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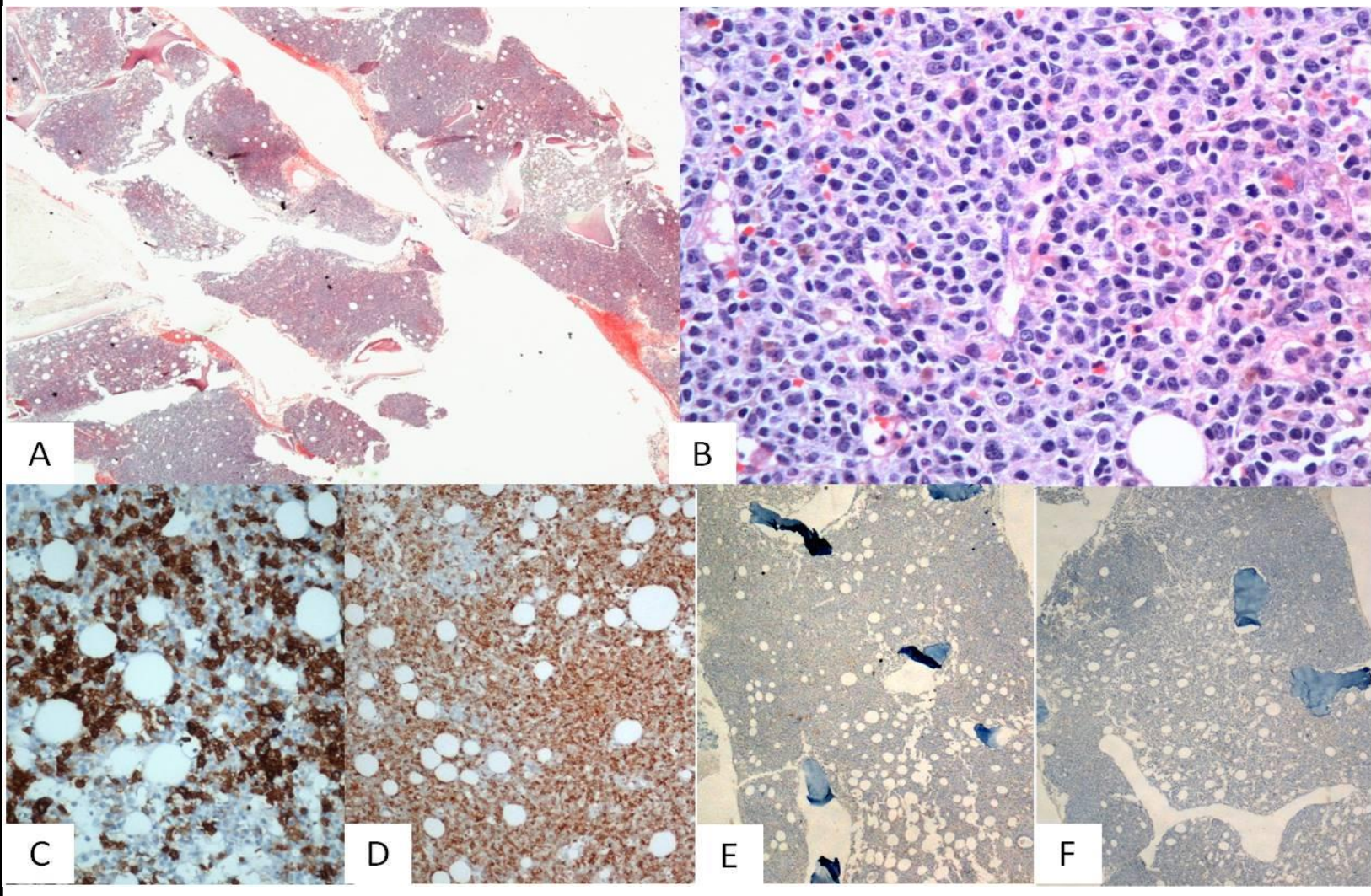

- **Rat brain synaptosomes: In vitro neuroprotective effects of betaine against fluoride toxicity**
- **Sexual abuse and accepting attitudes towards intimate partner rape in Uganda**
- **T-cell Non-Hodgkin lymphoma associated with myelodysplasia**

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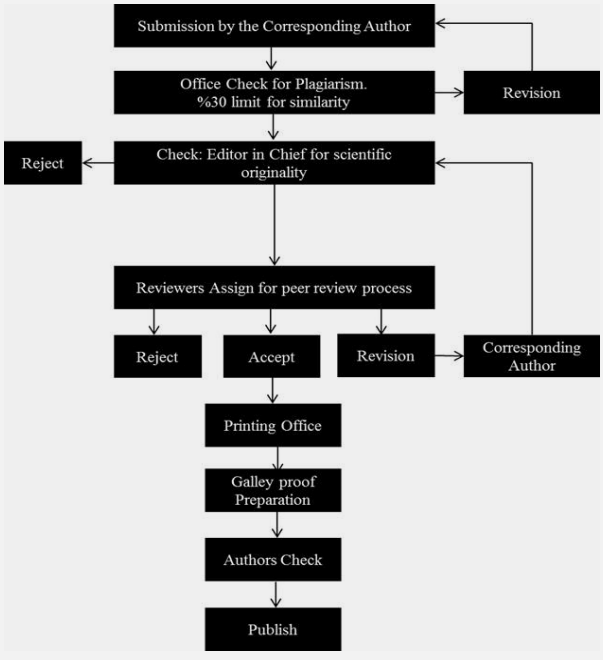

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Case Reports

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Case Report Doi: 10.17546/msd.413622

Developing Hemophagocytic Syndrome during the transformation of Chronic Lymphocytic Leukemia: Case reports of t(7;14), t(14;19) and deletion of 17p

Tarik Onur Tiryaki[1*], Sezen Genc[1] , Gulcin Bagatir Ozan[2], Kivanc Cefle[2], Sukru Palanduz[2], Gulcin Yegen[3], Oner Dogan[3], Meliha Nalcaci[1]

Abstract

Chronic lymphocytic Leukemia (CLL) is the most common form of leukemia in adults. Clinical findings may broad range vary. Detecting some deletions using the FISH method may help us to foresee the progression of the disease and to choose a better treatment method in healing the patient. In this case report, we will present you a CLL patient with t(7;14), t(14;19) and 17p deletions, which are known for bad prognosis, developing autoimmune hemolytic anemia which is refractor to the treatment. This patient unfortunately died due to a clinical form of hemophagocytic syndrome including prolymphocytic transformation.

Keywords: Chronic lymphocytic leukemia, hemophagocytic syndrome, t(7;14), t(14;19), 17p, deletion

Introduction

Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults. It's a type of cancer that starts in cells that become certain white blood cells (lymphocytes) in the bone marrow. The cancerous starts in the bone marrow then cancer cells passes into the blood. The frequency of chronic lymphocytic leukemia (CLL) increases in elderly people. Only the 10 % of these patients are younger than 55. This disease is a hematological malignancy which has varying clinical findings. Some genetical abnormalities are associated with bad prognosis. Especially, 11q, 13q, 17p deletions and trisomy 12 are important for prognosis (1, 2). Firstly, 17p deletion was diagnosed in our patient using FISH method. The patients with 17p deletion show bad prognosis and do not respond well to the conventional treatment (2). Chemotherapy of FC was implemented to patients with 17p deletion, high grade disease and showing systematic symptoms. For those patients fludarabine - cyclophosphamide and rituximab (FCR) is accepted as the first line therapy (2). However conventional chemotherapy is poor for those patients having 17p deletion and bad prognostic features (2). In our patient, FC therapy was ceased due to AIHA development after the 2nd cure. The response to therapy is poor for those who develop AIHA during CLL. For those CLL patients, chemotherapy or monoclonal antibody is necessary (3). Bone marrow evaluation and conventional cytogenetic analysis which are not necessary for diagnosis of CLL, should be implemented for the refractory and recurring diseases (1).

Case

A-51-year-old male patient appealed to the clinic with dyspnea with effort, headache, fatigue and pain in the left hypochondria on April, 2015. Patient had type 2 Diabetes Mellitus and hypertension. In physical examination, the patient was pale, had giant splenomegaly and 1 cm lymphadenopathy on right cervical lymph node. The laboratory findings were; Hb:10.6 g/dl, Hct:%33, MCV:87 fl, leukocyte:78.400/mm^3, platelet:44.100/mm^3, monocyte:22.400/mm^3. On the peripheral smear examination there were small mature lymphocytes, big lymphocytes with wide cytoplasm and smudge cells. In addition; reticulocyte rate was %0.8, glucose:89 mg/dl, creatinine:1mg/dl, urinary acid:11.6 mg/dl, LDH: 479 U, total protein:6.5 g/dl, albumin: 3.95 g/dl, gamma globulin:0.46 g/dl. On abdominal ultrasonography spleen was 260 mm and the flow cytometry revealed B cell chronic lymphocytic leukemia with CD5 (+), CD19 (+), CD20 (+), CD23(+). Cytogenetic examination showed %10 of 17p deletion. PET-CT showed cervical, axillar, mediastinal, inguinal and multiple lymphadenopathies in abdomen and splenomegaly. It was evaluated as RAI grade 3 and fludarabin- cyclophosphamide (FC) chemotherapy medication was implemented to the patient. Herpes virus and pneumocystis jiroveci pneumonia prophylaxis was added to the treatment. Despite two cures of chemotherapy leukocytosis and lymphocytosis were still present. FC treatment was stopped due to coombs positive autoimmune hemolytic anemia (AIHA) before the third cure of

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chemotherapy dated on July 2015. Bone marrow aspiration was made and showed hypercellular marrow with 20 % of fat tissue, hyperplasia of erythroid series, regression of granulocytic series, neoplastic tiny lymphocytic infiltration which showed us interstitial type of CLL. t(7;14), t(14;19) were diagnosed by conventional karyotype. Addition of these two translocations to 17p deletion was evaluated as complex karyotype.

No relative donor was found for allogenic bone marrow transplantation. The patient disagreed to have transplantation from a non-relative donor. Ibrutinib treatment was planned. While this drug was waiting for approval, cyclophosphamide-vincristine and prednisolon (COP) chemotherapy was given to this patient. Partial recovery was observed after this treatment. However, hemolytic anemia developed again after the second cure of COP. The patient's general situation was not suitable for splenectomy. Therefore we gave pulse steroid for three times and rituximab weekly. However, no improvement on AIHA was observed. On November 2015; treatment of 420 mg/day ibrutinib was given via IV.

On the first month of ibrutinib, the spleen length reduced to 11 cm. There was no transfusion demand anymore.Steroid treatment was stopped gradually when hemoglobin level was over 9 g/dl. At that time; some laboratory findings were; leukocyte:4900/mm^3, neutrophil:3800/mm^3 and platelet:186000/mm^3. On the 45th day of ibrutinib treatment; obvious anemia and thrombocytopenia occurred. Due to cytopenia, refractory infections developed. Ibrutinib treatment was stopped. The spleen got bigger in size very quickly; ferritin level progressed to 28000 ng/ml, triglyceride was 350 mg/dl, fibrinogen was 176 mg/dl. Fever, splenomegaly, cytopenia, hypertriglyseridemia and elevated ferritin levels were evaluated as hemophagocytic lymphocytosis (HLH). High dose of dexamethasone treatment. On the control bone marrow smear; transformation of prolymphocytic leukemia was diagnose was started. At this time, 17p deletion was 76% positive, t(7;14) and t(14;19) were still present, as well. The patient died after the first week of the diagnoses of HLH related CLL. The survival life span was estimated as 10 months after the disease was diagnosed.

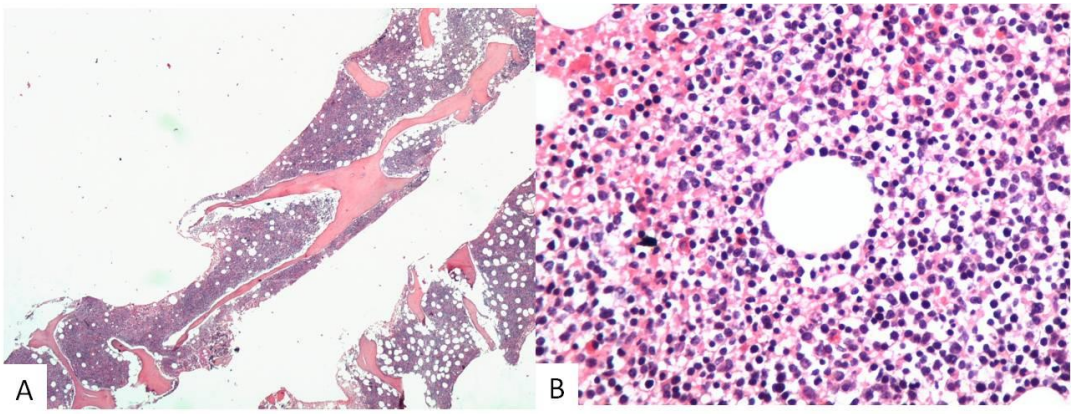

Figure 1: Low magnification view of hypersellulary bone marrow (A). At high magnification interstitial small lymphocytic infiltration is seen (B)(H&E, 4X and 40X magnification respectively)

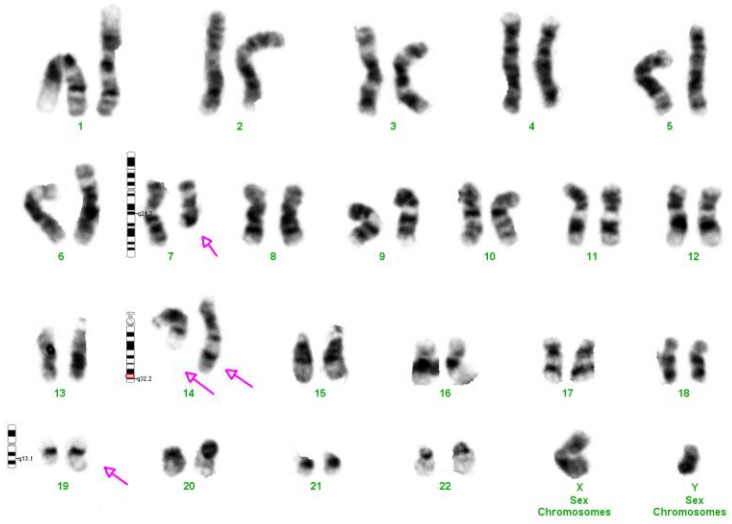

Figure 2: 46, XY, t(7;14) (q22; q32), t(14;19) (q32; q13)

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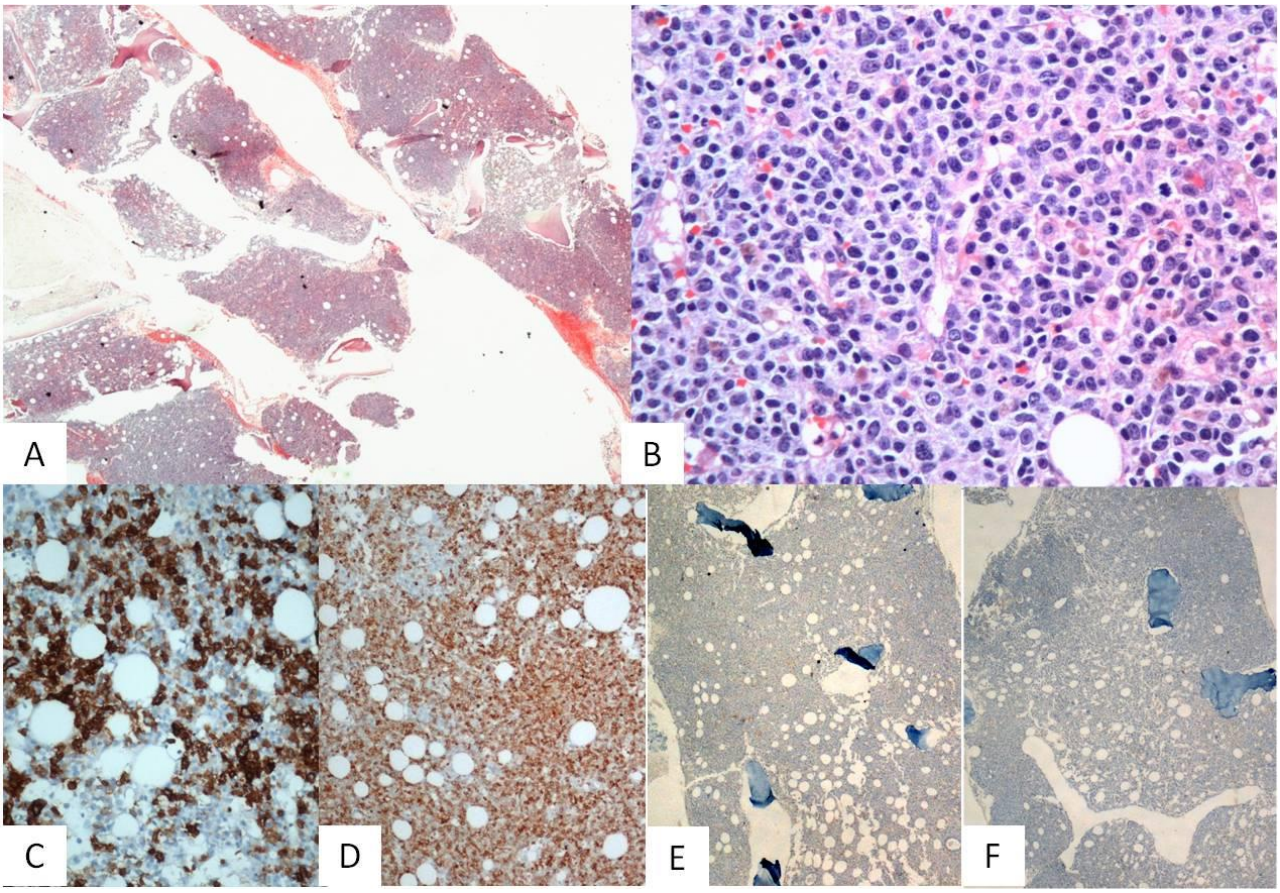

Figure 3: Bone marrow is hypercellulary (A) (H&E, 4X). High power view of H&E stained section reveals nearly diffuse infiltration of prolymphocytes (B). Immunohistochemically neoplastic cells were positive with CD20 (C), CD5 (D) and negative with CD23 (E) and cyclinD1 (F)

Discussion

In our patient, bone marrow was evaluated on the refractory period of the disease. Conventional cytogenesis, t(7;14) and t(14;19) was revealed in addition to signs consistent to chronic lymphocytic leukemia. It was reported that t(14;19) is rare in B cell malignancies. It was again reported that the frequency of the togetherness of young patients, aggressive clinical prognosis and complex karyotype is high. It was announced that this translocation was present in one CLL patient during transformation period to prolymphocytic leukemia (4,5,6,7). The t(7;14) is a rare disorder mostly seen in T cell malignities (8). The genetic features evaluated as complex karyotypes in our patient suggested that the disease would show bad clinical progress. To determine the prognosis of the disease in relapsing refractory CLL patients, complex karyotype is much more meaningful compared to 17p deletion that is presented with FISH method.

Treating those patients who have bad prognostic features with intense treatments and bone marrow transplantation are being discussed (9). Because of this, it was planned to change the medication to ibrutinib. It is well known that ibrutinib is the first line therapy for those who have bad prognostic features (2). In our patient, CLL and AIHA developing seconder to CLL were partially controlled using ibrutinib. Treatment with ibrutinib was permanently stopped due to refractory infections and severe thrombocytopenia, although the patient showed partial response to treatment with aforementioned agent. Lack of treatment caused progression of the disease resulting in prolymphocytic transformation and hemophagocytic syndrome in a very short time. Hemophagocytic syndrome is usually seen in malignancies and inflammations in elderly patients. HFH accompanying CLL had been reported in two patients before (10). The total survival period was 10 months in our patient.

Conclusion

For convenient treatment approaches, the 17p deletion should be searched in newly diagnosed CLL patients using FISH method. If response can not be obtained orin early recurrences it is beneficial to analyze the patient for conventional cytogenetic abnormalities. It would be suitable to start the new treatment methods when complex karyotype is found. The refractory CLL patients should be evaluated for HLH when they develop unexplained fever and in declining cytopenia.

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Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: TOT, SG, GBO, KC, SP, GY, OD, MN: Research concept and design; Patient examination, data collecting, biochemical analysis and interpretation of data. **GBO, KC, SP:** Genetic analysis, FISH, **TOT:** Preparation of article and revisions. All authors approved the final version of the manuscript,

Ethical issues: All Authors declare, Originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

References

1. Puiggros A, Blanco G, and Espinet B. Gene genetic abnormalities in chronic lymphocytic leukemia: where we are and where we go. BioMed Research International 2014; 435983 1-13.

2. Deeks ED. Ibrutinib: a review in chronic lymphocytic leukaemia. Drugs . 2017; 77:225–236.

3. Visco C, Barcellini W, Maura F, Neri A, Cortelezzi A, and Rodeghiero F. Autoimmune cytopenias in chronic lymphocytic leukemia. Am. J. Hematol. 2014; 89:1055–1062.

4. Michaux L, Dierlamm J, Wlodarska I, Bours V, Berghe H, and Hagemeijer A. t(14;19)/BCL3 rearrangements in lymphoproliferative disorders: a review of 23 cases cancer genet cytogenet 1997; 94:36-43.

5. Shin SY, Park CJ , Lee KH, Huh J ,Chi HS, Seo EJ. An illustrative case of t(14;19)/BCL3 rearrangement as a karyotypic evolution of chronic lymphocytic leukemia. Ann Hematol 2013; 92:1717–1719.

6. Chapiro E, Radford-Weiss I, Bastard C, Luquet I, Lefebvre C, Callet-Bauchu E et al. The most frequent t(14;19)(q32;q13)-positive B-cell malignancy corresponds to an aggressive subgroup of atypical chronic lymphocytic leukemia. Leukemia 2008; 22, 2123–2127.

7. Huh YO, Schweighofer CD, Ketterling RP, Knudson RA, Vega F, Kim JE et al. Chronic lymphocytic leukemia with t(14;19)(q32;q13) Is characterized by atypical morphologic and immunophenotypic features and distinctive genetic features. Am J Clin Pathol 2011;135:686-69.

8. Sugimoto KJ , Shimada A , Wakabayashi M , Sekiguchi Y , Izumi H , Ota Y et al. T-cell lymphoblastic leukemia/lymphoma with t(7;14)(p15;q32) [TCRγ-TCL1A translocation]: a case report and a review of the literature. Int J Clin Exp Pathol 2014;7(5):2615-2623.

9. Thompson PA, O'Brien SM, Wierda WG, Ferrajoli A, Stingo F, Smith SC et al. Complex karyotype is a stronger predictor than del(17p) for an inferior outcome in relapsed or refractory chronic lymphocytic leukemia patients treated with ibrutinib-based regimens. Cancer 2015;121:3612-21.

10. Kilari D, Venci N , Friedberg J , Bennett JM. Hemophagocytic lymphohistiocytosis masquerading as progressive chronic lymphocytic leukemia. Leukemia Research Reports 2013;2: 4–6.

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Rat brain synaptosomes: In vitro neuroprotective effects of betaine against fluoride toxicity

Ceyhan Hacioglu[1]*, Fatih Kar[1], Gungor Kanbak[1]

Abstract

Objective: Fluoride increases the production of reactive oxygen and nitrogen derivatives, causing oxidative stress and cellular damage. Betaine, an antioxidant and an important methyl donor, has been reported to have potential protective effects on cardiovascular and neurodegenerative diseases in recent years. In this study, we aimed to investigate the neuroprotective effects of betaine treatment against cellular damage caused by fluoride exposure on rat synaptosomes.

Material and Methods: In the experimental period, 8 Wistar albino rats were decapitated and then their frontal cortices removed and divided into 4 equal cuts (total-32 cuts). Subsequently, an appropriate experimental procedure was applied to obtain the synaptosomal fractions. The determined doses of sodium fluoride (NaF) and betaine were administered in vitro at 37°C for 30 min. Synaptosomal glutathione (GSH), malondialdehyde (MDA) and nitricoxide (NO) levels, and also catalase (CAT) and Ca+2/Mg+2 ATPase activities were measured spectrophotometrically.

Results: According to our results, NaF exposure caused a decrease in GSH levels, CAT and Ca+2/Mg+2 ATPase activities, and also an increase in MDA and NO levels, significantly. MDA levels, NO levels and CAT activities were closed to the control group depends on the betain doses. The best recovery in terms of synaptosomal GSH levels and Ca+2/Mg+2 ATPase activities was found at 0.5 mM betaine concentration ($P < 0.001$).

Conclusion: Our results showed that betaine could be a potential neuroprotective therapeutic agent against synaptosomal fluoride toxicity.

Keywords: Fluoride, Betaine, Synaptosomes, Neurodegeneration, Oxidative stress

Introduction

Fluoride (F), one of the most abundant elements on earth, can be found in rock and soil as well as in combination with other elements. Excessive F uptake causes fluorosis, which is an important health problem and characterized by defects in skeletal and tooth structure (1). The main cause of fluorosis is contaminated drinking water with organic and inorganic wastes. Since F in drinking water has an ionic structure, it is absorbed rapidly through the intestinal epithelium and interferes with metabolic processes by accumulating in the different organs of the biological systems (2). F intake at daily mean doses of 0.05 mg/L does not pose a risk to humans, while F exposure at doses ranging from 3 to 10 mg/L can cause significant health problems in various age groups (3).

Due to the electronegative character of F, which means that it is negatively charged and tends to form fluorine ions, it can pass through cell membranes via ion channels (4).

In vivo studies have found that F added to drinking water of rats causes toxic effects and accumulates in soft tissues such as lung, liver, heart, brain and kidney (5). F is a molecule with an anionic structure, which is easily permeable from membranes by binding to cations such as calcium and magnesium (6). F combined with cations has been shown to have direct effects on apoptotic processes characterized by impaired intracellular signaling mechanisms. It damages cell integrity by binding to Na+/K+ ATPases and Ca+2/Mg+2 ATPases found in the membranes, and also leads to enzyme degradations, reduction of intracellular calcium levels, deterioration of cell energy metabolism, depolarization of membranes and signal transduction (7). Calcium (Ca+2) is an element that plays a significant role in both metabolic processes and their interaction with the environment of the cell, and regulates various cellular responses.

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Therefore, Ca+2 plays a central role in maintaining viability, since it is an integrative component of many different signaling pathways in cells (8). F exposure disturbs the continuity of intracellular Ca+2 homeostasis by causing inhibition of Ca+2 pumps localized to cell membranes (9). Furthermore, F exposure has been shown to cause genotoxic effects with chromosome anomalies and DNA damage (10).

F can produce free oxygen and nitrogen species (ROS and RNS, respectively) by affecting the antioxidant metabolism (11). Furthermore, it has been shown that ROS and RNS interact with disulfide bonds in proteins, causing degradation of their synthesis and activities. In vitro and in vivo animal studies showed that F exposure causes oxidative stress by weakening the antioxidant defense system (12). F-induced ROS production reduces glutathione (GSH) levels as well as inhibition of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) (13). Increasing lipid peroxidation is also an important biomarker of oxidative stress. In vivo studies have been shown that F exposure enhances lipid peroxidation due to increased ROS production in rat brain tissues (14).

Betaine, which is known as trimethylglycine, is an important methyl donor derived from glycine amino acid. Betaine as an important osmolyte, can protect protein synthesis, enzyme activity and membrane integrity against the biotic and abiotic environmental stress conditions (15). Homocysteine, an important risk factor for cardiovascular diseases and neurodegenerative diseases, is converted to methionine by betaine. Moreover, betaine provides single carbon units for DNA synthesis and assists in the synthesis of choline, an important neurotransmitter (16). Metabolic anomalies linked to lack of betaine have been found to cause a variety of diseases such as cancer, cardiovascular disease and neurodevelopmental disorders (17). In animal model studies, increased lipid peroxidation, reduced GPx and GSH levels owing to oxidative stress have been shown to have neuroprotective effects by providing a significant improvement with betaine administration (18).

In this study, we aimed to investigate the neuroprotective effects of betaine against F toxicity, as studies related with fluorosis have become important in recent years. To verify our hypothesis, GSH contents, malondialdehyde (MDA) levels, CAT activities, nitric oxide (NO) levels and Ca+2/Mg+2 ATPase activities were measured to test the neuroprotective effects of betaine following sodium fluoride (NaF) exposure of rat brain synaptosomes.

Material and Methods

Animals and Experimental Design

Eight healty male Wistar albino rats weighing 300±50 g was supplied by Medical and Surgical Experimental Research Center, Eskisehir. Experimental procedures were carried out according to the decision of Animal Experiments Local Ethics Committee of Eskisehir Osmangazi University (Approval number: 651).

The rats were maintained under controlled conditions at 25°C ± 5°C and 50% ± 5% relative humidity with 12-hour periods (dark / light). Anesthesia was performed by intra-muscular injection at 45±10 mg/kg ketamine + 10±5 mg/kg xylazine doses, and then the unconscious rats were decapitated. Rats' frontal cortex was removed and divided into 4 equal cuts (total 32 cuts) and the cuts were stored at -80°C until the day of the experiment.

In our previous work, we found that 80 mg/L NaF gave rise to toxicity on synaptosomes. In this study we also investigated the neuroprotective effects of betaine at concentrations of 0.25, 0.5 and 1 mM versus the toxicity caused by 80 mg/L NaF. The experimental group design was as described in Table 1.

Table 1. Design of experimental groups (n=6).

Group 1	**Control**
Group 2	80 mg/L NaF
Group 3	80 mg/L NaF + 0.25 mM Betaine
Group 4	80 mg/L NaF + 0.5 mM Betaine
Group 5	80 mg/L NaF + 1 mM Betaine

Preparation of synaptosomal fractions

Rat brain synaptosomes were prepared according to Whittaker et al. (19). 32 brain cortical pieces obtained from healthy rats were distributed randomly into 4 experimental groups as it will be 6 pieces in each group (n=6). The cortical pieces were homogenized on ice in a solution containing 10 mM 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) and 30 µM sucrose. The homogenates were first centrifuged at 3000xg for 10 minutes at 4°C and then the supernatants were taken and centrifuged once more at 15000xg for 20 min at 4°C. The remaining pellets were re-suspended in saline and rat brain synaptosomal fractions were obtained. According to the determined experimental groups, synaptosomes were exposed to at 80 mg/L NaF and betaine at 0.25, 0.5 and 1 mM concentrations for 30 minutes at 37°C. Synaptosomal protein levels were measured according to the biuret method (20). This method is used to demonstrate the presence of peptide bonds in the samples. The reaction of copper (Cu+2) with the two peptide bonds is based on the principle of purple color formation, and the colored product was measured spectrophotometrically at 540 nm.

Glutathione (GSH) contents

The Ellman reaction is based on the principle that the p-nitrophenol anion formed by reaction of thiol compounds with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) in an alkali environment is spectrophotometrically measured. GSH levels were measured spectrophotometrically at 412 nm according to Srivastava and Beutler method (21). Briefly, Reaction medium contains 0.1 ml sample, 2 ml 100 mM Tris HCl pH 8.4 and 0.1 ml Ellman's reagent (60 mg/100 ml, 0.1 M Tris-HCl buffer pH 7.0). The data for GSH levels were expressed as µmol/mg protein.

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Malondialdehyde (MDA) levels

The quantitative determination of lipid peroxidation is based on the color reaction between MDA and thiobarbituric acid (TBA). Synaptosomal MDA levels were measured at 532 nm according to the method reported by Ohkawa et al (22). In short, 0.6 ml rat synaptosomal fraction was added to sample 4 ml of sodium dodecyl sulphate (8%), and then 2 ml of acetic acid (%0.6, pH 6.5) and 2 ml of thiobarbituric acid solution (% 20, pH 4) was added to the reaction medium. The final concentration was adjusted to be 5 ml and heated in a water bath at 100°C for 60 minutes. After this process, it was centrifuged at 4000 rpm for 10 minutes and then spectrophotometric measurement was performed. The results were expressed as nmol/mg protein.

Catalase (CAT) activities

Hydrogen peroxide (H2O2), which is harmful to the cell, is converted to water and oxygenase by CAT. CAT activity measurement was based on the decrease in absorbance of H2O2 at 240nm (23). In brief, the final concentration of the reaction medium adjusted to be 1 ml as follows: 60 mM sodium potassium phosphate (pH 6.5), 20 mM H2O2 and 20 µL of sample. CAT was activated by the addition of H2O2 to the reaction medium and the absorbance changes were spectrophotometrically monitored. The results were expressed as Unit/mg protein.

Nitric oxide (NO) levels

The determination of NO levels is based on the measurement of nitrite and nitrate as a result of nitric oxide oxidation. The amounts of nitrite and nitrate in the samples are determined by two consecutive reactions. Initially, Nitrate is reduced to nitrite by means of enzymatic or non-enzymatic conversion. In the acidic reaction medium, the nitrite is diazotized with sulfanilamide and subsequently forms a purple azo compound with N-(1-naphthyl) ethylenediamine. The amount of nitrite was measured precisely according to the method also known as the Griess reaction (25). The results were expressed as µmol/mg protein.

Ca+2/Mg+2 ATPase activities

This method is based on the fragmentation of ATP at 340 nm in the presence of pyruvate kinase, phosphoenolpyruvate, lactate dehydrogenase and reduced nicotinamide adenine dinucleotide (NADH) in the reaction medium (25). To initiate the reaction, 0.5 M CaCl2 was added to the medium containing 1 M ouabain. NADH oxidation was measured spectrophotometrically at 340 nm every 20 seconds for 5 minutes. The results were expressed as Unit/mg protein.

Statistical analysis

All data were assessed for the normality by using the Kolmogorov-Simirnov and Shapiro-Wilk tests. One-way ANOVA was performed to determine the difference between the experimental groups and P <0.05 was considered significant. For multiple comparisons, the Tukey test was used when the variances were equal, whereas the Tamhane's T2 test was used for analysis when the variances were not equal. Statistical analysis was performed by SPSS Version 21.

Results

NaF exposure was found to cause a statistically significant decrease in GSH levels on synaptosomes compared to the control group (0.57±0.12) (Figure 1). All betaine doses provided protective effect against NaF toxicity and increased GSH levels. 0.5 mM betaine concentration among all doses resulted in the most improvement in GSH levels (0.54±0.11) against 80 mg/L NaF toxicity group (0.31±0.34) (P <0.001). GSH levels improved a statistically significant improvement when 0.5 and 1 mM betaine groups were compared with the 80 mg/L NaF group (P <0.001 and P <0.01, respectively). However, the 0.25 mM betaine concentration did not provide a statistically significant difference in GSH levels (0.35±0.16) compared with the 80 mg/L NaF group (P >0.05).

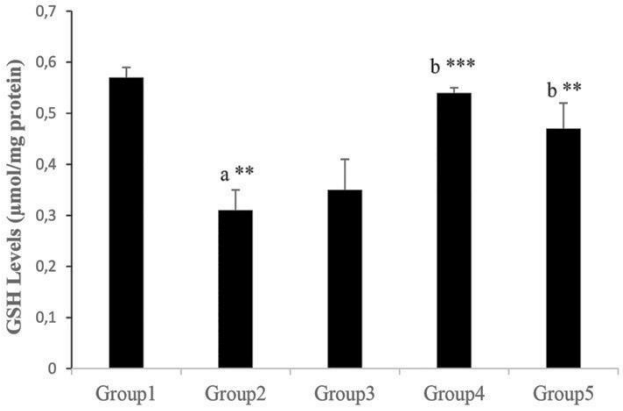

Figure 1. The protective effects of betaine on GSH levels against NaF-induced toxicity on rat brain synaptosomes. a: Comparison with the control group. b: Comparison with the 80 mg/L NaF group. *P <0.05, **P <0.01, ***P <0.001. All data are described as mean ± SEM.

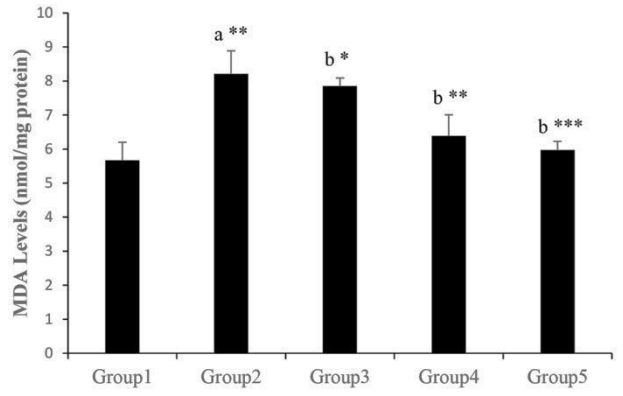

Figure 2. The protective effects of betaine on MDA levels against NaF-induced toxicity on rat brain synaptosomes. a: Comparison with the control group. b: Comparison with the 80 mg/L NaF group. *P <0.05, **P <0.01, ***P <0.001. All data are described as mean ± SEM.

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As shown in Figure 2, NaF exposure caused an increase in lipid peroxidation on synaptosomes, thus increasing MDA levels. MDA levels of 80 mg/L NaF group (8.21±2.68) were significantly higher than control group (5.76±1.53). All treatment doses of betaine has provided an amelioration by reducing effect at MDA levels and at 1 mM betaine concentration treatment group (5.97±1.26) was almost obtained similar results to the control group (P <0.001). In addition, we can say that the increase in MDA levels against fluoride toxicity showed a dose-dependent decrease with betaine treatment.

NaF exposure showed a significant decrease in synaptosomal CAT activities compared to the control group (17.53±2.44) (P <0.001). Reduced CAT activities were normalized by betaine treatment. The closest improvement to the control group was achieved at 1 mM betaine concentration treatment group (17.81±2.35) (P <0.001). In addition, a statistically significant increase in CAT activities was found in 0.25 and 0.5 mM betaine concentrations treatment groups (12.25±1.61 and 17.28±1.89; P <0.05 and P <0.01, respectively). According to our results, decreased CAT activities owing to NaF toxicity showed a dose-dependent increase with betaine treatment (Figure 3).

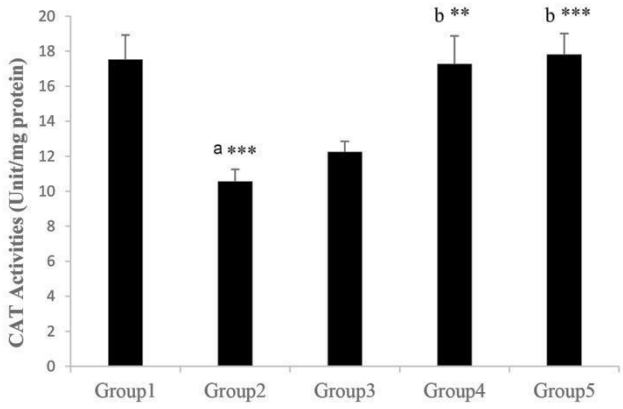

Figure 3. The protective effects of betaine on CAT activities against NaF-induced toxicity on rat brain synaptosomes. a: Comparison with the control group. b: Comparison with the 80 mg/L NaF group. *P <0.05, **P <0.01, ***P <0.001. All data are described as mean ± SEM.

The 0.5 and 1 mM betaine groups resulted in a statistically significant reduction in NO levels (7.49±1.43 and 7.03±1.43, respectively; P <0.05) when compared with the 80 mg/L NaF group (8.48±2.77). On the other hand, 0.25 mM betaine group (8.95±1.71) did not cause a statistically significant decrease in NO levels (P >0.05). As shown in Figure 4, the highest decrease/improvement in NO levels after NaF exposure was obtained at 1 mM betaine concentration (P <0.001). 0.25 mM betaine concentration did not suppress the increase in NO levels caused by NaF. The 1 mM betaine concentration provided the closest ameliorating to the control group as compared to the 80 mg/L NaF group.

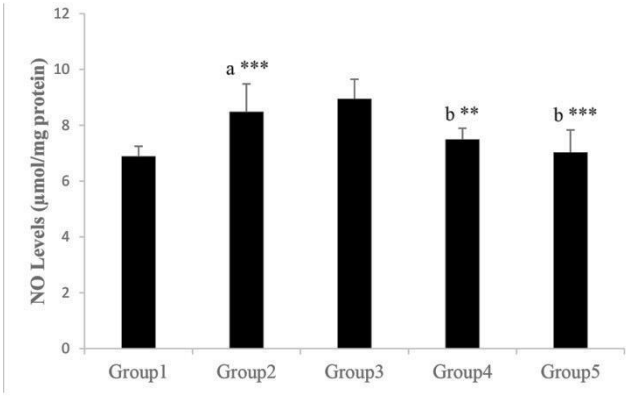

Figure 4. The protective effects of betaine on NO levels against NaF-induced toxicity on rat brain synaptosomes. a: Comparison with the control group. b: Comparison with the 80 mg/L NaF group. *P <0.05, **P <0.01, ***P <0.001. All data are described as mean ± SEM.

NaF exposure caused a decrease in Ca+2/Mg+2 ATPase activities compared to the control group (0.062±0.015) (Figure 5).

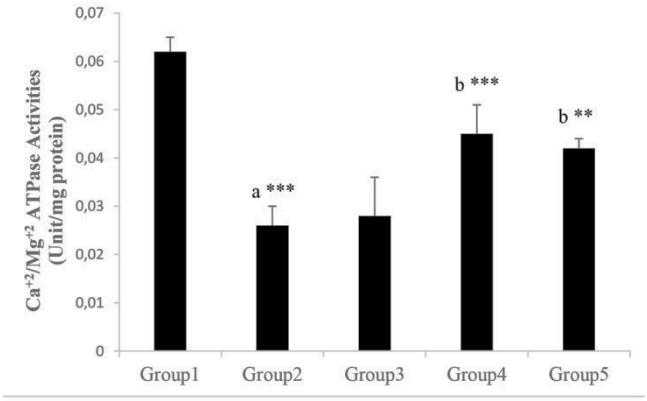

Figure 5. The protective effects of betaine on Ca+2/Mg+2 ATPase activities against NaF-induced toxicity on rat brain synaptosomes. a: Comparison with the control group. b: Comparison with the 80 mg/L NaF group. *P <0.05, **P <0.01, ***P <0.001. All data are described as mean ± SEM.

The 0.5 and 1 mM betaine groups (0.045±0.007 and 0.052±0.013, respectively) showed a statistically significant improvement as compared to the 80 mg/L NaF group, but 0.25 mM betaine group (0.038±0.008) did not provide a significant increase in Ca+2/Mg+2 ATPase activity caused by the NaF. In this study, the best amelioration in Ca+2/Mg+2 ATPase activities was found at 0.5 mM betaine treatment group.

Discussion

In this study, neuroprotective effects of betaine against NaF toxicity on synaptosomes were investigated. The effects of NaF exposure on antioxidant/oxidant parameters and Ca+2/Mg+2 ATPase activities were investigated and found to cause cellular damage by triggering oxidative stress. It has been determined that the antioxidant capacity also reduce due to increased reactive oxygen and nitrogen derivatives. Additionally, we determined that Ca+2/Mg+2 ATPases, which play an important role in intracellular and extracellular signaling, were inhibited by NaF-induced oxidative stress. Given that the brain consumes oxygen much more than other organs and contains too much unsaturated fatty acids in its structure, it is extremely defenseless to oxidative stress. Therefore, we examined the neuroprotective effects of betaine, an important antioxidant and methyl donor, against NaF toxicity.

GSH, a component of the antioxidant mechanism, plays an essential role in protecting cellular integrity against peroxidative damage resulting from reactive oxygen species (26). Studies with Sprague-Dawley rats have been reported that betaine contributed to antioxidant mechanism by providing upregulation of GSH against increased oxidative stress in brain (27). In our result parallel with the literature, the protective effect of betaine contributes to the methylation pathways by increasing the formation of S-adenosylmethionine and provides the glycine requirement, which is the essential component for GSH synthesis (18).

The central nervous system has greatly polyunsaturated fatty acids, which are the main target of free oxygen radicals. In our previous study, we found that ethanol exposure increased MDA levels in synaptosomes by increasing oxidative stress and that betaine administered at 0.5% dose significantly reduced MDA levels (28). Our results of betaine treatment against NaF exposure are consistent with the literature.

H2O2 derived from the superoxide anion is catalyzed to the water by CAT, so that the cell membranes are protected against oxidative damage (26). Since the F chemical structure is highly electronegative, in vitro and in vivo studies have been shown to cause oxidative stress-induced cellular damage by up-regulation of reactive oxygen species (12). It has been reported that F added to the drinking water of rats caused a decrease in GSH levels and CAT activities in brain tissue (13). Decreased CAT activity due to increased oxidative stress resulted in a significant improvement with betaine treatment (27). In other words, betaine helps protect cellular integrity by supporting antioxidant defense system.

NO is an important biological initiator of functional and metabolic processes for almost any organ. This gas modulates endothelial function, neurotransmission, immunity and cell death by activating intracellular cyclic guanosine monophosphate (cGMP) levels and guanylate cyclase, which increases cGMP-dependent protein kinase (29). Elevation of NO and cGMP has been associated with many pathophysiological processes. Consistent with the literature, we have found that NaF exposure significantly increased NO levels compared to the control group (30). NO is formed by the activation of N-methyl-D aspartate (NMDA) receptors in neuronal cell membranes and causes biochemical effects by altering cGMP and intracellular calcium concentrations. It has been reported that NMDA receptors was stimulated by F (31), and based on this result, it is thought that NO synthesis may increase. Our data indicated that betaine treatment resulted in the inhibitory effect on NOS leading to a decrease in NO levels.

F binds to the proteins of ion channels in cell membranes and inhibits them, causing the deterioration of membrane potential. Calcium, which plays an important role in intracellular and extracellular signaling, triggers apoptosis either directly or indirectly by impairment of cellular calcium homeostasis with F exposure (32). Consistent with our previous study, Ca+2/Mg+2 ATPase activities in erythrocyte membranes were reduced after ethanol exposure, and then betaine treatment was improved in Ca+2/Mg+2 ATPase activities (33).

Conclusion

F exposure has been shown to cause oxidative stress-induced neurodegeneration. In addition, experimental studies about fluorosis, which is the result of excessive F exposure, is increasing day by day. In this study, we found that betaine has neuroprotective effects against cellular damage caused by F. Betaine is taking significant steps towards becoming a new therapeutic agent, especially by giving positive results on neurodegenerative diseases. Nevertheless, there is a need to further study the action mechanisms of betaine in molecular and biochemical processes.

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Author's Contributions: CH, FK, GK: Research concept and design, data collecting, biochemical analysis and interpretation of data. **CH:** Preparation of article and revisions. All authors approved the final version of the manuscript,

Ethical issues: All Authors declare, Originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

References

1. Domingo JL. Health risk of dietary exposure to perfluorinated compounds. Environ Int. 2012;40:187-195.

2. Urbansky ET. Fate of fluorosilicate drinking water additives. Chem Rev. 2002;102:2837-2854.

3. Ozsvath DL. Fluoride and environmental health, a review. Rev in Environ Sci and Bio/Tech. 2009;8:59-79.

4. Whitford GM, Bawden JW, Bowen WH, Brown LJ, Ciardi JE, Clarkson TW, et al. Report for Working Group I: strategies for improving the assessment of fluoride accumulation in body fluids and tissues. Adv Dent Res. 1994;8:113-115.

5. Basha PM, Rai P, Begum S. Evaluation of fluoride-induced oxidative stress rat brain: a multigeneration study. Biol Trace Elem Res. 2011;142:623-637.

6. Whitford GM, Pashley DH, Garman RH. Effects of fluoride on structure and function of canine gastric mucosa. Dig Dis Sci. 1997;42:2146-2155.

7. Murao H, Sakagami N, Iguchi T, Murakami T, Suketa Y. Sodium fluoride increases intracellular calcium in rat renal epithelial cell line NRK-52E. Biol Pharm Bull. 2000;23:581-584.

8. Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signaling. Nat Rev Mol Cell Biol. 2000;1:11-21.

9. Narayanan N, Su N, Bedard P. Inhibitory and stimulatory effects of fluoride on the calcium pump of cardiac sarcoplasmic reticulum. Biochim Biophys Acta. 1991;1070:83-91.

10. Tiwari H, Rao MV. Curcumin supplementation protects from genotoxic effects of arsenic and fluoride. Food Chem Toxicol. 2010;48:1234-1238.

11. Hassan HA, Yousef MI. Mitigating effects of antioxidant properties of black berry juice on sodium fluoride induced hepatotoxicity and oxidative stress in rats. Food Chem Toxicol. 2009;47:2332-2337.

12. Zhang M, Wang A, He W, He P, Xu B, Xia T, et al. Effects of fluoride on the expression of NCAM, oxidative stress, and apoptosis in primary cultured hippocampal neurons. Toxicol. 2007;236:208-216.

13. Bharti VK, Srivastava RS. Fluoride-induced oxidative stress in rat's brain and its amelioration by buffalo (bubalus bubalis) pineal proteins and melatonin. Biol Trace Elem Res. 2009;130:131-140.

14. Shanthakumari D, Srinivasalu S, Subramanian S. Effect of fluoride intoxication on lipid peroxidation and antioxidant status in experimental rats. Toxicol. 2004;204:219-228.

15. Ross AB, Zangger A, Guiraud SP. Cereal foods are the major source of betaine in the Western diet–analysis of betaine and free choline in cereal foods and updated assessments of betaine intake. Food Chem. 2014;145:859-865.

16. Lever M, Slow S. The clinical significance of betaine, an osmolyte with a key role in methyl group metabolism. Clin Biochem. 2010;43:732-744.

17. Ganesan B, Buddhan S, Anandan R, Sivakumar R, Anbinezhilan R. Antioxidant defense of betaine against isoprenaline-induced myocardial infarction in rats. Mole Biol Rep. 2010;37:1319-1327.

18. Alirezaei M, Jelodar G, Niknam P, Ghayemi Z, Nazifi S. Betaine prevents ethanol - induced oxidative stress and reduces total homocysteine in the rat cerebellum. J Physiol Biochem. 2011;67:605-612.

19. Whittaker VP, Michaleson IA, Jeanette R. The separation of synaptic vesicles from nerve-ending particles (synaptosomes). The Biochem J. 1964;90:293-303.

20. Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. J of Bio Chem. 1949;177:751-766.

21. Srivastava SK, Beutler E. Accurate measurement of oxidized glutathione content of human, rabbit, and rat red blood cells and tissues. Anal biochem. 1968;25:70-76.

22. Okhawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. (979;95:351-358.

23. Aebi H. Catalase in vitro. Methods Enzymol. 1984;105:121-126.

24. Dejam A, Hunter CJ, Schechter AN, Gladwin MT. Emerging role of nitrite in human biology. Blood Cells Mol Dis. 2004;32:423-429.

25. Niggle V, Adunyah ES, Penniston JT, Carafoli E. Purified Ca-Mg ATPase of the erythrocyte membrane. J Biol Chem. 1981;256:395-401.

26. Ganesan B, Buddhan S, Anandan R, Sivakumar R, Anbinezhilan R. Antioxidant defense of betaine against isoprenaline-induced myocardial infarction in rats. Mole Biol Rep. 2010;37:1319-1327.

27. Alirezaei M, Khoshdel Z, Dezfoulian O, Rashidipour M, Taghadosi V. Beneficial antioxidant properties of betaine against oxidative stress mediated by levodopa/benserazide in the brain of rats. J Physiol Sci. 2015;65:243-52.

28. Kanbak G, Arslan OC, Dokumacioglu A, Kartkaya K, Inal ME. Effects of chronic ethanol consumption on brain synaptosomes and protective role of betaine. Neurochem Res. 2008;33:539-544.

29. Knowles RG, Palacios M, Palmer RM, Moncada S. Formation of nitric oxide from L-arginine in the central nervous system: a transduction mechanism for stimulation of the soluble guanylate cyclase, Proc Natl Acad Sci. 1989;86:5159-5162.

30. Liu G, Chai C, Cui L. Fluoride causing abnormally elevated serum nitric oxide levels in chicks. Environ Toxicol Pharmacol. 2003;13:199-204.

31. Woodward JJ, Harms J. Potentiation of N-methyl-D-aspartate-stimulated dopamine release from rat brain slices by aluminum fluoride and carbachol. J Neurochem. 1992;58:1547-54.

32. Adamek E, Pawłowska-Goral K, Bober K. In vitro and in vivo effects of fluoride ions on enzyme activity. Ann Acad Med Stetin. 2005;51:69-85.

33. Kanbak G, Akyüz F, Inal M. Preventive effect of betaine on ethanol-induced membrane lipid composition and membrane ATPases. Arch Toxicol. 2001;75:59-61.

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A single group, pretest-posttest clinical trial for the effects of botulinum toxin injection using dual guidance into the upper extremity muscles for the treatment of focal spasticity in patients with chronic stroke

Emre Ata[1]*, Umit Dincer[2]

Abstract

Objective: This study aimed to determine if ultrasonography and electrical muscle stimulation-guided botulinum toxin injection into the upper extremity muscles increases the efficacy of the treatment of focal spasticity in patients with chronic stroke.

Materials and Methods: This study included 22 chronic hemiplegic stroke patients with grade 2 and 3 spasticity in the upper extremity muscles, based on the Modified Ashworth Scale. The study hypothesis was that ultrasonography and electrical muscle stimulation-guided botulinum toxin injection would increase the efficacy of the treatment of spasticity. The Modified Ashworth Scale, Tardieu Scale, Barthel Index and Fugl-Meyer Motor Assessment Scale were administered at baseline, and at 2 weeks and 2 months post treatment.

Results: All parameters were improved significantly at 2 weeks post treatment, as compared to baseline, and the observed improvement persisted at 2 months post treatment ($P < 0.05$).

Conclusion: Ultrasonography and electrical muscle stimulation-guided botulinum toxin injection significantly improved spasticity and functional recovery in chronic stroke patients with upper extremity spasticity.

Keywords: spasticity, botulinum toxin, injection, ultrasonography, stimulation

Introduction

Spasticity is a cause of disability in 38% of stroke patients during the first year post stroke (1). Spasticity can negatively affect daily activities and a patient's physical appearance, balance, and gait pattern (2). Botulinum toxin (BTX) injection is a safe, effective, and commonly used method for the treatment of focal and multifocal spasticity. Correct muscle group selection and injection technique are the primary factors associated with successful treatment. BTX is reported to be most effective when injected correctly and into the deep-seated motor end plates in muscles (3). Intramuscular BTX injection can be performed using several types of guidance, including manual needle placement (MNP), electromyography (EMG), electrical muscle stimulation (EMS), and ultrasonography (US). EMS- or US-guided injection is recommended, especially for deep-seated and small muscles (4-6). The present study aimed to determine if US and EMS-guided BTX injection into the upper extremity muscles increases the efficacy of the treatment of focal spasticity in patients with chronic stroke.

Material and Methods

The study included 22 chronic hemiplegic stroke patients with grade 2 and 3 spasticity in upper extremity muscles, based on the Modified Ashworth Scale (MAS). Inclusion criteria were as follows: Spastic hemiparesis secondary to ischemic or hemorrhagic stroke; time from stroke onset at least 6 months; aged 20-75 years; grade 2 or 3 spasticity of the all affected upper extremity muscles, including: the biceps, pronator teres, flexor carpi radialis, flexor carpi ulnaris, flexor digitorum superficialis and flexor digitorum profundus, based on MAS. Exclusion criteria were as follows: Fixed contractures (tone grade 4, according to MAS); tumor or severe trauma in the affected arm; ongoing treatment with oral anti-spastic medication; BTX treatment within 3 months of the study start date; history of surgical treatment of fixed contractures affecting the arm; pregnancy; lactation; formation of neutralizing antibodies against BTX (previous 2 injections ineffective). All the patients were evaluated as 1 group. All the patients provided written informed consent and the study protocol was approved by the local Ethics Committee.

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Procedures

Abobotulinumtoxin-A was injected (Dysport, Ipsen, France) (500 U diluted with 2 mL of 0.9% saline) into all of the affected upper extremity muscles including: the biceps, pronator teres, flexor carpi radialis, flexor carpi ulnaris, flexor digitorum superficialis and flexor digitorum profundus. The dose was 125 U for all muscles on the purpose of standardization. Patients were placed in the supine position, the shoulder was abducted, the elbow was extended, and the forearm was placed in supination. The injection area was covered with sterile drape, and skin antisepsis was provided. Firstly, each targeted muscle was observed via US, using an M-Turbo system (Sonosite, USA) with a linear transducer (scanning frequency 7-12 MHz) and sterile gel. The transducer was positioned for the transverse view, perpendicular to the arm and forearm surface. A 25-G EMS needle (50 □ 0.5 mm, hypodermic, single use, Teflon coated, Technomed Europe, Netherlands) was inserted into the targeted muscle at a 30° angle to the transducer via the outline method under US guidance. The depth of the needle tip inside each target muscle was determined via the gentle and reciprocating movements of the needle. After confirming that the needle was in the target muscle, electrostimulation was administered (Dantec CLAVIS, REF-9015A0012, Denmark). The stimulating current intensity was set at 10 Ma. EMS was stopped when the best contractility was observed and felt, and then 125 U of BTX was delivered into the each target muscle under US guidance. The same physiatrist (E.A.) who was blinded to the assessments scores conducted all the scans and injections. All patients performed post-treatment stretching exercises 60 min d–1 for 2 weeks. In addition, each patient used an inhibitory wrist splint 6 h d–1 for 2 months. Patients were instructed not to use oral anti-spastic medications for 3 months after the treatment.

Clinical and Functional Assessment

Spasticity was evaluated using the MAS (7) and Tardieu Scale (TRS) (8), and functional ability was assessed using the Barthel Index (BI) and Fugl-Meyer Motor Assessment Scale (FMS) at baseline, and at 2 weeks and 2 months post treatment. All patients were examined by the same physiatrist (U.D.), who was blinded to the treatment.

Statistical Analysis

Statistical analysis was performed using SPSS v.22.0 for Windows (IBM, Corp., Armonk, NY). The Kolmogorov Smirnov test was used to analyze normal distribution of variables. Friedman's test was used for multiple intergroup comparisons and the Wilcoxon signed-rank test were used for single intergroup comparisons. The level of statistical significance was set at $P < 0.05$.

Results

The study included 22 patients with upper limb spasticity that were recruited from among 43 stroke patients that presented to our outpatient clinic (Figure 1). The mean age of patients who treated with BTX (eight women and fourteen men) was 60.5 ± 11 years. All of the 22 patients completed the study and none of them declared adverse events or complications of injections. Patient demographics are shown in Table 1.

A significant reduction in the degree of spasticity (based on MAS and TRS) was observed at 2 weeks post treatment and persisted at 2 months post treatment ($P < 0.05$) (Table 2). Additionally, functional scores (based on BI and FMS) at 2 weeks and 2 months post treatment were significantly better than at baseline ($P < 0.05$) (Table 2).

Table 1. Patient characteristics

Age (yrs) (Mean ± SD)	**60,5 ± 11**
Sex (male / female)	14/8
Type of stroke (ischemic / hemorrhagic)	14/8
Duration of stroke (month) (mean ± sd)	44,3±68,9
Hemiplegic side (right / left)	13/9
SD: standard deviation, yrs: years	

Table 2. Clinical and functional assessments

Parameters	Before Tx	After Tx (at 2 weeks)	After Tx (at 2 mos)	*p**	*p #*	*p ł*
MAS						
Biceps	2,45 ±0,50	0,81 ±0,54	1,18 ±0,52	***0,01***	***0,01***	***0,01***
Pronator	2,72 ±0,45	0,68 ±0,47	1,11 ±0,34	***0,01***	***0,01***	***0,01***
Wrist	2,77 ±0,42	0,70 ±0,57	1,18 ±0,39	***0,01***	***0,01***	***0,01***
Finger flexors	2,72 ±0,45	1,00 ±0,46	1,25 ±0,42	***0,01***	***0,01***	***0,01***
Tardieu degree						
Wrist	2,54±0,59	0,90 ±0,75	1,22 ±0,68	***0,01***	***0,01***	***0,01***
Tardieu angle						
Wrist	52,04±6,29	15,22 ±6,45	22,27 ±6,67	***0,01***	***0,01***	***0,01***
Barthel Index	52,50±12,32	58,63 ±13,01	58,86 ±12,33	***0,01***	***0,01***	***0,01***
Fugl-Mayer Assessment	8,54±9,26	16,95 ±11,61	15,22 ±10,71	***0,01***	***0,01***	***0,01***

**Triple comparison of BT, 2 weeks AT and 2 months AT; #BT vs. 2 weeks AT; łBT vs. 2 months AT; p values in boldface are statistically significant*

Discussion

The present findings show that significant improvement in spasticity and functional recovery was achieved using dual-guided BTX injection (US and EMS) for the treatment of upper extremity spasticity in chronic hemiplegic stroke patients. BTX injection in patients with stroke is performed via several methods, MNP, EMG, EMS, and US. The most important factor associated with the success of BTX injection is accurate needle placement into the targeted muscle (6). MNP is widely used for superficial and major muscles, and requires a good anatomical knowledge. A study in which the accuracy of MNP was monitored via US reported accuracy of 92.6% for m. gastrocnemius medialis and 64.7% for m. gastrocnemius lateralis (9). Another study monitored the accuracy of MNP via EMS, and reported accuracy of 13% for m. flexor carpi radialis and 16% for m. flexor carpi ulnaris (10). It can be considered that the achievement drive and reliability of MNP method is low, particularly for injections into small and deep-seated muscles such as forearm muscles, even when performed by experienced physicians (11).

It was reported that BTX injection performed close to the motor endplates might be most effective and EMG and EMS are reliable for muscle and motor endplate localization (12).

When performing BTX injection under EMG guidance it is easy to know when the needle is in a spastic muscle, but it is difficult to know if the needle is in the targeted muscle (13). Loss of selective muscle activation and over-activity of neighboring muscles negatively affects the accuracy of EMG. Additionally, needle EMG is associated with pain, which limits its use. Even though significant improvement was reported in patients with cervical dystonia following EMG-guided BTX injection (14), the technique may not be sufficient when used alone.

EMS is widely considered a reliable technique for the localization of a targeted muscle and motor endplate, although it is associated with the following disadvantages: it is a blind method, time is lost while guiding the needle into the targeted muscle, and it causes pain (11). In contrast, US is a more reliable, time-efficient, and painless method for muscle localization. It provides real-time imaging during injections and it can help physicians avoid accidental injection into neurovascular structures (15). Although it was reported that US-guided BTX injection is an alternative for BTX injection via EMG or EMS guidance, US is not reliable for motor endplate localization.

Several studies compared BTX injection methods, including MNP, EMG, EMS, and US, and US and EMS were reported to be superior to the other methods (4, 5, 9, 10, 16). Kwon et al. compared US and EMS guidance and reported a significant decrease in MAS and TRS scores in both groups of children with cerebral palsy at 1 month post injection; however, they also reported that the significant decreases in MAS and TRS scores persisted at 3 months post injection only in the US group (16). In the present study a significant reduction in the degree of spasticity (based on MAS and TRS) was observed at 2 weeks post treatment and persisted at 2 months post treatment.

Picelli et al. reported that EMS and US guidance provided better results for all parameters (based on MAS, TRS and fingers passive range of motion) in stroke patients with wrist and finger flexor spasticity than MNP. In addition, they reported there weren't any significant differences between the EMS and US groups (4).

In an earlier study by Picelli et al. the accuracy of MNP-guided and EMS-guided BTX injection was monitored via US to determine if the needle was inserted into the correct muscle (5). They reported the accuracy of each method as follows: proximal part of m. gastrocnemius medialis: 88.09% in the MNP group versus 92.30% in the EMS group; distal part of m. gastrocnemius medialis: 92.86% in the MNP group, versus 94.87% in the EMS group; proximal part of m. gastrocnemius lateralis: 64.28% in the MNP group, versus 87.17% in the EMS group; distal part of m. gastrocnemius lateralis: 73.80% in the MNP group, versus 92.30% in the EMS group. These findings indicate that BTX injection via MNP is much less accurate than via EMS for muscles smaller than m. gastrocnemius lateralis, and in particular for forearm muscles. Despite observing that EMS was more accurate than MNP, they also reported that EMS is a blind method, as is MNP, and may cause neurovascular damage if it is the only method used (5).

It was reported that BTX injection into the upper extremity muscles in stroke patients can improve functional disability.2 Shaw et al. compared BTX injection and physiotherapy, in terms of functional improvement, and reported that 25.1% of the BTX group and 19.5% of the control group exhibited functional improvement (17). Although better results were achieved in BTX group at 1, 3, and 12 months post treatment, there wasn't a significant difference between the groups (17). In the present study BI and FMS scores at 2 weeks and 2 months post treatment were significantly better than at baseline. We think the significant improvement in functional scores observed in the present study was due to dual US and EMS guidance of BTX injections, and misapplications can be prevented with dual-guidance, particularly for injections into forearm muscles. In addition to improvement in spasticity and functional recovery, the dual-guided injection method minimized the occurrence of complications.

The present study has some limitation foremost the lack of a control group. The study's small sample size is another limitation, as is the short (2 months) follow-up period.

Conclusion

In conclusion, significant improvement in spasticity and functional recovery were achieved via dual-guided (US and EMS) BTX injections; this method might also be used to reduce the risk of neurovascular complications. Based on the present findings, we think US and EMS-guided BTX injections should be used in eligible patients for the treatment of spasticity and that larger-scale randomized clinical trials with longer follow-up periods are needed to

further delineate the effectives of the dual-guided BTX injection method described herein.

Conclusion

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Author's Contributions: Research concept and design **(UD, EA);** botulinum toxin injections **(EA);** data collecting **(UD),** All authors approved the final version of the manuscript

Ethical issues: All Authors declare, Originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

References

1. Monaghan K, Horgan F, Blake C, et al: Physical treatment interventions for managing spasticity after stroke (Protocol), 2011 The Cochrane Collaboration, Published by John Wiley & Sons Ltd., The Cochrane Library 2011, Issue 7.

2. Ozcakır S, Sivrioğlu K. Botulinum toxin in poststroke spasticity. Clinical medicine and Research 2007;5(2):132-8.

3. Çeliker R. Bölüm 58, Spastisite Tedavisinde Kullanılan İlaçlar, Fiziksel Tıp ve Rehabilitasyon, 2. Baskı, (Ed: Beyazova M, Kutsal YG), 2011;2:901-17.

4. Picelli A, Lobba D, Midiri A, et al: Botulinum toxin injection into the forearm muscles for wrist and fingers spastic overactivity in adults with chronic stroke: a randomized controlled trial comparing three injection techniques, Clin Rehabil 2014; 28(3): 232-42.

5. Picelli A, Bonetti P, Fontana C, et al: Accuracy of botulinum toxin type A injection into the gastrocnemius muscle of adults with spastic equinus: manual needle placement and electrical stimulation guidance compared using ultrasonography. J Rehabil Med 2012; 44(5): 450-2.

6. Wissel J, Ward AB, Erztgaard P, et al. European consensus table on the use of botulinum toxin type A in adult spasticity. J Rehabil Med 2009; 41:13-25.

7. Bohannon RW, Smith MB: Interrater reliability of a modified Ashworth scale of muscle spasticity. Phys Ther 1987;67:206-7

8. Paulis WD, Horemans HL, Brouwer BS, Stam HJ. Excellent test-retest and interrater reliability for Tardieu Scale measurements with inertial sensors in elbow flexors of stroke patients. Gait Posture 2011; 33(2): 185-9

9. Yang EJ, Rha DW, Yoo JK, et al. Accuracy of manual needle placement for gastrocnemius muscle in children with cerebral palsy checked against ultrasonography. Arch Phys Med Rehabil 2009; 90:741-4.

10. Chin TY, Nattrass GR, Selber P, et al. Accuracy of intramuscular injection of botulinum toxin A in juvenile cerebral palsy: a comparison between manual needle placement and placement guided by electrical stimulation. J Pediatr Orthop 2005; 25:286-91.

11. Schroeder AS, Berweck S, Lee SH, Heinen F. Botulinum toxin treatment of children with cerebral palsy- a short review of different injection techniques. Neurotoxicity Research 2006; 9:189-96.

12. Shaari CM, Sanders I. Quantifying how location and dose of botulinum toxin injections affect muscle paralysis. Muscle Nerve 1993;16 (9):964-9.

13. G. Sheean, N. A. Lanninb, L. Turner-Stokes, et al. Botulinum toxin assessment, intervention and after-care for upper limb hypertonicity in adults: international consensus statement, European Journal of Neurology 2010;17:74–9.

14. Comella CL, Buchman AS, Tanner CM, et al. Botulinum toxin injection for spasmodic torticollis: increased magnitude of benefit with electromyografic assistance. Neurology 1992;42(4):878-82

15. Henzel MK, Munin MC, Niyonkuru C, et al. Comparison of surface and ultrasound localization to identify forearm flexor muscles for botulinum toxin injections. PM & R: the journal of injury, function, and rehabilitation 2010;2:642-6.

16. Kwon JY, Hwang JH, Kim JS. Botulinum toxin a injection into calf muscles for treatment of spastic equinus in cerebral palsy: a controlled trial comparing sonography and electric stimulation-guided injection techniques: a preliminary report. Am J Phys Med Rehabil 2010; 89:279-86.

17. Shaw LC, Price CI, van Wijck FM, et al. Botulinum Toxin for the Upper Limb after Stroke (BoTULS) Trial: effect on impairment, activity limitation, and pain. Stroke 2011;42:1371-9.

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T-cell Non-Hodgkin lymphoma associated with myelodysplasia: A case report in a child

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Abstract

Myelodysplastic syndrome (MDS) is a clonal bone marrow disease characterized by ineffective erythropoiesis. MDS patients have also cytopenia. The risk of acute leukemia, and particularly of acute myeloblastic leukemia (AML), is the most important characteristic of the disease .

Myelodysplasia associated with Non Hodgkin's lymphomas (NHL) has been rarely described in the literature. This is suggested to be due to the defect in the immune system, an up-regulation of some cytokines, and a common molecular origin.

In this article, we reported a 7-year-old pediatric NHL case with a normal karyotype and myelodysplasia in the bone marrow and discussed the pathogenesis of the association of NHL and myelodysplasia.

Keywords: Lymphoma, myelodysplasia, relation

Introduction

Myelodysplastic syndrome (MDS) is a clonal bone marrow disease characterized by ineffective erythropoiesis. MDS patients have cytopenia. The risk of acute leukemia, and particularly of acute myeloblastic leukemia (AML), is the most important characteristic of the disease (1).

MDS is defined as primary of de novo MDS if it develops in a child who has no other diseases, and who has not received chemotherapy or radiotherapy for any other reason, while it is considered as secondary MDS if there is a factor which promotes the development of myelodysplasia, especially a history of chemotherapy or radiotherapy (2).

Genetic factors, ionized radiation, chemotherapy, benzene, smoking, alcohol, hair dyes and over-consumption of foods especially rich in phenol are risk factors for the development MDS (2).

In primary MDS cases, cytogenetic abnormalities are found in 50-70% of the patients, while this rate rises over 85% in cases with treatment-related secondary MDS (2).

Non-Hodgkin's lymphomas (NHL) are a group of malignant diseases originating in the organs and cells of the immune system. Childhood NHL exhibits diffuse and extranodal involvement. Childhood NHL generally arises from lymphoid precursors and B-cell type is found in 80% of the cases (3).

Non-Hodgkin's lymphomas typically metastasize early and the risk of leukemic presentation and central nervous system relapse is high in NHLs (4).

Myelodysplasia associated with NHL has been rarely described in the literature. This is suggested to be due to the defect in the immune system, an up-regulation of some cytokines, and a common molecular origin (5-10).

In this article, we reported a 7-year-old pediatric NHL case with a normal karyotype and myelodysplasia in the bone marrow and discussed the pathogenesis of the association of NHL and myelodysplasia.

Case

A 7-year-old female patient admitted with a swelling on the right side of the neck which had been noted 5 days ago. She had had no complaints such as fever, weight loss, or night sweating. The past medical history and family history of the patient revealed no significant findings. The physical examination of the patient revealed multiple lymphadenopathies in both cervical chains in the submandibular region, with the largest on the left measuring 2.5x1.5 cm and the largest on the right measuring 3.5x1.5 cm, and a 2x2 cm lymphadenopathy in the right inguinal region.

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The laboratory examinations revealed a hemoglobin value of 10.1 g/dl, a WBC count of 1.2x109/l, and a platelet count of 159x109/l . Peripheral blood smear revealed no blasts. The lactate dehydrogenase 705 U/l and B 12 levels and the other biochemical test results were considered as normal. The immunoglobulin A 133.0 mg/dl, G 955.0 mg/dl, M 44.0 mg/dl and E 49.25 IU/ml levels were consistent with the age of the patient.

The results of the direct Coombs's test and the ELISA-based Parvovirus PCR, EBV, and CMV assays were negative.

Abdominal ultrasonography revealed multiple ovoid and round lymphadenopathies with loss of echogenic hilus in the para-aortic, parailiac and mesenteric regions, with the largest measuring 21x13 mm and neck ultrasonography revealed multiple reactive lymphadenopathies with echogenic hilus and hilar blood flow in both cervical chains in the submandibular region, with the largest one on the left measuring 23x10 mm and the largest one on the right measuring 32x14 mm.

The bicytopenia of the patient continued for 4 days in the clinical follow-up and bone marrow aspiration was performed. The bone marrow aspiration smear revealed 4% monocytes, 35% normoblasts, 30% lymphocytes, 15% myelocytes, 6% metamyelocytes, 6% neutrophis, and 4% blasts. Dysplasia was found in bone marrow cells.
Diffuse hypogranular myeloid cells, dysplastic megakaryocytes and erythroblastic cells were observed (Figure 1a,1b,1c). The cervical lymph node biopsy result was consistent with diffuse NHL with a high-grade malignancy (Figure 2).

Immunohistochemical examination of cervical lymph node was consistent with T-cell. Analyses of 17p13.1, p53, 20q12, 5q31, 7q31 gene deletions and monosomy/trisomy 7, and monosomy/trisomy 8 chromosomes were performed with bone marrow cytogenetic and FISH (fluorescence in situ hybridization) studies. The results were accepted as normal. The patient left our hospital to continue her diagnostic studies and treatment in another institution.

The cervical lymph node biopsy result was consistent with diffuse NHL with a high-grade malignancy (Figure 2).

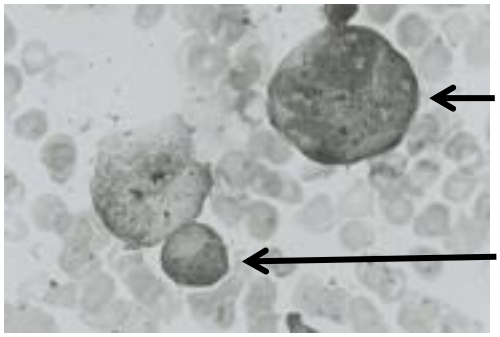

Figure 1A: A giant erythroblast with multiple nuclei and a larger-than-normal dysplastic metamyelocyte with hypogranular cytoplasm

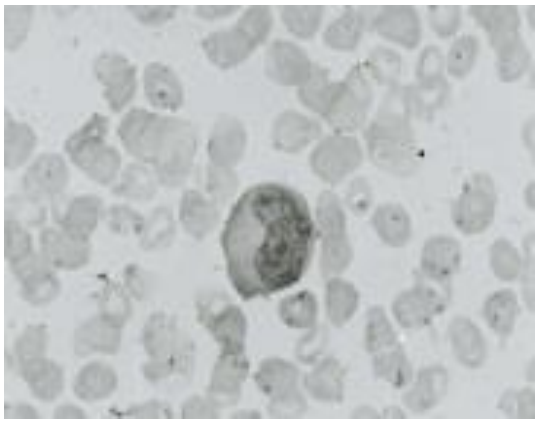

Figure 1B: A hypogranular metamyelocyte

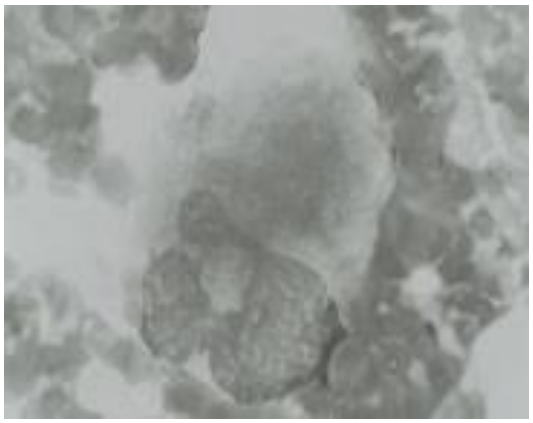

Figure 1C: A megakaryocyte with a pyknotic nucleus and a dysplastic appearance

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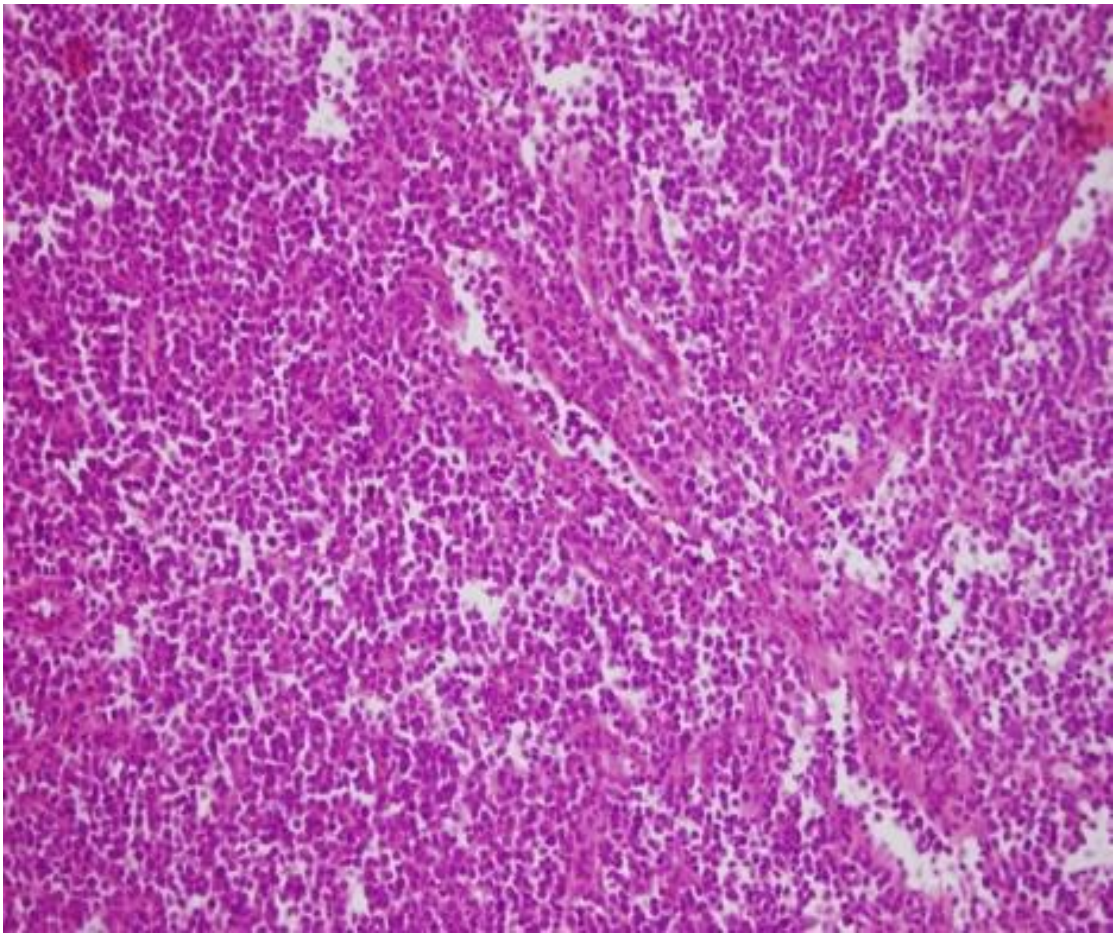

Figure 2: Diffuse NHL with high-grade malignancy

Immunohistochemical examination of cervical lymph node was consistent with T-cell. Analyses of 17p13.1, p53, 20q12, 5q31, 7q31 gene deletions and monosomy/trisomy 7, and monosomy/trisomy 8 chromosomes were performed with bone marrow cytogenetic and FISH (fluorescence in situ hybridization) studies. The results were accepted as normal. The patient left our hospital to continue her diagnostic studies and treatment in another institution.

Discussion

MDS is known to be related to a process in tumor differentiation. A high incidence of MDS was reported in relation with solid tumors, such as lung, colon, prostate and liver cancers (11). The same relationship was described between MDS and lymphoid neoplasms, such as acute lymphoblastic leukemia, chronic lymphocytic leukemia, and NHL (12-15). In 1996, a group of Spanish investigators studied the association of lymphoid malignancy in patients with primary MDS and found an association rate of only 1% and concluded that this association could be a coincidence (14).

The association of MDS and lymphoma was described in 21 cases in the literature. However, in 9 of these 21 cases MDS and NHL were diagnosed simultaneously.

In 12 cases, MDS diagnosis was made primarily before the onset of lymphoma and the time between the two diseases ranged between 5 months and 4 years (5,13,16-27).

The mechanisms responsible for the development of NHL in MDS patients have not been cleared yet. MDS is generally considered as a clone disorder with pluripotent stem cell origin and with a potential to differentiate into lymphoid and myeloid cells. Some authors suggest that the two diseases are caused by the same neoplastic process or a common origin (6).

Another opinion is that MDS plays a predisposing role in the development of lymphoid neoplasms. (5). MDS is associated with abnormal immunological functions. Abnormal lymphocyte count and function (especially natural killer cells) induce growth of neoplastic cells. The immune system defect underlying the development of myelodysplasia is also present in NHL (8,9).

Shimanoto et al. related the association of MDS and NHL to the up-regulation of particular cytokines, such as IL-6 and vascular endothelial growth factor (VEGF) and reported a case of anaplastic large-cell lymphoma with presence of high IL-6 and VEGF levels and bone marrow myelodysplasia at the time of diagnosis (10).

Chromosomal anomalies are common in both MDS and lymphomas. The questions, whether there are other

cytogenetic abnormalities not known yet, and whether the association of MDS with NHL is caused by these common cytogenetic anomalies, still remain to be answered.

In 1998, Mori A et al. found bone marrow dysplasia simultaneously with the diagnosis of angiocentric lymphoma in a 46-year-old male patient. They thought that the association might be caused by cytokines, such as interleukin-2, -4, and-6 (28).

Huang HH et al. also reported a case with the association of bone marrow dysplasia and lymphoma in 2009. They suggested that this association might be caused by a common chromosomal anomaly (del (20q)) based on the fact that the patient had 20q deletion in both myeloid and lymphoid cell lines (29).

Conclusion

In conclusion, the association of MDS and lymphoma is very rare. Only 21 cases have been reported to date. However, de novo MDS and lymphoma were simultaneously identified in 8 of these cases. We think that this association may be caused by a common molecular origin, common chromosomal anomalies and cytokines. Large scale studies including many cases are needed on this subject.

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Author's Contributions: GPR, AB, EE: Research concept and design; Patient examination, data collecting, histochemical, biochemical analysis and interpretation of data. **GPR:** Preparation of article and revisions. All authors approved the final version of the manuscript,

Ethical issues: All Authors declare, Originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

References

1. Steensma DP, Tefferi A. The myelodysplastic syndrome (s) : a perspective and review highligh-ting current controversies. Leuk Res. 2003; 27 : 95-120

2. Elghetany MT.Myelodysplastic syndromes in children: a critical review of issues in the diagnosis and classification of 887 cases from 13 published series. Arch Pathol Lab Med. 2007;131:1110-6

3. Link MP and Weinstein HJ: Malignant No-Hodgkin Lymphomas in children.In:Pizzo PA, Poplark DG, eds. Principles and Practive of Pediatric Oncology.Fifth Edition, Lippincott Williams and Wilkins, Philedelphia, 2006, pp:722-747

4. Percy CL, Smith MA, Linet M, et al. Lymphomas and reticuloendothelial neoplasms. In: Ries LAG, Smith MA, Gurney JG, et al., eds. Canser indicence and survival amon children and adolescents. United States SEER Program 1975-1995. Bethesda, MD: National Cancer Institute, SEER Program. NIH Pub. No. 99-4649, 1999:35-49.

5. Uematsu M, Ochi H, Ueda Y, et al: Coexistent myelodysplastic syndrome and Non-Hodgkin's lymphoma. Report of a case and review of the literature. Int J Hematol 1995;62:45-51.

6. Weimar IS, Bourhis JH, De Gast GC, et al: Clonality in myelodysplastic syndrome. Leuk Lymphoma 1994;13:215-221.

7. Brezinova J, Zemanova Z, Randsdorfova S, et al: Prognostic significance of del(20q) in patients with hematological malignancies. Cancer Genet Cytogenet 2005;160:188-192.

8. Bynoe AG, Scott CS, Ford P, et al: Decreased T helper cells in the myelodysplastic syndromes. Br J Haematol 1983;54:97-102.

9. Colombat PH, Renoux M,Lamagnere JO, et al: Immunologic indices in myelodysplastic syndromes. Cancer 1988;61:1075-1081.

10. Shimamoto T, Hayashi S, Ando K, et al: Anaplastic large-cell lymphoma which showed severe inflammatory status and myelodysplasia with increased VEGF and IL-6 serum levels after long-term immunosuppressive therapy. Am J Hematol 2001;66:49-52

11. Fong GH, Rossant J, Gertsenstein M, et al: Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. Nature 1995;376:66-70.

12. Abruzzese E, Buss D, Rainer R, et al: Progression of a myelodysplastic syndrome to pre-B lymphoblastic leukemia. A case report and cell lineage study. Ann Hematol 1996;73:35-38.

13. Copplestone JA, Mufti GJ, Hamblin TJ, et al: Immunologic abnormalities in myelodysplastic syndromes II. Coexistent lymphoid or plasma cell neoplasms. A report of 20 cases unrelated to chemotherapy. Br J Haematol 1986;63:149-159.

14. Florensa L, Vallespi T, Woessner S, et al: Incidence and characteristics of lymphoid malignancies in untreated myelodysplastic syndromes. Leuk Lymphoma 1996;23:609-612.

15. Hamblin TJ: Immunologic abnormalities in myelodysplastic syndromes. Semin Hematol 1996;3:150-162.

16. Anzai T, Hirose W, Nakane H, et al: Myelodysplastic syndrome associated with immunoblastic lymphadenopathy-like T-cell lymphoma: simultaneous clinical improvement with chemotherapy. Jpn J Clin Oncol 1994;24:106-110.

17. Takaku T, Miyazawa K, Sashida G, et al: Hepatosplenic αβ T- cell lymphoma with myelodysplastic syndrome. Int J Hematol 2005;82:143-147.

18. Xu B, Meng FY, Li YQ T- cell non-Hodgkin lymphoma with myelodysplastic syndrome: A case report. Zhonghua Zhong Liu Za Zhi 2005;27:320.

19. Sato T, Shiga Y, Takeda H, et al: IBL-like T-cell lymphoma with helper T-cell phenotype in a case of myelodysplastic syndrome. Rinsho Ketsueki 1986;27:612-616.

20. Auger MJ, Nash JRG, Mackie MJ, et al: Marrow involvement with T cell lymphoma initially presenting as abnormal myelopoiesis. J Clin Pathol 1986;39:134-137.

21. Kawashima K, Furukawa Y, Akutu M, et al: Primary non- Hodgkin's lymphoma, small lymphocytic type, in the spleen complicating myelodysplastic syndrome. J Jpn Soc Intern Med 1988;77:1051-1055.

22. Breccia M, Petti MC, D'Elia GM, et al: Cutaneous pleomorphic T-cell lymphoma coexisting with myelodysplastic syndrome transforming into acute myeloid leukemia: Successful treatment with a fludarabine-containing regimen. Eur J Haematol 2002;68:1-3.

23. Dalamaga M, Karmaniolas K, Chavelas C, et al: Coexistence of primary refractory anemia with ringed sideroblasts and T-cell lymphoblastic non- Hodgkin lymphoma. Acta Haematol 2004;111:171-172.

24. Cheng HB, Luo MZ, Zhang SM: A case of acute myelomonocytic leukemia transformed from myelodysplastic syndrome coincident with malignant lymphoma. Mod Diagn Treat 2004;15:191.

25. Liu GX, Li X: Coexistence of myelodysplastic syndrome and malignant lymphoma in the peritoneal cavity: a case report. Henan Zhong Liu Xue Za Zhi 2001;14:172.

26. Rostoker G, Raphael M, Boisnick S, et al: Coexistence of Sezary syndrome and dysmyelopoiesis with an excess of myeloblasts. J Am Acad Dermatol 1986;15:1296-1298.

27. Kazakov DV, Mentzel T, Burg G, et al: Blastic natural killer-cell lymphoma of the skin associated with myelodysplastic syndrome or myelogenous leukaemia: A coincidence or more? Br J Dermatol 2003;149:869-876.

28. Mori A, Hashino S, Imamura M, et al: Bone marrow dysplasia with basophilic cells in a patient with angiocentric lymphoma. Acta Haematol.1998;99(2):98-101.

29. Huang HH, Zhu JY, Han JY, et al: Co-existent de novo myelodysplastic syndrome and T-cell non- Hodgkin lymphoma: a common origin or not? J Int Med Res. 2009 Jan-Feb;37(1):270-6

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Can non-stress test predict meconium stained amniotic fluid presence without performing an amniotomy?

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Abstract

Objective: Meconium stained amniotic fluid is a frequently encountered situation, it can really disturb an obstetrician, as it increases the rates of neonatal morbidity and mortality and it is difficult to prevent meconium passage in utero. Noninvasive tests are needed to predict the meconium staining of the amnion fluid without making amniotomy or making a fetal invasive procedure. Non stress test is a commonly used method to determine the status of intrapartum fetal wellbeing. The purpose of this study was to predict fetal meconium release during labor by examining the fetal heart rate traces without performing an amniotomy procedure.

Materials and Methods: A total of 280 patients who have been diagnosed with active labor were included in the study. The 140 of them have demonstrated meconium stained fluid and 140 of them have clear amniotic fluid. The patients' labor courses have been watched and non-stress test results have been recorded besides obstetric outcomes.

Results: Non-stress tests performed before amniotomy; 52 (37.1%) of the non-stress tests in the meconium group were non-reactivated, whereas in the control group this count was 19 (13.5%) before amniotomy. When we accepted the deceleration entity as fetal distress; fetal distress was seen in 62(44.3%) of the patients in the meconium group and in 21(15.1%) of the patients in the control group.

Conclusion: In the presence of non-reactive non stress test pattern; we should be suspicious of meconium-stained amniotic fluid. In this case, caution should be taken in terms of fetal distress.

Keywords: Meconium stained amniotic fluid, Non stres test

Introduction

Presence of meconium stained amniotic fluid (MSAF) is seen in 12-16 % of deliveries. Meconium aspiration syndrome (MAS) which occurs in 2% to 36% of meconium-stained neonates is characterized with respiratory distress syndrome (1). Meconium output normally occurs within the first 24-48 hours after birth. It is not uncommon for amniotic fluid to be stained with meconium. The most serious complication of meconium-stained amniotic fluid is meconium aspiration syndrome (2,3). The incidence of meconium-stained amniotic fluid increases with gestational age and reaches 30% in postterm pregnancies. Regardless of fetal maturation, a significant increase in the incidence of meconium transmission in the amniotic cavity is evident in the presence of fetomaternal stress factors such as hypoxia and infection (4). Meconium stained amniotic fluid is associated with higher rate of caesarian delivery, increase the need for neonatal resuscitation and the need for neonatal intensive care (5,6).

Although meconium stained amniotic fluid is a frequently encountered situation, it can really disturb an obstetrician, as it increases the rates of neonatal morbidity and mortality and it is difficult to prevent meconium passage in utero. In utero, passage of meconium may simply represent the normal gastrointestinal maturation or it may indicate an acute or chronic hypoxic event. These fetuses are 100 times more likely to exhibit respiratory distress sendrome than those which are born through clear amniotic fluid (7). Meconium can cause umbilical vascular vasospasm and impair fetal-placental blood flow. Meconium presence may also have occurred with anal sphincter loosening resulting in intrauterine hypoxia. This is associated with fetal distress and non-reassuring fetal heart rate (FHR) (8,9). It is unclear why the meconium aspiration syndrome develops in one part of the babies painted with meconium and does not develop in others.

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Because of this reason if meconium is present in the amnion fluid, it should be regarded as a stimulant marker for fetal distress and the fetus should be closely evaluated for presence of fetal distress (10,11).

FHR monitorization and checking the presence of meconium in the amniotic fluid are the commonly used methods during labor. Electronic fetal heart rate monitoring helps to reduce fetal mortality and morbidity rates by detecting fetal hypoxia early but may increase unnecessary cesarean rates (12,13,14). The MSAF is a clinical diagnosis with no practical confirmatory test except diagnostic amniocentesis which is an invasive intervention.

Noninvasive tests are needed to predict the meconium staining of the amnion fluid without making amniotomy or making a fetal invasive procedure. Non-stress test is a commonly used method to determine the status of intrapartum fetal well being. Our leading purpose to investigate the difference between non-stress test patterns before and after amniotomy.

Material and Methods

A total of 280 patients who have been diagnosed with active labor were included in the study. Half (N:140) of them demonstrated meconium stained fluid and 140 of them clear amniotic fluid based on physical properties of the amniotic fluid following amniotomy procedures during close follow-up of labor. Patients whose gestational age was between 37 and 41 weeks were included to the study. Patients with high risk conditions were excluded. Informed consent was taken from all patients. The patients' labor courses have been watched and nonstress test results have been recorded besides obstetric outcomes. Caesarean section was liberally performed when fetal heart rate pattern has revealed late and/or variable decelerations. Newborns with respiratory distress were accepted to the intensive care unit as per the pediatrician's advice.

Statistical analysis was performed by using IBM SPSS Statistics Software (22.0, SPSS Inc., Chicago, IL). Patients's obstetric data has been evaluated for normal distribution by using the Kolmogorov-Smirnov test. The continuous variables were presented by means ± standard deviation and compared by using the independent samples t test based on normal distributionstatus. The non-parametric variables and data without normal distribution were compared by using the Mann- Whitney U test. Wilcoxon Signed Ranks test was used for comparing non-stress test patterns for two patient groups separately. The comparison of categoric variables was made by using Fisher's exact test, or the chi-square test. All p values <0.05 were considered statistically significant.

Results

A total of 280 patients were included in the study. Half (N:140) of them had meconium stained amniotic fluid. The amniotic fluid in the control group was clearly seen. Patients in the meconium group 74 of them were thin and 66 of them were thick meconium stained. Patients' age, parity, gestational age, body mass indexes, and birth weight results were similar. Also the need for oxytocin was similar in both groups (Table 1).

After amniotomy; non-reassuring non stress test pattern was observed in 6 (8.1%) of patients with thin meconium and 14 (21.2%) of patients with thick meconium. This difference was statistically significant (p= 0.021). Among patients with meconium stained amniotic fluid, 123 (87.9%) patients were delivered through normal vaginal delivery, while 17 (12.1%) were delivered by caesarean section. Caesarean rate was found significantly higher in the thick meconium group than in the thin meconium group (21% versus 4,2%) (p:0.003). A total of 5 newborns (3.7%) were transferred to intensive care unit due to meconium aspiration syndrome.

Table 1. Demographic and clinical characteristics of the groups (n:280)

Variables	Meconium group (n:140)	Control group (n:140)	P value
Age(yrs)	29.1±5.6	28.5±5.5	0.455*
BMI (kg/m2)	26.2±2.1	26.1±2.4	0.851
Birthweight(gr)	3313±329	3267±308	0.252*
Gestationalage (wks)	39.6±1.0	39.4±1.2	0.302*
Primipar	75(53.6%)	71(50.7%)	0.322¶
Vaginal Delivery	123(87.9%)	125(89.3%)	0.075¶
C-section	17(12.1%)	15(10.7%)	
Oxytocin	76(54.3%)	79(56.4%)	0.405¶
Nicuadmission	8(5.7%)	6(4.3%)	0.832¶
5th minuteApgar<7	9(6.4%)	7(5.0%)	0.226¶

Mean ±standard deviation and number(percentage).*Mann Whitney-U test, ¶Chisquare test. A p value<0.05 is considered statistically significant.

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Table 2. Distribution of non stress test results of groups.

	Nonstress test	Meconium(n:140)	Control (n:140)
Before amniotomy	Reaktive	72 (51.4%)	111 (79.3%)
	Non reaktive	52 (37.1%)	19 (13.6%)
	Early deceleration	16 (11.4%)	10 (7.1%)
After amniotomy	Reaktive	34 (24.3%)	89 (63.6%)
	Non reaktive	44 (31.4%)	30 (21.4%)
	Early deceleration	41 (29.3%)	6 (4.3%)
	Late deceleration	12 (8.6%)	11 (7.9%)
	Variable deceleration	9 (6.4%)	4 (2.9%)

Non-stress test patterns which were performed before amniotomy have been grouped as reactive, non-reactive and early deceleration (Table 2). Fifty two (37.1%) of the nonstress tests in the meconium group were nonreactive, whereas in the control group this number was 19 (13.5%) before amniotomy. This difference was statistically significant (p<0,001). Similarly non-stress test patterns were evaluated after amniotomy and grouped as reactive, non-reactive, early deceleration, late deceleration and variable deceleration (Table 2). When we accepted variable deceleration, late deceleration and non-reactive entity as non-reassuring test; non-reassuring pattern was seen in 62 (44.3%) of the patients in the meconium group and in 21 (15.1%) of the patients in the control group. Non-reassuring nonstress test incidence in the meconium group was statistically significantly higher (p:0.014). Within the meconium group itself; fetal distress was found to be higher in patients with thick meconium than thin meconium (22.8% versus 8.5% p<0,001).

We used the Wilcoxon Signed Ranks test to determine if the non-stress tests were different before and after amniotomy. Non-stress tests were found to be prone to transformed to worse patterns in terms of fetal distress and this statistical analysis was significant for both groups (for meconium group p<0,001, z:-6.6 and for control group p<0,001, z:-3.7). This statistically significant relationship was found to be stronger for the meconium group which is demonstrated with z values.

Incidence of vaginal delivery was 87.9 % in the study group and 89.3 % in the control group. normal birth rates in both groups were statistically similar (p:0.426). A total of 14 newborns (5.0%) were transferred to neonatal intensive care unit. Eight (57%) of these babies were in the meconium group. There was no difference in intensive care need between two groups (p:0.832). Apgar score at 1 and 5 min was lower in the meconium group than in the control group. This difference statistically significant (p <0.001) (Table 1).

Discussion

The presence of MAF during delivery varied between 10% and 16.6% in low risk pregnancies. Meconium Aspiration Syndrome (MAS) is a complication present in MAF and life-threatening disease affecting newborns with meconium staining (15). Meconium can cause umbilical vascular vasospasm and impair fetal-placental blood flow. This is associated with fetal distress and non-reassuring FHR (7,8,9). When we examined the non-stress test patterns of both groups before amniotomy; we have seen that the number of patients with nonreactive pattern was found to be higher in the meconium group (37.1% versus 13.5% p: 0,001).

There was a difference in non-stress test before and after amniotomy for both groups. We observed that the number of non-reassuring non stress tests increased after amniotomy. Also we found that the probability of non reassuring non stres test was higher in pregnant women with meconium-stained amnion fluid after amniotomy (44.3% vs. 15.1 %). Similary, Wong et al. found that the incidence of non-reassuring nonstres test with meconium-stained amniotic fluid was significantly higher (9.8% versus 6.4%) (12). Therefore if meconium is detected after spontaneous or artificial amniotomy clinicians must be carefull in terms of fetal distress following amniotomy. In our study, 87.9% patients in meconium group delivered through normal vaginal delivery, and 12.1% through cesarean section. Contrarily, in the study of Karim et al, 60% patients with meconium stained fluid were delivered through normal vaginal delivery, while 40 % were delivered by caesarean section (16). This may be due to false positive non-stress test results whereas there was no difference between the groups in terms of newborn perinatal outcome.

As a result; performing amniotomy increases the likelihood of non-reassuring non-stress test pattern due to lack of protective effect of surrounding amniotic fluid. Amniotomy

should not be done early during labor progress if possible. If the fetal membrane is spontaneously ruptured; clinicians must be careful with fetal distress establishment in pregnancies with meconium stained amnion fluid.

Conclusion

In our study, we have seen that amniotomy increases the likelihood of non-reassuring non-stress test. Also we found that the probability of fetal distress was higher in pregnant women with meconium-stained amnion fluid after amniotomy. In this case, caution should be taken in terms of fetal distress following spontaneous or artificial amniotomy. It would be better not to do early amniotomy during labor regardless of meconium presence or absence in amniotic fluid .

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References

1. Maymon E, Chaim W, Furman B, Ghezzi F, Shoham Vardi I, Mazor M. Meconium stained amniotic fluid in very low risk pregnancies at term gestation. Eur J Obstet Gynecol Reprod Biol. 1998; 80: 169-7.

2. Karatekin G, Kesim MD, Nuhoglu A. 1999. Risk factor for meconium aspiration syndrome. International Journal of Gynecology and Obstetrics 65:295 – 297.

3. Ahanya SN, Lakshmanan J, Morgan BL, Ross MG. Meconium passage in utero: mechanisms, consequences, and management. Obstet Gynecol Surv 2004; 60: 45-56

4. Cleary GM, Wiswell TE. Meconium-stained amniotic fluid and the meconium aspiration syndrome. An update. Pediatr Clin North Am 1998;45:511–529.

5. Ashfaq F, Shah AA, Effect of aminoinfusion for meconium stained amniotic fluid on perinatal outcome. J Pak Med Assoc 2004; 54: 322-5.

6. Shaikh EM, Mehmood S, Shaikh MJ. Neonatal outcome in meconium stained amniotic fluid- One year experience. J Pak Med Assoc. 2010;60(9):711-14.

7. Balchin I, Whittaker JC, Lamont RF, Steer PJ. Maternal and fetal characteristics associated with meconium-stained amniotic fluid. Obstet Gynecol 2011;117:828-35

8. Khazardoost S, Hantoushzadeh S, Khooshideh M, Borna S. Risk factors for meconium aspiration in meconium stained amniotic fluid. J Obstet Gynaecol 2007; 27(6):577-9.

9. Xu H, Wei S, Fraser WD. Obstetric approaches to the prevention of meconium aspiration syndrome. J Perinatol 2008;28 Suppl 3:S14-8.

10. Mundhra R, Agarwal M. Fetal outcome in meconium-stained deliveries. J Clin Diagn Res 2013; 7: 2874-2876.

11. Sunoo C, Kosasa TS, Hale RW. Meconium aspiration syndrome without evidence of fetal distress in early labor before elective cesarean delivery. Obstet Gynecol 1989;73:7079.

12. Wong SF, Chow KM, Ho LC. The relative risk of 'foetal distress' in pregnancy associated with meconium-stained liquor at different gestation. J Obstet Gynaecol 2002; 22: 594-9.

13. Paz Y, Solt I Zimmer EZ. Variables associated with meconium aspiration syndrome in labour with thick meconium. Eur J Obstet Gynaecol Reprod Biol 2001; 94: 27-30.

14. Becker S, Solomayer E, Dogan C, Wallwiener D, Fehm T. Meconium-stained amniotic fluid-perinatal outcome and obstetrical management in a low-risk suburban population. Eur J Obstet Gynecol Reprod Biol 2007; 132: 46-50.

15. Barbosa da Silva FM, Koiffman MD, Osava RH, Junqueira SMVO, Gonzalez Riesco ML. Centro de Parto Normal como estratégia de incentivo del parto normal: estudo descriptivo. Enferm Glob. 2008;(14):1-13

16. Karim R, Jabeen S, Pervaiz F, Wahab S, Yasmeen S, Raees M. Decreased amniotic fluid index and adverse foetal out come at term. J Postgrad Med Institute 2010; 24: 307-11.

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Sexual abuse and accepting attitudes towards intimate partner rape in Uganda

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Abstract

Objective: The aim was to study sexual abuse, accepting attitudes towards intimate partner rape and psychological concomitants in intimate partner relationships in Uganda.

Method: A questionnaire was completed by 315 respondents (174 females and 141 males). The mean age for females was 31.7 years (SD = 10.3) and 33.6 (SD = 12.4) for males.

Results: Females scored significantly higher than males on victimization from aggression due to denial of sex, victimization from sexual abuse, and psychological concomitants of intimate partner rape. The acceptance rate for rape in intimate relationships was high, only one percent among females and two percent among males reported zero tolerance. Victimization from sexual abuse as well as psychological concomitants of intimate partner rape were significantly higher among respondents who had completed only primary school compared to those with a higher education. Accepting attitudes towards rape in intimate relationships were positively correlated with age, no sex differences were found. Respondents with higher educational levels reported significantly lower levels of acceptance of intimate partner rape. For females, but not for males, accepting attitudes correlated positively with both victimization and psychological concomitants.

Conclusions: Victimization from sexual abuse, psychological concomitants and accepting attitudes towards intimate partner rape were all related to low educational level. Reasons for the high levels of accepting attitudes towards intimate partner rape especially among female victims are discussed.

Keywords: Sexual abuse, Intimate partner rape, Psychological concomitants, Uganda

Introduction

Intimate partner sexual abuse is still not recognised as such in many societies, because of the attitudes, values, and beliefs shaped by deep-rooted traditional norms. In Uganda, women are till commonly viewed as commodities belonging to their partners, and intimate partner sexual abuse is perceived as a bedroom matter that should be kept private. This often breeds among men a sense of entitlement to sexual favours without consent, and sex is viewed as an obligation (1).

Definitions of sexual abuse and rape

Sexual abuse can take on many forms and is defined as any attempted or actual sexual act or unwanted sexual advances or comments directed towards a person's sexuality by means of coercion, threats, blackmail, or psychological intimidation (2). Sexual abuse also involves acts that sexually degrade a person, e.g. intentional harm to someone during sex, such as the inserting of sharp objects vaginally or anally; proceeding to pursue sex even when the victim is not fully conscious; and coercion of individuals into sex without any means of protection against sexually transmitted diseases or pregnancy (3, 4).

There are three core elements characterising legal definitions of rape: (1) penetration, regardless of how slight it was and regardless of whether there was ejaculation or not; (2) lack of consent, or if was with a person who was not capable of giving consent due to mental incapacitation or intoxication; and (3) compelling involvement by either threat, force, or actual bodily harm (5, 3). It has been claimed that the most defining characteristic of rape is the lack of consent or choice by one of the parties during sexual intercourse (5, 3). Rozee (1993) prefers to use the word "choice" rather than consent, because it takes into account also unvoiced disapproval to engage in sexual intercourse.

Research has provided evidence that many non-consensual sexual acts take place within consensual unions such as marriage and long term cohabitation (6). Intimate partner rape is one of the most common types of sexual abuse

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within relationships. It is defined by the victim's relationship to the perpetrator and may include date rape, acquaintance rape, and marital rape (7).

Marital rape is in some cultures a new concept. The traditional definition of rape excluded that by intimate partners and it was only considered rape if the offender was unknown to the victim (8). Before the late 1970s, marital rape was not recognised by definitions in the United States which narrowed rape to "unconsented sexual intercourse by a man with a female who is not his wife" (9). For example in India, the penalty for rapists is the death sentence, yet there is no crime committed if a husband rapes his wife (10).

Marital rape includes acts of unwanted or forced sexual contact by a spouse, be it vaginal, anal, or oral; most times, it involves penetration. It can be achieved by means of physical aggression and force, by means of a threat, or when the spouse due to certain circumstances is unable to reject the sexual assault (11).

Cultural definition of marital rape

The biggest obstacle to the criminalisation of marital rape is the clash with cultural norms and values. Defining what "using force" entails in intimate partner relationships, especially in the context of wife rape, has proved challenging. This is because a woman's history of being dominated, intimidated, or battered by her husband may shape her beliefs regarding what and how a normal sexual encounter should be (12). Social coercion is reinforced by societal messages regarding appropriate sex roles for men and women within marriage. For instance, it has been found that many survivors of wife rape believed it was a wife's duty to submit to a sexual act or intercourse, regardless of their own desires (8).

Religion, family, and other cultural norms also play an important role in this context. When women attempt to discuss sexual assaults with friends, family, or service providers, they may be blamed, mocked, or not taken seriously (11). Non-consensual sex in marriage is in many cultures viewed as merely claiming a conjugal right and society considers it normal (3). Some of the arguments put forward over the years for exempting husbands from rape charges include: (a) it is a hard case to prove, (b) the exemption protects husbands from malicious and vindictive wives, (c) a marital relationship should be kept private, and (d) marriage has ups and downs, and a criminal charge like rape discourages reconciliation between spouses (13).

There are myths about masculinity and rape that are risk factors for marital rape, e.g. notions like, "boys will always be boys", and that men are in most cases at the "mercy of their sexual drives" (14). Such myths and beliefs create a conductive environment for rape to take place, and sexual coercion is seen as inevitable, therefore acceptable behaviour. In societies where such beliefs are promoted, there are more cases of marital rape (15). The aftermath of rape is commonly characterised by blame, confusion, and guilt felt by the victims. These feelings are also usually reinforced by the reactions they receive from family and friends, such as questions about how they were dressed, or whether they were intoxicated. The victims are often blamed even by the people they would normally rely on for support (16).

Prevalence of sexual violence and rape

In a survey carried out in the USA, nearly 61.9% of the adult female participants had been raped by a former or current boyfriend, cohabiting partner, spouse or date (17). In the United Kingdom, national surveys have estimated the prevalence of intimate partner sexual abuse among women to be 14.2% (18). In Uganda, a report by UN Women (2016) (19) estimated the prevalence of physical or sexual violence against women from an intimate partner to be 51%.

Most victims usually experience multiple forms of abuse, with a substantial fraction experiencing both physical and sexual abuse, as evidenced in a study carried out in the USA where an estimated 40–52% of women who experienced intimate partner physical violence also experienced sexual abuse (20). It has been argued that incidents of intimate partner sexual abuse are under-reported, and that the estimates are not even close to the actual prevalence rates (8).

Psychological concomitants of sexual abuse and rape

It is stated in DSM-5 that both experiencing and witnessing a traumatic event trigger feelings of horror, helplessness, and fear as response to actual or perceived threat of death or injury (21). Victims from intimate partner sexual abuse may experience severe and usually long-term psychological consequences due to being violated by someone they love and trust (22). They may experience symptoms such as panic attacks, generalised anxiety disorder, substance abuse, depression, and suicide attempts or actual suicide, all which can also be indications of Post-Traumatic Stress Disorder (PTSD) (23).

Finkelhor and Yllo (1985) (12) found that marital rape victims had developed similar or even more severe psychiatric disorders than stranger rape victims, a fact that dismisses the myth that marital rape is a less traumatic event. Like other types of rape, marital rape is a personal violation of the victim's body, but specific to marital rape is also a violation of trust at a deeper level. In cases of stranger rape, the perpetrator and the victim do not know each other, but in wife rape cases, the assault becomes personal to the victim (12).

Legal prohibition of marital rape in Uganda

For decades in Uganda, the Marriage and Divorce Bill, which also includes a clause on marital rape, has been awaiting to be approved by the parliament (24). The history of the bill is quite troubled. The first version of it was tabled in the 1970s, and about forty years later it was re-tabled in 2003, as it was again in 2006, 2009, and 2013, consecutively. The bill still remains unpassed. Some provisions in the bill are considered controversial, hence the conflicting interests are witnessed every time it is tabled in parliament. From its first tabling in the 1970s, the bill

was designed to help improve women's rights in marriage (25).

Material and Method

Sample

A questionnaire was filled in by 315 Ugandan respondents, 174 females and 141 males. The age range was between 18 and 80 years. The mean age for females was 31.7 years (SD = 10.3) and 33.6 (SD = 12.4) for males, the age difference was not significant. Of the respondents, 71.7% were born in urban areas, and 28.3% in rural areas; 33.3% were married, 24.1% were cohabiting, 18.1% were in a relationship but not married, and 24.4% were single but had previously had a partner.

The respondents with a partner had been cohabiting between one and 61 years (m = 8.9 years, SD = 9.8). Of the respondents, 16.2% had one child, 16.8% had two children, 8.6% had three children, and 6.2% had eight or more children. In the sample, 18 different tribes were represented: Muganda (54.3%), Musoga (11.1%), Munyankole (7.0%), Mugisu (6.0%), Mutooro (4.4%), Itesot (3.5%), Acholi (3.2%), Mukiga (2.2%), Munyoro (1.9%), Langi (1.3%), Alur (1.0%), and less than 1%: Mudaama, Munyole, Kakwa, Lugbala, Mugwere, Mukonjo, and Musamya. Of the respondents, 77.8% were Christians, 19.7% were Moslems, 1.0% adhered to a raditional African religion, and 1.6% were atheists.

The educational level of the respondents was as follows: no education (5.7%), primary school level (14.0%), lower secondary level (14.3%), upper secondary level (18.1%), diploma or vocational training (11.4%), Bachelor's degree (32.4%), and Master's degree or higher (4.1%).

Instrument

The questionnaire included scales for measuring (a) victimization from aggression due to denial of sex, (b) victimization from sexual abuse in intimate relationships, (c) psychological concomitants of intimate partner rape, and (d) accepting attitudes towards rape in intimate relationships (Table 1).

The response alternatives were on a five-point grading scale, ranging from never (0) to very often (4) for the scales of (a) victimization from aggression due to denial of sex, (b) victimization from sexual abuse in intimate relationships, (c) psychological concomitants of intimate partner rape, and strongly disagree (0) to strongly agree (4) for (d) accepting attitudes towards rape in intimate relationships.

The construction of the scales were inspired by articles by Rondenburg and Fantuzzo (1993) (26), Perilloux, Duntley and Buss (2012) (27), Faravelli, Giugni, Salvatori and Ricca (2004) (28), and Mahoney and Williams (1998) (1), although the scales were not fully based on any of them. Single items and Cronbach's alphas of the scales are presented in Table 1.

Procedure

An online questionnaire was constructed with Google Drive. Since the topic of the study was intimate partner sexual abuse, participation was not limited to married couples but also unmarried participants were invited. A link to the questionnaire was published electronically; a paper version was also used. The link was active between 13.12.2016 and 8.2 2017 and spread via snowball sampling on WhatsApp and Facebook. Email channels like Google mail and Yahoo were also used. The paper version was made available for distribution on 20.12.2016, and the questionnaires were hand delivered to participants in Uganda. The respondents were mainly found in a medical clinic in Kampala. The electronic version of the questionnaire was filled in by 40 respondents, and 275 respondents completed the paper version.

Ethical considerations

The study adheres to the principles concerning human research ethics of the Declaration of Helsinki (29) adopted by the World Medical Association, as well as guidelines for the responsible conduct of research of the Finnish Advisory Board on Research Integrity (2012) (30).

Table 1: Single items and Cronbach's Alphas for the scales in the study (N = 315)

Scales
Victimization from Aggression due to Denial of Sex (5 items, α = .79)
a) Have you ever been afraid to say no to sexual engagements with a partner?
b) Have you ever been called a horrible person after denying your partner sexual acts and favours?
c) Have you ever been humiliated in public because of denying sexual favors to a partner?
d) Has a partner ever been aggressive when demanding for sex?
e) Has a partner ever become very upset and hit, slapped or kicked you when you denied him/her sexual favors?
Victimization from Sexual Abuse in Intimate Relationships (5 items, α = .79)
a) Has a partner ever attempted to rape you but for some reason he/she was not successful?
b) Have you ever been forced by any partner into unwanted sexual acts?
c) Has a partner ever put their arms around your neck to choke you in order to forcefully have sex with you?
d) Has a partner ever sexually abused you after giving you alcohol and drugs?
e) Has a partner ever threatened to hurt you with a weapon/object or thrown objects at you in order to have sex with you?

Psychological Concomitants of Intimate Partner Rape (10 items, α = .96)
- a) Have you ever been depressed following forced sexual acts by a spouse?
- b) Have you ever felt hopeless and helpless following forced sexual acts from a partner?
- c) Have you ever felt "dirty" and worthless due to forced sexual acts by a partner?
- d) Have you ever experienced anxiety as a result of forced sexual acts from a partner?
- e) Have you ever experienced a feeling of constantly being on guard, watchful, or easily startled following a rape from a partner?
- f) Have you ever had nightmares following a rape from a partner?
- g) Have you ever experienced sleepless nights or bad sleeping habits following forced sexual acts from a partner?
- h) Have you ever experienced spells of terror and panic due to having experienced forced sex from a partner?
- i) Have you ever experienced a feeling numbness or being detached from others, activities, or surroundings after a rape from a partner?
- j) Have you ever thought about ending your life as result of forced sex from a partner?

Accepting Attitudes towards Rape in Intimate Relationships (14 items, α = .84)
- a) Having sex without consent is sometimes acceptable in a relationship.
- b) Rape by a partner only happens to women, men cannot be raped.
- c) When it comes to sex, women mean "yes" even when they say "no".
- d) There is no such thing as rape when in a relationship, since partners have sexual rights over each other.
- e) Sex without consent can be excused or forgiven if the partner is intoxicated.
- f) Sex without consent is more acceptable in marriage than when partners are not married or do not know each other.
- g) It stops being rape if it is enjoyed by both partners at the end of it all.
- h) Regular sex, even when sometimes against the wish of a partner, is after all healthy for the survival of a relationship.
- i) Some partners call for rape through teasing and dressing provocatively.
- j) It can only be considered a rape by a partner if excessive force and some sort of physical aggression was used.
- k) Using coercion or physical restraint is a legitimate way to acquire sex from a certain type of partners.
- l) Partners who lead others on deserve less sympathy when raped.
- m) Being legally prosecuted as a result of unwanted sexual acts with a long term partner like a wife is ridiculous.
- n) Respectable men and women do not experience intimate partner sexual abuse.

Results

Correlations between the scales

For females, all four scales of the study correlated significantly with each other at a $p < .001$-level (Table 2). The highest correlational coefficients were found between psychological concomitants of intimate partner rape and victimization from aggression due to denial of sex ($r = .88$), and victimization from sexual abuse in intimate relationships ($r = .85$). Accepting attitudes towards rape in intimate relationships was found to correlate positively with both victimization and psychological concomitants. For males, the scales measuring victimization and concomitants were intercorrelated, but in contrast to the results for females, accepting attitudes towards rape in intimate relationships did not correlate with the other scales (Table 2).

Correlations with age

Victimization from aggression due to denial of sex, victimization from sexual abuse in intimate relationships, and psychological concomitants of intimate partner rape did not correlate with age of the respondents, whereas accepting attitudes towards rape in intimate relationships was positively correlated with age ($r = .22$, $p < .001$).

Sex differences

It was found that among females, 36.2% were never victimized from aggression due to denial of sex, 47.7 % were never victimized from sexual abuse in intimate relationships, 50.6% did not suffer from any psychological concomitants of intimate partner rape, and 1.1% had zero tolerance for accepting rape in intimate relationships. Among males, 41.1% were never victimized from aggression due to denial of sex, 52.5% were never victimized from sexual abuse in intimate relationships, 75.2% did not suffer from any psychological concomitants of intimate partner rape and, 2.1% had a zero tolerance for accepting rape in intimate relationships.

A multivariate analysis of variance (MANOVA) was conducted with sex as the independent variable and the four scales as dependent variables. The multivariate test was significant. The univariate tests showed that females scored significantly higher on victimization from aggression due to denial of sex, victimization from sexual abuse in intimate relationships, and psychological concomitants of intimate partner rape. No sex differences were found regarding accepting attitudes towards rape in intimate relationships (Table 3, Fig. 1).

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Table 2: Correlations between the scales in the study, for females (N = 174) below and for males (N = 141) above the diagonal

	1.	2.	3.	4.
1. Victimization from aggression due to denial of sex		.77 ***	.58 ***	-.00 *ns*
2. Victimization from sexual abuse in intimate relationships	.85 ***		.49 ***	.01 *ns*
3. Psychological concomitants of intimate partner rape	.88 ****	.84 ***		.05 *ns*
4. Accepting attitudes towards rape in intimate relationships	.41 ***	.32 ***	.45 ***	

Table 3: Results of a multivariate analysis of variance (MANOVA) with sex as independent variable and four scales as dependent variables (N = 315). C.f. Fig. 1.

	F	*df*	$p \leq$	η_p^2	Group with higher mean
Effect of Sex					
Multivariate analysis	14.43	4, 309	.001	.157	
Univariate analyses					
Victimization from aggression due to denial of sex	10.43	1, 312	.001	.032	Females
Victimization from sexual abuse in intimate relationships	7.97	"	.005	.025	Females
Psychological concomitants of intimate partner rape	39.05	"	.001	.111	Females
Accepting attitudes towards rape in intimate relationships	0.54	"	*ns*	.002	-

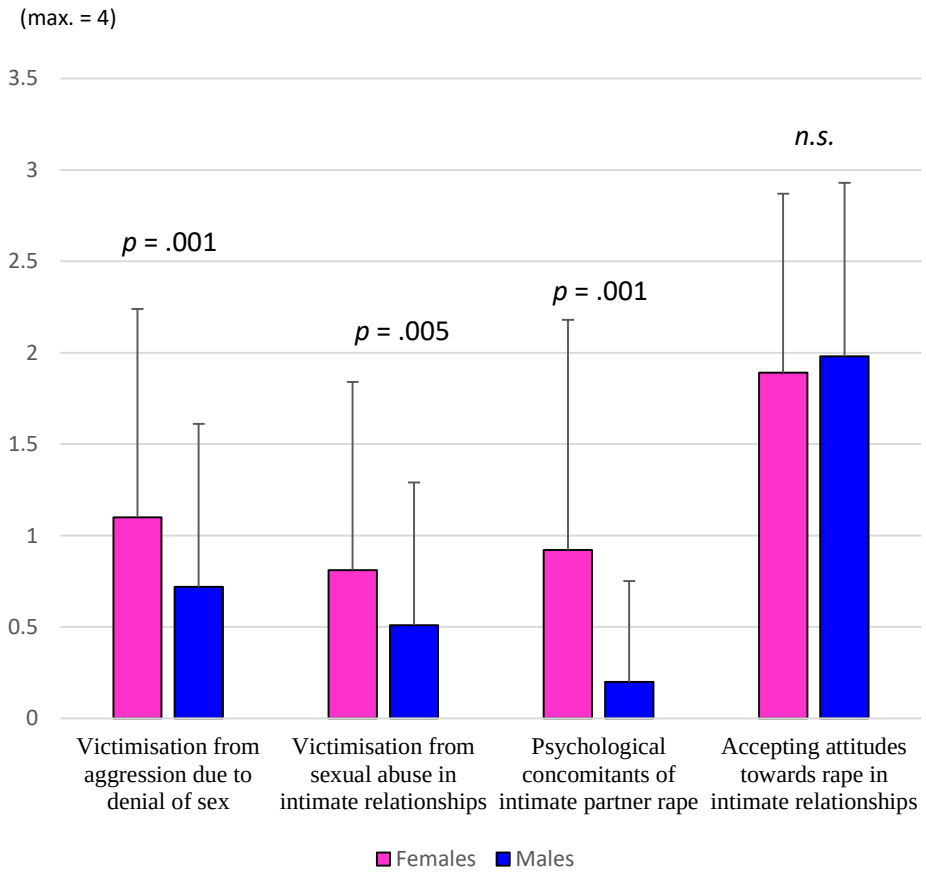

Figure 1: Mean values on four scales related to rape and sexual abuse in intimate relationships for females and males (*N* = 315). C.f. Table 3.

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Education

A multivariate analysis of variance (MANOVA) was conducted with educational level as the independent variable and the four scales as dependent variables. The multivariate test was significant (Table 4, Fig. 2).

Respondents with a Master's degree or higher reported significantly lower levels of accepting attitudes towards rape in intimate relationships than for respondents with no education, primary school level, or lower secondary level education. Respondents with a primary school education reported significantly higher accepting attitudes towards rape in intimate relationships as than for respondents with upper secondary level or higher education.

The univariate tests were significant for all four scales. Scheffé's test revealed no significant differences for victimization from aggression due to denial of sex between respondents of different educational levels. Victimization from sexual abuse in intimate relationships was significantly higher among respondents who had completed only primary school in comparison with those who had a Bachelor's degree. The level of psychological concomitants of intimate partner rape were significantly higher for those who had only primary school than for those with an upper secondary level, a Bachelor's, or a Master's degree or higher.

Table 4: Results of a multivariate analysis of variance (MANOVA) with level of education as independent variable and four scales as dependent variables (N = 315). C.f. Fig. 2).

	F	*df*	$p \leq$	η_p^2
Effect of Education				
Multivariate analysis	3.65	24, 1228	.001	.067
Univariate analyses				
Victimization from aggression due to denial of sex	2.62	6, 307	.017	.049
Victimization from sexual abuse in intimate relationships	2.87	"	.010	.053
Psychological concomitants of intimate partner rape	5.81	"	.001	.102
Accepting attitudes towards rape in intimate relationships	11.64	"	.001	.185

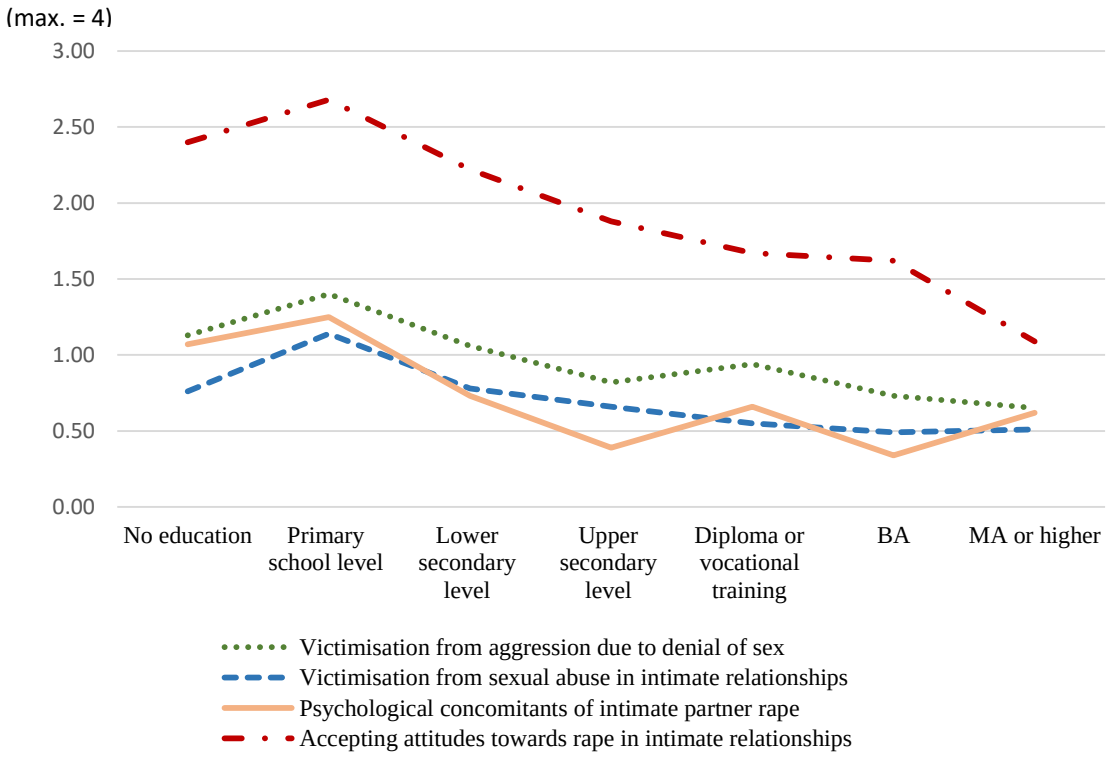

Figure 2: Mean values on four scales related to rape and sexual abuse in intimate relationships for respondents on different educational levels (N = 315). C.f. Table 4.

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Discussion

It was found that females were the main victims from sexual abuse in intimate relationships. Still, it should be noted that also males were frequently victimized. In the sample, more than half of the males (59%) had, to some degree been victimized by their female partner's aggression due to denial of sex, and almost half of them (48%) were also victimized from sexual abuse. Furthermore, one male in four (25%) suffered, to some extent, from psychological concomitants of intimate partner rape.

Victimization from aggression due to denial of sex

It was found that females scored significantly higher than males on victimization from aggression due to denial of sex. For both females and males, a correlation was found between denying an intimate partner sex and victimization from sexual abuse by the partner. In Uganda, it is common to think that denying a husband sex strips him of his masculinity, since a wife are seen as a mere personal possession. This circumstance may spark feelings of anger and threaten the husband's ego; hence he uses force to acquire sex regardless of whether his wife consents or not. Finkelhor and Yllo (1985) (12), found that denial of sex in intimate relationships was seen by men as emasculating them, and as a lack of appreciation by their spouses, which brought up feelings of anger.

The study, however, found that also females victimized their partner from sexual abuse when denied sex. In the cultural context of Uganda, a man is supposed to satisfy his spouse's sexual needs at all times, and denying a partner sex could be met with aggression. Denying a woman sex could signify that she is no longer physically attractive, sexually pleasing, or that the man is getting sex from elsewhere. In such instances, verbal attacks usually belittle the men, and they may be accused of having low libido, small penises or extra-marital affairs. If accusations are frequent, it may well lead to psychological consequences like stress and depression.

Education

The findings revealed that the higher the level of education, the less exposure to intimate partner sexual abuse. Victimization from sexual abuse in intimate relationships was significantly higher among respondents who had completed only primary school or had no education at all. Educated women are able to compete in the labor market, hence giving them economic empowerment and the ability to financially support their families. Financially stable women are an asset to their husbands, and they are usually respected and probably therefore less exposed to physical and sexual abuse. Because of the high victimization level among the less educated, psychological consequences of intimate partner abuse were significantly higher for those who had only a primary education than for those with a higher education. However, economic independence and education do not per se guarantee protection from intimate partner sexual abuse.

Accepting attitudes towards rape in intimate relationships

It was found that accepting attitudes towards rape in intimate relationships correlated with all the other scales for females, but not for males. This finding indicates that also victimized women held accepting attitudes towards sexual abuse, yet one would think they would be less tolerant and accepting towards such acts since they have experienced the abuse first hand, and therefore should know the pain and suffering associated to it. Some victims probably choose to cope with sexual abuse from a spouse by rationalizing it. In many cases, victims of sexual assault by an intimate partner are willing to accept the victimization due to the fear of being betrayed if she denies intercourse, especially if the victim relies on the perpetrator for economic and emotional support. They therefore minimise the assault in order to make it bearable. In a stranger rape scenario, the victim is only left with memories of the horrible encounter, while in the case of rape by an intimate partner, the victim has to deal with seeing and living with the perpetrator.

Contrary to expectations, no sex difference was found regarding accepting attitudes towards rape in intimate relationships. The finding that women had equally accepting attitudes might be explained by cultural beliefs. In Uganda, women are often advised by their parents to cater for their husbands' sexual needs at all costs, otherwise the husband will find another spouse. Terms such as "wifely duties" are a clear indication of seeing sexual relations between intimate partners as mere chores and obligations.

Religion also plays central role in this. Some married people believe that marriage vows oblige spouses to give in sexually at all times, therefore a sexual encounter within a marriage, regardless of its nature, can never be considered assault or rape. Furthermore, the concept of sexual abuse in the context of marriage is very unclear to many Ugandans, regardless of level of education or status in society. Many wonder if there is such a thing as marital rape at all, and they have a difficult time comprehending how a wife or a husband can be raped. Russell (1990) (8) received similar findings in his study on wife rape, noting that many victims were hesitant to label sexual coercion by spouses as sexual assault or rape.

Accepting attitudes towards rape in intimate relationships were found to correlate positively with age. The older the respondents were, the more accepting they were towards sexual abuse in a partnership. The failure to recognise it as abuse lies in longstanding cultural beliefs on sexuality and the self-image of men and women in Ugandan society. Women's sexuality is seen as a mere commodity, and it is also believed that rape in marriage is unnecessary if the woman plays her role of offering her husband his well-deserved sexual entitlement. The older generation is protective of cultural beliefs in a now seemingly Westernised society, while among the younger generation, many regard cultural traditions as outdated.

Conclusion

The study reaffirmed that sexual abuse in intimate partner relationships is prevalent in the Ugandan society and needs to be addressed. The marital rape clause in the domestic bill is therefore overdue.

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Ethical issues: All Authors declare, Originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

References

1 Mahoney P Williams LM. Sexual assault in marriage: Prevalence, consequences and treatment of wife rape. In: Jasinski JL, L. M. Williams LM, editors. Partner violence: A comprehensive review of 20 years of research (pp. 113-163). Thousand Oaks, CA: Sage; 1998.

2 World Health Organisation. World report on violence and health. Geneva, Switzerland: WHO Library Cataloguing-in-Publication Data; 2002.

3 Rozee PD. Forbidden or forgiven? Rape in cross-cultural perspective. Psychol Women Q 2002;17:499-514.

4 Searles P, Berger RJ. The current status of rape reform legislation: An examination of state statutes. Women's Rights Law Report 1987;10:25-43.

5 Minturn L, Grosse M, Haider S. Cultural patterning of sexual beliefs and behavior. Ethnol 1969;8:301-318.

6 Weiss E, Gupta GR. Bridging the gap: Addressing gender and sexuality in HIV prevention. 1998 Washington DC: International Center for Research on Women.

7 Koss M, Gidycz C, Wisniewski N (1987). The scope of rape: Incidence and prevalence of sexual aggression and victimization in a national sample of higher education students. J Consult Clin Psychol 1987;55:162-170.

8 Russell DEH. Rape in marriage. 1990 New York: Macmillan Press.

9 Barshis V. The question of marital rape. Women's Stud Int Forum 1983;6:383-393.

10 Toubia N. Female genital mutilation: A call for global action. 1993 New York: Women Ink.

11 Bergen RK. Wife rape: Understanding the response of survivors and service providers. 1996 Thousand Oaks, CA: Sage.

12 Finkelhor D, Yllo K. License to rape: Sexual abuse of wives. 1985 New York: Holt, Rinehart & Winston.

13 Augustine RI. Marriage: The safe haven for rapists. J Fam Law 1990-1991;29:559-590.

14 Frese B, Moya M, Megias J. Social perception of rape: How rape myth acceptance modulates the influence of situational factors. J Interpers Viol 2004;19:143-161.

15 Kirkpatrick C, Kanin E. Male sex aggression on a university campus. Am Soc Rev 1957;22:52-58.

16 Massaro T. Experts psychology, credibility and rape: The rape trauma syndrome issue and its implications for expert psychological testimony. Minnesota Law Review 1985;69:395-470.

17 Tjaden P, Thoennes N. Full report of the prevalence, incidence, and consequences of violence against women. Washington, DC, US Department of Justice, Office of Justice Programs; 2000.

18 Mooney J. The hidden figure: Domestic violence in north London. London, UK: Middlesex University; 1993.

19 UN Women. Global database on violence against women. 2016. http://evaw-global-database.unwomen.org/fr/countries/africa/uganda?formofviolence

20 Campbell JC, Soeken KL. Forced sex and intimate partner violence: Effects on women's risk and women's health. Viol Women 1999;5:1017-1035. doi:10.1177/10778019922181608

21 American Psychiatric Association. Supplement to diagnostic and statistical manual of mental disorders (5th ed.). Am Psychiatric Pub 2017. https://psychiatryonline.org/pb-assets/dsm/update/DSM5Update_October2017.pdf

22 Frieze IH. Investigating the causes and consequences of marital rape. Signs 1983;8:532-553.

23 Kessler RC, Sonnega A, Bromet E, Hughes M, Nelson CB. Posttraumatic stress disorder in the national comorbidity survey. Arch of General Psychiatr 1995;52:1048-1060.

24 Wanjala CW. Marital rape: Is it a crime or a conjugal right? Daily Monitor; 2013 March 16. http://www.monitor.co.ug/SpecialReports/Marital-rape--Is-it-a-crime-or-a-conjugal-right-/688342-1720960-item-00-wujcet/index.html

25 Polavarapu A. Uganda's Marriage and Divorce Bill on the table again. IntLawGrrls 2013. https://ilg2.org/2013/03/10/ugandas-marriage-and-divorce-bill-on-the-table-again/

26 Rondenburg FA, Fantuzzo JW. The measure of wife abuse: Steps toward the development of a comprehensive assessment technique. J FamViol 1993;8.203-228.

27 Perilloux C, Duntley JD, Buss DM. The cost of rape. Arch of Sex Behavior 2012;41:1099-1106.

28 Faravelli C, Giugni A, Salvatori S, Ricca V. Psychopathology after rape. Am J Psychiatr 2004;65:634-651.

29 World Medical Association. Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. JAMA 2013;310:2191-2194. https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/

30 Finnish Advisory Board on Research Integrity. Responsible conduct of research and procedures for handling allegations of misconduct in Finland. Helsinki: Finnish Advisory Board on Research Integrity. 2012.

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