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CONTENTS

Research Article

INVESTIGATION OF ENDOPLASMIC RETICULUM SIGNAL PROTEINS IN CUMULUS CELLS WITH NORMAL, HYPERRESPONSIVE, HYPORESPONSIVE AND POLYCYSTIC OVARY SYNDROMES

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Abstract: *The study aimed to investigate Endoplasmic reticulum dependent Unfolded Protein Response signal molecules in cumulus cells of the patients presenting normal, hyperresponsive, hyporesponsive, and polycystic ovary syndrome. Cumulus cells were provided during oocyte retrieval. Of the patients applying for in vitro fertilization treatment, three subtypes of patient groups were distinguished according to their response to follicle stimulations. The first group composed of normoresponsive (n=8) was considered as retrieval of 10-20 oocytes. The second group composed of hyporesponsive (n=8) as retrieval of 6 or less oocytes. Retrieval of 30 or more oocytes as a response to the same stimulus was considered hyperresponsive (n=8), which included polycyctic ovary syndromes. (n=8). We analyzed the protein expressions of glucose-regulated protein 78 and mRNAs levels of the X-box binding protein-1 and splicing X-box binding protein-1 by Western blot and Reverse Transcriptase PCR, respectively, in the cumulus cells from different patient groups. All data were loaded to the software Sigma Stat 3. Differences between groups were evaluated with one-way ANOVA post hoc TURKEY test. We found that the protein expression of glucose-regulated protein 78 was two-fold higher in cumulus cells from the hyporesponsive group than the other groups. Reverse Transcriptase PCR results showed us, the mRNA expression of splicing X-box / X-box binding protein-1 level was 1,5 fold higher in the cumulus cells from the hyporesponsive group compared to others. Furthermore, we have shown that when the cumulus cells were exposed to signal pathway molecules that related to endoplasmic reticulum stress; a decrease in cell proliferation and/or increase in apoptosis can occur. We have shown that an increased endoplasmic reticulum stress in the cumulus cells of the ovarian follicle from the patients forming the hyporesponsive group. We can conclude that increased endoplasmic reticulum stress or impaired protein folding mechanism in cumulus cells may affect oocyte maturation, therefore, the agents may be used to decrease pathological endoplasmic reticulum stress in the hyporesponsive patients.*
Keywords: *Endoplasmic Reticulum Stress, Unfolded Protein Response, Cumulus Cells*

1. Introduction

Endoplasmic reticulum (ER) is an essential organelle plays important roles in cells vital functions. ER regulates intracellular calcium concentration, protein synthesis, and homeostasis and it also enables lipid biosynthesis [1,2]. Several factors may affect the aggregation of unfolding proteins in the ER

lumen. The accumulation of unfolding or misfolding proteins in the ER lumen induces an ER-dependent cellular reaction cascade which is called Unfolding Protein Response (UPR) [3,4].

There are three ER stress transducers, inositol requiring enzyme (IRE) 1, protein kinase RNA-like ER kinase (PERK), and activating transcription factor (ATF) 6 that involve in UPR signaling pathway. These three ER stress sensors sense the levels of unfolded proteins in the ER lumen [5,6]. GRP78 is also called binding protein (BIP) is a major regulatory protein of UPR. Under normal conditions, BIP binds to the luminal domains of IRE1, PERK, and ATF6 receptors and keeps them in the inactive state. In response to the ER stress induced by accumulation of unfolded proteins in the ER lumen, BIP released from the luminal domains of PERK, IRE1, and ATF6 receptors to activate them [7,8].

PERK is an ER-related transmembrane protein kinase. Accumulation of unfolded proteins in the ER lumen, PERK dimerization and trans-autophosphorylation lead to activation of its eIF2α kinase activation [9]. PERK also induces transcription of about 1/3 of the UPR-dependent genes [10].

Under non-stress conditions, Ire1 protein kinase is maintained in an inactive state interaction with BIP. Upon aggregation of unfolded/misfolded proteins in the ER lumen, IRE1 is released from BiP and undergoes homodimerization and trans-autophosphorylation to activate its RNase activity [11] XBP1 mRNA is a substrate for the endoribonuclease activity of IRE1 [12]. Upon activation of the UPR, the IRE1 RNase activity initiates removal of a 26 nucleotide intron from XBP1 mRNA. Splicing X-box binding protein-1 (sXBP-1) is a transcriptional activator and activates transcription of the genes that involve in UPR. sXBP-1 increases protein folding capacity in the ER lumen [13,14].

Under unstressed conditions, ATF6 is localized at the ER membrane and bound to BIP. In response to ER stress, BIP dissociation permits ATF6 to translocate to the Golgi where ATF6 is cleaved by two proteases the serine protease site-1 protease (S1P) and S2P. S1P cleaves ATF6 in the luminal domain and N terminal portion is cleaved by the S2P [15]. Two ATF6-like molecules that are cleaved by S1P and S2P to activate UPR transcription [16].

Tunicamycin (TM) is highly toxic for mammalians [17]. TM induces ER stress in plants and eukaryotic cells. Tauroursodeoxycholic acid (TUDCA), is a hydrophilic bile acid found in small amounts in humans. TUDCA has been found to reduce the apoptotic effect [18,19].

The study aimed to investigate ER-dependent UPR signal molecules in the cumulus cells (CCs) of the patients presenting normal, hyperresponsive, hyporesponsive and polycystic ovary syndrome (PCOS).

2. Materials and Methods

2.1. Tissue Samples

CCs were provided during oocyte retrieval from patients under 35 years old without endometriosis. Informed consent was obtained from all individual participants included in the study. 32 patients were distinguished according to their response to follicle stimulations. Follicle stimulation was performed according to the procedure described previously [20]. Normoresponsive (n=8) and hyporesponsive (n=8) groups were considered as retrieval of 10-20 and 6 or fewer oocytes, respectively. Retrieval of 30 or more oocytes responded to the same stimulus was considered as hyperresponsive (n=8), which included PCOS (n=8). Human experimental procedures were carried out under protocols approved by Gazi University Medical Faculty Institutional Review Board Ankara, TURKEY.

2.2. Cumulus Cell Culture

5ml of DMEM: F12 medium was added on the CCs and distributed evenly in petri dishes. The cell medium was changed every two days. The CCs were checked until they reached a density of 60% -

70% saturation. CCs were washed with PBS and 5 ml trypsin was added into the CCs dishes and incubated at 37^{0}C for 5 minutes to separate the cells from the adherent surface. After checking the cells under phase-contrast microscopy, 5 ml of DMEM: F12 medium was added to eliminate trypsin activity and transferred to the falcon tube. Then it was centrifuged at 1800 rpm for 10 minutes. After centrifugation, the supernatant was removed and 5 ml of DMEM: F12 medium was added to the remaining cell pellet, and homogenized by pipette, and then distributed in equal amounts to the cell culture dishes. The cell medium was changed every two days and trypsinized when the cells reached a density of 80%-90%. To eliminate trypsin activity, the medium was added again and transferred to the falcon tube. Then it was centrifuged for 10 minutes at 1800 rpm. After the supernatant was removed and homogenized by adding 5 ml of medium to the pellet, the cells were transferred to 96-well culture dishes. Serum-free medium was applied to the cells for 24 hours. At the end of 24 hours, proliferation and apoptosis indices were determined by treatment with different UPR inhibitors and ER stress stimulating substances.

2.3. MTT Proliferation Technique

Cell proliferation was performed by using Cell Titer 96 Aqueous non-radioactive cell proliferation kits (Promega, Madison, WI, USA). 500,000cells / 100μl of cultured CCs were transferred into each well of the 96-culture plate. CCs were divided into four groups. Control: CCs were treated with serum-free medium; TUDCA: CCs were treated with 0.001 μl / ml TUDCA ; TM: CCs were treated with 0.5 μg/ ml TM (Sigma Aldrich St. Louis MO, USA) and TM+TUDCA: CCs were treated with both 0.5 μg/ ml TM and 0.001 μl/ ml TUDCA for 48 h [18,21]. The cells were incubated with 100 ml media contained 10 ml MTS solution for 4 h at 37ºC. The plates were read at 490 nm wavelength and the mean of their optic density was calculated.

2.4. TUNEL

Cultured CCs were transferred into 4-well culture plates. CCs were divided into two groups. Control: CCs were treated with 1 ml serum-free media for 48 h and TM: CCs were treated with 0.5 μg/ ml TM for 48 h. Apoptosis index was determined by using TUNEL kit (Roche, In Suti Cell Death Detection Kit, Manheim, Germany). CCs were washed with PBS and incubated with permeabilization solution (0.1% sodium citrate solution containing 0.1% triton X-100) for 5 minutes at +4ºC. The slides were incubated with the TUNEL solution for 1h at 37ºC. Cells were incubated with streptavidin-alkaline phosphatase enzyme for 30 minutes. The cells were incubated with Fast-Red substrate for 10 minutes to visualize the TUNEL positive cells. The discrimination and count of positive and negative cells were performed, and the apoptosis rates of cells after inhibition of the UPR mediated signal pathway estimated.

2.5. Western Blot

Total protein from CCs was extracted by using lysis buffer and protease inhibitors. 20 ug of protein was loaded into each hole. Samples were separated on 10% polyacrylamide gel at 80V in 1X running buffer for 1h. Samples were transferred to the nitrocellulose membrane inside the transfer buffer for one night at 32 mA +4° C. The membrane was incubated in TBS-T (0.05% Tween- in TBS) prepared with 5% oil-free dry milk for 1 h at room temperature. GRP78 (Cell Signaling Technology, Beverly, MA, USA) primer antibody was incubated for overnight at +4 °C. Secondary antibody (Peroxidase Labeled Anti-Rabbit, Vector Labs, Burlingame, CA, USA) stained with peroxidase was incubated. After being immunofixation with ECL for 1 minute, it was exposed to Bio Max film. The results were normalized by taking the ratio of GRP78 to β-actin expression levels.

2.6. Reverse Transcriptase PCR (RT-PCR)

CCs from each group put in the 100 µl lysis buffer of RNAqueous Micro kit (Ambion, Austin, TX, USA). We obtained total RNA from CCs according to the RNAqueous Micro kit protocol. The genomic DNA is removed by using DNase1 (Ambion, Austin, TX, USA). The RNA quality and concentration were determined by measuring absorbance at 260 and 280 nm. Amplifications first applied after denaturation at 94 °C, 20 µl of 1X PCR buffer, each 0.125 mM dNTP, 0.5 µm each primer and 2 units of Super Taq polymerase. Semi-quantitative RT-PCR was performed using specific XBP1 and sXBP1.

The primers are human XBP1: sense, 5'-ACACGCTTGGGAATGGACAC-'3 antisense5'-CCATGGGAAGATGTTCTGGG'-3 and human β-actin: sense, 5'-CGGATGTCAACGTCACACTT -'3, antisense, 5' - TGCGTGACATCAAAGAGAAG-'3. PCR products and molecular weight markers were carried out, 1.5 % for the β-actin and 3.5% for XBP-1 in agarose gel that contains 10 mg ml^{-1} ethidium bromide and visualized under ultraviolet light. The intensity of each band was normalized with β-actin band of each sample and compared in the semi-quantitative analysis between samples.

2.7. Statistical Analysis

All data were loaded to the software Sigma Stat 3. Data were shown as mean ± SD. Difference between groups was evaluated with one-way ANOVA post hoc TUKEY test. P-value of less than 0.05 was considered statistically significant.

3. Results

3.1. MTT assay results

As a result of the MTT analysis, the proliferation index of the cumulus cells of the control group was (0.142 ± 0.01). In the TM-treated group, the proliferation index of CCs was significantly decreased $(P < 0.05)$ compared to the control group (Figure 1). No significant difference was observed between the proliferation index of CCs-treated with TUDCA and of the cells from the control group $(p > 0.05)$ (Figure 1). The proliferation index was (0.04 ± 0.002) reduced in the cumulus cells induced by TUDCA and TM, compared to the control group. On the other hand, the proliferation index of the CCs was treated with both TUDCA and TM was two-fold higher than the CCs were treated with only TM $(p > 0.001)$ (Figure 1).
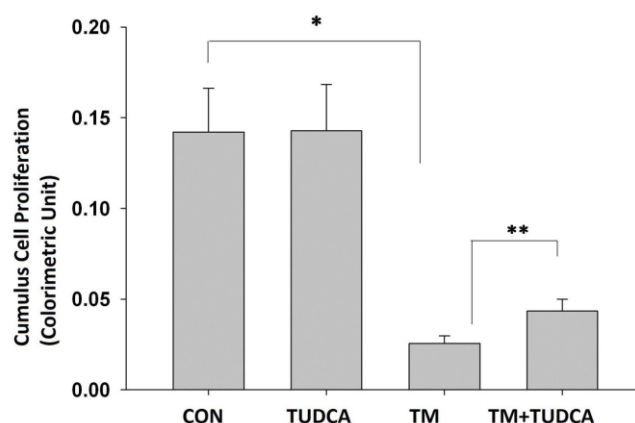
Figure 1. MTT cell proliferation in CCs culture. Control (CON): CCs were treated with serum-free media; TUDCA: CCs were treated with TUDCA; TM: CCs treated with TM; TM + TUDCA: CCs treated with both TM and TUDCA. *P < 0.05 and **P > 0.001. Bars indicate mean ± standard error.

3.2. TUNEL results

According to the TUNEL results, we found that the apoptosis index of CCs from the control group was (0.108 ± 0.017). On the other hand, when CCs were stimulated with ER stress inducer TM, there was a significant increase of apoptosis index of the CCs were treated with TM (0.334 ± 0.027), compared to those of the control group $(P < 0.001)$ (Figure 2a - c).
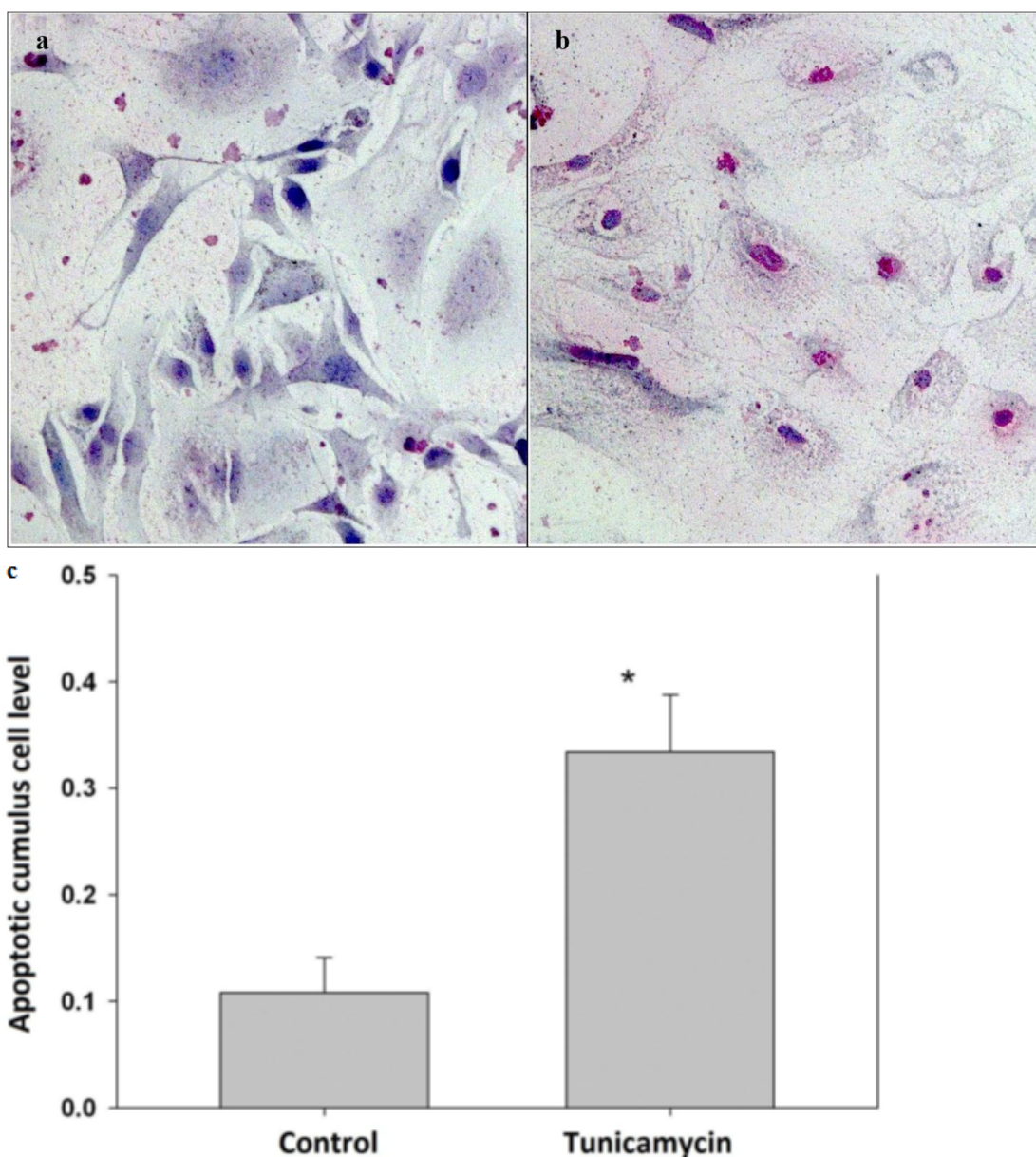
Figure 2a-c. The apoptotic effect of Tunicamycin in CCs culture.Control: CCs were treated with serum-free media (a). TM: CCs were treated with TM. Red color indicates TUNEL positive cells. Blue color indicates TUNEL negative cells (b). The quantitative analysis of CCs is observed. (c).*P < 0.001. Bars indicate mean ± standard error.3.3. Western Blot results

The protein expression of GRP78 was analyzed by Western blot method in the CCs of the normal, PCOS, hyporesponsive and hyperresponsive patients (Figure 3a). The results were normalized by taking

the ratio of GRP78 to β-actin expression levels. GRP78 expression levels, in CCs from normal, PCOS and hyperresponsive groups were 0.921 ± 0.048, 0.835 ± 0.155 and 0.892 ± 0.118 respectively and no significant difference was observed between the groups (Figure 3b). On the other hand, the GRP78 expression level was two-fold higher (1.706 ± 0.288) in CCs from the hyporesponsive group than the other groups ($p<0.05$).
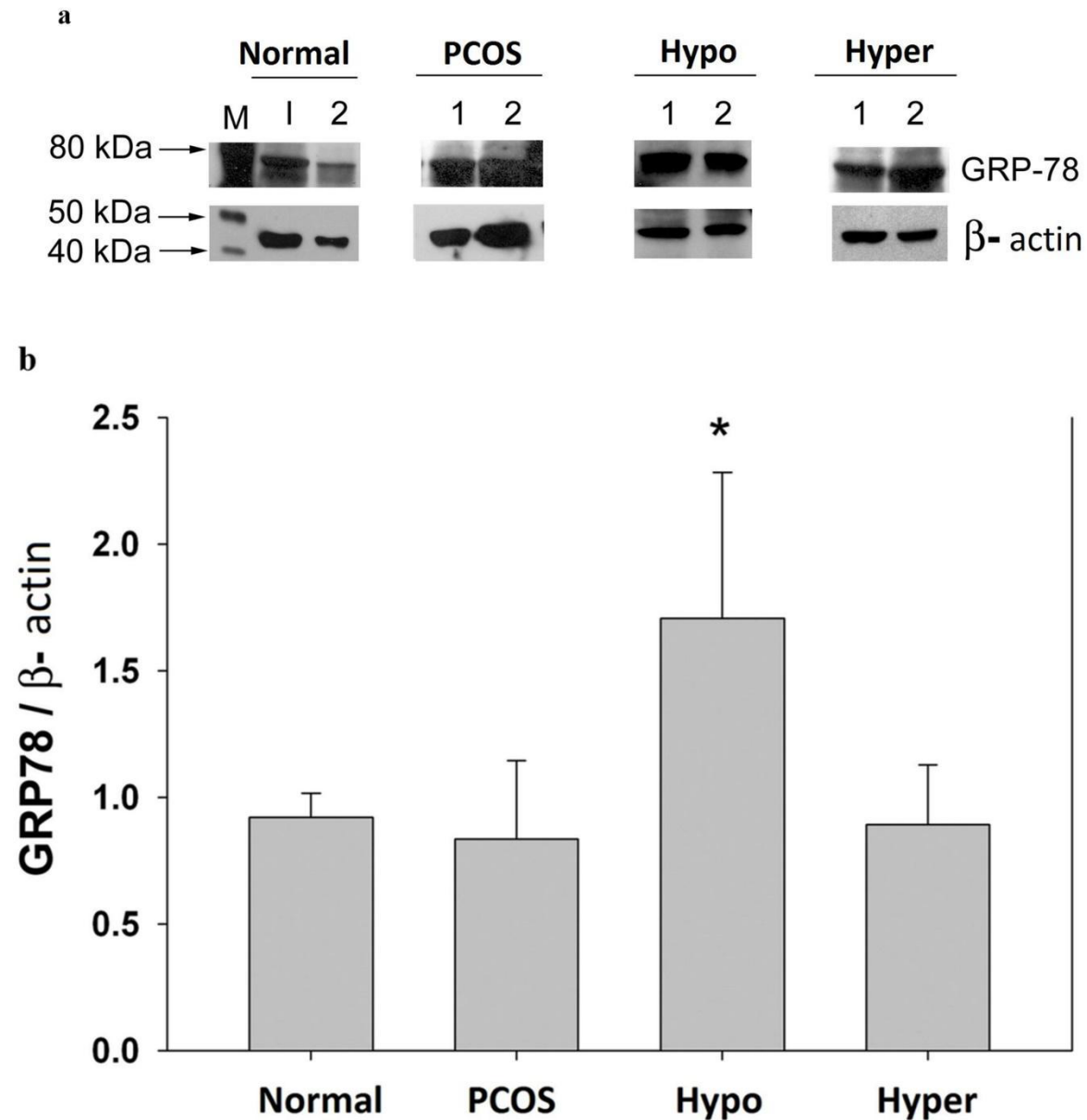
Figure 3a-b. GRP78 protein expression in CCs from normal, PCOS, hyporesponsive and hyperresponsive patients. Western blot analyzed of GRP78 and β - actin in CCs from different patient groups (a). Semi-quantitative analysis of GRP78 / β - actin levels of patient groups (b). PCOS, Hypo: hyporesponsive, Hyper: hyperresponsive. n = 8 patients per group. * P < 0.05. Bars indicate mean ± standard error.

3.4. RT-PCR results

The mRNA levels of XBP-1 and sXBP-1 were analyzed by RT-PCR method in CCs of normal, polycystic ovary syndrome, hyperresponsive and hyporesponsive patients (Figure 4a).

sXBP-1/XPB-1 mRNA expression levels were 0.712 ± 0.256, 0.622 ± 0.102 and 0.681 ± 0.291 in the CCs from normal, PCOS and hyperresponsive patients, respectively. No significant changes were observed in CCs between the groups (Figure 4b). On the other hand, sXBP-1/XBP-1 mRNA expression level was 1.5 fold higher (1.043 ± 0.165) in the CCs from the hyporesponsive group compared to others.

a
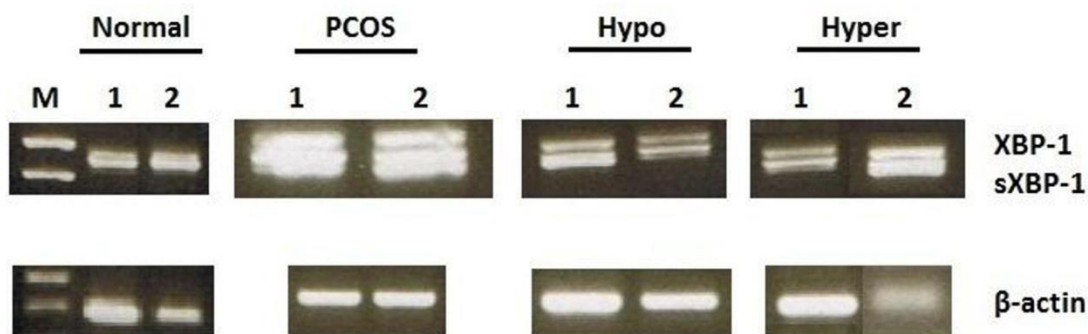
b
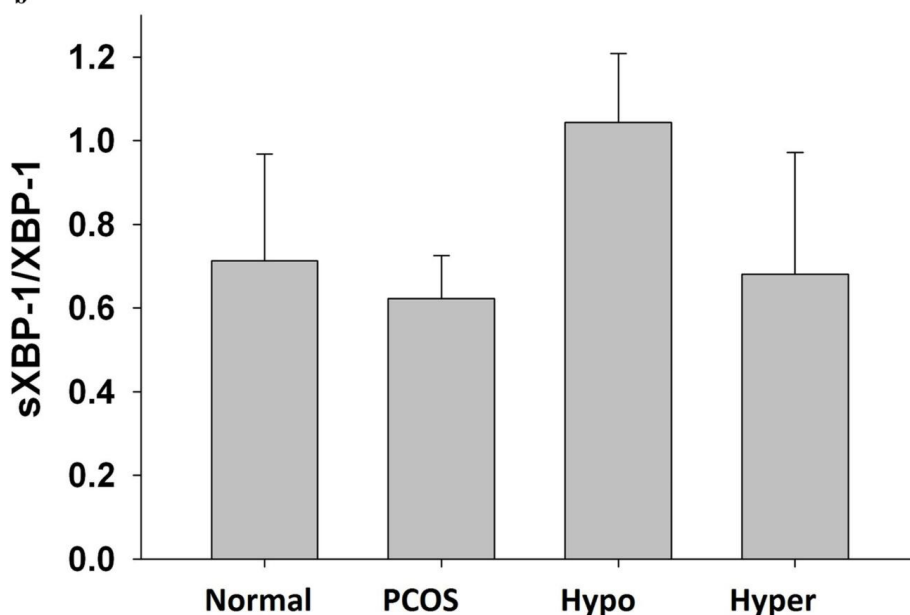
Figure 4a-b. sXBP-1/XBP-1 mRNA levels in CCs from normal, PCOS, hyporesponsive and hyperresponsive patients. RT - PCR samples of sXBP1, XBP1, and β-actin obtained from the groups (a). The semi-quantitativeve analysis of the ratio of sXBP1 / XBP1 to β - actin mRNA levels (b). PCOS, Hypo: hyporesponsive, Hyper: hyperresponsive. n = 8 patients per group. The bars indicate mean ± standard error.

4. Discussion

Cell to cell communication between oocytes and CCs coordinates ovarian follicles development [10]. Modification, folding and transportation of newly synthesized secretion and transmembrane proteins take place in the ER lumen. Some conditions such as hypoxia, glucose deficiency, oxidative

stress, and viral infections, can cause the accumulation of unfolding proteins in the ER lumen. The accumulation of unfolded proteins in the ER lumen activates UPR [22].

It has been reported that excess production of vascular endothelial growth factor A (VEGFA) induces ovarian hyperstimulation syndrome (OHSS) and TUDCA administration prevented the OHSS development by reducing VEGFA production in the ovary of OHSS model rats. Additionally, TUDCA has been used in preimplantation embryos culture [23-25].

In our study, we investigated the effects of ER stress on CCs proliferation, we determined cell viability by MTT assay. Cultured CCs were treated with UPR inhibitor TUDCAand ER stress stimulator TM, for 48 h. We found that cell proliferation was decreased in CCs treated with TM compare to other groups.

When we suppressed ER stress by treating CCs with both TUDCA and TM, we showed a slight increase in cell proliferation compared to TM-treated group. These data indicate that the longer-term or high- dose use of the TUDCA may increase cell proliferation. We used the TUNEL assay, to understand the apoptotic effect of TM in CCs. We showed that apoptosis was significantly increased in CCs treated with TM compared to control.

It has been reported that fatty acid-induced ER stress disrupts protein secretion and mitochondrial membrane potential in mouse cumulus-oocyte complex [26]. It has been indicated that ER stress may be involved in OHSS development [27].

GRP78 play important roles in many cellular processes, including folding and combining of newly synthesized proteins, orienting misfolded proteins to ERAD, regulation of calcium homeostasis, and activation of ER stress sensors [10,19].

In our study, protein expression of GRP78 which is the major regulatory protein of UPR was analyzed by Western Blot. We showed that protein expression of GRP78 in CCs of the hyporesponsive group was two times higher than the other groups. According to these findings, we can suggest that ER stress may be significantly degraded in the protein folding mechanism in hyporesponsive patients.

XBP-1 is a transcriptional activator gene which prevents the accumulation of misfolded proteins, by activating transcription of the genes that involves in the UPR [28]. Recent studies investigated the role of UPR in granulosa cells (GC) and CCs. It has been shown that both sXBP1 and HSPA5 expressed in GC of the later stage than large secondary follicles. It was suggested that UPR was involved in the process of follicular growth and maturation [29].

To evaluate the ER stress at mRNA level; we analyzed XBP1 and sXBP1 mRNA level by the RT–PCR. In our study, when the quantity of sXBP1 mRNA in the whole form XBP1 mRNA compared to the other groups, it has only shown an increase in the CCs from hyporesponsive patients. We have demonstrated that an increased ER stress in the CCs of the ovarian follicle of the patients forming the hyporesponsive group and we have shown this stress by sXBP1 at the mRNA level.

To obtain healthy folliculogenesis and quality oocyte, we can suggest that the ER stress and UPR signaling pathway should be kept within physiological limits and a new treatment approach with the use of agents to reduce the level of pathological ER stress in hyporesponsive patients.

Disclosure of interest

The authors report no conflict of interest.

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JOB SATISFACTION AS AN OBJECTIVE INDICATOR OF WORK MOTIVATION AMONG INTENSIVE CARE NURSES

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Abstract: *Job satisfaction is a complex psychological phenomenon and an extremely important factor in work quality and employees' work motivation. Considering that factors contributing to job (dis)satisfaction also affect the work motivation, the level of job satisfaction is used in this psychometric cross-sectional study as an indicator of work motivation among a sample of 71 intensive care nurses in one of the university hospitals in Belgrade, Serbia. The level of job satisfaction was assessed by an adapted and customized survey in Serbian language, based on famous Spector's Job Satisfaction Survey (JSS). Analyzing collected data, a high level of job dissatisfaction among participants of our study was founded. Considering obtained results in the light of the competent literary sources' allegations which suggest that job satisfaction level reliably indicate the level of work motivation at the same time, we concluded that work motivation in the observed sample was very poor at the moment of conducting this study, clearly indicating the need for a more comprehensive research of work motivation among Serbian nursing practitioners in future.*

Keywords: *intensive care nurse; job; motivation; satisfaction*

1. Introduction

Job satisfaction is a complex psychological phenomenon and an extremely important factor in work quality and employees' work motivation. In essence, job satisfaction represents a subjective perception of opportunities to achieve important aspects of work and life while performing a particular job, and can be defined as a pleasant, positive emotional state that arises as a result of work experience and motivates an employee to continue with work in a particular profession, or at particular workplace [1-3]. In the past, determining the potential correlation between job satisfaction and work motivation has been the subject of numerous studies in the field of applied psychology and related sciences. In many available studies, researchers have found that factors contributing to job (dis)satisfaction also affect the work motivation of employees, at the same time [4-6]. Considering this scientific fact, the level of job satisfaction is used nowadays as an objective indicator of work motivation in all professions, as well as in nursing, one of the most stressful professions of the contemporary society that requires simultaneous mental, physical and emotional engagement, especially in intensive care units (ICU) [7, 8].

Regardless of the type of professional activity, the subjective experience of job satisfaction is not affected only by the nature of performed work and conditions of the work environment, but also by personal expectations of the employee in relation to a particular job [1]. Although the assumed factor of work motivation among all health workers, and therefore of nurses, primarily should be the desire to help another human being, researches has shown that job satisfaction among this type of helping professionals objectively depends on several independent or interrelated factors - belonging to a particular gender, age of employees, degree of formal education, length of service, working place conditions, type of work, length and organization of working hours, manner of management, promotion opportunities, as well as the amount of material compensation for the work performed [9]. Various studies have shown that, compared with other humanist-oriented professions, all healthcare professionals are exposed to a significantly greater risk of professional dissatisfaction, especially those who are exposed to the prolonged influence of occupational stressors, like ICU nurses. In intensive care units, nurses are dealing daily with different needs, problems, sufferings, and, finally, the death of critically ill patients. Over time, the synergistic effect of all these factors inevitably leads to exhaustion of nurse's emotional potentials, which greatly affects both long-term job satisfaction and work motivation [10-12]. Because of that, job satisfaction among ICU nurses cannot be observed only as an isolated individual attitude in regard to the current job, but only as a complex phenomenon based on the synergistic psycho-emotional effect of all specific workplace factors, strongly influencing employees in the motivational sphere and in overall attitude toward work. [13].

Assessing the level of job satisfaction is of great importance for improving the general quality of professional activity and achieving better professional performance. In countries with the strongly developed health system, it is not uncommon for management of healthcare institutions to use the job satisfaction test as an objective instrument for assessing employee work motivation, general efficiency of work organization, and to identify failures in managing the institution or its smaller organizational segments [1]. The level of job satisfaction among ICU nurses is directly related to the presence of professional burnout syndrome, the frequency of work absenteeism, the quality of professional communication and overall quality of interpersonal relationships among employees at different levels of the organizational scale, but also the degree of professional efficiency of the institutional work organization which is expected to satisfy current and potential needs of healthcare users. Based on different researches, intensive care units are often marked as workplaces with a low level of job satisfaction among employees, especially members of the nursing profession [14]. Due to the non-fulfillment of personal expectations regarding work and various forms of psycho-emotional pressure on the workplace, an employee often experiences a subjective feeling of exhaustion, fatigue and emotional gaps that lead not only to professional dissatisfaction and loss of work motivation in a very short time, but also to creation of ideal base for development of burnout syndrome – a specific psycho-emotional construct that can have unpredictable and potentially dangerous consequences, especially if patient-related activities of health professionals directly affect life and death issues, such as nurses employed in intensive care units [14,15]. Available researches among participants from the nursing profession show that "nurse job dissatisfaction has been the primary predictor of intent to leave" [16]. Unfortunately, in low-income societies, the inability to leave a permanent job and the long-term job dissatisfaction often lead to permanent loss of work motivation among ICU nurses; professional errors can become more and more dangerous over time, and even can endanger patient' life in some situations [7,17]. However, despite all the above, there is almost no available data in the literature regarding job satisfaction among

ICU nurses in Serbia and the surrounding countries, which was the main reason for conducting this cross-sectional study. The aim of this study was to assess the level of work motivation among ICU nurses by analyzing the level of ten different domains of job satisfaction.

2. Methods

2.1 Sample and Design of the Study

This research was designed in the form of a psychometric cross-sectional study, which included a sample of 71 ICU nurses in total. The study included nurses employed in currently existing intensive care units of the clinics and hospitals for surgery, urology, internal medicine, pediatrics, gynecology and obstetrics, and otorhinolaryngology in one of the university hospitals in Belgrade, Serbia, in July of 2017.

2.2 Research Instrument

In this study, only factors related to the process of working and workplace were considered in assessing the level of job satisfaction. Regarding sociodemographic data, the respondents are required only to indicate the affiliation of a particular gender and age. The instrument of the research survey in Serbian language (Mladenovic, 2013.), based on Job Satisfaction Survey (JSS), originally created by American psychologist, Professor Paul E. Spector from University of South Florida (18). The survey is constructed in order to determine universal domains of job satisfaction. According to domestic and foreign literature, the original survey developed a Cronbach-alpha coefficient higher than 0.70, which makes it relevant for job satisfaction testing in different professions. The original survey with scoring instructions is available to researchers free of charge, only for non-commercial purposes, on following web page: http://www.shell.cas.usf.edu/pspector/scales/jsspag/html.

The survey contains 36 statements grouped in 10 domains: general job satisfaction (1), salary (2), promotion (3), supervision (4), benefits (5), rewarding (6), working conditions (7), associates 8), nature of work (9) and communication (10). The respondents were ordered to choose only one of the offered responses on the Likert scale which best describes their stand about the above statement, namely: 1 (totally disagree), 2 (partially disagree), 3 (slightly disagree) , 4 (slightly agree), 5 (partially agree), and 6 (completely agree). The number in front of the statement also indicates the number of points the study participant received for this question, except for the negatively formulated statements, under the ordinal number: 2, 4, 6, 8, 10, 12, 14, 16, 18, 19, 21, 23, 24 , 26, 29, 31, 32, 34, 36, for which the points of the participant are calculated in the following way: 1 = 6; 2 = 5; 3 = 4; 4 = 3; 5 = 2; 6 = 1. The unique score is calculated for each domain, obtained by adding points for statements related to a certain domain, after converting points for negatively formulated statements, as follows:

1. general job satisfaction (all statements in the survey):
   - 36 – 108 points (dissatisfied),
   - 109 – 144 points (ambivalent stand),
   - 145 – 216 points (satisfied);
2. salary (statements: 1, 10, 19, 28),
3. promotion (statements: 2, 11, 20, 33),
4. supervision (statements: 3, 12, 21, 30),
5. benefits (statements: 4, 13, 22, 29),
6. rewarding (statements: 5, 14, 23, 32),
7. working conditions (statements: 6, 15, 24, 31),
8. associates (statements: 7, 16, 25, 34),

9. nature of work (statements: 8, 17, 27, 35),
10. communication (statements: 9, 18, 26, 36):
   - 4 – 12 points (dissatisfied),
   - 13 – 16 points (ambivalent stand),
   - 17 – 24 points (satisfied).

2.3 Ethical Consideration

Purpose, ethics, scientific basis and other relevant issues related to this research have been considered and approved both by the Ethical Committee of the Faculty of Medicine, University of Novi Sad and by the Ethical Committee of the institution where the research has been carried out, documented by a written decision available. Participants have been included in the research on the voluntary basis exclusively, and the obtained information warranted secret. Each participant was informed about the aim of the study in the survey preamble.

2.4 Statistical Analysis

In order to generate descriptive statistics, results were statistically analyzed by using the SPSS Statistical Package for Windows, version 16. The obtained results are presented in tables and, depending on their type, expressed by absolute and relative numbers, calculated mean values, (the arithmetical mean, the median) and variability measures (standard deviation - SD, coefficient of variation - CV, range). As the research is dealing with attributive characteristics, the difference between expected and observed frequencies in all investigated categories has been determined using the Chi-square (x^2) independence test. Minimum significance level in this study was set to 0.05.

3. Results

3.1 Basic sociodemographic data

In the examined sample, statistically significant differences have been registered in the subjects' gender distribution - 23 males (32.4 %), and 48 females (67.6 %), *p<0.05*. The mean age of the examined subjects was 33.8 years, with a slightly younger mean age of males - approximately 31.3 years (SD, 5.9; CV<30 %) as compared to the mean females' age - approximately 36.3 years (SD, 9.5; CV<30 %); however, by testing, these differences have been established as statistically insignificant *(p>0.05)*. Gender and age distribution of the study participants is presented in Tab. 1.

Table 1. Participants 'gender and age distribution

GENDER	N	%	$\overline{x}$	SD	CV %	Med	Min.	Max.
					AGE			
males	23	32.4	31.3	5.9	18.8	31	22	43
females	48	67.6	36.3	9.5	26.2	34	20	53
∑	71	100.0	33.8					
p	<0.05		>0.05					

Note: N - subjects in total; $\overline{x}$ - mean arithmetic value, SD – standard deviation, CV% - variation coefficient, Med – median, Min. – minimum value, Max. – Maximum value

3.2 Job satisfaction analysis

The distribution of study participants based on general job satisfaction points to the statistically significant predominance of those who are not satisfied with the current job (p <0.05). General job dissatisfaction was registered in a total of 53 or 74.6 % of study participants (SD, 13.6,

CV<30 %); the ambivalent stand towards the general job satisfaction was observed in seven, i.e. 9.9 % of examined subjects (SD, 6.3, CV<30 %), while only 11 of them, or 15.5 % (SD, 16.1, CV<30 %) are satisfied with the current job in general sense (Tab. 2.)

Table 2. Distribution of general job satisfaction (GJS) among participants

GJS level (x)	N	%	$\overline{x}$	SD	CV%	Med	Min.	Max.
Dissatisfied (36-108 p.)	53	74.6	79.7	13.6	17.1	79	51	107
Ambivalent (109-144 p.)	7	9.9	125.4	6.3	5.01	123	116	135
Satisfied (145-216 p.)	11	15.5	160.9	16.1	10.0	157	145	192
∑	71	100.0						
p	<0.05							

Note: N - subjects in total; $\overline{x}$ - mean arithmetic value, SD – standard deviation, CV% - variation coefficient, Med – median, Min. – minimum value, Max. – Maximum value
*Chi-square (x^2) independence test

Analyzing collected data about subjects' salary satisfaction as a particular domain of job satisfaction, statistically significant dissatisfaction was found in total of 68, i.e. 95.8 % of study participants (SD, 1.9); the ambivalent stand towards satisfaction with salary was registered in two or 2.8 % of examined subjects (SD, 0.7), while satisfaction within this domain was confirmed only by one participant, i.e. 1.4 % (*p <0.05*). Considering participants' satisfaction with the possibility of promotion, a statistically significant dissatisfaction was found in total of 60, i.e. 84.5 % of subjects (SD, 1.8); related to this particular domain of job satisfaction, the ambivalent stand was registered in two or 2.8 % of participants (SD, 0.7), while statistically significant satisfaction with possibility of promotion was observed in nine, i.e.12.7 % of examined subjects (SD, 2.4), p <0.05. Regarding workplace benefits, statistically significant dissatisfaction was found in total of 66, i.e. 93.0 % of subjects (SD, 2.60) and the ambivalent stand took five, or 7.0 % of participants (SD, 0.9); at the same time, satisfaction was not registered with any of the examined subjects towards this domain of job satisfaction (p <0.05). Furthermore, statistically significant dissatisfaction with the possibility of rewarding was registered in a total of 52 study subjects, i.e. 73.2 % (SD, 0.7), while ambivalent stand took 13 or 18.3 % of participants (SD, 1.0); six subjects, i.e. 8.5 % (SD, 0.7) were satisfied with the rewarding possibilities they have at the workplace (p <0.05). Statistically significant majority of subjects, 62 in total or 87.3 % (SD, 2.4), expressed their dissatisfaction with working conditions; the ambivalent stand was registered in five i.e. 7.1 % of study participants (SD, 0.7) and satisfaction within this domain was observed in four or 5.6 % subjects (SD, 2.5), p<0.05. Further analysis of the collected data revealed also statistically significant dissatisfaction of subjects in the domain of nature of work, with a total of 36 i.e. 50.7 % dissatisfied participants (SD, 2.1); ambivalent attitude towards this aspect of job satisfaction was found in total of 11 or 15.5 % of participants (SD, 0.9), so the remaining 24 subjects, i.e. 33.8 %, expressed their satisfaction in relation to the nature of work (SD, 2.7), p <0.05. Finally, by testing subjects' distribution based on satisfaction with professional communication, statistically significant dissatisfaction in total of 46 or 64.8 % participants was found (SD, 2.3); ambivalent stand toward communication at the workplace took seven i.e. 9.9 % of participants (SD, 0.8), while the satisfaction within this domain of job satisfaction was observed in total of 18 or 25.3 % of examined subjects (SD, 2.7), p<0.05. The statistical significance of obtained distributions in domains "supervision" and

"associates" was not established in this study (p >0.05). Statistically significant distributions of participants in different domains of job satisfaction are presented in Tab. 3.

Table 3. Statistically significant distributions of participants in different domains of job satisfaction

SALARY, *p<0.05*							
JS level (x)	N	%	$\overline{x}$	SD	Med	Min.	Max.
Dissatisfied (4-12 p.)	68	95.8	4.9	1.9	4	4	12
Ambivalent (13-16 p.)	2	2.8	15.5	0.7	15.5	15	16
Satisfied (17-24 b.)	1	1.4	-	-	-	-	-
∑	71	100.00					
PROMOTION, *p<0.05*							
JS level (x)	N	%	$\overline{x}$	SD	Med	Min.	Max.
Dissatisfied (4-12 p.)	60	84.5	5.6	1.83	5	4	11
Ambivalent (13-16 p.)	2	2.8	13.5	0.7	13.5	13	14
Satisfied (17-24 b.)	9	12.7	21.0	2.4	21.0	18	24
∑	71	100.00					
BENEFITS, *p<0.05*							
JS level (x)	N	%	$\overline{x}$	SD	Med	Min.	Max.
Dissatisfied (4-12 p.)	66	93.0	6.8	2.6	6	4	12
Ambivalent (13-16 p.)	5	7.0	13.6	0.9	13	13	15
Satisfied (17-24 b.)	0	0	-	-	-	-	-
∑	71	100.00					
REWARDING, *p<0.05*							
JS level (x)	N	%	$\overline{x}$	SD	Med	Min.	Max.
Dissatisfied (4-12 p.)	52	73.2	9.4	0.7	9	8	11
Ambivalent (13-16 p.)	13	18.3	13.8	1.0	13	13	16
Satisfied (17-24 b.)	6	8.5	18.1	0.7	18	17	19
∑	71	100.00					
WORKING CONDITIONS, *p<0.05*							
JS level (x)	N	%	$\overline{x}$	SD	Med	Min.	Max.
Dissatisfied (4-12 p.)	62	87.3	6.4	2.4	5	4	12
Ambivalent (13-16 p.)	5	7.1	15.0	0.7	15	14	16
Satisfied (17-24 b.)	4	5.6	20.5	2.5	21	17	23
∑	71	100.00					
NATURE OF WORK, *p<0.05*							
JS level (x)	N	%	$\overline{x}$	SD	Med	Min.	Max.
Dissatisfied (4-12 p.)	36	50.7	8.2	2.1	8	5	12
Ambivalent (13-16 p.)	11	15.5	14.1	0.9	14	13	15
Satisfied (17-24 b.)	24	33.8	20.5	2.7	19.5	17	24
∑	71	100.00					
PROFESSIONAL COMMUNICATION, *p<0.05*							
JS level (x)	N	%	$\overline{x}$	SD	Med	Min.	Max.
Dissatisfied (4-12 p.)	46	64.8	7.5	2.3	7	4	12
Ambivalent (13-16 p.)	7	9.9	13.7	0.8	14	13	15
Satisfied (17-24 b.)	18	25.3	21.0	2.7	22	17	24
∑	71	100.00					

Note: N-subjects in total; $\overline{x}$-mean arithmetic value, SD – standard deviation, CV% - variation coefficient, Med – median, Min. – minimum value, Max. – Maximum value

*Chi-square (x^2) independence test

4. Discussion

Intensive care units represent specific departments of secondary level healthcare institutions, intended for hospitalization of critically ill patients [19]. In accordance with the purpose of intensive care units, it is quite understandable that those clinical departments are marked as exceptionally stressful working environment [7, 20]. Considering the well-known fact that level of job satisfaction basically correlates with the workplace conditions and the nature of a particular job, it is expected that nurses working in intensive care units will be more dissatisfied comparing with their colleagues from other organizational units of healthcare institutions [14, 21]. This cross-sectional, non-experimental psychometric study included 71 critical care nurses in total, all working in different intensive care units of one Belgrade's university hospital at the moment of entering the research. The examined subjects mean age was 33.8 years, with slightly lower mean values in males, as compared to females - 36.3 years. Similar to other researches that involve nurse population, in this study female participants, have been found to prevail over males [22]. By choosing a traditionally female occupation, males can experience role conflict and consequent job dissatisfaction [23]. Although some studies indicate that job dissatisfaction is more common in male nurses, this claim could not be proven by our study, as well as the results of some researches suggesting an increase in job satisfaction among nurses with aging [24-26].

In general, terms, job satisfaction can be seen as an affective attitude of an individual to its own work, degree of commitment to work, or the subjective importance of work for a particular individual. Depending on the contribution degree of different work factors to the satisfaction when performing professional activities, general job satisfaction can be also defined as a sum of satisfaction with certain aspects of the particular job [27]. Unlike similar researches that established the middle level of general job satisfaction among the examined population of nurses [28, 29], a statistically significant strong predomination of general job dissatisfaction among participants has been established in our study, amounting to more than three-quarters of dissatisfied subjects in total. Increasing knowledge about the importance of health professionals' job satisfaction should have a global social significance, because professional dissatisfaction may seriously affect the quality of performed patient care [14, 30]. Although authors of studies from different world countries suggest that the degree of job satisfaction among ICU nurses is always lower compared to nurses of other hospital departments because of higher levels of perceived job stress [31,32], such a significant representation of general dissatisfaction among participants always points to the existence of serious problems and low level of work motivation in particular profession, even though it is an employee sample from one institution, like in our study.

As expected, almost all subjects of the examined sample were most dissatisfied with their salary - a factor of work that does not depend on the institution management but reflects the economic situation in the country and the organization of its entire health system. Despite confirmed significant correlation between job satisfaction and monthly salary, material aspects of work are still one of the main problems in achieving job satisfaction in the nursing profession, not only in Serbia but also in the surrounding countries [33, 34]. Adequate material compensation for realized work is the essential prerequisite of job satisfaction and efficient work of health workers, since the time of Hippocrates [35]. In a few former Yugoslav countries, inadequate salary is nowadays the main reason of losing work motivation in the nursing profession and recruiting to other EU countries. The divergence between the contemporary system of nursing education and the existing institutional work systematization represents another important reason of job dissatisfaction, loss of work motivation and intention to leave among

nurses in these countries [34]. The global social underestimation of healthcare professions, especially the nursing profession, can be seen by further analysis of the data collected in our study. Besides low salary, study participants also indicated next reasons for job dissatisfaction: absence of benefits for performed work (no satisfied subject), inadequate working conditions and small opportunities for promotion (more than four-fifths of examined subjects in total), as well as absence of rewarding for achieved work results (approximately three-quarters of dissatisfied subjects in total). All the above-mentioned job satisfaction domains have a significant impact on the level of work motivation, and therefore on the efficiency and quality of the performed professional activities [3, 36].

However, besides above-mentioned domains of job satisfaction that are regulated by legal acts and mostly cannot be easily influenced, there are also those whose correction can easily increase the satisfaction of doing a particular job. By analyzing collected data, it is found that approximately half of our study participants are not satisfied with the nature of the work they are performing. If we exclude the objectively large influence of professional stress in intensive care units and impact of all the previously mentioned job satisfaction domains, it is obvious that adequate professional orientation and choosing a profession in accordance with individual characteristics of personality represent the key factors for a later higher level of job satisfaction. Implementation of educative programs for achieving an assertive way of communication could also contribute in increasing level of job satisfaction among participants of our study, as approximately two-thirds of them in total expressed dissatisfaction with the current way of professional communication. Quality of communication as a factor of increasing job satisfaction has been the subject of many different studies. The results of domestic research conducted among intensive care units staff indicate that even 37% of professional errors have been caused by poor communication between physicians and nurses [37].

4.1 Study limitations

The limitation of the study originates from a relatively small sample and the fact that the study included ICU nurses of a single healthcare institution during a particular period. In order to reach more scientifically significant results, it is necessary to carry out prospective research on a larger sample of subjects from several healthcare institutions.

5. Conclusion

Based on the analysis of collected data, a high level of job dissatisfaction among participants of our study was founded. Participants were most dissatisfied with the salary, but they also expressed dissatisfaction in other domains of job satisfaction – work benefits, working conditions, promotion opportunities, rewarding, nature of work and the way of professional communication. Considering obtained results in the light of the competent literary sources' allegations which suggest that job satisfaction level reliably indicate the level of work motivation at the same time, we concluded that work motivation in the observed sample was very poor at the moment of conducting this study, clearly indicating the need for a more comprehensive research of work motivation among Serbian nursing practitioners in future.

Conflict of interests

Authors have no conflicts of interest to declare.

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Research Article

THE STUDY OF THE LONG-LIVERS DYNAMICS IN THE POPULATIONS OF ADJARA IN 2012-2017

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Abstract: *The life expectancy of human is increasing in almost all over the world, accordingly population aging. Long-liver studies and research about longevity have increased considerably in recent decades and are currently recognized as providing important contributions to the understanding of what can be "successful aging." Population aging is a multidimensional and complex phenomenon and depends as a social, medical as well as genetic factors. The aim of our study was to reveal and statistically process the number of centenarians in the region of Adjara, Georgia since it was known that the mentioned region is famous of long-livers comparing to the other regions of Georgia. At the same time, we were interested in how is the ranging the number of centenarians in 5 years dynamics, in concrete, statistical data of 2012-2017 has been processed. The study has shown that the increase of the long-livers concentration can be observed in all five municipalities of Ajara in the five-years dynamics. Special increase of live longer was observed in Batumi, Khelvachauri and Kobuleti municipalities; The number of centenarians apart from Keda and Shuakhevi municipalities has increased as well. Obtained data have shown that longevity of Ajarian population has been increased and the fact will be useful for further research to identify the main reasons for the multifactorial process of successful aging in Adjara populations.*

Keywords: *longevity, centenarian, long-liver, aging.*

1. Introduction

In the last decades, it is obvious that the longevity and life expectancy of the human population is increasing in almost all over the world, accordingly it is increasing the median age and population aging. Most of the countries have a rising life expectancy and aging of populations. The aged population is currently at its highest level in human history.[1-2] The United Nations (UN) predicts the rate of population aging in the 21st century will exceed that of the previous century. [2] The number of people aged 60 years and over has tripled since 1950, reaching 600 million in 2000 and surpassing 700 million in 2006. It is projected that the combined senior and geriatric population will reach 2.1 billion by 2050. [3][4] Countries vary significantly in terms of the degree and pace of aging, and the UN expects populations that began aging later will have less time to adapt to its implications. [2] Globally, considering the total world population, the estimated number of centenarians in 2013 was 441,000; 3.4 million are expected in 2050, and 20.1 million are expected in 2100 [5]. Long-lived studies and research about longevity have increased considerably in recent decades [6] and are currently recognized as

providing important contributions to the understanding of what can be "successful aging." [7] Human population aging is a multidimensional phenomenon and it differs from aging at the level of individuals. Each additional year individuals are alive, they grow one year older, but defining how populations age is much more complex [1–2]. Through a review of the results of international centenarians' studies, it is possible to determine their main sociodemographic characteristics, health, functionality, and psychosocial features, that can affect or aging. Within the range of various studies, it is possible to be mentioned an extensive number of countries that have already profiled their centenarian population, such as Denmark [8], Greece [9], Italy [10], USA [12], Japan [13], Germany [14], Sweden [15], and Australia [16].

The aim of our study at the first stage was to reveal and statistically process the number of centenarians in the region of Adjara, Georgia since it was known that the mentioned region is famous of long-livers comparing to the other region of Georgia. At the same time, we were interested in how is the ranging the number of centenarians in 5 years dynamics, in concrete, the data of 2012-2017 has been processed. Profiling the Adjarian populations in its individuals who have reached 100 years of age is thought to be an important step forward in understanding the longevity process in our country, it would improve the next stages of study connected of long-livers and would challenge the service delivery and various programming for the further considerations.

2. Material and method

In order to study the data for long-livers in the Adjara Population and survey the numerical dynamics we have found at social service offices of the Autonomous Republic of Adjara and statistically processed the data of long-livers living in Batumi, Khelvachauri, Kobuleti, Keda, Shuakhevi and Khulo municipalities on the five-year dynamics (2012-2017) including the ones with more than a hundred years of age.

3. Research outcomes

3.1. The Dynamics of the long-livers in Batumi Municipality in 2012-2017

According to the Adjara Agency of Social Services, the number of people in Batumi municipality is distributed according to the following: 162 people in 2012, 181 – in 2013, 2014-288, 2015-323, 2016-287, and in 2013 - 411. According to the data of the Social Agency of Adjara, the number of centenarians in Batumi municipality according to years is as follows: 2012-2, 2013-3, 2014-7, 2015-13, 2016-13, 2017-17.

As shown in the Fig.1 in 2012-2017, the concentration of long-livers in Batumi municipality is increasing by the years, the exception is 2016 year. Fig.2 shows that in the municipalities of Batumi along with the increase in the number of long-livers, the number of centenarians is also increasing.

Figure 1. Dynamics (in percentage) of Long-livers in Batumi Municipality in 2012-2017

Figure 2. Dynamics (in percentage) of the Centenarians in Batumi municipality in 2012-2017

3.2.The dynamics of long-livers in Khelvachauri Municipality in 2012 – 2017

According to the data of Adjara Agency of Social Services, the number of long-livers in Khelvachauri municipality is as follows: 136 people in 2012, 145 in 2013, 2014-207, 2015-212, 2016-227 and in 2017- 242. According to the data of Adjara Agency of Social Services, the number of centenarians in Khelvachauri municipality is increasing as follows: 2012-5, 2013-6, 2014-8, 2015-10, 2016-11, 2017-12.

In Khelvachauri municipality, in the dynamics of 2012 - 2017 years, the number of the long-livers is moderately growing according to years and the concentration of the centenarians is quite high (Fig.3 and 4)

Figure 3. Dynamics (in percentage) of long-livers in Khelvachauri Municipality in 2012-2017

Figure 4. Dynamics (in percentage) of the Centenarians in Khelvachauri Municipality in 2012-2017

3.3.The Dynamics of long-livers in Kobuleti Municipality in 2012-2017

According to the data of Adjara Agency of Social Services, the number of the long-livers in Kobuleti municipality is as follows: 141 people in 2012, in 2013 - 156, 2014-253, 2015-256, 2016-292, and in 2017 - 303.

According to the Ajara Agency of Social Services, the number of centenarians in Kobuleti municipality according to years is as follows: 2012-13, 2013-16, 2014-16, 2015-16, 2016-15, 2017-14.

In Kobuleti Municipality in the dynamics of 2012- 2017, despite the increasing number of the long-livers, the number of the centenarians is reduced (Fig.5 and 6).

Figure 5. Dynamics (in percentage) of long-livers in Kobuleti Municipality in 2012-2017

Figure 6. Dynamics (in percentage) of the Centenarians in Kobuleti Municipality in 2012-2017

3.4.The Dynamics of long-livers in Keda Municipality in 2012-2017

According to the data of Adjara Agency of Social Services, the number of the long-livers in Keda municipality is as follows: 90 people in 2012, 85 in 2013, 85-585, 2015-93, 2016-91, and in 2017-97.

According to the Ajara Agency of Social Services, the number of the centenarians in Keda municipality according to years ranges in the following numbers: 2012-8, 2013-8, 2014-7, 2015-7, 2016-5, 2017-3.

The number of long-livers in Keda municipality decreased in 2013 – 2014 years and in recent years the number is increased. The number of centenarians is decreased by the years (Fig.7 and 8).
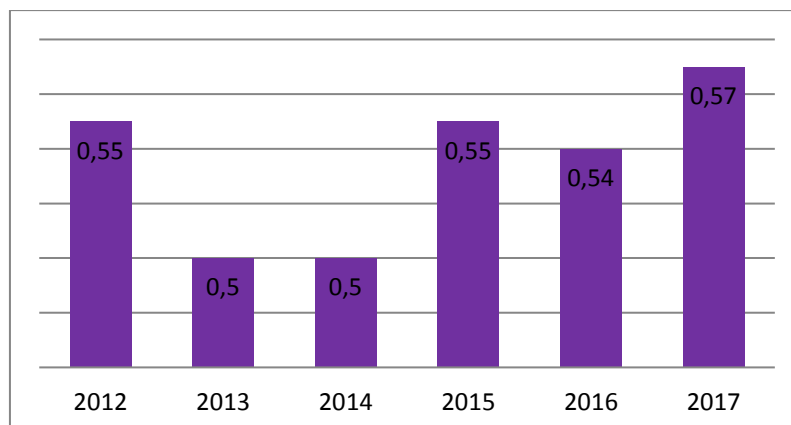
Figure 7. Dynamics (in percentage) of long-livers in Keda Municipality in 2012-2017
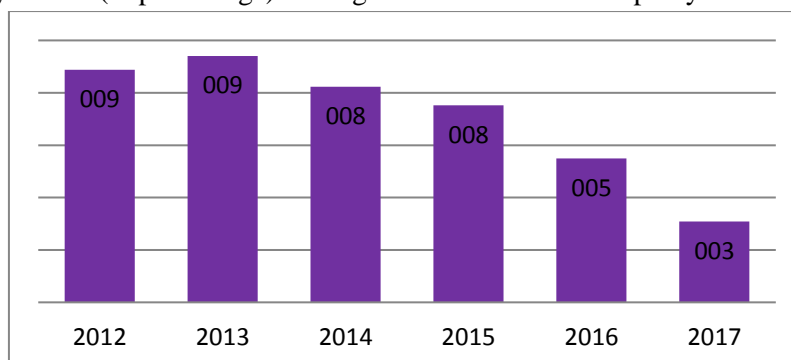
Figure 8. Dynamics (in percentage) of the Centenarians in Keda Municipality in 2012-2017

3.5.The Dynamics of long-livers in Shuakhevi Municipality in 2012-2017

According to the data of Adjara Agency of Social Services, the number of the long-livers in Shuakhevi municipality is as follows: 121 people in 2012, in 2013 - 123, 2014-224, 2015-113, 2016-123, and in 2017 - 125.

According to the data of Adjara Agency of Social Services, the number of centenarians in Shuakhevi municipality is distributed according to years as follows: 2012-12, 2013-11, 2014-10, 2015-7, 2016-7, 2017-8.

In Shuakhevi municipality there is a slight increase in the concentration of long-livers, the exception is 2015, as for the centenarians in the abovementioned municipality, their concentration in the five-year dynamics is reduced (Fig 9,10).
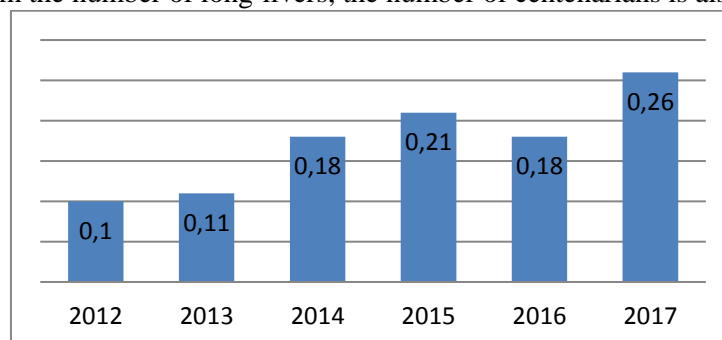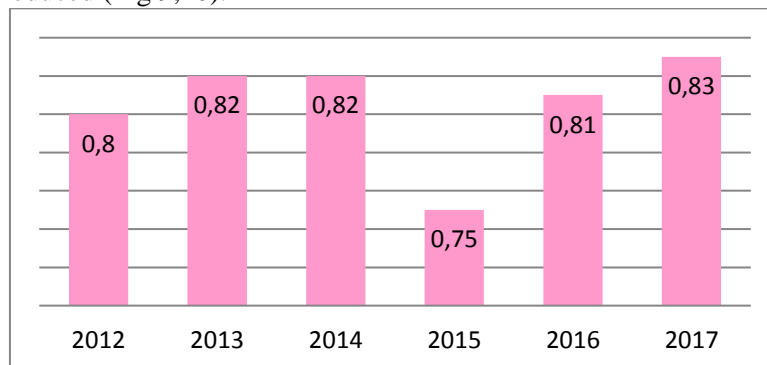

Figure 9. Dynamics (in percentage) of long-livers in Shuakhevi Municipality in 2012-2017
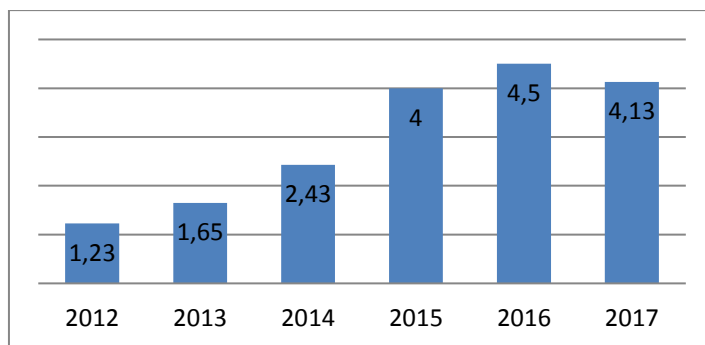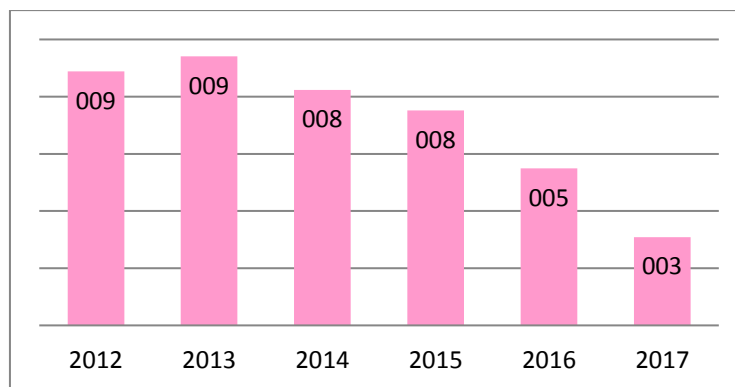
Figure 10. Dynamics (in percentage) of the Centenarians in Shuakhevi Municipality in 2012-2017

3.6 The Dynamics of long-livers in Khulo Municipality in 2012-2017

According to the data of Adjara Agency of Social Services, the number of long-livers in Khulo municipality is as following: 173 people in 2012, in 2013 - 166, 2014-163, 2015-168, 2016-181, and in 2017 - 182.

According to the data of the Adjara Agency of Social Services, the number of centenarians according to the years in Khulo municipality is distributed as follows: 2012-11, 2013-13, 2014-12, 2015-12, 2016-15, 2017-17.

The number of long-livers in the five-year dynamics in Khulo municipality slightly increases, except for the year 2014. In the mentioned municipality the number of centenarians in 2012 – 2017 years was increased (Fig.11, 12).
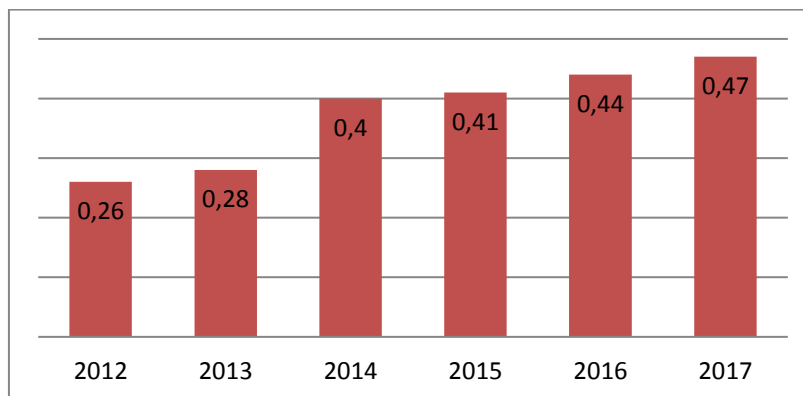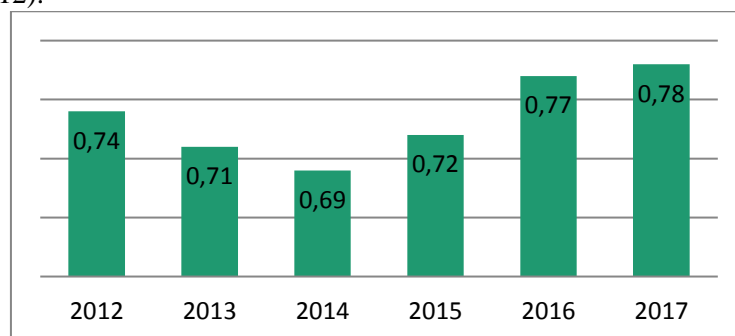

Figure 11. Dynamics (in percentage) of long-livers in Khulo Municipality in 2012-2017

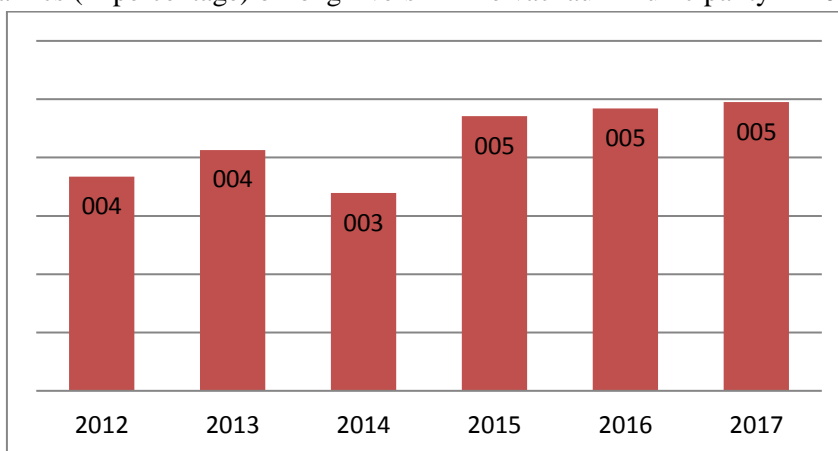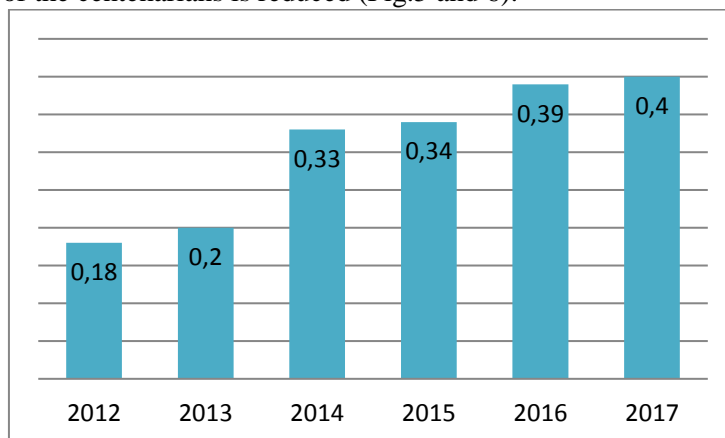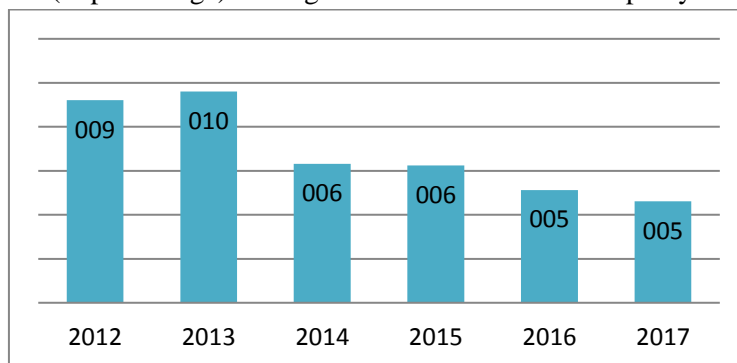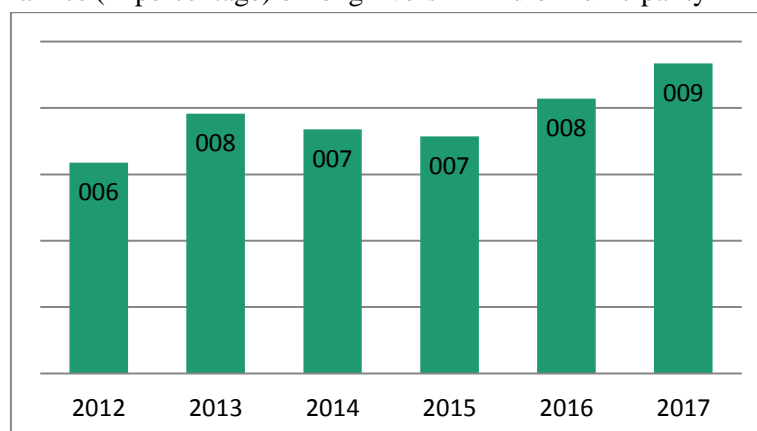**Figure 12.** Dynamics (in percentage) of the Centenarians in Khulo Municipality in 2012-2017

3.7.The Dynamics of long-livers in Adjara Region in 2012-2017

According to the data of Adjara Agency of Social Services, the number of the long-livers in the Autonomous Republic of Adjara is as follows: 823 people in 2012, 856, 2014-1120, 2015-1165, 2016-1201, and in 2013 - 1360.

According to the data of the Adjara Agency of Social Services, the number of centenarians in the Municipality of Adjara Autonomous Republic is distributed as follows: 2012-51, 2013-57, 2014-60, 2015-65, 2016-66, 2017-71.

In the five-year dynamics in the Autonomous Republic of Adjara, the quantitative increase of the long-livers was observed, in particular, in 2012 it was 823 (0,24%), and in 2017 - 1360 (0.4%). The number of centenarians has also been increased; in 2012 it was 51 (6.19%), while in 2017 - 71 (5,22%). The lowest rate is due to the fact that the total number of long-livers is increased.
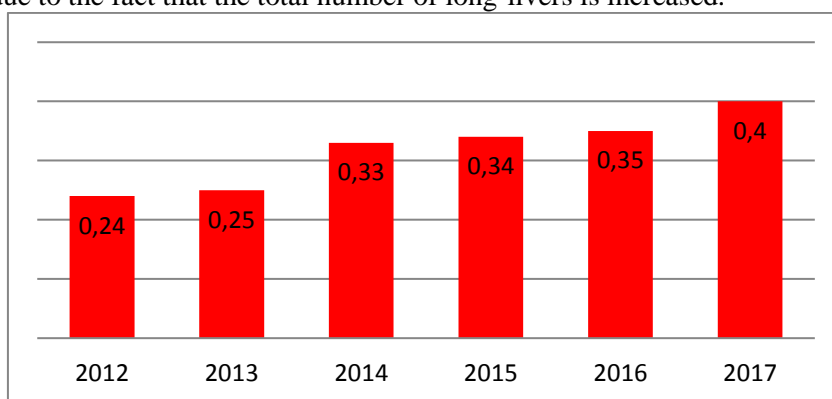
Figure 13. Dynamics (in percentage) of long-livers in Adjara A. R. in 2012-2017
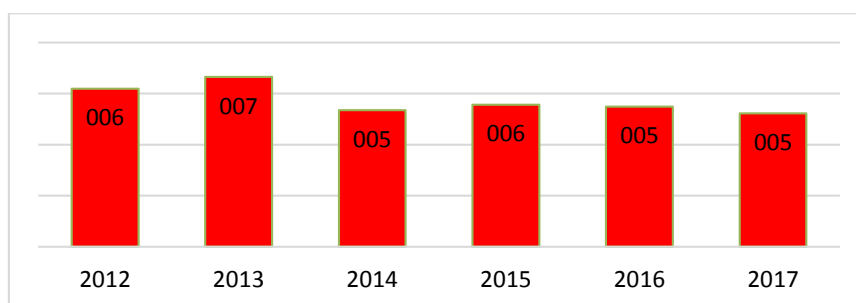
Figure 14. Dynamics (in percentage) of the Centenarians in Adjara A. R. in 2012-2017

Thus, in 2012 -2017, in the five-year dynamics in the Autonomous Republic of Adjara the long-livers concentration was increased; The number of centenarians, apart from Keda and Shuakhevi municipalities is growing according to years; These data coincide with the data taken in different parts of the world, where there is also an increase in the number of long-livers [7,8,9,10, 11,12,13,14,15].

4.Conclusions

In five years of dynamics (2012-2017) it has been studied the longevity rate in six municipalities of Autonomous Republic of Adjara. With the obtained results we conclude:

* Growth of the long-livers concentration can be observed in five municipalities in the five-year dynamics. The special increase was observed in Batumi, Khelvachauri and Kobuleti municipalities;

* The number of centenarians apart from Keda and Shuakhevi municipalities are growing in 5-year duration;

* The abovementioned data allows the implementation of the next steps of the research, where it will be possible to search and identify the main causes of the multifactorial process of aging which led to the increase the lifespan and the longevity 5 years duration in the Adjara populations.

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Research Article

FRAILTY AMONG THE BENEFICIARIES OF BATUMI ST. CATHERINE NURSING HOME

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Abstract: *Frailty is defined as "medical syndrome with multiple causes and contributors that is characterized by diminished strength, endurance, and reduced physiologic function that increases an individual's vulnerability for developing increased dependency and/or death. An assessment of Frailty in various populations, especially in the elderly population, has medical outcomes. The present work deals with the study of frailty in the beneficiaries of Batumi St. Catherine Nursing Home of. In total 70 beneficiaries of both sexes, were over 60 years of age, were investigated. The study was conducted in two years 2016-2017. Frailty phenotypes were assessed within five criteria: weight loss, self-reported exhaustion, weakness, slowness, and low physical activity. The existence of three and more criteria from these listed criteria are assessed as frail phenotype; the existence of one or two criteria - prefrail, and if a person does not have any criteria, it is assessed as robust. 25.7% of the elder beneficiaries turned out to be frailty phenotype, 12,9 % - prefrail phenotype, 61.4% - healthy or sustainable. Total frailty phenotype was identified in 18 samples, including 11 women and 7 men. Hard cognitive disorders from the 18 frailty phenotypes were found in only 3 individuals. Among the three most commonly researched populations in the frailty criteria are low physical activity, weakness, and slowness. In the group of people older than 80 years, the syndrome of frailty was higher (35.7%) than in the group of people older than 60 years. (19%). The gender difference was not observed. As the research has shown frailty syndrome is revealed in beneficiaries in both investigated groups. It is important to pay special attention to assess frailty syndrome in the nursing community, to be used timely medical, social or other activities.*

Keywords: *Frailty, nursing home, beneficiaries, elderly*

1. Introduction

Individuals of the same age may not age at the same rate. Quantitative biomarkers of aging are valuable tools to measure physiological age, assess the extent of 'healthy aging', and potentially predict health span and life span for an individual [1]. Aging is a major risk factor for most chronic diseases and functional impairments. Within a homogeneous age sample, there is a considerable variation in the extent of disease and functional impairment risk, revealing a need for valid biomarkers to aid in characterizing the complex aging processes. [2] Biomarkers are increasingly employed in empirical studies of human populations to understand physiological processes that change with age, diseases whose onset appears linked to age, and the aging process itself [3-5]. There is a lot of attention to studying longevity phenotypic markers. Phenotypic markers are relatively simple and non-invasive,

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although less accurate. Integration of these methods is essential for the complete analysis of healthy aging mechanisms [2].

In recent years, frailty syndrome spread among elderly populations has been actively studied. Frailty has long been considered synonymous with disability and comorbidity, to be highly prevalent in older age and to high risk for falls, hospitalization, and mortality. However, it becomes recognized that frailty may be a distinct clinical syndrome with a biological basis [6].

There are two opinions about the development of frailty: the first - the frailty of the elderly as a phenotype, the genotypically conditioned [7] and the second - the unity of acquired deficiencies developed on the background of polymorbidity [8].

In human populations, frailty may be revealed during different ages. The obtained results of research in age groups of different populations are not universal. According to the data available in scientific publications, frailty is prevalent in older people [9], the probability of syndrome is significantly increased after the age of 80 [10]. There are contradictory data, and some authors believe that the frailty is not an age-dependent process, and often the long-livers are not asthenic [11].

Among current operational definitions of frailty, the criteria proposed by Fried have attracted great scientific interest [12]. Fried and colleagues point out at five basic characteristics of frailty: weight loss, self-reported exhaustion, weakness, slowness, and low physical activity [7].

Thus, frailty can be considered as an adaptive response of the body which is followed by the slowdown in metabolism and the decrease of the action intensity of the body and body system. At the same time, frailty is considered a dynamic process that can be improved or worsened after a certain period of time [10, 13]. It is noteworthy that the risk of deterioration is higher, followed by incapacitation, hospitalization, and mortality [14].

Hence, it is important to identify the pre-asthenic prefrailty status and persons with a frailty risk group that will enable practitioners to determine further development and management capabilities.

The demographic aging rate is very high in the population of Georgia. In addition, several tens of thousands of older citizens are deprived of permanent residence (forcibly displaced people). Most of these people live in nursing homes. Thus, elderly population research is quite relevant in this direction. Especially in places where the concentration of older people is particularly high, it is important not only to detect and evaluate the phenotypes of frailty syndrome but also to find a resource that an elderly person has in a given case and to direct it to help them [2].

The aim of our research was to study frailty prevalence in the community of nursing homes. The target group was selected because of that the majority of beneficiaries are socially vulnerable people with various health problems.

2. Materials and methods

The research was conducted at Batumi St. Catherine nursing home. We have studied 70 beneficiaries from ages 60 to 93. The study was conducted for two years between 2016 and 2017.

In our research, we used a questionnaire (Brief Assessment Protocol Centenarians) modified by Porto University, which was approved in the study of Portuguese centenarian population [15]. The questionnaire includes demographic, social, health, functional, psychological and cognitive aspects. The questionnaire was translated and adapted to the Georgian reality.

The survey was conducted in the form of individual visits and face-to-face with researchers. One or two (seldom three) visits were made with each member of the study group. The study was conducted by the World Medical Association Declaration of Helsinki, Ethical Principles for Medical

Research Involving Human Subjects. All participants of the research have confirmed the willingness of voluntary participation in the experiment. The inquiry was attended by the representative of the nursing home. The obtained results were processed statistically.

During the survey, 122 beneficiaries lived in a nursing home. Since the target group was composed of people with the age of more than 60, 19 of them were excluded at the beginning of the research because of age incompatibility, 33 people did not participate in the study for various reasons: disability, tiredness or lack of desire to participate in the study. Finally, we have studied and analyzed the populations of 70 people of more than 60 years of age.

Identification of phenotypes of frailty syndrome was conducted according to the following criteria and procedures:

Socio-demographic characteristics included assessment and data analysis of age, sex, education, marital status, and living conditions; for determining the cognitive status we applied MMSE (Mini-Mental State Examination) [16], the questionnaire includes an assessment of cognitive, functional and behavioral disorders. Each component is measured by points (1 to 5); **Cognitive status** assessment also includes the evaluation of dementia range. Finally, according to cognitive status the study groups were divided into three groups: severe (0-17 points); moderate (18-23 points); mild (24-30 points); ADLs (Activities of Daily Livings) - This criterion assessment is based on the Duke University's Center for Disease and Human Development Research, [17] developed for American Population. The questionnaire was adapted for Georgian populations - taking into consideration the lifestyle and mentality of the population. The questions included daily activities: nutrition, bath taking, walking, personal hygiene protection, etc. Totally seven types of activities were estimated from 0 to 14 points; **Morbidity and Medical Treatment** - this criterion includes 14 different chronic diseases of cardiovascular, urinary, parenteral and other systems. Due to the high statistics of goiter in the local population, we added this disease to the questionnaire. We also indicated how many medicines a beneficiary daily received; **Subjective health** - this criterion was assessed based on the subjective response (feeling) of the survey. The questionnaire was divided into two categories: 1) excellent / very good / good; 2) fair / poor.

Finally, we have identified frailty phenotype by five criteria defined by Freid and coauthors: weight loss, self-reported exhaustion, weakness, slowness, and low physical activity. Three and more criteria from the listed criteria were assessed as frail; The existence of one or two criteria indicates frailty on the previous period and is defined as prefrail phenotype, which represents a high-risk group and is highly likely to progress. And if a person does not have any criteria, was assessed as robust [7].

3. Results and Discussion

We analyzed the findings of the research conducted due to the above-listed procedures and criteria in the study population, as well according to gender. A total of 70 samples including 43 (61.4%) female and 27 (38,6%) male. Since there is evidence that the probability of frailty detection significantly increases after the age of 80 [10], we also analyzed the frailty distribution by age groups. We separated two age groups: first from 60 to 80 years; second – more than 80 years old. The first table represents social-demographic and health survey results (Tab. 1).

Marital Status: Only three women from the study group have never been married; The majority of the study group are widows, including 21 females (30%) and 9 males (12,9%); 15,7% of respondents were divorced, from them - seven female and four male. At the moment of the survey, 37,1% of the respondents were married (12 female 14 male).

Among the first age groups (60-80) were 42 samples, among them 26 females (61,9%), 16 males (38.1%) were married, only two women were never married (4,8%), widow - 21,4%, divorced - 19,7%, living with a partner - 0%. It is noteworthy that the percentage of widows among the beneficiaries of the nursing home is twice more than men widows, while the married beneficiaries almost half in amount and almost equal in both sexes (26,2% female and 24% male), while there live more woman in the nursing home than man.

The second age group (80+) included 28 samples, among them 17 (60,7%) females and 11 (39,3%) mails. The percentage of widows in this age group is twice high compared to the first age group that is logical since this group is more elder. From the whole research group, married people were 17,9% (including the majority of men). One woman never was in marriage. All the respondents rejected the fact of co-existence with an informal (unmarried partner) partner. It seems that the reason is local traditions and elderly mentality.

Table 1. Results of Socio-demographic and health characteristics of the sample

	first age group (60-80)				second age group (80 +)				In total			
	Female		Male		Female		Male		Female		Male	
1. Living arrangements	n	%	n	%	n	%	n	%	n	%	n	%
Living alone	-	0	-	0	-	0	-	0	-	0	-	0
Living with others	-	0	-	0	-	0	-	0	-	0	-	0
Living in nursing homes	26	61,9	16	38,1	17	61	11	39	43	61,4	27	38,6
2. Marital status												
Single	2	4,8	-	0	1	3,6	-	0	3	4,3	-	0
Married	11	26,2	10	23,8	1	3,6	4	14,3	12	17,1	14	20
Widowed	6	14,3	3	7,1	15	53,5	6	21,4	21	30	9	12,9
Divorced	7	16,7	3	7,1	-	0	1	3,6	7	10	4	5,7
Non-marital partnership	-	0	-	0	-	0	-	0	-	0	-	0
3. Education												
High school education	11	26,2	11	26,2	8	29	7	25	19	27,1	18	25,7
Secondary school education	14	33,3	5	11,9	9	32	4	14	23	32,9	9	12,9
Illiterate	1	2,4	-	0	-	0	-	0	1	1,4	-	0
4. Subjective health												
Excellent	1	2,4	-	0	1	3,6	-	0	2	2,9	-	0
Very good	1	2,4	-	0	1	3,6	-	0	2	2,9	-	0
Good	7	16,7	3	7,1	-	0	1	3,6	7	10	4	5,7
Fair	8	19	8	19	6	21,4	3	10,7	14	20	11	15,7
Fair	9	21,4	5	12	9	32,1	7	25	18	25,7	12	17,1

Education: By this parameter, the survey group showed that more than half of the respondents (52.8%) have higher education (higher or technical), and almost half of them have finished the school (45.8%).

Only one female was not able to read and write; the people with the highest education were in equal number in both sexes.

Subjective health: We have set this query with the standard questions asked in the questionnaire: "How do you feel? What do you think about your health condition?" Most of the respondents estimated their health condition as satisfactory and bad. The assessment "good" was reported by 11 (26%), a "very good" – by two beneficiaries, "excellent" - also by two beneficiaries, it should be mentioned that last two, the most positive respondents were women.

Most of the respondents in the first age group evaluated their health condition as a "satisfactory" and "bad" - 71.5%. A "good" and "very good" assessment was made by a quarter of respondents, most of them were women. In the second age group, more respondents fixed the assessment "bad" comparing to the first group. However, despite biological age, 7.2% feel "good" or "very good", while one person evaluated his health as an "excellent".

Cognitive disorders were revealed in 40% of individuals. Dementia was found in 44,3% of respondents (20 women and 11 men), And without dementia - 35.7%. In addition, people with dementia are equally divided into first and second age groups (Tab. 2). It is noteworthy that the oldest people from the second age group did not show severe cognitive status and dementia, which once again proves that biological age is not a definitive determinant of human cognitive status and it is quite possible that a very old person was mentally healthy [18].

Table 2. Cognitive status and dementia index in the study population

	first age group (60-80)				second age group (80 +)				In total			
Cognitive status	Female		Male		Female		Male		Female		Male	
	n	%	n	%	n	%	n	%	n	%	n	%
Severe (0-17)	8	19,1	5	11,9	9	32	6	21,4	17	24,3	11	15,7
Moderate (18-23)	7	16,6	3	7,1	4	14,3	2	7,1	11	15,7	5	7,2
Mild (24-30)	11	26,2	8	19,1	4	14,3	3	10,7	15	21,4	11	15,7
Dementia												
Dementia stages (0-21)	10	23,8	5	11,9	10	35,7	6	21,4	20	28,6	11	15,7
Pre-dementia stages (21-24)	5	11,9	4	9,5	3	10,7	2	7,2	8	11,4	6	8,6
Without dementia stages (25-30)	11	26,2	7	16,7	4	14,3	3	10,7	15	21,4	10	14,3

From the defining criteria of the frailty syndrome (Tab. 3), the three most common criteria in our study population were: **low physical activity, loss of power and slowing down movement**. Based on the analysis of the data obtained, 25.7% of the researched population is frailty phenotype, 12,9% - prefrail phenotype, 61.4% - healthy or sustainable. There were 18 individuals in total with frailty, including 11 women and 7 males.

Table 3. Frailty dissemination in the study population

	first age group (60-80)				second age group (80 +)				In total			
	Female		Male		Female		Male		Female		Male	
	n	%	n	%	n	%	n	%	n	%	n	%
Weight loss	6	14,3	1	2,4	5	17,9	2	7,1	11	15,7	3	4,3
Weakness	4	9,5	2	4,8	7	25,0	5	17,9	11	15,7	7	10,0
Exhaustion	3	7,1	2	4,8	4	14,3	4	14,3	7	10,0	6	8,6
Slowness	-	0,0	3	7,1	9	32,1	7	25,0	9	12,9	10	14,3
Low physical activity	4	9,5	3	7,1	8	28,6	6	21,4	12	17,1	9	12,9
0 criteria	20	47,6	12	28,6	7	25,0	4	14,3	27	38,6	16	22,9
1 criterion	1	2,4	1	2,4	1	3,6		0,0	2	2,9	1	1,4
2 criteria	-	0,0	-	0,0	3	10,7	3	10,7	3	4,3	3	4,3
3 criteria	4	9,5	2	4,8	-	0,0	-	0,0	4	5,7	2	2,9
4 criteria	1	2,4	1	2,4	4	14,3	2	7,1	5	7,1	3	4,3
5 criteria	-	0,0	-	0,0	2	7,1	2	7,1	2	2,9	2	2,9

According to the obtained results, frailty syndrome is higher in the group of people over 80 years (35.7%) than in the first age group (19%). As we see, the frailty syndrome is correlated with age, the more the biological age of the age group, the more frailty phenotype can be found. The obtained data coincides with the findings of other European populations [8]. We could not find a significant difference in sexes.

The researchers often refer to cognitive disorders and frailty connections [15]. In our case, only three individuals were found from 18 frailty phenotypes of severe cognitive disorder. Perhaps the relationship could not be revealed because of the lack of sample amount, and on the other hand, the features of the population should be taken into account as well.

4. Conclusions and recommendations

As the research has shown frailty syndrome is revealed in beneficiaries in both investigated groups. The spread of frailty in various age group in nursing homes requires the study of their physical condition. It is important to be assessed frailty syndrome in nursing community for timely medical and social assistance and other activities. Complex emerging programs would help beneficiaries to keep their independent daily activity and delay their disability.

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Research Article

INVESTIGATION OF THE EFFECTS OF OCCUPATIONAL HEALTH AND SAFETY BEHAVIOURS ON THE CLIMATE SAFETY OF WORKERS ACCORDING TO PLANNED BEHAVIOUR THEORY

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Abstract: *The study was conducted to examine the relationship between safety climate and factors affecting occupational health and safety behaviors of workers. The research was carried out at the Mus Sugar Factory in Mus city center between July 2015 and December 2016. The researchers' universe was formed by 321 workers in the Mus Sugar Factory. To collect data: "Questions about socio-demographic characteristics of workers", 'The scale used to evaluate workers' behavior' and 'Security Climate Scale' were used. The average points of the scale used to evaluate the behavior of the workers were determined to be 122.04±21.840. The average points of workers' safety climate were found to be 53.87 ± 11.430. It was seen that the behavior of the workers was higher than the average of the total points, the gender, the family type did not affect the behavior (p>0.05), the norms of the chefs, the norms of the workmates and the security behavior affected the security climate (p<0.05).*

Keywords: *Safety behavior, Safety climate, Planned behavior theory, Worker.*

1. Introduction

Because people are a part of the society in which they live, they are in continuous interaction with their environment. Occupational health and occupational safety concepts addressing all types of physical, psychological and social effects of working order, which is part of everyday life, on people, have arisen. As a result of researches on the healthy and safe working of employees, the subject of protection and development of employees' health has become an extremely important issue [1].

The concept of occupational health first arose from the need to take the relevant measures towards the relationship between occupational life, occupational health, and wellbeing after the wars, and afterward state laid the burden on employers in this field.   Thus, the state has begun to make the necessary regulations to provide workers with minimum health and safety measures in the work environment. However, the acceptance of these rights at the constitutional level and their inclusion in international texts had been in the periods following the industrial revolution [2, 3].

The employer must provide a safe work environment and is responsible for protecting employees from any hazards (occupational accidents and occupational diseases) that may occur in the workplace [4, 5]. Occupational health and safety behavior refers to "employees' adaptation to behavioral safety

routines". These behaviors include safety activities that are a part of legal business roles and procedures and provided by the application of safety policies and procedures, such as the proper use of personal protective equipment, the application of proper business practices to reduce exposure to potential damage and risks [6]. Despite all kinds of technological progress facilitating the living conditions of today, it has not been possible to completely eliminate the probability of accidents and disease [7].

It is accepted that 80-90% of accidents occurred due to human errors [8]. Human errors are also explained by human behavior and it is considered that defining the components of individual behaviors will help to develop accident-preventing applications and to direct individual behavior to a safe dimension [9].

There are many socio-cognitive models that examine health and safety behavior under behavioral groups which are motivational and based on behavioral animation and multi-layered [10]. Planned behavior theory model that is one of these models is one of the most effective methods used in studies to understand human behavior [11].

Planned behavior theory and its antecedent theory of reasoned action provide cognitive and motivational effects on behavior and also a prediction of behavior. According to the theory, human behavior is under the influence of three factors. These factors are beliefs (behavioral beliefs) about similar behavioral consequences or other features of behavior, beliefs about normative expectations of other individuals (normative beliefs) and beliefs called as "control beliefs" related to the existence of obstructive or progressive factors in the realization of behavior [12, 13].

Safety climate is the perception that employees related to the safety of their workplaces and constitutes one of the distinctive features of organizations [14-16].

The safety climate provides a framework for interpreting organizational events and processes related to individual and organizational safety values and for fulfilling daily tasks [17]. It is mentioned that the mutual agreement provided related to the subjects of the safety climate and all the measures to be taken in the work environment positively affects the ability of the employees to properly analyze the accidents and gain experience from the accidents [18]. Efforts to protect, develop and maintain the health of employees require a multi-professional approach. The nurse included in the multi-professional team is the most suitable person to initiate practices in the workplace by monitoring the work environment and the employees during the working together with any changes and by planning any attempts about reason [19].

Therefore, there is a need for an occupational health nurse to know the occupational health and safety behavior of employees working in a factory well, to understand the crux of the problems and to develop approaches for increasing adaptation of workers to work depending on the information obtained.

This study was conducted to determine the relationship between factors affecting occupational health and safety behaviors of workers and safety climate.

2. Methods

2.1. Objective

The purpose of this study is to determine the relationship between the occupational health and safety behaviors of the employees, their perceptions about the security climate, their attitudes towards occupational health and safety, their defining subjective norms towards occupational health and safety, their perceived behavioral controls on occupational health and safety.

2.2. Place and Time of the Study

It was carried out as a descriptive and correlational study between July 2015 and December 2016 in a factory in Mus city

2. 3. Population and Sample of Study

The population of the study consists of 321 workers working in the Sugar Factory of Mus City. All the population formed the research sample. 303 people could be contacted due to some reasons such as sickness of workers, their being on leave, being out of shift and not willing to participate in the study.

2. 4. Data Collection Tools

Question form including the socio-demographic characteristics created by the researcher, scales used in the evaluation of the Behaviour Assessment Scale, Safety Climate scale was used.

2. 5. Socio-Demographic Question Form

It consists of 9 questions, including gender, age, marital status, family type, educational background, income status and information on working Period.

2. 6. Behaviour Assessment Scale for Workers

It is basically aimed to determine the occupational health and safety behaviors of the participants in the study and in the direction of this purpose, it has been evaluated the participants'

• Attitudes of workers towards occupational health and safety,

• Descriptive and preventive subjective norms towards the occupational health and safety of the chiefs and their colleagues,

• Behavioral controls perceived towards occupational health and safety,

• Occupational health and safety behaviors.

In the measurement of all the variables mentioned, different scales used by Fugas, Silva and Melia [6], Fugas et al [20], Davis et al [21], Conner and McMillan [22], Burke et al. [23], Hofmann, Morgeson and Gerras were gathered and the scale created was used [24].

• Attitudes of workers towards occupational health and safety:

The attitude scale towards occupational health and safety was measured with a 3-item scale obtained by Fugas et al. [20], Davis et al. [21]. By averaging the scale in their study. Cronbach's Alpha Value of Scale for Attitude is 94. In our study, Cronbach's Alfa value is determined to be 91.

Descriptive and preventive subjective norms towards the occupational health and safety of the chiefs and their colleagues: It was measured by four 3-item scales developed in the study of Fugas et al. [20]. Cronbach's alpha value of scale; Norms of Chiefs are 92, Norms of Colleagues are 95 dir. Cronbach's alpha value in our study; Norms of the Chiefs are 88 Norms of the Colleagues are 92.

Behavioral controls perceived towards occupational health and safety: It was measured by a 3-item scale adopted in the studies of Fugas, Silva and Melia [6], Conner and McMillan [22], Cronbach's Alpha Value of Scale is 86. Cronbach's Alpha Value in our study is determṣned to be 88.

Occupational health and safety behaviours: behavior was measured by a scale adapted to measure from 6-items under two subtitles of "adaptation behaviour to safety" and "preventive Safety Behaviour" created by "General-Safety Performance Scale" of Fugas, Silva ve Melia and by the scales of "Safety Citizenship Role Definitions and Behavioural Expressions" of Hofmann, Morgeson and Gerras [24] Cronbach's alpha value of scale for Safety Behaviour is 92. Cronbach's alpha value in our study is determined to be 88. They were measured according to the "strongly disagree" - "strongly agree" attitudes in 7'Likert scale as the interval. The high score suggests safe behaviour. Lowest 24, highest 168 points are received.

The scale consists of five sub-dimensions. These are **my Department Chief** (questions 1-2-3-4-5-6), which refers to the Norms of the Chiefs, **My Colleagues** (questions 7-8-9-10-11-12), which refers to the Norms of the Colleagues, **During my Work** (questions 13-14-15), which refers of Attitude, **at my Workplace** (16-17-18), which refers to Perceived Behavioural Control, and **Soon** (questions 19-20-21-22-23-24), which indicates the Safety Behaviour.

2.7. Safety Climate Scale

A scale has been recommended that was developed by Choudhry, Fang, and Lingard [25] applied in construction companies in Hong Kong and adapted by shortening the safety climate scale. The main reason for this shortening can be explained as follows: while the interest of the management that is the first of two dimensions of the scale of Choudhry, Fang and Lingard [25] and the dimension of employees' participation include positive points related to subject; the second dimension named inappropriate safety and business processes include negative points, and in general terms, the fact that the variables of these two dimensions are opposite expressions to each other. Because the opposite expressions in two different dimensions are approved in terms of the scale development methodology, 8 items from the 22 items have been eliminated and 14-items which are semantically different from each other were determined and used. Validity and reliability of scale have been made by Türen et al., [26] Scale consisting of 14 questions is 5' Likert type one. Options are as follows: "strongly agree", "agree", "neutral" "disagree" "strongly disagree". Two samples have been studied in the reliability and validity study of the scale. Cronbach's alpha coefficients were 0.84 and 0.93 for the first sample; and 0.93 and 0.91 for the second sample, respectively. In this study, the Cronbach's alpha value was found to be 92. Lowest 14, highest 70 points are received. A high score indicates a high security climate.

2.8. Research Model of Employees Converted According to the Planned Behaviour Model

In the model, it is seen that the descriptive and preventive norms of the chiefs merge under the factor of "the Norms of Chiefs"; the descriptive and preventive norms of the Colleagues under the factor of "the Norms of Colleagues" and adaptation to safety behaviour and preventive safety behaviour under the factor of "safety behaviour" (Figure 1.) [27]
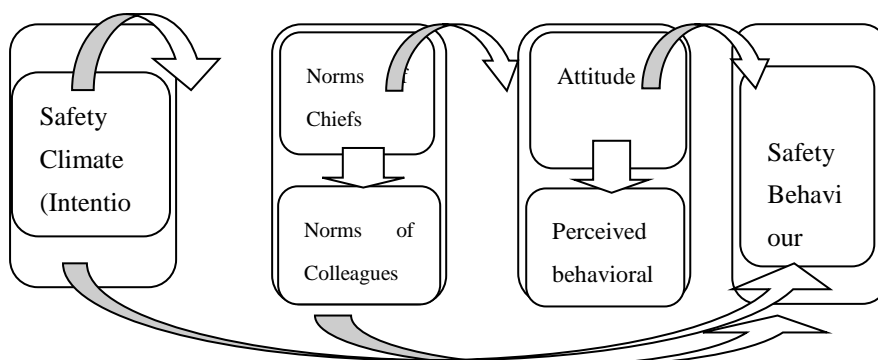
Figure 1. Research Model of Employees converted according to the Planned Behaviour Model

2.9. Variables of the Study

Independent Variables; Gender, age, marital status, family type, educational background, income status, working Period in the institution, total working Period, training at workplace related to the occupational health and safety are the independent variables of the workers who participated in the study. Dependent Variables; "Scales used in evaluating workers' behaviour", "Responses to questions related to the Safety Climate Scale" are dependent variables of this study.

2.10. Data Collection

The study data were gathered by the researcher during rest and break times between July 2015 and December 2016 and through approximately 15-20 minutes of face-to-face interview method with workers, when the time could not be enough.

2.11. Evaluation of Data

Data codification and their statistical analysis were performed by using Statistical Package for Social Sciences for Windows (SPSS) 22.0,   ready statistical package program. Methods used in the evaluation of data obtained as a result of research. Cronbach α reliability coefficient, Percentage distribution and mean, Dunnet Post Hoc test, Kruskall Wallis H test, Mann Whitney U test, t-test in Independent groups, Variance analysis, Pearson Moments Multiplication Correlation.

2.12. The Ethical Principles for the Study

Before starting the research, the ethics committee approval and the study permission from the research institution were taken. Workers participating in the study were informed about the study and their informed written consents were received before starting data collection**.**

2.13. Limitations of the Study

Taking workers serving at the factory in study and limitation of the population to the related factory is the most important limitation of this study.

3. Findings

The distributions of workers included within the scope of study according to their descriptive features are given in Table 1.

It is seen that 96.4% of workers participated in study were male, 37% were 35-44 years old, 81.5% were married, 59.4 % had extended family type, 47.2% were secondary education graduates, 53.8% had low income, working period of 39.9%   in institution was between 1-3 years, 35.6% of them was more than 10 years and 64% of them received training related to the occupational health and safety at the workplace (Table 1).

Table 1. Findings related to the Descriptive Features of Workers included in Study (n=323)

		S	%
Gender	Female	11	3.6
	Male	292	96.4
Age	17-24	19	6.2
	25-34	75	24.8
	35-44	112	37.0
	45-54	72	23.8
	55 years old and above	25	8.2
Marital Status	Married	247	81.5
	Single	56	18.5
Family Type	Extended Family	181	59.4
	Nuclear Family	119	39.3
	Broken family	3	1.3
Educational Background	Elementary Education	37	12.2
	Secondary Education	143	47.2

	Upper Secondary Education	93	30.7
	Undergraduate Education	24	7.9
	Postgraduate Education	6	2.0
Income status	Very Low	5	1.7
	Low	163	53.8
	Middle	48	15.8
	High	87	28.7
Working Period in the institution	Less than 1 year	12	4.0
	Between 1-3 years	121	39.9
	Between 3-6 years	38	12.5
	Between 6-10 years	28	9.2
	More than 10 years	104	34.3
Total working Period	Less than 1 year	17	5.6
	Between 1-3 years	87	28.7
	Between 3-6 years	63	20.8
	Between 6-10 years	28	9.2
	More than 10 years	108	35.6
training at the workplace related to the occupational health and safety at workplace	Yes	194	64.0
	No	109	36.0

It is seen that the mean score of the Norms of Chief that is a sub-dimension of the Assessment of Workers' Behaviour was 30.04±7.973, mean score of the Norms of Colleagues was 28.74±8.326, mean score of Attitude is 15.77±4.723, mean score of Perceived Behavioural Control was 14.59±4.886, mean score of Safety Behaviour was 30.23±7.759, mean score of Total Score of Scale used to assess Behaviour of Workers was 122.04±21.840 and Total of Safety Climate Scale was 53.87±11.430 (Table 2).

Table 2. The arithmetic mean and standard deviation values related to the scores of the Workers' Behaviour Assessment Scale and Safety Climate Scale.

	Min	Max	Mean ± SD
The dimension of Norms of Chief	6	42	30.04±7.973
The dimension of Norms of Colleagues	6	42	28.74±8.326
Attitude Dimension	3	21	15.77±4.723
Perceived Behavioural Control Dimension	3	21	14.59±4.886
Safety Behaviour Dimension	6	42	30.23±7.759
Total Score of Scale used to assess Behaviour of Workers	24	168	122.04±21.840
Total of Safety Climate Scale	14	70	53.87±11.430

From Table 3 the characteristics of the workers, it was found that there was a direct relationship between their age, their working time, and "the Norms of the Chiefs aspect", "the Norms of Co-workers aspect", "the Attitude aspect", "the Perceived Behavioral Control aspect" and "the Safety Behavior aspect" among the components of Planned Behavior Theory. In terms of "the Safety Climate Scale"

scores, no relationship was found. From the characteristics of the workers, it was found that there was a relationship between their educational status, income status and total working time, and "the Norms of the Chiefs aspect", "the Norms of Co-workers aspect", "the Attitude aspect", "the Perceived Behavioral Control aspect", "the Safety Behavior aspect" and "the Safety Climate Scale" among the components of Planned Behavior Theory, and it was found to be significant at p<0.05 significance level. From the characteristics of the workers, it was found that there was a relationship between their traineeship status related to occupational health and safety, at workplace, and "the Attitude aspect", "The Perceived Behavioral Control aspect", among the components of Planned Behavior Theory, and it was found to be meaningful at p<0.05 significance level. The attitudes during work and behavior Control status of those, who had occupational health and safety-related training at the workplace, are observed to be higher according to those who did not have.

Table 3. Comparison of Workers' Behavioural Assessment Scale and Safety Climate Scale Scores according to their Descriptive Features

		The dimension of the Norms of Chiefs	The dimension of the Norms of Colleagues	Attitude Dimension	Perceived Behavioural Dimension	Safety Behaviour Dimension	Total of Safety Climate Scale
		X± SD	X± SD	X± SD	X± SD	X± SD	X± SD
Gender	Female	26.73±5.985	25.45±7.699	15.55±5.502	13.00±4.733	26.27±7.577	46.64±12.675
	Male	30.16±8.020	28.86±8.336	15.78±4.702	14.65±4.889	30.38±7.739	54.15±11.314
	Test and p value	U=1119.50 P=0.088	U=1211.50 P=0.166	U=1585.50 P=0.942	U=1279.50 P=0.250	U=1120.50 P=0.088	U=1063.00 P=0.057
Age	1- 17-24	27.74±7.423	28.00±6.716	14.53±5.125	13.32±5.991	28.11±7.923	55.21±11.830
	2-25-34	29.24±7.168	27.63±8.183	15.17±5.295	14.00±5.181	28.91±8.326	52.39±11.550
	3-35-44	29.29±7.825	26.89±8.826	15.08±4.776	13.79±4.513	29.36±7.812	52.53±11.000
	4-45-54	31.46±7.838	31.28±7.792	17.29±3.891	16.03±4.654	32.32±6.794	56.42±10.868
	5-55 years old and	33.44±10.409	33.60±5.292	17.20±3.266	16.84±3.944	33.76±6.260	56.04±13.324
	Test and p value	**KW=17.076 P=0.002**	**KW=24.204 P=0.000**	**KW=12.363 P=0.015**	**KW=17.337 P=0.002**	**KW=14.122 P=0.007**	KW=8.516 P=0.074
Marital Status	Married	30.67±7.953	28.85±8.566	15.96±4.587	14.91±4.815	30.55±7.805	53.61±11.543
	Single	27.25±7.515	28.25±7.224	14.91±5.241	13.21±5.001	28.84±7.461	55.04±10.943
	Test and p value	**t=2.933 p=0.004**	t=0.486 p=0.627	t=1.509 p=0.132	**t=2.358 p=0.019**	t=1.493 p=0.136	t=0.842 p=0.401
Family Type	Extended Family	30.64±8.378	28.92±8.561	15.92±4.811	14.92±4.993	30.61±7.634	53.70±11.855
	Nuclear Family	29.12±7.242	28.47±8.077	15.46±4.624	14.19±4.663	29.83±7.935	54.10±10.945

	Nuclear Family	29.12±7.242	28.47±8.077	15.46±4.624	14.19±4.663	29.83±7.935	54.10±10.945
	Broken Family	29.66±10.214	28.00±3.605	15.66±3.785	10.33±5.507	23.00±5.291	54.66±1.527
	Test and p-value	KW=5.743 P=0.057	KW=1.499 P=0.779	KW=5.113 P=0.573	KW=4.005 P=0.135	KW=3.903 P=0.142	KW=.254 P=0.881
Educational Background	Elementary Ed	33.19±6.983	31.57±4.879	16.78±3.092	15.76±3.869	31.43±5.810	56.16±10.849
	Secondary Ed	30.71±7.834	29.48±8.843	16.80±4.662	15.38±5.036	31.77±7.669	51.74±11.834
	Upper Sec. Ed	27.01±8.101	25.18±7.695	13.22±4.660	12.59±4.550	27.15±7.786	55.23±10.488
	Undergraduate	31.21±6.547	32.50±6.447	16.96±3.951	14.88±4.812	30.00±7.940	55.63±12.122
	Postgraduate	36.67±4.967	33.83±10.265	19.67±2.338	18.67±1.966	35.00±5.899	62.67±7.448
	Test and p-value	**KW=27.279 P=0.000**	**KW=33.563 P=0.000**	**KW=43.280 P=0.000**	**KW=26.926 P=0.000**	**KW=25.552 P=0.000**	**KW=11.715 P=0.020**
Income status	Very Low	31.80±8.758	34.40±7.057	18.00±5.196	18.80±1.924	28.20±8.408	52.00±10.700
	Low	27.65±7.177	25.63±7.648	14.09±4.705	12.82±4.636	28.34±7.615	52.96±11.196
	Middle	31.52±7.565	32.04±6.919	17.31±4.273	16.13±4.527	31.33±7.171	53.08±11.725
	High	33.59±8.149	32.43±8.044	17.93±3.707	16.84±4.305	33.13±7.400	56.14±11.612
	Test and p-value	**KW=43.555 P=0.000**	**KW=58.748 P=0.000**	**KW=46.582 P=0.000**	**KW=51.001 P=0.000**	**KW=25.486 P=0.000**	**KW=6.351 P=0.000**
The working period in the institution	Less	33.33±7.127	31.17±6.658	16.58±4.502	14.08±6.022	32.58±4.379	51.33±13.963
	Betwee	26.77±6.957	25.30±7.966	14.02±4.596	12.72±4.646	28.05±7.937	52.72±11.129
	Betwee	30.63±6.578	27.42±7.108	14.76±5.000	14.18±4.632	29.47±7.062	53.89±10.755
	Betwee	30.64±9.378	31.82±7.124	16.36±4.801	16.29±4.353	31.32±7.803	55.43±11.157
	More	33.08±7.913	32.12±7.988	17.91±3.854	16.53±4.417	32.49±7.434	55.09±11.802
	Test and p-value	**KW=46.938 P=0.000**	**KW=49.751 P=0.000**	**KW=43.300 P=0.000**	**KW=39.506 P=0.000**	**KW=22.311 P=0.000**	KW=3.279 P=0.512
Total working period	Less	29.59±9.925	31.59±8.277	17.41±4.542	16.65±4.554	30.35±6.103	48.47±14.938
	Betwee	25.45±7.539	24.08±8.691	13.87±5.002	12.89±5.038	27.47±9.001	50.82±11.996
	Betwee	30.35±5.908	27.97±5.509	13.81±4.173	12.54±4.208	29.84±5.528	57.92±7.722
	Betwee	30.75±8.168	29.71±7.517	16.18±4.456	15.50±4.299	30.54±7.946	54.00±10.530
	More	33.44±7.277	32.24±7.807	18.07±3.696	16.61±4.370	32.59±7.324	54.80±11.594

		KW=53.243 P=0.000	KW=53.297 P=0.000	KW=56.055 P=0.000	KW=45.919 P=0.000	KW=20.790 P=0.000	KW=15.184 P=0.004
	Test and p-value						
Training status related to occupational health and safety at the workplace	Yes	29.85±9.002	28.89±9.205	16.27±4.761	15.21±4.963	30.65±8.310	54.55±12.098
	No	30.37±5.726	28.47±6.506	14.88±4.543	13.50±4.568	29.50±6.638	52.67±10.073
	Test and p value	t=0.609 p=0.543	t=0.467 p=0.641	**t=2.474 p=0.014**	**t=2.946 p=0.003**	t=1.324 p=0.187	t=1.377 p=0.169

Table 4. Relationship between Worker's Behaviour Assessment Scale and Safety Climate Scale Score

		Total of Safety Climate Scale
The dimension of the Norms of Chiefs	r	**,181****
	p	**,002**
The dimension of the Norms of Colleagues	r	**,295****
	p	**,000**
Attitude Dimension	r	,083
	p	,148
Perceived Behavioural Dimension	r	,077
	p	,179
Safety Behaviour Dimension	r	**,233****
	p	**,000**

(*) p<0.05   (**) p<0.001

From Table 4, when we evaluate the relationship between occupational health and safety behavior and safety climate, it is determined that there is no effect of person's attitude and perceived behavioral control in the relationship between safety behavior and safety climate, and it has a direct effect from the descriptive and preventive norms of the supervisors and colleagues at the workplace. In the study, it was determined that the safety climate had an indirect effect on the preventive safety behavior and compliance with the safety behavior, the effect of the security climate on behavior is provided entirely through planned behavioral theory components; the strongest effects on preventive safety behavior were the definitive norms of co-workers (r = 0.295 and p < 0.0001) and the preventive norms of the chiefs (r = 0.181 and p < 0.0001), and these variables had a fully mediated effect.

4. Discussion

Behaviour Assessment Scale for Workers participating study (BASW) was found to be 122.04 ± 21.840. This finding suggests that workers exhibited positive health behaviors, and workers' safe behavior levels were above the median. This result shows that workers have high occupational health and safety behaviors. (Table 2). In the study conducted by Avcı in the rest area, it is also stated that the mean scores for BASW were above the median [27]. In our study, mean scores of Total of Safety Climate Scale were found to be high as 53.87±11.43. This result shows that workers perceive the security climate as high. In the study with healthcare employees by Gül, total safety climate levels were

found to be high [28]. It was determined that there was a statistically significant difference between the ages of the workers participating study and the mean scores of the BASW sub-dimension, and similar results were found in the studies conducted by Yurtçu and Gyekye and Salminen [29, 30]. In this study, when the workers' Educational Background is discussed; A significant difference was found between the mean scores of all the sub-dimensions of the BASW and this finding indicates that the educational background of the workers is influenced by the chiefs and colleagues and by their exhibition of positive safety behaviors. Studies performed show similar results [28-30]. When the mean scores of the BASW sub-dimension are evaluated according to the educational background of the workers participating in the study; we can say that workers have shown safer behavior as their educational status increases.

It was found that the difference between the mean score of the safety climate scale and education was significant. In the studies of Garcia, Boix and Canosa [31], Hahn and Murphy [32] there was no significant relationship between safety culture/safety climate variables and Educational Background, but in the studies of Gül, Yegin, and Külekçi, it is indicated that educational level effected that safety climate [33-35]. There was a significant relationship between the mean scores of BASW sub-dimension and Income status. It is seen that the workers with high incomes status perceive the norms of the chiefs and the safety behaviors at a higher level. We can say that this result, that is good income status, is effective on sayings of their bosses and on their own behaviors. In the study of Ocaktan with the workers working in the automotive factory, he stated that satisfaction of employees having good and high-income status about the workplace and their perception of occupational safety are influenced positively [36].

A significant relationship was also found between the mean score of the safety climate scale and Income status. It was found that the total mean scores of the workers in the high-income group who participated in the study were higher than the other income groups. In the direction of these results, it can be said that employees who are financially good perceive themselves as safer the workplace. In the study of Ocaktan, it was also stated that more increase in income status, more increase also in safety perceptions [36]. It was determined that the difference between the mean score of BASW sub-dimension of workers and their total working periods was significant. When assessing the sub-dimension mean of THE Safety Behaviour, we can say that those working more than 10 years have higher mean scores than other employees and in the direction of this result, workers exhibited safer behaviors with the more increase in working period. Similar results are also given in the studies of Koç and Kaplancan [37]. The difference between the total score of the safety climate scale and the total working period was found to be statistically significant. In the study of Tüzüner and Özaslan [38] any difference between the total working period and the occupational safety perception at the workplace could not be found, but it was stated in the studies of Ocaktan, Vinodkumar and Bhasi that more working year, more increase in the perception of safety climate [36, 39]. When discussing about receiving training related to occupational health and safety for the mean scores of BASW sub-dimension of workers, a significant difference was found between the mean scores of attitude and perceived behavioral control sub-dimension. (Table 3). It can be said in the direction of these findings that individuals trained on occupational health and safety, in their attitudes about it and in their negative behavioral control, could provide it in a more desired level. While a significant relationship is found in the comparison of the Worker's Behaviour Assessment Scale (BASW) sub-dimension, Dimension of the Norms of Chiefs, Dimension of the Norms of Colleagues and Safety Behaviour Dimension; found that employees have more reliable behavior as their Chiefs and colleagues increase norms and Total of Safety Climate Scale scores, any significant relationship could not be found between Attitude Dimension and Perceived Behavioural Dimension

(Table 2). It can be said that the Dimension of the Norms of Chiefs, Dimension of the Norms of Colleagues and safety behavior dimension scores shall increase when the score of Total of Safety Climate Scale increases. As the perception of the security climate in the workplace increases, the safety of employees increases. When we evaluate these findings in the direction of the planned behavior model, we can say that the relationship between the safety climate perception and occupational health and safety behavior is not the effect of attitude and perceived behavioral control, but the dimension of the norms of chiefs and safety behavior were affected significantly. (Table 4) We can say that the safety behaviors of workers, Chiefs and colleagues affect both themselves and the safety perception of the workplace. It has been indicated in studies on this subject that safety behavior was effected by the safety climate. We can say that with an increase in safety culture in enterprises, safe behaviors will also increase. In direction of the results of our study, it was determined in the study of Avcı [27] that the safety climate influenced the norms of chiefs, norms of colleagues, attitude, perceived behavioral control and safety behavior.

5. Conclusions and Recommendations

The following results were obtained in the study conducted to examine the effects of occupational health and safety behaviors on safety climate according to workers' planned behavior theory. It was found that

• Workers exhibited moderately safe behaviors,

• Workers had high safety climate perceptions,

• Educational Background, Income status and total working periods of workers affected safety behaviors and safety climate,

• Workers' training status related to occupational health and safety affected attitude and perceived behavioral control,

• Workers with high incomes were found to have a high score in the dimension of the Norms of Chiefs, dimension of Safety behavior and safety climate,

In the direction of these results, it can be recommended;

• to increase the effectiveness of training for occupational health and safety,

• to have personal, especially occupational health nursing staff at the workplaces, for providing counseling services in terms of some features such as safety behaviors of the workers and their educational levels, working period, income status influencing the safety climate

• to do initiative studies towards increasing occupational health behaviors and safety climate

• the organization of supportive programs such as rewarding safe behaviors because of the the behavior of colleagues is important for employees to show safe behavior

• It can be suggested that the personnel working in the field of occupational health should be in the works supporting the safe behavior of the occupational health physician and the occupational health nurse.

Acknowledgments

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HISTOLOGICAL OF CHANGES IN THE AMNIOTIC MEMBRANE AND PLACENTAL VILLOUS BASAL LAMINA IN COMPLICATED PREGNANCIES

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Abstract: *This study aimed to histological changes compare placental villous basal lamina and amniotic membrane changes in complicated pregnancy. Studies were performed on the human placentas, delivered between 24-39 weeks of gestation. Patients were separated equally into 4 groups (Control, preeclampsia (PE), gestastional diabetes (GD), and HELLP syndrome groups). Placental tissue samples were dissected and fixed in 10% neutral formalin buffer. Routine paraffin tissue protocol was followed. Some of the sections were stained with Periodic Acid Schiff. Remaining sections were stained with integrin alpha-6 antibody. To define expression percentage, mean of the staining area/total staining area ratio were calculated. The statistical significance of the expression percentages was compared by One Way ANOVA and Tukey tests with SPSS Statistics V24 software. In PAS-stained preeclamptic, HELLP and gestational diabetes groups placental villous basal lamina and vasculo-syncytial membranes were thicker than the control group. A significant difference was observed in all 3 groups compared to the control group the placental villous basal lamina thickness of the HELLP group was found to be significantly different from all three groups. In chorionic villi of HELLP group, dense integrin expression was found in placental villous basal lamina similar to that in GD and preeclampsia groups. The HELLP group was significantly different from all groups. In preeclampsia, gestational diabetes and HELLP placentas, the placental viiöz basal lamina and amniotic membrane significantly thickened and structural changes were observed.*

Key Words: Placenta, Basal lamina, Preeclampsia, Gestational diabetes, HELLP syndrome

1.Introduction

The placenta develops where complex and coordinated interactions undergo between fetal embryonic tissue and pregnant endometrial tissues. Throughout the pregnancy, even on the 10th day of pregnancy, a large number of placental macrophages were detected in the placenta [1]. Placental macrophages consist of Hofbauer cells in the fetal chorionic villi and decidual macrophages in the maternal decidua basalis [2]. Among the functions, placental macrophages produce factors that regulate local immune reactions (factors that regulate maternal immunological tolerance and protect the fetus) and stimulate placental angiogenesis during placental development [3].   The integrin alpha 6/beta 4 complex is a member of the integrin family of adhesion receptors. It is found in a variety of epithelial cell types but is most strongly expressed in stratified squamous epithelia. It is a receptor for laminin in epithelial cells and it plays a critical structural role in the hemidesmosome. The alpha 6 subunit is also strongly localized to the basal region but also, is present over the entire surfaces of basal cells and

some cells in the immediate suprabasal region. Localization is generally cell surface and hemidesmosome [4].

This study aimed to histological changes compares placental villous basal lamina and amniotic membrane changes in complicated pregnancy.

2. Materials and methods

The Ethics Committee of Research Center of Dicle University Hospital approved the protocol. Studies were performed on the human placentas, delivered between 24-39 weeks of gestation. Patients were divided equally into 4 groups (Control, preeclampsia, gestastional diabetes, and HELLP syndrome groups) with a total of 40 full-term human placentas. All women recruit were singleton pregnancy, between 24- 39 weeks of gestastional age. These patients were aged between 20 and 37 years (mean ±SD: 27.2±3.1 years). The birth weight of the 40 live births was 1850 -3300 grams. The average placenta weight of control group was 672 g, PE group 387 g, HELLP group 340 g, and in GD group 491 g respectively. For grouping, the World Health Organization (WHO) criteria were considered:

Diagnostic criteria for preeclampsia are systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg after 20 weeks of gestation. Proteinuria ≥0.3 g in a 24-hour urine specimen.

In the Tennessee Classification System diagnostic criteria for HELLP (hemolysis, elevated liver enzymes, and low platelet count) are hemolysis with increased LDH (> 600 U/L), AST (≥ 70 U/L), and platelets < 100,000 cells/mm.

 Criteria for diabetes mellitus in pregnancy should be diagnosed with fasting plasma glucose 7.0 mmol/L (126 mg/dL).

Placental tissue samples were dissected and fixed in 10% neutral formalin buffer. Routine paraffin tissue protocol was followed. 5 µm paraffin sections were stained with Periodic Acid Schiff (PAS). In these sections, the thickness of the plasental villous basal lamina and the amniotic membrane was measured by ocular micrometer and the differences between the groups were evaluated. Remaining sections were stained for immunohistochemistry. To evaluate the integrin expression level of basal membrane, sections were incubated with anti-integrin alpha-6 antibody (cat no: ab133386, dilution rate: 1/750, abcam). 5 areas were randomly chosen where the terminal villi were densely stained. To define expression percentage, mean of the staining area/total staining area ratio were calculated. The statistical significance of the expression percentages was compared by One Way ANOVA and Tukey tests with SPSS Statistics V24 software (IBM, USA) and $p<0.05$ was considered statistically significant. Samples were evaluated by Zeiss Imager A2 light microscope and micrographed.

3. Results

3.1. Histochemical Findings: In PAS-stained preeclamptic, help and gestational diabetes group placentas Placental villous basal lamina and vasculo-syncytial membranes were thicker than the control group (Figure-1). In some sections, there were amniotic membrane abnormalities observed in preeclampsia and HELLP group samples (Figure-2).

Placental villous basal lamina thickness measured by 400x magnification in the microscope is shown in Table-1. According to the One-Way Anova, Post-hoc Tukey tests, Placental villous basal lamina thickness of the control group was found to be 1.35 ± 0.24 µm. A significant difference was observed in GD and PE groups ($p <0.05$) compared to the control group. There was no significant difference in Placental villous basal lamina thickness of GD group (2.02 ± 0.13 µm) and preeclampsia group (1.99 ± 0.14 µm) ($p> 0.05$). The placental villous basal lamina thickness of the HELLP group was

found to be 2.70 ± 0.44 µm and found to be significantly different from GD and preeclampsia groups (p <0.05) and control group (p = 0.00).
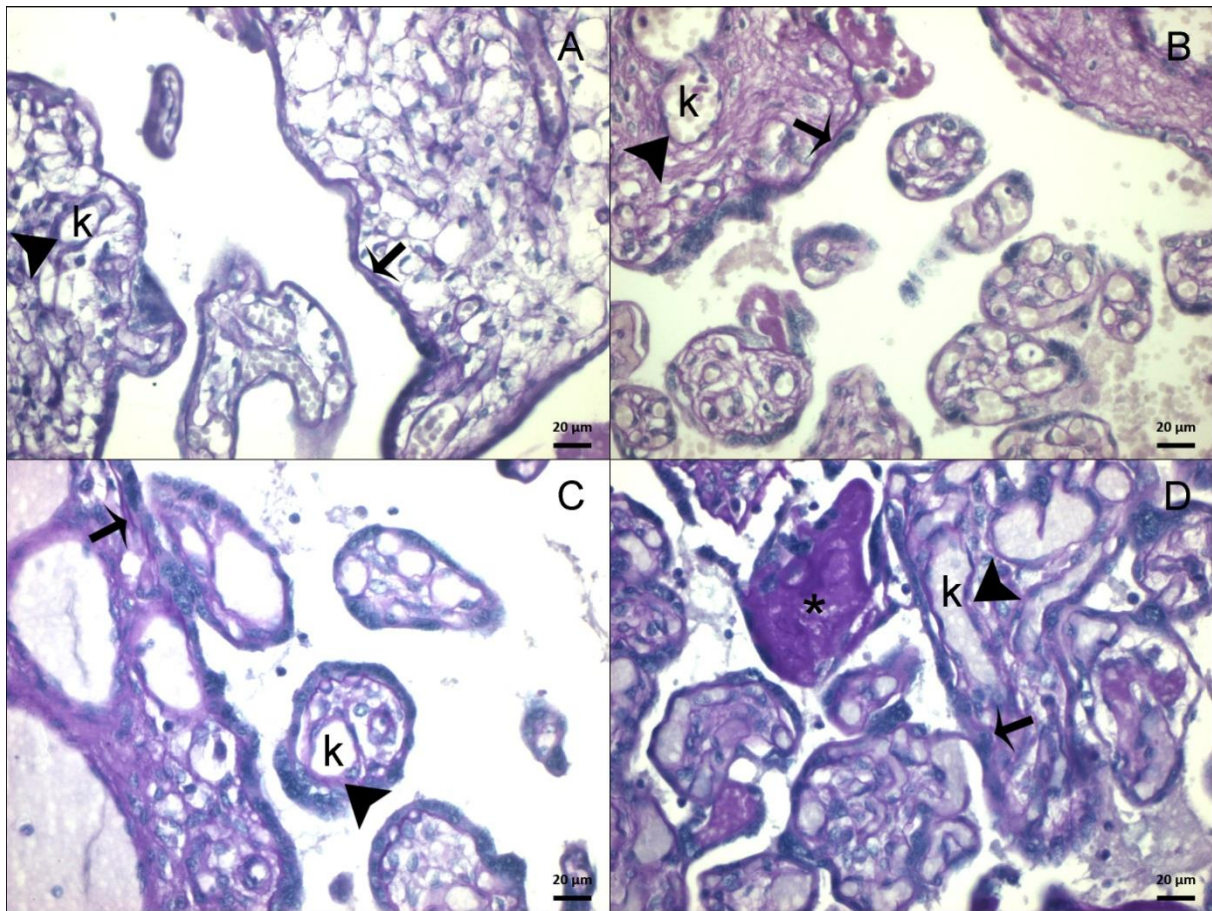

Figure-1: Placental sections stained with PAS. Note thickening of the placental villous basal lamina. **A)** Control group, **B)** Preeclampsia group, **C)** Gestational diabetes, **D)** HELLP group. **Arrow:** placental villous basal lamina. **Arrowhead:** Capillary endothelial basal lamina, **asteriks:** Fibrin, **k**: Capillary, (PAS, Bar: 20 µm).
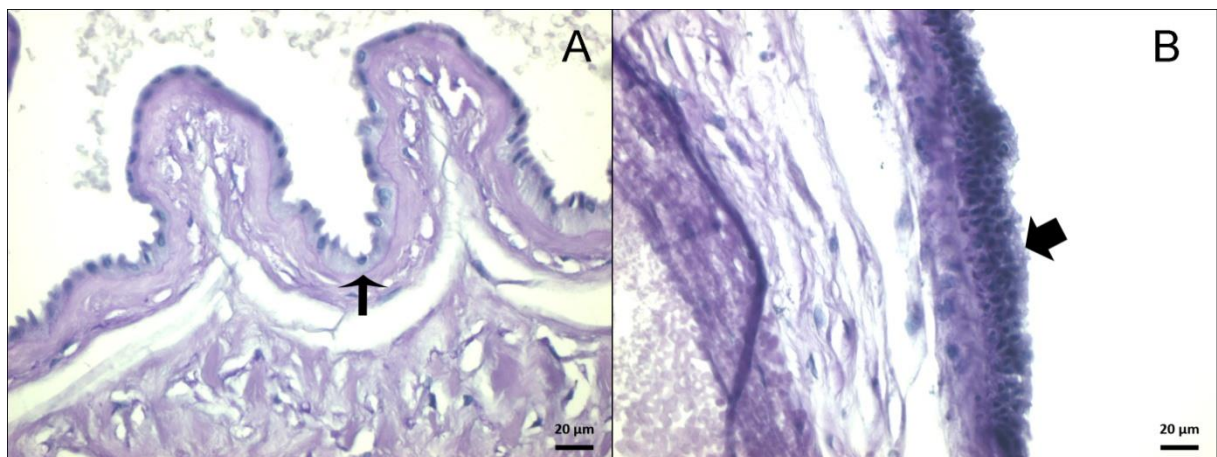

Figure-2: Thickening of the amniotic membrane and squamous metaplasia in the epithelium. **A)** Preeclampsia group, **B)** HELLP group, **thin arrow:** Amniotic membrane, **thick arrow:** Squamous metaplasia (PAS, Bar 20 µm).

Table 1: Placental villous basal lamina thickness and Standard Deviation. Values with different superscripts in the same column are significantly different (a-b: p <0.05, b-c: p <0.05, a-c: p = 0.00).

Groups	Mean (µm)± S.D.	Maximum (µm)	Minimum (µm)
Control	1,35±0, 24[a]	1,92	1,06
GD	2,02±0, 13[b]	2,20	1,79
PE	1,99±0,14[b]	2,17	1,73
HELLP	2,70±0, 44[c]	3,54	2,14

3.2. Immunohistochemical findings: We observed that integrin is expressed in various areas in control, PE, (GD) and HELLP groups. In the control group, while expression level was normal in the placental villous basal lamina, we observed increased expression in the placental villous basal lamina of GD and PE groups. In chorionic villus of HELLP group, dense integrin expression was found in placental villous basal lamina similar to that in GD and PE groups. Additionally, integrin expression was detected in the basal membrane of capillaries of all groups. Microscopic evaluation revealed that GD, PE, and HELLP groups had more intense expression levels in vasculo-syncytial membranes (Figure-3).

According to the results of the One Way ANOVA, Post-hoc Tukey tests of the expression analysis performed with the Image J software: The control group basal membrane integrin expression was 16.20 ± 2.10% in the randomly selected areas and the expression area in the GD and PE groups (p <0.05) and HEELP group (p = 0,00) observed significantly different. %29,60± 1,71 GD group and %27,30 ± 2,21 PE group were similar expression area (p>0.05). It was determined that the %35,30 ± 2,54 expression area of the HELLP group was significantly different from GD and PE groups (p <0.05) and control group (p = 0,00) (Table-2).
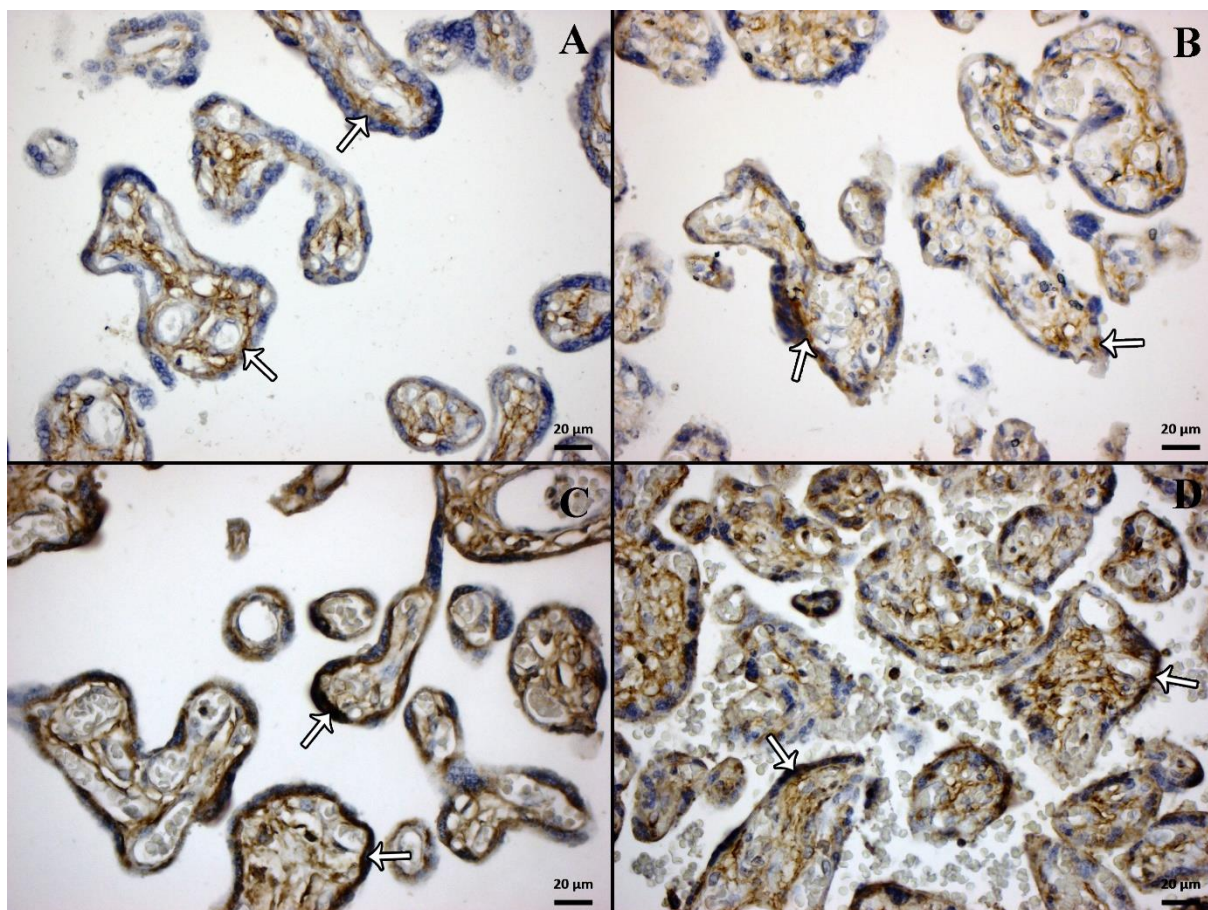
Figure-3: Placental sections stained with integrin alpha-6 antibody. While normal levels of integrin expression (arrow) were observed in the control group, it was increased in expression areas (arrow) of GD, preeclampsia and HELLP groups. **A:** Control group, **B:** Preeclampsia group, **C:** Gestasyonal diabetes group, **D:** HELLP group (Bar: 20 μm, Counterstain: Mayer Hematoxylin, integrin alpha-6).

Table 2: Covered area of integrin expression. Values with different superscripts in the same column are significantly different (a-b: p <0.05, b-c: p <0.05, a-c: p = 0.00).

Groups	Mean (%)±S.D	Maximum (%)	Minimum (%)
Control	19,20±2,10[a]	22,00	16,00
GD	27,60±1,71[b]	30,00	24,00
PE	27,30±2,21[b]	31,00	23,00
HELLP	35,30±2,54[c]	41,00	32,00

4.Discussion

The placenta is an essential organ for the exchange of nutrients and metabolites between the mother and the fetus. The thickness of the placenta depends on the length of the stem villi [5] Placenta develops in two phases: hyperplasia and subsequent hypertrophy [6-8]. Fetal hyperglycemia may disrupt the osmotic environment leading to cell damage and cell death [9]. This process also involves capillary endothelium. Because the damaged capillary endothelium is present in the former basal laminae, the endothelium regenerates itself [10]. As the newly formed endothelial cells synthesize their basal

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laminae, the endothelial basal laminae of the fetal capillaries in the chorionic villi become extremely thick. Since chorionic capillary basal lamina is a part of the Placental villous basal lamina, it makes placental basal membrane thickened. This thickening means the reduction of oxygen and nutrient transport to the fetus. The organism responds with an increase in placental weight with hyperplasia and basal lamina thickening in terminal villi. In a study by Salvatore et al. [11], Placental villous basal lamina of normotensive placentas was considerably thicker compared to HELLP and diabetic group placentas. Our study supports this evidence. Normal fetal development and growth depend on the function of the placenta. Some forms of intrauterine growth retardation result from placental blood flow failure and insufficient transport of nutrients from mother to fetus [12]. In this study, significantly increased cell hyperplasia and placental villous basal lamina were observed in preeclamptic, HELLP and gestational diabetic placentas (Figure-1) but not observed in placentas of the control group. The Placental villous basal lamina thickness may be the result of the accumulation of mucopolysaccharides. This condition occurs as a result of intrauterine growth retardation and uteroplacental circulation insufficiency [13]. Also, there was a significant thickening of the vasculo-syncytial membranes of the gestational diabetic group and this may be due to the increase in the diffusion distance between mother and fetus blood [12].

In our study, we observed Placental villous basal lamina and vasculo-syncytial basal membrane thickness of placentas in all group except for the control group, congestion in intervillous space, villous edema and thinning in a decidual basal membrane. These findings are similar to previous studies [15]. Placental villous basal lamina and amniotic membrane have significantly thickened and underwent structural changes in groups of preeclampsia, GD and HELLP groups.

In conclusion, in preeclampsia, gestational diabetes, and HELLP syndrome placentas, Placental villous basal lamina and amniotic membrane significantly thickened and structural changes were observed.

Acknowledgment

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Research Article

EFFICIENCY, EQUITY, AND EFFECTIVENESS OF MALARIA FUNDING IN UGANDA: MAKING THE CASE FOR REFORMING PUBLIC FINANCING FOR HEALTH

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Abstract: *Decreasing external funding for malaria requires high burden countries such as Uganda to increase domestic public financing to control and eliminate malaria. This study aimed to examine how the Government of Uganda, in collaboration with development partners and private sources of funding, has directed public resources in the recent past to help address the malaria burden. Data search and collection included national, regional and global reports and global databases. Government reports including National Development Plans, national health accounts, national demographic health surveys, and other reports such as the Uganda Medicines Price Monitor, were included in the review. The findings indicate that government funding for malaria is limited (10% of the total budget for malaria programming). Households (67%) are the main source of funds which makes malaria control in Uganda unsustainable. Public allocations are also inefficient with only 30% of funds allocated for preventive services contrary to global and national malaria control priorities. Evidence of inequity in the implementation of malaria programming is closely linked to poverty and ownership of preventive materials such as ITNs. Poor regions also report high malaria case incidence. Unavailability of antimalarials is reported in lower-level facilities serving mainly the poor. Overall progress in improving key malaria indicators is poor. There is also no correlation between expenditure on malaria and the availability of ITNs ($R^2=0.6631$). In conclusion, public financing for primary services such as malaria requires reforms to strengthen health sector performance in terms of sustainability, efficiency, effectiveness, and equity.*

Keywords: Malaria, Public Finance, Uganda

1. Introduction

The Uganda Vision 2040 aims to transform the Ugandan society from a peasant to a modern and prosperous country by the year 2040. To realize this vision, the Uganda National Development Plan (NDP) II is meant to steer the country toward middle-income status by 2020[1]. The pursuit of this goal has called for the transformation of the health sector to provide access to quality health care for all Ugandans through the delivery of promotive, preventive, curative, palliative and rehabilitative health care, with particular focus on primary health care. The drive toward Vision 2040 has precipitated a relatively rapid expansion of government spending catalyzed by a strong population growth rate and inflation. The increased spending by the Government of Uganda (GOU) has highlighted a number of high priority sectors but has been less beneficial to the health sector; i.e., of the Uganda Shillings (UGX)

7.8 trillion increase in government spending between FY 2012/13 and FY 2016/17, the health sector got a mere 6% of the total share compared to about 21% for Works and Transportation sector, Education (13%) and Justice, Law and Order (10%), among other priorities[2]. Noting that the health sector is increasingly facing a heavy double burden of communicable and non-communicable diseases (NCDs) yet is critical to the achievement of Vision 2040, the expectation was that the sector would be accorded high priority in government spending.

To contain the growing burden of disease in Uganda, one of the key priorities for the government is malaria control. Malaria is one of the deadliest diseases in human history and its increasing burden in Uganda requires significant effort in funding and infrastructural improvements to bring it under control and set the country on the path of elimination. Based on the World Health Organization (WHO) guidelines, Uganda is still at the first stage of controlling malaria, let alone elimination. The high malaria burden in Uganda is self-evident: the World Malaria Reports of 2017 and 2018 state that Uganda is one of the 15 countries that contribute 80% of the global malaria burden[3, 4] and one of the five countries that accounted for nearly half of all malaria cases worldwide[4]. The main contributors to the malaria burden globally are Nigeria (25%), the Democratic Republic of the Congo (11%), Mozambique (5%), India (4%) and Uganda (4%)[4]. In 2016, Uganda accounted for 4% of the global share of malaria cases and 3% of total deaths from the disease. Besides, Uganda reported a twofold increase in confirmed cases during 2015–2016 compared to 2013, which was largely attributed to inadequate vector control, improved reporting and climatic factors[5]. The World Malaria Report [3] confirms that Uganda is one of the countries experiencing increases in case incidence accounting for 17% of estimated malaria cases in East and Southern Africa.

As a major public health problem that is endemic in approximately 95% of Uganda, clinically diagnosed malaria is the leading cause of morbidity and mortality in the country, accounting for 30% to 50% of outpatient visits at health facilities, 15% to 20% of all hospital admissions, and up to 20% of all hospital deaths[5]. Deaths that occur at home, especially among children under five years of age, remain unreported. According to the MOH, Uganda has the sixth-highest number of annual deaths from malaria in Africa, as well as some of the highest reported malaria transmission rates in the world, with approximately 16 million cases reported in 2013 and over 10,500 deaths annually. Besides, there are socioeconomic implications including high out-of-pocket expenditure estimated to be between USD 0.41 and USD 3.88 per person per month (equivalent to USD 1.88 and USD 26 per household). Household expenditure for malaria treatment is also a high burden to the Ugandan population, consuming a larger proportion of the incomes in the poorest households[5, 6].

With the intervention of the President's Malaria Initiative (PMI) in 2005, Uganda has had some improvement in malaria control; however, a lot still needs to be done to build the capacity of the National Malaria Control Program to be able to progressively work toward reducing the malaria burden and establish structures for elimination. In this respect, the Ugandan Government policy goal is to reduce mortality due to malaria by 80% of the 2010 levels and reduce morbidity due to malaria by 75% of the 2010 levels. The key policy strategies toward this goal include timely and effective malaria case management, national scale-up of long-lasting insecticide-treated bed-nets, prevention of malaria in pregnancy, and indoor residual spraying[5].

The strategies outlined above require the significant mobilization of domestic funding and the consistency to improve national malaria outcomes, something which is not happening currently. In line with the 2001 Abuja Declaration and global estimates, Uganda's domestic financial contribution to

malaria reduction needs to increase if this reduction strategy is to succeed. The Global Technical Strategy (GTS) for Malaria 2016 - 2030 estimated that global malaria spending would rise from about USD 3.9 billion in 2016 to USD 6.6 billion in 2020 to scale-up and sustain the control and elimination of malaria globally[4]. Sadly, these estimates have not been met; e.g. there was a funding shortfall of about USD 1.0 billion in 2016 which increased to USD 1.3 billion in 2017[4].

Based on these high global shortfalls, the response from high burden countries such as Uganda is critical because national financing is crucial to the financial requirements in malaria control and elimination. As much as malaria is preventable and treatable, progress worldwide has installed and more-so in high-burden countries. Responses to end malaria need to be more efficient, equitable and effective to be sustainable. This review aimed to examine how the Government of Uganda has directed public resources of the past four years or so to help address the malaria burden, in collaboration with development partners and private sources of funding.

2. Methods

This was part of a public expenditure review (PER) exercise for Uganda's Ministry of Health that took place from December 2017 to June 2018. The aim was to assess the performance of public expenditure planning, allocation, and execution processes in Uganda.

The main data sources were government reports including planning documents such as National Development Plan (I & II), various national health accounts (NHAs), national demographic health surveys, and other reports such as the Uganda Medicines Price Monitor (2015). Regional reports included those from Medicines for Malaria Venture (2008), Coalition for Health & Social Development (HEPS 2014) and Access, Bottlenecks, Costs & Equity (ABCE) survey. Several national reports were downloaded from the webpages of the Uganda Bureau of Statistics (UBOS) and the Ministry of Health. Some of the global reports included in the review were the World Health Statistics (2017), World Malaria Report 2017 and the Global Fund report on malaria. Search strategies involved accessing specific webpages such as the Ministry of Health, Uganda Bureau of Statistics, World Health Organization as well as accessing data from Uganda's health management information system (HMIS). Additional data were searched using a combination of terms such as "malaria financing Uganda", "malaria budget Uganda", "malaria indicators Uganda" and "malaria report Uganda". A few data gaps were filled through key informant interviews although this was very limited. The analysis was mainly descriptive and involved looking at trends in budgetary allocations and financing of malaria and making correlations and inferences concerning sustainability, efficiency, effectiveness, and equity in financing and health outcomes. Findings were peer-reviewed by senior officials from the Ministry of Health (MOH), officers from the United States Agency for International Development (USAID) and the World Bank.

Ethical approval

Secondary data sources were used in the article so no ethical approval was required.

3. Results

3.1. Financing sources and potential for sustainability

Figure 1. and 2 present findings from an analysis of sources of funding for malaria in Uganda [7-10]. GOU funding has been relatively limited over the whole period, representing on average 10% of total malaria funding. Households represented about 67% of funding, while donors represented about 23% on average, signaling potential future problems for sustainability.
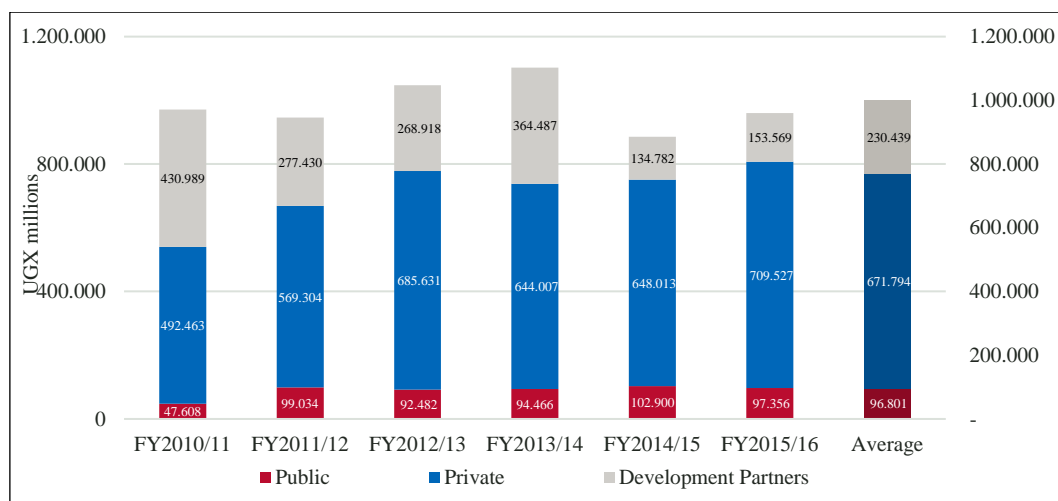
Figure 1: Sources of financing for malaria in Uganda Shillings (UGX) (2010 – 2016).

The NHAs suggest declining donor support for the malaria program yet there is no indication that the government is replacing donor funds with its funds. While government allocations to malaria programming rose from FY2010/11 through FY 14/15, they decreased in FY 15/16 in absolute figures and as a share of government budget; i.e. from USD 30.6m (UGX 102,900m) in FY2014/15 to USD 29m (UGX 97,400 million) in FY2015/16. Other sources of data such as the World Malaria Report [3] suggest increasing donor support for malaria programs in Uganda (Figure 2). The contradiction may be as a result of the difficulty to trace off-budget funds because of weaknesses in health information systems that fail to capture and standardize all data sources, and routinely monitor data quality. The main external malaria funders- Global Fund, PMI/USAID and the UK, increased their disbursement for malaria programs in Uganda from about USD71.3 million (UGX 239.6 billion) in 2014 to about USD125 million (about UGX 420 billion) in 2016, contrary to what is reported by the NHA for FY2015/16. Despite the stated increase in donor support, households still contribute significantly to malaria financing in Uganda which jeopardizes access to quality treatment for households
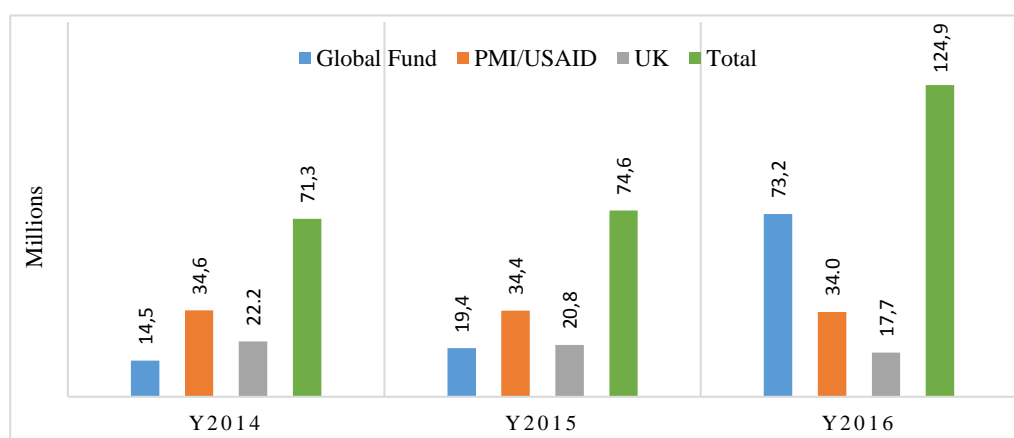

Figure 2: Main sources of donor financing for malaria Uganda- 2014 - 2016 (USD).

3.2 Efficiency in financing malaria

There is relative allocative efficiency as about 52% of the entire PMI budget goes into funding preventive activities which are in line with government policy to prioritize malaria prevention. About

29% funds curative care and 19% of funds go towards other activities[11]. Global trends on allocative efficiency for malaria indicate that governments largely fund human resources for health which account for a global average of about 39% of government resources, planning, administration and overheads (17%) and vector control other than insecticide-treated nets (ITNs) (18%). The Global Fund, on the other hand, funds mainly ITNs (43%) and treatment (21%), among others. The PMI emphasizes expenditure on ITNs (35%), other vector control measures (25%) and treatment (20%), among others [3]. These trends are consistent with the government strategy to put more emphasis on preventive measures. Figure 3 shows global malaria financing priorities until 2025[12].
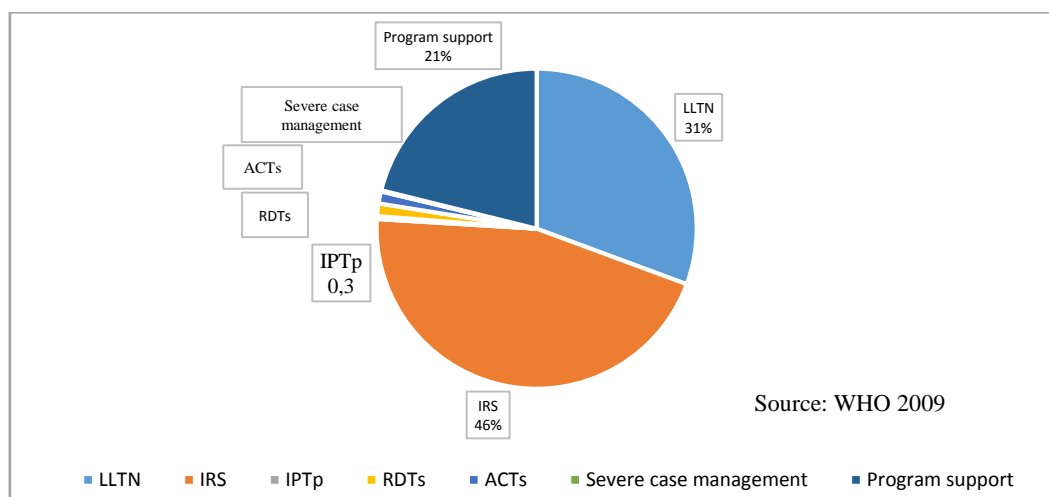

Figure 3: Global priorities in malaria financing by 2025.

As has been shown, Uganda government funding for malaria remains critical but minimal. The WHO[12] estimates that nearly 70% of total government funding for malaria goes into human resources and technical assistance, with medicines, insecticide and spray materials taking much of the remaining 30%; meaning that very limited funds are allocated to the number priority area of prevention. Moreover, the World Malaria Report 2017 shows that per capita domestic spending on malaria across the East and Southern Africa region (excluding staff salaries and out-of-packet payments) is very low with Uganda spending USD 0.2 per capita. Overall, per capita funding for populations at risk decreased by 25% between 2011 and 2016 in Uganda[3], indicating that with decreased funding allocated for more deserving malaria-prone areas, the chances of a resurgence of malaria as already reported are real.

In technical efficiency, the cost of malaria medications (ACTs) represents an opportunity for improved efficiency. The main driver of malaria treatment cost is the number of players in the supply chain including the importer, wholesaler and outlet/retailer (e.g., pharmacy, drug store or clinic). The mark-ups added at each supply chain step contribute significantly to price variations by sector and region. In some cases, the overall mark-up in private clinics compared to other outlets is as high as 325%.

Malaria treatment unit costs and prices also vary in public facilities; e.g. cost and prices in hospitals vary from about USD 4.46 to USD 9.52 respectively compared to Health Centre II where costs and prices vary between USD 2.52 and USD 3.57 respectively. The high variations represented by hospitals are likely due to payments for high cadre health workers such as doctors and other specialists.

Elsewhere, failure to effectively implement a private sector co-payment mechanism has affected access to subsidized anti-malarial medicines in private health facilities, pharmacies, and drug shops. As

a result, medicines are sold at USD 1.49 (UGX 5,000) beyond the recommended price of USD 1.04 (UGX 3,500) [13]. Overall, even though all health facilities (public and private) report that up to 90% and above have ACTs available[14] (Figure 4), being a first-line treatment for uncomplicated malaria means that many Ugandans still cannot access treatment as some facilities have not stocked ACTs. The other antimalarials are poorly stocked in all sectors; however, the public sector has the lowest availability of quinine injections with only 38% of facilities stocking the medicine.

Anti-malarial sales in private facilities remain unaffordable for ordinary Ugandans; e.g. as demonstrated in Figure 4, the lowest-paid Ugandan government worker spends 80% and 72% of a day's wage to be able to purchase ACTs from private-for-profit and private-not-for-profit facilities, respectively. There is also remarkable inefficiency in the use of anti-malarials especially first-line ACTs for treatment of uncomplicated malaria. The MoH's management information system indicates that up to 43% of patients reporting fever are treated with malaria without confirmed diagnosis and/or after negative laboratory results. This also raises the risk of resistance. A policy revision to allow testing using rapid diagnostics kits at private drug outlets has been initiated to reduce presumptive treatment for malaria[15].
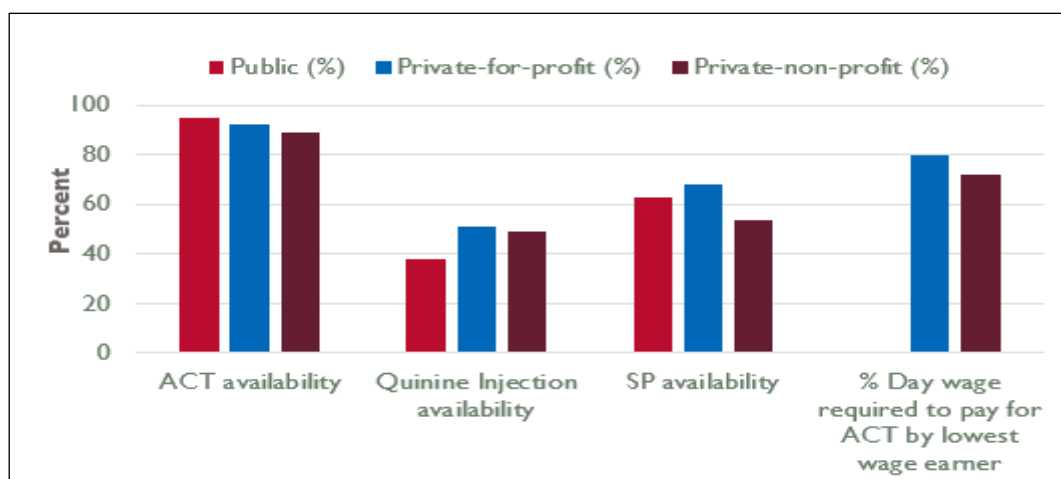

Figure 4: Availability and affordability of anti-malarials.

3.3 Equity

The Uganda Malaria Quarterly Bulletin 2016[16], indicates inequities in malaria incidence across the fifteen regions. The worst affected are Eastern, Karamoja, North and southwest regions which experienced increases in malaria incidence ranging from 19% in the East to 120% in the North. Karamoja had 44% increase and the North West experienced a 100% increase. The rest of the regions experienced decreases in malaria incidence between -3% and -72%. There is a likely correlation between high malaria incidence rate and poverty as the regions reporting the high incidence rates also have the highest poverty rates ranging from 70% to 80% of the population living below the international poverty line[17]. Other reasons to explain the differences in malaria control strategies could be linked to community responses to these strategies including ownership and actual use of ITNs. Karamoja region, in particular, has the lowest ownership of ITNs (55%) compared to the rest of the regions where ITN ownership range from 72% of households in Bugisu to 92% in West Nile[18].

Differences in ITN ownership are also reported between rural and urban households and between wealth quintiles. Per the most recent household survey[19], ITN ownership in the poorest households

stands at 71% compared to 84% in the richest households. In terms of treatment for malaria, higher costs in hospitals than in lower-level facilities likely benefit the poor and rural who tend to utilize the lower-level facility services. However, a report by Medicines Transparency Alliance[20] indicates that all types of health facilities in rural areas where the majority of poor Ugandans live have lower availability of medicines than urban areas.

In areas where there is not a public facility nearby, or when medicines might not be available at public facilities, private (for-profit and non-profit) health facilities may be the main service providers. Because services from such facilities are based on ability to pay rather than need, the poor may not be able to access malaria treatment services. Data from the Uganda Medicine Price Monitor demonstrate that the prices for a round of malaria treatment (Artemether/Lumefantrine tab 20/120mg) has fallen in private facilities from more than an average day's wage in 2013 to about 0.72 days' wages in 2015 but costs in mission facilities rose from 0.6 days' wages to about 0.8 days' wages (Figure 5) [16]
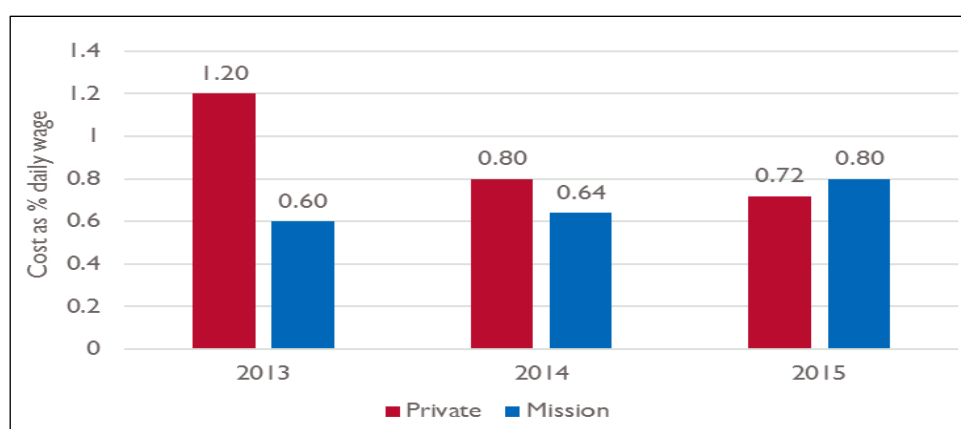

Figure 8: Costs of Malaria treatments in non-GOU facilities (% daily wage).

Price decreases in private facilities will likely improve access to malaria commodities but the opposite is true in mission facilities where prices have been on an upward trend between 2013 and 2015 [14, 21, 22].

3.4 Effectiveness

As noted above, much of the envelope of government funding for malaria control and treatment is directed toward human resources and technical assistance at the expense of treatment and prevention programs. While this support has resulted in strong improvement in malaria reporting[18], progress on improving key indicators such as case incidence, admissions, and mortality is mixed (Figure 6).
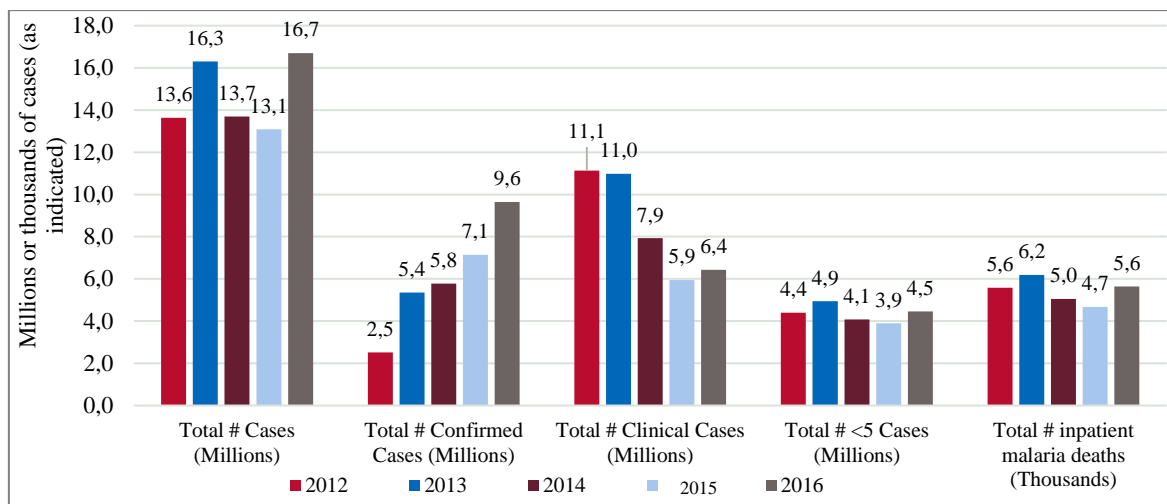
Figure 6. Key malaria case indicators.

From Figure 6, the malaria control interventions have not been effective in reducing malaria cases and related mortality to set the path for elimination. The total number of malaria cases increased from 13.6 million in 2012 to 16.7 million in 2016. spiked in 2013, rising from about 14 million in 2012 to 16.3 million. The rapid decline to 13.1 million cases reported in 2015 down from 16.3 million cases in 2013 was due to significant investments in vector control. These gains were reversed in 2016, when malaria cases spiked again to about 17 million, signaling lack of investments in malaria control and inconsistency in the rollout of interventions. Over the same period, the total number of confirmed cases also rose from 2.5 million in 2012 to 9.6 million in 2016. Although this could be partly due to improved diagnostics, there is no concrete evidence to support this suggestion because investment in diagnostics has been very limited. Other indicators such as inpatient mortality have been variable; e.g. in 2012 inpatient mortality was about 5,600 deaths, which declined to 4,700 in 2014 but then rose again to about 5,600 in 2016. It is noteworthy that the negative outcome indicators correspond with a period of declining government funding for malaria programs where key interventions such as ITNs and IRS did not seem to get priority in funds allocation. There is a positive relationship between increased spending and the reported availability of ITN, as demonstrated in Figure 7.
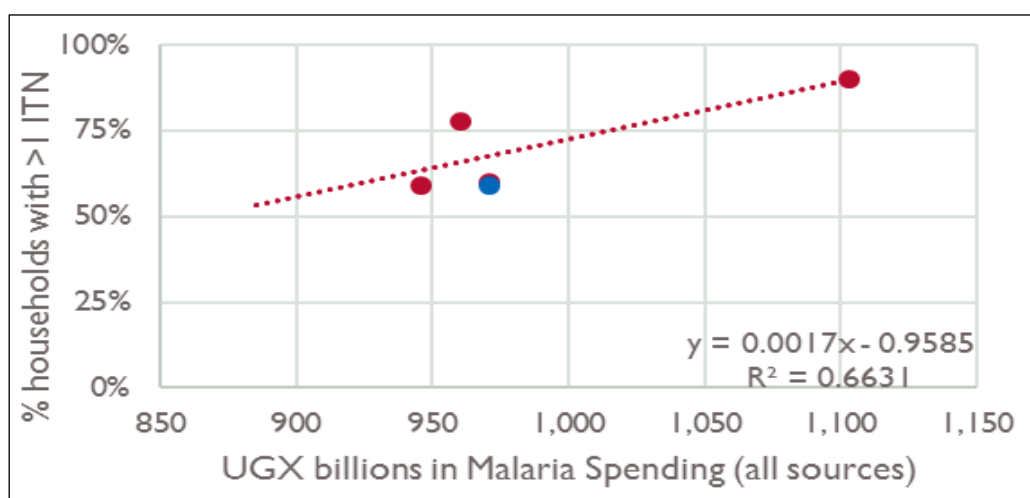

Figure 7. Correlation between spending on malaria and the availability of ITNs.

Increased spending does not, however, have a strong direct relationship with reduced inpatient malaria deaths as there appears to be a slight positive relationship between the two factors (Figure 8). This may indicate that spending tends to be responsive to increases in malaria mortality rather than the inverse.
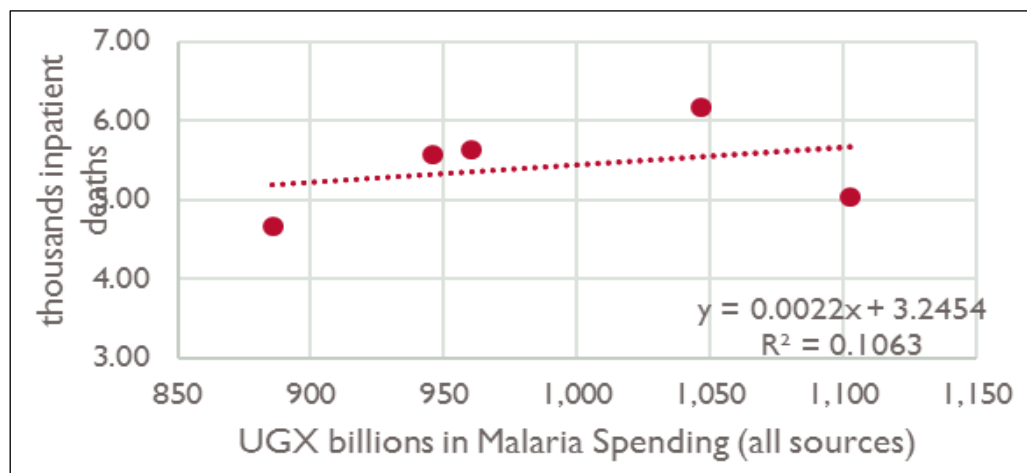

Figure 8. Correlation between spending on Malaria and in patient malaria deaths.

Gaps in the availability of medicines and testing materials at the facility level may explain some of the mixed results on the effectiveness of malaria prevention and treatment programs. Effective case management of malaria requires that health facilities have both malaria treatment and diagnostics on hand to identify cases of malaria and provide treatment. The 2014 report on the ABCE survey [23] found that while malaria diagnostics were widely available, there appear to be gaps in the availability of either ACTs or diagnostics –, particularly at lower-level facilities (Figure 9).
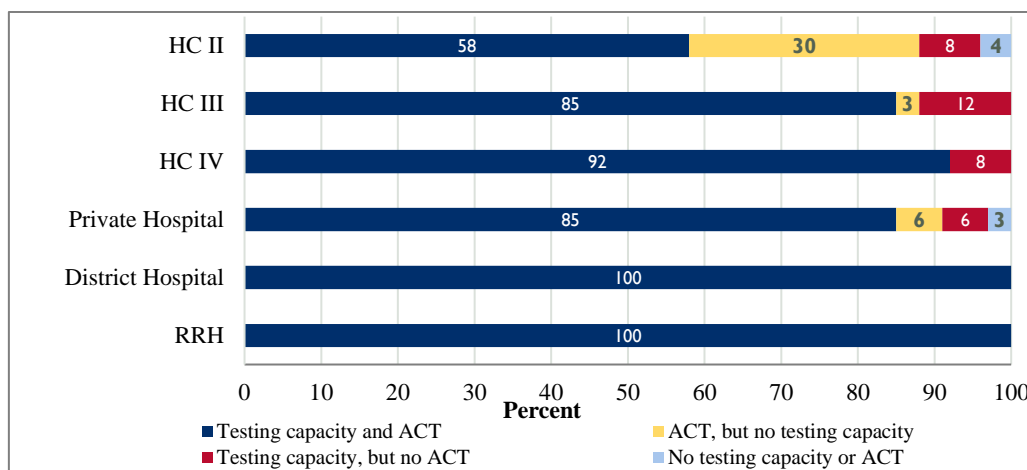

Figure 9. Availability of equipment and supplies to provide malaria health services.

Effectiveness of malaria control would benefit from strengthening primary care level facilities as well as ensuring that preventive interventions reach and are utilized by communities in rural areas.

4. Discussion

The Government of Uganda has clear policy objectives with regard to malaria control and work toward the elimination of the disease. These objectives are articulated in the long-term health sector strategy outlined under Vision 2040. Part of the planned reforms under Vision 2040 includes improvements in primary health care as a basis for achieving universal health coverage (UHC), which is key to addressing some of the noted health disparities in Uganda. There is, however, a disconnect between government professed policy reforms in malaria control and elimination which emphasize preventive measures, and the trajectory of public funding for malaria and the health sector as a whole; for instance, the government allocates limited funds for malaria programming most of which go into salaries and wages. To effectively control malaria, it is strongly recommended that the government undertakes reforms in domestic resource mobilization and allocation towards malaria in particular and the health sector as a whole. At the global level, financing for malaria has consistently failed to meet its funding targets in the recent past, which should be a wake-up call for Uganda to initiate fiscal reforms to increase domestic funding for malaria. In 24 out of 41 high-burden countries which rely heavily on donor support for malaria programs, the per capita funding for at risk populations declined in 2015–2017 compared to 2012–2014[4]. Although Uganda experienced the lowest decrease in per capita funding (1.0%) over the stated period, the main sources for funding for malaria in the country (donors and household out-of-pocket) are unsustainable, inequitable and inefficient. They are not only unpredictable for programming purposes but also fragmented to place the financing burden on individual households and therefore cannot meet the long-term UHC goals. Fragmentation in health financing has been variously discussed as detrimental to health financing goals[24-27]. To reduce the fragmentation in health financing in Uganda, there is an urgent need to reinforce domestic funding for malaria activities, both by increasing the level of GOU funding and by allocating more of the GOU resources toward direct service delivery activities to reduce dependence on external funding and OOP payments. The need for improved domestic financing for the health sector has been emphasized as a means of mobilization additional resources and financial sustainability[28, 29].

Globally, about 77% of funds for malaria control and elimination go into purchasing long-lasting treated nets (LLTN) and indoor residual spraying (IRS)[12]. This is consistent with the goal to strengthen primary care delivery systems and position countries for UHC. The GOU needs to realign its expenditure on malaria with national and global priorities in malaria financing. Such realignment will improve efficiency in malaria programming. Also, improvements in technical efficiency, for example, would require strategic reforms in the commodity purchasing including a reduction in the number of players and price monitoring to ensure availability of essential commodities, particularly in lower-level facilities. Price monitoring will also promote the affordability of malaria drugs outside government facilities. In addition, the GOU is encouraged to make funding at facility level available for the purchase of equipment and supplies to provide malaria services, particularly for health center (HC) III and below. Strongly encouraged are initiatives to reduce the unit costs of malaria medicines and treatments, particularly for ACTs, in both public and private facilities and promote more targeted use of anti-malarials to improve treatment efficiency.

Targeted use of anti-malarials includes test diagnosis before treatment to eliminate presumptive treatment. Treatment without test in public facilities is a common problem in sub-Saharan Africa but one that has remarkably improved over the years; e.g. in 2010 less than 40% of patients were treated after a diagnostic test but this improved to about 87% as of 2016[3]. However, in Uganda, the proportion

of suspected cases in public facilities receiving a diagnostic test before treatment is below 60% [18]. Improvements in diagnostic testing before treatment of malaria, therefore, constitutes a significant cost-saving area for the GOU.

As part of the reforms to ensure efficient and sustainable health programs and systems, the GOU needs to implement strategic purchasing of health services. Strategic purchasing, as an effort to improve health system performance, is an important reform objective in LMIC that aspire to achieve UHC[30, 31]. It involves three key elements, namely; aligning population health needs with services provided whilst accounting for national priorities and evidence on cost-effectiveness, effective provider contractual mechanisms, and payment systems, and accounting for provider levels of quality and efficiency[30]. The current status of purchasing in Uganda is passive involving mainly the government paying the bills without any contractual engagements on provider performance. Strategic purchasing coupled with increased domestic funding for malaria would help in addressing the inequities noted in malaria control in Uganda, particularly the malaria case incidence and ownership of ITNs.

Whilst medicine prices favor the poor, i.e. prices are lower in primary health facilities than in hospitals, these medicines are often unavailable in these facilities serving the poor. Lack of commodities in facilities that serve the poor expose this population group to catastrophic costs with potential for further impoverishment. Besides, the cost of purchasing a full dose of anti-malarials from non-government facilities is prohibitive for low-income earners, as it constitutes up to 80% of the daily wages of the lowest-paid worker. Improving access to medicines for the poor is a health system priority and has been variously emphasized in health system reforms [32-35].

As far as the effectiveness of malaria control interventions is concerned, viewed in terms of processes and health outcomes, there is a need for drastic improvements beyond the first stages of malaria control current in Uganda. Government investments have contributed to improved malaria reporting but not much else. Key malaria outcome indicators including case incidence, admissions, and mortality have not changed and in some cases have worsened.

In conclusion, malaria control structures in Uganda and specifically public financing at 10% of total malaria funding, remain too weak to allow the country to make progress toward elimination. All the indicators including those for sustainable financing, efficiency, equity, and effectiveness, are fragile suggesting that even at the early stages of malaria control, Uganda is still not making much progress. With 67% of malaria funding coming from households, the financing mechanism is not sustainable. A re-prioritization of malaria control efforts needs to be considered as a step toward strengthening primary health care delivery systems and contribute to UHC. As data shows, on average, national governments globally are the second largest contributor to malaria financing, which confirms the capability of most countries to mobilize the financial resources to attain some level of universality, particularly of most essential services such as malaria control and other primary health care services. For Uganda and many other developing countries, while the economic growth rate is essential, they are particularly encouraged to invest more in policy reforms to expand domestic resources and invest in providing quality primary health care services.

Acknowledgment

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Case Report

CHROMOSOMAL TRANSLOCATIONS IN MEN WITH AZOOSPERMIA

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Abstract: *Male infertility is liable for half of the genetic infertility cases. Robertsonian and Reciprocal translocations are the major chromosomal rearrangements in the infertile population. In this study, we aimed to submit a Robertsonian and two Reciprocal translocations in three couples with a history of male infertility with azoospermia. Chromosomal analysis of the one couple in the male partner appeared with an abnormal karyotype with 45,XY,rob(13;14) chromosomal constitution, while the female partner revealed normal 46,XX karyotype. The other two couple revealed in the male partner with reciprocal translocations, while the female partners showed normal 46,XX karyotype; one of the infertile males has karyotype with 46,XY,rcp(19;10), and another infertile male with 46,XY,rcp(6;14) chromosomal constitution. The cytogenetic analysis is mandatory to identify any probable chromosomal anomalies for couples with primer infertility. Couples with repeated abortions should be offered a prenatal diagnosis in the case of future pregnancies. Chromosome translocation carriers should be counseled to use advanced technologies such as assisted reproductive technology such as PGD.*
Keywords: male infertility, chromosome abnormalities, oligozoospermia, azoospermia translocation

1. Introduction

Infertility is a disease-defined incapability to imagine within 12 months of regular and insecure sexual interaction. Men are responsible for only 20-30% of infertility cases, but 50% of total infertility cases are male infertility [1]. Male infertility is associated with genetic factors such as chromosomal structural abnormalities and specific genetic conditions (ie CFTR gene of cystic fibrosis, Y chromosome microdeletions) [2]. The q11 part on the long arm of the Y chromosome has AZF (AZFa, AZFb, AZFc) parts linked to sperm production. It is known that microdeletions in these regions cause oligospermia or azoospermia and thus cause fertility problems [3]. Therefore, microdeletion of a Y chromosome should be investigated by molecular genetic methods in patients with non-obstructive or harsh oligospermia. Translocation is one of the chromosomal abnormalities seen as the loss of a part of a chromosome or a part of it that breaks to another chromosome. If there is no loss of chromosome material during the exchange between chromosomes, it is called balanced translocation. These individuals are usually clinically normal. However, if chromosome material loss occurs during this change, it is called 'unbalanced translocation and may present with serious clinical findings [4]. There are generally 2 types of translocation; Reciprocal and Robertsonian. Fragment exchange between non-homologous chromosomes is a chromosome abnormality called Reciprocal translocation. Two detached fragments of two different chromosomes are switched. Patients can be phenotypically normal when no genetic material gained or lost and if the breakpoints do not result in truncation of a gene. Reciprocal translocations are known to be a structural chromosomal abnormality that is common in the population

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(1 in 600) [5]. Chromosomal reorganizations in the infertile males are about approximately 10 times as frequently as normal population [6]. The most widespread structural chromosomal anomaly observed in the population is Robertsonian translocations with a frequency of approximately 1.23 / 1000 live births [7]. These special types of translocation are formed by the centric fusion of the long arms of the acrocentric chromosomes 13, 14, 15, 21 and 22, and the long arms that fuse at the centromeric part and lose their short arms. Instantaneous loss of both short arms is usually observed [8]. This translocation transmitted by one of the parents or can be occurred de novo. Robertsonian translocation is one of the major chromosomal anomalies, with the pervasiveness of 1% of the infertile population and 0.1% of the general population. Because of oligoasthenoteratozoospermia men carrying this translocation have more frequent infertility problems (OAT). The most frequent type of translocations in infertile men are der(13;14) (0.97 per thousand), followed by der(14;21)(0.20 per thousand) [9]. In infertile men 5.3%) somatic chromosome abnormalities in the karyotypes have 10 times as high as in the general population. The incidence of male infertility with synaptic chromosome anomalies, which limited to the germ cell line and only visible by meiotic studies is 4–7.7 % [10]. Numerical and structural chromosome abnormalities play an important role in azoospermic and strictly oligoasthenozoospermic men [11]. In this study, we aimed to present a Robertsonian and two Reciprocal translocations in three couples with a history of male infertility with azoospermia. We believe that phenotype-genotype correlations of our cases provide important contributions to the literature.

2.Materials and methods
2.1.Case 1

A 35 year-old-male whose parents were non-consanguineous referred to our Medical Biology and Genetics laboratory for karyotype analysis because of primary infertility. According to information of his family, he had one sister who had died. He was married for 3 years and had no children. His 30year-old-wife had no apparent fertility problem. Physical examination showed male habitus with normal adult pubic and axillary hair. Either malformations or gynecomastia was not seen. Severe oligoasthenozospermia (sperm account $0.5*10^6$ ml, 10% normal morphology, and 90% continuous motility) was established in examinations. Laboratory examination of hormone tests as measured FSH: 8.74 mlU / ml, LH: 3.34 mlU / ml, Estradiol (E2): 35 pg / mL, Testosterone 4.41ng / mL, Prolactin 0.86 ng / ml within normal levels. The patient had never received hormone therapy. His Y chromosome microdeletion test was positive.

Lymphocyte culture performed in peripheral blood and 45,XY,rob(13;14) chromosomal constitution were found in all metaphases plates and diagnosed as Robertsonian Translocation. Karyotype indicated 45 chromosomes with deficient chromosomes of 13 and 14, together with an extra chromosome that did not suit into any group of the chromosomes in the karyotype. The banding pattern of the short and long arms of the extra chromosome was akin to chromosome 13 and 14, thus showing the existence of a non-homologous Robertsonian Translocation
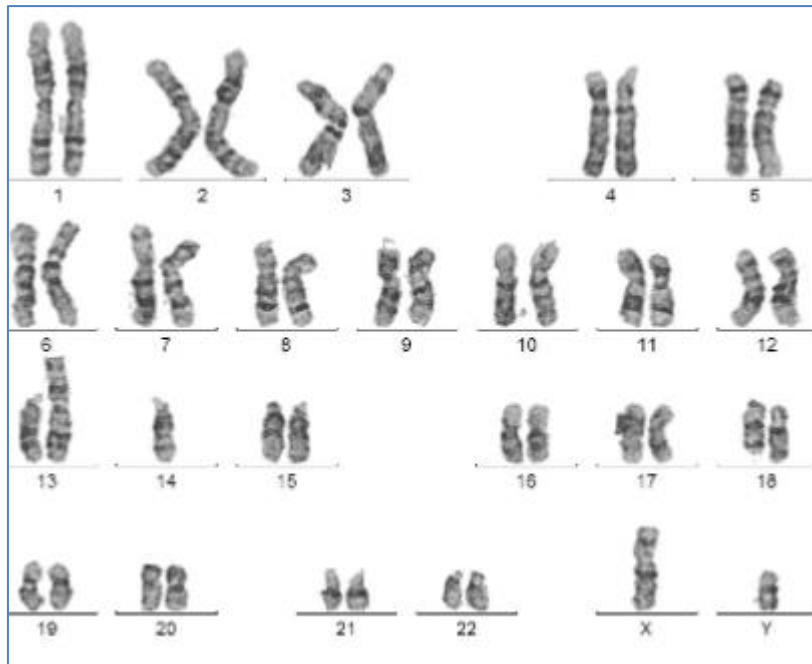
Figure 1. Robertsonian translocation karyotype analysis [45,XY,rob(13;14)]

2.2. Case 2

A 35-year-old man with fertility problems despite 14 years of sexual intercourse was referred to our Medical Biology and Genetics laboratory for karyotype analysis. His 32- year- old wife had no apparent fertility problem. The couple had one unsuccessful IVF trials. Laboratory examination of hormone tests as measured FSH: 21.97 mlU / ml, LH: 6.04 mlU / ml, Estradiol (E2): 15.56 pg / mL, Testosterone 1.73 ng / mL, Prolactin 4.18 ng / ml within normal levels. The patient had never received hormone therapy. His Y chromosome microdeletion test was positive. Semen analysis demonstrated normal volume azoospermia. Lymphocyte culture was performed in peripheral blood and an average of 2 preparations were made. Praperates were stained by Giemsa banding technique (GTG), [46,XY,rcp(19;10)] chromosomal constitution were found in 25 metaphases plates and diagnosed as Reciprocal Translocation. Karyotype analysis of mother, father, and sibling had done. Karyotyping of the mothers was normal (46,XX). The chromosome analysis of father also showed balanced Reciprocal translocation. Therefore, we considered the reciprocal translocation in the man transmitted from the father.
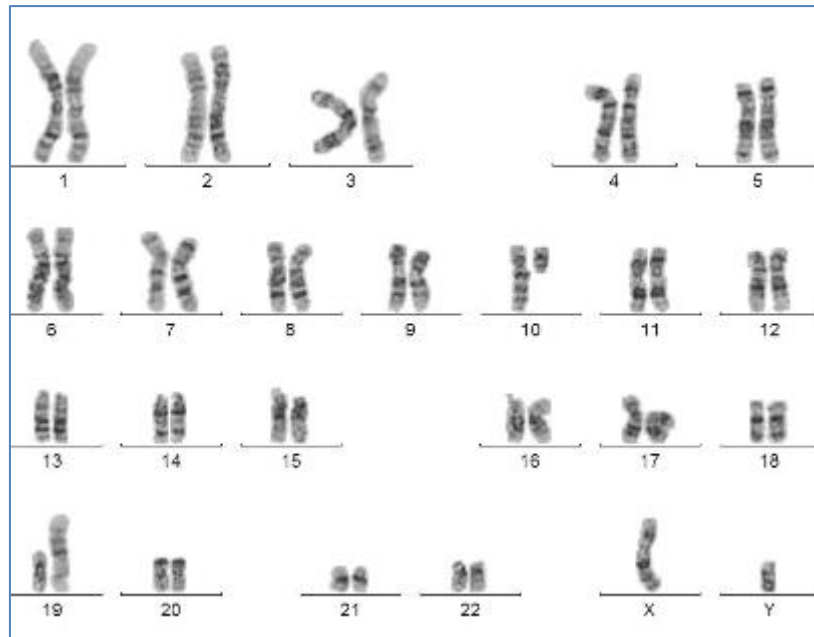
Figure 2. Reciprocal Translocation karyotype analysis [46,XY,rcp(19;10)]

2.3. Case 3

A 40-year-old male whose parents were consanguineous referred to our Medical Biology and Genetics laboratory for karyotype analysis after 6 years of sexual interaction without beginning his 33-year-old-wife had no apparent fertility problem. His brother was married for 25 years and had primary infertility problems. Semen analysis verified normal volume oligoasthenoteratozoospermia. His Y chromosome microdeletion test was positive. Laboratory examination of hormone tests as calculated FSH: 212.03 mlU / ml, LH: 5.34 mlU / ml, Testosterone 4.88 ng / mL, Prolactin 6.2 ng / ml within normal levels. Karyotype analysis revealed 46,XY,rcp(6;14).
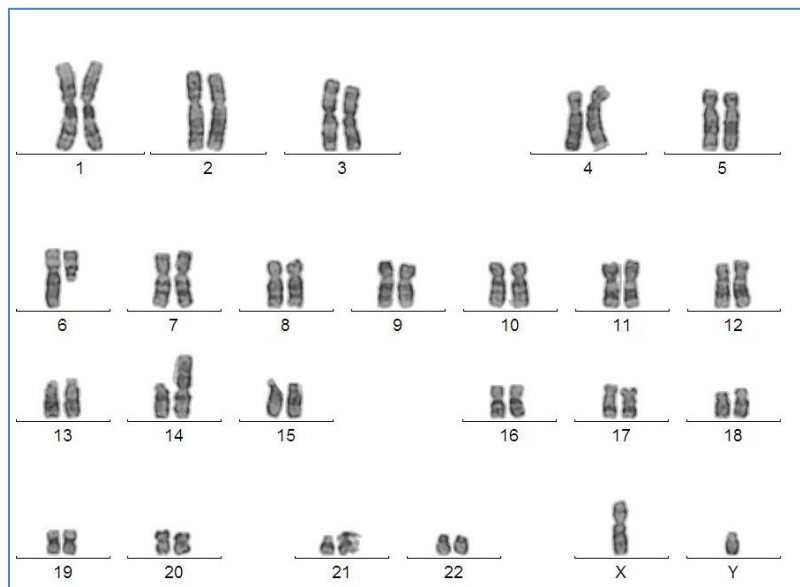

Figure 3. Reciprocal Translocation karyotype analysis [46,XY,rcp(6;14)]

3.Discussion

Infertile men have a higher frequency of structural chromosomal abnormality than the general population [12]. Loss of chromosome material in reciprocal translocation in the form of a balanced translocation and the clinical problem usually does not appear. However, although translocation carriers do not have any problems in themselves, they cause unstable chromosomal formations during parental gamete formation. Their infertility problems may be caused by more or less severe oligospermia [13]. In our infertile male cases with reciprocal translocation have a normal phenotype, but have troubles of infertility with azoospermia.

The number of chromosomes in Robertsonian translocation is 45. While most Robertsonian translocation is inborn from a parent, up to 40% can be de novo. We found that Robertsonian translocation had de novo. De novo translocations could be formed with rearrangements in meiosis. Robertsonian translocation linked to azoospermia is a rare condition [14]. Unlike the literature, karyotype analysis revealed 45,XY,rob(13;14) associated with azoospermia. Cytogenetic assessment should be a part of the evaluation of infertile male presenting to the reproductive clinic. Carriers of chromosome translocation should be counseled to utilize the diverse technologies obtainable through assisted reproductive technology, such as PGD.

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