% (72/112)
	2. No	27.1% (75/277)	44.3% (81/183)	13.8% (22/159)	35.7% (40/112)
Q12	Does your household have oil today?				
	1. Yes	85.1% (355/417)	73.5% (211/287)	81.9% (254/310)	81.3% (205/252)
	2. No	14.9% (62/417)	26.5% (76/287)	18.1% (56/310)	18.7% (47/252)
Q13	If No, did your household have oil any day in the last 7 days?				

1. Yes	8.1% (5/62)	88.2% (67/76)	85.7% (48/56)	53.2% (25/47)
2. No	91.9% (57/62)	11.8% (9/76)	14.3% (8/56)	46.8% (22/47)

RESULTS AND INTERPRETATION:

Rapid market survey to assess the availability of staple foods and condiments in some markets in remote communities in three provinces:

This section of the technical report presents the data obtained from the questionnaires completed by the owners (or representatives) of stalls in markets in Jimi district in Jiwaka province, Kerowagi and Sina-Sina districts in Simbu province, and Okapa district in EHP.

In Jimi district 110 questionnaires were from traders in the market stalls in the different villages that participate in the study. The questionnaires for Kerowagi district and Sina-Sina district were in one box. A total of 163 questionnaires were completed by traders in market stalls in the different villages in the two districts. In Okapa district 145 questionnaires were completed by traders in market stalls in the various villages. The response rates for each of the districts were not calculated because only the completed questionnaires were submitted for data entry and analysis.

Socio-demographic characteristics of the Traders:

The mean ages of the respondents in Jimi, Simbu and Okapa were 39.0 years, 37.6 years and 34.8 years respectively. The age range for respondents in Jimi, Simbu and Okapa were 15 - 77 years, 14 – 69 years and 17 – 75 years respectively. Gender was not indicated in the questionnaires.

Availability of Salt, Staple Foods, Condiments and Flavourings:

This section of the questionnaire contains three questions. The summary of the results obtained for each of the districts are presented in Table 3.

The respondents were asked **question 1** (**Q 1**) "***Is this product available at this stall?***" The list of nine products indicated in Table 3 was shown to each of the respondents.

"***Commercial packaged salt***" was the first product in the list. It was available in 98.2% (108/110), 98.2% (160/163) and 100% (145/145) of the market stalls in Jimi, Simbu and Okapa respectively on the day of the survey.

The next in the list was "***Stock / bouillon cubes***". They were available in 94.5% (104/110) of the market stalls in Jimi, 67.8% (110/163) of the market stalls in Simbu and 97.2% (141/145) of the market stalls in Okapa.

The next was **"*Seasoning Salt/Powder***": They were available in 13.6% (15/110) of the market stalls in Jimi, 16.6% (27/163) in Simbu and 44.1% (64/145) in Okapa. Results for "***Seasoning sauce***" and "***Traditional salt / local salt***" are presented in Table 3.

Next was "***Wheat flour***": They were available in 64.5% (71/110), 49.7% (81/163) and 67.6% (98/145) of the market stalls in Jimi, Simbu and Okapa respectively.

These results were in contrast to the availability of "***Wheat flour products***" in the market stalls (Table 3). In Jimi 97.3% (107/110), in Simbu 87.1% (142/163) and in Okapa 100% (145/145) of the market stalls had wheat flour products on the day of the survey.

The next was **"*Rice***". They were available in 99.1% (109/110) of the stalls in Jimi, 84.7% (138/163) of the stalls in Simbu and 98.6% (143/145) of the market stalls in Okapa.

The results show that **"*Cooking Oil*"** was available in 96.4% (106/110), 96.9% (158/163) and 98.6% (143/145) of the market stalls in Jimi, Simbu and Okapa respectively.

The next question (Q 2) was to assess the different brands of products available in the market stalls. The respondents were asked:

Q 2: "*For all the products available, indicate the different brands available. For traditional salt indicate the type available*"

"***Brands of Commercial Packaged salt***":

Five brands of commercial packaged salt were available in market stalls in Jimi district. Star salt, True-cook salt and Pacific salt were available in 98.1% (106/108), 47.2% (51/108) and 22.2% (24/108) respectively in market stalls in Jimi district. The others are presented in Table 3.

In Simbu, eight brands of commercial package salt were available in market stalls. Star salt, True-cook salt and Jumbo-salt brands were available in 63.1% (101/160), 29.4% (47/160) and 28.1% (45/160) respectively. The other brands are indicated in Table 3.

In Okapa, five brands of commercial packaged salt were in the market stalls. Of the five brands, Star salt was available in 79.3% (115/145), followed by True cook salt available in 47.6% (69/145) market stalls. The other brands are indicated in Table 3.

"***Brands of Stock / bouillon cub****es*":
Four brands were available in market stalls in Jimi, six brands in Simbu and only one brand in Okapa.
Maggie Kakaruk Stock cubes were available in 86.5% (90/104), 68.2% (75/110) and 100% (141/141) of the market stalls in Jimi, Simbu and Okapa respectively. The other brands are shown in Table 3.

"***Brands of Seasoning Salt / Powder***":
Four different brands were available in market stalls in Jimi. Topmi (VC – Tsin) was available in 33.3% (5/15), Curry powder was available in 26.7% (4/15) and Teisti Rais was also available in 26.7% (4/15) of market stalls.

In Simbu, six different brands were available in market stalls. Curry powder, Maggie salt and Teisti Rais were available in 55.6% (15/27), 33.3% (9/27) and 22.2% (6/27) of market stalls respectively.

Five different brands were available in market stalls in Okapa. Curry powder was in 89.1% (57/64) and Zesi-1 in 28.1% (18/64) of the market stalls. The other brands are shown in Table 2.

"***Brands of seasoning sauce***":
Two different brands of *seasoning sauce* were available in market stalls in Jimi and Simbu, compared to five different brands in market stalls in Okapa.
In Jimi, ***Soy sauce*** was available in 75.0% (3/4) market stalls and ***Mushroom sauce*** was available in 25.0% (1/4) market stalls.

In Simbu, ***Mushroom sauc****e* was available in 66.7% (4/6) market stalls and ***Soy sauce*** was available in 33.3% (2/6) market stalls.

In Okapa, ***Soy sauce*** was in 87.0% (20/23) market stalls, ***Mushroom sauce*** in 47.8% (11/23) and ***Maggie sauce*** in 8.7% (2/23) market stalls. The others are indicated in Table 3.

"***Types of Traditional / Local salt***": One type (Rock salt) was available only in market stalls in Okapa.

"***Brands of Wheat flour***": Four different brands were available in markets in Jimi, ten different brands in Simbu and five different brands in Okapa.
Plain flour was available in 74.6% (53/71), ***Flame flour*** in 33.8% (24/71) and the ***3-Rose flour*** in 23.9% (17/71) of market stalls in Jimi. In Simbu, ***Plain flour*** was in 48.1% (39/81), ***Flame flour*** in 23.5% (19/81) and ***3-Rose flour*** in 38.3% (31/81) of market stalls. The other brands are shown in Table 3. In Okapa market stalls ***Plain four*** was in 37.8% (37/98), ***Flame flour*** in 58.2% (57/98) and ***3-Rose flour*** in 31.6% (31/98). The other

brands available in the markets stalls are presented in Table 3.

"***Types of Wheat flour products***": ***Instant Noodles/2-minutes Noodles*** were available in 99.1% (106/107), 100% (142/142) and 98.6% (143/145) of the market stalls in Jimi, Simbu and Okapa respectively. ***Bread/buns/rolls/crackers/biscuits*** were available in 93.5% (100/107), 57.7% (82/142) and 92.4% (134/145) of the market stalls in Jimi, Simbu and Okapa respectively.

Brands of Rice: The number of different brands of rice in market stalls in Jimi, Simbu and Okapa were eleven, ten and eleven brands respectively.

Roots rice, ***Skel rice*** and ***Trukai rice*** were available in 90.8% (99/109), 43.1% (47/109) and 30.3% (33/109) of the market stalls respectively in Jimi. The other brands are shown in Table 3.

In Simbu, ***Root rice, Skel rice*** and ***Trukai rice*** were available in 79.0% (109/138), 68.1% (94/138) and 18.1% (25/138) respectively of the market stalls. Other brands are shown in Table 3.

For markets in Okapa, ***Root rice, Skel rice*** and ***Trukai rice*** were available in 46.9% (67/143), 90.9% (130/143) and 20.3% (29/143) of the stalls. The other brands are shown in Table 3.

"***Brands of cooking oil***": Eleven different brands of cooking oil were available in markets in Jimi. ***Golden sun oil, Voila oil***, and ***Flame oil*** were in 64.2% (68/106), 50.0% (53/106) and 33.0% (35/106) of the market stalls respectively. The other brands are shown in Table 3.

In Simbu, a total of fifteen brands of oil were available in the markets. ***Mamas cooking oil (Mamas choice)*** was available in 45.6% (72/158), ***Flame oil*** in 28.5% (45/158) and ***Golden sun oil*** in 25.9% (41/158) of market stalls. The other brands are shown in Table 3.

Eight different brands of cooking oil were on sale in market stalls in Okapa. ***Flame oil*** was in 62.9% (90/143), ***Golden sun oil*** in 43.4% (62/143) and ***Mamas cooking oil*** in 35.7% (51/143) of the market stalls.

The market stall owners were asked Q 3, to determine the most popular brands used in the community.

Q3: "Of the brands listed ask the stall owner which is the brand most frequently purchased?"

"Most frequently purchased brand of Commercial Packaged salt":

The most frequently purchased brand of commercial packaged salt was ***Star salt*** with the frequency of 88.0% (95/108) in Jimi, 56.9% (91/160) in Simbu and 69.7% (101/143) in Okapa.

"***Most frequently purchased brand of Stock / bouillon cubes"***:

Maggie Kakaruk Stock cubes were the most frequently purchased in Jimi (77.9%; 81/104), in Simbu (72.7%; 89/110) and in Okapa (100%; 141/141).

"Most frequently purchased brand of Seasoning Salt / Powder":

In Jimi, ***Topmi (VE-Tsin)*** was the most frequently purchased (33.3%; 5/15). In Simbu, the most frequently purchased was ***Maggie salt/powder*** (33.3%; 9/27). ***Curry powder*** was the most frequently purchased in Okapa (81.3%; 52/64).

"Most frequently purchased brand of seasoning sauce":

Soy sauce was the most frequently purchased in Jimi (75.0%; 3/4) and Okapa (60.9%; 14/23). ***Mushroom sauce*** was the most frequently purchased in Simbu (66.7%; 4/6).

"Most frequently purchased brand of wheat flour":

Plain flour was the most frequently purchased brand in Jimi (73.2%; 52/71) and in Simbu (45.7%; 37/81). In Okapa, ***Flame flour*** (50.0%; 49/98) was the most frequently purchased brand of wheat flour'

"Most frequently purchased type of wheat flour product":

Instant noodles/2-minutes noodles were the most frequently purchased type of wheat flour products in Jimi (99.1%; 106/107), Simbu (100%; 142/142) and Okapa (70.3%; 102/145).

"Most frequently purchased brand of rice":

Roots rice was the most frequently purchased brand in Jimi (59.6%; 65/109). ***Skel rice*** was the most frequently purchased brand of rice in Simbu (46.4%; 64/138) and in Okapa (74.8% (107/143).

"Most frequently purchased brand of cooking oil":

In Jimi markets the most frequently purchased brand of cooking oil was ***Golden sun oil*** (40.6%; 43/106). ***Mamas cooking oil*** (27.2%; 43/158) was the most frequently purchased brand in Simbu markets. In Okapa markets ***Flame oil*** (45.4%; 65/143) was the most frequently purchased brand.

Table 3: Availability of Salt, Staple Foods, Condiments and Flavourings in the markets

	DISTRICT / PROVINCE	JIMI	SIMBU	OKAPA
	N	110	163	145
Q1	Q 1: Is this product available at this stall?			
	Commercial packaged salt	98.2% (108/110)	98.2% (160/163)	100% (145/145)
	Stock / bouillon cubes	94.5% (104/110)	67.5% (110/163)	97.2% (141/145)
	Seasoning salt / powder	13.6% (15/110)	16.6% (27/163)	44.1% (64/145)

	Seasoning sauce	3.6% (4/110)	3.7% (6/163)	15.9% (23/145)
	Traditional / local salt	0	0	0.7% (1/145)
	Wheat flour	64.5% (71/110)	49.7% (81/163)	67.6% (98/145)
	Wheat flour products	97.3% (107/110)	87.1% (142/163)	100% (145/145)
	Rice	99.1% (109/110)	84.7% (138/163)	98.6% (143/145)
	Oil	96.4% (106/110)	96.9% (158/163)	98.6% (143/145)
Q2	Q 2: For all the products available, indicate the different brands available. For traditional salt indicate the type available (*NB: multiple brands were available in the stalls*)			
	Brands of Commercial packaged salt:	N = 108	N = 160	N = 145
	1 Star salt	98.1% (106/108)	63.1% (101/160)	79.3% (115/145)
	2 Five star salt	0.9% (1/108)	7.5% (12/160)	0
	3 True cook salt	47.2% (51/108)	29.4% (47/160)	47.6% (69/145)
	4 Pacific salt	22.2% (24/108)	0	0
	5 Jumbo salt	0	28.1% (45/160)	0
	6 Supa salt	1.9% (2/108)	6.3% (10/160)	0
	7 Ezy salt	0	0.6%) (1/160)	14.5% (21/145)
	8 Saxa salt	0	0.6% (1/160)	8.3% (12/145)
	9 Repackaged salt	0	6.9% (11/160)	0
	10 Kakaruk salt	0	0	1.4% (2/145)
	Brands of Stock bouillon cubes	N = 104	N = 110	N = 141
	1 Maggie cubes	25.0% (26/104)	28.2% (31/110)	0
	2 Maggie Kakaruk Stock cubes	86.5% (90/104)	68.2% (75/110)	100% (141/141)
	3 Chicken flavour cubes	1.9% (2/104)	8.2% (9/110)	0
	4 Super flavor Maggie cubes	0	8.2% (9/110)	0
	5 Beef cubes	0	4.5% (5/110)	0
	6 Stock cubes	1.9% (2/104)	6.4% (7/110)	0
	Brands of Seasoning salt / powder:	N = 15	N = 27	N = 64
	1 Teisti Rais	26.7% (4/15)	22.2% (6/27)	3.1% (2/64)
	2 Topmi (VE-Tsin)	33.3% (5/15)	7.4% (2/27)	0
	3 Coconut milk powder	26.7%	0	0

		(4/15)		
	4 Curry powder	26.7% (4/15)	55.6% (15/27)	89.1% (57/64)
	5 Vetgur / Vetzin	0	14.8% (4/27)	10.9% (7/64)
	6 Zesi-1	0	14.8% (4/27)	28.1% (18/64)
	7 Maggie	0	33.3% (9/27)	0
	8 Yellow egg	0	0	3.1% (2/64)
	Brands of Seasoning sauce:	N = 4	N = 6	N = 23
	1 Soy sauce	75.0% (3/4)	33.3% (2/6)	87.0% (20/23)
	2 Mushroom soy sauce	25.0% (1/4)	66.7% (4/6)	47.8% (11/23)
	3 Chilli sauce	0	0	4.3% (1/23)
	4 Tomato sauce	0	0	4.3% (1/23)
	5 Maggie sauce	0	0	8.7% (2/23)
	Type of Traditional / Local salt:	N = 0	N = 0	N = 1
	1 Ash	0	0	0
	2 Rock salt	0	0	100% (1/1)
	3 Sea water	0	0	0
	Brand of Wheat flour:	N = 71	N = 81	N = 98
	1 Plain flour	74.6% (53/71)	48.1% (39/81)	37.8% (37/98)
	2 Flame flour	33.8% (24/71)	23.5% (19/81)	58.2% (57/98)
	3 3-Rose flour	23.9% (17/71)	38.3% (31/81)	31.6% (31/98)
	4 Whole meal flour	38.0% (27/71)	18.5% (15/81)	22.4% (22/98)
	5 Wheat scone flour balls	0	2.5% (2/81)	0
	6 Scone flour	0	8.6% (7/81)	0
	7 Self-Raising plain flour	0	1.2% (1/81)	11.2% (11/98)
	8 Bakers flour	0	4.9% (4/81)	0
	9 Brown wheat bakers flour	0	3.7% (3/81)	0
	10 Skai flour	0	0	0
	11 Skow flour	0	1.2% (1/81)	0
	Do you have wheat flour products:			
	Yes	97.3% (107/110)	87.1% (142/163)	100% (145/145)

	No	2.7% (3/110)	12.9% (21/163)	0
	Types of wheat flour products:	N = 107	N = 142	N = 145
	1 dried noodles/macaroni/pasta	18.7% (20/107)	2.1% (3/141)	2.1% (3/145)
	2. Instant noodles/2-minute noodles	99.1% (106/107)	100% (142/142)	98.6% (143/145)
	3. Bread/buns/rolls/Crackers/Biscuits	93.5% (100/107)	57.7% (82/142)	92.4% (134/145)
	4. Cake/scones/donuts	0	28.2% (40/142)	1.4% (2/145)
	Brands of Rice:	N = 109	N = 138	N = 143
	1 Roots rice	90.8% (99/109)	79.0% (109/138)	46.9% (67/143)
	2 Skel rice	43.1% (47/109)	68.1% (94/138)	90.9% (130/143)
	3 Trukai rice	30.3% (33/109)	18.1% (25/138)	20.3% (29/143)
	4 Long grain rice	54.1% (59/109)	46.4% (64/138)	0.7% (1/143)
	5 Star rice	1.8% (2/109)	2.2% (3/138)	41.3% (59/143)
	6 Wantok rice	0	0.7% (1/138)	6.3%) (9/143)
	7 Jasmine rice	3.7% (4/109)	4.3% (6/138)	3.5% (5/143)
	8 Ezy cook rice	0	1.4% (2/138)	0
	9 Super A1 rice	18.3% (20/109)	0.7% (1/138)	11.9% (17/143)
	10 Frangipani rice	3.7% (4/109)	0	18.9% (27/143)
	11 Sun long rice	10.1% (11/109)	0.7% (1/138)	0
	12 Ori rice	11.0% (12/109)	0	2.8% (4/143)
	13 Kumul rice	11.9% (13/109)	0	3.5% (5/143)
	Brands of cooking oils:	N = 106	N = 158	N = 143
	1 Mamas cooking oil (Mamas choice)	22.6% (24/106)	45.6% (72/158)	35.7% (51/143)
	2 Jumbo oil	0	0.6% (1/158)	0
	3 Vegetable oil	0	11.4% (18/158)	0
	4 Sun shine oil	0.9% (1/106)	4.4% (7/158)	0.7% (1/143)
	5 Sunflower oil	0	10.1% (16/158)	0
	6 Flame oil	33.0% (35/106)	28.5% (45/158)	62.9% (90/143)
	7 Patna oil	0	3.8% (6/158)	0
	8 Sun Rise oil	4.7% (5/106)	3.8% (6/158)	0

	9 Golden Sun oil	64.2% (68/106)	25.9% (41/158)	43.4% (62/143)
	10 Highlands Meadow	4.7% (5/106)	8.2% (13/158)	0
	11 Voila oil	50.0% (53/106)	6.3% (10/158)	18.9% (27/143)
	12 Fresh sun oil	22.6% (24/106)	5.7% (9/158)	21.0% (30/143)
	13 Best oil	10.4% (11/106)	2.5% (4/158)	7.0% (10/143)
	14 Chefs oil	2.8% (3/106)	0.6% (1/158)	0
	15 Mothers choice	24.5% (26/106)	0	0
	16 King oil	0	0.6% (1/158)	0
	17 Bimoli oil	0	0	3.5% (5/143)
Q3	Of the brands listed ask the stall owner which is the brand most frequently purchased (check the listing)			
	Most frequently purchased commercial package salt brand:	N = 108	N = 160	N = 145
	1 Star salt	88.0% (95/108)	56.9% (91/160)	69.7% (101/145)
	2 Five star salt	0.9% (1/108)	3.7% (6/160)	0
	3 True cook salt	18.2% (11/108)	20.0% (32/160)	17.9% (26/145)
	4 Pacific salt	0	0	0
	5 Jumbo salt	0	10.0% (16/160)	0
	6 Supa salt	0.9% (1/108)	1.9%) (3/160)	0
	7 Ezy salt	0	0	9.0% (13/145)
	8 Saxa salt	0	0.6%) (1/160)	3.4% (5/145)
	9 Repackaged salt	0	6.9% (11/160)	0
	10 Kakaruk salt	0	0	0

	Most frequently purchased brand of bouillon cubes:	N = 104	N = 110	N = 141
	1 Maggie cubes	22.1% (23/104)	18.2% (20/110)	0
	2 Maggie Kakaruk Stock cubes	77.9% (81/104)	72.7% (80/110)	100% (141/141)
	3 Chicken flavour cubes	0	6.4% (7/110)	0
	4 Super flavor Maggie cubes	0	0.9% (1/110)	0
	5 Beef cubes	0	0	0
	6 Stock cubes	0	1.8% (2/110)	0

	Most frequently purchased brand of Seasoning salt / powder:	N = 15	N = 27	N = 64

	1 Teisti Rais	26.7% (4/15)	18.5% (5/27)	3.1% (2/64)
	2 Topmi (VE-Tsin)	33.3% (5/15)	0	0
	3 Coconut milk powder	26.7% (4/15)	0	0
	4 Curry powder	13.3% (2/15)	29.6% (8/27)	81.3% (52/64)
	5 Vetgur / Vetzin	0	7.4% (2/27)	0
	6 Zesi-1	0	11.1% (3/27)	15.6% (10/64)
	7 Maggie	0	33.3% (9/27)	0

	Most frequently purchased brand of Seasoning sauce:	N = 4	N = 6	N = 23
	1 Soy sauce	75.0% (3/4)	33.3% (2/6)	60.9% (14/23)
	2 Mushroom soy sauce	25.0% (1/4)	66.7% (4/6)	34.8% (8/23)
	3 Chilli sauce	0	0	0
	4 Tomato sauce	0	0	4.3% (1/23)
	5 Maggie sauce	0	0	0

	Most frequently purchased type of Traditional / Local salt:	N = 0	N = 0	N = 1
	1 Ash	0	0	0
	2 Rock salt	0	0	100% (1/1)
	3 Sea water	0	0	0

	Most frequently purchased brand of Wheat flour:	N = 71	N = 81	N = 98
	1 Plain flour	73.2% (52/71)	45.7% (37/81)	25.5%% (25/98)
	2 Flame flour	19.7% (14/71)	14.8% (12/81)	50.0% (49/98)
	3 3-Rose flour	5.6% (4/71)	24.7% (20/81)	13.3% (13/98)
	4 Whole meal flour	1.4% (1/71)	4.9% (4/81)	10.2% (10/98)
	5 Wheat scone flour balls	0	2.5% (2/81)	0
	6 Scone flour	0	2.5% (2/81)	0
	7 Self-Raising plain flour	0	0	1.0% (1/98)
	8 Bakers flour	0	2.5% (2/81)	0
	9 Brown wheat bakers flour	0	1.2% (1/81)	0
	10 Skai flour	0	0	0
	11 Skow flour	0	1.2% (1/81)	0

	Most frequently purchased type of wheat flour products:	N = 107	N = 142	N = 145

	1 dried noodles/macaroni/pasta	0.9% (1/107)	0	0
	2. Instant noodles/2-minute noodles	99.1% (106/107)	100% (142/142)	70.3% (102/145)
	3. Bread/buns/rolls/Crackers/Biscuits	0	0	29.7% (43/145)
	4. Cake/scones/donuts	0	0	0

	Most frequently purchased brand of Rice:	N = 109	N = 138	N = 143
	1 Roots rice	59.6% (65/109)	39.1% (54/138)	10.5% (15/143)
	2 Skel rice	23.9% (26/109)	46.4% (64/138)	74.8% (107/143)
	3 Trukai rice	0	6.5% (9/138)	4.2% (6/143)
	4 Long grain rice	16.5% (18/109)	7.3% (10/138)	0
	5 Star rice	0	0.7% (1/138)	7.7% (11/143)
	6 Wantok rice	0	0	0
	7 Jasmine rice	0	0	0
	8 Ezy cook rice	0	0	0
	9 Super A1 rice	0	0	0.7% (1/143)
	10 Frangipani rice	0	0	1.4% (2/143)
	11 Sun long rice	0	0	0
	12 Ori rice	0	0	0.7% (1/143)
	13 Kumul rice	0	0	0

	Most frequently purchased brand of cooking oils:	N = 106	N = 158	N = 143
	1 Mamas cooking oil	6.6% (7/106)	27.2% (43/158)	11.2% (16/143)
	2 Jumbo oil	0	0.6% (1/158)	0
	3 Vegetable oil	0	10.8% (17/158)	0
	4 Sun shine oil	0	3.2% (5/158)	0
	5 Sunflower oil	0	0.6% (1/158)	0
	6 Flame oil	1.9% (2/106)	22.8% (36/158)	45.4% (65/143)
	7 Patna oil	0	3.8% (6/158)	0
	8 Sun Rise oil	0	0.6% (1/158)	0
	9 Golden Sun oil	40.6% (43/106)	21.5% (34/158)	30.8% (44/143)
	10 Highlands Meadow	0.9% (1/106)	4.4% (7/158)	0
	11 Voila oil	29.2% (31/106)	1.9% (3/158)	7.7% (11/143)
	12 Fresh sun oil	12.3% (13/106)	1.3% (2/158)	2.1% (3/143)

	13 Best oil	1.9% (2/106)	0.6% (1/158)	2.1% (3/143)
	14 Chefs oil	0.9% (1/106)	0	0
	15 Mothers choice	5.7% (6/106)	0	0
	16 King oil	0	0.6% (1/158)	0
	17 Bimoli oil	0	0	0.7% (1/143)

RESULTS AND INTERPRETATION:

Results for Quantitative Assay of Iodine Content in Various Brands of Salt Collected from Markets in Kerowagi & Sina-Sina, Jimi and Okapa during the Rapid Market Survey in 2022:

This section of the technical report presents the results of the quantitative assay of iodine content in the various brands of salt, salty condiments and flavourings purchased in the various markets in Jimi district in Jiwaka province, Kerowagi and Sina-Sina districts in Simbu province, and Okapa district in EHP.

The information on the enclosed sheets of papers in each of the large zip-locked polythene bags containing a number of smaller zip-locked bags with salt samples, were checked and recorded. The salt samples with (brand names) were collected from markets in Kerowagi & Sina-Sina districts (total of 90 samples), Jimi district (total of 35 samples) and Okapa district (total of 19 samples). In addition, the brands of salt and some flavourings sold in shops and supermarkets in the National Capital District (NCD) were purchased and analyzed.

The quantitative assay of iodine content in each of the salt samples was carried out, using the WYD Iodine Checker, which is specifically used to measure the iodine content in iodized salt. The iodine was expressed in *parts per million (ppm)* which is equivalent to one *mg of iodine per kilogram of salt (mg/kg)* {*mg/kg = ppm*}. The WYD Iodine checker measures the concentration of iodine in salt iodized with either *Potassium Iodate or Potassium Iodide* [12, 13].

The amount of salt used per assay was 1.0g. Proportional amounts of reagents were used for the assay of 0.5 g of salt [12, 13]. Each of the salt samples were analyzed in duplicate.

Calibration of the WYD Iodine checker:

The iodine working standard solution supplied by the manufacturer was used routinely to calibrate the WYD iodine checker used for the analysis. After calibration of the WYD checker, the special Grey Glass supplied with the WYD checker was used

routinely as the internal quality control QC [12, 13].

Internal Quality Control (QC) monitoring:
The internal (bench) QC used for the analysis was the "Westgard" QC system and "Westgard" QC rules. QC Pool Tracking Levy-Jennings Chart, prepared using the Grey Glass, was used for daily routine monitoring of the performance characteristics of the WYD checker. The intra-assay Percent Coefficient of Variation (CV) was 0.9%.

Criteria for Interpretation of the results on salt iodization:
The criteria used for interpretation of the salt iodine results were based on the PNG Salt Legislation [14, 15]. According to the legislation all salt must be iodised with Potassium Iodate; the amount of Iodine in table salt should be 40.0 to 70.0mg/kg (ppm); the amount of Iodine in other salt should be 30.0 to 50.0mg/kg. These levels of Iodine should be present at production or at the point of import.

WHO recommendations for Iodine levels of food grade salt aim to provide 150µg Iodine per day, assume 92% bioavailability, 30% losses from production to household level before consumption [14, 15].

If 30% of Iodine is lost from salt iodised as per PNG Salt Legislation, Iodine content of table salt at the retail or household (HH) level should be between 28.0mg/kg (40mg/kg minus 30%) and 49.0mg/kg (70mg/kg minus 30%). This implies that in PNG the Iodine content in salt in retail outlets or at the time of consumption should be between 28.0mg/kg and 49.0mg/kg.
Some recent publications on salt iodisation in PNG have rounded up the cut-off points by using "30.0 to 50.0mg/kg". In this report, for the purpose of comparison, the rounded up cut-off points (30.0 to 50.0mg/kg) have been used for presentation of the results. Salt with Iodine levels of less than 5.0 mg/kg is considered non-iodised salt [14, 15].

The format (Table 4) recommended in the recent UNICEF guidelines [14, 15] for the presentation of results on the monitoring of salt iodization programs is used in this report.

RESULTS:
It is important to note that the salt samples were not from the households in the districts, they were collected from the salt sold in the markets.
{*NB: Please note that the mean values presented in the results in Tables 4 to 10 have been sorted from lowest to highest*}
In the present report the adjusted cut-off points (Table 4) are used to define "Inadequate", "Adequate" and "Excess"

Iodine in the salt samples. This is important for the purpose of comparing the salt iodine results with other recently published data on salt iodization in PNG [6 - 10].

Salt samples from Kerowagi and Sina-Sina districts Simbu province:

A total of 90 salt samples were received. Seven different salt brands were recorded (Jumbo, 5-Star salt, Super salt, Star salt, Tru-cook salt, Saxa salt, Gold & Black salt). The corresponding iodine content (Mean ± standard deviation) in the salt from each of the brands are presented in Tables 5. According to the criteria for interpretation of results on salt iodization (Table 4), the iodine content in 18.9% (17/90) of the salt samples was inadequate, 45.6% (41/90) was adequate and 35.5% (32/90) contain excess iodine.

Salt samples from Jimi district Jiwaka province:

A total of 35 salt samples were from Jimi district. Three different salt brands were recorded (Super salt, Star salt, Tru-cook salt). The corresponding iodine content (Mean ± standard deviation) in the salt from each of the brands of salt are presented in Table 6. The iodine content in 2.9% (1/35) of the salt samples was inadequate, 68.6% (24/35) was adequate and 28.5% (10/35) contain excess iodine.

Salt samples from Okapa district EHP:

Of the 19 salt samples from Okapa, one was traditional salt and 18 were commercial salt samples. Two different salt brands were recorded (Star salt, Tru-cook salt). The corresponding iodine content (Mean ± standard deviation) in the salt from each of the brands of salt are presented in Table 7. According to the criteria for interpretation of results on salt iodization, for commercial salt, no iodine was in 5.6% (1/18) of the commercial salt samples, Iodine content in 94.4% (17/18) of the salt samples was adequate.

Salt samples from National Capital District (NCD):

For the purpose of comparison, the iodine content in the different brands of salt sold in trade stores and supermarkets in NCD were analyzed. There were 20 different brands of salt in NCD. A total of 53 salt samples were purchased and analyzed. The mean iodine content in the salt samples from the different brands is presented in Table 8. Iodine content was below 5.0 mg/kg in 9.4% (5/53) of the salt samples, the iodine content in 20.8% (11/53) of the salt brands was inadequate, 32.1% (17/53) was adequate and 37.7% (20/53) of the salt contains excess iodine.

Table 4: Criteria for interpretation of iodine content in salt [14, 15]

				Percentage (n) of salt with Iodine content		
				Rounded-up cut-off points in PNG Salt Legislation		
Name of district	**Name of Provinces**	**Number of salt**	**<5.0 mg/kg**	**Inadequate: 5 – 29.9 mg/kg**	**Adequate: 30 – 50 mg/kg**	**Excess: > 50 mg/kg**
Kerowagi	**Simbu**	90	0	18.9% (17/90)	45.6% (41/90)	35.5 (32/90)
Jimi	**Jiwaka**	35	0	2.9% (1/35)	68.6% (24/35)	28.5% (10/35)
Okapa	**EHP**	18	5.6% (1/18)	0	94.4% (17/18)	0
NCD		53	9.4% (5/53)	20.8% (11/53)	32.1% (17/53)	37.7% (20/53)

While it is recommended that the definition of "no iodine" be maintained in different settings, the definitions of Inadequate, Adequate, and Excess Iodine should be modified based on national standards, which is the PNG Salt Legislation [4, 5]

One of the generally acceptable concepts is that it is better to consume salt containing more iodine that less iodine [1].

A recalculation of the results for Kerowagi and Sina-Sina districts, indicates that the iodine content in 81.1% (73/90) of the salt samples was over 30.0 mg/kg. For Jimi, of the 35 salt samples collected the iodine content in 97.1% (34/35) was over 30.0 mg/kg. For Okapa, of the 18 salt samples collected 94.4% (17/18) contained iodine content over 30.0 mg/kg. For NCD, the iodine content in 69.8% (37/53) of the salt samples was over 30.0 mg/kg.

Flavourings and Seasonings available in shops in National Capital District (NCD):

The different brands of flavourings and seasonings available in trade stores and supermarkets in NCD were also purchased and analyzed. A total of 14 different brands were purchased. The corresponding iodine content (Mean ± standard deviation) in each brand is presented in Table 9.

There are no acceptable criteria for interpretation of the iodine content in flavourings and seasonings that are added to foodstuffs. However, the results in Table 9, show that the mean iodine content (7.8mg/kg) in the New Maggi Kakaruk stock cube with "Iodized" on the label was about twice the amount (4.5mg/kg) in the regular Maggi Kakaruk cube. The iodine content in the regular Kakaruk salt (6.7mg/kg) was almost twice less than the iodine content in the New Kakaruk salt (11.0mg/kg) with "iodized" on the label.

The flavourings with the highest iodine content was "*Tru-cook good pela taste*" with iodine content of 33.5 mg/kg. This locally produced flavoring is not popular among residents in the NCD.

Table 5: Iodine content in the brands of salt collected from markets in Kerowagi and Sina-Sina districts Simbu province

Salt brands	Number of samples	Mean Iodine content (mg/kg)	Standard Deviation	95% CI (mg/kg)	Range (mg/kg)
Jumbo	22	42.3	12.6	36.7 – 47.9	19.4 – 54.1
5 Star salt	16	39.0	6.8	35.4 – 42.6	30.4 – 57.6
Super salt	24	53.1	4.8	51.1 – 61.5	41.3 – 61.5
Star salt	18	35.3	10.7	30.0 – 40.6	17.1 – 55.5
Tru cook & two others	10	35.4	15.2	26.3 – 44.6	21.1 – 60.7

Table 6: Iodine content in the brands of salt collected from markets in Jimi district Jiwaka province

Salt brands	Number of samples	Mean Iodine content (mg/kg)	Standard Deviation	95% CI (mg/kg)	Range (mg/kg)
Super salt	9	58.8	1.99	57.3 – 60.3	55.5 – 61.0
Star salt	10	43.0	6.51	38.3 – 47.7	30.0 – 54.1
Tru cook & others	16	39.9	4.9	37.3 – 42.5	25.4 – 44.9

Table 7: Iodine content in the brands of salt purchased from markets in Okapa EHP

Salt brands	Number of samples	Mean Iodine content (mg/kg)	Standard Deviation	95% CI (mg/kg)	Range (mg/kg)
Tru Cook	9	37.8	3.93	34.8 – 40.8	31.8 – 43.4
Star salt	9	32.8	11.62	23.9 – 41.7	4.5 – 45.7
Traditional salt	1	2.7	0.8	--	--

Table 8: Iodine content of brand of salt purchased in various stores and supermarkets in NCD

SALT BRAND	Number of samples	Mean Iodine content (mg/kg)	Std Dev	Range (mg/kg)
Super salt	14	64.1	8.98	46.5 – 76.5
Star salt	3	36.2	1.51	35.1 – 37,9
5 Star salt	6	74.6	15.31	63.4 – 96.0
Jumbo salt	1	35.7	0.6	35.3 – 36.1
Tru cook salt	1	29.3	1.2	28.4 – 30.1
Saxa salt	5	15.9	0.57	0.5 – 39.5
Saxa Iodized Table Salt	9	42.9	19.52	27.7 – 92.0
Sazza Fine Salt Iodized Salt	2	14.0	0.55	12.0 – 16.3
Fine Salt	1	0.3	0.1	0.2 – 0.3
Mermaid Table Salt	1	6.8	1.8	5.5 – 8.0
Kakaruk Salt	1	7.8	0.3	7.6 – 8.0
Nature Salt Refined Iodized salt	1	10.0	1.1	9.2 – 10.7
Silver Iodized Free Flow salt	1	17.8	0.7	17.3 – 18.3

Cerebos Iodized Table salt	1	20.5	0.7	20.0 – 21.0
National No.1 Iodized Salt	1	25.0	0.5	24.6 – 25.3
Ezy -Cook Pure Iodized Table Salt	1	37.4	0.9	39.0 – 40.0
Horizon Pure Cooking Iodized Salt	1	43.7	1.3	42.7 – 44.6
Tata Salt	1	46.8	2.2	45.2 – 48.3
Black & Gold	1	49.0	1.4	48.0 – 50.0
Best Choice Table Salt	1	53.5	2.1	52.0 – 55.0

One of the brands of salt (Star salt) was available in the markets in the three provinces and in NCD. The mean iodine content of Star salt in the markets in Kerowagi and Sina-Sina was 35.3 mg/kg, in the markets in Jimi it was 43.0 mg/kg, in Okapa it was 32.8 mg/kg and in NCD it was 36.2 mg/kg. There was no statistically significant difference in the mean iodine content in the Star salt from the markets in the four locations. This may suggest minimal loss of iodine in this brand of salt, which is very popular in the remote communities.

Table 9: Iodine content of some flavorings and seasonings from various shops and supermarkets in NCD

Brand Names	Mean iodine content (mg/kg)	Std Dev
Star fresh	1.1	0.1
Maggie Kakaruk stock cube	4.5	0.2
Packet of seasoning in 2-minutes noodles	6.1	0.7
Kakaruk Maggi Salt	6.7	1.4
Knorr Pork Broth cube	8.0	0.7
Knorr Chicken Broth cube	8.2	0.7
Teisti Rais Beef flavor	11.0	0.8
Maggi Kakaruk Stock Cube (Iodized)	7.8	0.5
Super Chicken Stock Cube	16.8	1.1
Maggi Teisti Raise	16.1	0.8
Maggi 2 Minute Noodle Flavor	10.3	0.8
Kakaruk Salt (Iodized)	11.0	0.7
Star Fresh	0.0	0.0
Tru Cook Good Pela Taste	33.5	1.8

CONCLUSION:

In each of the four districts that participated in this study, the findings show that commercial packaged salt was available in more that 85% of the households. Similarly, over 85% of respondents in the households were aware of the importance of iodized salt. Stock / bouillon cubes were not popular among the households. Over 75% of the respondents had wheat flour products available in the households within the last seven days. Within the last seven days, rice

was available in over 75% of households. In addition, cooking oil was also available in over 85% of the households within the last seven days.

Salt was available in over 95% of the market stalls in all the three districts assessed. In addition most of the salt available in the market stalls were adequately iodized according to the PNG salt legislation. Unlike in Jimi district, stock / bouillon cubes were available in about 95% of the market stalls in Kerowagi, Sina-Sina and Okapa district. Wheat flour products were available in over 85% of the market stalls in the districts in the three provinces. Different brands of Rice and Cooking Oil were available in about 85% and over 95% respectively of the market stalls in the districts in the three provinces that participated in this study.

The iodine content in over 80%, 97% and 94% of the salt samples purchased from market stalls in Kerowagi and Sina-Sina districts, Jimi district and in Okapa district respectively were adequately iodized according to PNG Standards. These results are better than those obtained for the salt samples purchased from trade stores in the National Capital District that yielded results of only 69.8% of the salt samples meeting the national standard iodization standard of 30.0 mg/kg. The National Capital District is the capital city of PNG.

The availability of about 30 percent of salt that is inadequately iodized in the NCD is a concern that requires action by relevant Government authorities and stakeholders. This is more critical considering that the national capital is highly populated hence the probable effect at population level could be pronounced. One explanation for this discrepancy may be because of the very high number of salt brands available in the NCD. Multi-strategic interventions are required to remedy the situation in the NCD, in particular effective monitoring of the iodine content in the various brands of salt imported and sold in the NCD. Effective implementation of the PNG salt legislation is required to improve the access to and availability of adequately iodized salt in the NCD. Commitments at relevant levels of government are essential for successful implementation of such strategies.

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SHORT COMMUNICATION:

EVALUATION OF A HYBRID DEMENTIA CARE SKILLS WORKSHOP IN MALDIVES AND BRUNEI

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ABSTRACT:
Dementia is a global public health priority. In Brunei, Geriatric Medicine provides dementia teaching sessions to undergraduate health science students at Universiti Brunei Darussalam (UBD). It was found that more knowledge of dementia management was needed. Thus, a dementia care skills workshop was provided. Content from a dementia care skills workshop for care workers was given in a hybrid format. UBD nursing students attended in person, while participants from Maldives joined online via zoom. Feedback on the workshop was obtained via an online form at the end of the session. There were 27 undergraduate medical students from UBD and 43 participants from Maldives, with representation from hospitals and health centres, government ministries and non-government organisations. Feedback was positive in terms of satisfaction with format and structure, content relevance and effectiveness of learning experience. Participants felt that future sessions on effective communication and how to manage caregiver burden would be useful. The dementia care skills workshop held in a hybrid format was well-received. Further sessions to improve the knowledge and understanding of dementia care skills are warranted.

Keywords: Brunei, Caregiver Burden, Dementia, Education, Maldives

INTRODUCTION:

Dementia is a global public health priority with significant health and social implications, not just for the person with dementia but also their families and societies. Dementia is the major cause of disability in older people and is a concern in Brunei, given the rapidly ageing population and high rate of non-communicable diseases in the country [1]. Thus, there is much national effort to raise public awareness about dementia, especially the warning signs or symptoms of the disease and risk reduction measures. During the pandemic, the use of social media and online presentations were utilised to promote dementia awareness [2].

In Brunei, the Geriatric Medicine specialty provides teaching sessions on dementia to

undergraduate health sciences students at Universiti Brunei Darussalam (UBD). A survey done in 2022 showed that the majority of these students had good awareness about dementia. However, more knowledge on management approaches for dementia was needed, including psychosocial interventions [3]. Thus, an additional session on dementia care skills was provided in 2023. This paper describes the feedback from participants regarding the dementia care skills workshop delivered through a hybrid approach, with suggestions for future teaching sessions on dementia care.

METHODS:

A Dementia Care Skills (DCS) Workshop Train the Trainers session was held in Brunei in 2017, with subsequent workshops provided by the trainers to various audiences [4]. The actual DCS workshop for care workers was 18 hours and consists of six modules. The modules are nature of dementia, impact of dementia / person-centered care, effective communication, behavioural and psychological symptoms of dementia (BPSD), purposeful and meaningful engagement, and application to care practices. Given the limited slots available to fit this additional session into the undergraduate students' timetable, the workshop content was compressed into 2.5 hours, focusing on highlighting the key clinical aspects of dementia, care management and support.

At that time, the local dementia association (Demensia Brunei) was also collaborating with the Alzheimer's Society of Maldives (ASM) on improving dementia awareness in their respective countries. It was decided to share this workshop session online, which the Maldives attendees would join via zoom. Thus, the actual workshop took place on 16th March 2023; 2:00 to 4:30pm, where undergraduate nursing students attended physically in a seminar room in UBD, while participants selected by ASM joined virtually through Zoom. An online link and QR code were provided to all participants to complete a feedback form at the end of the session.

RESULTS:

There were 27 undergraduate medical students from UBD and 43 participants from Maldives. From the Maldives, there were 23 (53.5%) from hospitals and health centres, 11 (25.6%) from government ministries (Ministry of Gender, Family and Social Services as well as Ministry of Health) and 9 (20.9%) from non-government non-profit organisations related to aged care, dementia and nursing. Table 1 summarises the responses from participants based on the feedback form given to attendees.

Table 1: Feedback responses from participants regarding the DCS workshop

	Score	UBD (n=27)	ASM (n=43)
Satisfaction with format and structure	1 (Poor)	0	0
	2	1 (3.7%)	0
	3	6 (22.2%)	1 (2.3%)
	4	11 (40.7%)	15 (34.9%)
	5 (Excellent)	9 (33.3%)	27 (62.8%)
Relevance of content for your needs	1 (Poor)	0	0
	2	0	0
	3	4 (14.8%)	2 (4.7%)
	4	9 (33.3%)	16 (37.2%)
	5 (Excellent)	14 (51.9%)	25 (58.1%)
Effectiveness of learning experience	1 (Poor)	0	0
	2	0	0
	3	8 (29.6%)	1 (2.3%)
	4	12 (44.4%)	20 (46.5%)
	5 (Excellent)	7 (25.9%)	22 (51.2%)

There were two main areas the participants felt needed more time or that future sessions would be beneficial: how to effectively communicate with people with dementia and how to cope as a caregiver in terms of mental health and stress management.

Some of the additional comments provided by the participants were as follows:

'Very informative session. Learnt a lot and good reflections from past scenarios.'

'It was very comprehensive, compact and the topics were well-covered.'

'It was very detailed and the way the teacher used his personal experience is wonderful.'

'An eye opener with knowledge through life experiences.'

'More interactive sessions. Would like to do some activities regarding the information provided.'

DISCUSSION:

The dementia care skills workshop was held in a hybrid format, with attendees from two organisations. Based on the feedback given, the session was viewed positively by the participants. There were several strengths to this teaching approach. The online format enabled attendees from other localities to join, removing the barrier of travel time and distance. It was possible to share knowledge and expertise across two different settings and provide the educational session to a larger number of people. Such collaborative working is essential for coming up with creative ideas on

how to solve issues with significant community impact such as dementia.

It was quite ambitious to compress a lot of content into the one session. While this workshop served its purpose in providing an overview and introduction to dementia care skills, further detailed workshops for each of the modules may be required. Similar shorter workshops should still be planned in the future as it provides a solid foundation for understanding dementia, after which participants may register for more advanced sessions covering each module in detail.

Based on the feedback forms, the participants felt that workshops focusing on communication and caregiver coping should be prioritized. A flipped classroom approach may be applied to future sessions to consolidate learning and maximise the limited contact time. This means that participants should be given resource materials to look through beforehand, with the session utilised for interactive discussions to clarify uncertainties and apply the knowledge in group activities [5]. As the participants are relevant stakeholders in raising dementia awareness, it may also be worthwhile planning how they can apply this knowledge after the workshop, including volunteering with the local dementia association and contributing to their community [6].

CONCLUSION:

The dementia care skills workshop held in a hybrid format was beneficial and well-received by the participants. Further educational sessions to improve the knowledge and understanding of dementia care skills are warranted.

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OUTCOME OF EMERGENCY CAESAREAN SECTIONS IN A SAUDI GENERAL HOSPITAL: A COMPARISON OF LOWER VERSUS HIGHER NUMBER OF REPEAT SURGERY

Running title:
Emergency Caesarean Section in a Saudi General Hospital: A Comparison of Lower versus Higher number of Repeat Surgery.

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ABSTRACT:
Caesarean section (CS) is frequently employed in the delivery of the New born as alternative route to otherwise problematic vaginal birth. National and regional CS rates as well as the number of surgeries the individual woman may be exposed to are on the increase. This retrospective comparative study carried out in a Saudi Arabian General Hospital, compared the foetal and maternal outcome in lower number ≤3 with higher number ≥4 repeat caesarean section.
Results: Out of Maternal population of 364 women, those who are age group 31 to 40 years accounted for 234 (64.3%). 188 (51.7 %) were unbook. 56 (15.3 %) of surgeries were in preterm. In 145 (43.7 %) previous CS was the sole indication and mother refusing vaginal birth is the reason in 96 (26.9 %). Foetal distress and antepartum haemorrhage are the other common indications. Only 32 (8.8%), 66 (18.1%) and 10 (2.7%) of mothers respectively stayed 3 days post operation or more, had blood transfusion and had wound sepsis. For the New born, out of a total of 364, Twelve (3.3 %) and 134 (36.8%) of the babies had low APGAR (appearance, pulse, grimace, activity, respiration) at 1st minute and Neonatal intensive care unit (NICU) admission respectively. Blood transfusion is statistically correlated with number of CS ($p < 0.001$). Low APGAR at 1st minute, Low birth weight and NICU admission were significantly correlated with the number of CS, each has ($p < 0.001$).
Conclusion: The results obtained in this study, indicate that emergency Caesarean Section was safe for both mother and baby. Higher repeat Caesarean Section was associated with blood transfusion in the mother and Neonatal intensive care admission in the New born.

KEY WORDS: Caesarean Section; Emergency; Out-Come

INTRODUCTION

Emergency caesarean section is a major obstetric operation that is carried out on a pregnant woman to deliver the fetus per abdomen whenever there is a threat to either the life of the fetus or mother and sometimes both [1]. This implies that indications for the surgery must be defined and cannot be trivialised.

Generally, caesarean section is one of the commonest surgeries globally [2], the rate of which had progressively increased from 7% in the early 1990s to around 20% in the year 2014 [3, 4]. The increase in the developed world is even higher. The World Health Organization WHO set Caesarean section (CS) rate of 10-15 % for the global community [5].

The secondary healthcare level is a significant part of the health management system in the Kingdom of Saudi Arabia (KSA) which happens to be a welfare state. The lower level refers patients needing expert care to this level, domiciled at the general hospitals, the majority of which are spread across the districts in the regional directorates of KSA [6].

The operation of Caesarean section would yield favourable outcomes for the foetus and the mother when it is employed rightly including timeliness [7]. Hence, hospitals rendering maternity services should be prepared for Emergency Obstetric Cares (EOC) which include emergency CS and must be able to meet up with such emergencies within the allowed time frame [8, 9].

The American College of Obstetricians and Gynaecologists (ACOG) along with the Royal College of Obstetricians and Gynaecologist (RCOG) have both recommended a decision – delivery interval (DDI) of 30 minutes, [10, 11], but unfortunately many hospitals in several countries, especially in the developing world are yet to attain this standard [12]. These failures may contribute to unfavourable outcomes on the long run. In some instances, these unfavourable outcomes ended up in medico – legal suits against the facilities and the practitioners [13].

A number of studies have been carried out, mostly in developing countries to access how the decision – delivery intervals affected the outcomes of emergency Caesarean section especially in relations to the newborn babies. In a study from Ethiopia, on “effects of decision to delivery interval on perinatal outcomes during emergency cesarean deliveries” it was discovered that the average decision to delivery time was 43.73 ±10.55 minutes [14]. The elongated decision to delivery interval was found to have a significant association with adverse perinatal outcomes [14]. In another study titled “decision to delivery interval and associated factors for emergency cesarean section” which was a cross-sectional study, a decision-to-delivery interval below 30 min was found in only 20.3% of emergency cesarean section [15]. The

results showed that referral status, time of the day the emergency cesarean section occurred, status of surgeons, type of anesthesia, and transfer time were factors significantly associated with the decision to delivery interval [15]. Furthermore, a similar study titled "the decision delivery interval in emergency caesarean section and its associated maternal and fetal outcomes at a referral hospital in northern Tanzania: a cross-sectional study", the median decision to delivery interval was one hour [16]. The authors reported that just 12% of the cases was the interval within 30 minutes. In the study there was no significant relationship between decision delivery interval (DDI) and the transfer of the neonates, Apgar scores at first and fifth minutes, maternal blood loss, and the duration of hospital admission [16]. The impact of DDI on the outcome of ECS has been well researched. In the KSA the Ministry of Health Obstetrics Protocal prescribed DDI of half an hour and most health institutions in the country observed the protocol. However, the effects of many repeat surgeries on the feto-maternal outcome has not been evaluated.

This study was carried out in a facility that has been able to eliminate wide variation in decision delivery interval (DDI) in cases of emergency caesarean section. This was a retrospective study. The maternal and perinatal outcomes of emergency Caesarean sections were reviewed, comparing the lower ≤ 3 versus higher number of repeat ≥4 surgeries.

METHODOLOGY:

Study site: The study was carried out at a Maternity and Children Hospital Hafer Al-Batin, Eastern Province Kingdom of Saudi Arabia KSA. Location: Latitude 28.446959 and Longitude: 45.948944 coordinates.

The hospital is a secondary level referral health facility, with annual delivery of about 4000.

Through a retrospective comparative study, the records of all women who had emergency CS carried out from August 2017 to November 2019 in a general hospital were accessed. Using a Pro forma data instrument information were extracted on socio-demography, indications for surgery, number of CS per woman, Gestational Age, and feto-maternal outcome.

Data Processing: The data were entered into the computer in a double entry fashion and cleaned. Analysis was carried out with SPSS version 20.

Outcome measures.

Maternal: Number of days on admission, blood transfusion requirement and post op infection.

New born: APGAR score at 1st minute, gestational age, admission into Neonatal intensive care units (NICU) and birth weight.

Ethics: Ethical approval for the study was obtained from ethic research committee of College of Health Sciences, Osun State University.

RESULTS.

Table 1 depicts socio-demography typical of women in their reproductive age group with age group 31 to 40 years constituted 64.3 %. Non-Saudis made up only 15.9 %. Un-booked status

constituted 48.4 %, and high parity, ≥P4 accounted for 75.3 % of cases.

Preterm birth accounted for 29.1 %. Previous Caesarean section (CS) profile showed that 69.8 % was repeat surgery with 20.0% classified as higher order repeat C/S i.e. more than 3 previous scars. Only 3 in 10 are primary C/S.

Table 2: The detailed individual indications for the emergency c/s were depicted. The four leading indications were previous CS, Foetal distress, Ante Partum Heamorrhage and Severe Preeclampsia PET, together these four accounted for 82.2 % of cases.

Maternal outcomes are presented in Table 3. Of the 364 cases, three hundred and thirty two (91.2 %) were discharged within 3 days of surgery, 66 (18.7 %) had blood transfusion while 10 (2.7 %) had postoperative wound infections; most of them minor being limited to the skin and subcutaneous levels.

The New born outcome (Table 4) showed 250 (68.7 %) had good APGAR score of 8/10 at 1st minute, and 134 (36.5 %) were admitted in to NICU. Only 30 (8.2 %) had low birth weight.

The correlation of maternal out come and the number of repeat CS are presented in table 5. Only blood transfusion was significantly associated the number of previous c/s, $p < 0.001$

The outcome in the New born correlated with the number of repeat surgeries, showed that APGAR score at 1st minute, $p < 0.001$ lower foetal weight $p < 0.001$ and admission in to Neonatal intensive care unit (NICU) $p < 0.001$, all were significantly associated with the number of surgery (Table 6).

Table 1: socio demography.	**Frequency: N = 342 (%)**
Age in year	
Mean ± SD	33±5.9
≤19 years	8 (2.2)
20-29 years	98 (26.9)
30-39 years	234 (64.3)
≥40 years	2 (6.6)
Nationalist of respondents (N = 364)	
Saudi	306 (84.1)
Non-Saudi	58 (15.9)
Booking status (N = 364)	
Booked	188 (51.6)
Unbooking	176 (48.4)
Parity (N = 364)	
P 0	30 (8.24)
P 1-3	60 (16.48)
P 4-6	162 (44.51)
≥ P 7	112 (30.77)
Gestational age (364)	

26-30 weeks	14 (3.85)
31-36 weeks	92 (25.27)
37-40 weeks	246 (67.58)
>40 weeks	12 (3.30)
Number of Previous C/S (364)	
0 (Primary)	110 (30.21)
1-3	180(49.45)
≥4	74(20.24)

Table 2: INDICATIONS FOR THE EMERGENCY C/S (N 364)	
Diagnosis	**Frequency (%)**
Previous ≥2 c/s	159 (43.7)
Foetal distress.	20 (5.5)
Antepartum Heamorrhage.	25 (6.9)
Breech presentation.	13 (3.6)
Failure to progress in labour.	14 (3.8)
Severe Hypertension (PET).	15 (4.1)
Refuse VBAC	95 (26.1)
Transverse lie in labour	4 (1.1)
Bad obstetric history	11 (3.0)
Multiple indications	8 (2.2)
Total	364

Table 3. Maternal outcome.	
Variables	**Frequency (%)**
Duration of Hospital stay (N = 364)	
Mean ± SD	3±0.7
1-3 days	332 (91.2)
>3 days	32(8.8)
Blood transfusion	
Negative	298 (81.9)
Positive	66 (18.1)
Post-op infection	
Negative	354 (97.3)
Positive	10 (2.7)

Table 4: Foetal outcome.	
Variables	**Frequency (%)**
Apgar score at 1st minutes of life	
Mean ± SD	7±1.7
8-10	250 (68.7)
5-7	102 (28.0)
<5	12 (3.3)

Birth weight	
Mean ± SD	3.1±0.4
Low birth weight (<2.5 kg)	30 (8.2)
Normal birth weight (2.5–4.49 kg)	330 (90.7)
Big baby (≥4.5 kg)	4 (1.1)
Nicu admission	
Negative	230 (63.2)
Positive	134 (36.8)

Table 5: Association between number of Previous C/S and Maternal outcome

Variables	Numbers of previous C/S			Chi-Square	df	p-value
	None	1-3 times	≥4 times			
Discharge day				3.182	2	0.204
1-3 days	96(26.4)	168(46.2)	68(18.7)			
>3 days	14(3.8)	12(3.3)	6(1.6)			
Blood transfusion				22.690	2	<0.001*
Negative	88(24.2)	162(44.5)	48(13.2)			
Positive	22(6.0)	18(4.9)	26(7.1)			
Clinical infection				4.086	2	0.130
Negative	106(29.1)	178(48.9)	70(19.2)			
Positive	4(1.1)	2(0.5)	4(1.1)			

Table 6: Association between number of Previous C/S and foetal outcome

Variables	Numbers of previous C/S			Chi-Square	df	p-value
	None	1-3 times	≥4 times			
Apgar score at 1st minutes of life						
8-10				61.784	4	<0.001*
5-7	48(13.2)	154(42.3)	48(13.2)			
<5	52(14.3)	26(7.1)	24(7.1)			
	10(2.7)	0(0.0)	2(0.5)			
Birth weight						
Low birth weight (<2.5 kg)	18(4.9)	10(2.7)	2(0.5)	24.394	4	<0.001*
Average birth weight (2.5–4.49 kg)	88(24.2)	170(48.7)	72(19.8)			
Big baby (≥4.5 kg)	4(1.1)	0(0.0)	0(0.0)			
Nico admission				43.227	2	<0.001*
Negative	42(11.5)	130(35.7)	58(15.9)			
Positive	68(18.7)	50(13.7)	16(4.4)			

DISCUSSION:

The current practice in KSA as recommended by the Ministry of Health MOH is to offer Bilateral tubal ligation BTL to a woman who is going for the 5th C/S. However, if such woman declined, BTL should not be done. This liberal policy on the number of C/S a woman can undergo has led to cases of women coming for 8th even 9th repeat C/S, sometimes in emergency situation. Delivery after 2 previous CS were by elective repeat CS. However, many of these multiple previous CS do present at the facility for the first time in labour, with imminent uterine rupture or bleeding per vaginal Ante partum Haemorrhage

(APH) from undiagnosed placental previa and other co-morbidities.

Socio-demography revealed 3 in 5 of the women are in the 4th decade of life. This may be a reflection that women continue procreation till the end of reproductive age in this environment, the fact that 3 in 4 of them are grand multipara corroborated this observation. About one in six are non-Saudis. Almost half 176 (48.4%) of the women presented as unbook emergency at Maternal and Child Hospital MCH, this is quite worrisome. Some of the women might have been receiving ANC at lower level facilities and sometimes in private hospitals only to show up at the public secondary health facility for delivery and in emergency conditions. These senario created unbook emergency at the receiving health facility and constitute risk factor for adverse obstetrics outcome (17). In order to reverse the situation women with multiple previous C/S and their Husbands should be counselled to book for ANC at facilities with capacity for surgical deliveries. The Clinicians running private health facilities should refer such patients early enough if the women will not be delivered in their facility.

The patterns of indications were shown in tables 1 and 2. The leading reasons for surgeries are Previous CS in labour, Antepartum haemorrhage, Hypertensive disorders in pregnancy and Foetal distress. This is typical of emergency CS in Obstetrics (18). However, the large number of previous CS in this review is worth reporting as it constituted sole indication in more than 4 in 10, and contributed in another 3 in 10 caesarean section. This may be a reflection of high CS rate in the Kingdom and the reason for this may not be different from what obtains else with higher CS rates. This is connected to high litigation rate, attendant defensive obstetrics practice and high background primary CS. .

Among the factors impacting on the feto maternal outcome of emergency CS are indication, pre-operative patient preparation, post-operative care and competency of the surgeon, the number of previous surgeries the woman had, could be an important determinant of feto-maternal outcome of the procedure [10]. The pattern of indications are outlined in table 2. There is relative uniformity in the other factors listed above at the facility of study and elsewhere except the number of previous CS exposure, which is higher in KSA, therefore, the possible impact of the number of previous CS on the outcome of the emergency surgery for both the mother and the baby was analysed in this study. The finding may enable us recommend a further research into the liberal use of CS with a view to putting a upper limit to the number.

The maternal outcome endpoints of interest are duration of hospital stay, blood transfusion and post-operative infection. The baby outcome endpoints were APGAR score at first minutes of life, admission to neonatal intensive care unit (NICU). In this study the number of surgeries were grouped in to lower, i.e. 1 to 3, and higher i.e. 4 and more CS.

Table 3, showed that 9 out of 10 women were discharged within 72 hours of the procedures and in table 5 only blood transfusion was significantly associated with the number of CS, $p < 0.001$ This showed that emergency CS is safe for mothers even with multiple repeat procedures.

The baby outcome from this study showed that 7 out of 10 had good APGAR score at 1st minutes which is good record. Correlating to number of CS the higher the number of CS a woman is exposed to the higher the likelihood of baby being admitted to NICU and low 1st minute APGAR, the $p < 0.001$ in both cases, not-withstanding, emergency CS is safe for the New born in this study.

This study is limited by the small number of cases and other confounding variables that may not have been accounted for such as duration of surgery, indication, gestational age and method of anaesthesia.

Conclusion: Multiple repeat caesarean section is safe for both mother and baby, with relative risk of neonatal intensive care admission for the New born.

Implication: Emergency Obstetrics care services (EmOC) complimented with New born critical care services (NICU) should be strengthened at all maternity care facilities.

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