TOJSAT
The Online Journal of Science and Technology

ISSN 2146-7390

The Online Journal of Science and Technology

Volume 10 Issue 2
April 2020

www.tojsat.net
April 2020

Published in TURKEY

Contact Address:

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Editor-in-Chief
April 01, 2020
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Message from the Editor

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This is the volume 10, issue 2 of the Journal of Science and Technology which covers the whole areas of Science and Technology all over the World. Studies either applied or social sciences that are used to fill in the gap between science and technology. Globally, this century scientific and technological developments are going very fast and importance of the journal will be most useful for the papers.

The journal, which covers all scientific and technological subjects, is published 4 times a year. Selected papers are accepted from the science and developing technologies which is published in the journal.

April 01, 2020
Assoc. Prof.Dr. Tolgay KARANFİLLER
Cyprus International University

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EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR AFTER LOW DOSE CAPSAICIN ADMINISTRATION IN RAT DUODENUM

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Abstract: Growth factors are essential for the development, growth and homeostasis of multicellular organisms and play an important role in the development and the maintenance of the gastrointestinal tract. Capsaicin (CAP) with neurotoxic properties has been shown to have a protective effect against experimental gastric mucosal injury in animals and humans. Epidermal growth factor (EGF) and ıt's receptore has been shown to exert gastric hyperemic and gastroprotective effects via capsaicin-sensitive afferent neurons, including the release of calcitonin gene-related peptide (CGRP). The. aim of the present study was to investigate the effects of low dose capsaicin (CAP) on epidermal growth factor receptor (EGFR) expression in the duodenum. In this study, 21-day-old rats were divided into two groups as CAP-treated and vehicle. CAP prepared in a solvent and injected subcutaneously to CAP-treated group (0.5 mg/kg/d) and vehicle group was injected with only solvent for 20 days. At the end of the experiment, tissue samples were collected and paraffin-embedded tissues were processed for standard immunohistochemistry by the labelled streptavidin-biotin technique. The EGFR localizations were identified on the surface epithelium of the villi, Lieberkühn crypts, in the Brunner's glands and smooth muscles layer of the duodenumIn the experimental group, the expression of EGFR in surface epithelial cells was not different compared to the control group, while the expressions in the Lieberukhn crypts, Brunner's glands and smooth muscle layer were stronger than the control group. As a result, the low dose capsaicin increased EGFR expression in the digestive system and probably have positive effects on the digestive system
Key Words: Capsaicin, duodenum, epidermal growth factor, rat.

Introduction

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is the pungent ingredient in red peppers and chillies that has been a pharmacological tool for the study of thin afferent fibres for almost half a century (Hwang et. al 2010). Capsaicin at a low dose stimulates release of neuropeptides such as catecholamines, neurokinin A (NKA), vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP), and substance P (SP) from sensory neurons endings (Holzer 1991, Surh and Lee, 1996). In contrast, high dose capsaicin shows neurotoxic effect and induces an irreversible long-standing inactivation of the capsaicin-sensitive nerve endings with a loss of their sensory-afferent functions and their ability to release sensory neuropeptides (Holzer 1991).

Sensitive primary afferent neurons of capsaicin participate in the regulation of gastrointestinal (GI) motility (Barthó et. al 2002). The effects of CAP are dependent on the concentration of capsaicin and of the mode of application. Also, Capsaicin induces the apoptosis of cancers cells, including myeloid leukemia (Ito et. al 2004), human hepatoma (Lee et. al 2004), and colon cancer (Kim et. al 2007). However, epidemiologic and animal experimental evidence suggests that capsaicin also acts as a carcinogen or cocarcinogen, particularly during the tumor promotion stage (Surh and Lee, 1996).

Growth factors are essential for the development, growth and homeostasis of multicellular organisms (Wieduwilt et. al 2008). The epidermal growth factor (EGF) is a polypeptide of 53 amino acids originally isolated from the rodent submaxillary gland (Cohen, 1962). EGF is continuously secreted from the salivary glands and the duodenal Brunner's glands (Konturek et. al 1995, Konturek, 1990). Intragastric EGF has been shown to enhance the healing of gastric mucosal injury (Konturek et. al 1995, Konturek, 1990, Olsen et. al 1986) and to protect the gastric mucosa against various stimuli such as stress, ethanol, hypertonic saline, and aspirin (Hui et al. 1993, Kang et al. 1998, Konturek, 1988, Konturek et. al 1981a, Konturek et. al 1981b). Therefore, it has therapeutic potential in digestive system disorders. The protective effects of EGF-family members are most likely related to their ability to modulate epithelial cell migration (Dignass et. al 1993), mucosal blood flow (Hui et al 1993) gastrointestinal motility (McLeay et al.1990), mucus production and secretion (Kelly and Hunter 1990), and gastric acid secretion (Rhodes et. al 1986). In addition, EGF and related peptides are potent mitogens for cells of the gastrointestinal tract in vivo (Al-Nafussi and Wright 1982) and in vitro (Carpenter and Cohen 1979)

The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that mediates intracellular signaling in response to various extracellular stimuli (Cohen, 2003). The epidermal growth factor family of receptor tyrosine kinases plays essential roles in regulating cell proliferation, survival, differentiation and migration (Wieduwilt and Moasser 2008).

EGF and EGFR have been shown to exert gastric hyperemic and gastroprotective effects via capsaicin-sensitive afferent neurons, including the release of calcitonin gene-related peptide (CGRP) EGF has a protective role in gastric mucosal injury, via capsaicin-sensitive afferent neurons involving calcitonin gene-related peptide (CGRP) mechanisms (Matsumoto et. al 2001**).**

Overexpression of epidermal growth factor receptor (EGFR) is common in many tumors. Specifically in colon and rectal cancer, EGFR is estimated to be overexpressed in 60%-80% of tumors, and is associated with a poor prognosis (Cohen, 2003).

In previous studies, EGFR immunolocalization is in the lung, stomach, duodenum, pituitary gland, thyroid gland, mammary gland, ovary, smooth muscle cells and small intestinal epithelium cells were determined (Carpenter and Cohen 1979, Carpenter 1987, Gómez-Pinilla et al.. 1988, Thompson 1988, Kajikawa et al. 1991, Massagué and Pandiella 1993, Playford et al. 1996, Kelly et al. 1997, Jeffrey et al. 2001, Zeineldin and Hudson 2006)

However, the effects of capsaicin on the small intestine, the role of EGFR is not fully understood. The aim of the present investigation was to determine the localization of EGFR in rat duodenum by immunohistochemistry and to identify different EGFR immunoreactivity after the application of a low dose of capsaicin in the rat's duodenum.

Material and Methods

Thirty immature female Sprague- Dawley rats (21 d old) were used throughout the experiments. The rats were obtained from the Experimental Animals Breeding and Research Centre, Uludag University, Turkey. The animals were housed five per cage, in temperature controlled conditions of (20–24ºC), humidity (60–70%), and lighting (12 h light/dark cycle), and were provided with feed and water *ad libitum*. The experimental protocols were approved by the Animal Care and Use Committee of the Uludag University and were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Experimental protocol. The rats were divided at random into 2 groups of 20 animals each. The first group (control) remained without any treatment. The second group (experimental) received subcutaneous injection of CAP (Sigma Chemical Co.) (0.5mg/kg/d), prepared in a solvent consisting of 10% of ethanol, 10% of Tween 80, and 80% of distilled water, for 20 consecutive days, Following 20 d of capsaicin treatment, the animals were euthanasised by the injection of sodium pentobarbital and the abdominal walls were opened. The proximal part of the duodenum was removed and fixed in alcoholic formaldehyde. Tissue samples were embedded in paraffin blocks according to routine histological procedures. Five micrometres thick sections were cut and immunostained for EGFR localisation (Inoue et. al 2002).

Immunohistochemistry analysis

After dewaxing and rehydration, slides were carried out antigen retrieval by boiling sections in microwave oven at 750 W in sodium citrate buffer (1 M, pH 6.1). After cooling, slides were rinsed with PBS and endogenous peroxidase activity was blocked by 10 min incubation at room temperature in 3% H_2O_2 solution in distilled water. After blocking with non-immune serum into kit for 30 minute to reduce nonspecific antibody binding, sections were incubated with primary antibodies, a rabbit polyclonal antibody to EGFR (sc 03, Santa Cruz, CA, USA) diluted to 1:100 for overnight at 4°C. The sections were stained using ImPRESS IgG-peroxidase kits (Vector Labs) (cat. No. MP-7401), according to the supplier's instructions. Finally, 3.3'-diaminobenzidine (DAB) was used for colour development. After counterstaining with haematoxylin, specimens were dehydrated and mounted.

Quantitative evaluated according to the staining intensity as follows: no staining (negative, −), slight brown (weak, +), brown-yellow (moderate, ++), and dark brown (strong, +++). The accumulated score of the positive staining represented the relative expression of the protein (Fromowitz et al. 1987, Ergin et al. 2008).

Results and Discussion

The EGFR localizations were identified on the surface epithelium of the villi, Lieberkühn crypts, in the Brunner's glands and smooth muscles layer of the duodenum (Fig. 1a, 2a, 3a). EGFR expression differences between groups are presented in Table 1. In the regions,

In the duodenum of the control group, EGFR expression in the surface epithelium of the tunica mucosa layer showed a strong immunoreactivity. EGFR expression also demonstrated strong immunoreactivity in the surface epithelial cells of the experimental group. (Fig. 1a, 1b) (Table 1.). In the control group, a moderate EGFR immunoreactivity was observed in the Lieberkühn crypts in the Tunica mucosa, while a strong EGFR expression was observed in the experimental group (Fig. 2a, 2b,3b) (Table 1.).

EGFR immunoreaction in Brunner's glands of the submucosa showed intracytoplasmic location. A weak EGFR imunoreactivity was observed in the control group, while a strong immunoreaction was observed in the Brunner's glands of the experimental group (Fig. 2a, 2b) (Table 1.).

Muscle layer. both the circular and longitudinal muscle layers, the dose of CAP administered increased EGFR expression. A weak EGFR expression was determined in the circular muscle layer in the control group, while a moderate EGFR immunoreactivity was observed in the circular muscle layer in the experimental group (Fig 2a 3a, 3b) (Table 1.).

EGFR expression was moderate in the longitudinal muscle layer of the control group, while it showed a strong immunoreactivity in the experimental group. (Fig 2a 3a, 3b) (Table 1.).

Table 1. Semiquantitative observations of the EGFR immunoreactivity in the rat duodenum.

	T. Mucosa		**T.Submucosa**	**T. Muscularis Externa**	
	S. Epithelial Cells	Liβ. Cryps.	Brunner's glands	C.Muscle Layer	L. Muscle Layer
Control	+++	++	+/-	+	++
Experimental	+++	+++	+++	++	+++

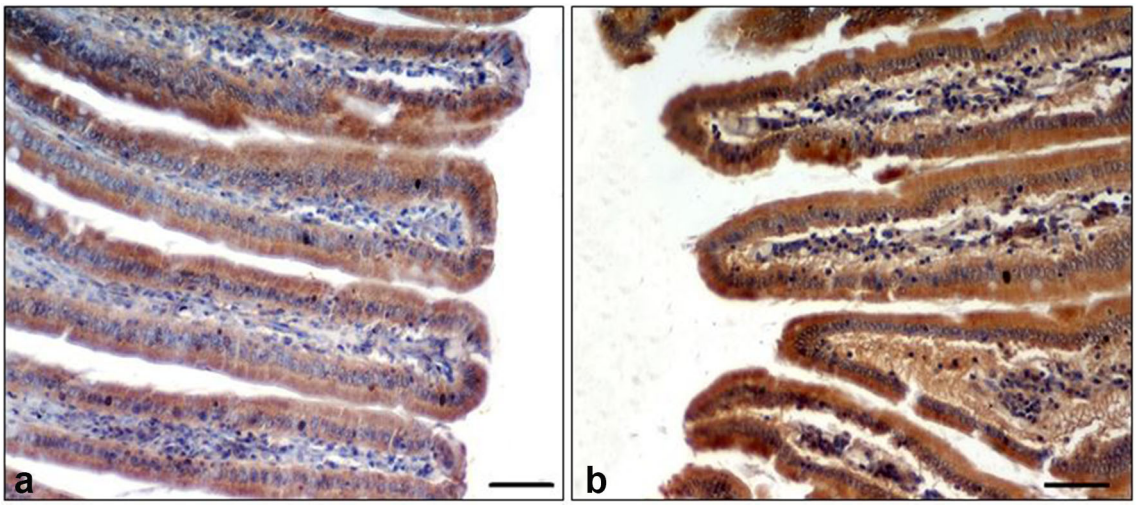

Figure 1. EGFR expression in surface epithelia cells of (a) control group, (b) experimental group. Bar 50 µm.

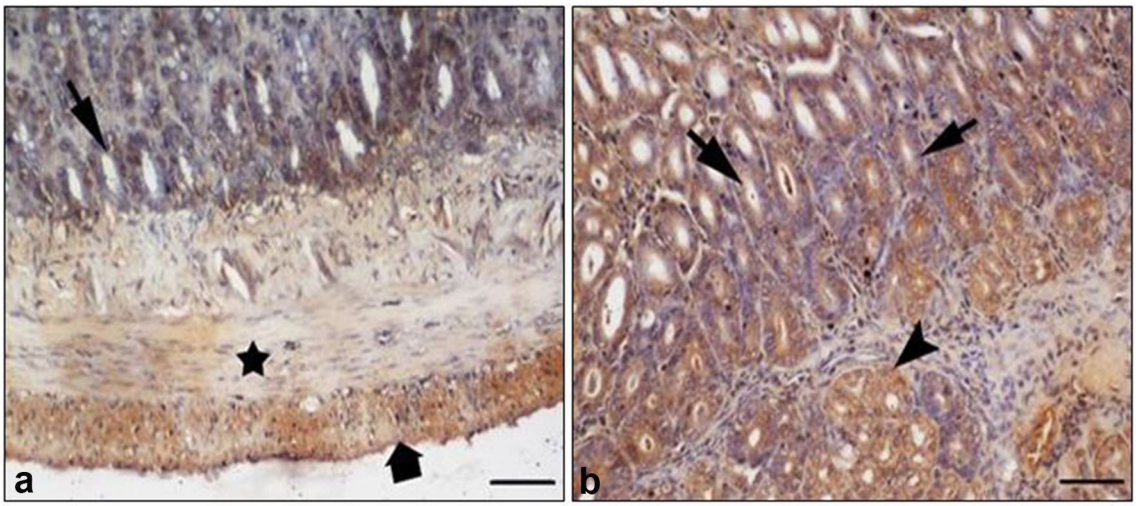

Figure 2. . EGFR expression in Liβ. Cryps, circular and longitudinal muscle layers of (a) control group, (b) experimental group. Bar 50 µm.

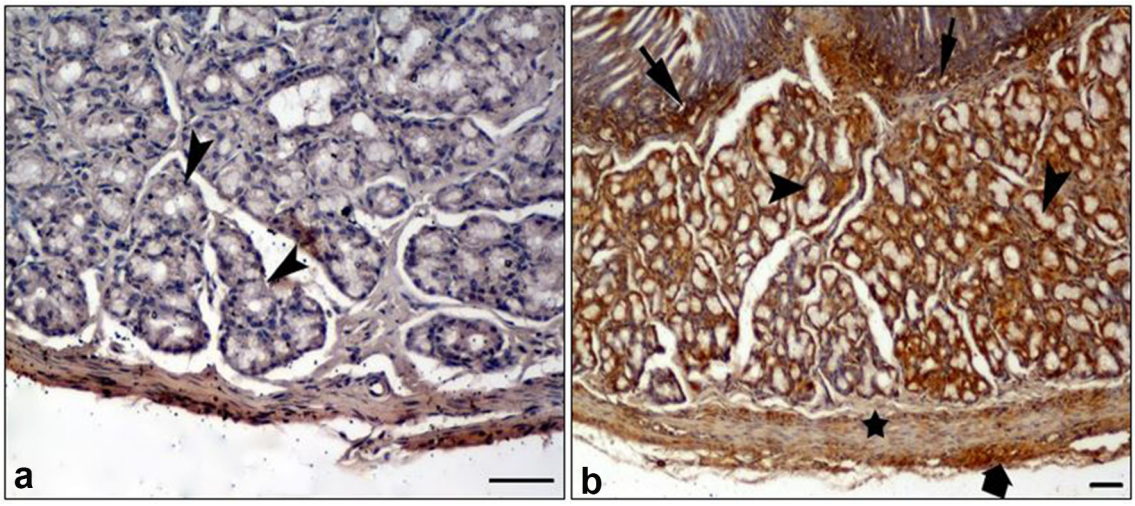

Figure 3. EGFR expression in Brunner's gland, circular and longitudinal muscle layers of (a) control group, (b) experimental group. Bar 50 µm.

The EGFR family members have roles in a broad spectrum of human diseases and are a paradig m for the translation of fundamental biological discoveries into therapeutics for human disease (Wieduwilt and Moasser 2008), and numerous studies have shown the existence of capsaicin sensitivity neurons in the gastrointestinal tract (Holzer, 1991, Nozawa et al. 2001, Patterson et. al 2003). In the present study, we observed that the given low-dose CAP increased EGFR expression in the duodenum's Lieberkühn cryptes, Brunner's glands and smooth muscle layer. Capsaicin has been reported to be effective on growth and development by increasing the release of growth factors. On the other hand EGFR plays essential roles in regulating cell proliferation, survival, differentiation and migration (Szalasi and Blumberg 1999, Yıldız et. al 2013, Bakır and Sarı 2015).

Aberrant regulation of EGFR activates downstream signals including ERKs and Akt resulting in increased tumor cell proliferation, survival, and invasiveness. Thus, modulation of EGFR signalingis key in preventing cancers. Capsaicin enhanced the metastasis of murine breast cancer cells by reducing the expressionof apoptosis-related genes (Erin et al. 2006) and induced LNCaP prostatecancer cell proliferation by increasing androgen receptor expression through the activation of ERKs and Akt (Malagarie-Cazenave et al. 2009). Also, another studies have shown that capsaicin-sensitive afferent neurons do not have a possible role in induction of EGFR in the duodenum (Bulut et al. 2008).

The biological effects of capsaicinoids are dependent on the dose of these compounds administered and the time of exposure (Bley et al. 2012).

Studies have shown that high doses of capsaicin (over 100 mg capsaicin per kg body weight) over a long period of time cause peptic ulcers, accelerate the development of prostate, stomach, duodenal and liver cancers and improve breast cancer metastasis (Mózsik et al. 2009, Bley et al. 2012).

Several convergent studies indicate that low-doses of capsaicin display a cancer-chemopreventive, anti-neoplastic activity (Lau et al. 2012, Aggarwal et al. 2008). Capsaicin induces robust apoptosis in multiple types of human cancer cells both in vitro and in mice models. Recent studies have focused on the potential of capsaicin as a viable anti-cancer drug applicable to the management and treatment of human breast cancer, and colon cancer (Lau et al. 2012, Lau et al 2014). The intestinal absorption of low capsaicin in vitro was done using everted intestinal sacs isolated from rats (Monsereenusorn, 1980). It was observed that capsaicin was robustly absorbed both into intestinal tissues, jejunum and serosal fluid. Kawada et al. (1984) studied the in situ metabolism of capsaicin using ligated loops of stomach, jejunum and ileum (Kawada et al. 1984). The application of 1 mM capsaicin led to rapid absorbance of the compound in the lumen within one hour; the absorbance rate was 50% in the stomach, 80% in the jejunum and 70% in the ileum. This indicated that capsaicin was absorbed better in the jejunum and ileum as compared to the stomach. The authors repeated these studies with dihydrocapsaicin and obtained similar results (Kawada et al. 1984).

Intragastric EGF has been shown to enhance the healing of gastric mucosal injury (Konturek et al. 1990, Konturek et al. 1995, Olsen et al. 1986) and to protect the gastric mucosa against various stimuli such as stress, ethanol, hypertonic saline, and aspirin.

Conclusion

Considering all these data, the fact that low dose CAP application increased the expression of EGFR which plays an important role in cell proliferation, differentiation and migration on dudodenum, suggests that it affects the intestinal activities positively and has a more facilitating effect on digestion.

Acknowledgements

This study was funded by a grant from TUBITAK- TOVAG (The Scientific and Technological Research Council of Turkey) project no: 104 O 372. .

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GENERATION OF CELLULAR CLONES FOR APOBEC1 ENZYME AND AUXILIARY COFACTORS

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Abstract: RNA editing is defined as any site-specific alteration in RNA sequence excluding the post-transcriptional modifications namely 5' 7-methylguanosine capping, 3' polyadenylation and pre-mRNA splicing. Almost all of the RNA editing processes so far describe the changes in mRNA sequences which result in the production of altered protein products. In this study, generation of some cellular clones for the analysis of APOBEC1 (apolipoprotein B mRNA editing enzyme, catalytic polypeptide)-induced RNA editing has been aimed. In one hand, conditions for the cytodifferentiation of Caco-2 cells to allow them to resemble phenotypically the small intestine enterocytes where the mRNA editing of apolipoprotein B takes place in vivo has been set. On the other hand, inactivation of the genes that code for APOBEC1, ACF (APOBEC-1 Complementation Factor) and RBM47 (RNA Binding Motif47) has been performed. To achieve this, a very recent and popular genome editing tool derived from the bacterial CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) / Cas9 (CRISPR-associated 9) system was utilized.
Key words: Caco-2, RNA Editing, Cytodifferentiation, CRISPR / Cas9, Gene Silencing

Introduction:

RNAs transcribed from most eukaryotic genes can undergo a variety of posttranscriptional RNA processes (splicing, capping, polyadenylation). Besides, some novel RNA editing and modification events have recently been described (Gott and Emesson, 2000; Licht and Jantsch, 2016). RNA editing is a term associated with structural changes in an RNA strand that alter its coding properties. Deamination type of editing changes the identity of a base by deaminating cytidine to uracil or adenosine to inosine by cytidine and adenosine deaminases respectively. This benefits the organisms by forming protein isoforms that are cell-type specific, developmentally regulated or environmentally-induced (Tariq and Jantsch, 2012; Baysal et al., 2017).

APOBEC1 enzyme (Apolipoprotein B mRNA editing enzyme catalytic polypeptide 1) is the catalytic subunit of RNA editing complex, editosome, which deaminates C_{6666} to U in the pre-mRNA of human apolipoprotein B (ApoB). Consequently, two protein isoforms, ApoB100 and ApoB48 are formed which are involved in the metabolism of lipids. The full length of apolipoprotein B, ApoB100, is synthesized in the human liver whereas the short form, ApoB48, is produced in the human small intestine as a result of a premature stop codon introduced by C-to-U deamination (CAA→UAA) (Saraconi et al., 2014).

Initially, the minimal structure of this editosome assumed to contain two proteins; APOBEC-1 and a cofactor APOBEC-1 Complementation Factor (ACF) (Mehta et al., 2000; Smith 2007). Later, a novel protein, RNA Binding Motif47 (RBM47) was identified that interacts with them (Fossat et al., 2014).

Genome editing is a way used to manipulate the genome. These days, a very popular tool called the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) / CRISPR associated 9 (Cas9) targets a nuclease protein Cas9 to a specific genomic region by means of a guide RNA (gRNA) (Çetintaş et al., 2017, Lau and Davie 2017). Despite many advantages of this tool, the targeting efficiency can still be a burdening factor. To overcome it, the "surrogate reporter system" that enables efficient enrichment or selection of gene-modified cells has been described. According to it, a surrogate target sequence homologous to that of the gene of interest is used to maintain a reporter gene which is located out of the ORF (Open Reading Frame). The repair of the induced double-strand break shifts the reporter gene into the ORF and activates it. Reporter can code for selection characteristics such as surface antigens, fluorescence or antibiotic resistance (Niccheri et al., 2017).

The human Caco-2 cell line has been widely used as a model of the intestinal epithelial barrier. One of its most advantageous properties is its ability to differentiate to resemble morphologically and functionally into the small intestine enterocytes which is the site for deamination of ApoB by APOBEC-1 (Tor, 2015, p. 103).

In this study, the generation of a cellular model for the analysis of RNA editing was aimed. The first aspect regards the setting up of the conditions for the cytodifferentiation of Caco-2 cells. The other regards to the work to inactivate the genes that code for the elements of editosome by using CRISPR / Cas9.

Materials and Methods

1. Culturing of Caco-2 cells

Caco-2 cells purchased from ATCC & LGC Standards S.r.l, Milan, Italy were used. Caco-2 WT and Caco-2 R / G (Red / Green) cells obtained by transfecting Caco-2 WT cells with plasmids encoding the "mCherry-ApoB-GFP" chimeric protein were cultured according to the protocol as described by Natoli et al. (2012) in an incubator at 37 ^{0}C with 5% CO_2. Cell culture medium DMEM (Dulbecco modified Eagle's Medium, EuroClone) supplemented with 20% FBS (Fetal Bovine Serum; Carlo Erba), 200 mM L-glutamine (Carlo Erba), and 1 mM penicillin / streptomycin solution (EuroClone) was used. Different from the above-mentioned protocol, instead of 20%, 10% heat-activated FBS was added.

2. Differentiation of Caco-2 cells

Caco-2 WT and Caco2 R / G cells were seeded on polycarbonate filters, 12 mm diameter, 0.4 µm pore diameter (Transwell, Corning Inc. Lowell, MA, USA) at a density of 3.5×10^5 cells / cm^2 in complete medium in apical compartment and in 10% FBS supplemented medium in basolateral compartment (asymmetric) as described in the protocol of Ferruzza et al. (2012). Cells were allowed to differentiate for 21 days with regular medium changes three times a week.

3. Preparation of samples for FACS analysis

200 µl of cells were resuspended in 4 ml of PBS in each of the 3 test tubes. Flow cytometric analysis of the samples has been performed as to the following;

- the first test tube contained Caco-2 WT cells grown on filter (to be used as blank),
- the second test tube contained Caco-2 R / G cells grown on filter and,
- the third test tube contained Caco-2 R / G cells grown without filter (to be used as negative control).

4. RNA extraction and cDNA synthesis from differentiated Caco-2 cells

3×10^6 Caco-2 WT and Caco-2 R / G cells, after the differentiation process, were applied to RNA extraction process by using 500 µl of TRIzol. cDNA synthesis was performed (for Caco-2&Caco-2 R / G cells grown with / without filter) using the iScript cDNA Synthesis Kit (Bio Rad). Manufacturer's protocol and recommendations were followed.

5. Preparation of ApoB cDNAs from Caco-2 cells for sequence analysis

Apolipoprotein-B cDNAs derived from Caco-2 WT cells grown on filter (blank) and Caco-2 R / G cells grown on filter during cytodifferentiation and undifferentiated Caco-2 R / G cells (as the negative control) were amplified by PCR. PCR products were run on 2% ultrapure agarose gel. Gel extraction purification was performed.

6. Amplification of APOBEC1 cDNAs from Caco-2 and Caco-2 R / G cells for expression analysis

APOBEC1 cDNAs from Caco-2 and Caco-2 R / G cells were amplified using the related primers for expression analysis. PCR products were run on 1% agarose gel by electrophoresis. Gel purification and sequence analyses of PCR products could not be performed due to insufficient findings.

7. Knock-out of APOBEC1 gene in Caco-2 cells

10^6 Caco-2 WT and 10^6 Caco-2 R / G cells, after reaching the desired confluence were transfected with the plasmids listed in Table 1 by electroporation using a Gene Pulser II electroporator (Bio-Rad). Its voltage was adjusted to 250 V, capacitance to 1000 uF, resistance to ∞ and cuvette size to 4 mm. Caco-2 WT and Caco-2 R / G strains were transiently co-transfected with plasmids expressing CRISPR / Cas9 and sgRNA system, as well as blasticidin and puromycin resistance reporter plasmids.

Table 1: List of plasmids and their corresponding functions used in the electroporation of Caco-2 cells to knock-out hA1, RBM47 ve ACF genes

Plasmid	Function
pX330-hA1 (3 exon)	Codes for CRISPR / Cas9 elements to knock-out APOBEC1 gene
pBML5-mcherry-hA1-bsrR	Includes the mCherry-hA1-BSR cassette which codes for Blasticidin resistance for selection
pX330-RBM47	Codes for CRISPR / Cas9 elements to knock-out RBM47 gene
pBML5-mcherry-RBM47-bsrR	Includes the mCherry-RBM47-BSR cassette which codes for Blasticidin resistance for selection
pX330-A1CF	Codes for CRISPR / Cas9 elements to knock-out ACF gene
pBML5-mcherry-A1CF-bsrR	Includes the mCherry-ACF-BSR cassette which codes for Blasticidin resistance for selection
pBML4	Includes the reporter gene for Puromycin selection

After electroporation, cells co-transfected with sgRNA / Cas9 construct and pBSR construct were placed under selection with BlasticidinS (1 µg / ml) for Caco-2 WT and Caco-2 R / G cells at 24 hours of transfection. After 48 hours, the antibiotic selection was removed and all cells were seeded in four 96-well plates per type.

Cells co-transfected with sgRNA / Cas9 construct and pBML4 control plasmid were treated with Puromycin (0.1 µg / ml) for Caco-2 WT and Caco-2 R / G cells to select transfected cells. At 48 hours of puromycin treatment, all cells were seeded in four 96-well plates in four dilutions (1 cell / well, 10 cells / well, 50 cells / well and 100 cells / well).

8. Knock-out of ACF and RBM47 genes in Caco-2 cells

In this part of the study, 4×10^5 Caco-2 WT and 4×10^5 Caco-2 R / G cells were transfected with the plasmids listed in Table 1 by electroporation using a Gene Pulser II electroporator. The voltage was adjusted to 250 V, capacitance to 1000 uF, resistance to ∞ and cuvette size to 4 mm. After electroporation, cells transfected with the pBSR construct were placed under selection with BlasticidinS (1 µg / ml) for Caco-2 WT and Caco-2 R / G cells at 48 hours of transfection due to the insufficient confluence observed. After 48 hours of applying BlasticidinS, the antibiotic selection was removed and all cells were seeded in three 96-well plates per type.

4×10^5 Caco-2 WT and 4×10^5 Caco-2 R / G cells were transfected with the plasmids listed in Table 1 above by electroporation using a Gene Pulser II electroporator. The voltage was adjusted to 250 V, capacitance to 1000 uF, resistance to ∞ and cuvette size to 4 mm. After electroporation, the cells co-transfected with sgRNA / Cas9 construct and pBML4 control plasmid were treated with Puromycin at 48 hours of transfection due to the insufficient confluence observed. Caco-2 R / G KO ACF sample was treated with 0.1 µl / ml and Caco-2 KO ACF, Caco2 R / G KO RBM47, Caco-2 KO RBM47 samples were treated with 0.03 µl / ml puromycin due to the less confluence than expected.

After 48 hours of puromycin treatment, Caco-2 KO ACF and Caco-2 KO RBM47 cells were seeded in one 96-well plate and Caco-2 R / G KO ACF, Caco-2 R / G KO RBM47 cells were seeded in three 96-well plates in three dilutions (10 cells / well, 50 cells / well and 100 cells / well).

9. DNA extraction from knocked-out Caco-2 and Caco-2 R / G cells

DNA Extraction from knocked-out Caco-2 WT and Caco-2 R / G cells for APOBEC1, ACF and RBM47 was performed using the DNA extraction kit by Promega. Recommendations and protocol defined by the manufacturer were followed.

10. PCR amplification of knocked-out genes in Caco2 and Caco2 R / G cells

Genomic DNAs obtained from genomic DNA extraction were amplified via PCR. In our study, PCR amplifications were performed by a PCR Kit (AB Analitica; AB Taq Polymerase (5 U / µl), Buffer Solution10X and 50 mM $MgCl_2$). Recommendations and protocol defined by the manufacturer were followed. PCR tubes were transferred to a thermocycler with the block preheated to 95°C. Thermocycling conditions for the PCR are listed on Table 2.

Table 2: Thermocycler conditions for PCR

Step		Temperature	Time
Initial Denaturation		95 °C	30 seconds
Denaturation	× 30-35	95 °C	20 seconds
Annealing		Depends on primer	15-30 seconds
Extention		Depends on length of fragment	1 minute / kb
Final Extention		72 °C	5-7 minutes
Hold		4 °C	∞

PCR products are purified by using the Macherey-Nagel PCR Clean-up kit. Recommendations and protocol defined by the manufacturer were followed. PCR products, after the purification process, were sent for sequencing to Macrogen Europe (Netherlands).

Results

In this study, preparation of Caco-2 cell clones for the analysis of RNA editing on ApoB mRNA was aimed. Therefore, Caco-2 cells were subjected to 21 days of cellular differentiation (cytodifferentiation). After that, cDNAs of both apolipoproteinB-48 and APOBEC-1 got obtained by reverse transcription of the respective mRNAs obtained from differentiated Caco-2 cells. However, due to the insufficient findings as shown in Figure 1 and Figure 2, whether RNA editing was carried out or not couldn't be well-observed.

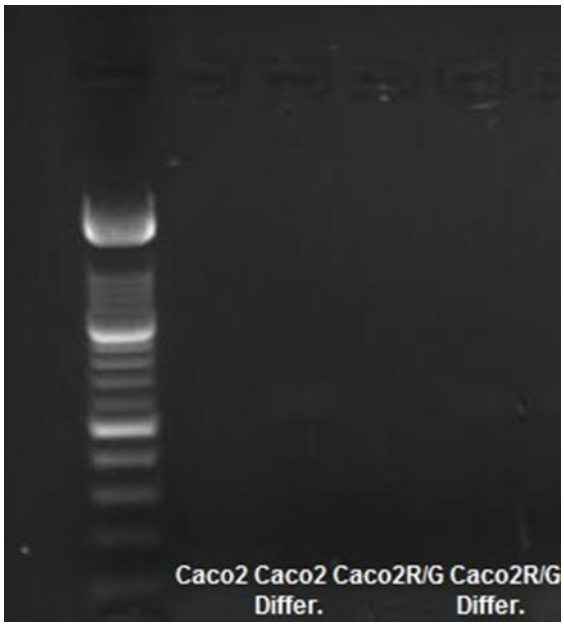

Figure 1. APOBEC1 expression in differentiating Caco-2 cells. DNA ladder (100 bp) used as standard

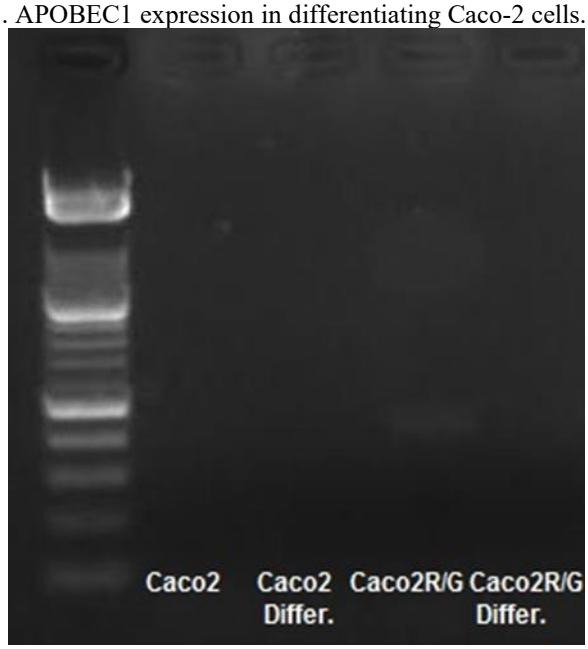

Figure 2. ApoB mRNA editing in differentiating Caco-2 cells. DNA ladder (100 bp) used as standard

1. Analysis of RNA editing by flow cytometry

Furthermore, Caco-2 WT and Caco-2 R / G cells were grown on specific filters to allow them to differentiate into small intestine enterocytes and FACS (Fluorescence-Activated Cell Sorting) analysis was performed after 21 days. Results were displayed in Figure 3 where wild type of Caco-2 cells cultured on filter are blank, Caco-2 R / G strains not cultured on filter are negative control and Caco-2 R / G cells cultured on filter are shown as experimental group.

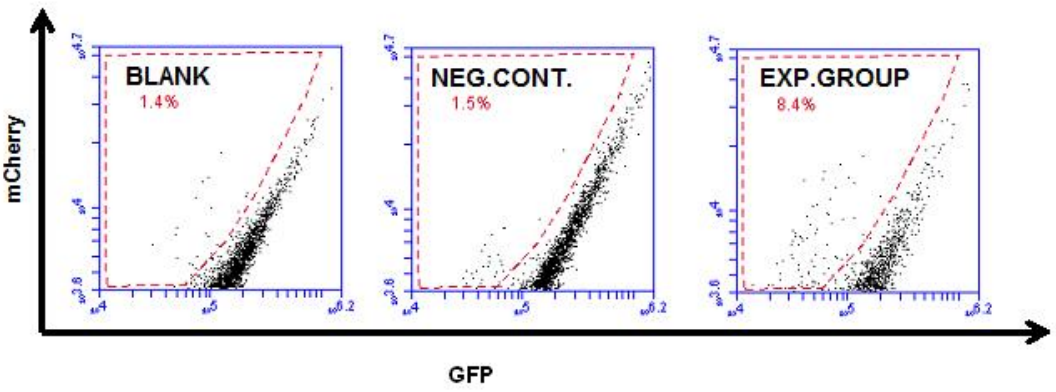

Figure 3. Analysis of RNA editing by flow cytometry

According to Figure 3, FACS analysis of both blank and negative control groups show a very low deviation from mCherry / GFP diagonal at a value near zero. On the other hand, Caco-2 R / G strains cultured on filter, which are experimental group, have a higher deviation compared to other groups. However, this deviation was observed to be quite low compared to the results of a previous study of Severi and Conticello (2015).

2. Sequence analysis of knocked-out cells

In this part of our study, knock-out of the genes coding the APOBEC-1 enzyme and its cofactors, ACF and RBM47, was performed using the CRISPR / Cas9 genome editing tool. In in vitro and eukaryotic applications of gene silencing studies, crRNA and tracrRNA are fused into a single guide RNA (sgRNA) and form a minimal functional system with Cas9, a RNA-driven nuclease. This complex leads to double stranded DNA breaks in the target region identified with the help of guide RNA. However, as shown in Figure 4, Figure 5 and Figure 6, the nucleotide sequences in the target regions are identical and show similarities to the nucleotide sequence in wild type. Referring

to Niccheri et al. (2017), it is thought that Cas9 nuclease constitutes an indel on the surrogate target in the stable clones following the 48-hour of Blasticidin selection. However, the fact that the same nuclease has no effect on the target DNA in the genome suggests the presence of one or more factors that inhibit the binding of the guide RNA to the target site.

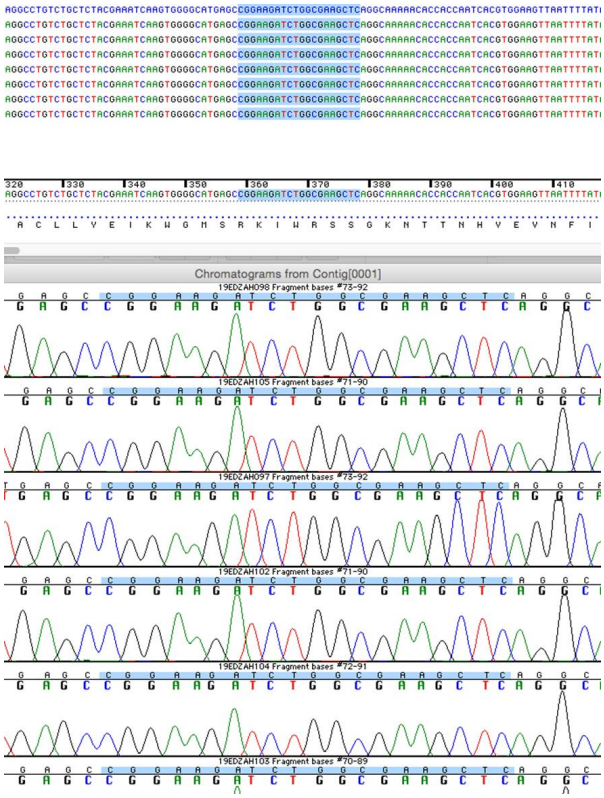

Figure 4. Sequence analysis of knocked-out *hA1*gene

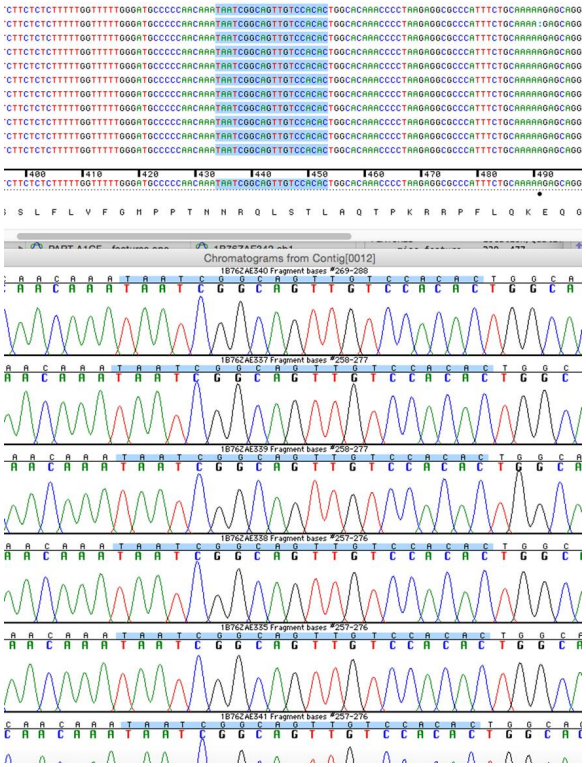

Figure 5. Sequence analysis of knocked-out *acf* gene

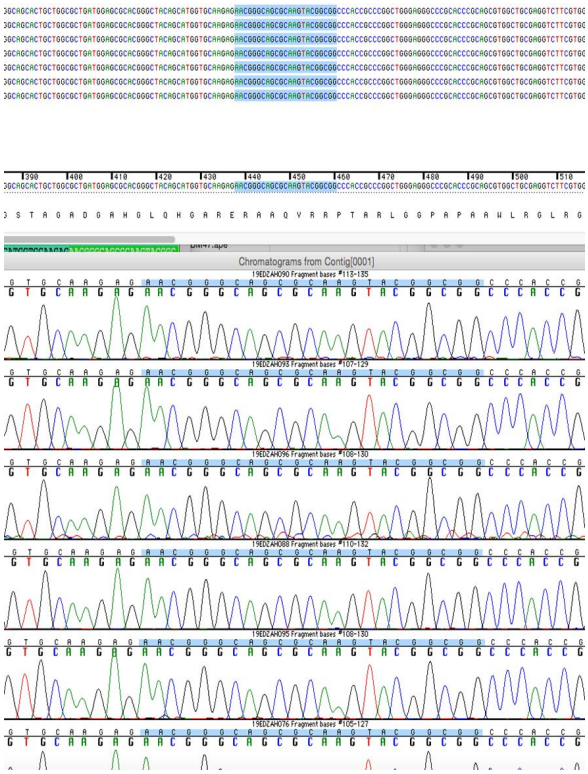

Figure 6. Sequence analysis of knocked-out *rbm-47* gene

Discussion

According to Figure 1 and Figure 2, mRNAs extracted from Caco-2 WT cells and Caco-2 R / G strains after 21 days of differentiation are thought to dissipate. Accordingly, bands on the gel that are expected to correspond to complementary DNAs are not visible. This is thought to be due to an undesired situation/experimental error at the stage of cell differentiation or mRNA extraction of both APOBEC-1 and ApoB. One more reason could have been that the integration of mCherry-ApoB-GFP reporter chimera into the genome within the cell may have been disrupted due to long-time subculturing of the Caco-2 R / G strains used.

1. Analysis of RNA editing by flow cytometry

The reason of the low amount of gene-edited Caco-2 R / G clones is most probably due to the problem encountered during the cytodifferentiation of these cells on filter. Caco-2 cell line is heterogenous and some lab-specific features such as culture conditions and cell line properties can vary from lab to lab. Due to the cellular heterogeneity of the Caco-2 cell line, the differentiation process occurs in a mosaic pattern, with some areas expressing fully differentiated cells while less differentiated cells in other areas (Tor, 2015, p. 103).

2. Sequence analysis of knocked-out cells

We primarily discuss that the sgRNA activity, according to a previous, relevant study by Liu et al. (2018), can potentially influence the cleavage activities of Cas9-sgRNA complexes. The parameters listed by Çetintaş et al. (2017), such as the definition of PAM sequences and target site selection that should be considered in the formation of the guide RNAs couldn't have been realized. Therefore, Cas9-sgRNA complex hasn't performed.

Moreover, in a study on the effectiveness of the CRISPR / Cas9 system, Thyme et al. (2016) stated that two possible factors that may hinder the efficient functioning of the system. These are either an inability to establish an effective Cas9-gRNA system or the inability to recognize the target site in vivo. More specifically, it has been stated that in vitro, the primary structure of the guide RNA can be altered and have a secondary structure, which will affect the operation of the Cas9-gRNA complex. They also discuss that sequence-specific genomic factors such as transcriptional repressor proteins and the chromatin structure in the target region may prevent the active complex from recognizing this region. In the same study, it was mentioned that CTCF, a transcriptional repressor

protein, affects the binding of Cas9-gRNA complex to the target region which should be considered in future studies.

In another study Yuen et al. (2017) expressed that the binding and directing capacity of guide RNA to Cas9 affects the knock-out process. In addition, they stated also that sgRNA and Cas9 would affect the operation of the complex at expression level. As mentioned in the methodology section before, the desired carrying capacity could not be reached in the cells after electroporation. This could suggest that a problem at expression levels of gRNA and Cas9 after transfection may have been encountered.

Genomic instability of cancer cells has been reported to be one of the most important barriers to CRISPR knock-out imaging (Yuen et al., 2017). They reported that chromosomal instability is encountered especially in cancer cells obtained from epithelial tumors. In this case, in such cancer cell lines where increasing copies of the target gene are present, it may be difficult to direct the guide RNA to the target region. Because, Caco-2 cells originate from adenocarcinomal cells of colon, one would expect the regulation of Cas9-mediated gene editing to be affected by chromosomal instability.

Conclusion

In conclusion, the AID / APOBEC family, among which APOBEC1 is present, contains proteins that convert DNA sequences to deoxyuridines by deamination of deoxycytidines. Although AID / APOBECs are powerful tools that enhance immune response, counteract genomic invaders, and alter gene regulation by removing epigenetic modifications from genomic DNAs, their ability to add mutations to nucleic acids resembles a double-edged sharp sword in cellular metabolism. When uncontrolled, overexpression of these potent agents may threaten genome stability and eventually lead to cancer. This suggests that APOBEC protein family genes are proto-oncogenes due to their DNA deamination activities. Therefore, there has to be future studies to search for additional sites different from small intestine enterocytes where APOBEC1 is still active. CRISPR / Cas9 complex stands as an effective tool for silencing of these genes where they are non-locally expressed or overexpressed.

Acknowledgement: This study has been performed at Conticello laboratory at the Instituto Toscano Tumori, Florence, Italy within the budget of the Italian Ministry of Health (GR-2008-1141464; PE-2013-02357669). We wish to thank to the group leader Prof. Silvestro Conticello and PhD student Martina Chieca for their valuable contribution to this study.

Authors indicate no conflicts of interest to disclose.

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READING THE STREETS OF BALAT AND URBAN IDENTITY

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Abstract: Being one of the biggest metropolises in the world, İstanbul is a peerless city with its natural beauties, history, archaeological heritage and socio-cultural life. These properties both create the rich identity of the city and provide a strong urban image. The skyline composed of the historical texture of the city and physical elements offers an extremely impressive view. Fener-Balat is an important element of the physical structure of the city of Istanbul. It plays an important role for historic and cultural values of Istanbul to take a shape. As an unforgettable part of the metropolis of Istanbul, Balat where Turkish, Rum, Armenian, Jewish and Bulgarian identities and mosques, churches and synagogues co-exist has the characteristic of a sub-region with strong elements forming its identity. It is observed that big changes have occurred in social textures and population compositions of these regions. The innermost parts of the city, where particularly the urbanized, settled upper-middle class families have left became the areas where poor or immigrant population became dense. The world continuously keeps changing within the frame of socio-cultural, physical, economical, political and technological dynamics and this change reflects on urban areas. The urban area which takes shape as a result of mutual interaction of these dynamics involves some differences and similarities in every age, every country and every city. Therefore no city is being shaped in the same way as others.

In the study, the relationships established by various social groups with the region are examined through printed texts and news on Internet. All sources are compared in the study and analyses of language, wording and ideological approaches are made and the meaning of Fener-Balat area for different groups is discussed. It s observed that the memory generated in the news on Internet regarding Fener-Balat region is composed of images generated by the groups from their ideological positions. In printed sources, on the other hand, it is observed that some kind of a popular history is narrated rather than generating a memory.

Keywords: Istanbul, Balat, Urban Identity, Roman, Culture.

Introduction

The geography, space, or human variant in the universe is unique to the entire world, the existing world, the understanding of human behavior may not be taken for many years, the reasons for neglecting the best interview, is actually looking for their own problems. In the field of psychology, it approaches the 60s, in its place of contents, or as a “relevant, confounding variable gereken that needs to be controlled, or at best, as a passive background to describe how it affects human behavior (Göregenli, 2010).

In the process of shaping the urban space, morphological, functional, visual or contextual features change in the urban built environment. Some of these changes cover a large area and may not be possible to observe. However, it is possible to observe concretely the changes taking place especially on the scale of single parcel or building block. In other words, the changes that can be observed directly in urban space appear as changes in morphological characteristics (building height, construction order, parcel dimensions, building form, etc.) (Ünlü, 2006).

How do spatial variables affect human behavior? In the broader sense, the science of psychology has always wondered and researched the effects of the environment on human behavior. However, to Kunt Lewin (1890-1947), the turning point in defining the environment-human relationship, the environment was generally criticized by psychology with a Skinner box or well-structured laboratory environment. The world of the 60s has shown that the world can no longer be "explained" by familiar epistomologies, so that it can no longer be "controlled". Life was much more complex than the one that could be controlled in a laboratory independent of time and space, and simulation was impossible (Göregenli, 2010).

Beginning in the 1960s, with the influence of major paradigms such as phenomenology, aesthetics, linguistic theory (semiotics, structuralism, poststructuralism, deconstruction), Marxism and feminism, which were internalized from other disciplines and reformed the disciplines of literature, philosophy, sociology, anthropology as well as architectural theories. and the interdisciplinary nature of architectural theory is strengthened. Architects have been working on determining the meaning in the linguistic field and adapting the acquired knowledge to architecture through linguistic analogy (Çağlar and Ultav, 2004).

In recent years, the relationship between human behavior and the physical environment has attracted the attention of researchers in the social sciences and environmental design disciplines and the field of study is rapidly evolving. The multidisciplinary character of environment - behavior studies and its application to real - world problems related to environmental design played an important role in the development of this field (Turgut, 1990).

Environmental Components

Environment and human relations are discussed in many disciplines such as sociology, psychology, philosophy, architecture and geography, especially social psychology, environment-behavior studies, human-environment relations, environmental psychology, environmental design research. The city, which is the stage of social relations networks, intellectual and cultural images, is also one of the basic elements of the social processes. Therefore, the history of mankind or world history is generally accepted as the history of cities and urban life and environment-human relations are examined through urban and urban spaces (Solak, 2017).

Different definitions and classifications of the concept of environment have been made in the Human - Environment interaction, which is dealt with from different perspectives in Environment - Behavior studies. For example, Rapoport defines the environment in the broadest sense as any condition or effect outside the ornagism, group or system being studied. Ittelson describes the environment as a system with seven components, such as perceptual, semantic, aesthetic, adaptive, integrative objective and general ecological relations of all these components. Lawton considers the environment as an ecological system with five components. Individual ; physical environment; personal environment, including important sources of behavior control such as family and friends; age, class, lifestyle, ethnic origin; social environment consisting of social norms and institutions (Turgut, 1990).

According to Rapoport, 'In human-environment relations, culture defines three different points of view that define each other. According to the first approach, culture is the lifestyle of a typical group. The second is based on the fact that culture is a system of cognitive schemes, symbols and meanings formed by symbolic codes. Thirdly, it is the point of view that culture is a set of adaptation strategies for survival in relation to ecology and resources. " (Batmaz, 2013).

Social rules, shared values, common experiences such as people's environmental experience, knowledge, learning and attitudes that determine the decisions of people in environmental choices or reshaping the environment reveal the inevitability of human-cultural interaction by creating a verse within themselves.

Kevin Lynch highlighted the use of five main components in creating the image of the environment in people's minds by highlighting urban images in identifying urban components: roads, boundaries, regions, nodes and sign elements. Lynch's work (1960) is based on cognitive interaction between urban space and users (Köseoğlu, 2011). The author wrote in his book: en I went back to the past when I was writing this book, and I met both myself and those old people again in the narrow, curved streets. I wandered again through the streets of my childhood and youth, fascinating with the people who served in the chirping bazaar of Balat, the music and the sounds of people coming from the taverns, the guilds, the summer cinemas and the unique ruins. Those relentless slopes stopped breathing again. I've always been proud to have a real value, a place that is truly unknown, and I am still talking about the general environmental fabric of balat (Tecim, 2017).

“The so-called Kant imaginary representation is the person who is reduced to the level of subjective representation of mental action and has said that the subject acting in the field of aisthesis cannot actually represent the idea. Images are fictions produced within the framework of priorities; instead of representing what they have sent, they rebuild it. In other words, the image of something is not its origin and this break between the image and what it refers to has unquestionably liberated the image. Kant, who no longer shows any sign, images by ıyla beautiful reflections ”(Schiller) - after the Kant independence - at least the Romantics call it independence and self-directionality - they only mark themselves” (Alemdar, 2009).

When I was talking about Balat, I wrapped up with pleasure in the mad wind that shuddered me and the cold that cut my face like a nail. My head is full of thoughts, my heart is full of emotions, my hands are in my pockets, I walk through the woods in the woods, narrow and pitch dark streets, hearing the weight of something both in my heart and my mind. It would give me a different melancholy when the stove walls that sprung out of the chimneys and swung in the wind wandered around me like ghosts and then disappeared. The blind pits of the houses that accumulate in my left and on my right would warm me a little. I have determined my route as Ayvansaray by avoiding the wrath of stray dogs and watching the garbage accumulated here and jumping over the puddles if there are any bal (Balim's socio-cultural structure clearly speaks of it) (Tecim, 2017).

The fact that the architectural environment started to be created, read and criticized by these "structured meaning biçim forms caused the image to be criticized in this field as well. For example, formal elements of historical buildings in Turkey (domes, minarets, eaves, bay windows, etc.), Hanging their tectonic and formal situations, they began to exist together with the means of the structure. These architectural elements are molded as forms and new forms produced for these meanings have started to be perceived as meaningless (Alemdar, 2009).

'Social And Spatial Restructuring In Balat"

Auge says that the space studied by anthropology is the space used by people, and that the legibility of that space is enhanced by the inhabitants, equipped with symbols that reflect their social existence and schemata. The three main themes symbolized - intertwined - are identity, relation and history. The way of symbolizing the place of a community is the factor that determines the personalities of individuals and constitutes their experiences (Kural et al., 2013).

Social relations, which are socially reproduced by forming a whole with the life style in the environment, and which are in constant change and development, cause the redefinition of urban identity. Social experiences, opinions, beliefs and behaviors constitute the socio-cultural structure of society. These elements, which direct one's social behaviors, also play an active role in the formation of urban identity. Identity affects the formation of the designer as much as society. Therefore, it can be defined as a cultural phenomenon in which completely new or past elements are reinterpreted. It is necessary to perceive and comprehend the city in a continuity from past to future.

The elements constituting the urban identity can be evaluated in terms of elements originating from natural, human and human-made environment.

Identity elements originating from human environment are individuals and society. The identity of the individual matures in the environment in which he lives. All conscious, unconscious perceptions, knowledge, experiences, thoughts, behaviors, expectations and predictions about the future, needs and desires of the individual, and also the identity of the community in which he lives are shaped. The individual identity forms the group and community identity. Accordingly, identity elements originating from the human environment are composed of sub-elements for demographic structure (population size, structure, density, age groups ...), institutional structure (political, administrative, legal, economic ...) and cultural structure (Importance). and Kılıçarslan, 2005).

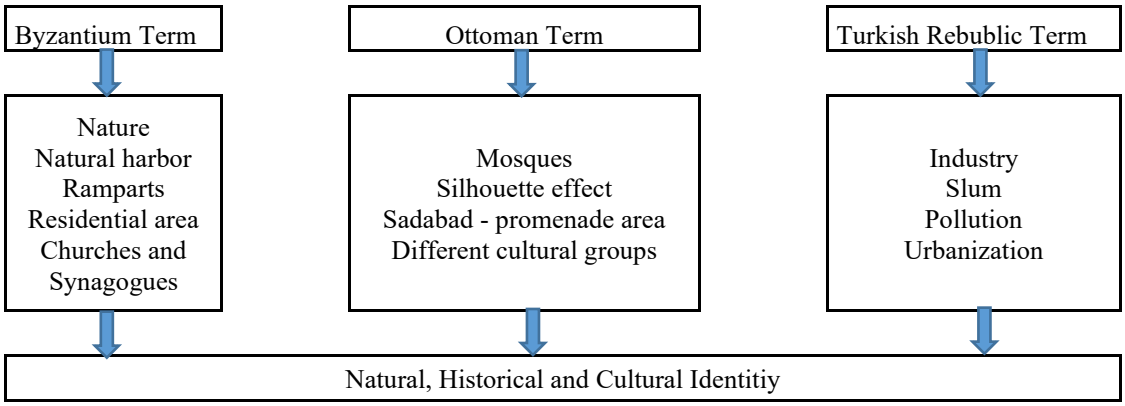

Figure 1. Identity formation of Haliç in historical process (Importance and Kılıçarslan, 2005).

Before defining space, Heidegger believes in the necessity of understanding it and describes it as an integral part of the existence of man and his world with the idea of being in the world (Hisarlıgil, 2008). Various writers emphasize that there are semantic differences between the creator / designer of the space and its use or distance

reading. For this reason, it is important to establish a language unity between the designer and the reader of the space. In the space-user communication environment, the language of space is a communication tool. The designer should be able to reach the user and express himself / herself while transforming the space he created and designed in his memory into a concrete product. In order to do this, he / she should evaluate and shape the spatial elements loaded with meaning that correspond to the concepts used in the project by choosing from the culture, expectations and traditions of the society with their own knowledge and experience. Thus, the user can easily detect and read space (Günal and Esin, 2007).

There is a close connection between people's places and identities. Because they look at the world from this “space / and“ home ve and try to give meaning to everything that is going on around them in proportion to their equipment in this “space”. As Gaston Bachelard puts it, evi the house of people opens up to the world. İ The connection of man with the “house” is in fact his connection with the ”world ((Tüzer, 2007).

Fener, Balat And Ayvansaray Neighborhood, What Makes This Place Special?

I think it would be a good place to say that Istanbul is the most surprising district. As you walk through the streets, you become aware that you are special; you cannot believe what you see, you find yourself in a visual and emotional feast. Here you can see what Istanbul, Constantinople, Bizantion mean. Turks, Greeks, Jews, Armenians, Bulgarians and Gypsies lived for years as neighbors. It is the most beautiful representation of the cultural mosaic of the 3000-year-old Istanbul city. On the other hand, now extinction, abandonment, deceit, forgotten, has become a symbol of ignorance, Fener, Balat, Ayvansaray. These streets are covered with the sadness of a faded, almost extinct wealth. Even though it may seem cute that the chirp of an impassive life that doesn't belong here, sometimes, you're buried and connected to this special world birden.When you say Fener, inevitably, the Patriarchate and Fener Greek High School, which welcomes people at the beginning of that breathtaking hill, comes to mind. Balat is the picture that comes to mind with its typical houses, synagogues and colorful bazaar. Ayvansaray is a place with more garden houses and walls.

	
Fener Greek orthodox patriarchate (frontage)	Fener Greek Ortodox patriarchate (rear facade)

	
Fener Greek High Scoll (frontage)	Fener Greek High Scoll (rear facade)
	
Typical Balat Houses	Typical Balat Houses
	

Balat Bazaar	Balat Bazaar
	
Balat Bazaar	Balat Bazaar
	
Ahrida synagogue (Inside)	Ahrida synagogue (frontage)

Fener, Balat and Ayvansaray Today

The districts of "Fener, Balat and Ayvansaray alan, located on the shores of the Golden Horn and constitute a great urban potential for Istanbul, are a great cultural heritage for Istanbul with its historical and socio-cultural richness extending along the historical Byzantine sea walls. The old Jewish quarter is one of the last settlements inhabited by Roma today. The gentrification process has continued in the region for the last 20 years. On the other hand, aside from the rehabilitation works currently underway, it is still an idle region and sanitary conditions have not been improved.

The region was first brought to the agenda in the early 2000s with the rehabilitation project started with Fatih Municipality-European Union cooperation and protection-improvement targets. In the second stage, Fener-Balat Renovation Project works are taking place. Thus, the restoration works carried out under the leadership of the European Union with Fatih Municipality were replaced by renovation works carried out in a public-private partnership. The region was declared a renewal area in 2006, based on the Law No. 5366 on the Conservation, Conservation and Use of Worn Historical and Cultural Immovable Assets.

About Law No. 5366

Law No. 5366 on the Kanun Renewal Protection and Survival of Immovable Historical and Cultural Immovable Assets', approved by the Council of Ministers Decision dated 17.11.2005 and numbered 2005/9668, published in the Official Gazette dated 14.12.2005 and numbered 26023. In Article 4 (f) of the Implementing Regulation titled başlıklı renewal area sit, the areas registered and announced as protected areas and protected areas and the areas within the protected areas belonging to these areas are defined and accepted by the Council of Ministers upon the proposal of the competent authority. (Ahunbay et al.2016).

Urban renewal, which can be defined as the process of reorganization of urban building stock, urban areas and social relations, often transformation, revival, revitalization, etc. with and / or in place of concepts. Different nomenclatures and definitions related to the process in question cause the evaluations to be intertwined (Şentürk, 2011).

Renovation and Generation Studies in Balat, Fener, Ayvansaray

In Fener, Balat, Ayvansaray region, in order to ask how the people in the region view the urban transformation and how they meet the gentrification processes, the inhabitants of this neighborhood were asked what problems they had and what they needed.

Many people who have participated in research with the local people have been living here for a long time. Some of the people living in the neighborhood are living, some of the region consists of leading artisans. Almost all of the researchers stated that the region was ignored. They state that even if they have heard that the problems will be solved before the local elections, the problems will continue exponentially. The participants say that many projects have been produced in the region and that in every project the authorities say Bal Balat will change, everything will be nice “but nothing has changed.

The main problems of the participants who live in the region are the neglect and weakness of the buildings in the neighborhood. The reason for this problem is that the buildings in the neighborhood are registered and because of this reason they can not maintain their buildings.

According to the opinions of the people living in the neighborhood, the reason for the lack of service to Balat is that the state considers the inhabitants of the municipality as suburbs, and that the majority of the nearby constructions are near nightlife. They also think that due to the low level of culture of the inhabitants, the district does not provide adequate services.

Since the buildings in the region could not meet the needs of the day, both the people living in Istanbul and the people who lived there preferred to settle in new places. As the physical conditions of the vacated houses are constantly deteriorating, they are preferred by low-income families. Balat people are poor due to unemployment. The poor neighborhood affects the tradesmen and prevents the development of the environment. The continuation of the tradesmen situation in the surrounding area leads to a homogenous structure with the presence of people from all walks of life.

There is not enough children's playground in the neighborhood. The lack of cultural activities (theater, cinema, exhibition hall, etc.) causes the streets to be vacated in the evening.

Original, Spatial and Social Texture

It has been determined that the buildings in the region need to be renovated. The weaknesses of the façades of some buildings and the internal structures of some buildings were determined. Within the scope of the study, it is necessary to ensure the restoration and rehabilitation of the buildings in accordance with the original, not with demolition and reconstruction. Thus, the neighborhood does not lose its own stance and preserves its historical texture. The shanty houses in the region must be demolished and constructed in accordance with the environmental texture that can reflect the spirit of the region.

The inability of the region to renew itself for many years caused periodical problems. The neighborhood continues to have hemeonomic, social and cultural problems both physical infrastructure today. Due to these problems, the residents of the district agree that the region will be in a much better condition than the old state regardless of the quality of the renovation works. According to the participants, the renewal of the region will prevent the mentioned problems and the values of the properties will increase and consequently the social, economic and cultural structure will change and the income of the owners and the artisans will increase.

In recent years, the movement of trades in the neighborhood real estate trades, traders, those who make the “old residents” (foreigners) who deal with real estate business non-Muslims, rich, high society, artists, intellectuals, expats, lawyers, doctors and engineers who have a professional profession.

A significant number of respondents who believe that building purchases and renovations in the region are good for the region perceive the fact that purchases are made silently by foreigners as a threat. According to the participants, the first of the reasons for my region to be preferred is that the region, which is the most beautiful place of Istanbul, will be a very valuable district in the future.

Outdoor Stage with Historical Decor; Start

The city is a multi-faceted phenomenon that has been in the interest of many disciplines and has been defined in many ways. Today, although it is argued that all the cities of the world are starting to resemble each other, it is a fact that the dynamics, socio-cultural interaction and development process of each city are different from each other. Therefore, each city has a different history, a silhouette, social life and identity formed with a different structure.

According to Al do Rossi, the city is hidden within its historicity that has created a collective consciousness over time. Rossi's approach is not to create new ideal forms, but to use them in the essence of existing and created forms in historicality (Hagur, 2005). There is a close connection between people's places and identities. Because they look at the world from this “space / and“ home ve and try to give meaning to everything that is going on around them in proportion to their equipment in this “space”. As Gaston Bachelard puts it, evi the house of people opens up to the world. I The connection of man with the “house” is in fact his connection with the ”world ((Tüzer, 2007). Rather than neglecting personal changes, Barker thought that they would be self-contained with the concept of behavioral environment and that environmental factors were more important. Individuals move every day in a wide range of behavioral environments and they control their behavior; but they are not personal characteristics. For example, the main street, post office, classroom, hair salon, cinema, where any person lives, are all behavioral environments. Each is described in terms of mutually dependent environmental characteristics that make it homogeneous (Göregenli, 2010).

According to Bailly, the space represented becomes the perceived space. In other words, it becomes what is seen, heard, felt and even in the life space. However, Bertrand thinks that the perceived space is generally larger than the experiential space (Göregenli, 2010).

The first connotation of the concept of mek third space an used by Oldenburg for the first time is an order of priority. The first space corresponding to the conceptualization of the home / private space has priority. This is followed by public spaces called the second space, such as workplaces or schools. The third spaces, which are considered to be outside the first and second spaces, but can also carry the characteristics of these spaces in a sense, are at the top of the list (Demir, 2017).

Third Space Features;
• People who use third places are not in their homes or workplaces. But they are as comfortable as at home. (Homes away home)
• These places; neutral, egalitarian, cheerful places whose main activity is chat.
• They are easily accessible to everyone.
• It has a simple profile.
• These places are regulars (Oldenburg, 1991)

Istanbul and even start having an important place for Turkey, Lantern, Ayvansaray, as we can see from Istanbul, and has become a place frequented by tourists. The unspoiled historical texture, mosque, church, synagogue, patriarchate, narrow and abundant slopes with organic curves and colorful buildings are enough to turn the balat into a center of attraction. Today, we observe that cafes are opened under almost every building of balat. Young people show much interest in the neighborhood. We can connect this to the history of the district, but it can be thought that the universities of Kadir Has and Ayvansaray, which are located next to balat, are influential.

One of the most characteristic features of Balat is the antique shop, auctioneer, and second-hand shop wearers. Some of them hold auction sales, some of them old, some of them new, some of which are recently fashioned, and some of them specialize in major fields such as toys and electronic devices that transport people to their childhood. They all have one thing in common: it gives people intravenous nostalgia.

Balat was always nice. Who knows, he would come again, but most of the young people were outside the radar. Balat stopped, stopped, with the concept spaces opened to become the new address of the old Cihangir and Galata goers. At the weekend, they started to come to Balat for breakfast and to get up from Sarıyer just to have a coffee. Every corner in Balat is full of colorful Balat houses with every bay, with cozy concept cafes, breakfasts, 3rd wave coffee shops, modern antique shops and vintage shops.

The Agora Tavern is a living legend. For almost 140 years, every raki-loving pub has been coming to the bar, like a pub with ossified goers. Even in the dull days of Balat, the neighborhood was a shining place.

Balat has become a place where art houses take place, where handmade, ceramic objects are made, cloth bags, badges of anime characters, handmade notebooks and postcards, and design products are produced.

The natural texture of the neighborhood is the backdrop for photographic photographers and newlywed couples to have their wedding photos taken on the streets that are open-air scenes.

The neighborhood, which used to be the venue for many films and series in the past, now creates a natural outdoor scene for a series that is completely owned by it. The first question asked by the residents of Balat is nerede Where is the Çukur withdrawn? Ken while some guides organize the 'Çukur' tour and respond to the request.

The 'Çukur' series brings thousands of people to the historical streets of Balat. Founded benches, street music played in the street, guiding children in return for the money and more. Here, step by step Balat and "Çukur" tourism

Balat, one of the oldest districts of Istanbul, where the series of 'Çukur' was shot, is experiencing historical days with series tours. The attraction of local and foreign tourists to the attraction places made the shopkeepers smile.

With its colorful houses and historical texture, Fener Balat, which is one of the popular sightseeing places of domestic and foreign tourists, is now experiencing 'Çukur' tourism.

In the places used in the series, in front of the graffiti and 'Çukur' tattoos, queues are formed especially on weekends to have photos taken.

The cafes carrying the name of the series characters and cafes close to the set where the 'Çukur' music plays are also full.

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STUDY ON SHUNT CURRENTS IN A MULTI-STACK VANADIUM FLOW BATTERY SYSTEM

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Abstract: An all-vanadium redox flow battery (VRFB) is an energy storage device that uses the redox reaction of vanadium ions with different oxidation states. It has the advantages of quick conversion between charge and discharge processes, long operation life, separate power and energy capacity designs; as a result, it has been considered as one of potential candidates as energy storage systems for renewable energies. Because the electrolyte is electrically conductive, shunt currents occur within a multi-cell stack and within the piping system that connecting stacks. Shunt currents are affected by cell number in a single stack, stack number, dimensions of flow channels. In this study, a mathematical model is developed to determine shunt currents in a multi-stack system. The effect of stack number on charge transfer efficiency are predicted by this model. Results show that central stacks in a VRFB system provide more current. The maximum cell current decreases with increasing stack number.

Keywords: All-vanadium redox flow battery, multi-stack, shunt current, flow rate, charge transfer efficiency

Introduction

All-vanadium redox flow batteries (VRFBs) are devices that charge and discharge by the redox reaction of vanadium with different oxidation states. VRFBs have the advantages of long cycle life, low self-discharge, low maintenance cost, flexible design on stack power and environmental-friendly; as a result, they are considered as a potential energy storage system for renewable energy sources [1-3]. In a VRFB stack, multiple cells are connected in series for high voltage requirement. To meet the system voltage requirement, multiple VRFB stacks are connected in series in a system.

Because the electrolyte is electrically conductive, shunt currents occur in the manifolds and flow channels within the stacks and also in the piping system. The existence of shunt currents results in the decrease of electric capacity and energy conversation efficiency. As a result, it is important to understand the effect of shunt current on the battery performance and the effect of design parameters on shunt currents.

Xing et al. [4] developed a model to study shunt current in a VRFB stack based on the electrical circuit analog method. Their results suggested that increase of single cell power, reduction of cell number, and increase of the resistances of electrolytes in the manifolds and channels can help reduce shunt current loss. Tang et al. [5] investigated the effect of shunt current on efficiency and temperature variation within a 40-cell stack during standby condition by combining the shunt current model and thermal model. Their results showed that stack temperature was significantly increased due to the existence of shunt current within the VRFB stack.

It is challenging to measure shunt currents within a VRFB stack because it is not easy to install any current sensor in the manifolds and flow channels. Fink et al. [6] installed current sensors on the external hydraulic system of a 5-cell stack for the measurement of shunt currents. A mathematical model was also developed for the investigation of shunt current on coulombic efficiency. Results showed that inner cells within a stack discharge faster than outer cells. Yin et al. [7] developed a three-dimensional model to investigate the effect of shunt current distribution on coulombic efficiency. The model was validated by the experimental data and the results showed that short channel design caused a coulombic efficiency loss of approximately 23%.

Shunt currents could be reduced by increasing the electrical resistance of electrolyte in the flow path between cells;

as a result, longer and smaller cross-sectional area of flow path are preferred in designing a VRFB. However, the increase of flow resistance will cause the increase of pump consumption. The trade-off between shunt current loss and pump power consumption is a key issue on stack and system design. In this study, a mathematical model that can investigate the effect of flow path design on shunt current of a multi-stack system is developed. The distribution of shunt currents in the stack and in the piping system is predicted and the charge transfer efficiency is analyzed.

Modeling Methods

In order to simplify the model, the following assumptions were made.
1. Electrical potential is uniform throughout the active area.
2. Shunt currents in the inlet paths and outlet paths are the same in a single cell and in the piping system.
3. Temperature and electrolyte concentrations within the system are uniform.

The shunt currents within a VRFB stack and in the piping system were modeled as an equivalent electrical circuit, as shown in Figure 1. In the system, there are z VRFB stacks connected in series and each stacks consists of K cells, resulting in $M = Kz$ cells totally. The parameters $R_{pc,S}$, $R_{nc,S}$, $R_{pm,S}$, and $R_{nm,S}$ represent the equivalent resistances of electrolyte within the positive channel, negative channel, positive manifold, and negative manifold, respectively. The resistances of the electrolyte in the piping system are represented by $R_{pc,P}$, $R_{nc,P}$, $R_{pm,P}$, and $R_{nm,P}$.

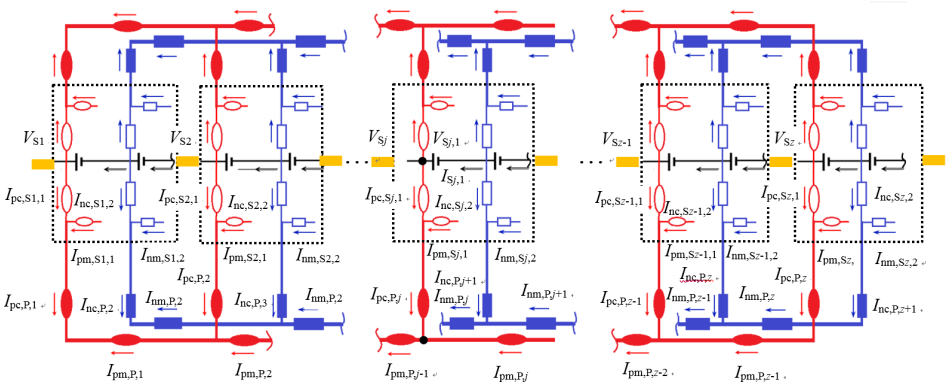

Figure 1. The equivalent electrical circuit of the system with multiple stacks in series.

Appling Kirchoff's law to the i-th cell of the equivalent electrical circuit for the stack results in linear equations.

$$I_{Sj,i} - I_{Sj,i-1} - 2I_{pc,Sj,i} - 2I_{nc,Sj,i} = 0 \quad (1)$$

$$I_{pc,Sj,i} + I_{pm,Sj,i} - I_{pm,Sj,i-1} = 0 \quad (2)$$

$$I_{nc,Sj,i} + I_{nm,Sj,i} - I_{nm,Sj,i-1} = 0 \quad (3)$$

$$V_{Sj,i} - R_{pc}I_{pc,Sj,i} + R_{pm}I_{pm,Sj,i} + R_{pc}I_{pc,Sj,i+1} = 0 \quad (4)$$

$$V_{Sj,i} - R_{nc}I_{nc,Sj,i} + R_{nm}I_{nm,Sj,i} + R_{nc}I_{nc,Sj,i+1} = 0 \quad (5)$$

where $I_{Sj,i}$ and $V_{Sj,i}$ represent the current and voltage of j-th stack in the system and i-cell in the stack.

In each stack, the positive electrolyte is connected with the positive channel of the positive piping system. The current and voltage relations can be modified as:

$$I_{Sj,1} - I_{Tj} - 2I_{pc,Sj,1} = 0 \quad (6)$$

$$I_{pc,Sj,1} + I_{pm,Sj,1} - I_{pc,Pj} = 0 \quad (7)$$

$$V_{Sj,1} - R_{pc}I_{pc,Sj,1} + R_{pm}I_{pm,Sj,1} + R_{pc}I_{pc,Sj,2} = 0 \quad (8)$$

where I_{Tj} is the overall current of a single stack. Similarly, the negative electrolyte is connected with negative piping system:

$$I_{nc,Sj,2} + I_{nm,Sj,2} - I_{nc,Pj} = 0 \quad (9)$$

The last single cell in each stack does not have a complete positive loop, the related equations are modified as:

$$I_{Sj,M} - I_{Sj,M-1} - 2I_{pc,Sj,M} - 2I_{nc,Sj,M} = 0 \quad (10)$$

$$I_{Sj,pc,M} - I_{Sj,pm,M-1} = 0 \quad (11)$$

$$I_{nc,M+1} - I_{nm,M} = 0 \quad (12)$$

In the piping system, similar equations for the current balance and voltage balance can be obtained by the same method:

$$I_{\mathrm{pc,P},j} + I_{\mathrm{pm,P},j} - I_{\mathrm{pm,P},j-1} = 0 \quad (13)$$

$$I_{\mathrm{nc,P},j+1} + I_{\mathrm{nm,P}j+1} - I_{\mathrm{nm,P}j} = 0 \quad (14)$$

$$V_{\mathrm{S}j} - R_{\mathrm{pc,S}} I_{\mathrm{pc,S}j,1} - R_{\mathrm{pc,P}} I_{\mathrm{pc,P}j} + R_{\mathrm{pm,P}} I_{\mathrm{pm,P}j} + R_{\mathrm{pc,P}} I_{\mathrm{pc,P}j+1} + R_{\mathrm{pc,S}} I_{\mathrm{pc,S}j+1,1} = 0 \quad (15)$$

$$\left(V_{\mathrm{S}j} - V_{\mathrm{S}j,1}\right) - R_{\mathrm{nc,S}} I_{\mathrm{nc,S}j,2} - R_{\mathrm{nc,P}} I_{\mathrm{nc,P}j+1} + R_{\mathrm{nm,P}} I_{\mathrm{nm,P}j+1} + R_{\mathrm{nc,P}} I_{\mathrm{nc,P}j+2} + R_{\mathrm{nc,S}} I_{\mathrm{nc,S}j+2,2} + V_{\mathrm{S}j+1,1} = 0 \quad (16)$$

The first and last stacks are special cases in this system:

$$I_{\mathrm{pc,P},1} + I_{\mathrm{pm,P},1} = 0 \quad (17)$$

$$I_{\mathrm{nc,P},j+1} + I_{\mathrm{nm,P}j+1} = 0 \quad (18)$$

$$I_{\mathrm{pc,P},z} + I_{\mathrm{pm,P},z-1} = 0 \quad (19)$$

$$I_{\mathrm{nc,P},z+1} - I_{\mathrm{nm,P},z} = 0 \quad (20)$$

The system voltage is the summation of stack voltage:

$$V_{\mathrm{SYS}} = \sum_{j=1}^{z} V_{\mathrm{S}j} \quad (21)$$

The system current is the same with the stack current:

$$I_{\mathrm{SYS}} = I_{\mathrm{T},j} \quad (22)$$

The cell voltage is assumed to be a function of operating current:

$$V_i = f(\mathrm{I}_i) = V_{\mathrm{cell}}^0 - R_{\mathrm{e}} I_i \quad (23)$$

The resistance of the electrolyte can be determined by the dimension and geometry of the flow path. Shunt current and cell current are determined by this model. The overall unknown of the system is 5*M*-2. The linear equations can be expressed in a matrix from:

$$\mathbf{V} = \mathbf{AI} \quad (24)$$

where **V** is the vector, consisting of cell voltages, **A** is the matrix with resistances, and **I** is the current vector to be solved. The solution process is similar to the previously published paper [8].

Results and Discussion

Voltage and current of each cell and shunt currents in the system are coupled together in this model. An iteration procedure was applied to solve this model. All cell currents were initially assumed to be the same; therefore, the initial cell voltages were the same. The cell voltages obtained from first and second calculations were very closed, as shown in Figure 2. In order to obtain accurate results, the results obtained by the second time calculation were used for analysis.

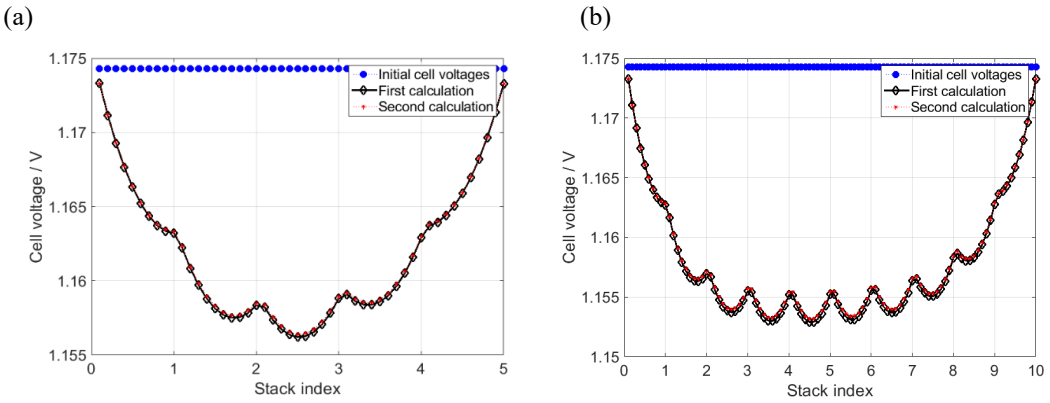

Figure 2. Convergence test of the model for (a) 5 20-cell stacks; and (b) 10 10-cell stacks.

There could be different configurations of cell number and stack number in a system. The following shows an example of designing an approximately 100-volt VRFB system with 100 cells. The stack number was designed as 5, 10, and 20 with cell numbers of 20, 10, and 5, respectively. The electrolyte conductivity and dimension of the flow channel and manifold in the stack can be obtained from our pervious study. The length of connected pipes varied with respect to stack size. Some distance was left between stacks for piping connection and maintenance, as shown in Fig. 3. The channel distance from the manifold of piping system to each stack is estimated as 15 cm.

Each cell thickness is estimated as 1 cm. The dimension of the piping system and corresponding electrolyte resistances are listed in Table 1.

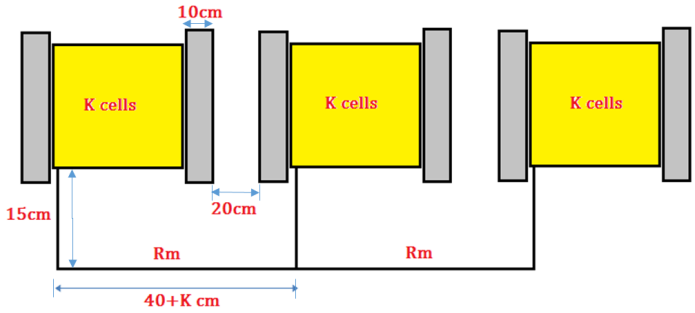

Figure 3. Dimension of piping system for electrolyte.

Table 1: Pipe length and resistance for various stack number.

Stack number, Z	Cell number in a stack, K	Pipe manifold length $L_m = 40+K$ (cm)	Pipe manifold resistance R_{pm}, R_{nm} (Ω)
5	20	60	39.52
10	10	50	32.93
20	5	45	29.64

Figures 4(a), 4(b), and 4(c) present cell current distribution in each stack for 5, 10, and 20 stacks in series when the system is charged at 54 A. It can be seen that the cell currents are lower than 54 A. Part of charge was lost due to shunt current. For the 5-stack system, the lowest cell current in each stack is located at the central cells. However, for the 10-stack and 20-stack systems, the cell current of the first and last stack gradually decreases. It is due to the shut current exiting in the flow channels of piping system. Figures 4(d), 4(e), and 4(f) present cell current distribution in each stack for 5, 10, and 20 stacks in series when the system is discharged at 54 A. The cell current distribution shows similar distribution as that in the charging process. Most central cells need to generate more current to compensate shunt current loss. It can also be observed that the cell current decreased with increasing stack number and the cell current distribution for most stacks became uniform. This is because the voltage difference between cells was reduced with decreasing cell number in a single stack.

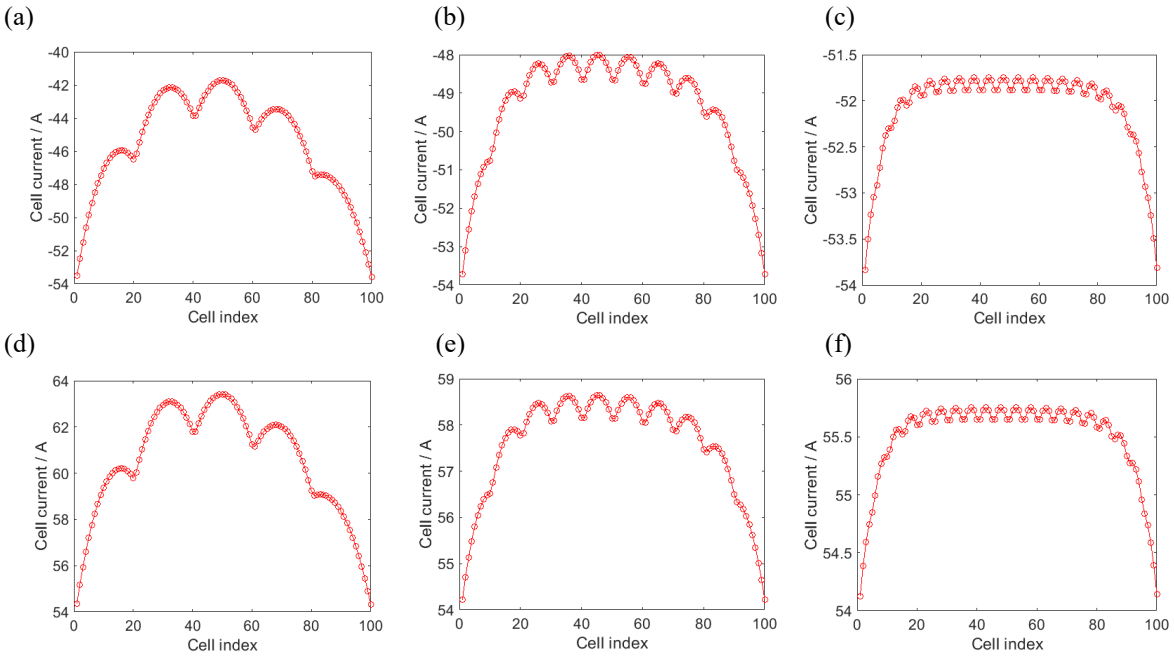

Figure 4. Cell current distribution during charge process for (a) 5 20-cell stacks; (b) 10 10-cell stacks; and (c) 20 5-cell stacks and during discharge process for (d) 5 20-cell stacks; (e) 10 10-cell stacks; and (f) 20 5-cell stacks.

Figures 5(a), 5(b), and 5(c) present shunt current in the flow channel and manifold of each stack for 5, 10, and 20 stacks in series when the system is charged at 54 A. In each stack, the maximum shunt current is located in the

manifolds due to the smallest electrolyte resistance. As the stack number increases, the shunt currents in the flow channel or manifold decrease. Due to the potential difference between cells and stacks. During the discharge process, the shunt current distribution shows similar trend.

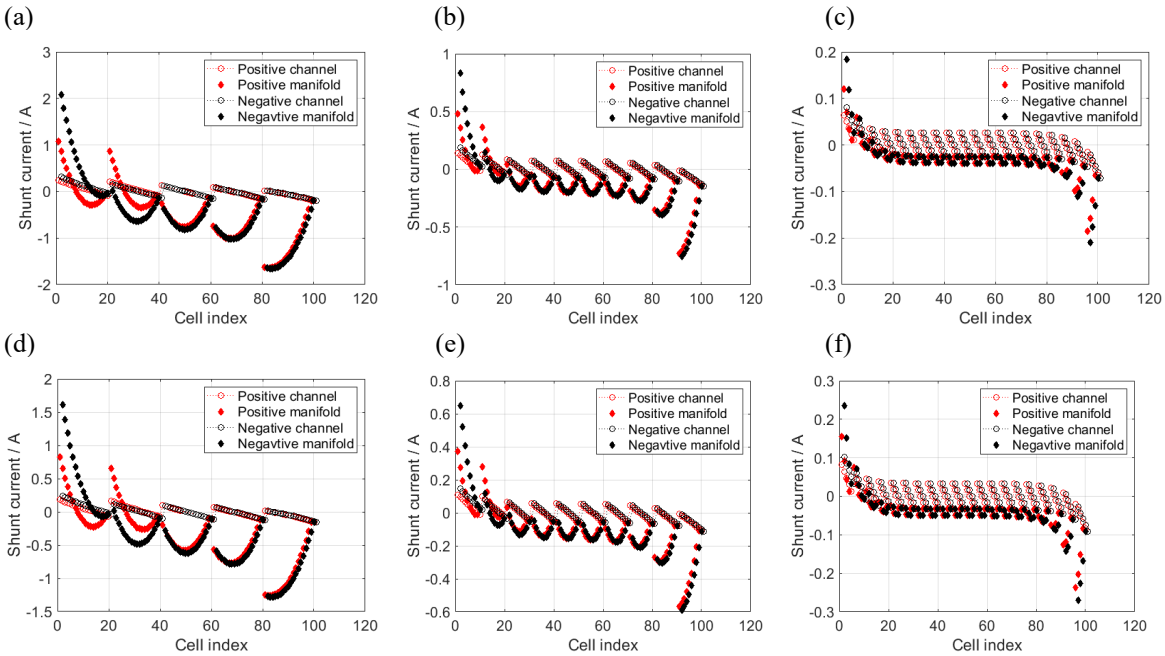

Figure 5. Shunt current distribution within the stacks during charge process for (a) 5 20-cell stacks; (b) 10 10-cell stacks; and (c) 20 5-cell stacks and during discharge process for (d) 5 20-cell stacks; (e) 10 10-cell stacks; and (f) 20 5-cell stacks.

Figures 6(a), 6(b), and 6(c) present shunt current in the flow channel and manifold of the piping system for 5, 10, and 20 stacks in series when the system is charged at 54 A. The maximum shunt current is located in the central manifold. In addition, the shunt currents in the first flow channel and last channel also showed relatively larger than those in the other flow channels. Because of the special location of first flow channels, shunt currents in the first stack converged to the first flow channel and first manifold. Similar Explanation can be applied for the discharge process, as shown in Figures 6(d), 6(e), and 6(f).

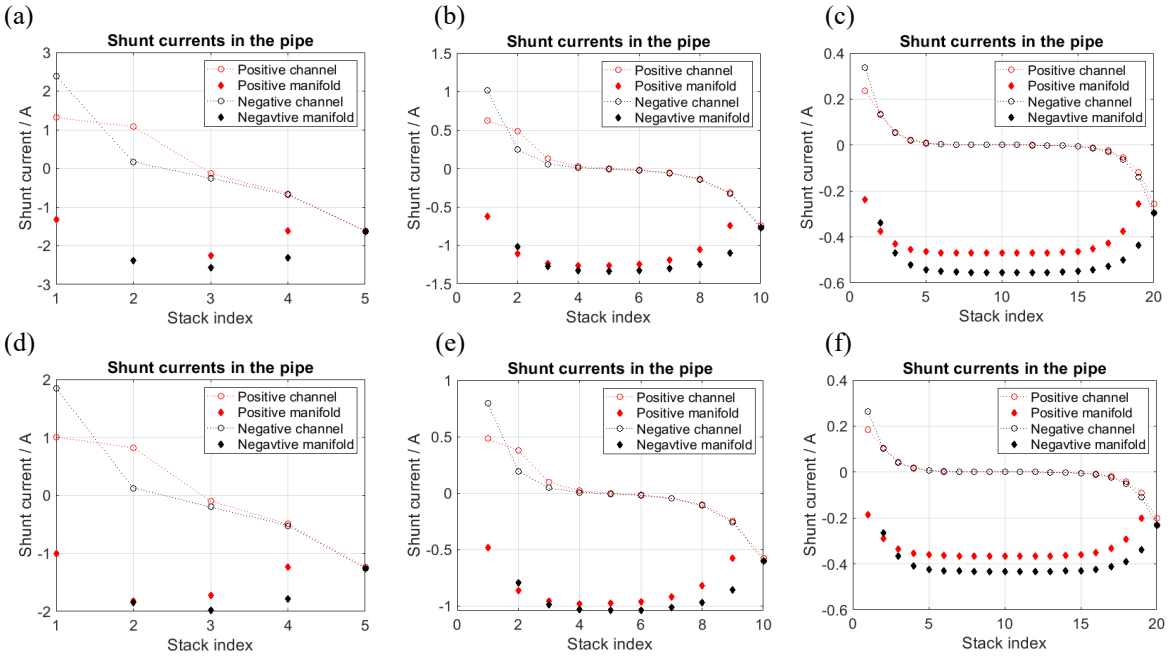

Figure 6. Shunt current distribution within the piping system during charge process for (a) 5 20-cell stacks; (b) 10 10-cell stacks; and (c) 20 5-cell stacks and during discharge process for (d) 5 20-cell stacks; (e) 10 10-cell

stacks; and (f) 20 5-cell stacks.

The charge efficiency is defined as the ratio of the power charged to each cell to the power applied to the system:

$$\varepsilon_{\text{charge}} = \frac{\Sigma\left(V_{\text{cell}} I_{\text{cell}}\right)}{V_{\text{sys}} I_{\text{sys}}} \tag{25}$$

whereas the discharge efficiency is defined as the ratio of net power generated from the system to the power generated from each cell.

$$\varepsilon_{\text{discharge}} = \frac{V_{\text{sys}} I_{\text{sys}}}{\Sigma\left(V_{\text{cell}} I_{\text{cell}}\right)} \tag{26}$$

The power difference between cells and system results from shunt current loss. The effect of stack number in a system with overall 100 cells on the efficiencies is shown in Table 2. As can be seen, the efficiencies increase with increasing stack number. As cell number in a single stack decreases, the maximum cell potential difference also reduces, resulting in lower shunt current. In addition, the electrolyte resistances in the piping system are usually larger than those within a stack, causing less shunt current. Although shunt current in a system can be reduced by increasing stack number, the pump power also increases due to increased pipes. A trade-off between pump power and shunt current need further investigation.

Table 2: Comparison of efficiencies of the system with various stack number.

Stack number	Charge efficiency	Discharge efficiency	Overall efficiency
5	0.896	0.926	0.830
10	0.943	0.957	0.902
20	0.975	0.968	0.944

Conclusion

Electrolyte of the VRFB is electrically conductive, causing shunt currents in the manifolds and flow channels within the stacks and also in the piping system. The existence of shunt currents results in power loss and performance drop. Shunt current is affected by cell number in a stack and stack number in a system. In this study, the effect of system configuration on shut current is investigated by a mathematical model. The following conclusion can be drawn.

(1) Shunt current shows quasi-symmetrically distribution within the connected stacks and the maximum shunt current is located in the central manifold of the piping systems.
(2) Charge transfer efficiency increases with increasing stack number under a predetermined overall cell number in a system.

Acknowledgements

The authors would like to acknowledge the support from the Bureau of Energy (107-D0112), Ministry of Economic Affairs, Taiwan, ROC for this study.

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THE EFFICACY OF THE HERBAL IODINE SOLUTION CONCENTRATE FROM THE BRAND OF "JADRANKINA OTOPINA" (JADRANKA'S SOLUTION) IN THE TREATMENT OF THYROID DISEASES

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Abstract: This paper presents the results of the research on effectiveness of the Herbal Iodine Solution Concentrate "Jadranka's Solution" on diseases of thyroid gland. The research included 64 participants who fulfilled detailed online questionnaire about their socio-demographic data and experiences they had with the Herbal Iodine Solution Concentrate „Jadranka's Solution". Along with that, each participant sent us two laboratory findings of the blood' level of TSH and T4. The most important result of the research was confirmation of the hypothesis that regular use of the Herbal Iodine Solution Concentrate "Jadranka's Solution" improves clinical status of patients with disorders of thyroid gland. Pairwise' comparison has confirmed significant difference at the ,05 level for levels of TSH and T4 before and after the use of "Jadranka Solution". Statistically, the results of the study showed a significant decrease of the values of TSH, and an increase in FT3 and FT4 hormones to their mid-values and above the reference value, when comparing values at the point of diagnosed health issues.
These results confirms subjective evaluation of the participants who, in 98.5% of participants evaluated effectiveness of Herbal Iodine Solution Concentrate "Jadranka's Solution" as excellent and none of them evaluated Solution as ineffective. Speaking about socio-demographic data, most of the participants were 30 to 40 years old (56%), 49,2% of them have university' degree and 68.6% of them is employed. These data point that participants are better educated and informed than overage population in Croatia. Most of them use Herbal Iodine Solution Concentrate "Jadranka's Solution" because of diseases of thyroid gland, 73.4% because of hypothyroidism and 7.8% because of hyperthyroidism.

Introduction

Iodine is a trace element that is essential to the organism in small quantities. Adequate intake of iodine is irreplaceable in the synthesis of thyroid hormone, thyroxine (T4) and tri-iodothyronine (T3) necessary for growth, organ development and the use of nutrients in the body (1). Serious iodine deficiency increases the risk of various disorders in children and adolescents, but the most pronounced development of hypothyroidism and dyspnoea. Namely, thyroid hormones regulate various physiological processes such as protein synthesis, growth and development (2). The chronic iodine deficiency results in increased TSH synthesis in the pituitary gland that stimulates the thyroid to increase the use of available iodine, which over time leads to thyroid hypertrophy and the formation of one or more nodules on the thyroid, ultimately resulting in drowsiness. Health risks arise not only in severe iodine deficiency, but also in small or moderate iodine deficiency, resulting in decreased work capacity, metabolic disorders, disturbed thyroid function, and dyspnoea. In children, slower growth and decreased cognitive functioning resulted in reduced IQ.

In 1993, the World Health Organization therefore issued a decision on the obligatory iodination of kitchen salt, as it is a safe and inexpensive way to combat the lack of iodine. It seemed that this procedure would ensure optimal iodine intake for the entire world population. However, despite these efforts, iodine deficiency has become a public health problem again in developed countries such as Australia and the United Kingdom, which were previously considered to be iodine-sensitive countries. The World Health Organization estimates that 2 billion people, including 285 million school children, have a deficit of iodine (3).

Therefore, the measure of addition of iodine to the kitchen salt proved insufficient for the ingestion of sufficient amounts of iodine, and in many studies it proved extremely useful to add additional iodine Solutions. The fact is that in cases of mild to moderate iodine deficiency, increased thyroid activity may be retained by euthyroidism, that is, thyroid hormone levels are within normal limits, but chronic thyroid stimulation leads to the formation of nodules on thyroid glands and consequent clinical illnesses. Increased intake of iodine leads to a decrease in the incidence of subclinical hypothyroidism and to a reduction in risk for M. Graves (Graves' disease) and thyroid

carcinoma. Accordingly, iodine optimization is an important component of preventive health programs aimed at reducing the incidence of thyroid disease (1,4).

Opinions on the addition of iodine are split, some experts (mostly medical doctors working in practice with patients) insist that each patient with thyroid disease must take iodine supplements, some recommend only iodine from herbal remedies such as algae or seaweeds (4).

„Jadranka's Solution“ is precisely such a solution, i.e. the Herbal Iodine Solution Concentrate that contains exclusively iodine from seaweed, and is therefore completely natural. Jadranka's Solution has been on the market for two years, but the production has increased significantly in the last year and the product has a significant rise in the number of beneficiaries. This is exactly what triggered this research, the subjective sense of improvement and the feedback received from beneficiaries were extremely positive. Many stated that their subjective sense of improvement was accompanied by an objective improvement in laboratory findings (before and after the use of the Herbal Iodine Solution Concentrate), in particular TSH and FT4.

It was on this track that we designed a study with a starting hypothesis that the use of Herbal Iodine Solution Concentrate Jadranka's Solution leads to the normalization of the levels of TSH and FT4 in the blood.

Participants and methods

The study included 64 participants who used the Herbal Iodine Solution Concentrate **„**Jadranka's Solution“for a relatively long period of time. Participants were asked for consent to participate in the research and after that they had to fill out an on-line questionnaire containing general data (such as gender, age, educational level, employment) and allegations of Jadranka's Solution (reason of taking, physical illness and diagnosis, length and the daily dose of Jadranka's Solution and satisfaction with the efficiency of the Solution). Additionally, subjects were required to send scanned laboratory findings of TSH and T4 before and after use of the Herbal Iodine Solution Concentrate **„**Jadranka's Solution“ and the finding of a physician with a daily dose of Eutirox.

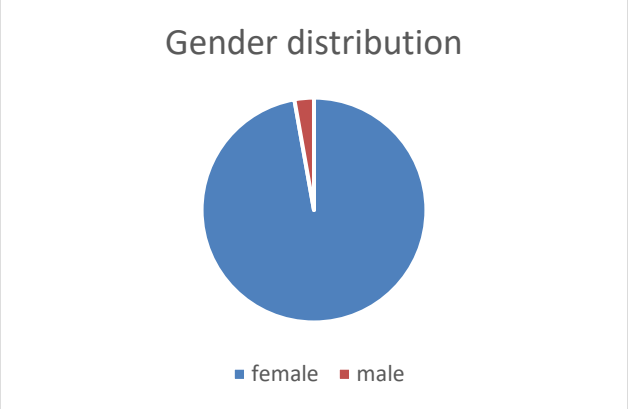
The results were processed first by a simple method of dividing the individual responses to the questions asked, and then the differences of the groups of subjects by different variants were tested with a one-way ANOVA test in order to simplify testing of the difference in TSH and T4 levels before and after the application of the tested agent. For the difference test before and after Repeated Measures ANOVA test was used. Nonparametric Spearman's rank between all variables and changes in the findings was also made. Changes to the findings are counted as a percentage change in relation to the starting value. Everything was analysed in the IBM SPSS Statistics 22.

Results and Discussion

The first group of results refers to general data on respondents and data on the illnesses they suffer from, the dose and duration of taking the Herbal Iodine Solution Concentrate „Jadranka's Solution“ and, as a matter of priority, subjective data on the effect of the Solution and its efficacy.

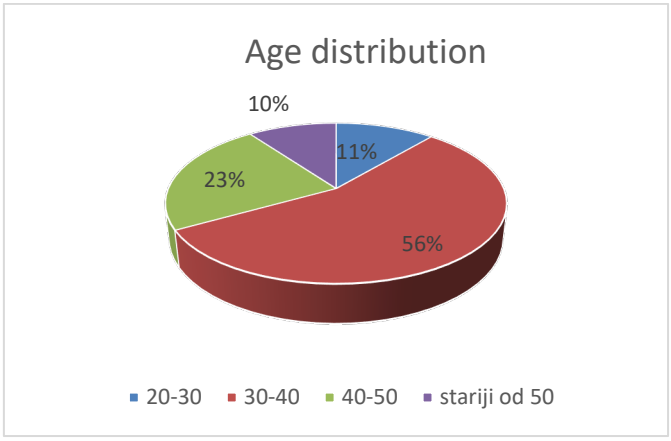
Socio-demographic data

1. Gender Distribution



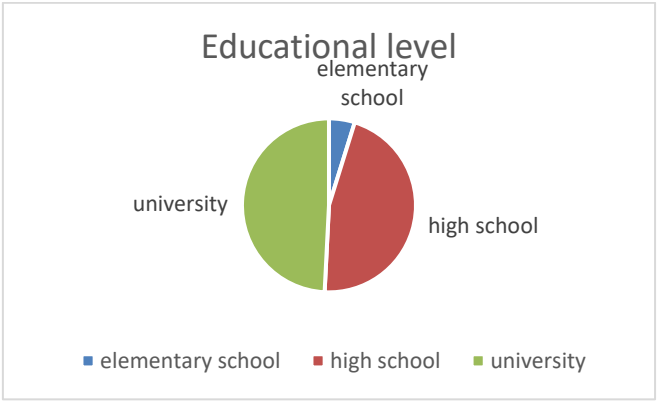
The study involved 96.8% of female respondents and only 3.2% of male respondents which was expected since the occurrence of hypothyroidism is significantly higher in women.

2. Age Distribution



The majority of respondents are aged 30 to 40 (56%), followed by persons aged between 40 and 50, while those younger than 30 and older than 50 contribute to 10%. This age distribution is expected because the disease most often occurs in people's thirties.

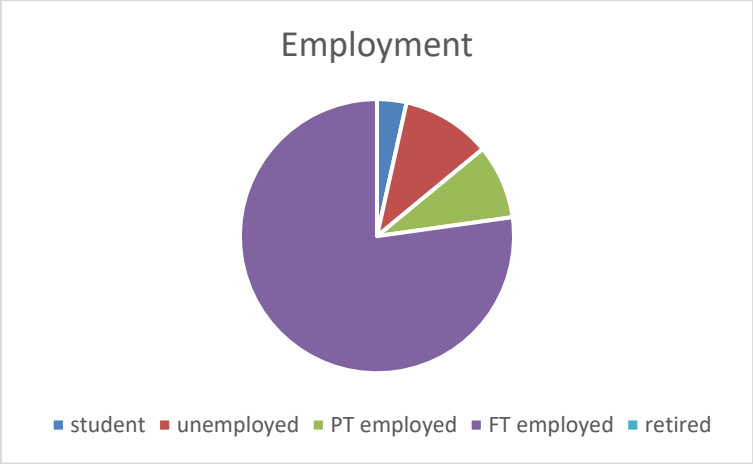
3. Education level



This distribution of educational level is interesting because it differs considerably from the general situation in the Republic of Croatia - as opposed to the general share of highly educated persons in the population of 17.8%, in our sample 49.2% are highly educated persons.

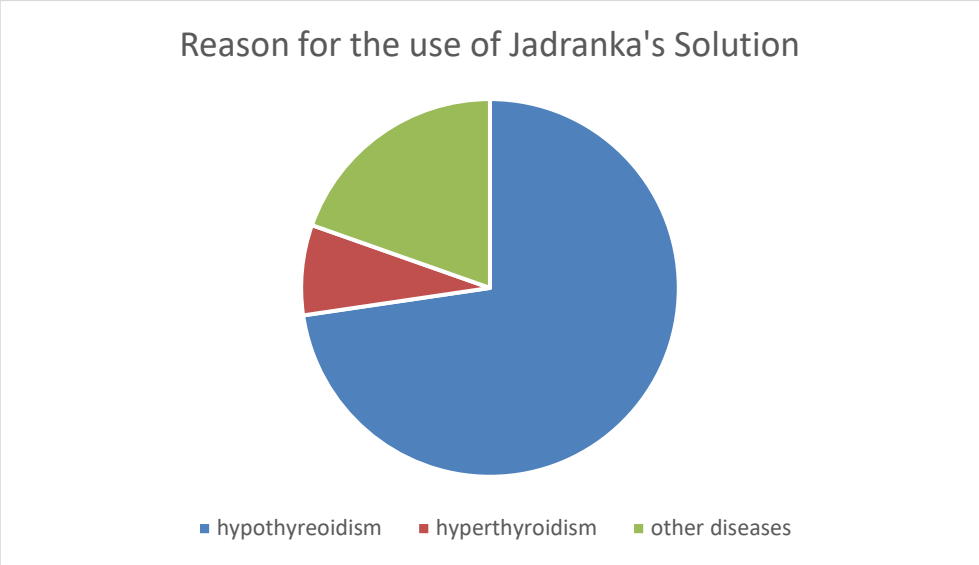
The explanation of this result lies in the fact that highly educated people have more interest in their own health, they try to be informed about the possibilities of treatment and, in particular, are more open to using non-pharmaceutical Solutions. Indeed, information on the long-term effectiveness of the replacement therapy is twofold, and the evidence of the importance of iodine in improving the thyroid function is unquestionable, and it is assumed that more educated persons use more sources of information, in particular they are more literate and use information in the right way.

4. Employment status



These results are very interesting, 68.8% of respondents are employed, which is more than the Croatian average, that mostly comes as high as 50%, but this is explained by the age distribution and educational level (the largest number of respondents is younger).

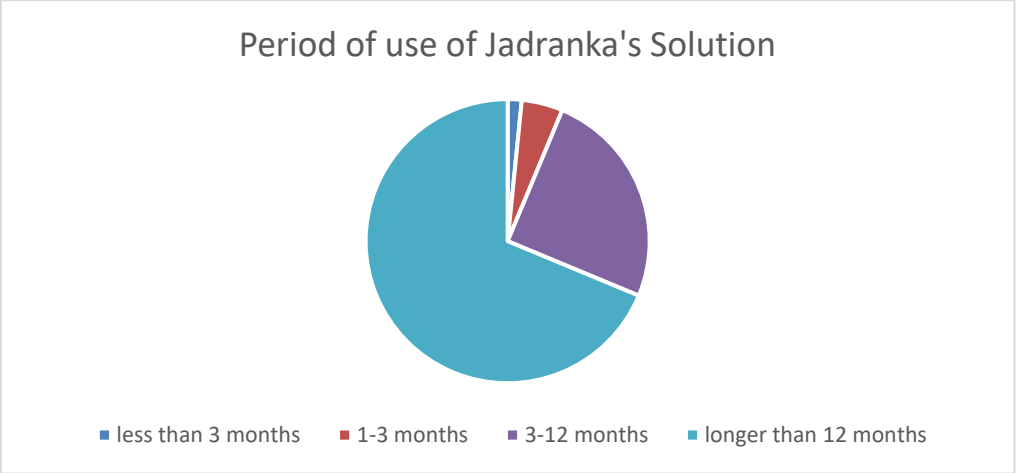
5. The reason for the use of the Jadranka's Solution



Among the participants, the largest share used the Herbal Iodine Solution Concentrate „Jadranka's Solution“for thyroid disease, far more for hypothyroidism (73.4%) and 7.8% for hyperthyroidism. The other respondents generally referred to some of the disorders of reproductive organs, most commonly cysts on the ovaries or menstrual cycle disorder.

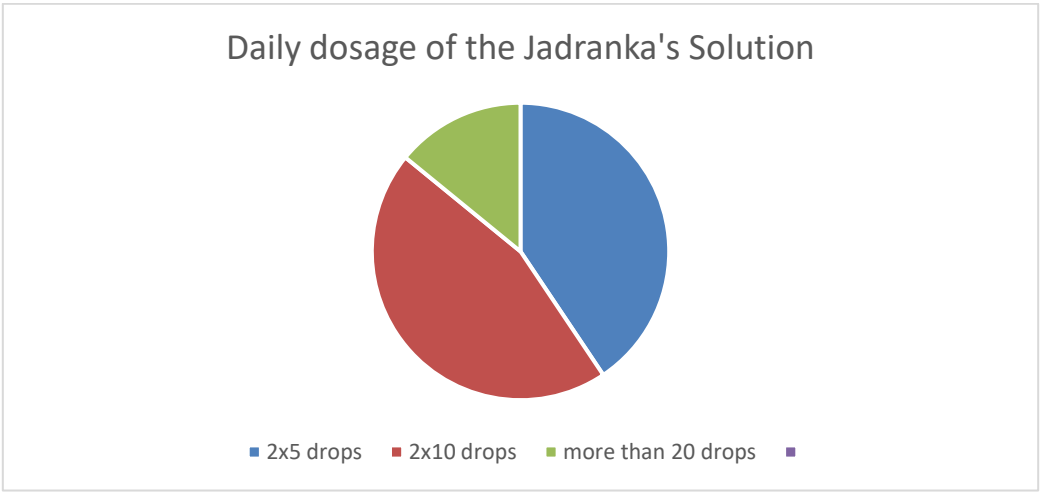
This distribution diagnosis is not a surprise because it is well-known that iodine deficiency affects the functioning of the thyroid and it is a clear need for patients to try to compensate for the deficiency, or, as they usually say, "feed thyroid with the iodine".

6. Period of use of the Jadranka's Solution



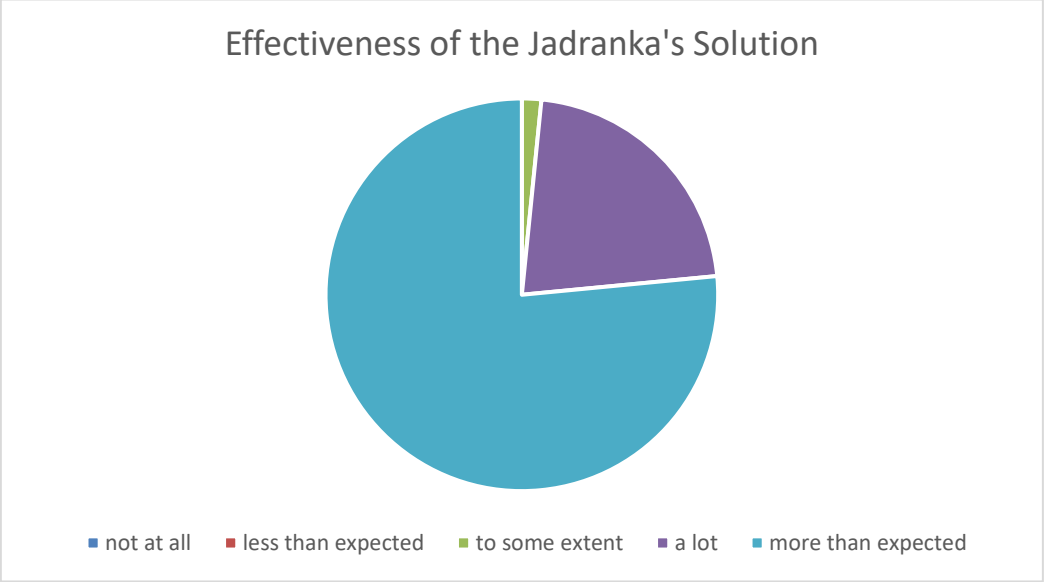
Most participants have taken the Solution for more than a year (68.8%), another 25% longer than three months, therefore, a total of 93.8% take the Solution regularly for more than three months. This information points to two facts, the first is that the subjective feeling of improvement leads to the regularity of taking the Solutions, and besides, those who take longer to take the Solution have rather taken part in the research.

7. Daily doses of the Jadranka's Solution



Similar number of participants have took 10 drops (40.6%) and 20 drops (45.3%) of Jadranka's Solution daily, divided into two doses. However, 14.1% of subjects received more than the recommended dose which potentially could cause adverse reactions. Subsequently, they were sent a message that they should not take doses larger than the recommended dose.

8. Self-evaluation of the effectiveness of the Herbal Iodine Solution Concentrate „Jadranka's Solution“



This graph shows the subjective feeling of satisfaction with the the Herbal Iodine Solution Concentrate „Jadranka's Solution“. As visible in the graph, participants were asked to evaluate satisfaction with results after taking the Herbal Iodine Solution Concentrate. Five responses were offered, on a scale from 1 to 5, where 1 indicates complete dissatisfaction with the efficacy of the Solution, while 5 indicates that the effects are better than expected. A total of 98.5% of respondents rated the efficacy of Jadranka's Solution 4 and 5, of which 76.6% estimated that Jadranka's Solution helped above expectations. Equally important is the fact that no respondent had estimated that her drops helped less than expected or not.

We have to observe these data in the light of the above-average educational level of younger subjects, therefore by definition, the least accessible people. According to this data, it is clear, even before the examination of laboratory findings, that it is difficult to attribute the placebo effect of the Jadranka's Solution.

Laboratory findings

Table 1.: Correlations of different variables of the questionnaire

			education	employment status	physical illness	length of application	dosage	satisfaction
Spearman's rho	education	Correlation Coefficient	1,000	,384**	,230	,204	,167	-,043
		Sig. (2-tailed)		,005	,104	,152	,242	,767
		N	51	51	51	51	51	51
	employment status	Correlation Coefficient	,384**	1,000	,144	,062	,081	-,193
		Sig. (2-tailed)	,005		,312	,668	,574	,175
		N	51	51	51	51	51	51
	physical illness	Correlation Coefficient	,230	,144	1,000	-,050	-,050	-,254
		Sig. (2-tailed)	,104	,312		,728	,729	,072
		N	51	51	51	51	51	51
	length of application	Correlation Coefficient	,204	,062	-,050	1,000	-,028	,319*
		Sig. (2-tailed)	,152	,668	,728		,847	,023
		N	51	51	51	51	51	51
	dosage	Correlation Coefficient	,167	,081	-,050	-,028	1,000	-,007
		Sig. (2-tailed)	,242	,574	,729	,847		,962
		N	51	51	51	51	51	51
	satisfaction	Correlation Coefficient	-,043	-,193	-,254	,319*	-,007	1,000
		Sig. (2-tailed)	,767	,175	,072	,023	,962	
		N	51	51	51	51	51	51

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

The presented results, those obtained by the correlation method, show the correlation of the examined variables. Table 1 shows the correlation of the individual variables and the correlation method results in a statistically significant association between the length of time taken and satisfaction with the Herbal Iodine Solution Concentrate „Jadranka's Solution“, i.e. the satisfaction increases with the length of taking the Solution. Table 2

shows the correlation of individual variables with changes in TSH and T4 values. A statistically significant difference was obtained with the association of the disease with the values of TSH and T4.

Table 2.: The correlation of the individual variables of the questionnaire with changes of TSH and T4

			TSH change	T4 change	EUTIROX change
Spearman's rho	education	Correlation Coefficient	,045	-,131	
		Sig. (2-tailed)	,785	,484	
		N	40	31	14
	employment status	Correlation Coefficient	,014	-,086	
		Sig. (2-tailed)	,930	,646	
		N	40	31	14
	physical illness	Correlation Coefficient	,338*	0,000	
		Sig. (2-tailed)	,033	1,000	
		N	40	31	14
	length of application	Correlation Coefficient	,097	-,164	
		Sig. (2-tailed)	,553	,378	
		N	40	31	14
	dosage	Correlation Coefficient	-,054	,352	
		Sig. (2-tailed)	,743	,052	
		N	40	31	14
	satisfaction	Correlation Coefficient	-,225	-,049	
		Sig. (2-tailed)	,162	,793	
		N	40	31	14

Table 3: Pairwise Comparisons for T4

Measure: T4

(I) TIME		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	25,152*	,870	,000	23,349	26,956
2	1	-25,152*	,870	,000	-26,956	-23,349

Based on estimated marginal means

*. The mean difference is significant at the ,05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Figure 1.

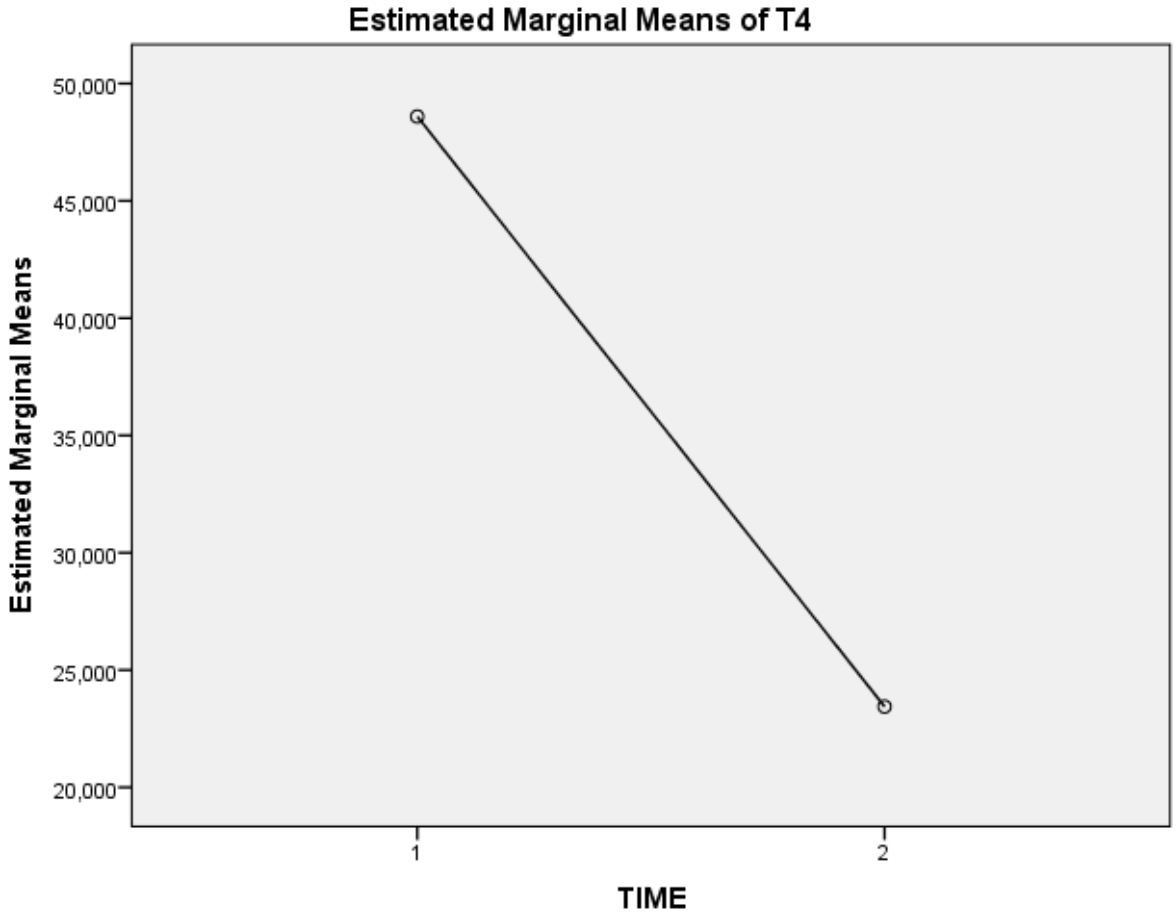


Table 4: Pairwise Comparisons for TSH

Measure: TSH

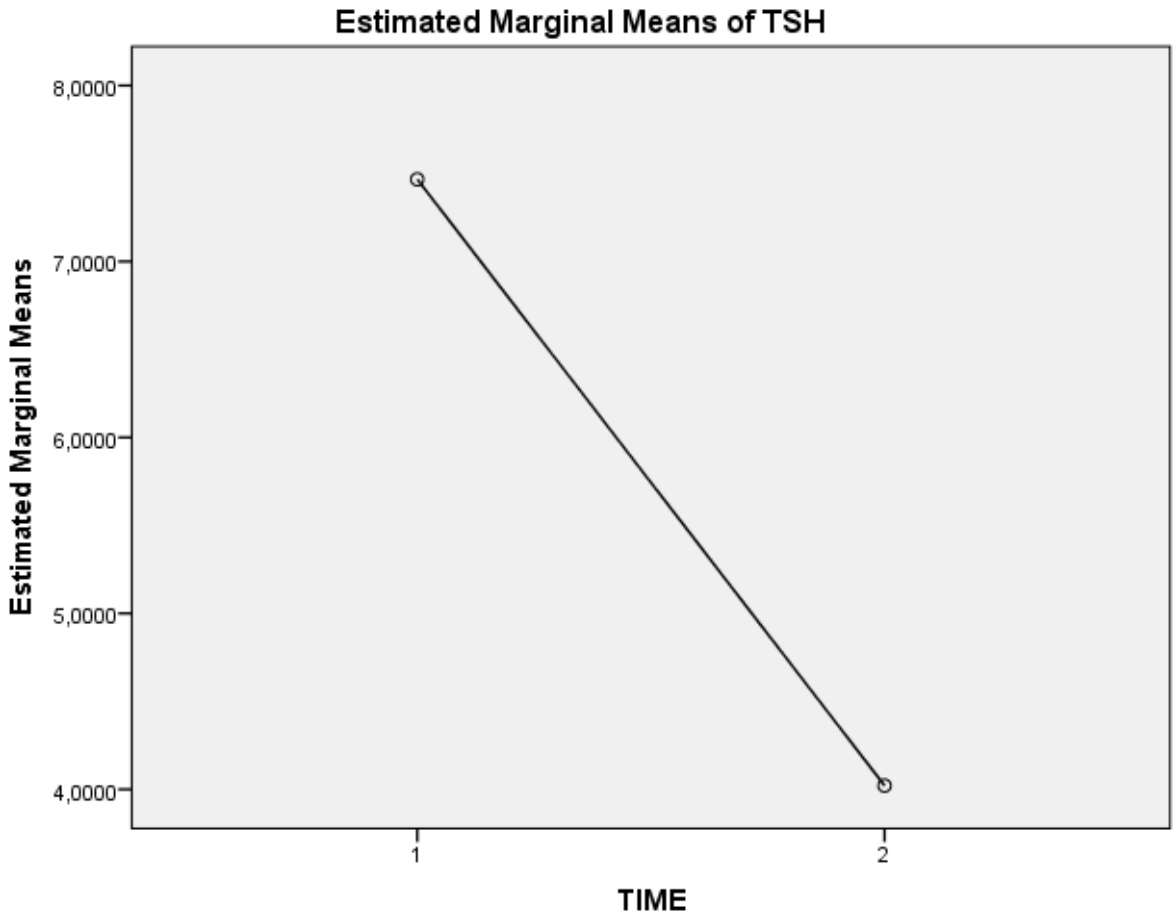
(I) TIME		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	3,446*	,865	,000	1,691	5,202
2	1	-3,446*	,865	,000	-5,202	-1,691

Based on estimated marginal means

*. The mean difference is significant at the ,05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Figure 2.



In fact, the most important research results were obtained by Pairwise comparison and are shown in Tables 3 and 4, and in two graphs. Namely, this method confirmed the hypothesis that the use of the Jadranka's Solution results in a positive change in TSH and T4 hormone levels. That is exactly what is shown in Figures 1 and 2 - a statistically significant drop in TSH values after using Jadranka's Solution.

Conclusion

The conducted study of the efficacy of the the Herbal Iodine Solution Concentrate of the brand „Jadranka's Solution“ in the treatment of thyroid diseases confirmed the hypothesis that regular taking of the Solution results in an improvement in the health status of the respondents. The results of the study showed not only that the subjects were subjectively satisfied with the Herbal Iodine Solution Concentrate „Jadranka's Solution“ but the correlation method showed a statistically significant association between the use of the Solution and the reduction of the TSH and T4 hormone levels in the blood and consequent improvement of the clinical image (reduction or complete disappearance of the thyroid node and withdrawal of hypothyroid symptoms).

Research results are an excellent foundation for a longitudinal study that will be conducted on a larger sample, under controlled conditions and with the monitoring of a large number of parameters. It is our intention to include a greater number of people using the Herbal Iodine Solution Concentrate „Jadranka's Solution“ for other reasons, not just for thyroid disease. Besides the laboratory findings in the longitudinal study we will also observe the clinical findings of the examinees.

Finally, we think that this research was a multiple challenge. In spite of the well-known fact of the importance of iodine for the preservation of health and the impact of iodine deficiency on the development of various diseases, there is almost no research in Croatia or in the world that has studied the influence of iodine on health and diseases. An additional challenge was to deal with a solution that belongs to the so-called "alternative solutions“ because there are still many prejudices in the medical community.

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THE USE OF A SENSOR NETWORK IN THE PROMOTION OF THE HEALTH OF THE DEPENDENT ELDERLY AT HOME

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Abstract: The aging population and the increasing longevity of individuals is a challenging reality for today's healthcare, as it brings increased dependency and need for continuous care, which is often left to informal caregivers. This will be an "almost experimental" study as the investigators will attempt to control some independent variables through the implementation of a therapeutic plan's assistance and vigilance program from a sensor network, which will monitor and assist (alarm sending) in complying with medication, raisings and positionings. 28.6% of the elderly cannot move alone in bed and 100% of the elderly take medication regularly. In the control group, there are 6 elderly people with total dependence, 3 moderately dependent elderly and 5 elderly with slight dependence. So, knowing that Portugal has a high rate of informal home care, it was relevant to develop this project for the application of information and communication technologies in the development of a prototype of a system focused on the monitoring and aid of the execution of the therapeutic plan in two strands, mobilization and medication.

Keywords: Sensor network; Health; Elderly

Introduction

The aging rate has been rising dramatically. In 2017 the aging rate reached 153.2%, accompanied by an increase in longevity, but also an increase in dependency (Pordata, 2017). Older people over the age of 60 are in the highest age range and it is estimated that over the next few years the number of people over 65 years old will exceed the number of children under the age of 5 (Le Deist & Latouille, 2016).

Considering the numbers of aging and dependency, and also knowing that, according to the Health Regulatory Entity, Portugal has the highest rate of informal home care provided by a person in the same residence, therefore, the development of this project was relevant to help these caregivers in the routines, that take practically their whole day, improving their quality of life.

Some elderly people, who are in home care carried out by informal caregivers, have difficulty in locomotion and are often immobile in bed. Immobility produces musculoskeletal pathophysiological changes that cause deformities and postural alterations, promoting the appearance of pressure lesions. If there is no regular care and dedicated interventions, these changes will affect the quality of life of the elderly and predispose to the appearance of diseases (Assis, Vidal, & Dias, 2015).

The pressure ulcers is defined as localized damage to the underlying skin and / or soft tissue, striking regions of bone prominence or in regions of prolonged contact with equipment or devices that cause prolonged or intense pressure, combined or not, with friction. The pressure on bone prominence affects the blood circulation promoting the cell death and consequent appearance of these lesions in places of greater risk, such as the occipital, scapular, elbow, sacral, malleolus and calcaneal regions (Silva, Santos, Zoche, Argenta, & Ascari, 2017).

Depending on the need for care, in its complexity or duration, caregiving may require from the caregiver a restructure of his life, often having to change customs, routines, habits, not always being easy and leading to feelings of tension and distress that lead to overload. For this reason, the caregiver may, on the one hand, ignore his own needs or, on the other, neglect the care of those for whom he is responsible (Nunes, Brito, Corona, Alexandre, & Duarte, 2018).

When we talk about the needs of the elderly, we must mention the need for therapeutic compliance at the medication level. Most of the elderly have several comorbidities and are polymedicated. Sometimes these schemes are complex, which hampers correct compliance and increases the risks of misuse of medicines (Marin, Rodrigues, Druzian, & Cecílio, 2010)

This project is being developed with the objective of applying information and communication technologies in the development of a prototype of a system focused on the monitoring and assistance of the execution of the therapeutic plan in two strands: mobilization and medication.

Materials and Methods

This will be an "almost experimental" study as the investigators will attempt to control some independent variables through the implementation of a therapeutic plan's assistance and vigilance program from a sensor network, which will monitor and assist (alarm sending) in complying with medication, raisings and positionings.

Therefore, 2 groups will be defined: the experimental group, on which the program will be applied, and a control group.

This study was authorized by the Ethics Commission of UTAD (nº 37/2019 of 20/02/2019) as well as the elderly and their caregivers who participated in the study gave their informed consent.

Results and Discussion

At the moment, in the experimental group (where the sensors will be placed), there are already 9 elderly people with total dependency, 1 elderly with severe dependence, 3 elderly with moderate dependence and 8 elderly with slight dependence, 10 elderly people in this group have a high risk of developing pressure ulcers, see (Table 1).

Table 1: Dependency – Experimental Group

	N	%
Total Dependency	9	42,8
Severe Dependency	1	4,8
Moderate Dependency	3	14,3
Slight Dependency	8	38,1
Total	21	100,0
High Risk of Developing Pressure Ulcers	**10**	**47,6**

In the control group, there are 6 elderly people with total dependence, 3 moderately dependent elderly and 5 elderly with slight dependence. In this group, 6 elderly people are at high risk of developing pressure ulcers, see (Table 2)

Table 2: Dependency – Control Group

	N	%
Total Dependency	6	42,9
Severe Dependency	0	0
Moderate Dependency	3	21,4
Slight Dependency	5	35,7
Total	14	100,0
High Risk of Developing Pressure Ulcers	**6**	**42,9**

In the experimental group there are more women than men, 57,1% are female and 42,9% are male.
28.6% of the elderly cannot move alone in bed, were 33,3 % never get out of bed and 23,8% don't even get seated during daytime. 100% of the elderly take medication regularly, see (Table 3)

Table 3: Gender, mobility and medication – Experimental Group

Variables		N	%
Gender	Male	12	57,1
	Female	9	42,9
Move alone in bed	Yes	15	71,4
	No	6	28,6
	Doesn't get seated during the day	5	23,8
	Bedridden	7	33,3
Does medication	Yes	21	100
	No	0	0
	Needs help	21	100
	Knows time and name of medication	0	0
AGE MEAN – 75,9 years old			

In the control group there are the same number of men and women, 28.6% of the elderly cannot move alone in bed, were 28,6 % never get out of bed and the same 28,6% stay bedridden. 100% of the elderly take medication regularly, see (Table 4)

Table 4: Gender, mobility and medication – Control Group

Variables		N	%
Gender	Male	7	50
	Female	7	50
Move alone in bed	Yes	10	71,4
	No	4	28,6
	Doesn't get seated during the day	4	28,6
	Bedridden	4	28,6
Does medication	Yes	14	100
	No	0	0
	Needs help	14	100
	Knows time and name of medication	0	0
AGE MEAN – 81,2 years old			

The selection of dependent elderly people at home has already been made (experimental group and control group). There have also been applied scales that determinate functional independence in the domains of personal care and mobility and scales that gives us the type of risk of development of pressure ulcers. We will also assess the difficulties and overload of the informal caregiver, and will apply sensors to test respiration, heart rate, humidity, temperature, change of position and detection of falls to implement programs of assistance and surveillance of therapeutic plans.

Regarding the sensors we will test:
- Bed sensors (placed in the bed of the elderly, allows the evaluation of breathing, heart rate, estimate the cardiac output and detects the occupation of the bed);
- RFID's (radio frequency identification for humidity and temperature detection);
- Accelerometer (this sensor will allow detection of falls);
- Fixtures (consisting of an LED rule that will be associated with an elderly person and that will serve as a way to quickly see some of the warnings regarding the elderly in question: detection of falls, heart rate and respiratory cycles, medication and change of position of the elderly);
- Two buttons (one will be to check if the medication is given by the informal caregiver, and another that would serve as an alarm, which is used by the elderly);
- Gateway FE - Central computing unit (which will have all the sensors connected to it, wireless, and will process all the data).

Conclusion

Technology application in healthcare has been arousing engineering's attention for a long time in support to health recovery and maintenance therapy practices.

In this regard, creating support systems to watch the fulfilling of the therapeutic plan, assisting healthcare professionals and informal caregivers on dependent elders' care may be an added value.

The advantages of the installation of a sensor network are endless, since the possibility of scheduling alarms to warn of positioning at predetermined times, alarm in case of forgetfulness or delay in positioning and warning of medication time. These written alarms will be available to the informal caregiver, as well as to health professionals, since there will be a web interface where health professionals and / or the family will be able to visualize the values of the sensors, the state of the elderly and warnings.

Acknowledgements

This work was supported by the project SAICT-POL/23428/2016 – IPAVPSI - The impact of an aid and surveillance programme backed up by a sensor network in the health care promotion of the dependent elder at their home, with reference NORTE-01-0145-FEDER-023428, project financed by the Foundation for Science and Technology (FCT) and co-financed by the European Regional Development Fund (FEDER) through the Northern Regional Operational Program (NORTE2020).

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